Mathematics in cancer as a population ecology problem– bridging scales

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Origins in a failed experiment

- IDEA – Use tumor cells obtained through CT guided biopsies of primary and metastatic tumor for diagnosis to develop primary cell cultures and test the inhibitory effects of chemotherapeutic agents in-vitro to predict clinical outcomes analogous to sensitivity testing of cultured bacteria to antibiotics
- METHODS- Disperse material from CT guided aspiration of probable metastatic colon cancer into small cellular aggregates and seed in culture flasks containing DMEM with 20% FBS plus antibiotics

- RESULTS-Initial good tumor growth forming monolayers in about 2 weeks but with observable “islands” of normal fibroblasts. Islands then expanded rapidly and invariably overgrew the culture dish destroying all the tumor cells.
- RESPONSE – kill fibroblasts!
- EVENTUAL RESPONSE- why?? No good answer – lots of data but no organizational framework.
- SOLUTION-Mathematical models
- FUNDAMENTAL FLAW – linear intuitive thinking, reasoning by analogy but bacterial infection is typically a short-term, linear disease, cancers are chronic and dominated by non-linear processes.

- “In the absence of consistent application of rigorous mathematical models, theoretical medicine will largely remain empirical, phenomenological and anecdotal, successful only in linear systems that can be defined by a single experiment or a few experiments.”

- Gatenby and Maini in *Nature*, 2002

Application of mathematics to biology and medicine is philosophically appealing

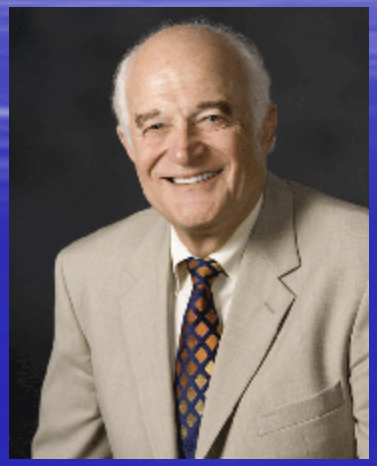
- "I believe the day must come when the biologist will - without being a mathematician - not hesitate to use mathematical analysis when he [sic] requires it". Karl Pearson (Nature, 1901)

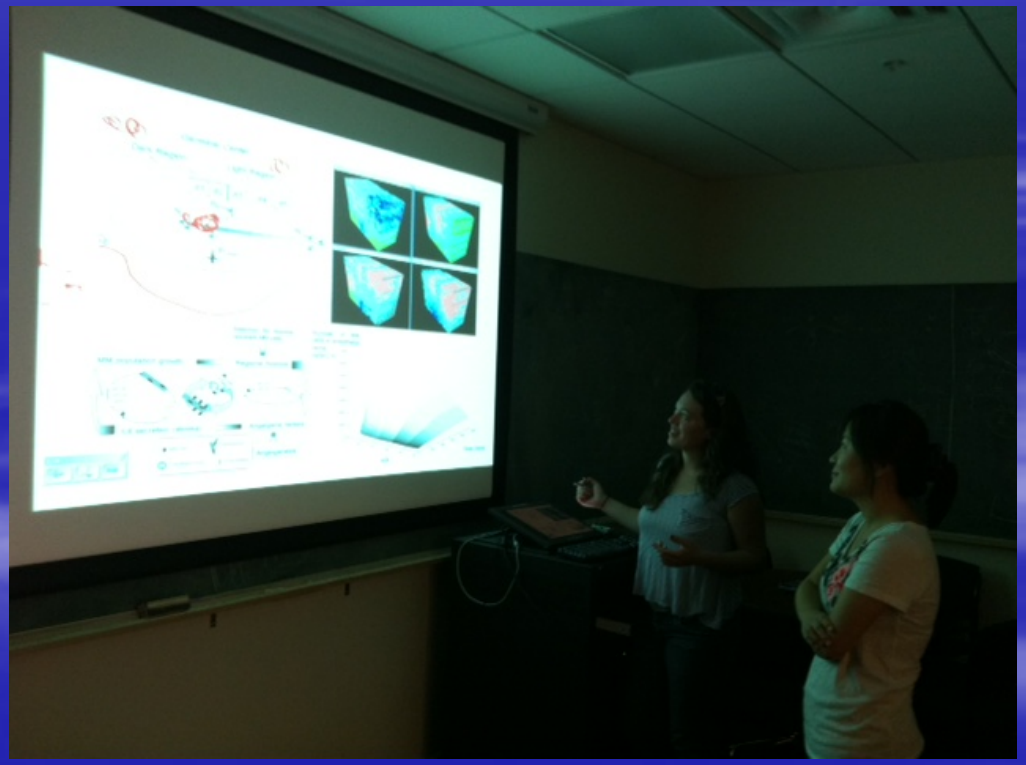
- “If cancer is ever to be understood properly, mathematical models such as these will surely play a prominent role. “ Economist, 2004

But enthusiasm is hardly universal…

- "I don't believe in mathematical modeling."
- "Mathematical models are for researchers too lazy to do the experiments."
- "The PI *opines* mathematical models can describe tumor invasion – this is patently absurd."
- "This paper does not belong in Clinical Cancer Research"

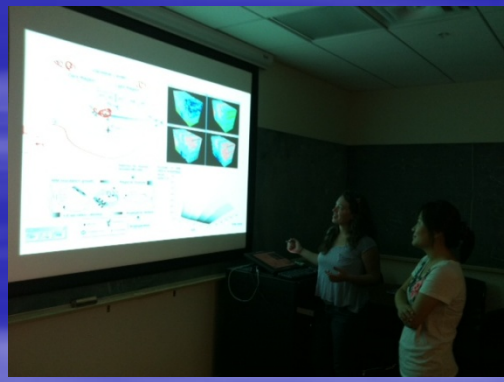
The Integrative Mathematical Oncology Program at Moffitt: Motive, Means, and Opportunity





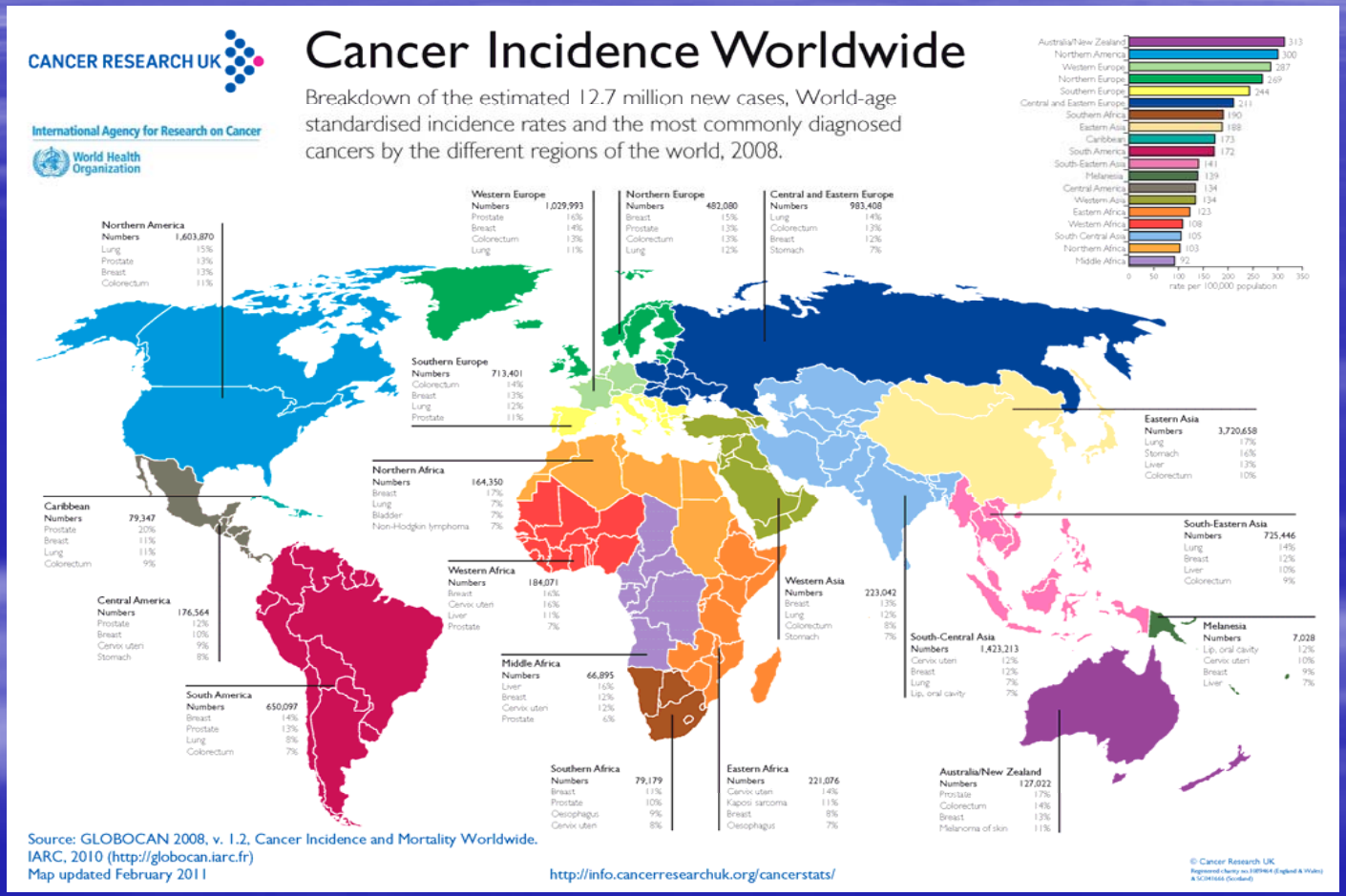
Moffitt Integrated Mathematical Oncology Dept. – The physics paradigm, finding first principles

- Cancer is complicated and complex but not incomprehensible!
- First principles will exist
- Quantitative models linked to experimental and clinical data are necessary to define tumor dynamics
- Evolution provides a unifying framework

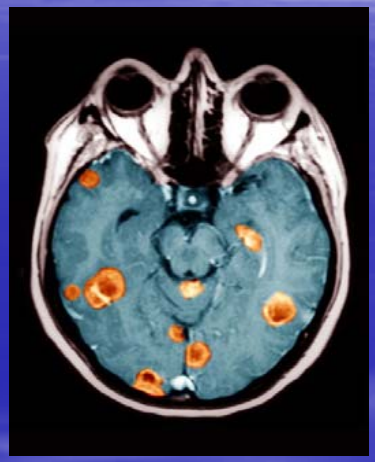


Cancer – a multiscale problem

Population: In 2008, world cancer deaths estimate at 7.6 million. Current projections: 17million in 2030

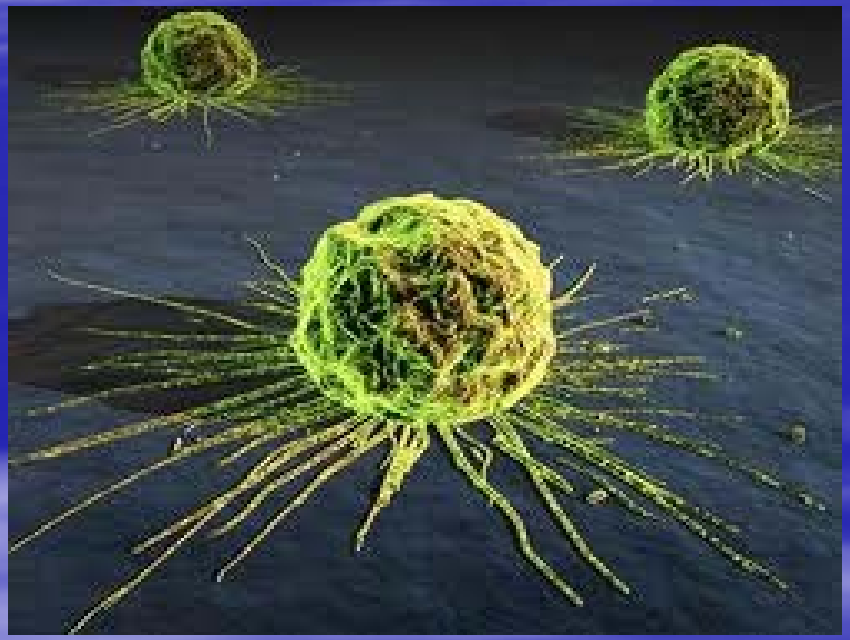


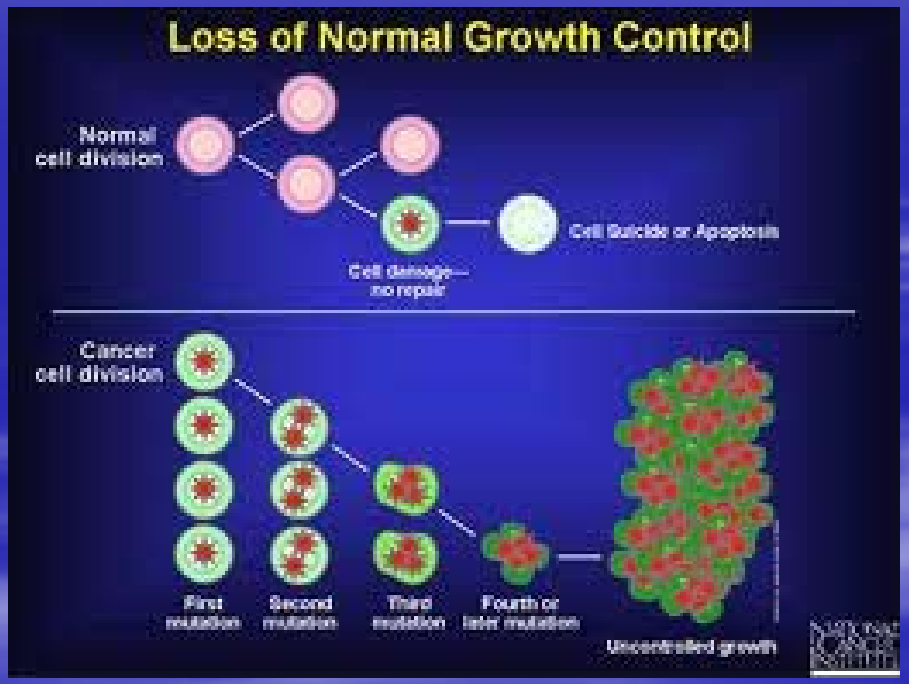
Individual patient – “the personal and societal burden of cancer.”



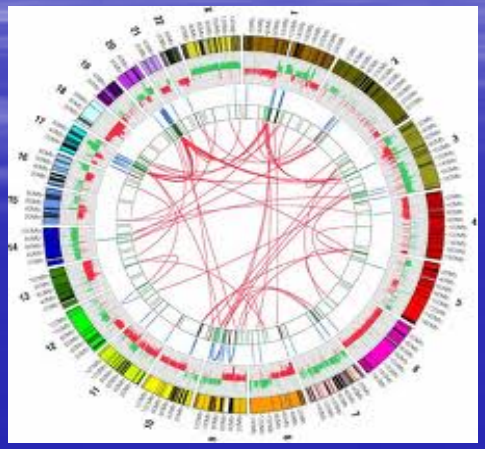
Site	All stages	Local	Regional	Distant
Breast (female)	86.6	97.0	78.7	23.3
Colon and rectum	62.3	90.1	65.5	9.2
Liver	6.9	16.3	6.0	1.9
Lung and bronchus	14.9	48.7	16.0	2.1
Melanoma	89.6	96.7	60.1	13.8
Ovary	53.0	94.7	72.0	30.7
Pancreas	4.4	16.6	6.8	1.6
Prostate	97.5	100.0	--	34.0
Testis	95.5	99.1	95.0	73.1

Individual cells

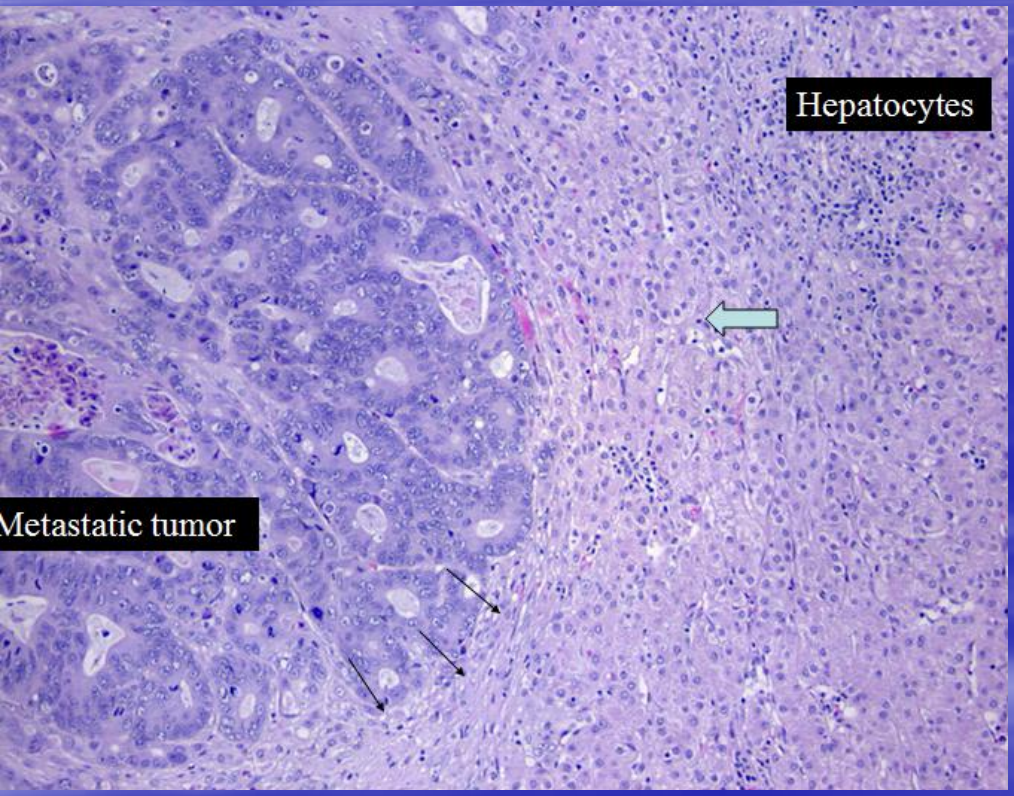


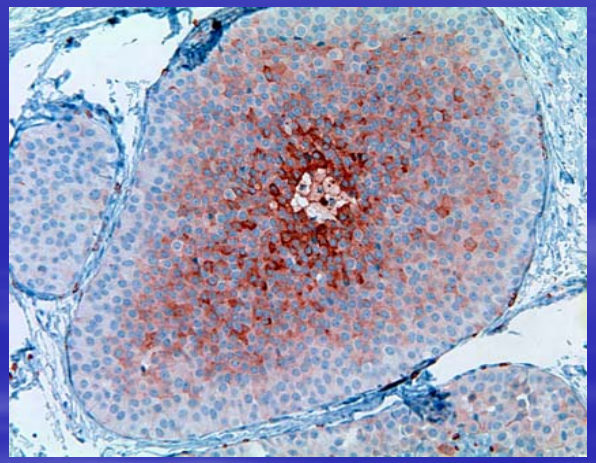


Individual genes



Focus on tissue level interactions





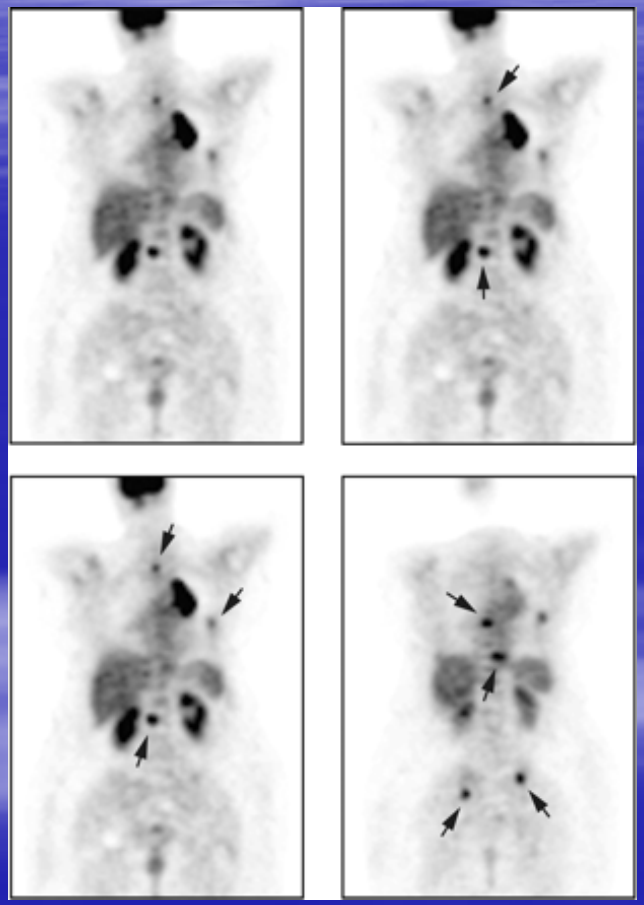
Strategy for developing mathematical models of tumor invasion

Treat tumor as a biological invasion. That is, the tumor cells represent a foreign "species" that begin as a small population of cells (perhaps one) but, because of competitive advantages over normal tissue, proliferate rapidly driving the normal cells to extinction.

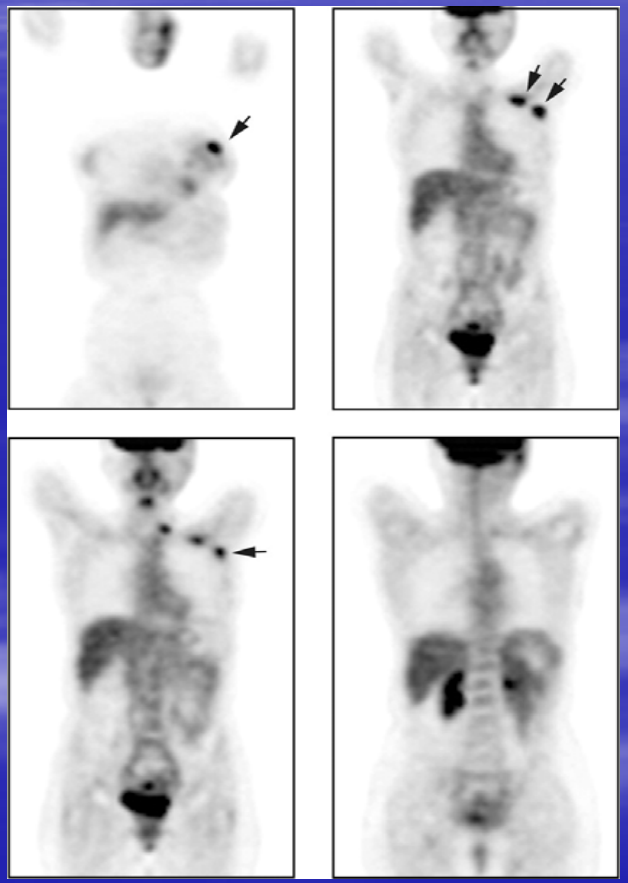
Apply models models from population biology to invasive cancer

What are the competitive advantages that transformation confers on tumor cells that allow unbounded growth? "One species invades another only by killing its young or stealing its food" - Schaefer

Speaking of food, consider glucose metabolism in cancers



Recurrant Melanoma



Breast w/ equivocal MRI

Cancers with elevated Fd Glucose uptake:

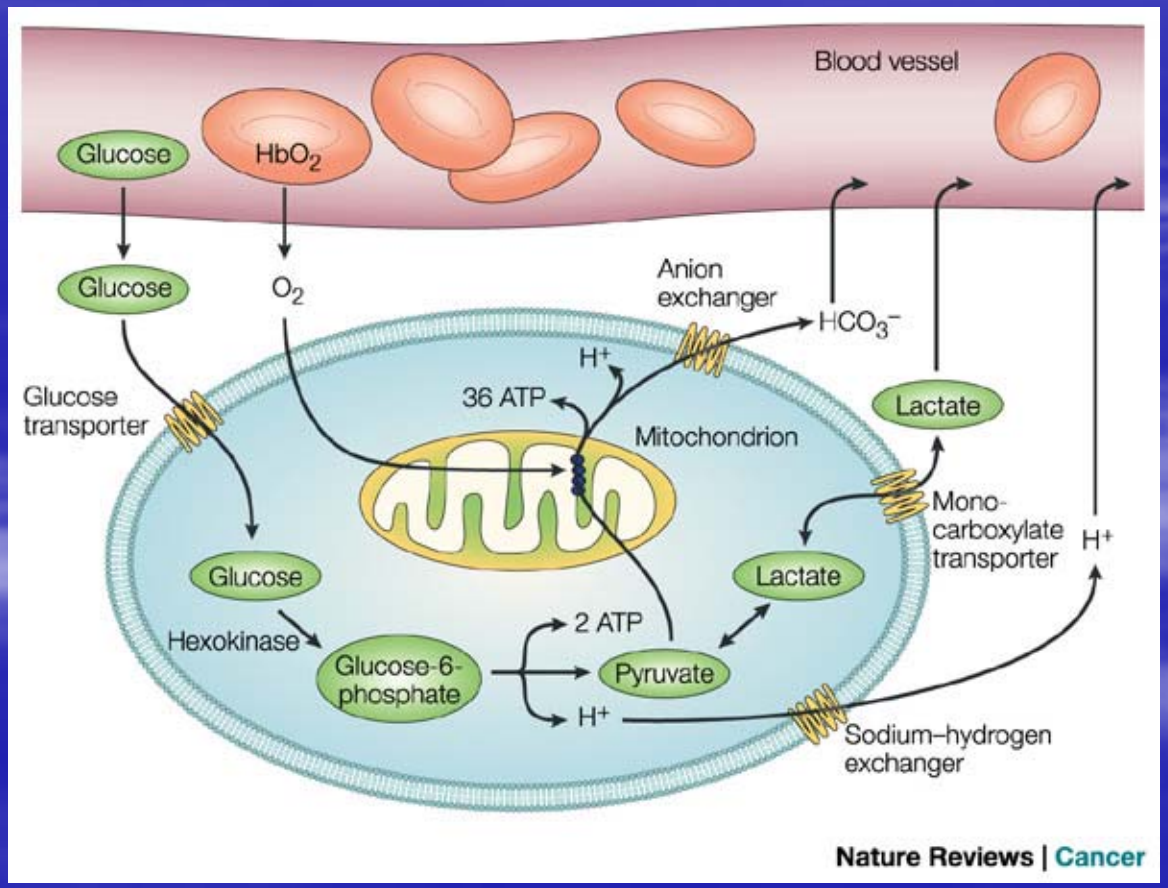
- Colorectal
- Brain
- Breast
- Melanoma
- Cervical
- Ovarian
- Lung
- Pancreatic
- Esophageal
- Lymphoma
- Leimyosarcoma
- H&N

Anderson & Price (2000) E.J. Cancer

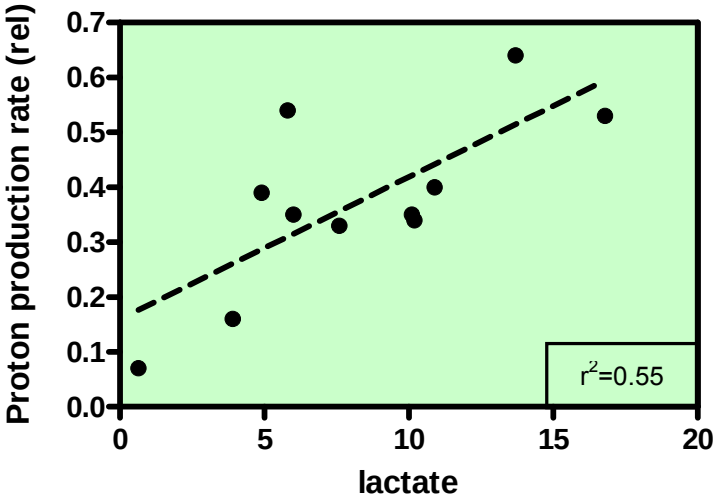
Czernin & Phelps. (2002) Ann. Rev. Med. 53:89-112

" tumors have a remarkable capacity to ferment glucose even in the presence of adequate oxygen"

- Otto Warburg, 1934



High glycolysis leads to increased acid production



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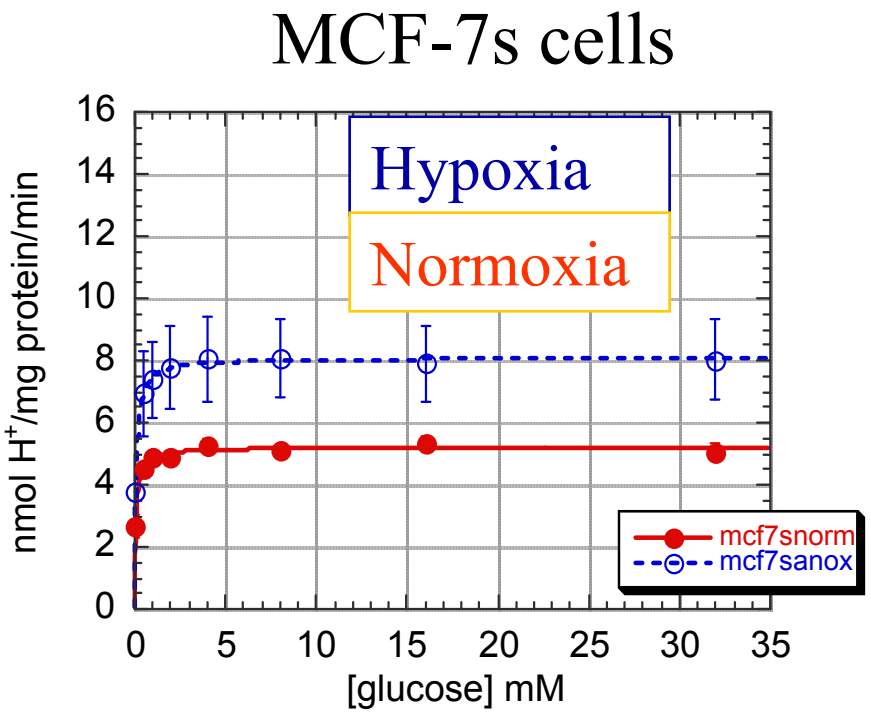
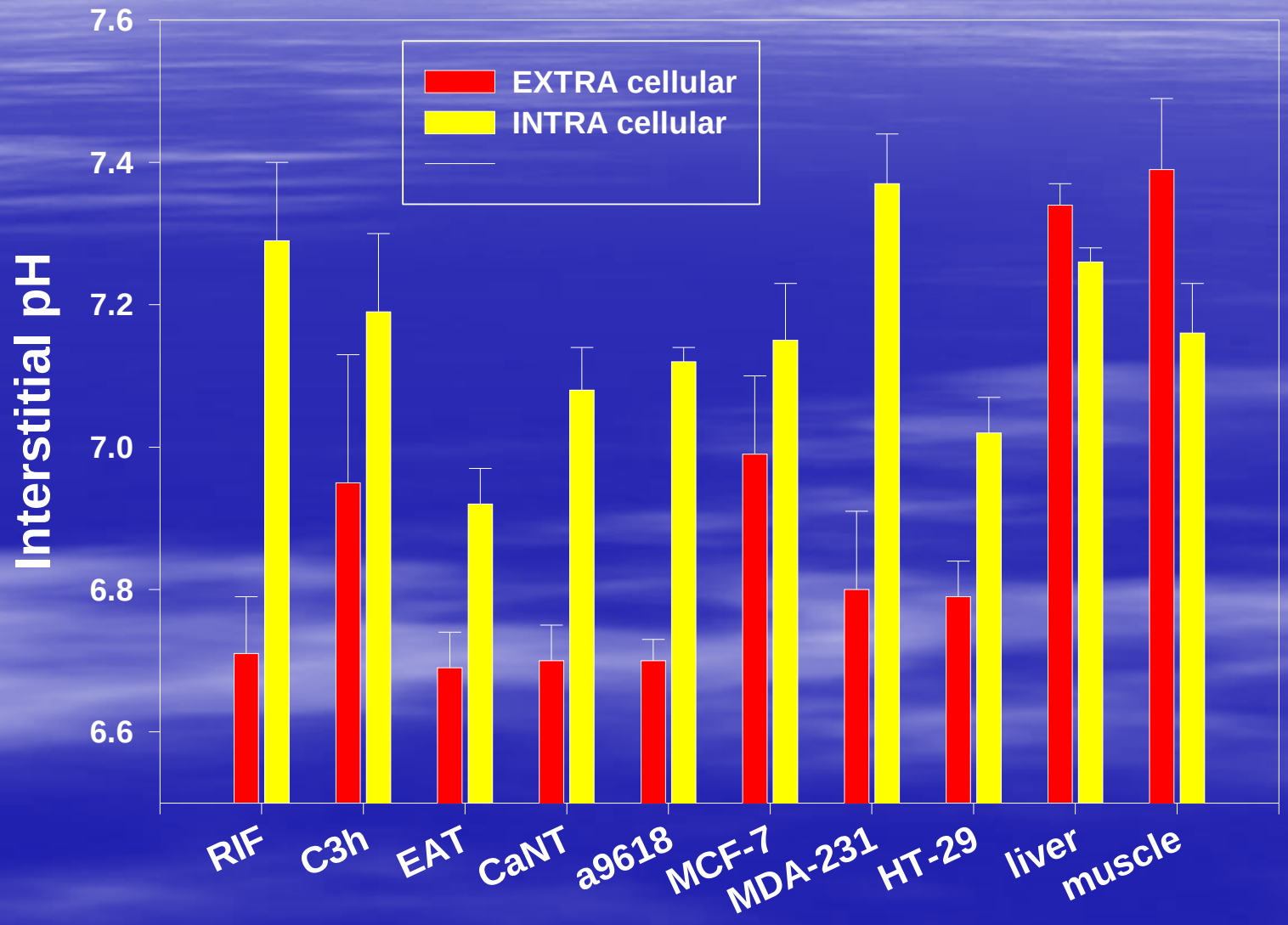
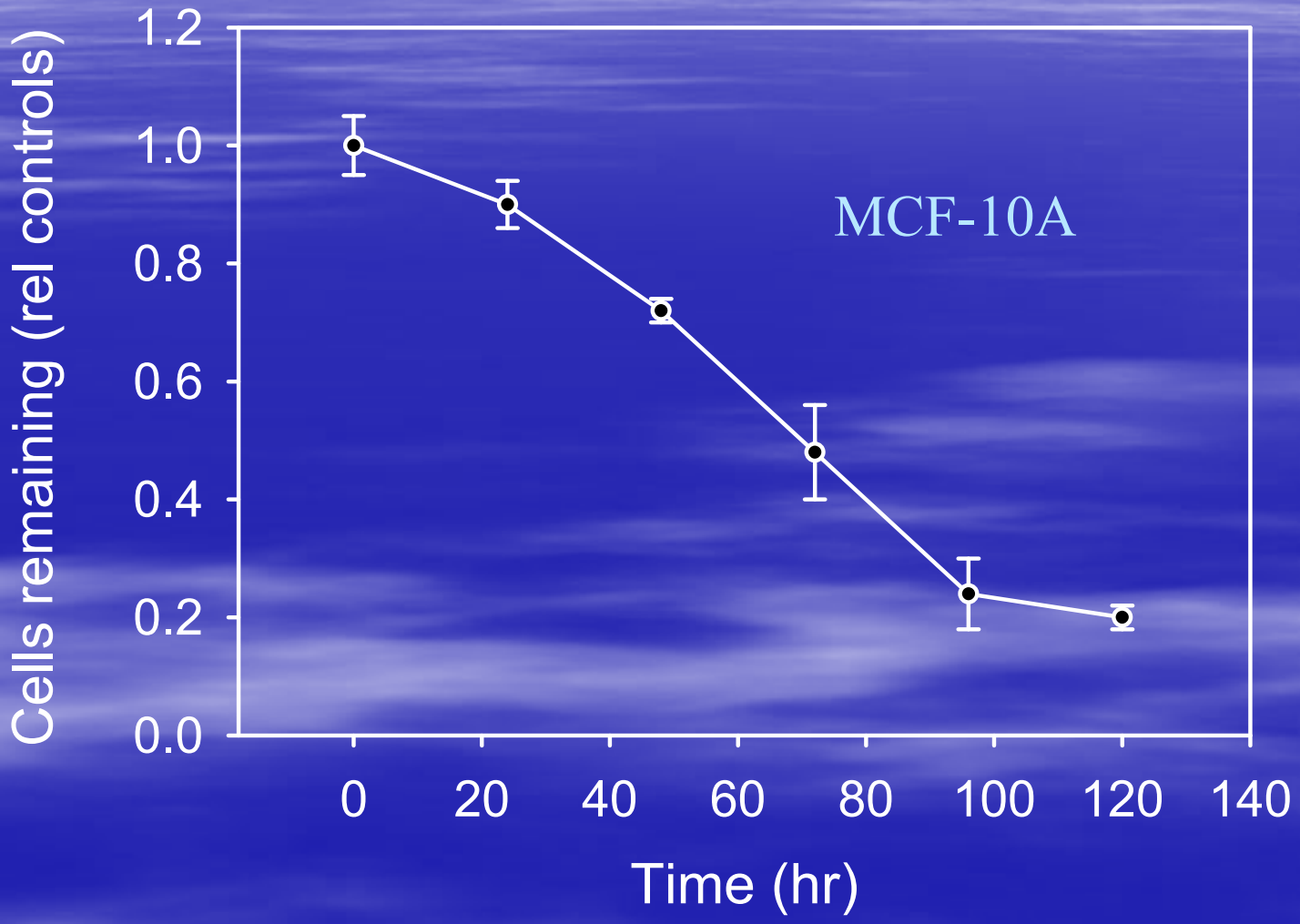


PA Schornack

A consequence of upregulated glycolysis is an acidic extracellular environment - here measured with ^{31}P NMR



Killing its young: Chronic exposure to acidic pH_e is toxic

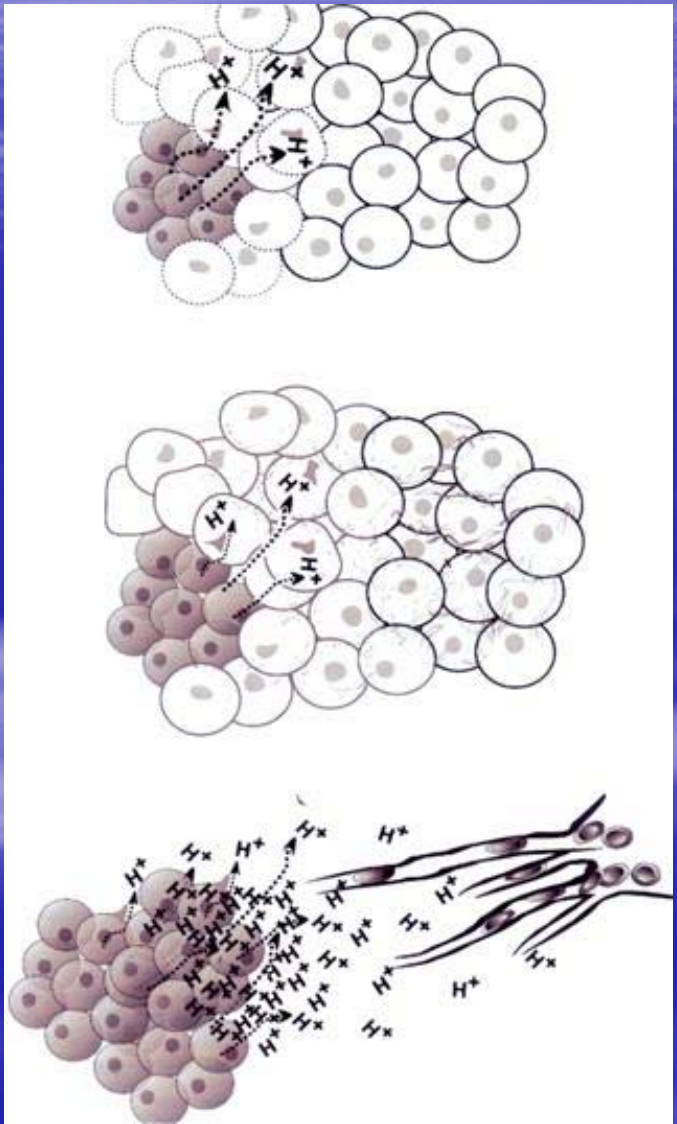


The Dilemma:

If carcinogenesis is somatic evolution, common phenotypic traits of invasive cancers emerge following competition and Darwinian selection and *must always* confer a selective growth advantage

Aerobic metabolism seems inconsistent with this principle since it is metabolically inefficient and produces a potentially toxic, acidic environment. Survival of the fittest??

Why does aerobic glycolysis persist in advance primary and metastatic tumors? Acid-Mediated Tumor Invasion Hypothesis



- General concept: Tumor-induced perturbations in the micro-environment are unfavorable to normal tissue and enhance tumor growth in a self propagating pattern
- Specific concept: Altered tumor metabolism results in an acidic pH_e both in the tumor and in a ring of surrounding normal tissue. Tumor cells have an ideal pH_e (i.e. maximum proliferation) of about 0.5 pH units lower than normal. This provides a selective growth advantage so that they continue to proliferate while normal cells die

Proposed Sequence:

- Altered glucose metabolism results in increased lactic acid production
- H^+ transport across the membrane is increased primarily through amplification of the Na^+/H^+ antiport
- This results in increased pH_i and decreased pH_e.
- H^+ ions in the extracellular space will diffuse along concentration gradients into peritumoral host tissue resulting in: normal cell death, ECM degradation, induction of angiogenesis, and blunting of immune response.
- Tumor cells (more tolerant of acid pH_e) continue to proliferate and invade into the disrupted normal tissue

Acidic pH_e causes p53 dependent apoptosis through increased caspase activity and confers relative growth advantage on cancer cells

- Williams, Collard, Paraskeva. An acidic environment leads to p53 dependent induction of apoptosis… Oncogene. **16**:3199-3204, 1999
- Park et al. Acidic environment causes apoptosis by increasing caspase activity. British J. Cancer **80**(12):1892-1897,1999.
- Dairkee et al. Selective culture of primary breast cancer. Cancer Res. **35**:2516-2519, 1995
- Isuishi et al. Remarkable tolerance of tumor cells to nutrient deprivation: possible new biochemical targets for cancer therapy. Cancer Res. **60**:6201-6206, 2000
- Gerweck and Fellenz. The simultaneous determination of intracellular pH and cell energy status. Radiation Res. **125**: 257-261, 1991

Extracellular matrix degradation due to acid-induced release of Cathepsin B and other proteolytic enzymes

- Rhozin et al. Pericellular pH affects distribution and secretion of cathepsin B in malignant cells. Cancer Res. 45:6517-6525. 1994
- Webb et al. Modeling tumour acidity and invasion. Novartis Found. Symp. 240:169-181, 2001

Acid-mediated angiogenesis through release of IL8 and VEGF

- Xu and Fidler Acidic pH-induced elevation in Interleukin 8 expression by human ovarian carcinoma cells. Cancer Res. 60:4610-4616, 2000
- Shi et al Regulation of vascular endothelial growth factor expression by acidosis in human cancer cells. Oncogene. 20(28):3751-3756. 2001

The hypothesis was initially framed mathematically

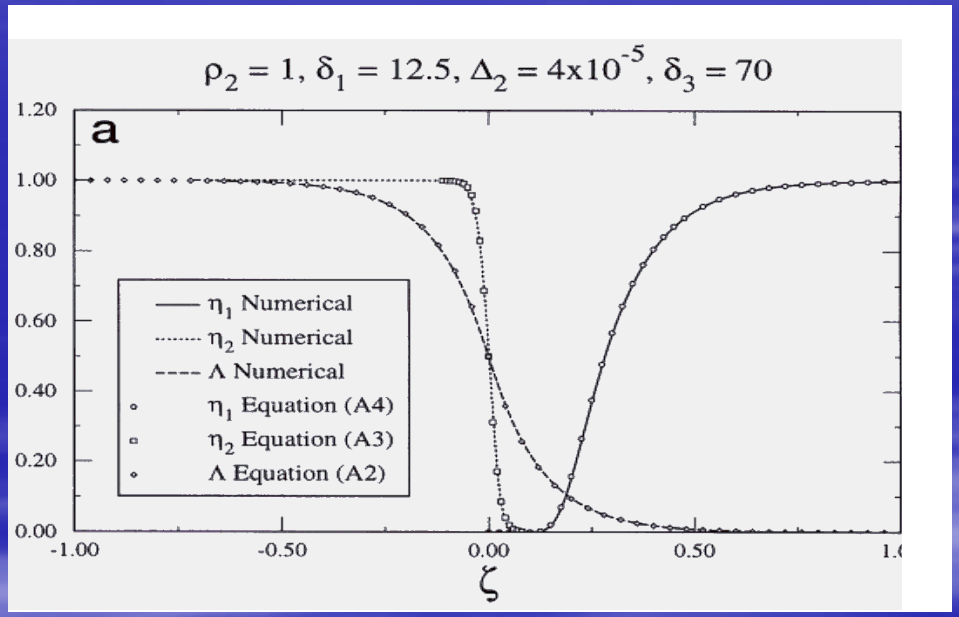
$$\frac{\partial N_1}{\partial t} = r_1 N_1\left(1 - \frac{N_1}{K_1} - \alpha_{12}\frac{N_2}{K_2}\right) - d_1 L N_1 + \nabla \cdot (D_{N_1}[N_2]\nabla N_1)$$

$$\frac{\partial N_2}{\partial t} = r_2 N_2\left(1 - \frac{N_2}{K_2} - \alpha_{21}\frac{N_1}{K_1}\right) - d_2 L N_2 + \nabla \cdot (D_{N_2}[N_1]\nabla N_2)$$

$$\frac{\partial L}{\partial t} = r_3 N_2 - d_3 L + D_3 \nabla^2 L$$

where

N_1= Normal cells
N_2= Tumor cells
L= Excess acid concentration (i.e. the acid above pH 7.4)
r_1 and r_2 = Maximal growth rate for the cellular populations respectively
K= Carrying capacity
α = Lumped interference term
D_1 and D_2= Invasion terms for each cell population
d_1 and d_2 = Death rate due to excess acid in the extracellular space
d_3= Removal of excess acid by tumor and peritumoral vasculature
r_3= Excess acid production by tumor cells
D_3 = Diffusion coefficient for H^+



Dimensionless Parameters

$$\eta_1 = \frac{N_1}{K_1} \qquad \eta_2 = \frac{N_2}{K_2} \qquad \Lambda = \frac{Ld_3}{r_3 K_2}$$

$$\tau = r_1 t \qquad \xi = \sqrt{\frac{r_1}{D_3}}$$

$$\frac{\partial \eta_1}{\partial \tau} = \eta_1(1 - \eta_1) - \delta_1 \Lambda \eta_1$$

$$\frac{\partial \eta_2}{\partial \tau} = \rho_2 \eta_2(1 - \eta_2) + \nabla_\xi \cdot [\Delta_2(1 - \eta_1)\nabla_\xi \eta_2]$$

$$\frac{\partial \Lambda}{\partial t} = \delta_3(\eta_2 - \Lambda) + \nabla_\xi^2 \Lambda$$

where

$$\delta_1 = (d_1/d_3) \times (r_3/r_1) \times K_1$$

$$\rho_2 = r_2/r_1$$

$$\Delta_2 = D_2/D_3$$

$$\delta_3 = d_3/r_1$$

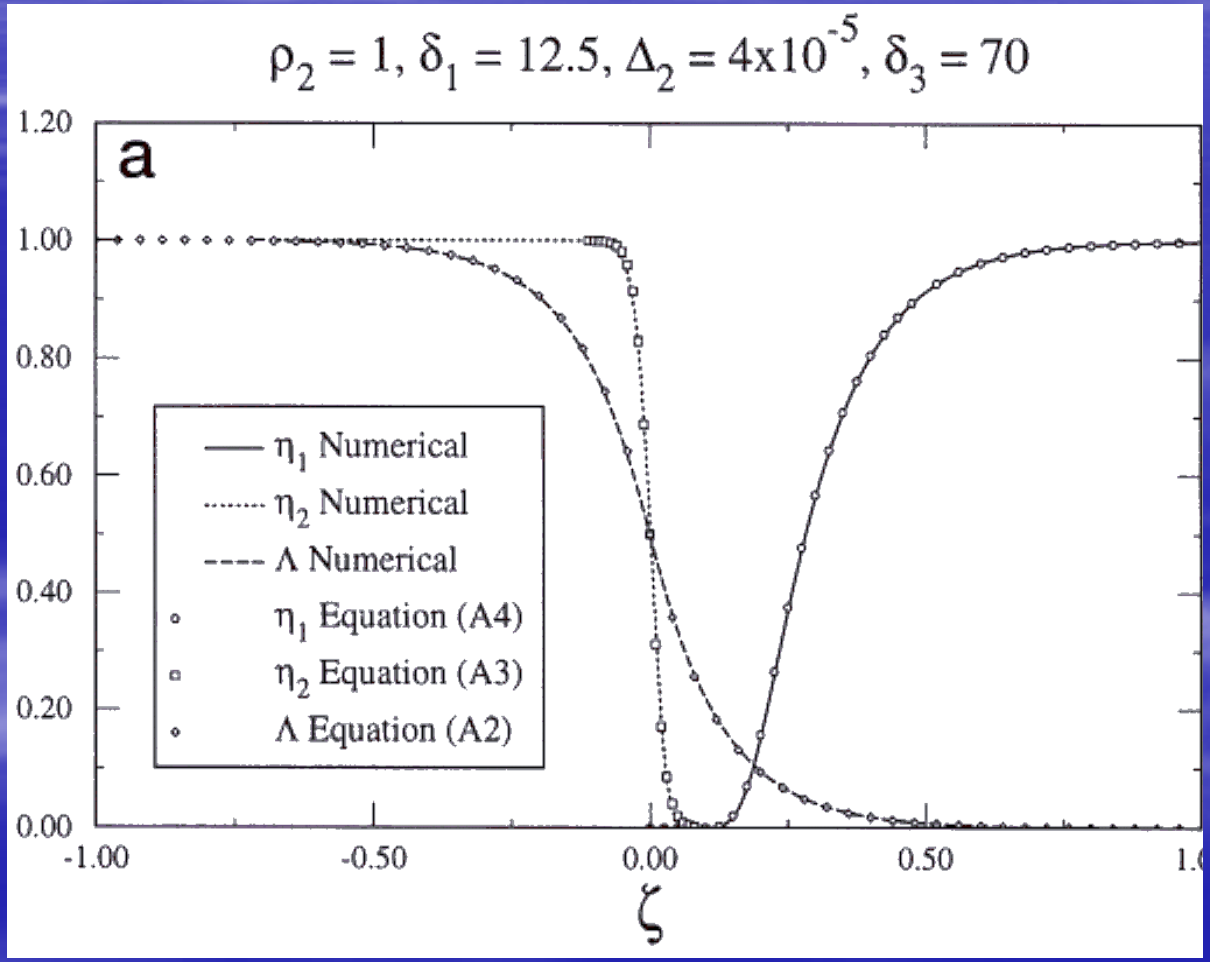
Fixed Points (Spatial Homogeneity and Temporal Invariance)

- FP#1 $N_N=0$ $N_T=0$ $H=0$
- FP#2 $N_N=K_N$ $N_T=0$ $H=0$
- FP#3 $N_N=K_N(1-\delta_1)$ $N_T= \delta_1 K_T$ $H=H_0$
- FP#4 $N_N=0$ $N_T=K_T$ $H=H_0$
- Where $\delta_1=(d_N/d_H) \times (r_H/r_N) \times K_T$

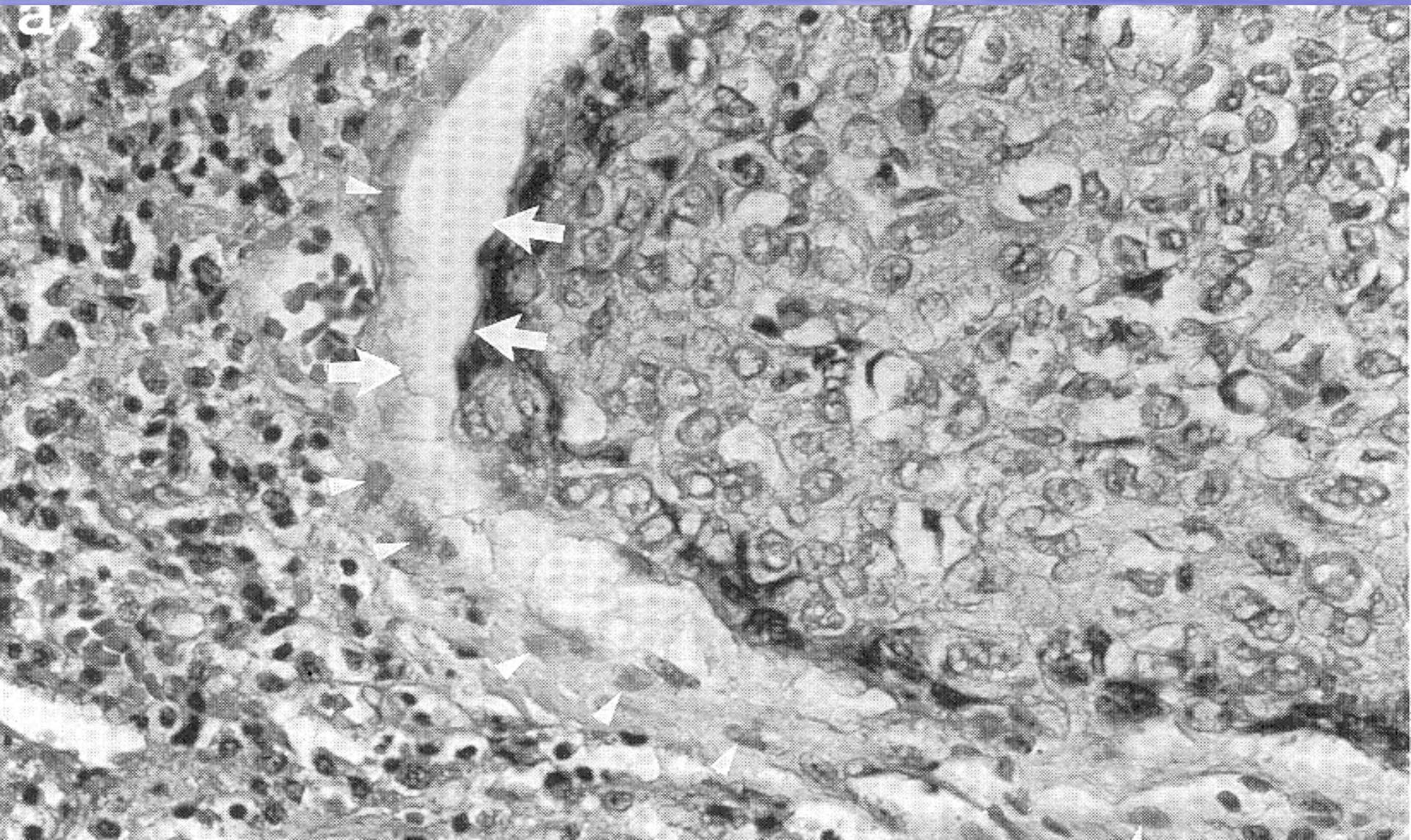
Linear Stability Analysis

- FP #1 and FP#2 are unconditionally unstable
- FP#4 is stable and FP#3 is unstable if $\delta_1 > 1$ and *vice versa*
- Recall $\delta_1 = (d_N/d_H) \times (r_H/r_N) \times K_T$

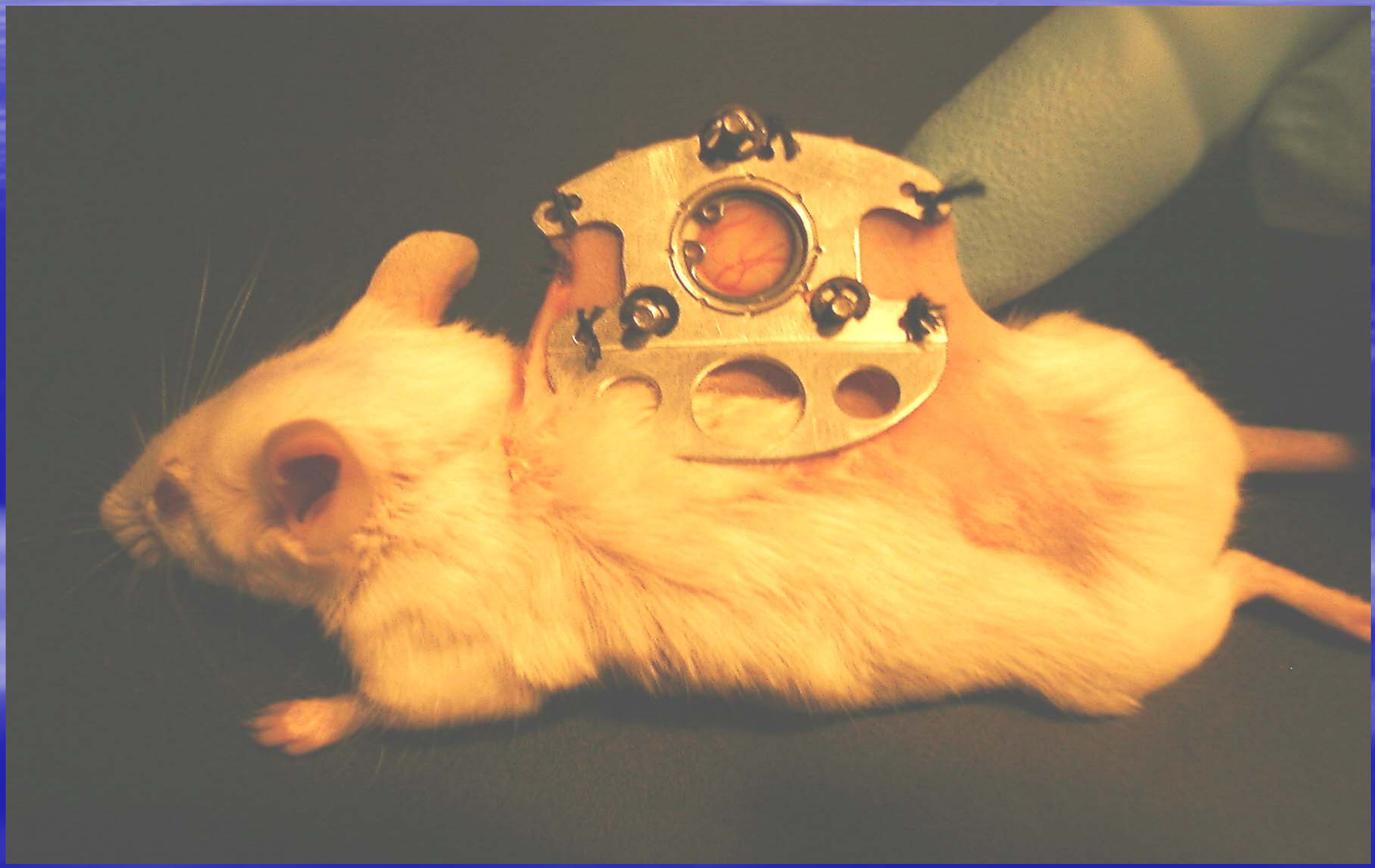
Traveling wave solution at the tumor-Host Interface



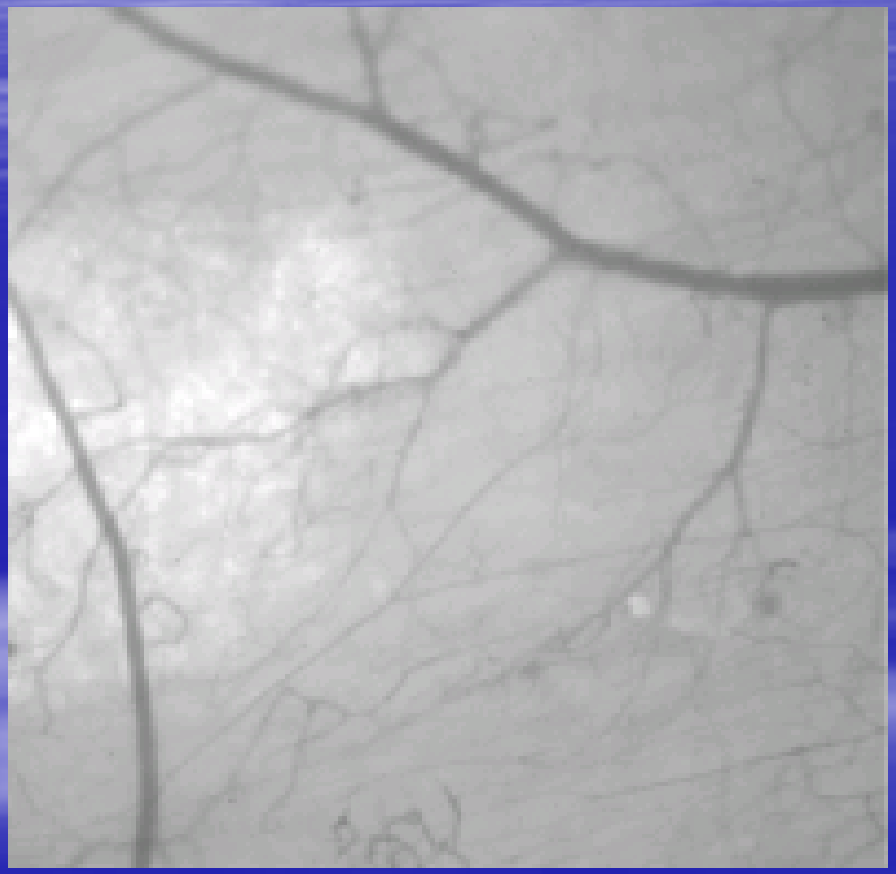
Acellular gap at the tumor-host interface in head and neck cancer

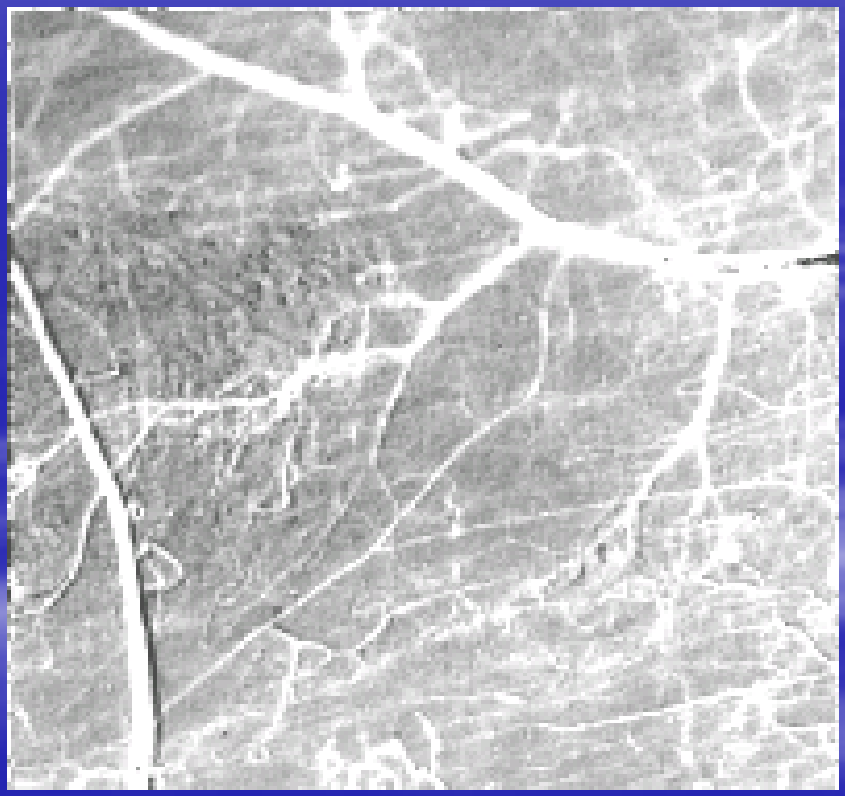


Mouse Dorsal Wound Chamber

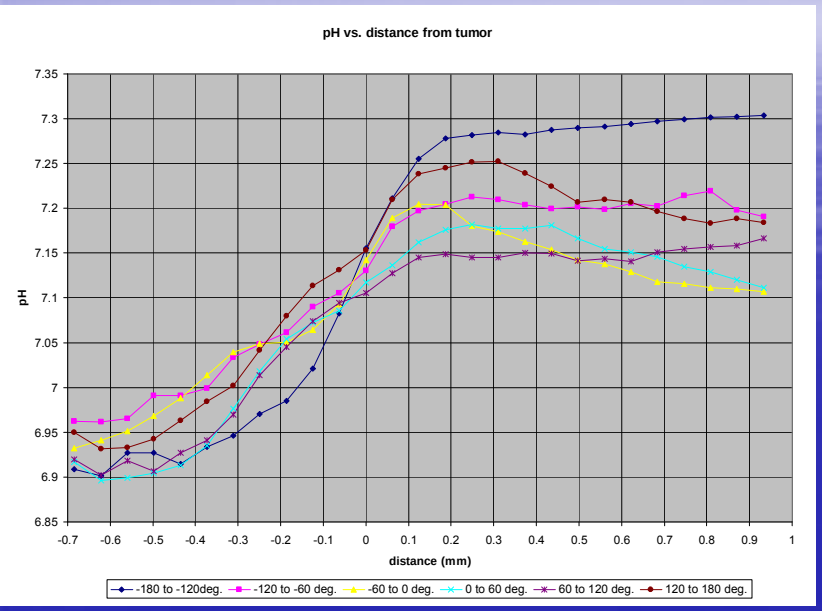


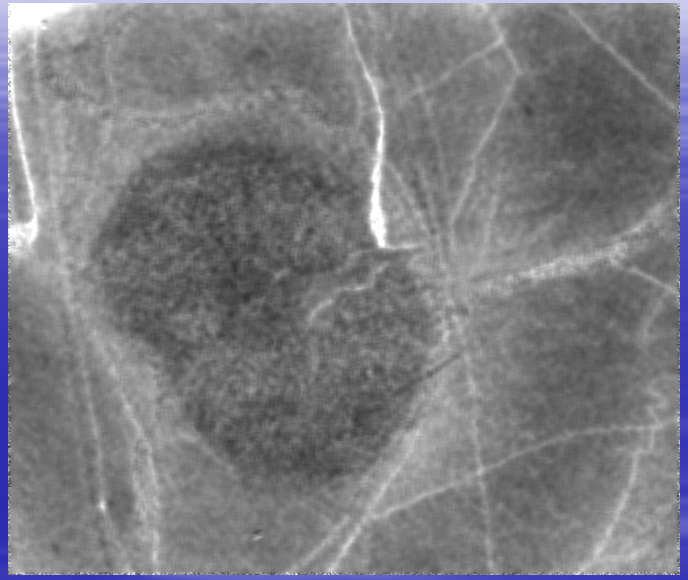
Simultaneous maps of tumor location and extracellular pH

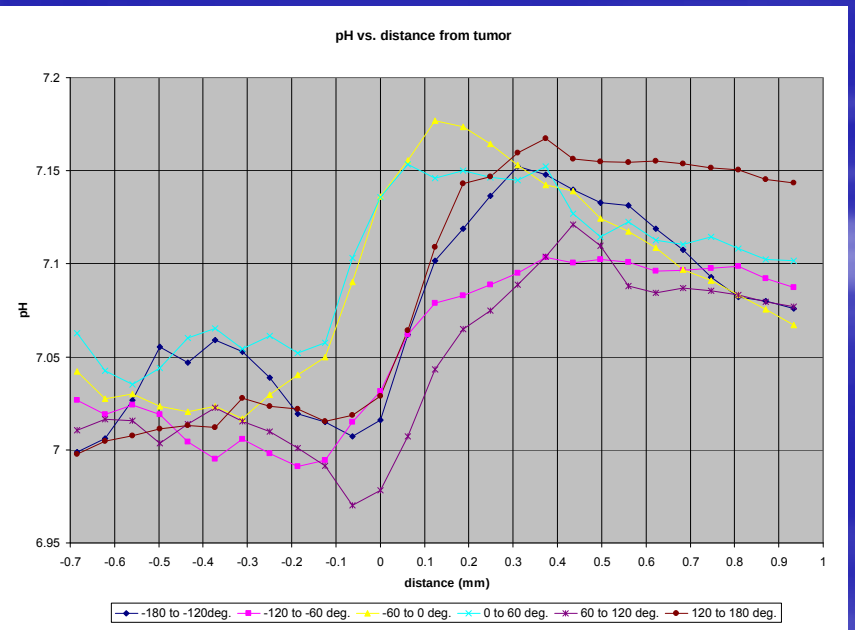


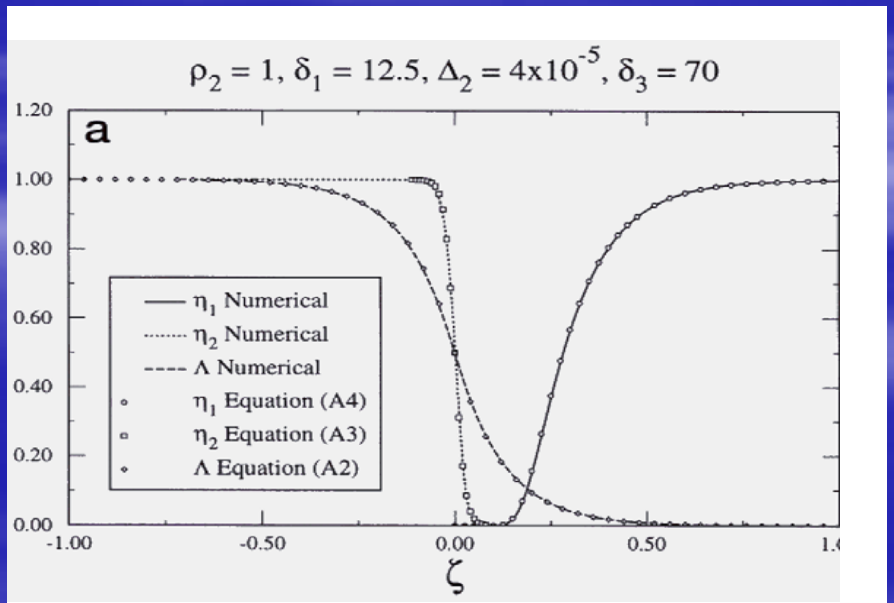


pHe gradients match model predictions

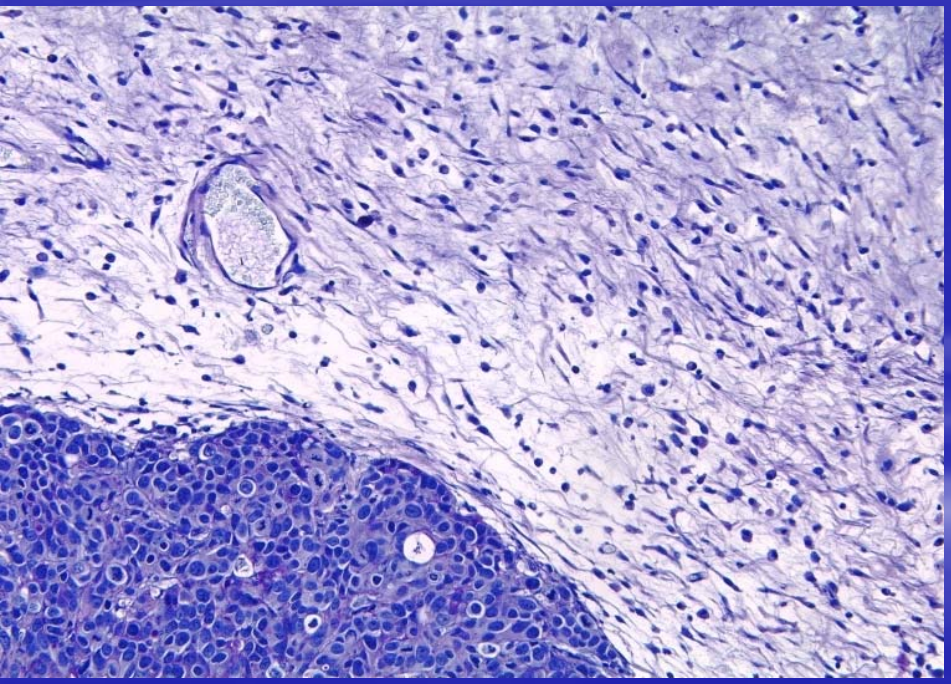


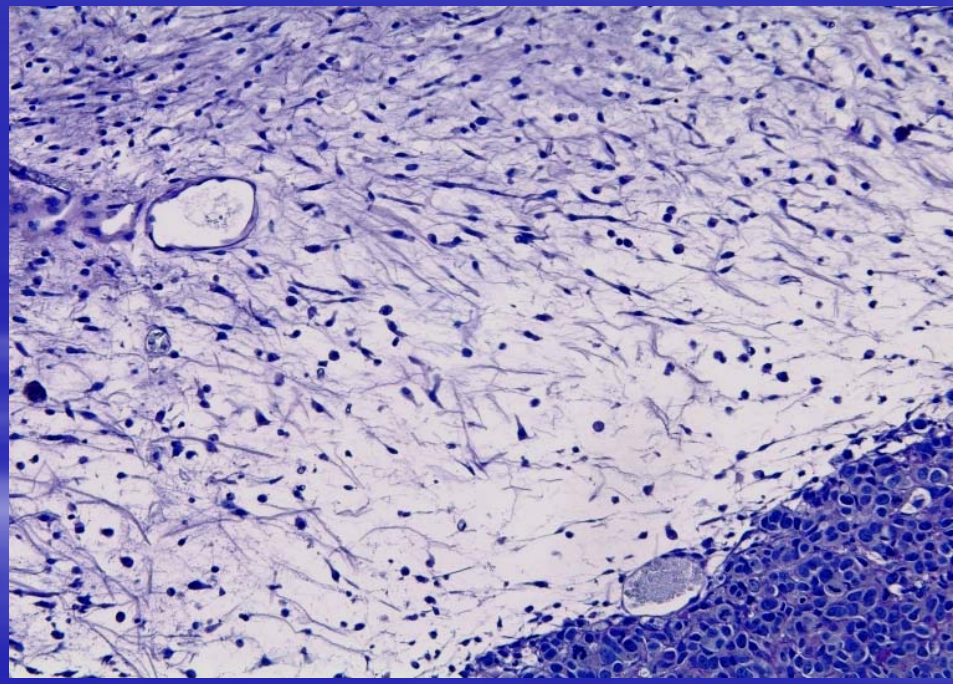




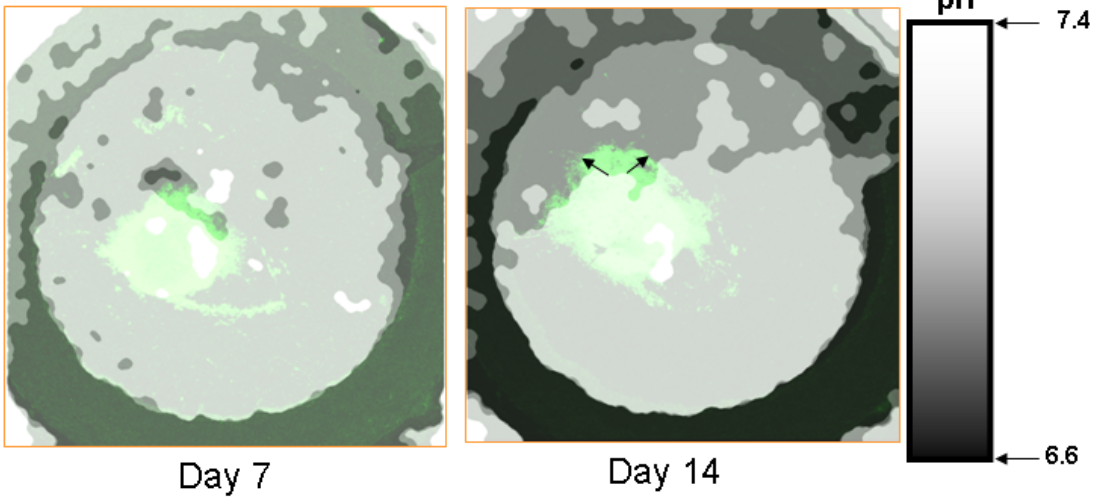


PAS staining shows degradation of the ECM around the tumor roughly corresponding to the acid gradient



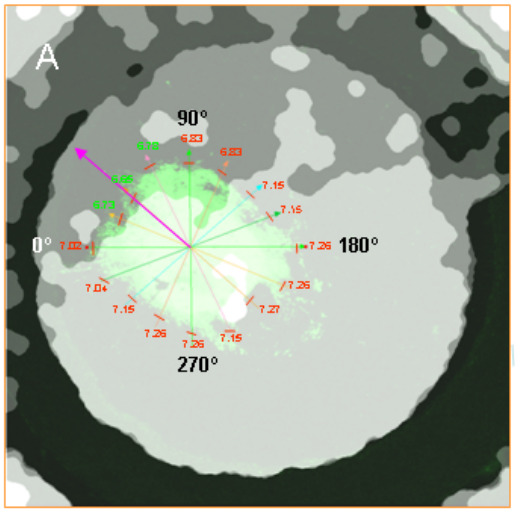


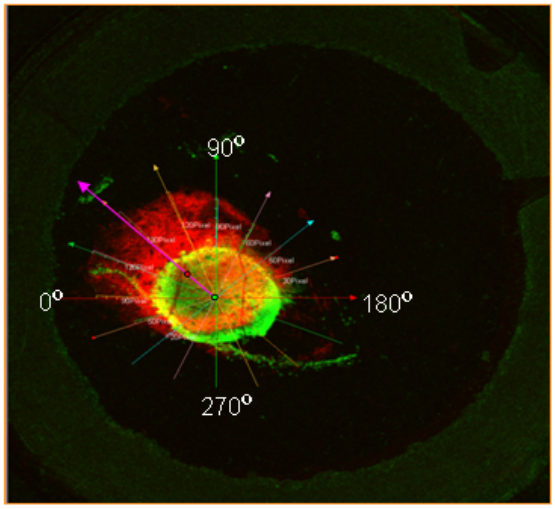
Does pH_e distribution around a tumor predict subsequent growth?



Day 7

Day 14

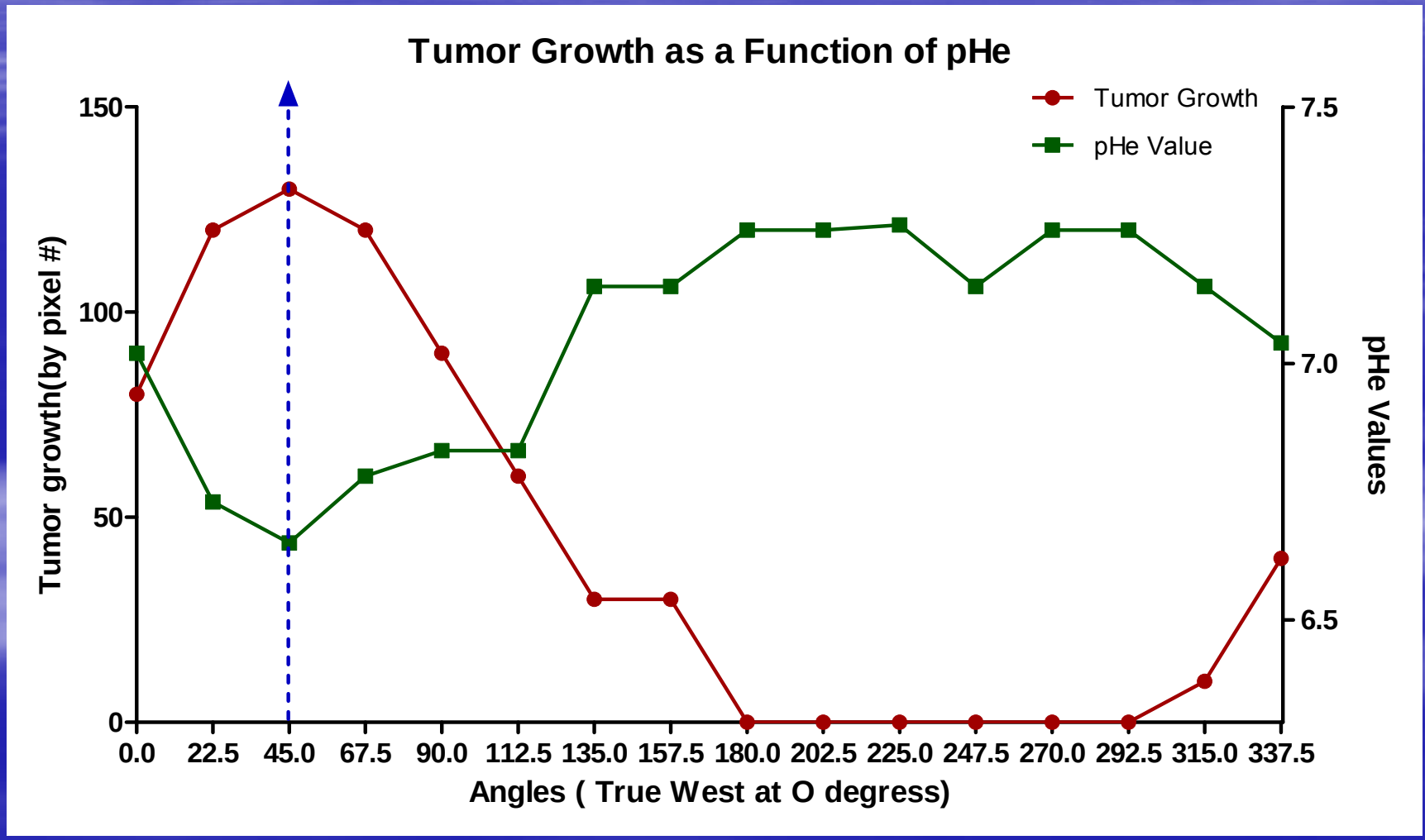




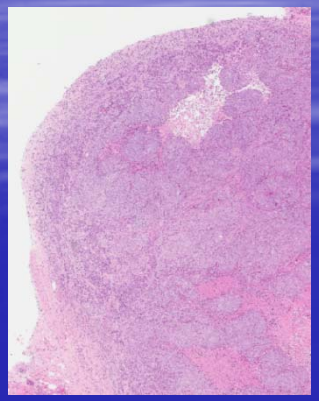
Day 21

Overlay of Day 7 and Day 21

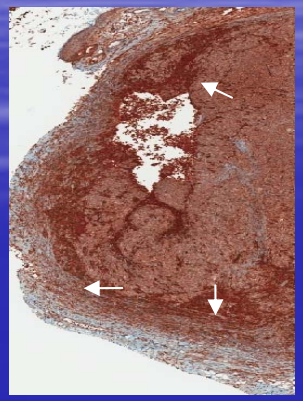
Comparison of pH_e distribution with tumor growth during subsequent 7 days



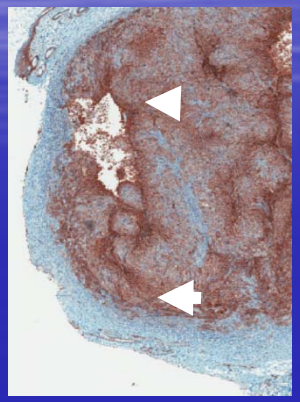
Regional selection pressures in breast cancers: Radial distribution of NHE-1 and GLUT 1



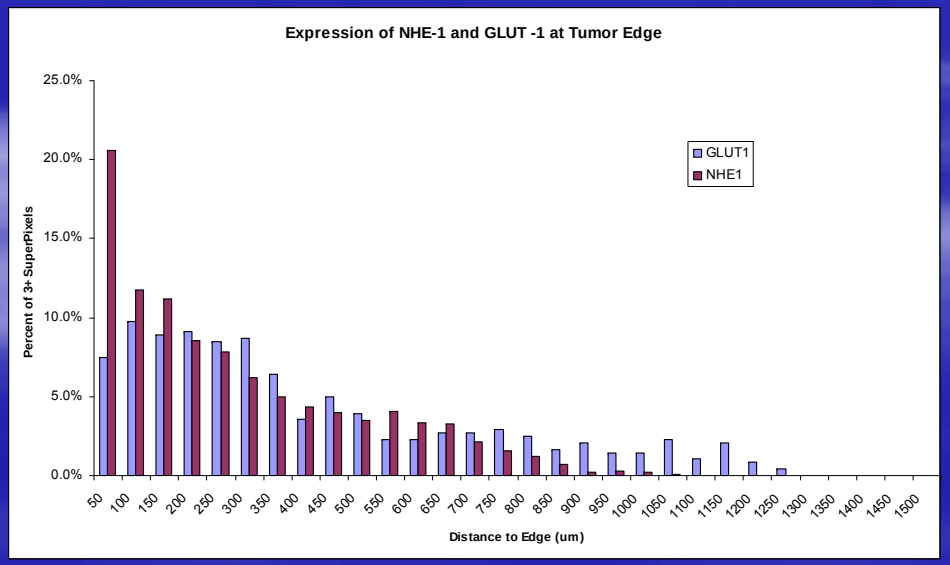
H & E

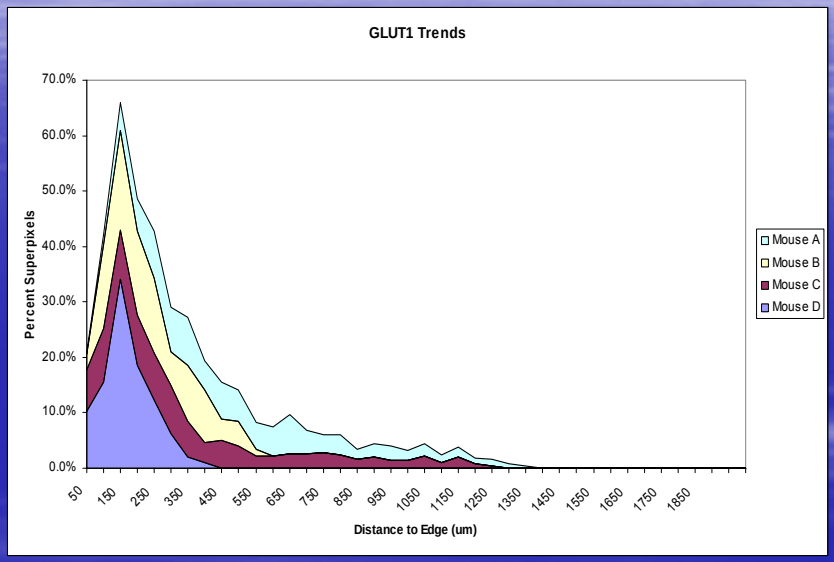


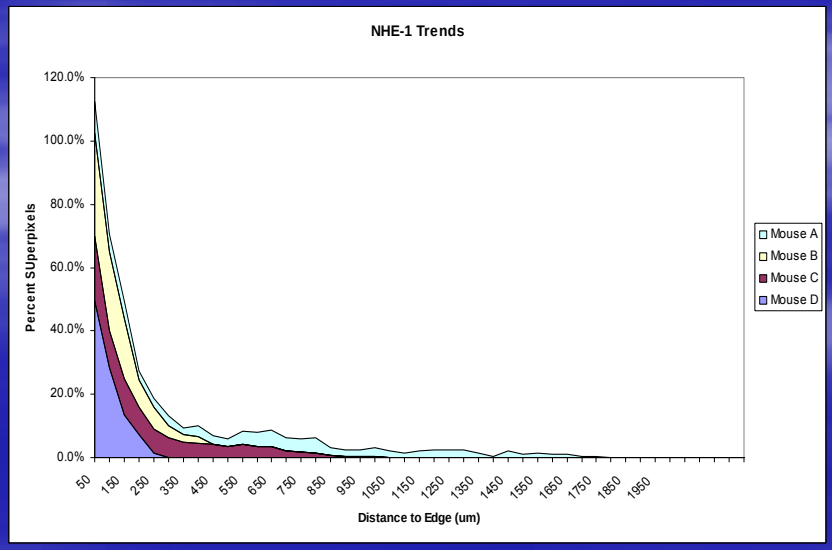
NHE-1



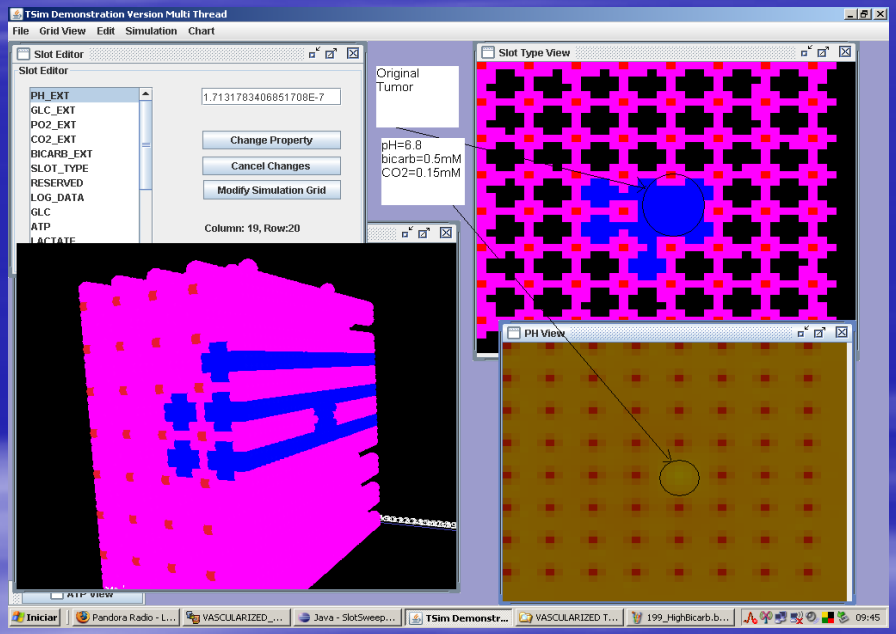
GLUT 1

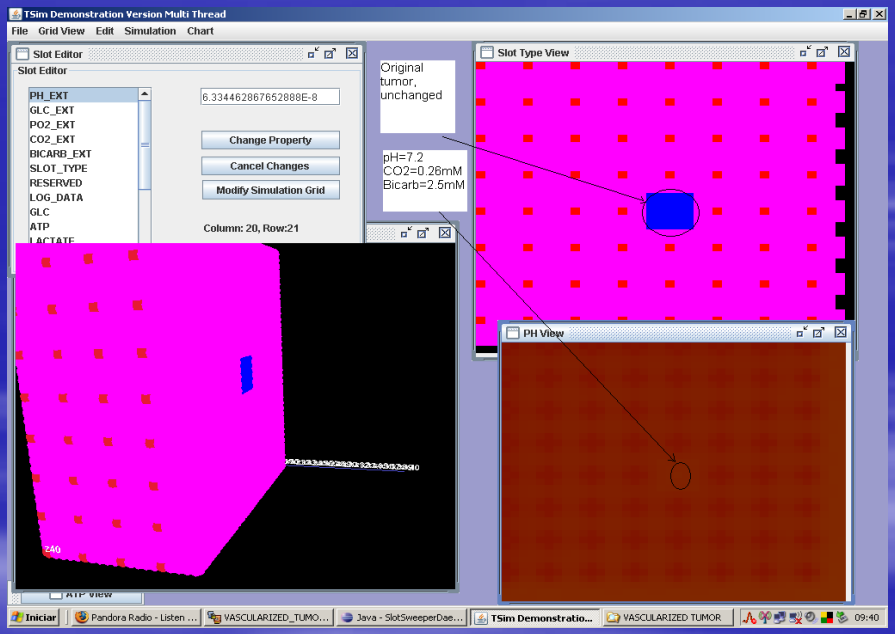






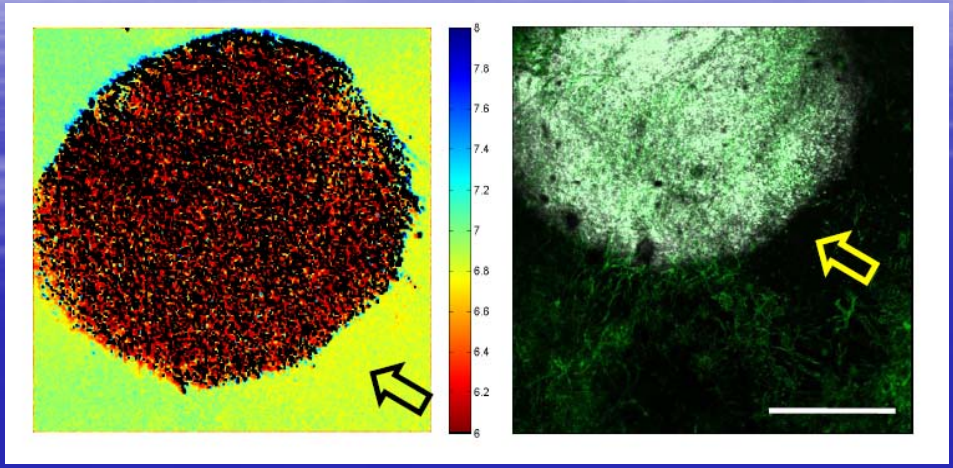
Hypothesis: Could an increase in serum buffer reduce the gradient and stop invasive tumor growth?

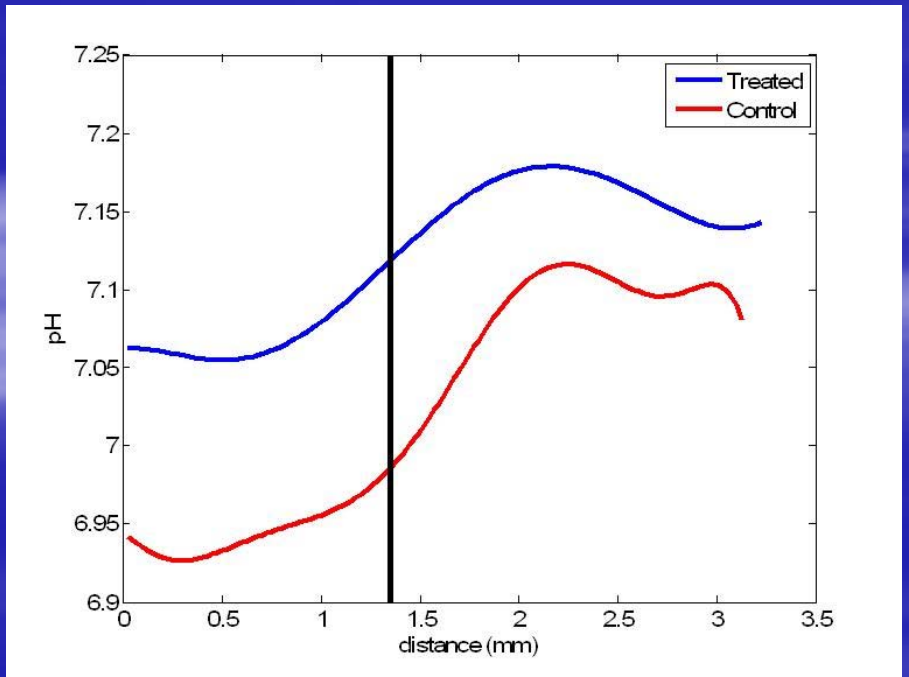




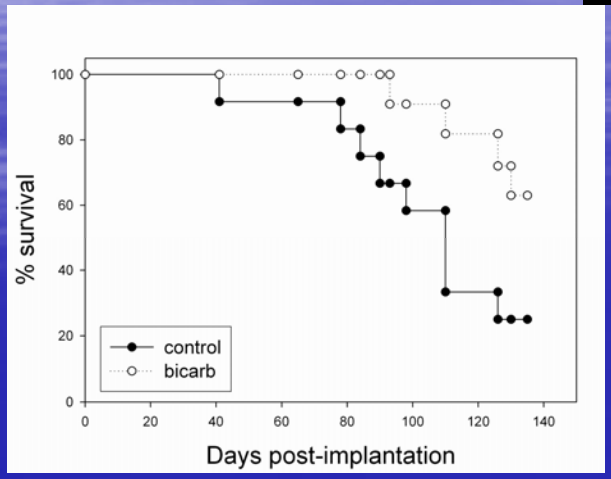
$NaHCO_3$ raises Tumor pH

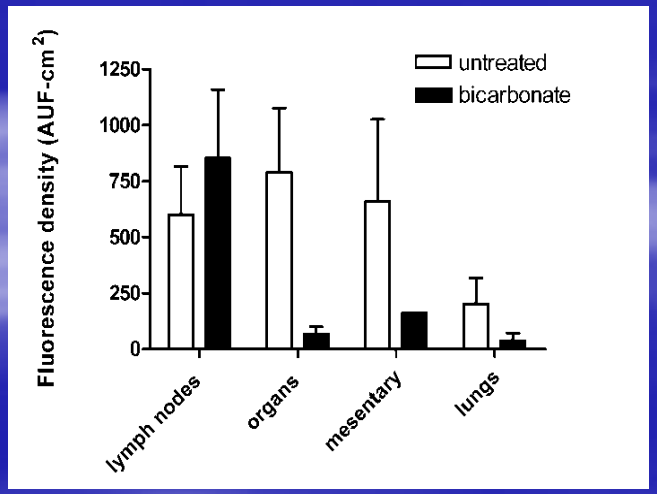
(200 mM ad lib; SCID mice)

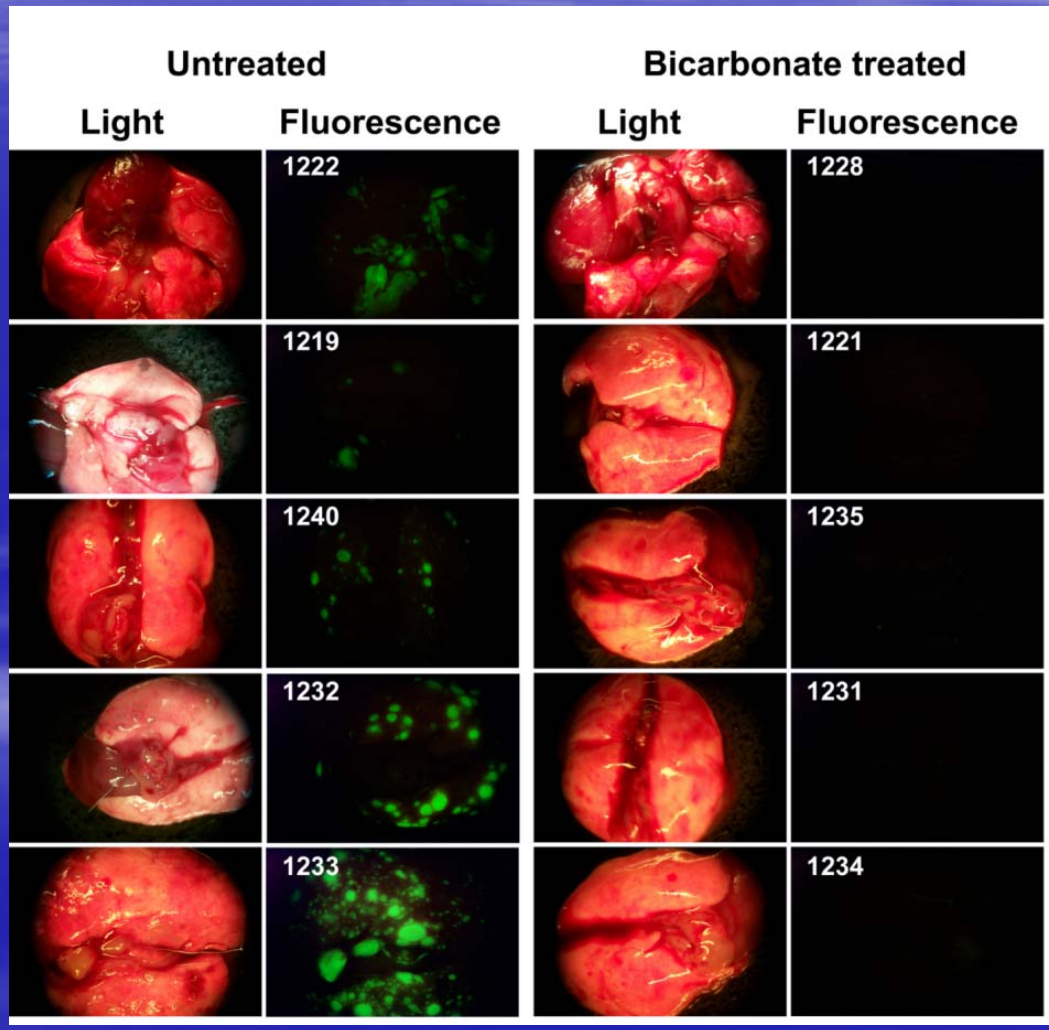




Bicarbonate inhibits metastases and prolongs survival in MDA-mb-231 cells







Does acid-mediated tumor invasion apply to early tumor growth?

- Model must include a discretized approach to describe individual cell history and its interactions with other cells while retaining the while retaining continues elements to describe the production, diffusion, and removal of H^+ ions (Aalpen Patel).

- Modified Cellular Automata Model:

  Establish N x N array of automaton cells with a one to one correspondence between the automaton cells and physical cells with size 20 x 20 microns.

Each automaton cell is described by a state vector with 3 components:

- The automaton is either a tumor cell, a normal cell, a microvessel, or vacant
- The local extracellular pH
- The local glucose concentration

Microvessels are randomly distributed throughout the distributed throughout the simulation space with density α

Where

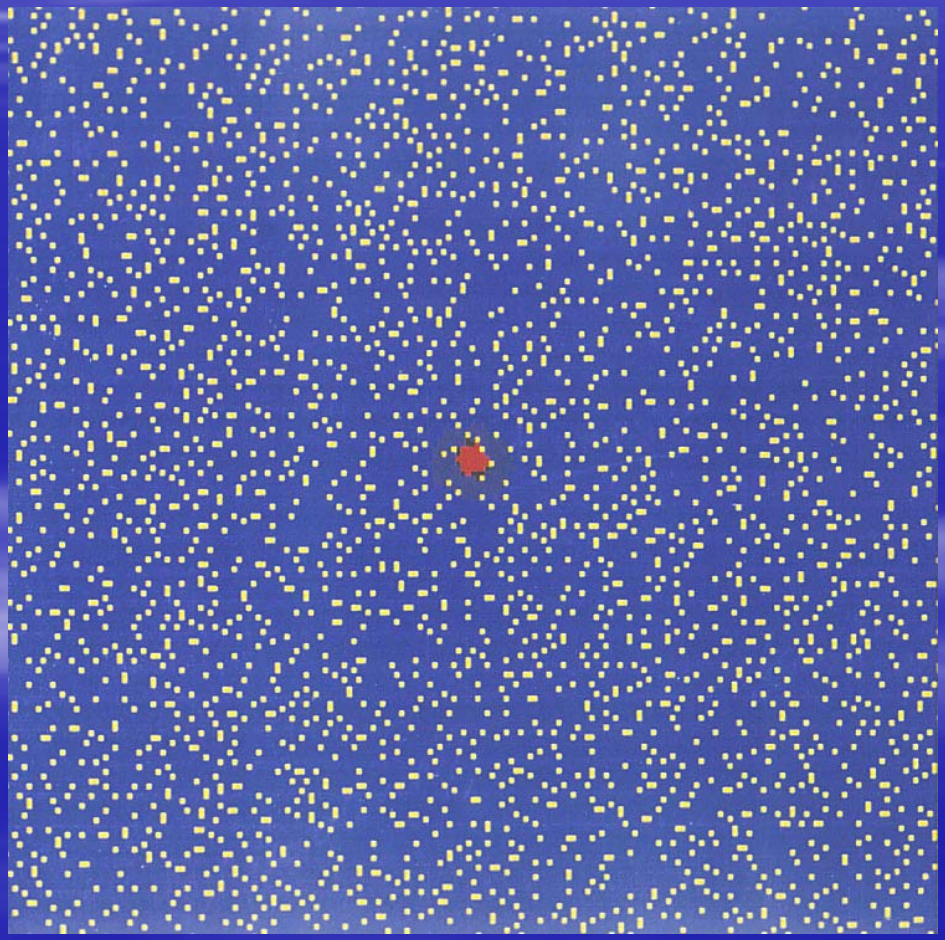
$\alpha = N_v/N^2$

Where

N_v is the number of automaton cells occupied by vessels and N is the total number of automaton cells

- H^+ and glucose concentrations form 2 continuous fields over the simulation space obeying suitable time-dependent diffusion equations with sinks, sources, and boundary conditions determined by cells and vessels

Typical 200x200 matrix showing normal cells (blue), randomly scattered microvessels (yellow) with a small tumor disc 5 cells in diameter (21 cells total) placed centrally



Automata Rules:

- Microvessels remain constant
- If automaton cell is either a tumor or normal then the value of the concentrations of H^+ or Glucose in its state vector are considered.
- If pH is lower than some critical threshold (pH_{DN} pH_{DT}), the cell dies and the automata becomes vacant.

  Typical values pH_{DN}=6.8 pH_{DT}=6.0

  If pH > pH_D but lower than some threshold pH_Q, the cell survives but in a quiescent state (ie. no mitosis occurs)

  Typical pH_{QN}=7.1 pH_{QT}=6.4

Automata Rules (cont.)

- If cell pH > pH_Q and glucose concentrations are adequate (threshold G_N and G_T assumed to be 2.5 mM), the cell may divide but only if an adjacent automata cell is vacant. If more than one is vacant it enters the cell with the largest value of G.

Diffusion equation for the time-dependent glucose field

$$D_G \nabla^2 G_t(\vec{r}) - k(\vec{r}) G_t(\vec{r}) = 0$$

where $G_t(\vec{r})$ is the glucose concentration at $\vec{r}$ after sub-generation t. The term $k(\vec{r})$ (having units $1/s$) is the glucose consumption rate at the location $\vec{r}$:

$$k(\vec{r}) = \begin{cases} k_N & \forall \vec{r} = Normal \quad Cells \\ k_T & \forall \vec{r} = Tumor \quad Cells \\ 0 & \forall \vec{r} = Vacant \quad Cells \\ 0 & \forall \vec{r} = Vessel \quad Cells \end{cases}$$

where $1 \times 10^{-6} / s < k_N < 5 \times 10^{-4} / s$ and $1 \times 10^{-5} / s < k_T < 1 \times 10^{-3} / s$ are ranges for glucose consumption by normal and tumor cells respectively

Acid profile governing equation

$$D_H \nabla^2 H_t(\vec{r}) + h(\vec{r}) = 0$$

where $D_H = 1.08 \times 10^{-5} cm^2/s$ is the diffusion constant for lactic acid, $H_t(\vec{r})$ is the H^+ concentration at position $\vec{r}$ after sub-generation t, and $h(\vec{r})$ is an acid production rate that is non-zero only at positions $\vec{r}$ where there is a tumor cell:

$$h(\vec{r}) = \begin{cases} \dot{H}_T^A & \forall \vec{r} = \textit{Active tumor cells} \\ \dot{H}_T^Q & \forall \vec{r} = \textit{Quiescent tumor cells} \\ 0 & \forall \vec{r} \neq \textit{Tumor cells} \end{cases}$$

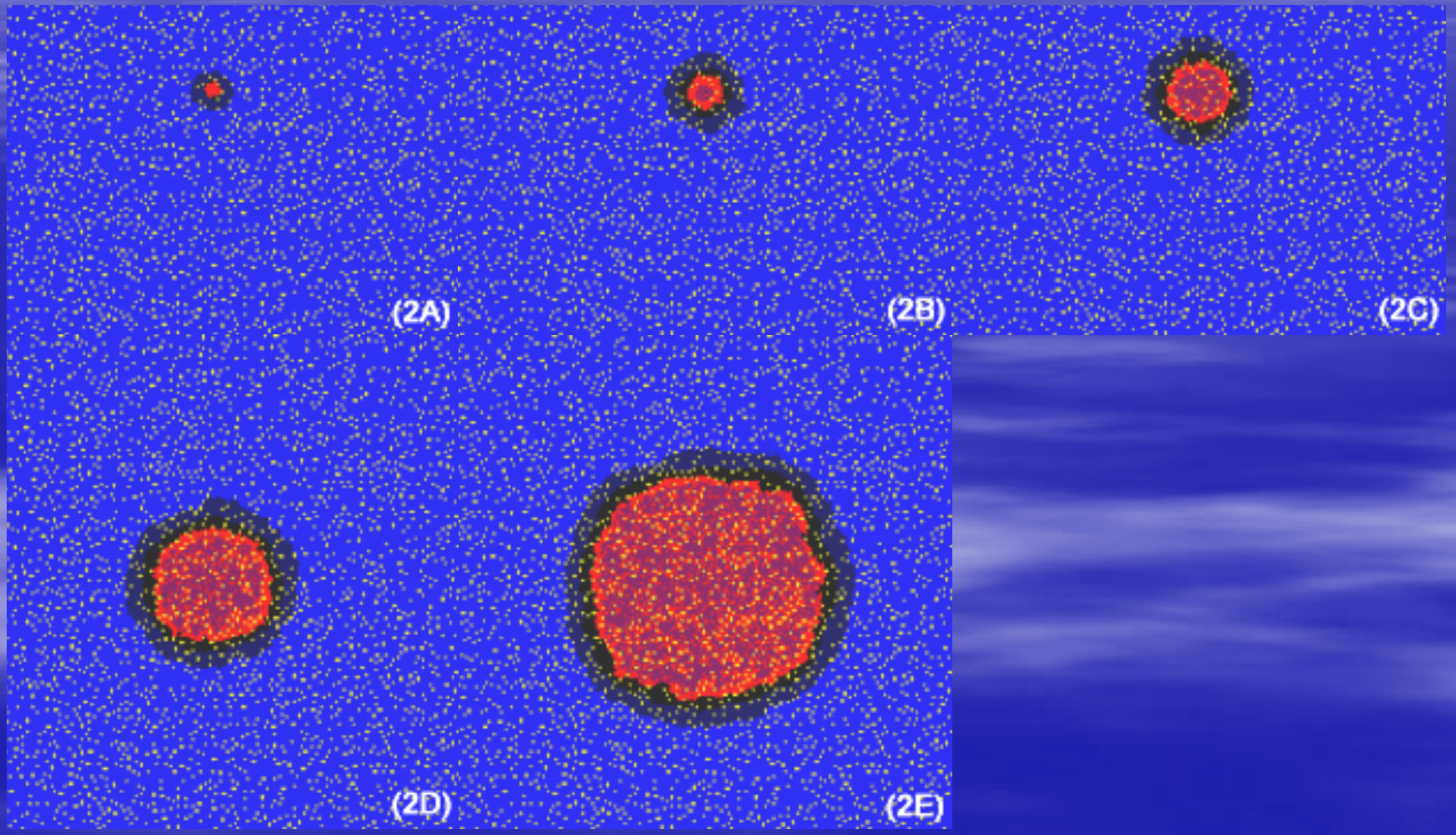
In our model $\dot{H}_T^A$ and $\dot{H}_T^Q$ are key variables adjusted to model tumor phenotypes expressing different metabolisms:
$1 \times 10^{-6} < \dot{H}_T^A < 1 \times 10^{-4} mM/s$ and $\dot{H}_T^Q = 5 \times 10^{-7} mM/s$.[3]

Strategy for Model Analysis

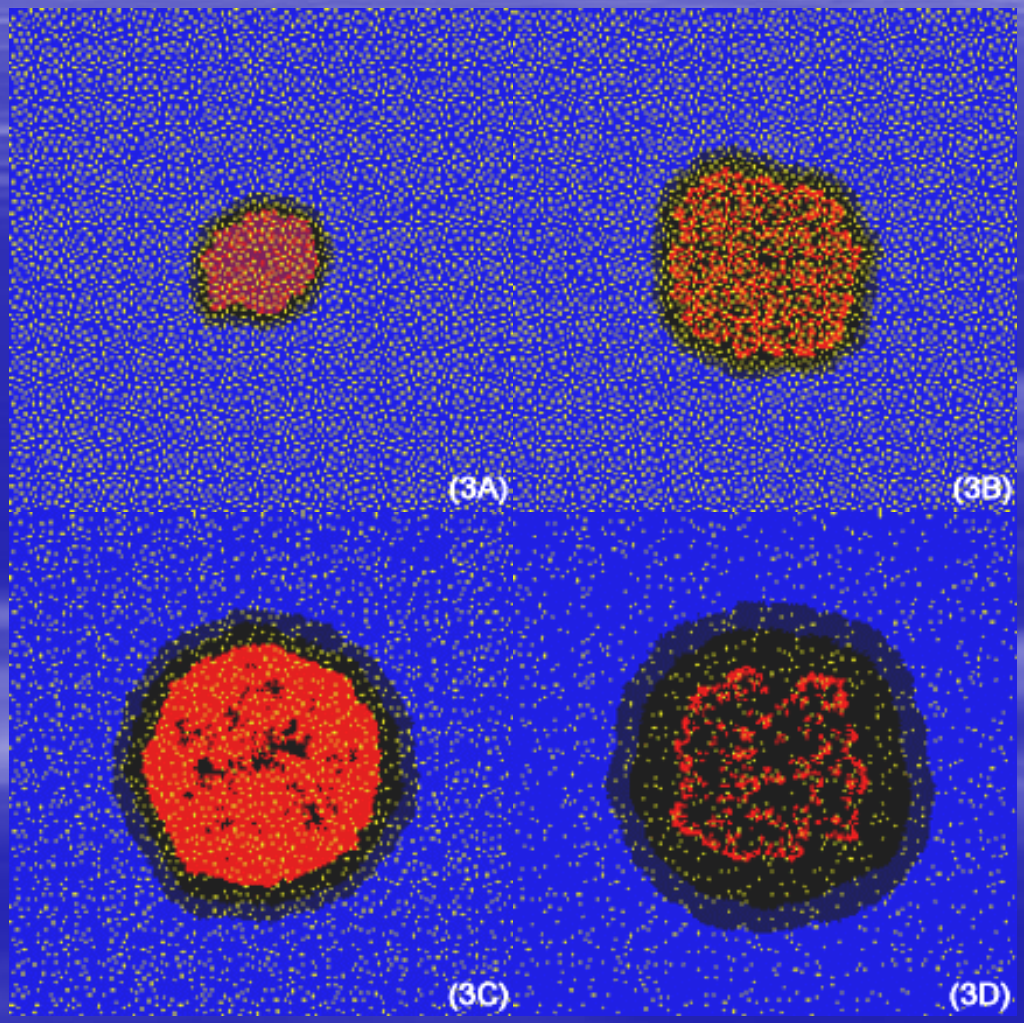
- Use large disparity in time scales of cell proliferation (about 10^2 hrs) and chemical diffusion (intervessel diffusion time 1 – 10 s).
- Cell distribution changes so slowly they can be treated as adiabatic perturbations on the chemical fields.

- Solve a series of equilibrium boundary-value equations on a coarse time scale. Chose a random subset f ($f < 1.0$) of automaton cells for updating. Then solve the equilibrium boundary-value problems to determine the resultant response of of the chemical fields. This is repeated $1/f$ times until all cells and chemical fields have been updated. This is one generation. No quantitative differences found in evolution of automata if $f<0.1$

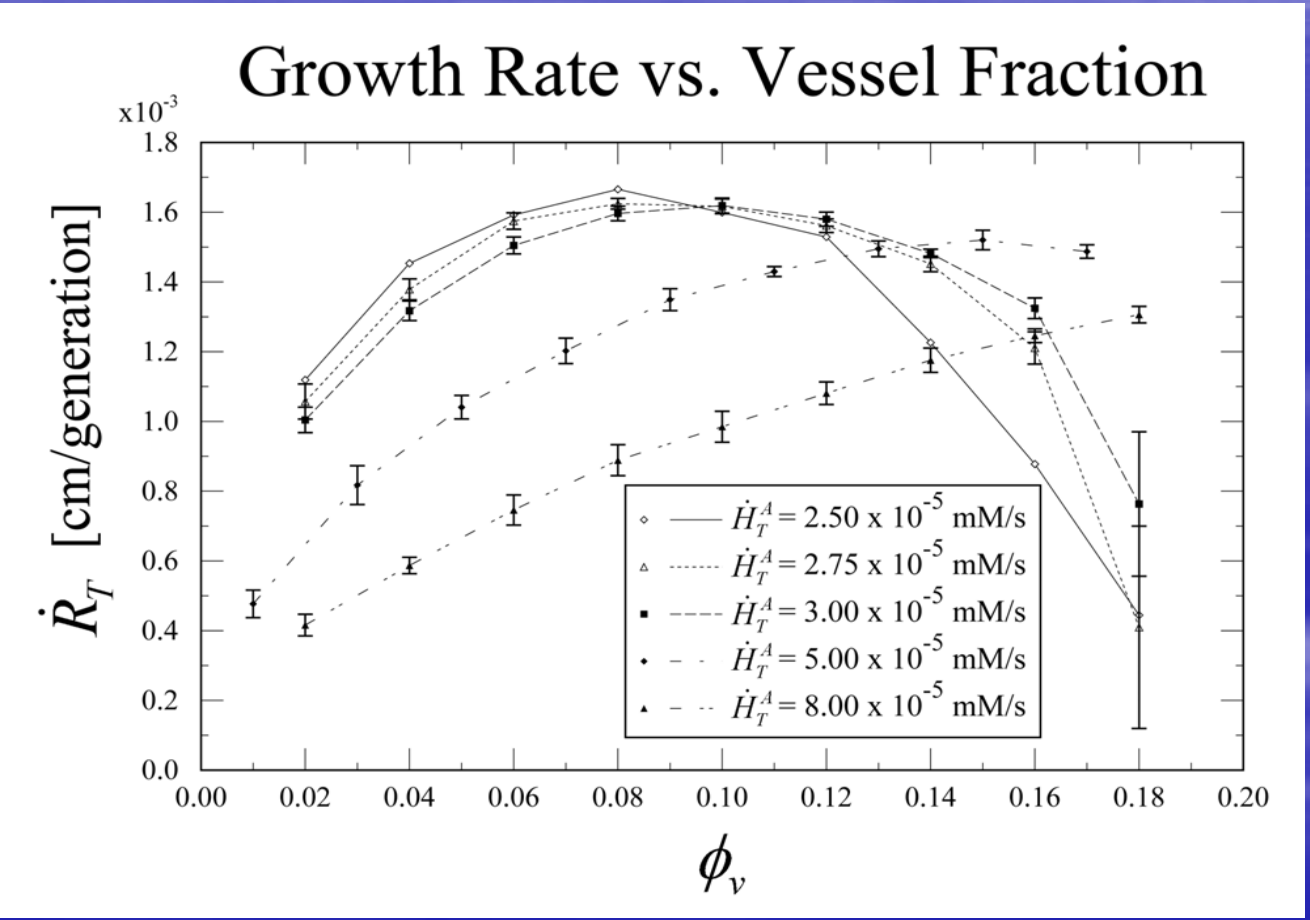
Simulated tumor growth using modified cellular automata model



Variations in Tumor Morphology



Variations in Growth Rate



Evolutionary Models of Carcinogenesis

- Carcinogenesis is often described as “somatic evolution”
- Cancer cells typically accumulate hundreds, thousand, and even hundreds of thousands of genetic mutations
- Each mutation will perturb the fitness of the individual. Those mutations that confer selective growth advantage will result in clonal expansion.
- What are the environmental selection parameters that govern the relative growth advantage of each new phenotype?

Summary of evolutionary model

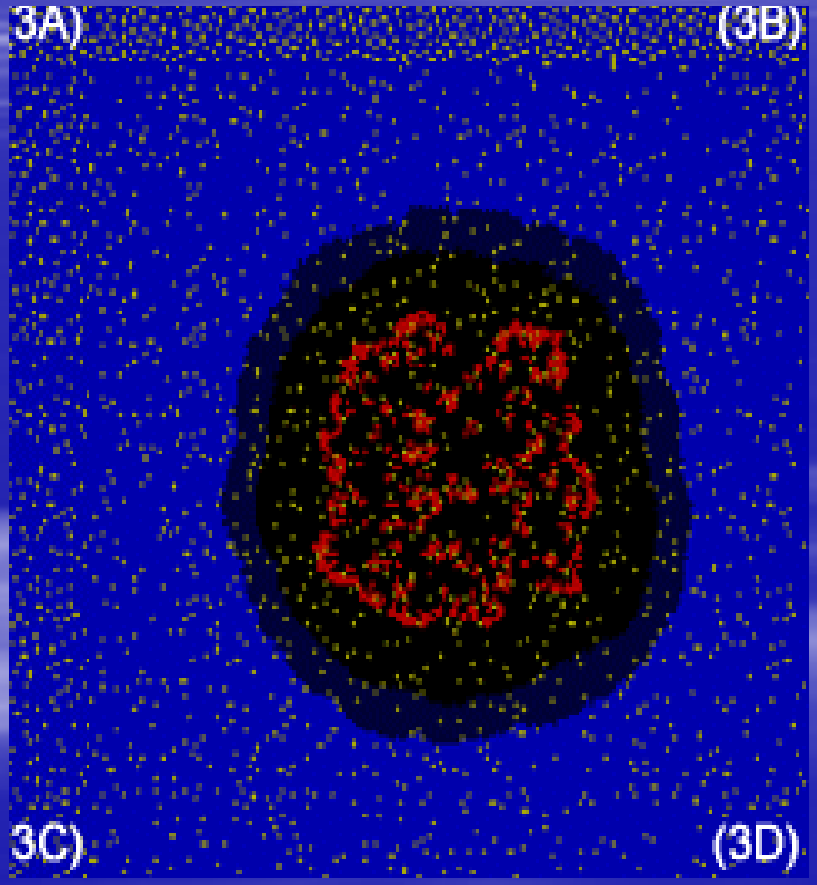
- Carcinogenesis is an inevitable consequence the normal tissue adaptive landscape. A clinical cancer emerges only if the evolutionary speed is sufficient to reach a fitness maximum during the lifetime of the host. Fundamental equation of carcinogenes is $\dot{u}_i = \sigma_{ii} \left| \partial G / \partial v \right|_{v=u_i}$
- Environmental selection parameters in early carcinogenesis promote mutations in oncogenes but these produce only self-limited clonal expansion
- Later carcinogenesis is dominated by substrate competition. This promotes the glycolytic phenotype.
- The resulting acidification of the environment will tend to result in p53 dependent induction of apoptosis. This produces a new environment selecting for p53 mutations to promote resistance the acidic pH_e.
- The invasive phenotype is the predictable result of the interaction of a plastic genome and a sequence of environmental selection forces.

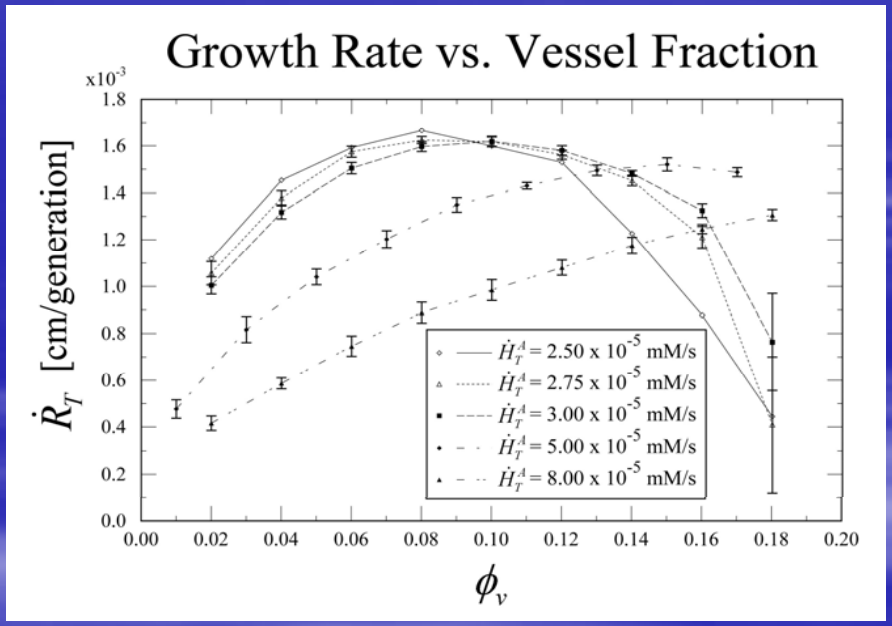
Does the acid-mediated tumor model suggest new therapeutic strategy?

- Recall that the tumor solution to the state equations is only conditionally stable. Critical parameter is δ_1 where $\delta_1 = (d_N/d_H) \times (r_H/r_N) \times K_T$

- Recall the phenomenon of “self-poisoning” in cellular automaton model

Tumor growth can be limited by self-poisoning





Simplistic equations relating tumor growth to vascular pH

$$\frac{dT}{dt} = r_T T\left(1 - \frac{T}{K_T}\right) - d_T f\left(H\right)T$$

where T is the concentration of tumor cells ($cells/cm^3$), r_T is the tumor growth rate ($1/s$), K_T is the tumor carrying capacity ($cells/cm^3$) and d_T is the maximum death rate ($1/s$) for either extreme acidification or alkalinization:

$$\frac{dH}{dt} = r_H T - d_H\left(H - H_S\right)$$

where r_H is the H^+ ion production rate by tumor cells ($M \cdot cm^3/\left(cell \cdot s\right)$), H_S is the serum H^+ ion concentration and d_H is the rate of removal (or addition) of H^+ ions if the local H^+ ion concentration is greater (or less) than that within the serum. Typically, $d_H = \alpha p$, where α is the blood vessel areal density ($1/cm$) and p is the vessel permeability (cm/s) but could also be viewed as having contributions due to buffering capacity

Where

$$f\left(H\right)=\frac{\left(H-H_{opt}\right)^{2}}{H^{2}+bH+H_{opt}^{2}}$$

H is the H^{+} ion concentration expressed as a molarity (M). This function has the desirable properties that $f\left(H_{opt}\right)=0$, $f\left(0\right)=1$, and $f\left(H\to\infty\right)=1$. The parameter b in equation (1) sets the width of the hospitable zone. If $H_{1/2}>H_{opt}$ is the half-maximum point on the acidic side, then $b=H_{1/2}\left[1-4H_{opt}/H_{1/2}+\left(H_{opt}/H_{1/2}\right)^{2}\right]$ such that $f\left(H_{1/2}\right)=1/2$.

Dimensionless form:

$$\frac{d\tau}{ds} = \tau(1-\tau) - \delta_\tau f(h)\tau$$

$$\frac{dh}{ds} = \rho_h \tau - \delta_h (h - h_S)$$

where

$\delta_\tau = d_T / r_T$

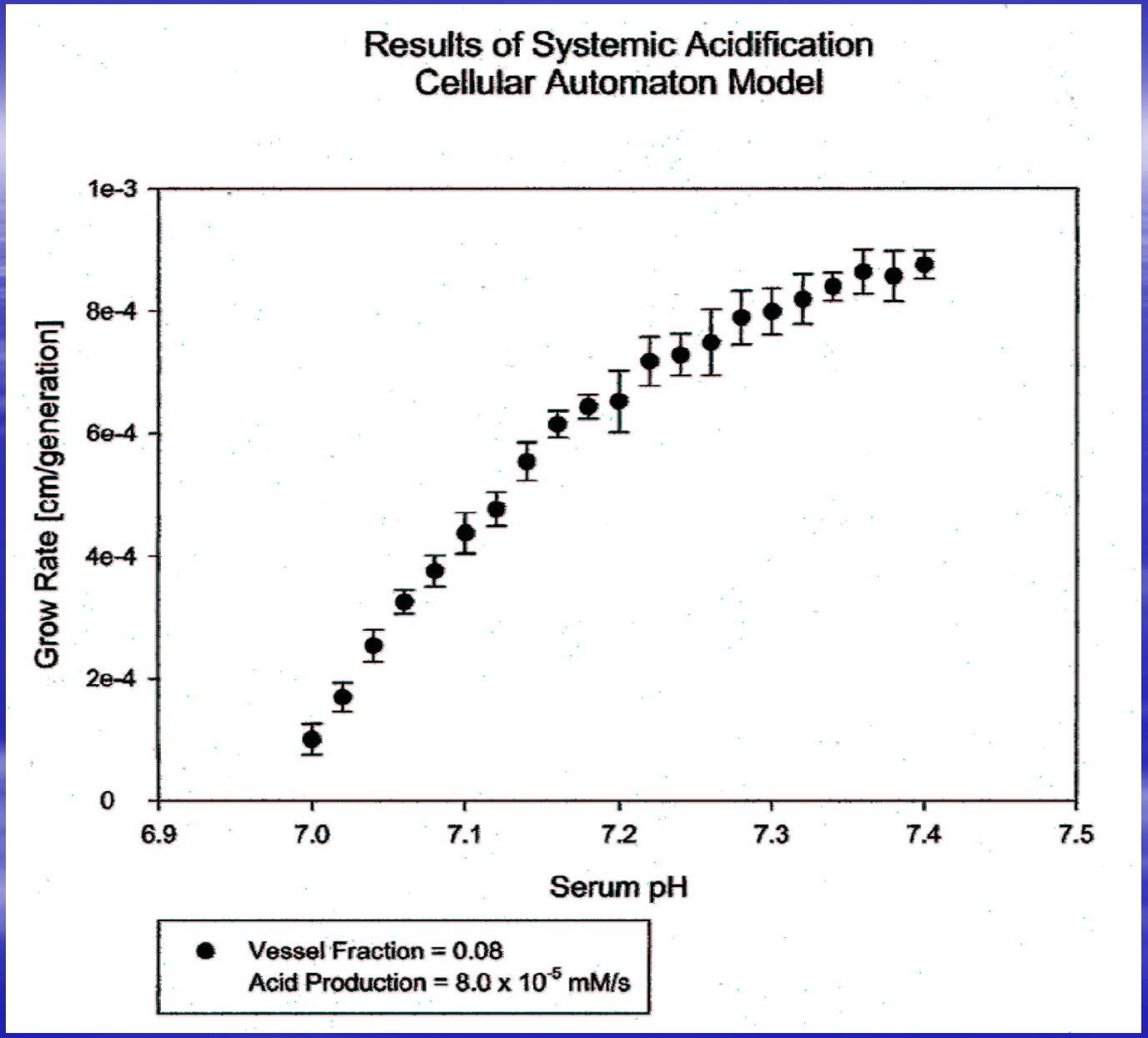
$\rho_h = r_H K_T / (r_T H_{opt})$

$h_S = H_S / H_{opt}$

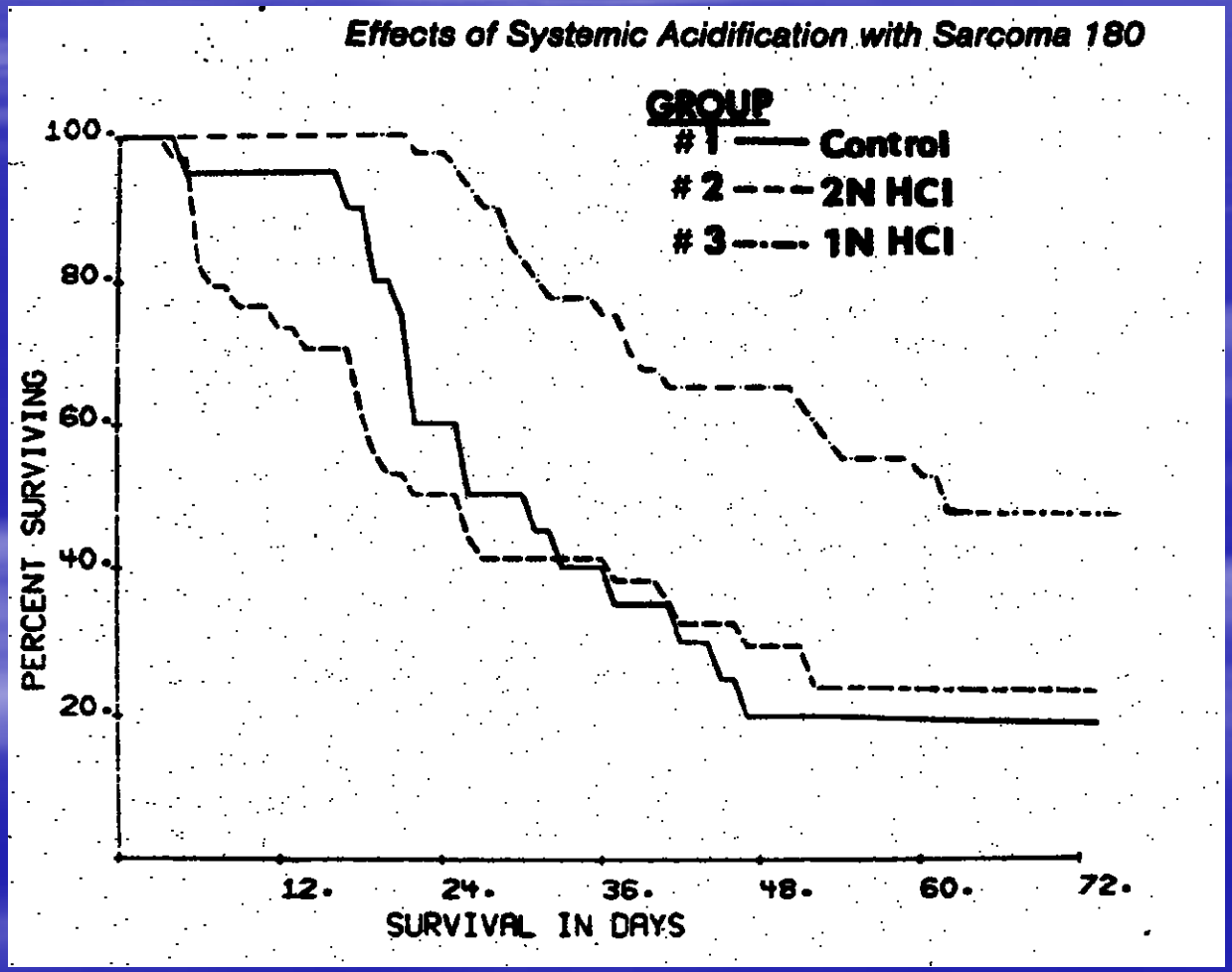
Two fixed-points (*i.e.*, (τ, h) values at which $d\tau/ds = 0$ and $dh/ds = 0$), one where $(\tau = 0, h = h_S)$ and the other where $(\tau > 0, h > h_S)$.

$$\frac{\delta_\tau h_{1/2} (h_S - 1)^2}{h_S + h_{1/2}\left[1 + h_S (h_S + h_{1/2} - 4)\right]} > 1$$

then absence of tumor is a stable state and vice-versa.


Results of Systemic Acidification
Cellular Automaton Model
Grow Rate [cm/generation]
1e-3
8e-4
6e-4
4e-4
2e-4
0
6.9
7.0
7.1
7.2
7.3
7.4
7.5
Serum pH
Vessel Fraction = 0.08
Acid Production = 8.0 x 10^-5 mM/s

From Harguindey SA, Henderson ES, Naeher. Effects of systemic acidification of mice with Sarcoma 180. Cancer Research 39:4634-4371, 1979



Clinical Benefits of cytoreductive nephrectomy in patients with metastatic renal cancer.

- “Spontaneous” regression observed in up to 6% of patients in large series following nephrectomy (usual number about 1%).
- Two recent studies (NEJM 23:1655-1659, 2001 and Lancet 358:966-970, 2001) showed statistically significant survival benefit in patients receiving cytoreductive nephrectomy prior to system therapy with interferon-alfa.
- Proposed mechanisms include reduction of tumor burden, removal of source for future metastases, enhanced immunologic response.

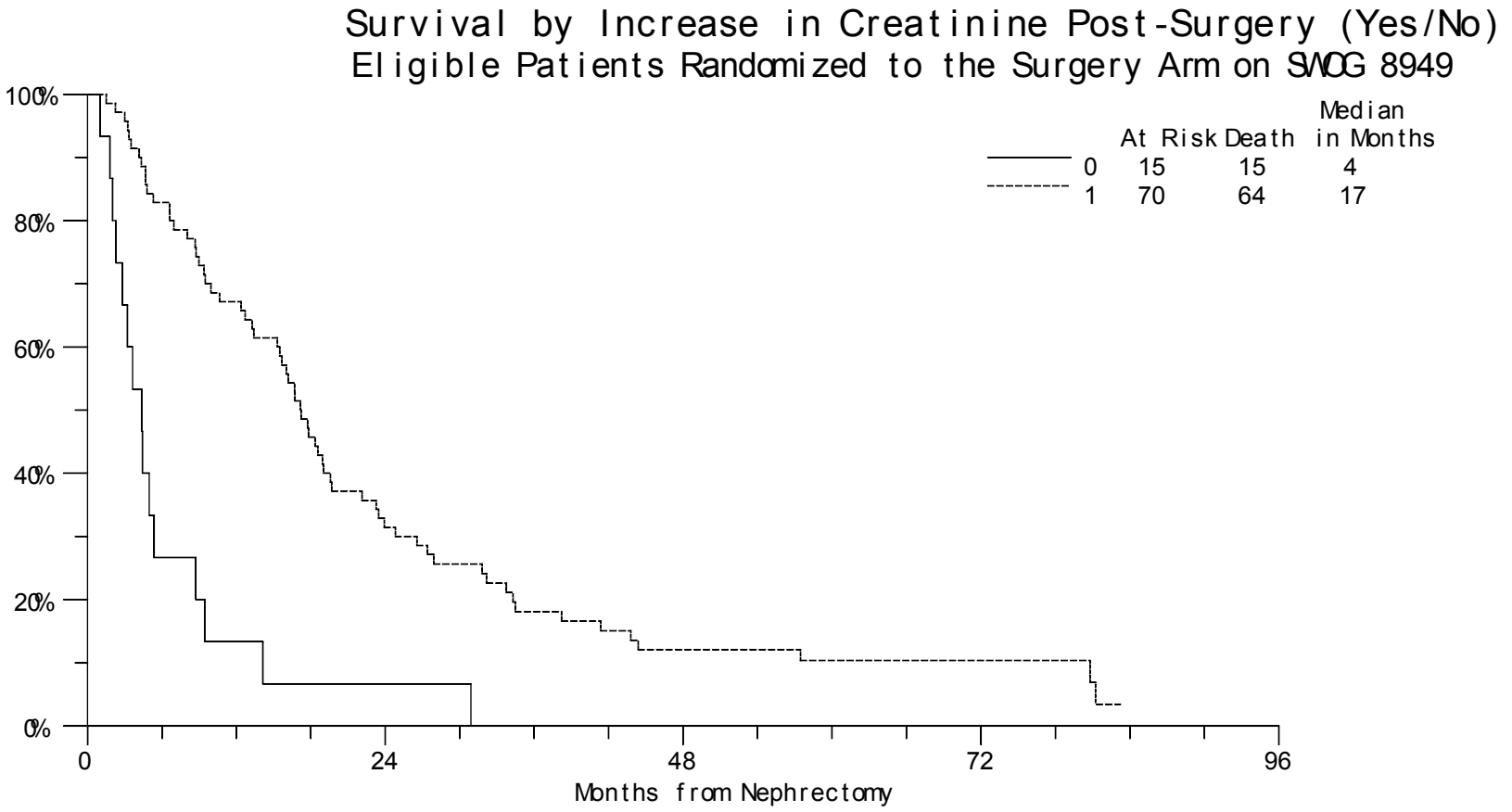
Alternative hypothesis: The benefits of cytoreductive nephrectomy are due to removal of the kidney rather than the cancer

- Proposed mechanism:
- Unilateral nephrectomy, by removing functioning nephrons, produces mild renal failure.
- Mild azotemia produces a graded metabolic acidosis
- The systemic acidification will be sufficient in many cases to reduce the velocity of the propagating tumor wave front prolonging survival
- In rare cases the degree of acidification is sufficient to destabilize the tumor solution of the state equations so that the system moves to the new stable solution (the null solution) with apparently “spontaneous” regression of the tumor.

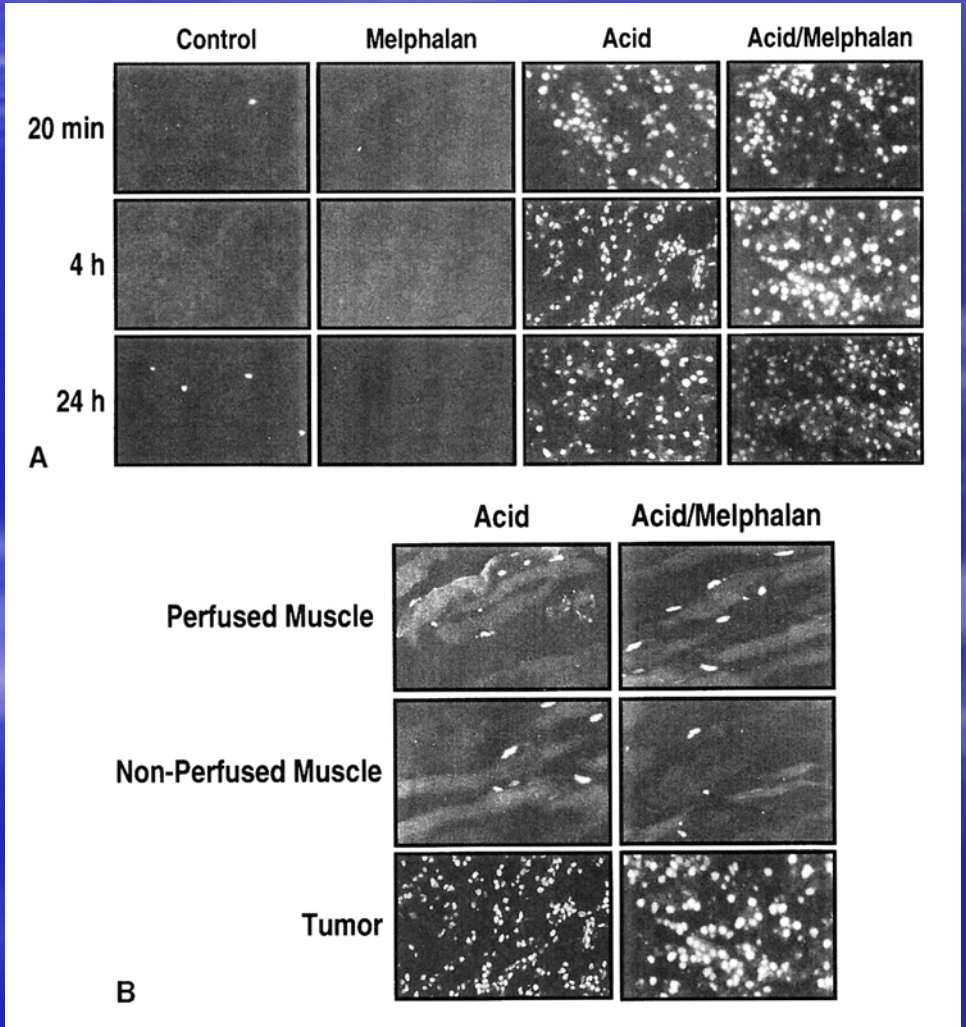
The hypothesis would be supported by a correlation between the degree of renal failure and survival following cytoreductive nephrectomy

- Clinical data from SWOG 8949 reviewed (see NEJM article)
- All patients received Interferon-alfa. Randomize into surgical (cytoreductive nephrectomy) and non-surgical arms.
- Survival for interferon alone 8.1 months, nephrectomy plus interferon 11.1 months. P=0.012
- BUN and creatinine obtained from records. “Pre-surgical” values at time of enrollment. “Post-surgical” values at time of first dose of interferon.

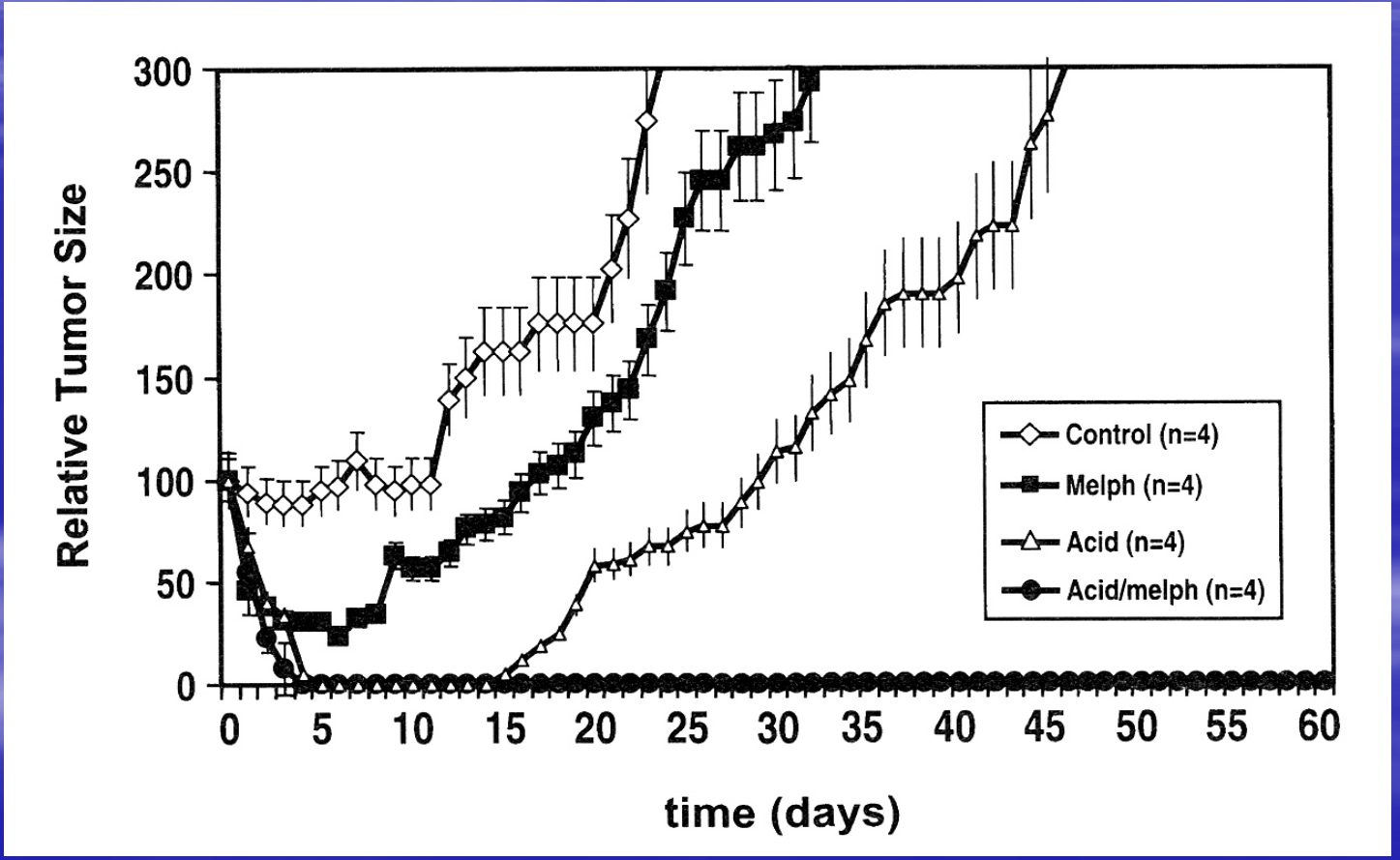
Patient Survival Following Cytoreductive Nephrectomy



Tumor cell apoptosis induced by 10 minute intraarterial infusion of melphalan, acid (pH 6.8) or acid plus melphalan (from Kelley ST et al , Surgery 132 (2):252-258, 2002.



Survival following 10 minute intraarterial infusion of acid (pH 6.8). Melphalan and acid plus melphalan (from Kelley et al Surgery 132 (2):252-258, 2002)



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Conclusions:

General:

Cancer is a non-linear disease!

Specific:

1. Mathematical models demonstrate that both early and late tumor growth may be explained by local microenvironmental perturbations that result from altered tumor metabolism with increased acid production
2. The glycolytic phenotype is the consequence of specific evolutionary selection pressures during the later stages of carcinogenesis.
3. The acid-mediated tumor invasion model predicts novel methods of treatment which are supported by some preliminary clinical data.

The 40 Years War

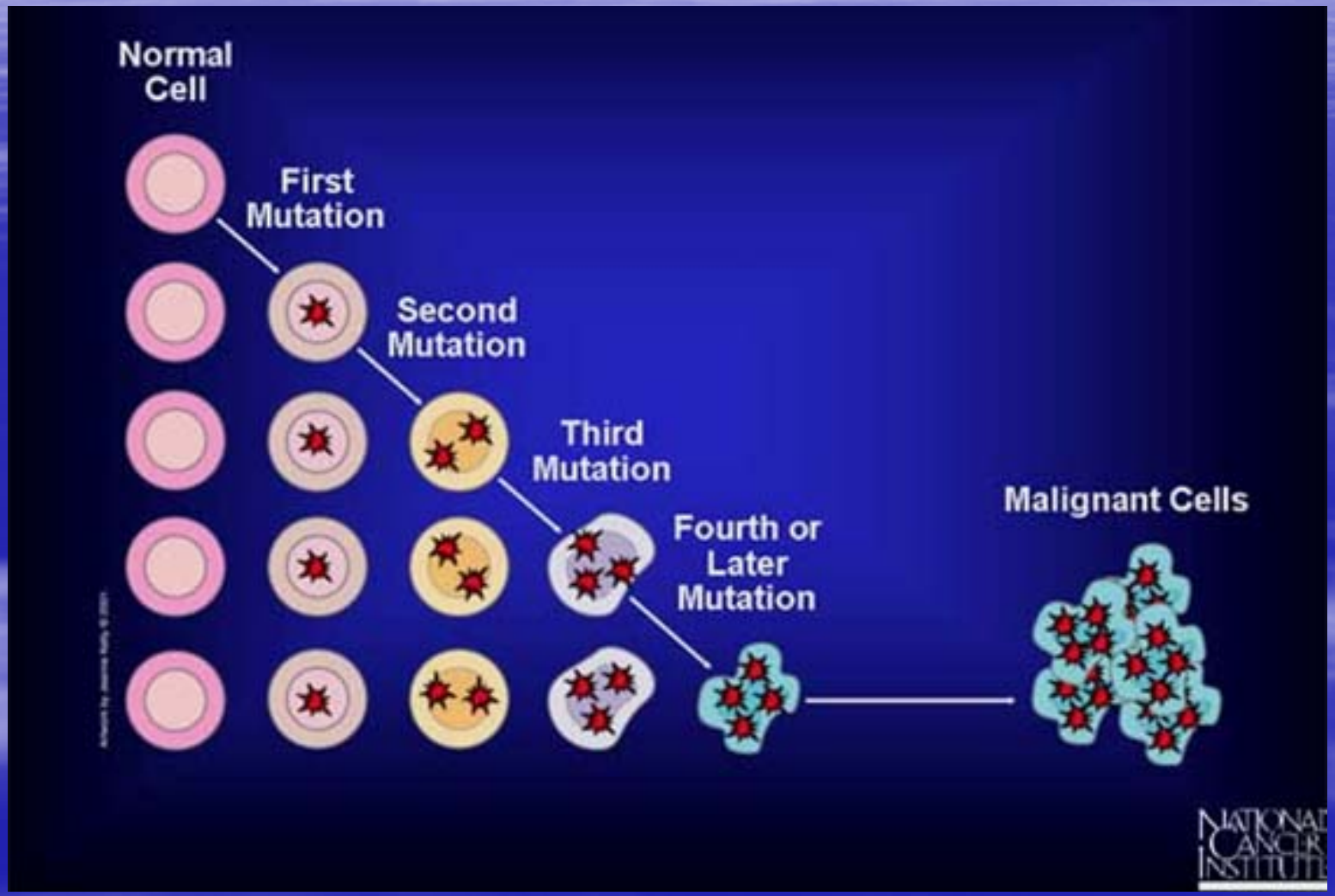
- "I will also ask for an appropriation of an extra $100 million to launch an intensive campaign to find a cure for cancer, and I will ask later for whatever additional funds can effectively be used. The time has come in America when the same kind of concentrated effort that split the atom and took man to the moon should be turned toward conquering this dread disease. Let us make a total national commitment to achieve this goal."
- Richard Nixon. State of the Union Speech, 1970

The “war” is not going that well

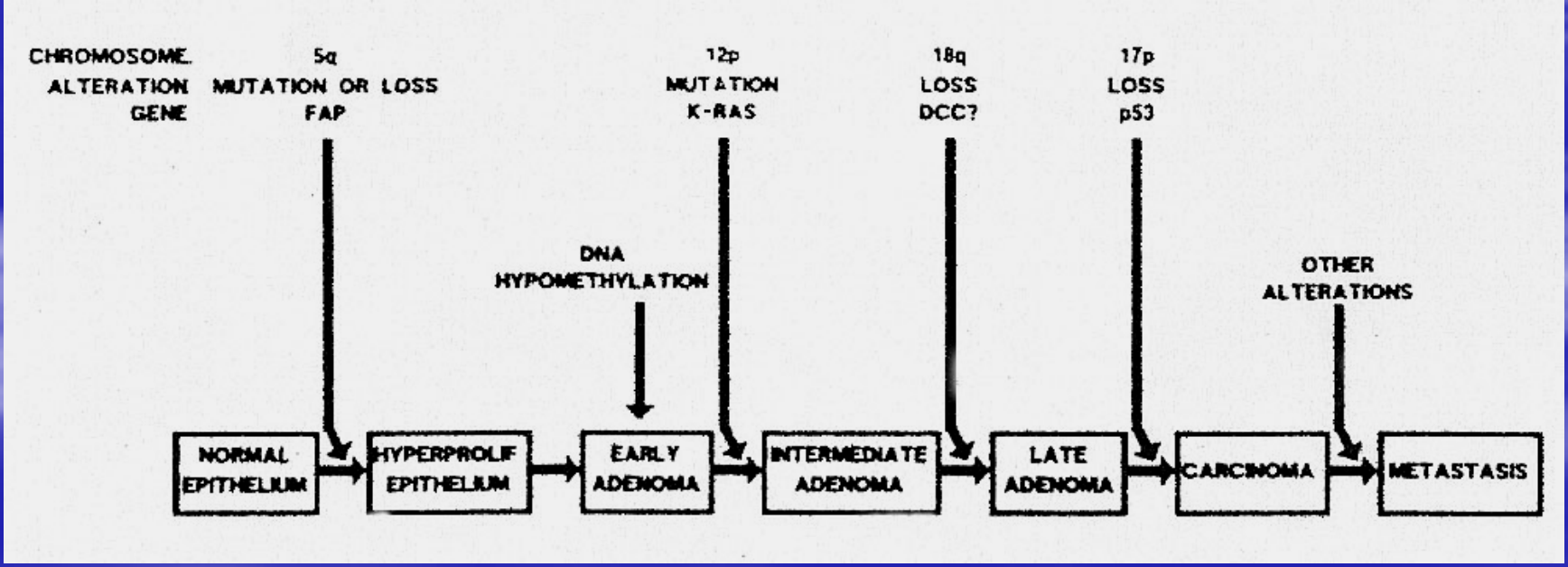
Rank	Cause of Death	No. of deaths	% of all deaths
1.	Heart Diseases	700,142	29.0
2.	**Cancer**	**553,768**	**22.9**
3.	Cerebrovascular diseases	163,538	6.8
4.	Chronic lower respiratory diseases	123,013	5.1
5.	Accidents (Unintentional injuries)	101,537	4.2
6.	Diabetes mellitus	71,372	3.0
7.	Influenza and Pneumonia	62,034	2.6
8.	Alzheimer’s disease	53,852	2.2
9.	Nephritis	39,480	1.6
10.	Septicemia	32,238	1.3

Source: US Mortality Public Use Data Tape 2001, National Center for Health Statistics, Centers for Disease Control and Prevention, 2003.

“Cancer is a disease of the genes”



The concept of “somatic evolution” was first proposed in the 1950’s but is embodied in Fearon-Vogelstein diagram.



What does genetics tell us and not tell us?

Darwin's Principles in cancer evolution

- ***Heritable Variation at several levels (population and individual phenotypic heterogeneity - reaction norms - in cancer and normal cells)***
- ***Struggle for Existence (only in cancer)***
- ***Fitness determines proliferation – fitness of any phenotype is dependent on environmental selection force***

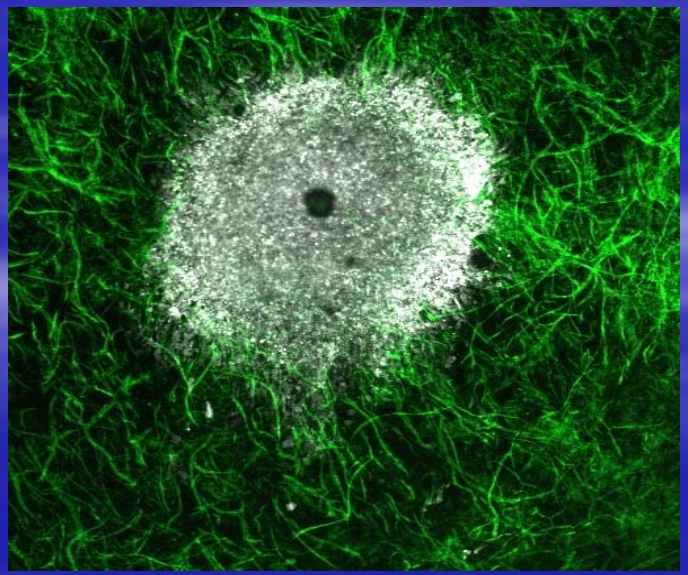
Bridging molecular biology with cancer evolution

How exactly does a mutation confer a proliferative growth advantage?

Evolution selects phenotypes not genotype. Fitter phenotypes proliferate at the expense of those less fit

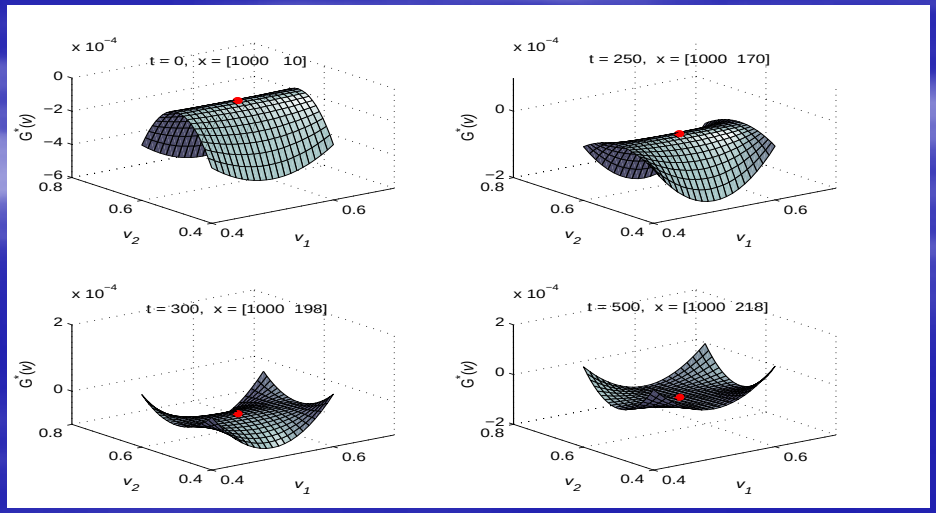
The fitness value of any genotype or phenotype is dependent on the extant environment

Which, in most tumors, is spatially and temporally heterogeneous



Evolutionary models in cancer

- **Ecological Dynamics**: $\partial x_i/\partial t = x_i G$ at $v = u_i$
- **Strategy Dynamics**: $\partial u_i/\partial t = \sigma^2 (\partial G/\partial v)$ evaluated at $v = u_i$ (Note combination of genetic and environmental influence)
- Small changes in a population or strategy can trigger dramatic changes in the adaptive landscape.



$$\dot{X}_i = 1 - \left(\frac{\sum_{i=1}^{n_s} a(v,\mathbf{u}) x_i}{K(v)} \right) \cdot B_n \left(\frac{E(v) R^2}{R_0^2 + R^2} - m \right)$$

Proliferation of Population x_i	**Is dependent on**	Normal tissue growth constraints	**and**	Substrate availability
Normal or abnormal growth		*The ability of cells in the population to detect and process signals from other cells, the ECM, and positive and negative growth factors*		*Substrate uptake must exceed basal demand for cell proliferation*

Normal cell

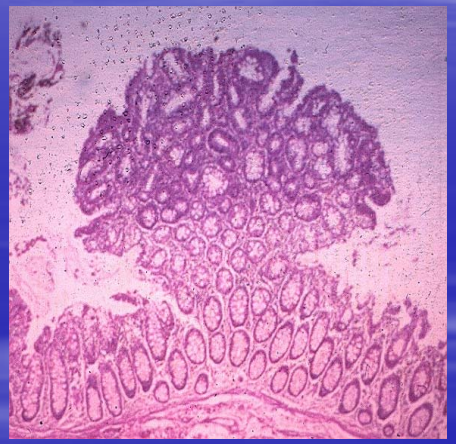
The math model demonstrates carcinogenesis requires mutations in genes that make, receive, or process growth and death signals

APC mutation (5q)

Proliferation

DNA hypomethylation

Adenoma I



K-ras mutation

Adenoma II

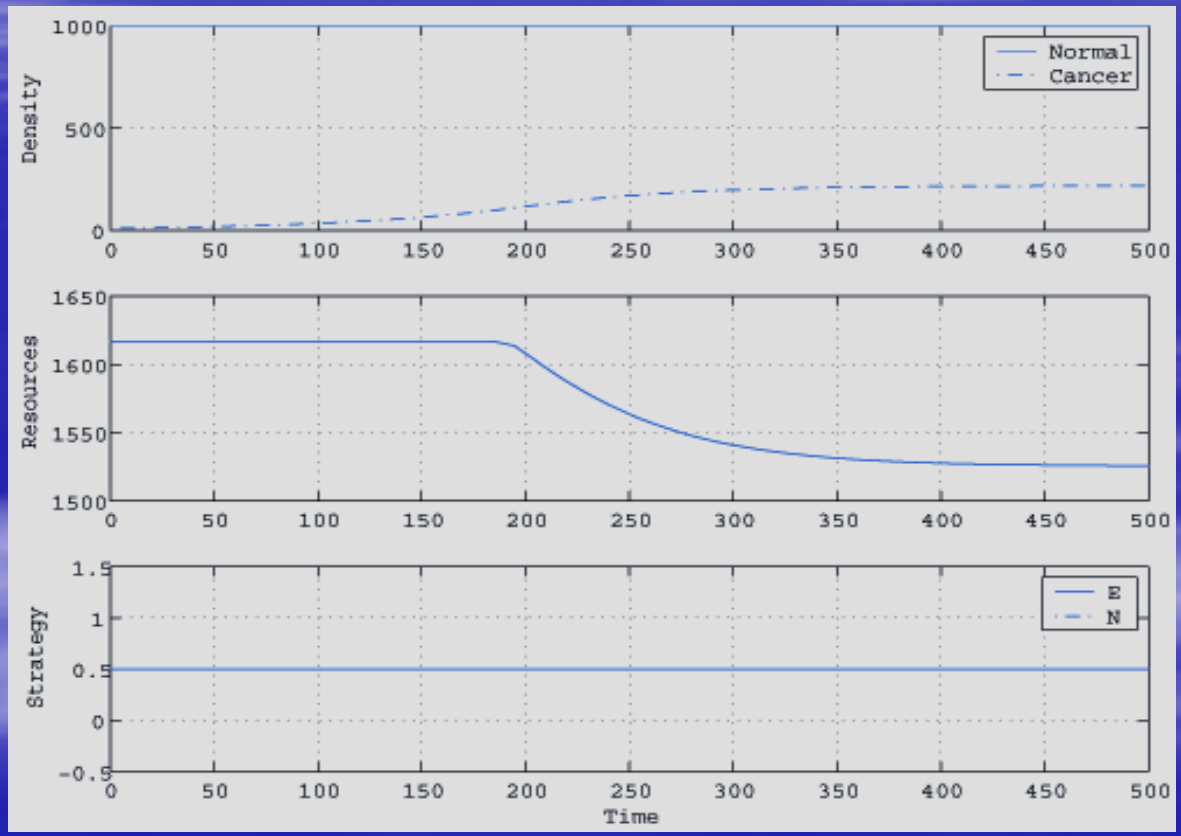
DCC deletion (18q21)

Adenoma III

p53 mutation (17p)

Carcinoma

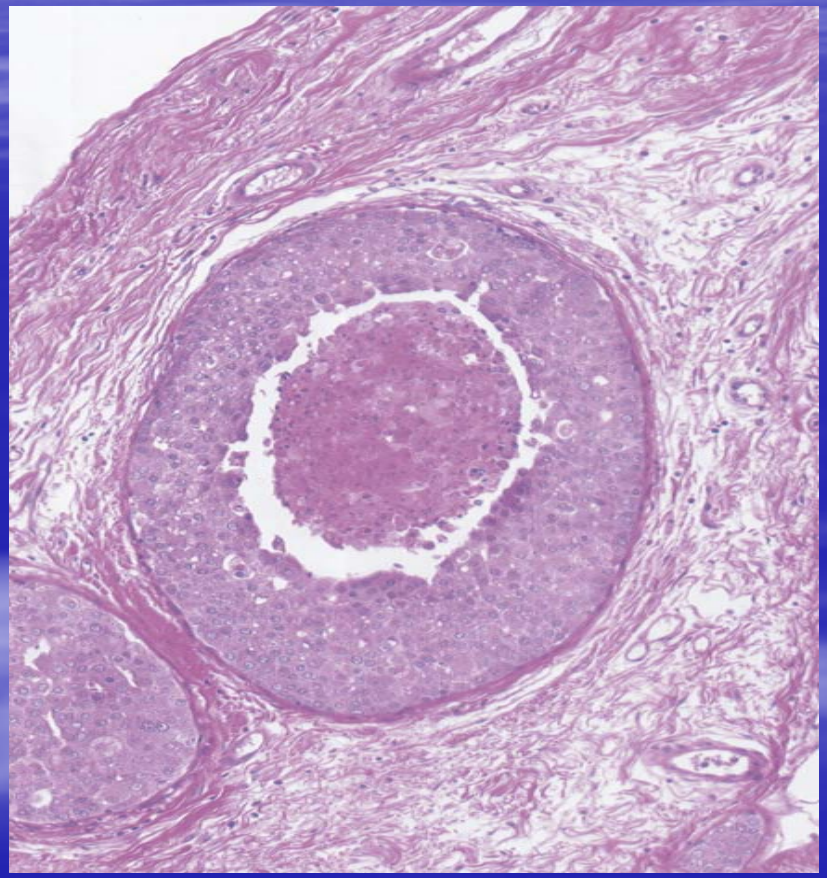
But this produced only self-limited growth

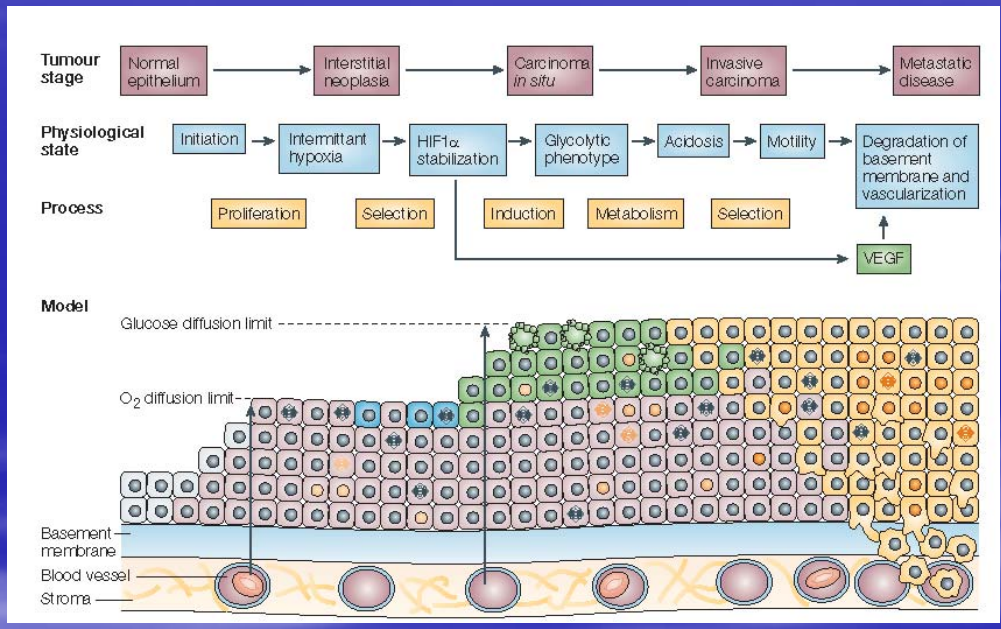


$$1 - \frac{\sum_{i=1}^{n_s} a(v,\mathbf{u}) x_i}{K(v)}$$

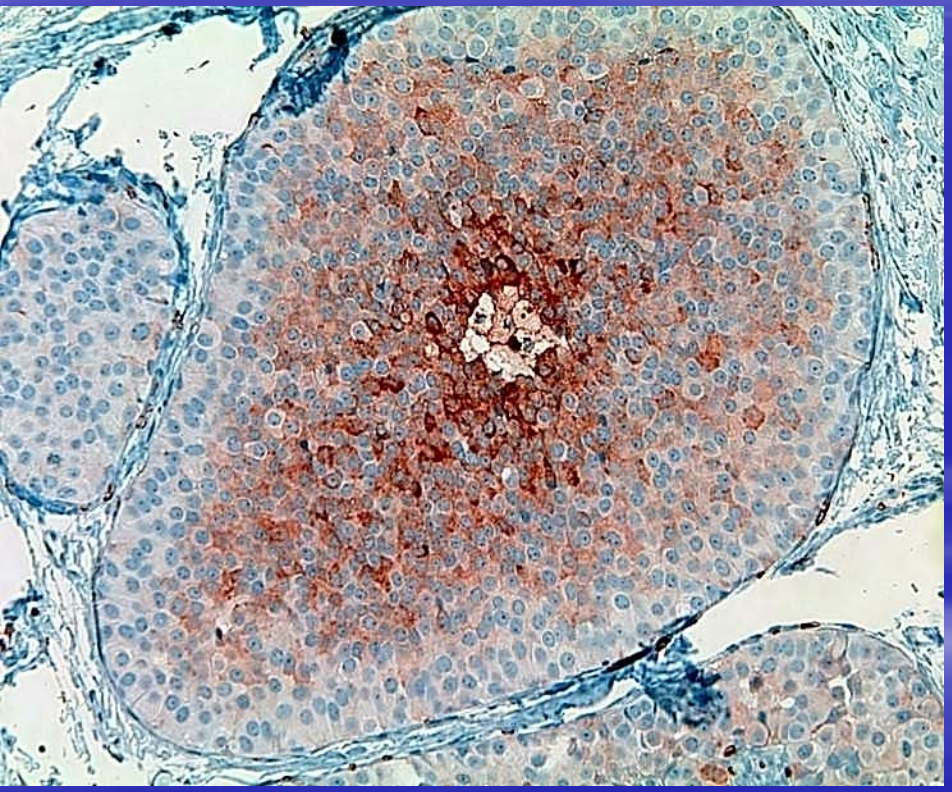
$$B_n \left(\frac{E(v) R^2}{R_0^2 + R^2} - m \right)$$

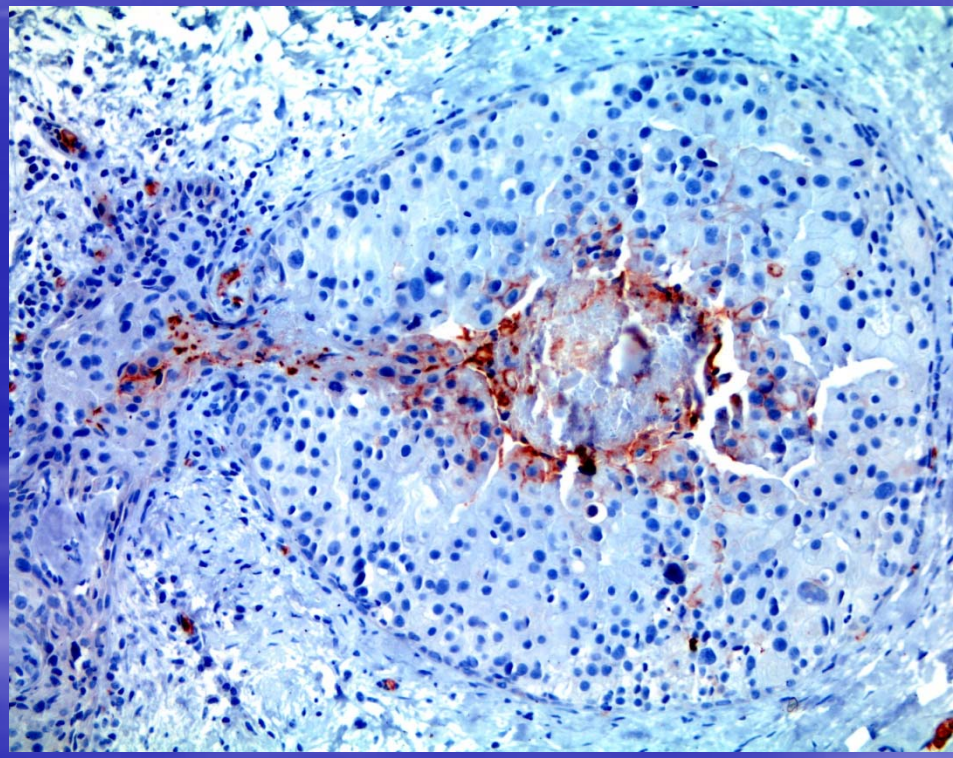
Re-focus on the anatomy and physiology of epithelial surfaces



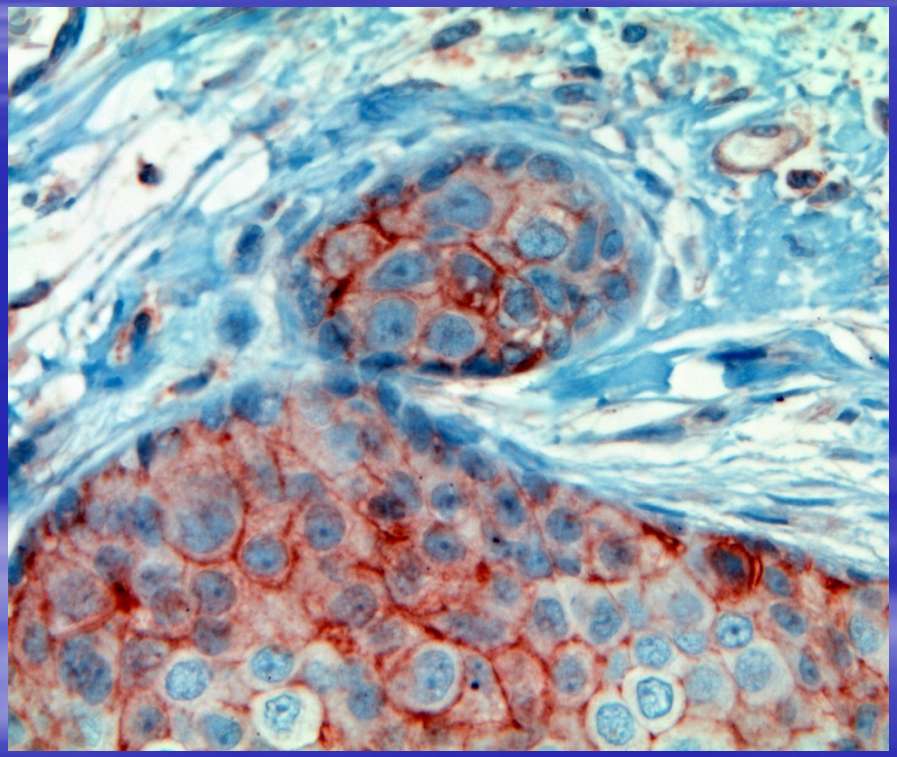


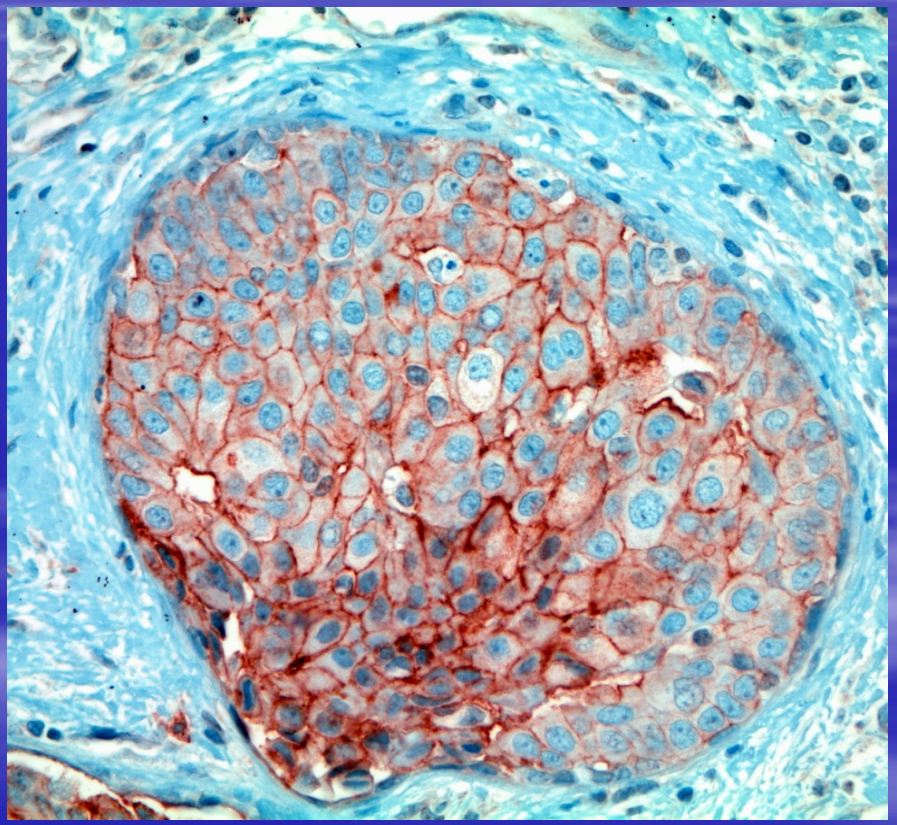
19 of 20 DCIS exhibited focal areas of increased GLUT-1 expression. Central distribution and in micro-invasion





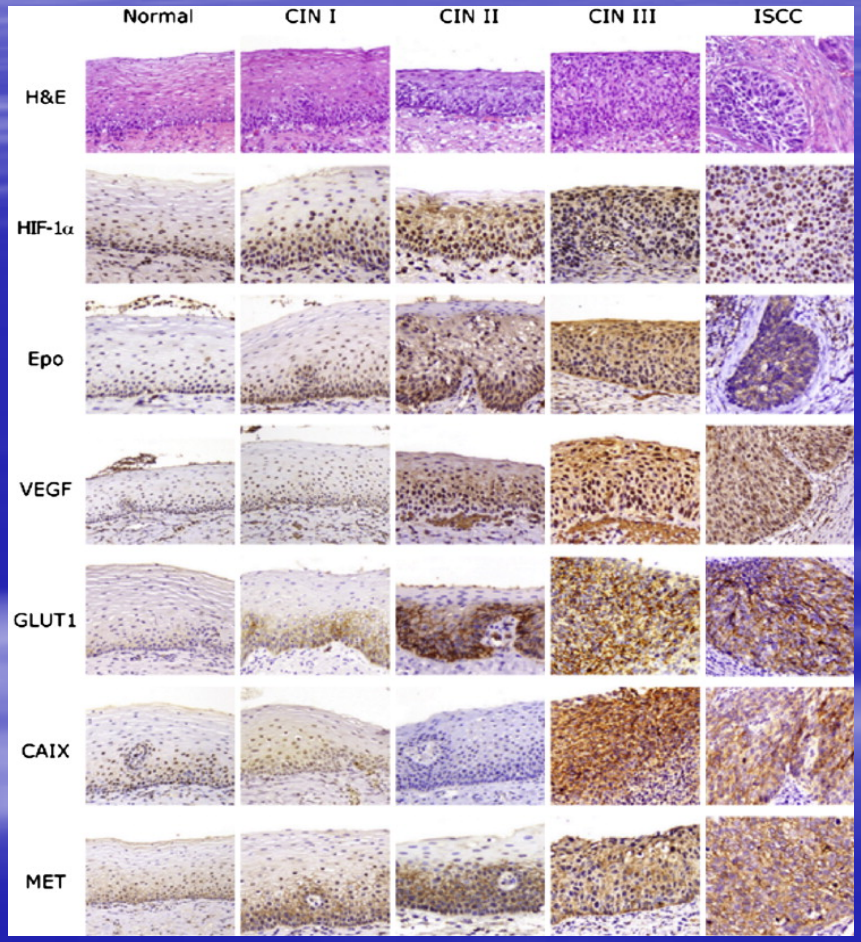
Upregulation of Na/H exchangers in regions of micro-invasion





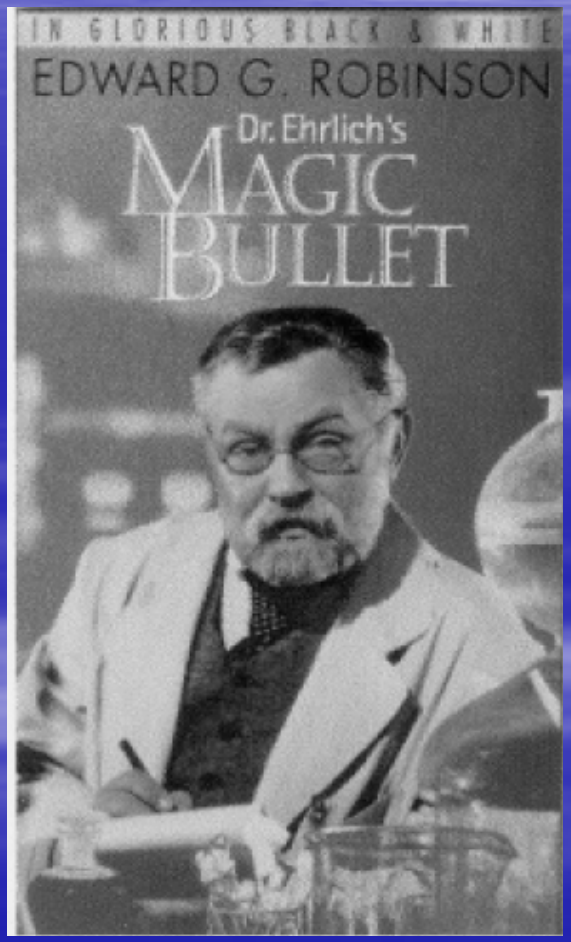
Similar results reported in cervical cancer

Lee et. al.Gynecol Oncol. 2008



- "Successful adaptation to the hypoxia-glycolysis-acidosis sequence in the microenvironment is crucial during carcinogenesis."

The concept of a cancer cure stems from Paul Ehrlich's magic bullet which proved prophetic in treating infectious diseases



The magic bullet concept is manifested by the desire to find "cancer antibiotics"

"In fact, a diagnosis of *cancer* would be similar to diagnosing *infection* in a patient. Both words represent a broad spectrum of illnesses. Using *infection* as an example, what kind of infection – strep, staphylococcus, e-coli, tuberculosis, anthrax? Each of those infectious bacteria responds to certain antibiotic treatments, but which antibiotic?

(from the Arizona Cancer Center website)

Expectations remain that a cure will be found if the medical community is sufficiently diligent, creative, and intelligent

“It (stimulus package) will launch a new effort to conquer a disease that has touched the life of nearly every American by seeking a cure for cancer in our lifetime.”

- President Obama

Will today be the day? -

Moffitt Cancer Center logo

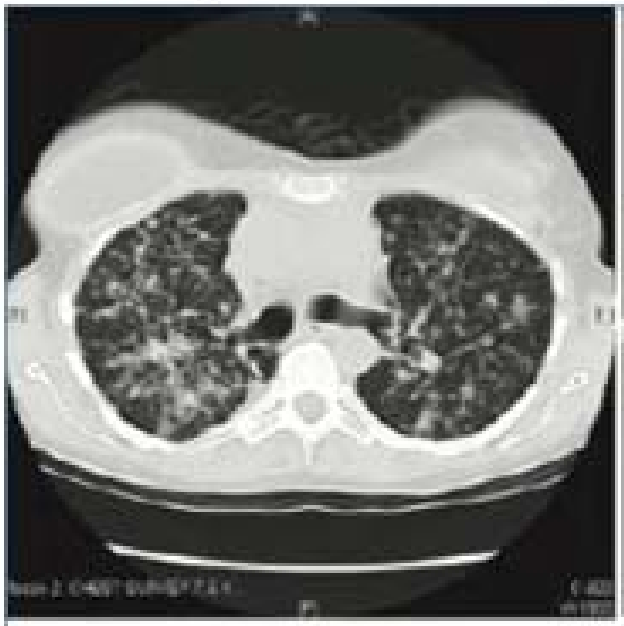
Saudis cure cancer with camel urine

AP news headline

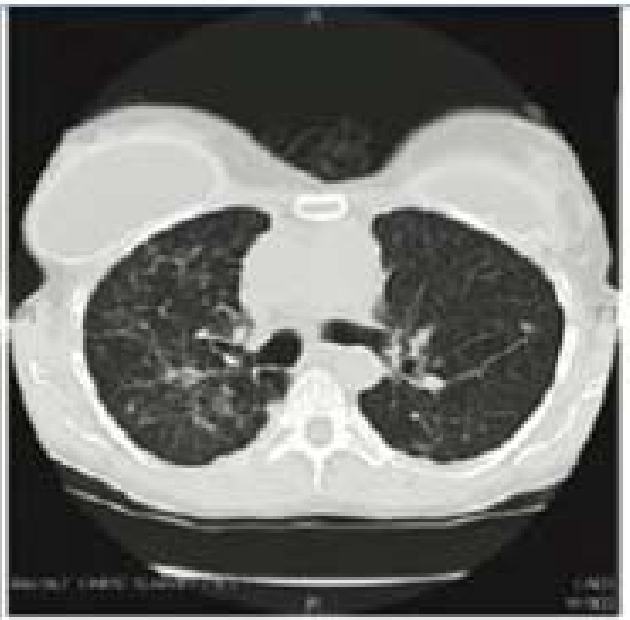
Cannabis linked to 'prostate cancer cure

LA Times

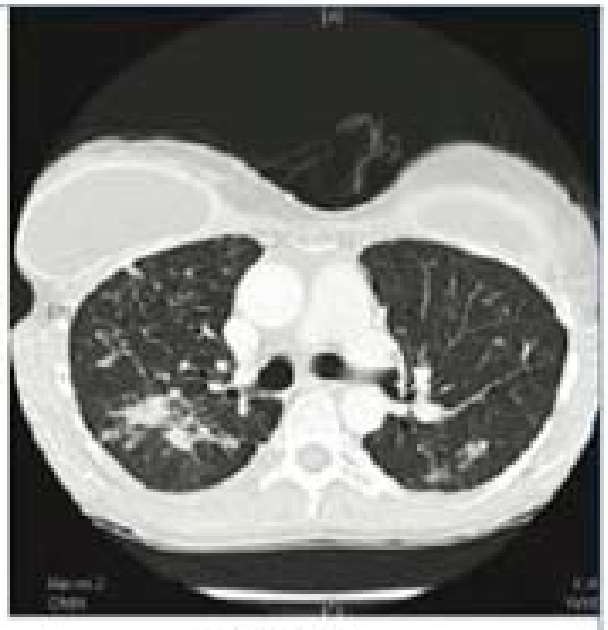
Cancers evolve resistance



Day 0



4 months



25 months

Although 50% died, 50% survived – some were sheltered, some were sistant

GENOMICS

CMJ

doi: 10.3325/cmj.2009.50.34

Historic, Demographic, and Genetic Evidence for Increased Population Frequencies of CCR5Δ32 Mutation in Croatian Island Isolates after Lethal 15th Century Epidemics

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Aim To assess the frequency of 32 base pair deletion in *CCR5* (CCR5Δ32), which has been shown to confer resistance to HIV infection in a homozygous form, in 10 isolated island communities of Dalmatia, Croatia, with different histories of exposure to epidemics during and since the medieval period.

Methods In 2002, DNA analysis of 100 randomly selected individuals from each of the 10 isolated communities of 5 Croatian islands (Susak, Rab, Vis, Lastovo, and Mljet) showed high levels of 3-generational endogamy, indicating limited gene flow. Five of the communities were decimated by epidemics of unknown cause between 1449-1456, while the other 5 villages remained unaffected. Genotyping of the *CCR5* gene was performed using the polymerase chain reaction method with primers flanking the region containing 32-bp deletion.

Results The frequency of CCR5Δ32 in the 5 villages affected by the epidemic was 6.1-10.0%, and 1.0-3.8% in the 5 unaffected villages. The Δ32 mutation was found in 71 of 916 alleles among the individuals from the affected villages (7.5%), and in 24 of 968 alleles in unaffected villages (2.5%, $\chi^2 = 27.3$, $P < 10^{-6}$). A previous study in 303 random Croatian blood donors showed the frequency of the CCR5 Δ32 of 7.1% in the general population. The difference remained significant after correcting for population structure using both STRAT and STRUCTURE software and the genomic control test, to ensure results do not arise from the background genetic differences.

Conclusion Our results and historical evidence, suggest that the mid-15th century epidemic could have acted as a selection pressure for the CCR5Δ32 mutation.

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The plague returned, but with much lower mortality

- In 1361 there was a second pestilence within England, which was called the mortality of children. Several people of high birth and a great number of children died.
- In 1374 the fourth pestilence began in England... In the following year, a large number of Londoners from among the wealthier and more eminent citizens died in the pestilence.

Overlooking evolution: A systematic analysis of cancer relapse and therapeutic resistance research

C. Athena Aktipis[1,2], Virginia S. Y. Kwan[1], Kathryn A. Johnson[1], Steven L. Neuberg[1], Carlo C. Maley[2]

- Cancer therapy selects for cancer cells resistant to treatment, a process that is fundamentally evolutionary. To what extent, however, is the evolutionary perspective employed in research on therapeutic resistance and relapse? We analyzed 6,228 papers about therapeutic resistance and/or relapse in cancers and found that the use of evolution terms in abstracts has remained at about 1% since the 1980s. However, detailed coding of 22 recent papers revealed a higher proportion of papers using evolutionary methods or evolutionary theory, although this number is still less than 10%. Despite the fact that relapse and therapeutic resistance is essentially an evolutionary process, it appears that this framework has not permeated research. This represents an unrealized opportunity for advances in research on therapeutic resistance.

Challenges: Overcoming barriers

What is evolution and how does it apply to cancer?

Depends on who you ask:

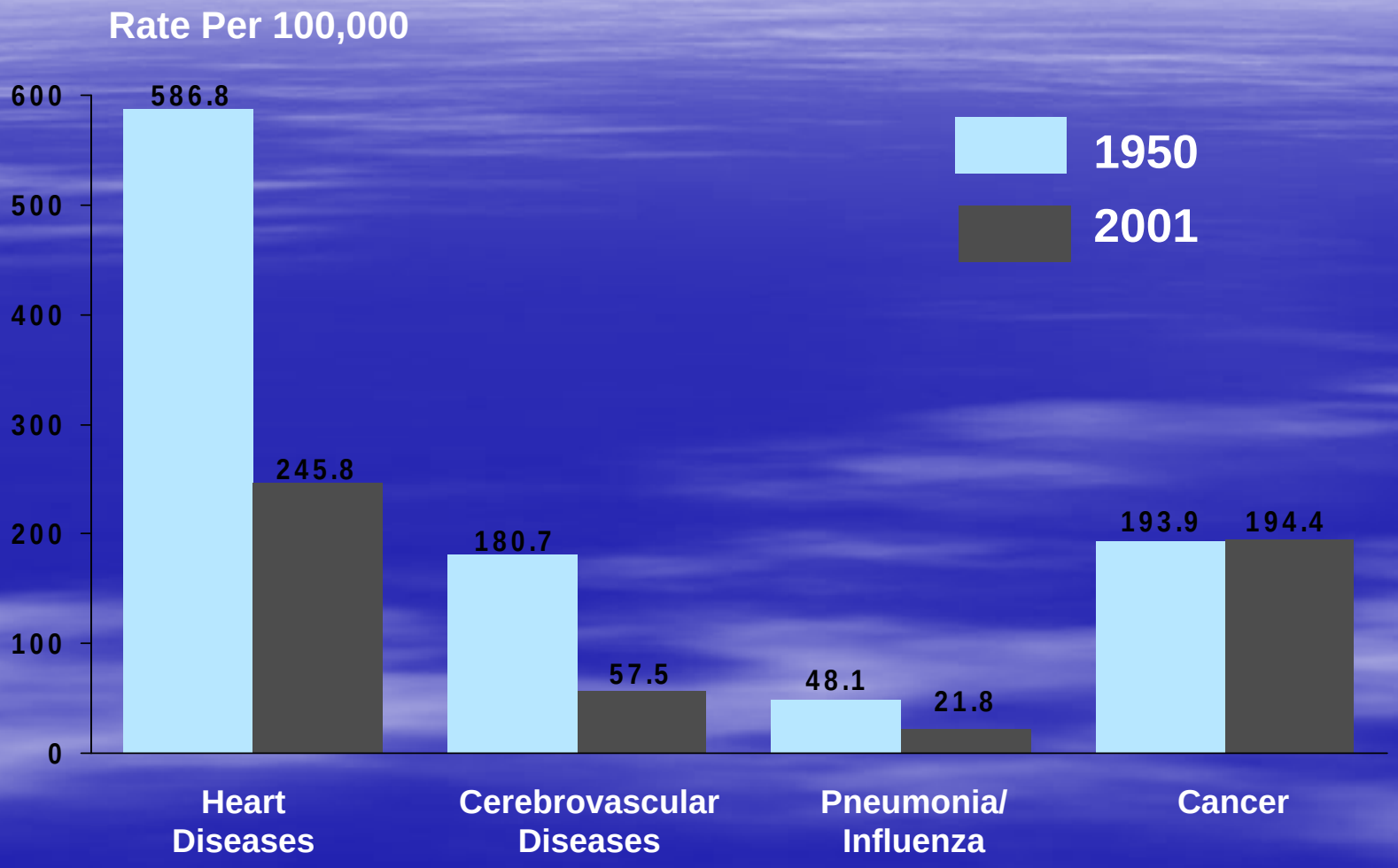
evolutionary biologists,

Oncologists, social scientists

New paradigms typically meet resistance

- "I don't believe in mathematical modeling."
- "Mathematical models are for researchers too lazy to do the experiments."
- "The PI *opines* mathematical models can describe tumor invasion – this is patently absurd."

Change in the US Death Rates* by Cause, 1950 & 2001



* Age-adjusted to 2000 US standard population.
Sources: 1950 Mortality Data - CDC/NCHS, NVSS, Mortality Revised.
2001 Mortality Data–NVSR-Death Final Data 2001–Volume 52, No. 3.
http://www.cdc.gov/nchs/data/nvsr/nvsr52/nvsr52_03.pdf

Tumor invasion models originated in a failed experiment

- IDEA – Use tumor cells obtained through CT guided biopsies of primary and metastatic tumor for diagnosis to develop primary cell cultures and test the inhibitory effects of chemotherapeutic agents in-vitro to predict clinical outcomes analogous to sensitivity testing of cultured bacteria to antibiotics
- METHODS- Disperse material from CT guided aspiration of probable metastatic colon cancer into small cellular aggregates and seed in culture flasks containing DMEM with 20% FBS plus antibiotics

- RESULTS-Initial good tumor growth forming monolayers in about 2 weeks but with observable “islands” of normal fibroblasts. Islands then expanded rapidly and invariably overgrew the culture dish destroying all the tumor cells.
- RESPONSE .
- – kill fibroblasts!
- EVENTUAL RESPONSE- why?? No good answer – lots of data but no organizational framework.
- SOLUTION-Mathematical models
- FUNDAMENTAL FLAW – linear intuitive thinking, reasoning by analogy but bacterial infection is typically a short-term, linear disease, cancers are chronic and dominated by non-linear processes

- “In the absence of consistent application of rigorous mathematical models, theoretical medicine will largely remain empirical, phenomenological and anecdotal, successful only in linear systems that can be defined by a single experiment or a few experiments.”

- Gatenby and Maini in *Nature*, 2002

General Goal:

- Search for a common, unifying mechanism that confers on cancer cells, despite their genotypic and phenotypic diversity and instability, the consistent ability to invade and destroy normal tissue.

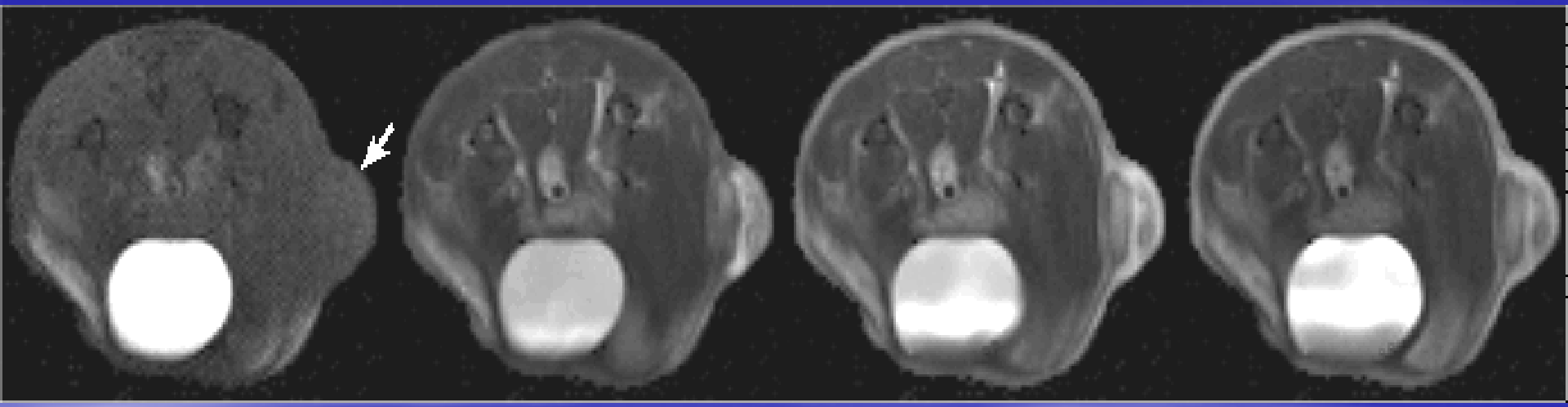
Strategy for developing mathematical models of tumor invasion

Treat tumor as a biological invasion. That is, the tumor cells represent a foreign "species" that begin as a small population of cells (perhaps one) but, because of competitive advantages over normal tissue, proliferate rapidly driving the normal cells to extinction.

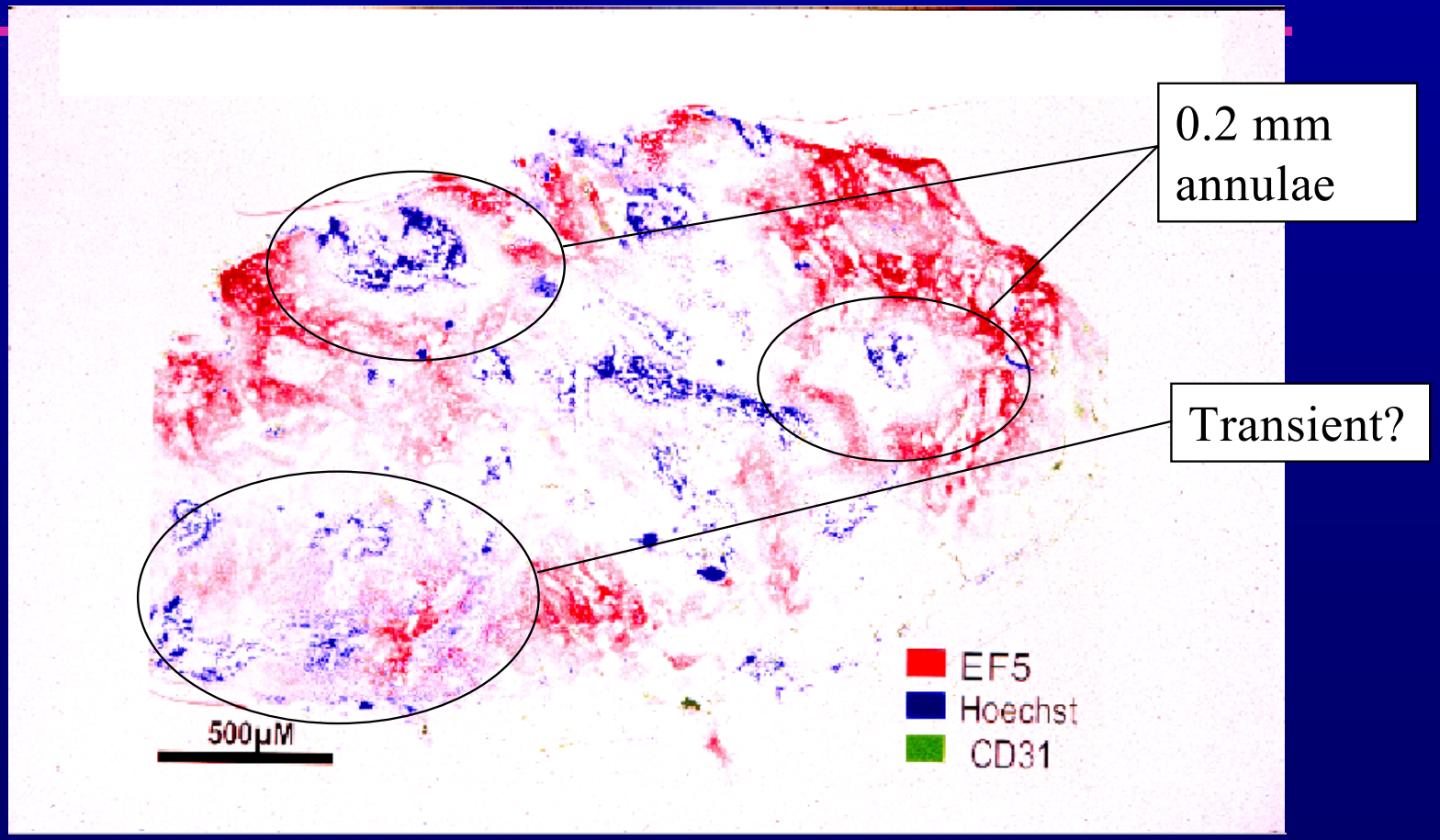
Apply models models from population biology to invasive cancer

What are the competitive advantages that transformation confers on tumor cells that allow unbounded growth? "One species invades another only by killing its young or stealing its food" - Schaefer

Focus on tumor in the context of altered tumor metabolism and microenvironment: vascularity is spatially and temporally heterogeneous



The tumor microenvironment



Courtesy R Hill

Selective use of glycolytic metabolism for energy production is a *hallmark* of transformed cells

Warburg (circa 1920's) first observed consistent increased glucose uptake in tumor tissue. This turned out to be due to preferential use of the glycolytic metabolic pathways even in the presence of abundant oxygen. Recall:

Aerobic metabolism

- Glucose + O_2 —> 36 ATP + H_20 + CO_2

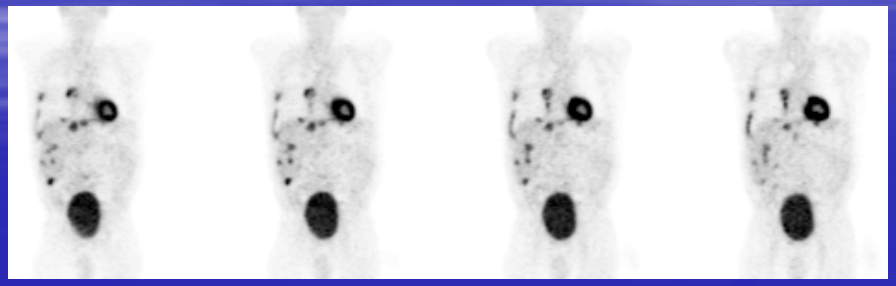
Anaerobic metabolism

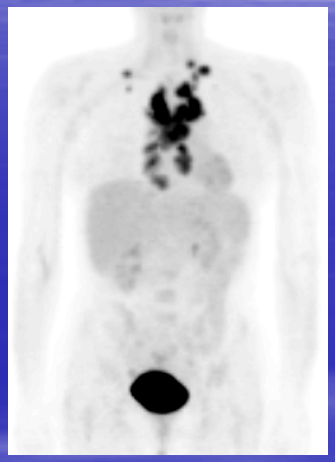
- Glucose —> 2 ATP + 2 lactic acid.

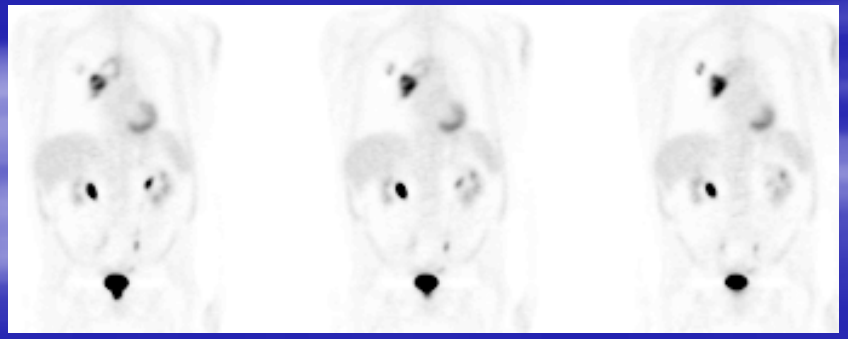
Tumors compensate for diminished yield with marked increased glucose flux (typically 5 to 10 fold). Increased acid is pumped into the extracellular space (pH_i high and pH_e low!)

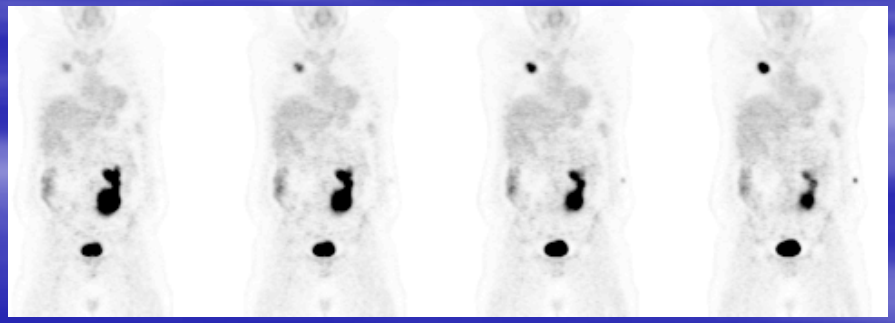
- Inefficient and have to get rid of acid load. Why???

FDG-PET imaging demonstrates increased glucose uptake in the vast majority of tumors and correlates uptake with prognosis.





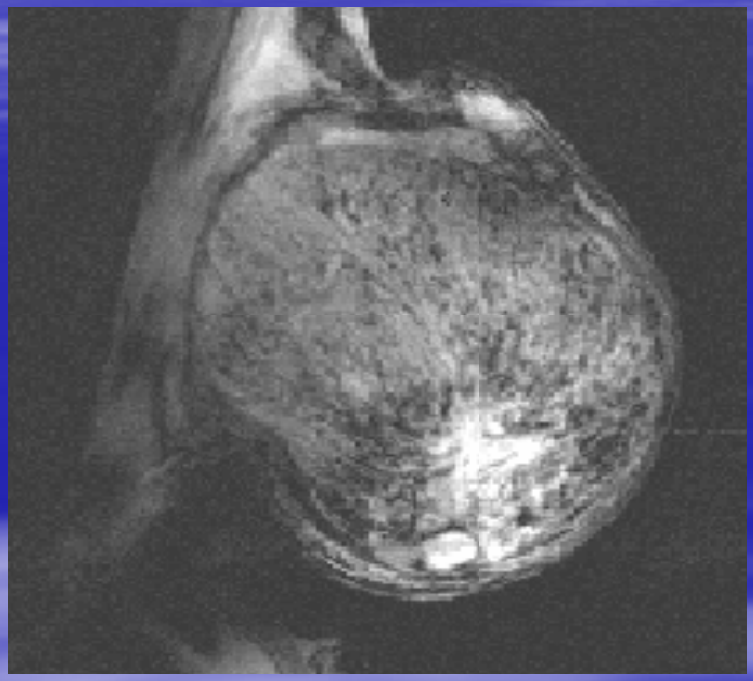




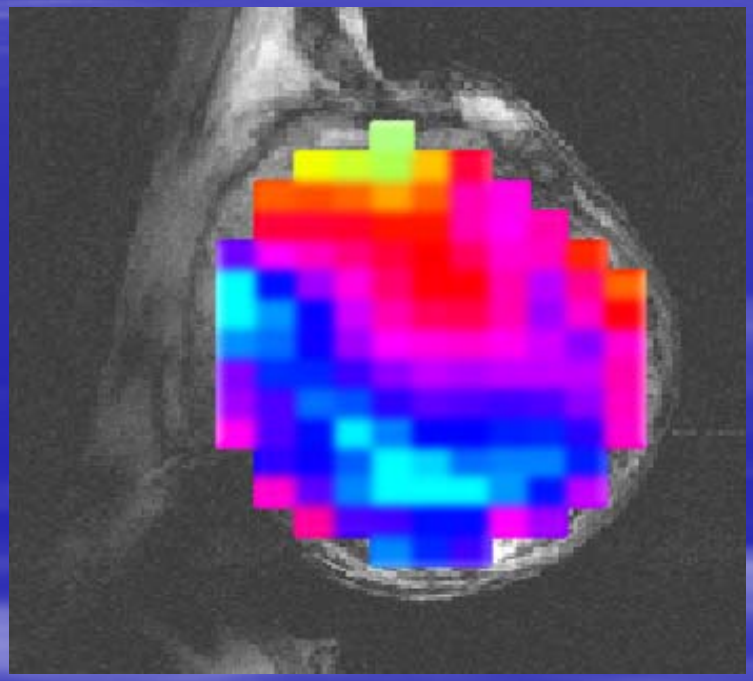
Tumor pH measured with 3-APP

Tumor	Species	Cancer	pHex	pHin
RIF	Mouse	Sarcoma	6.71±0.08	7.29±0.11
C3h	Mouse	Breast c	6.95±0.18	7.19±0.11
Ehrlich	Mouse	Breast c.	6.69±0.05	6.92±0.05
CaNT	Mouse	Adoeno c.	6.70±0.05	7.08±0.06
CONTROL	Mouse	Muscle	7.39±0.10	7.16±0.07
9618a	Rat	Hepatoma	6.70±0.03	7.12±0.02
Walker	Rat	Sarcoma	6.30±0.04	7.04±0.04
MNU	Rat	Breast c	6.80±0.07	7.16±0.07
CONTROL	Rat	liver	7.34±0.03	7.26±0.02
MCF-7/s	Human	Breast c	6.99±0.11	7.15±0.08
MDA-435	Human	Breast c	6.80±0.11	7.37±0.07
“+nm-23	Human	Breast c	7.17±0.10	7.16±0.05
HT-29	Human	Colon c.	6.79±0.05	7.02±0.05

Figure 6.



DCE of MDA-435



pHe map of MDA-435

Acid-Mediated Tumor Invasion Hypothesis:

- General concept: Tumor-induced perturbations in the micro-environment are unfavorable to normal tissue and enhance tumor growth in a self propagating pattern
- Specific concept: Altered tumor metabolism results in an acidic pH_e both in the tumor and in a ring of surrounding normal tissue. Tumor cells have an ideal pH_e (i.e. maximum proliferation) of about 0.5 pH units lower than normal. This provides a selective growth advantage so that they continue to proliferate while normal cells die
- Appeal: simple, based on properties found in virtually all tumors

Proposed Sequence:

- Altered glucose metabolism results in increased lactic acid production
- H^+ transport across the membrane is increased primarily through amplification of the Na^+/H^+ antiport
- This results in increased pH_i and decreased pH_e.
- H^+ ions in the extracellular space will diffuse along concentration gradients into peritumoral host tissue resulting in: normal cell death, ECM degradation, induction of angiogenesis, and blunting of immune response.
- Tumor cells (more tolerant of acid pH_e) continue to proliferate and invade into the disrupted normal tissue

Equations governing acid mediated invasion

$$\frac{\partial N_1}{\partial t} = r_1 N_1 \left(1 - \frac{N_1}{K_1} - \alpha_{12} \frac{N_2}{K_2}\right) - d_1 L N_1 + \nabla \cdot (D_{N_1}[N_2] \nabla N_1)$$

$$\frac{\partial N_2}{\partial t} = r_2 N_2 \left(1 - \frac{N_2}{K_2} - \alpha_{21} \frac{N_1}{K_1}\right) - d_2 L N_2 + \nabla \cdot (D_{N_2}[N_1] \nabla N_2)$$

$$\frac{\partial L}{\partial t} = r_3 N_2 - d_3 L + D_3 \nabla^2 L$$

where

N_1= Normal cells
N_2= Tumor cells
L= Excess acid concentration (i.e. the acid above pH 7.4)
r_1 and r_2 = Maximal growth rate for the cellular populations respectively
K= Carrying capacity
α = Lumped interference term
D_1 and D_2= Invasion terms for each cell population
d_1 and d_2 = Death rate due to excess acid in the extracellular space
d_3= Removal of excess acid by tumor and peritumoral vasculature
r_3= Excess acid production by tumor cells
D_3 = Diffusion coefficient for H^+

Dimensionless Parameters

$$\eta_1 = \frac{N_1}{K_1} \qquad \eta_2 = \frac{N_2}{K_2} \qquad \Lambda = \frac{Ld_3}{r_3 K_2}$$

$$\tau = r_1 t \qquad \xi = \sqrt{\frac{r_1}{D_3}}$$

$$\frac{\partial \eta_1}{\partial \tau} = \eta_1(1 - \eta_1) - \delta_1 \Lambda \eta_1$$

$$\frac{\partial \eta_2}{\partial \tau} = \rho_2 \eta_2(1 - \eta_2) + \nabla_\xi \cdot [\Delta_2(1 - \eta_1)\nabla_\xi \eta_2]$$

$$\frac{\partial \Lambda}{\partial t} = \delta_3(\eta_2 - \Lambda) + \nabla_\xi^2 \Lambda$$

where

$$\delta_1 = (d_1/d_3) \times (r_3/r_1) \times K_1$$

$$\rho_2 = r_2/r_1$$

$$\Delta_2 = D_2/D_3$$

$$\delta_3 = d_3/r_1$$

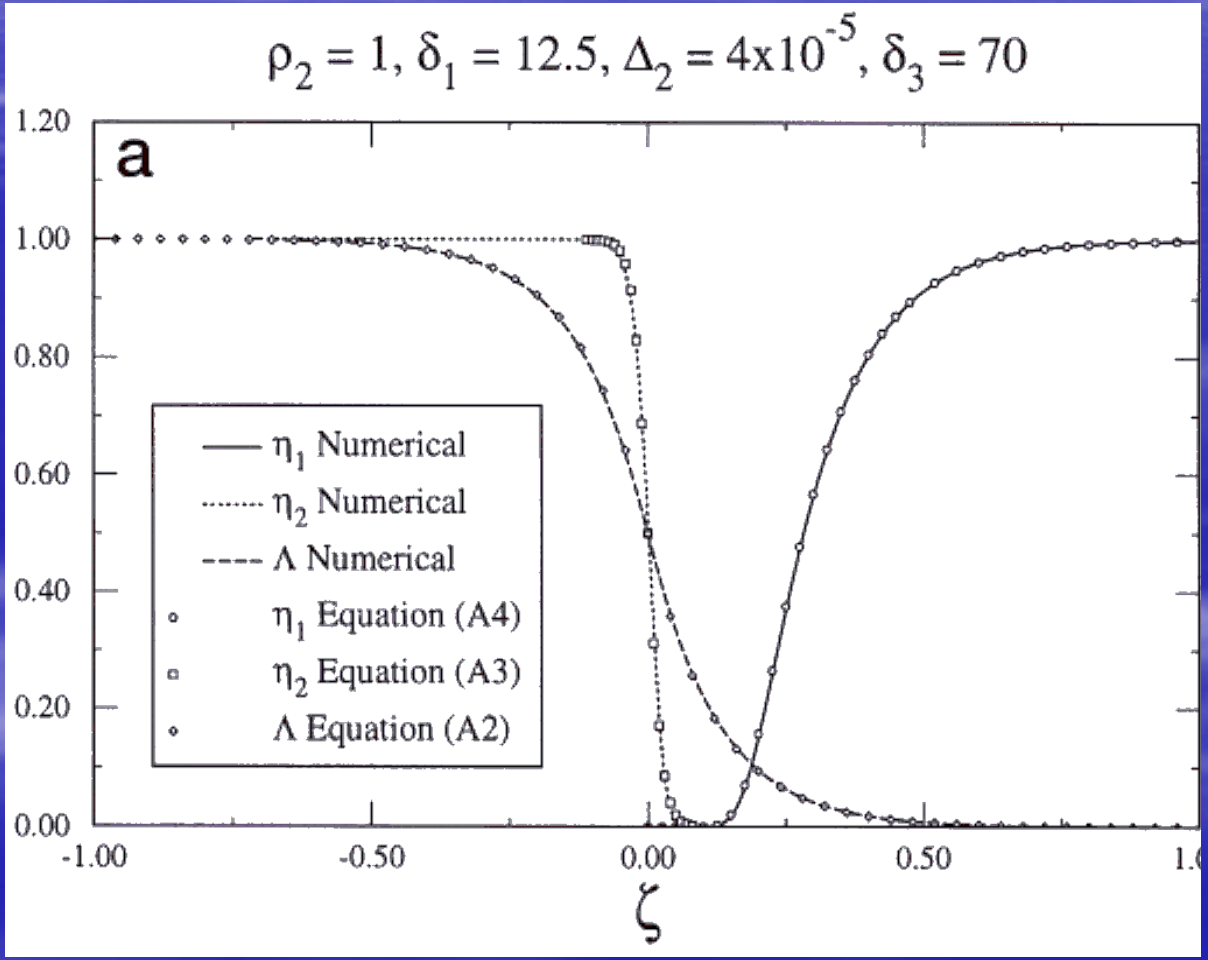
Fixed Points (Spatial Homogeneity and Temporal Invariance)

- FP#1 $N_N=0$ $N_T=0$ $H=0$
- FP#2 $N_N=K_N$ $N_T=0$ $H=0$
- FP#3 $N_N=K_N(1-\delta_1)$ $N_T=K_T$ $H=H_0$
- FP#4 $N_N=0$ $N_T=K_T$ $H=H_0$
- Where $\delta_1=(d_N/d_H) \times (r_H/r_N) \times K_T$

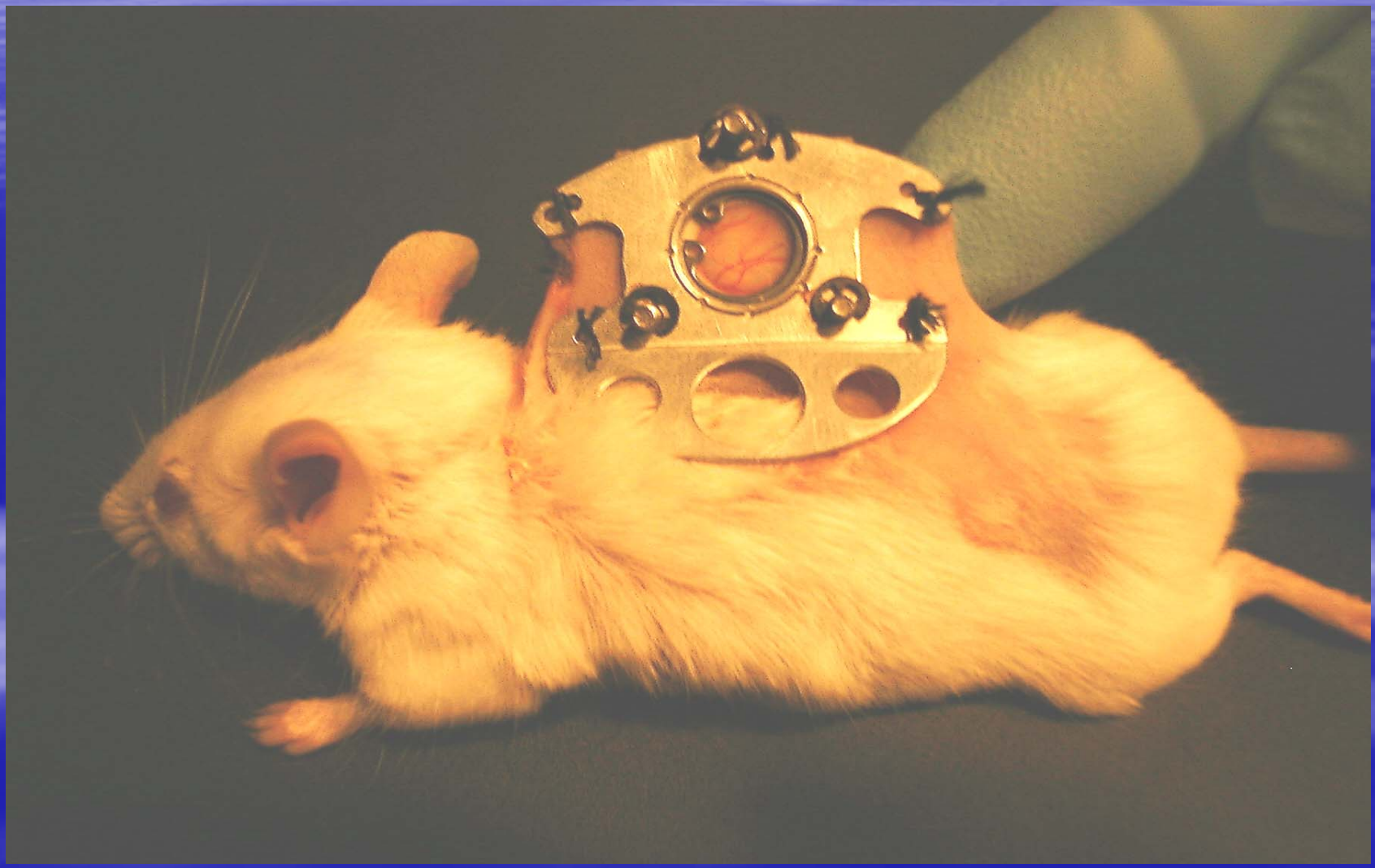
Linear Stability Analysis

- FP #1 and FP#2 are unconditionally unstable
- FP#4 is stable and FP#3 is unstable if $\delta_1 > 1$ and *vice versa*
- Recall $\delta_1 = (d_N/d_H) \times (r_H/r_N) \times K_T$

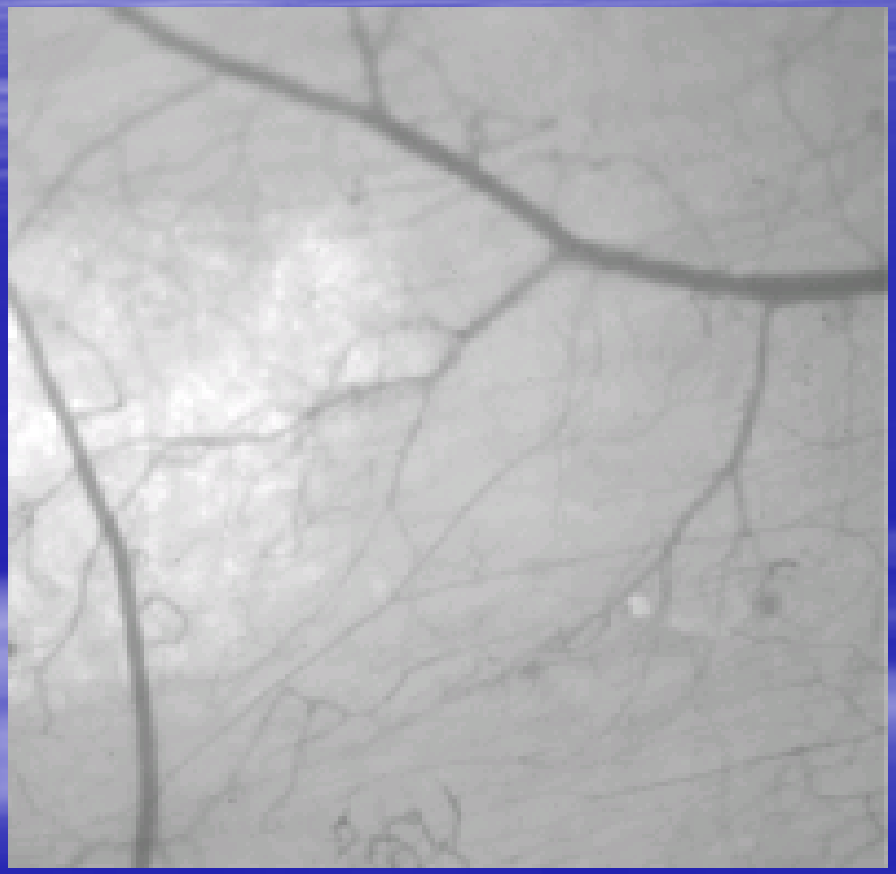
Tumor-Host Interface

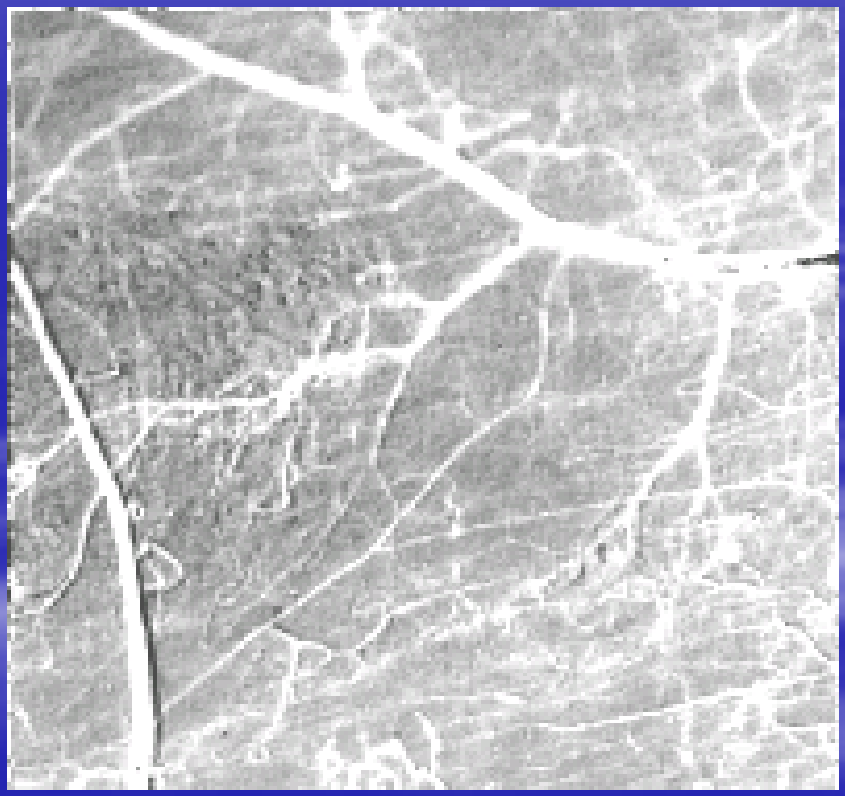


Mouse Dorsal Wound Chamber

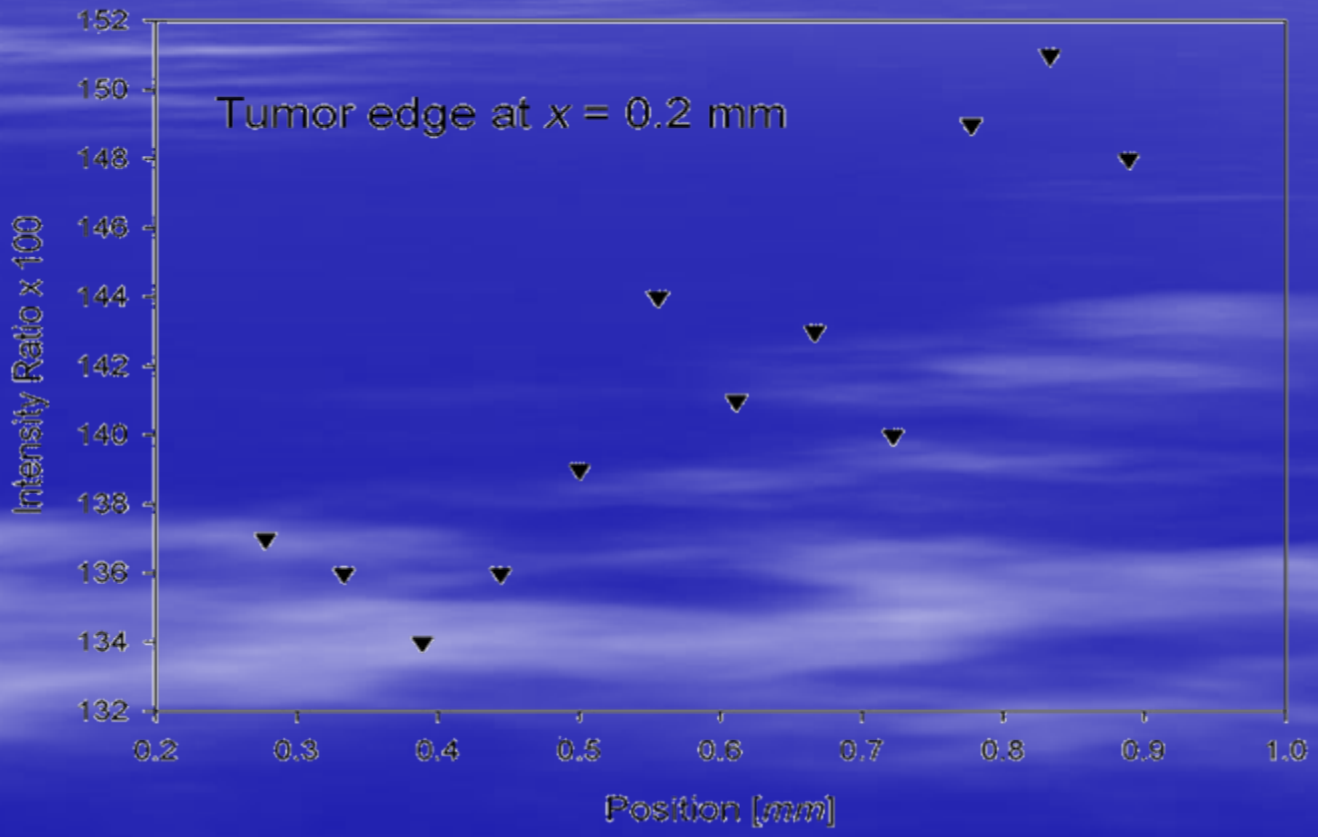


Simultaneous maps of tumor location and extracellular pH

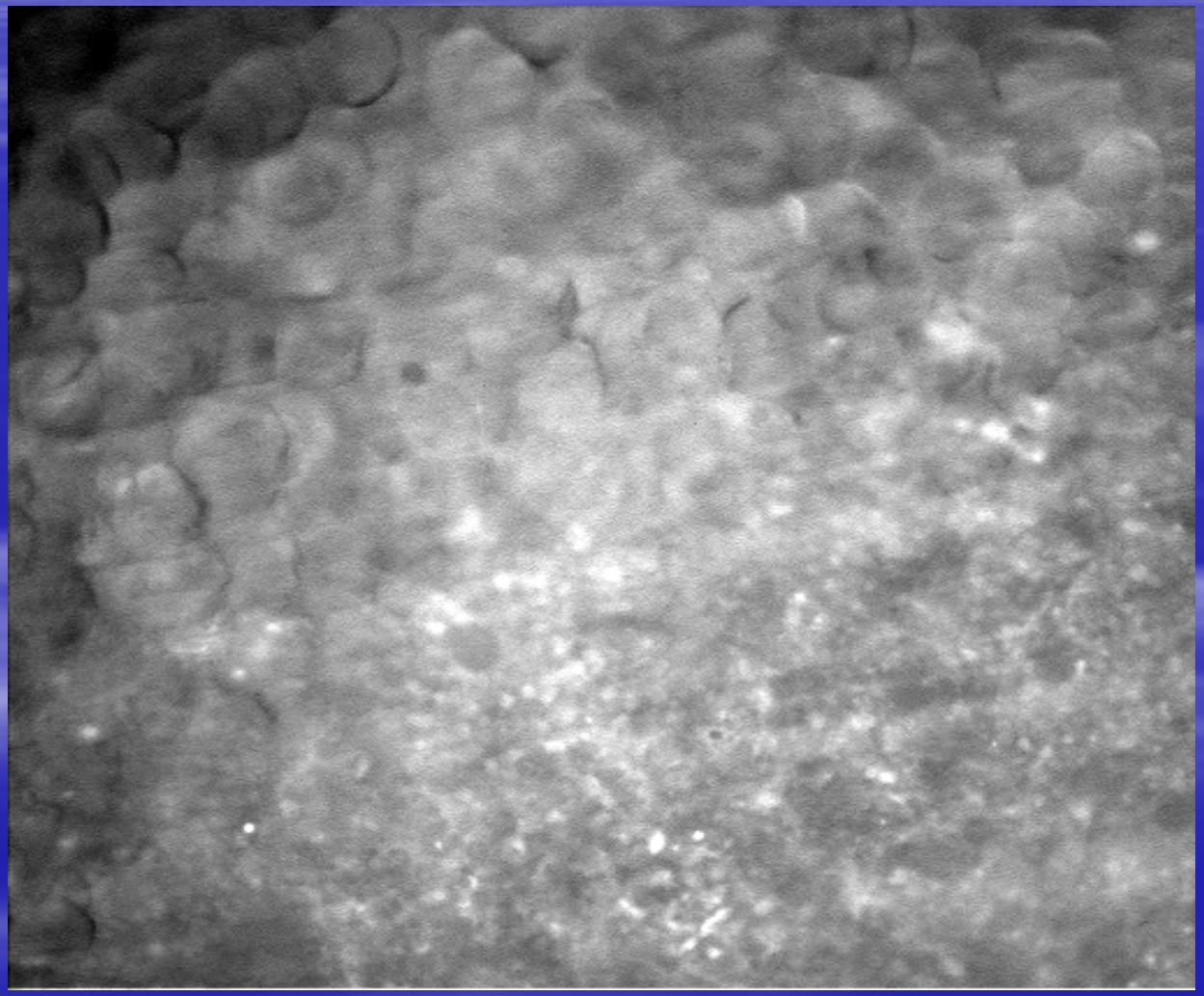




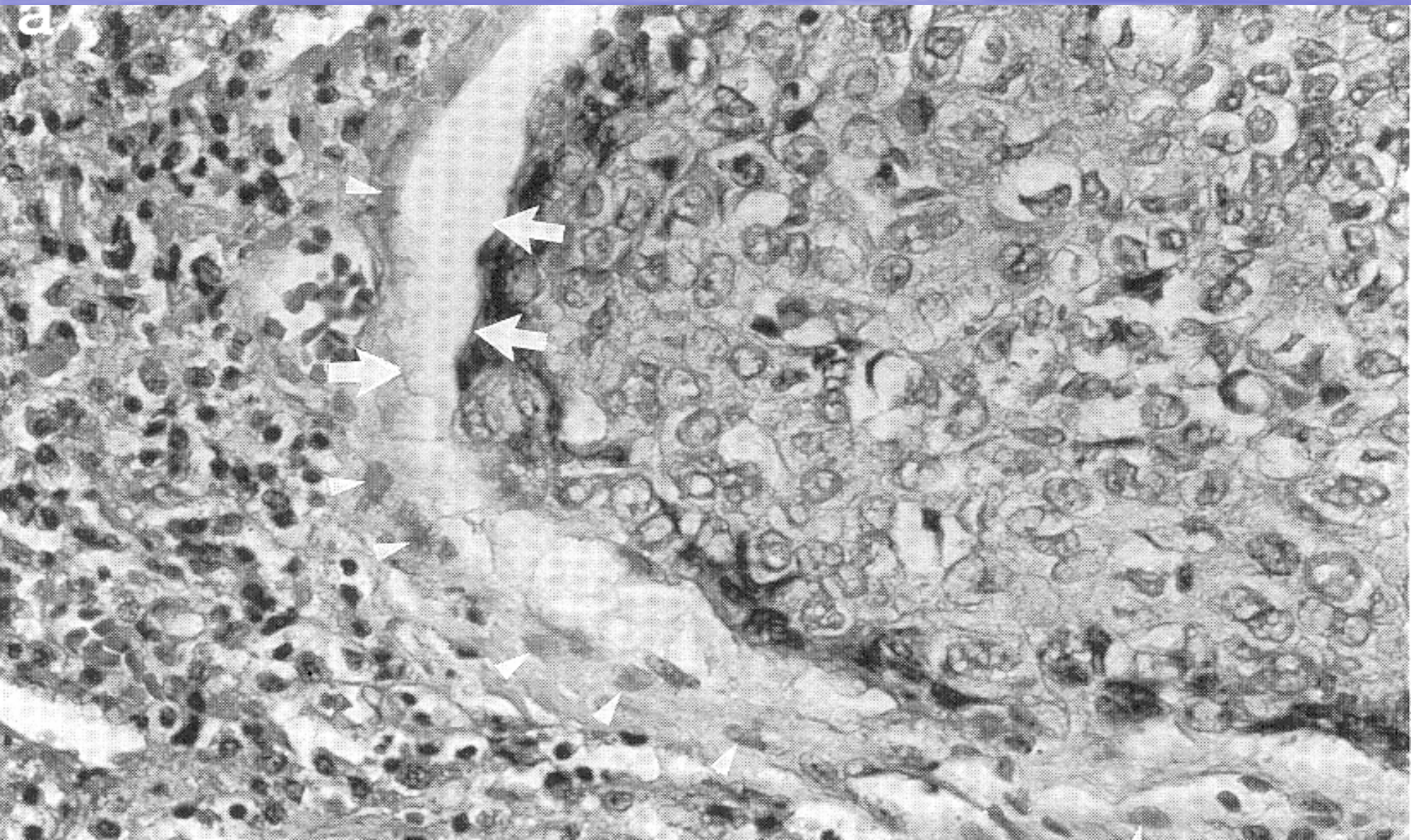
Typical Acid Profile using FRIM

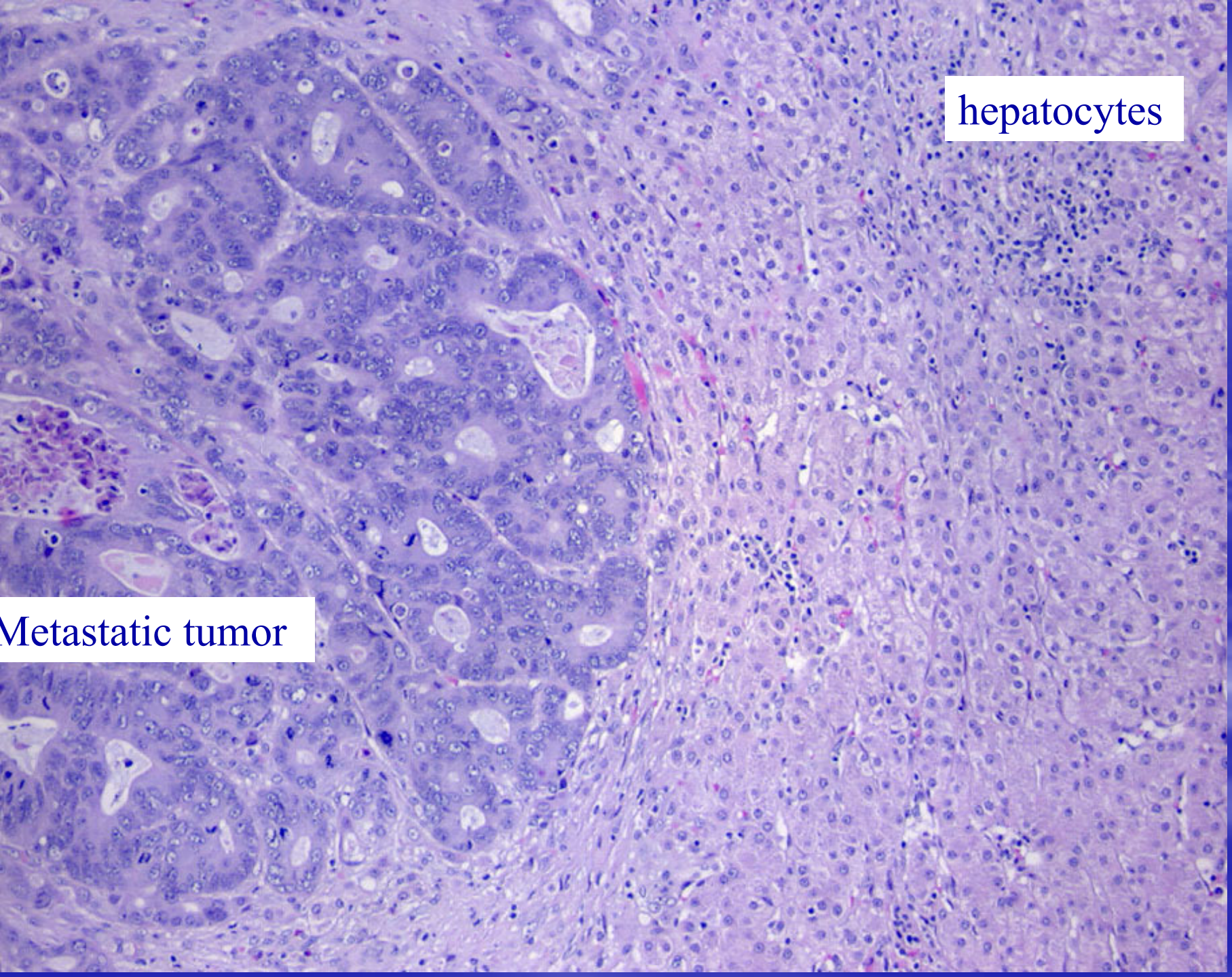


Cell viability Stain



Acellular gap at the tumor-host interface in head and neck cancer




hepatocytes
Metastatic tumor

Does acid-mediated tumor invasion apply to early tumor growth?

- Model must include a discretized approach to describe individual cell history and its interactions with other cells while retaining the while retaining continues elements to describe the production, diffusion, and removal of H^+ ions.

- Modified Cellular Automata Model:

  Establish N x N array of automaton cells with a one to one correspondence between the automaton cells and physical cells with size 20 x 20 microns.

Each automaton cell is described by a state vector with 3 components:

- The automaton is either a tumor cell, a normal cell, a microvessel, or vacant
- The local extracellular pH
- The local glucose concentration

Microvessels are randomly distributed throughout the distributed throughout the simulation space with density α

Where

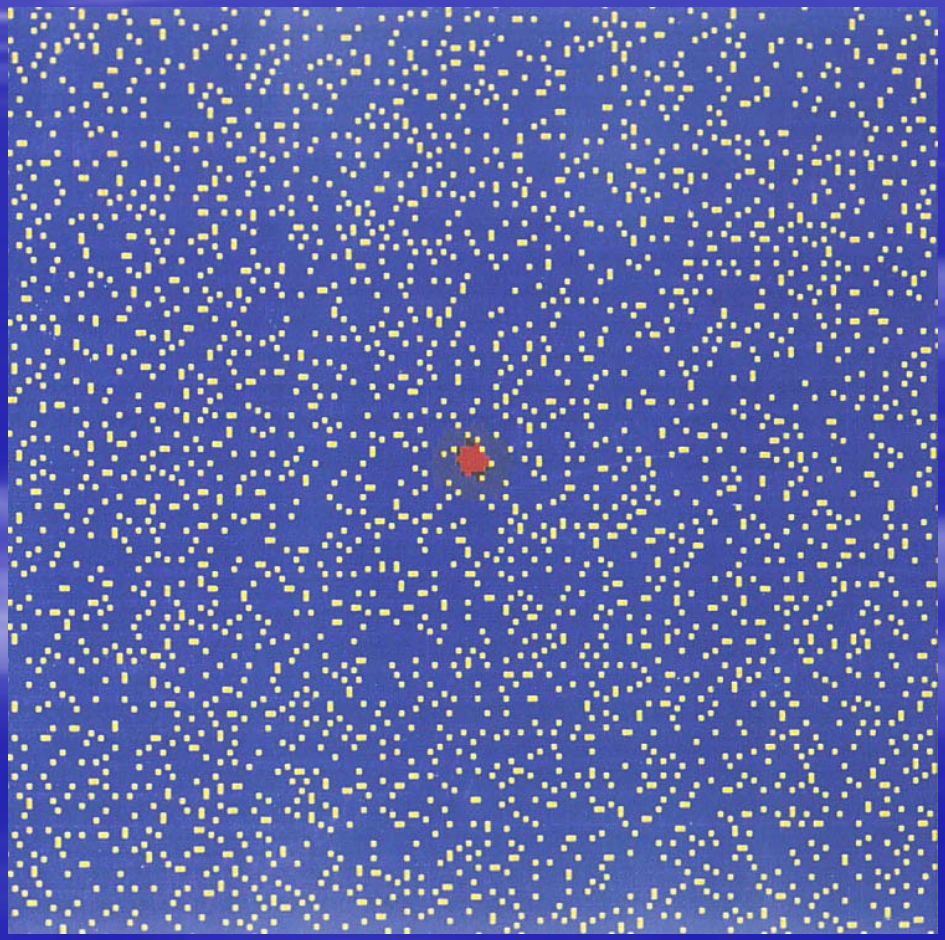
$\alpha = N_v/N^2$

Where

N_v is the number of automaton cells occupied by vessels and N is the total number of automaton cells

- H^+ and glucose concentrations form 2 continuous fields over the simulation space obeying suitable time-dependent diffusion equations with sinks, sources, and boundary conditions determined by cells and vessels

Typical 200x200 matrix showing normal cells (blue), randomly scattered microvessels (yellow) with a small tumor disc 5 cells in diameter (21 cells total) placed centrally



Automata Rules:

- Microvessels remain constant
- If automaton cell is either a tumor or normal then the value of the concentrations of H^+ or Glucose in its state vector are considered.
- If pH is lower than some critical threshold (pH_{DN} pH_{DT}), the cell dies and the automata becomes vacant.

  Typical values pH_{DN}=6.8 pH_{DT}=6.0

  If pH > pH_D but lower than some threshold pH_Q, the cell survives but in a quiescent state (ie. no mitosis occurs)

  Typical pH_{QN}=7.1 pH_{QT}=6.4

Automata Rules (cont.)

- If cell pH > pH_Q and glucose concentrations are adequate (threshold G_N and G_T assumed to be 2.5 mM), the cell may divide but only if an adjacent automata cell is vacant. If more than one is vacant it enters the cell with the largest value of G.

Diffusion equation for the time-dependent glucose field

$$D_G \nabla^2 G_t(\vec{r}) - k(\vec{r}) G_t(\vec{r}) = 0$$

where $G_t(\vec{r})$ is the glucose concentration at $\vec{r}$ after sub-generation t. The term $k(\vec{r})$ (having units $1/s$) is the glucose consumption rate at the location $\vec{r}$:

$$k(\vec{r}) = \begin{cases} k_N & \forall \vec{r} = Normal \quad Cells \\ k_T & \forall \vec{r} = Tumor \quad Cells \\ 0 & \forall \vec{r} = \quad Vacant \quad Cells \\ 0 & \forall \vec{r} = \quad Vessel \quad Cells \end{cases}$$

where $1 \times 10^{-6} / s < k_N < 5 \times 10^{-4} / s$ and $1 \times 10^{-5} / s < k_T < 1 \times 10^{-3} / s$ are ranges for glucose consumption by normal and tumor cells respectively

Acid profile governing equation

$$D_H \nabla^2 H_t(\vec{r}) + h(\vec{r}) = 0$$

where $D_H = 1.08 \times 10^{-5} cm^2 / s$ is the diffusion constant for lactic acid, $H_t(\vec{r})$ is the H^+ concentration at position $\vec{r}$ after sub-generation t, and $h(\vec{r})$ is an acid production rate that is non-zero only at positions $\vec{r}$ where there is a tumor cell:

$$h(\vec{r}) = \begin{cases} \dot{H}_T^A & \forall \vec{r} = \textit{Active tumor cells} \\ \dot{H}_T^Q & \forall \vec{r} = \textit{Quiescent tumor cells} \\ 0 & \forall \vec{r} \neq \textit{Tumor cells} \end{cases}$$

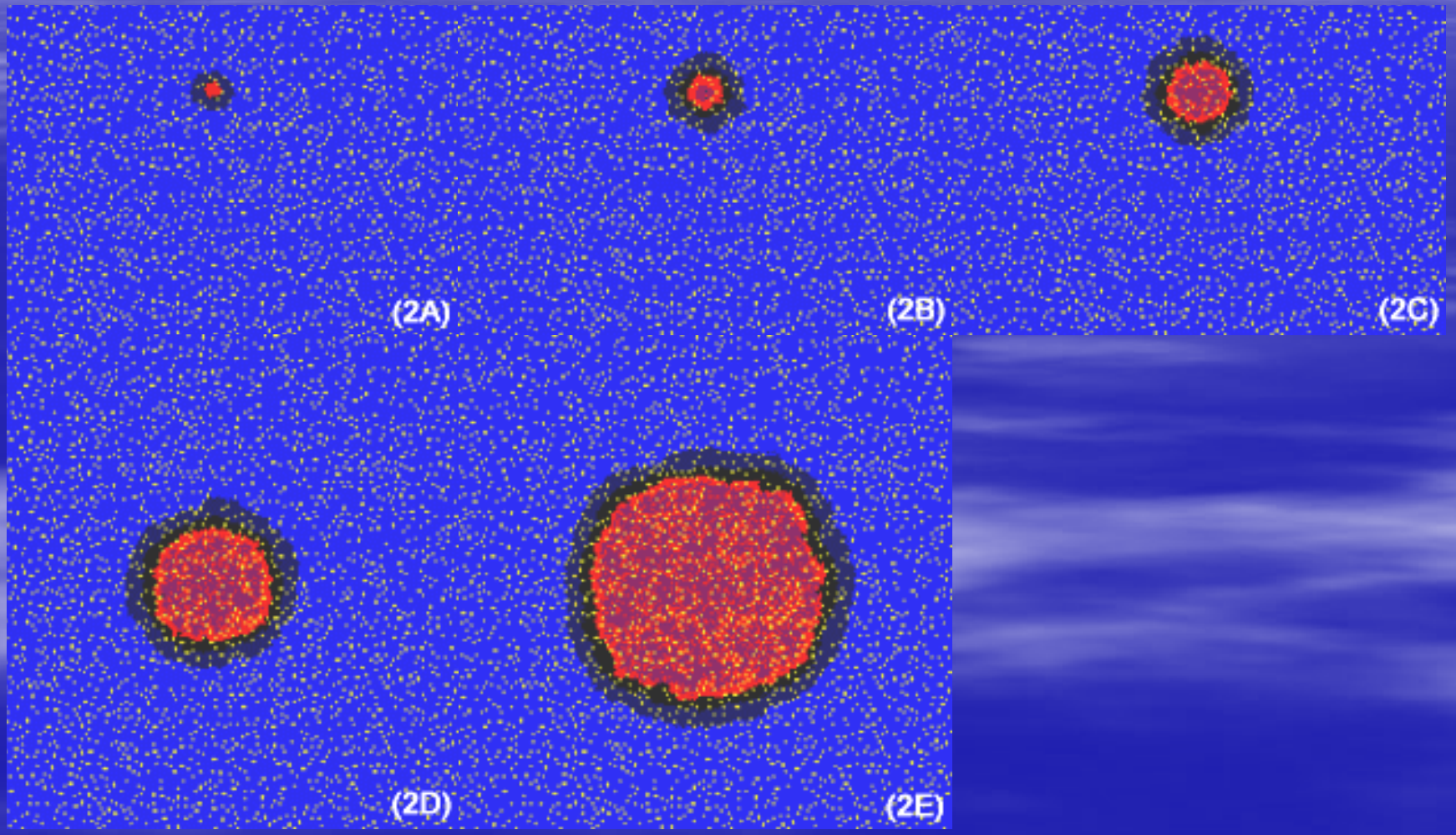
In our model $\dot{H}_T^A$ and $\dot{H}_T^Q$ are key variables adjusted to model tumor phenotypes expressing different metabolisms:
$1 \times 10^{-5} < \dot{H}_T^A < 1 \times 10^{-4} mM / s$ and $\dot{H}_T^Q = 5 \times 10^{-7} mM / s$.[3]

Strategy for Model Analysis

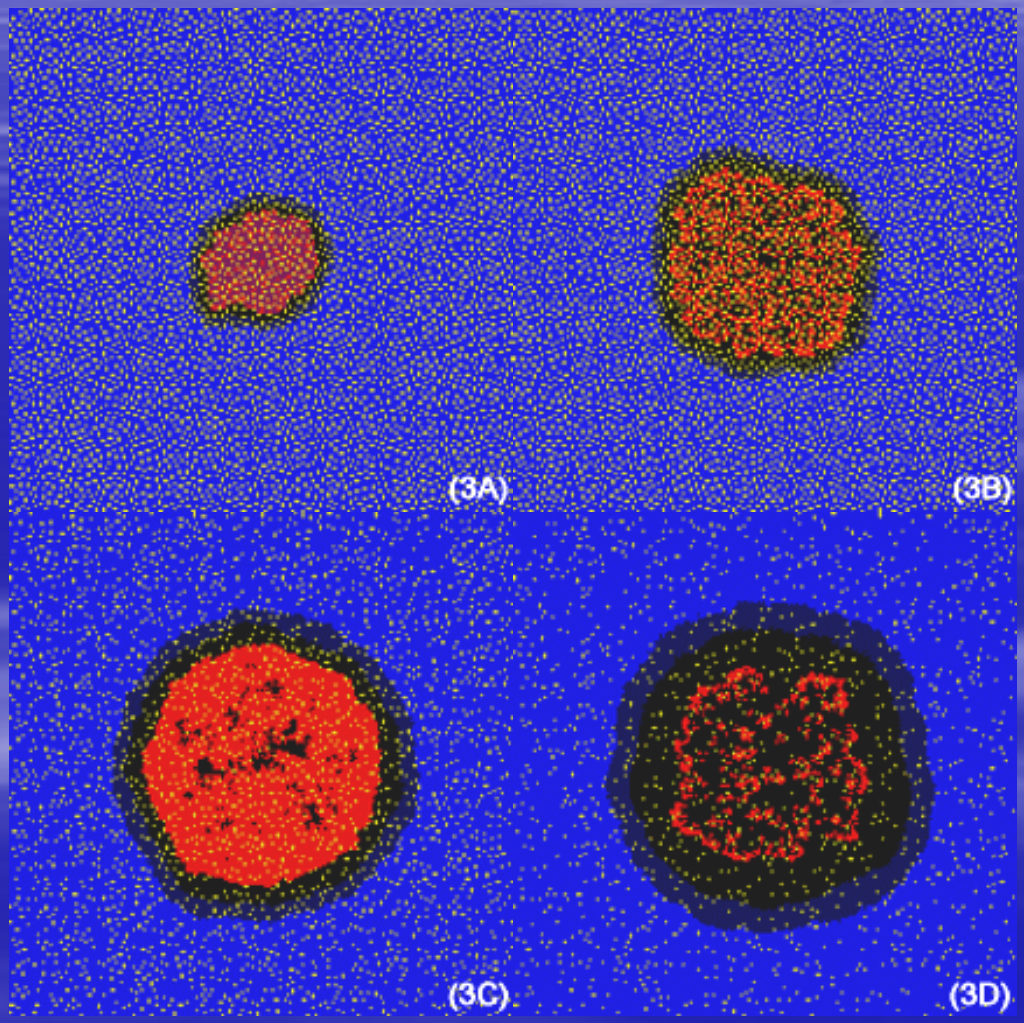
- Use large disparity in time scales of cell proliferation (about 10^2 hrs) and chemical diffusion (intervessel diffusion time 1 – 10 s).
- Cell distribution changes so slowly they can be treated as adiabatic perturbations on the chemical fields.

- Solve a series of equilibrium boundary-value equations on a coarse time scale. Chose a random subset f ($f < 1.0$) of automaton cells for updating. Then solve the equilibrium boundary-value problems to determine the resultant response of of the chemical fields. This is repeated $1/f$ times until all cells and chemical fields have been updated. This is one generation. No quantitative differences found in evolution of automata if $f<0.1$

Simulated tumor growth using modified cellular automata model



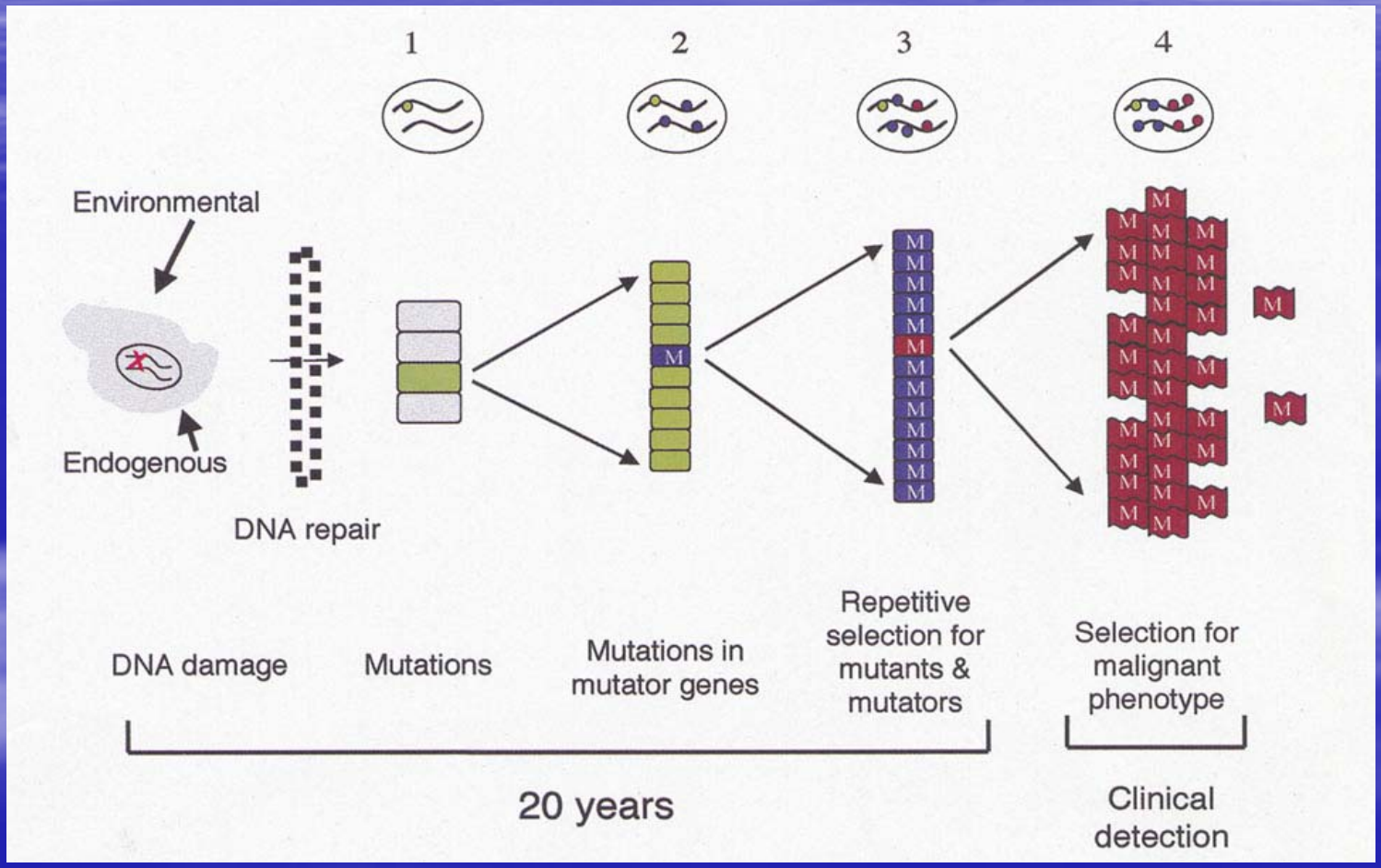
Variations in Tumor Morphology



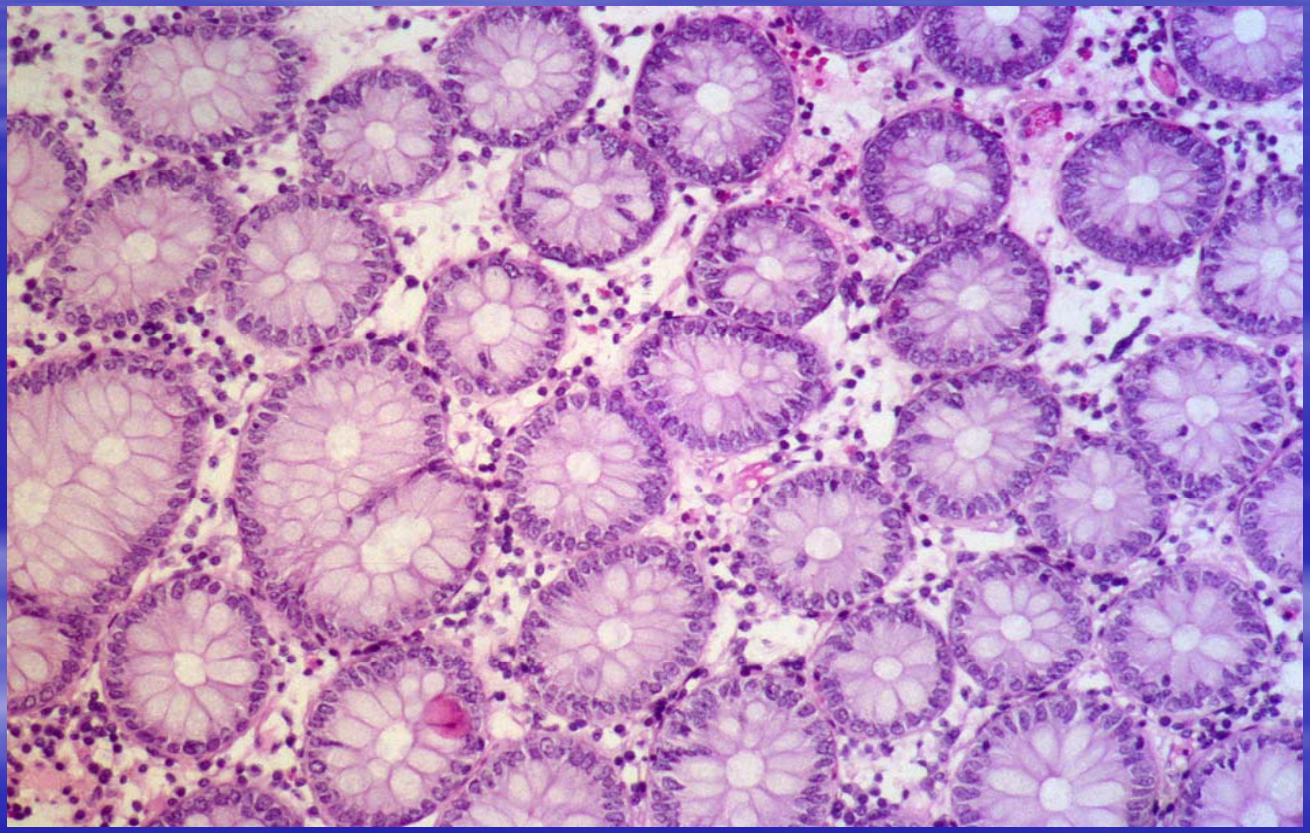
Background

- The development of sporadic cancer generally requires years or even decades with multi-step progression from normal tissue through increasingly disordered pre-malignant lesions such as colon polyps to invasive cancer.
- Carcinogenesis is often describe as “somatic evolution” driven by competition among different populations arising through random mutations with clonal selection determined by the properties of the tissue environment formally analogous to classical Darwinian dynamics.
- There is clear evidence of accumulating mutations during carcinogenesis – most transformed cells possess hundreds, thousands, or even hundreds of thousands of mutations. But, there is no prototypical cancer genotype – the genome of every sporadic cancer populations appears to be unique.
- The general conceptual model is that some genomic mutations confer “selective growth” advantage with clonal expansion. Over time these advantageous mutations accumulate until unconstrained growth results.
- Loeb and others hypothesize increased mutation rate due to chromosomal or microsattelite instability is necessary to drive carcinogenesis

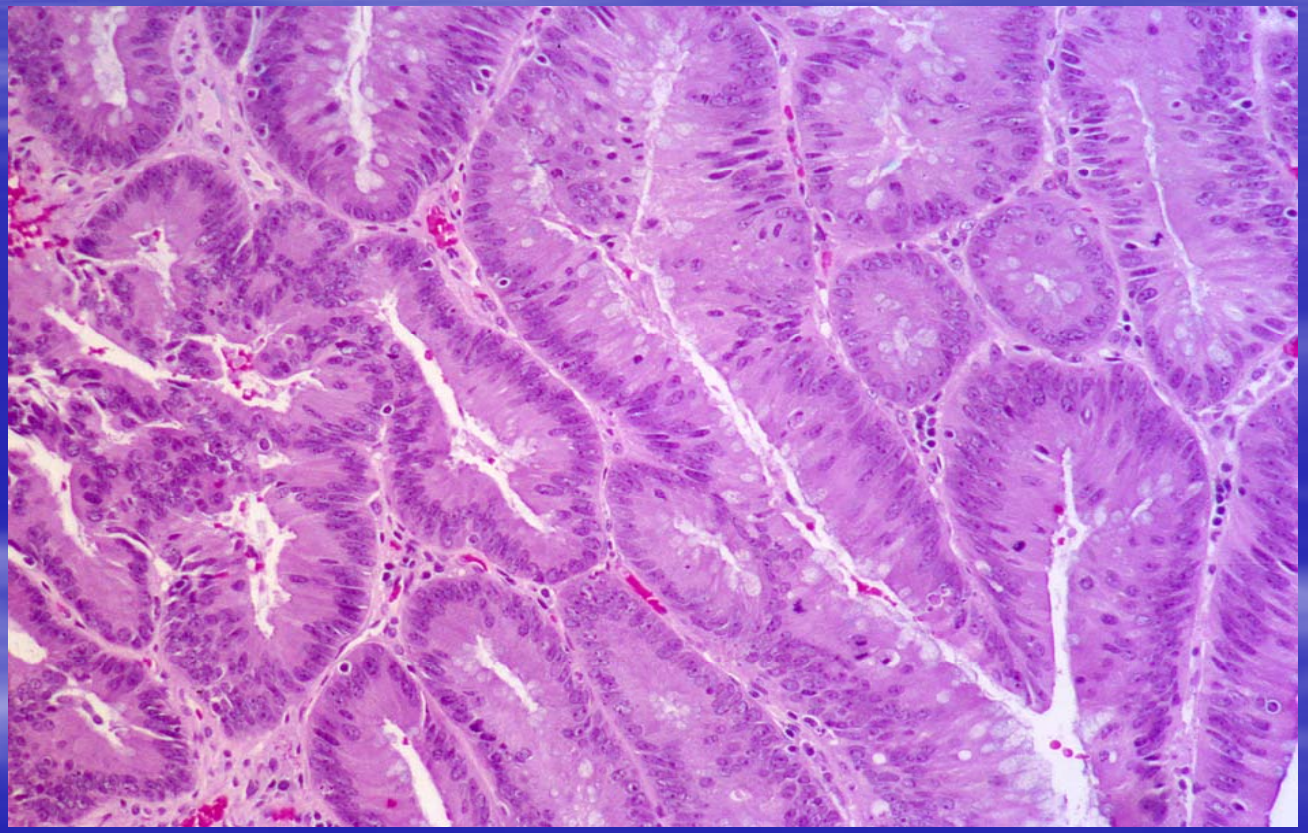
Accumulating mutations is a central component of virtually all carcinogenesis theoretical models



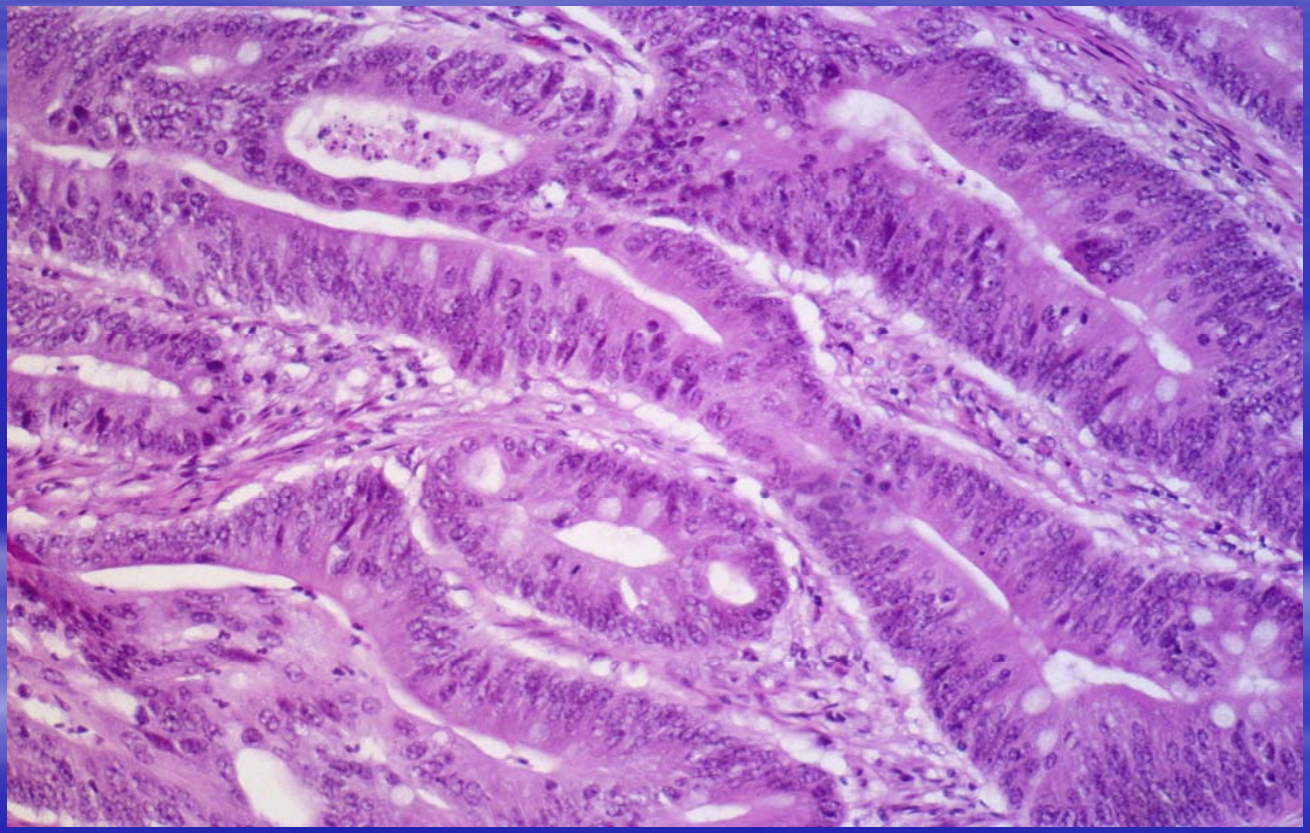
Normal colonic mucosa



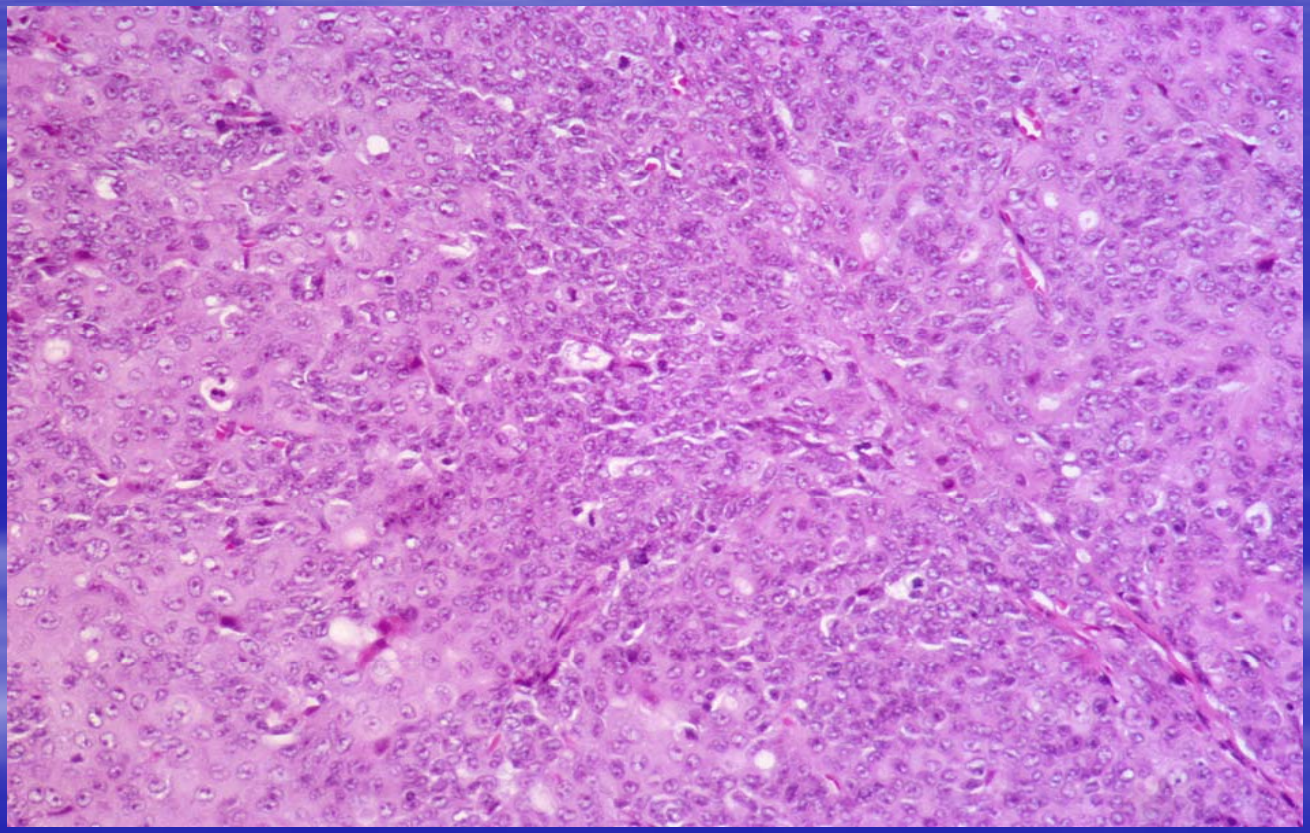
Colon polyp with low grade dysplasia



Colon polyp with high grade dysplasia



Colon cancer



Problems and Controversies in the Current Conceptual Model

What does “selective growth advantage” mean? Need to define the dynamics of environmental selection forces with the phenotypic expression of genetic mutations to understand the process.

Is the mutator phenotype necessary? Can invasive cancer evolve with the normal background mutation rate? There is evidence of non-random distribution of the mutations among different gene segment with complete degradation of some and stability of others (e.g. membrane transport proteins).

The role of the environment is poorly defined. Bissell et. al. have shown variations is the microenvironment can profoundly alter cellular phenotype and growth dynamics in the absence of alterations in the genome (“it takes a tissue to make a cancer”).

The role of the mutagenic phenotype can be evaluated using information theory since genomic information generates and maintains the transmembrane entropy gradient.

Shannon entropy in each codon where r is the probability of each of the 64 possible configurations:

$$H^i = -\sum_{j=1}^{N} r_j^i \log_b r_j^i$$

Total information in a gene with m codons:

$$I_g = mH^i$$

Total information in the genome with G genes

$$I_c = \sum_{g=1}^{G} I_g$$

Cellular fitness u_c is sum of contribution from all of the genes where u_g is the fitness

contribution from each gene and is a function of the information content (i.e. reduction in the information content of the gene reduces its contribution to fitness) and the total number of gene products within the celll k which may be controlled by other genes acting as repressors or promoters

$$u_c = \sum_{g=1}^{G} k(I_c) u_g(I_g)$$

Cellular proliferation r_c is determined by the cellular fitness compared to the mean

fitness of its competitors within the community

$$r_c = f(u_c - \bar{u}) = f[\sum_{g=1}^{G} k(I_c) u_g(I_g) - \ 1/M \sum_{m=1}^{M} u_m]$$

Applying the Eigen-Schuster limit, information degradation in a specific gene follows its contribution to fitness:

$$\frac{du_c}{dt} = \alpha(u_c - \bar{u}) \frac{d(I_{c\max})}{dt}$$

Results

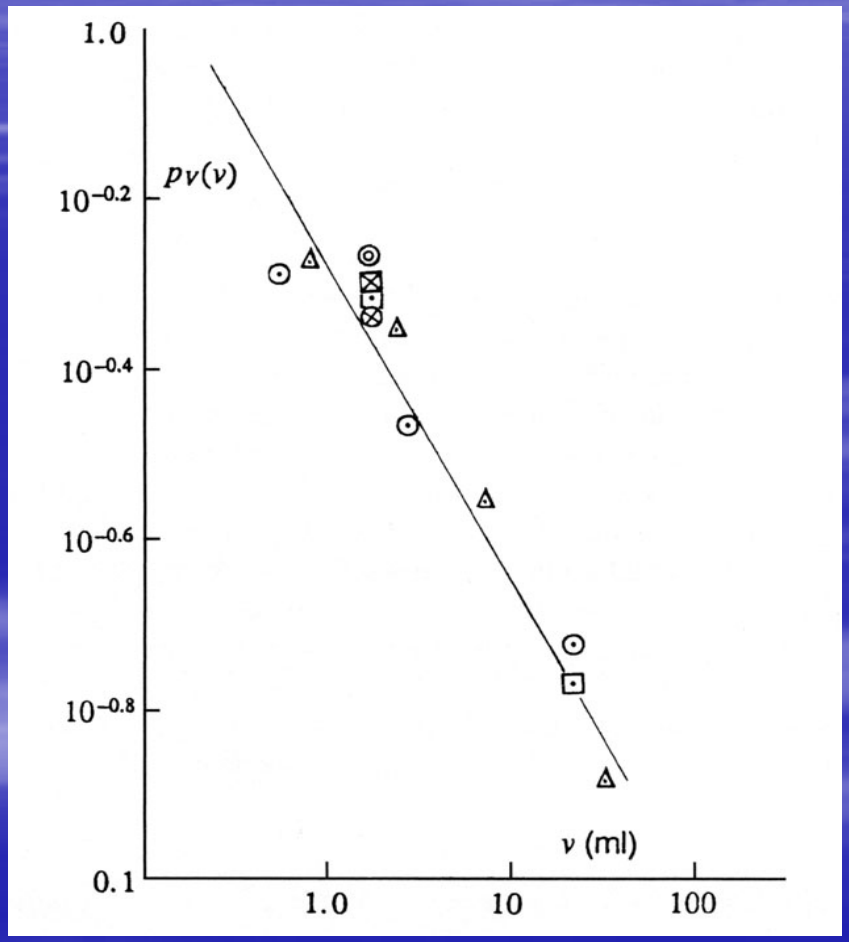
- Information loss because of accumulating mutations is constrained by the competitive stresses of the Darwinian environment in carcinogenesis protecting the genome from an “information crisis”
- Because of these dynamics, gene segments that decrease the fitness (proliferation) of the cells are subject to maximum degradation. This is manifested as loss of function of tumor suppressor genes and differentiation genes. The latter manifests as progressive de-differentiation
- Gene segments necessary for proliferation (such as oncogenes, membrane transporters, PFK) are protected by prompt clonally loss following a muation. The *observed* mutation rate in these genes will be minimal consisting primarily of gain-of-function mutations.
- The net effect is tumor cells will asymptotically approach a state of minimum information (minimal complexity) resulting in progressive loss of differentiated function but unbounded proliferation. This implies mechanism of tumor invasion must be simple

The role of the minimum information state in cancer biology can be determined using Extreme Physical Information (EPI)

- Define $p_c(x,t)$ as the probability that any observed cell in some volume of tissue (x) is a tumor cell. This marginal probability also represents, by the law of large numbers, the relative number of cancer cells in any space x. The time dependence of $p_c(x,t)$ defines tumor growth.
- In EPI data in any measurement is the result of flow of Fisher Information from an information source to a sink $J \rightarrow I$
- Here J is the extracellular information produced by the presence of the cancer cell about the age of the tumor. I is the quantity of information that reaches normal cells.
- Assuming only that cancer cells exist at a minimum information state and proliferate in a “free field” the prediction is:

$$p(t)=Ft^{\theta} \quad F=\text{constant} \quad \theta \approx 1.62$$

The EPI predictions can be compared to the growth rate of small breast cancers obtained using sequential mammograms. Six studies were found in the literature – all showed power law growth with θ of 1.72, 1.69, 1.47, 1.75, 2.17, and 1.61 (mean 1.73 ± 0.23)



Basic Evolution Equations

Assume a volume of tissue contains distinct cellular populations designated by $x_i, i = 1, \ldots, n_s$. Each population is defined by a phenotype vector $\mathbf{u}_i$ composed of multiple scalar components that include cellular properties and interactions with the microenvironment (other cells, ECM, nutrients etc.). We define population and mean phenotype vectors:

$$\mathbf{x} = \begin{bmatrix} x_1 & \cdots & x_{n_s} \end{bmatrix}$$

$$\mathbf{u} = \begin{bmatrix} \mathbf{u}_1 & \cdots & \mathbf{u}_{n_s} \end{bmatrix}$$

"mean phenotype" assumes limited diversity within each population due to the small background mutation rate and environmental perturbations. This has been observed in clonal populations of both normal and transformed cells .

Cellular fitness is defined by clonal proliferative capacity and determined by fitness-generating or G-functions with a virtual variable, v. Setting the virtual variable equal to the phenotype of a population produces the fitness for that population, which is a function of $\mathbf{x}, \mathbf{u},$ and substrate concentration R. The relationship between fitness and the G-function is given by

$$G(v, \mathbf{u}, \mathbf{x}, R)_{v=u_i} = H_i(\mathbf{u}, \mathbf{x}, R) \quad i = 1, \ldots, n_s.$$

The population dynamics maybe written either in terms of the fitness function or the fitness generating function

$$\dot{x}_i = x_i H_i(\mathbf{u}, \mathbf{x}, R) = x_i G(v, \mathbf{u}, \mathbf{x}, R)|_{v=u_i}$$

While the G-function does not provide a conceptual advantage from simply writing down equations of motion, it is critical for understanding how systems evolve.

A single G-function model with scalar strategies - defining "somatic ecology":

$$G(v, \mathbf{u}, \mathbf{x}, R) = B_n \left(1 - \frac{\sum_{i=1}^{n_s} a(v, \mathbf{u}) x_i}{K(v)} \right) \left(\frac{E(v) R^2}{R_0^2 + R^2} - m \right)$$

Cell populations in-vivo are subject to two general growth constraints:

1."Organizational" controls encompassed in $K(\mathbf{v})$ including intracellular processes such as senescence and interactions with the extracellular environment including other cells and environmental factors that resutl from their phenotypes such as ECM, growth promoters and suppressors $[a(v, \mathbf{u})]$. 2. Substrate availability (second term) - cells must obtain substrate in excess of basal metabolic demand m to supply energy and macromolecules for proliferation. B_n is a constant converting excess substrate into new cells.

Substrate dynamics

We assume population numbers for each phenotype x_1 are normally determined by $K(\mathbf{v})$. That is, normal cells under physiologic conditions are not subject to substrate limitations. Pathological exceptions include acute or chronic ischemia such as stroke, myocardial infarction or diabetic ulcers.

Substrate dynamics include Michaelis-Menten uptake:

$$\dot{R} = r - \sum_{i=1}^{n_s} \frac{E(v)R^2}{R_0^2 + R^2} x_i$$

where r is substrate delivery rate

$$r = r_e \left(m_n N_1 + m_t \sum_{i=2}^{n_s} N_i \right)$$

r_e represents local physiologic control that modulates flow through the vascular network and must be > 1 for cell proliferation (i.e. delivery must exceed basal demand). We assume maximum substrate delivery is limited by local vascularity:

$$\text{if } r > r_{\max} \text{ then } r = r_{\max}$$

The model becomes evolutionary by defining the critical growth parameters as distribution functions

$$E(v_1) = E_{\text{mean}} \exp\left(-\frac{(v_1 - u_{E_{\max l}})^2}{2\sigma_E^2} \right)$$

and the second component of v determines carrying capacity according to

$$K(v_2) = K_l^{mean} \exp\left(-\frac{(v_2 - K_{ij}^{\max})^2}{2\sigma_K^2} \right)$$

where

$K_1^{\max}$ = Maximum number of cells

K_l^{mean} = Mean tissue carrying capacity of tumor cells

$E_{n_{\text{mean}}}$ = Mean substrate uptake for normal cells

$E_{t_{\text{mean}}}$ = Mean substrate uptake for tumor cells

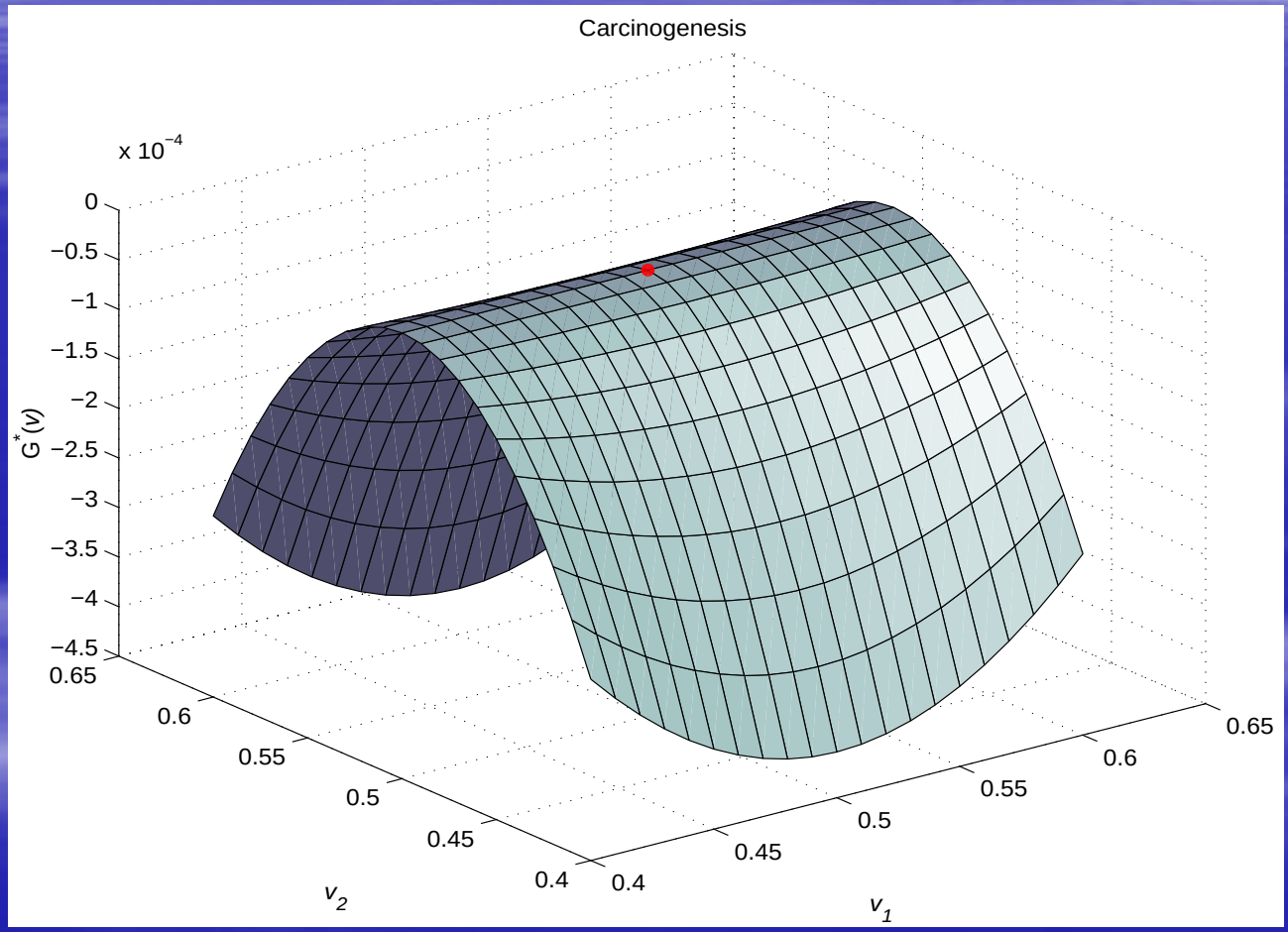
u_{x_t} = Value of u_{i2} for largest $x_l^{\max}$ $\quad t = 2, \ldots, n_s$

$u_{E\max}$ = Value of u_{i1} for maximum E

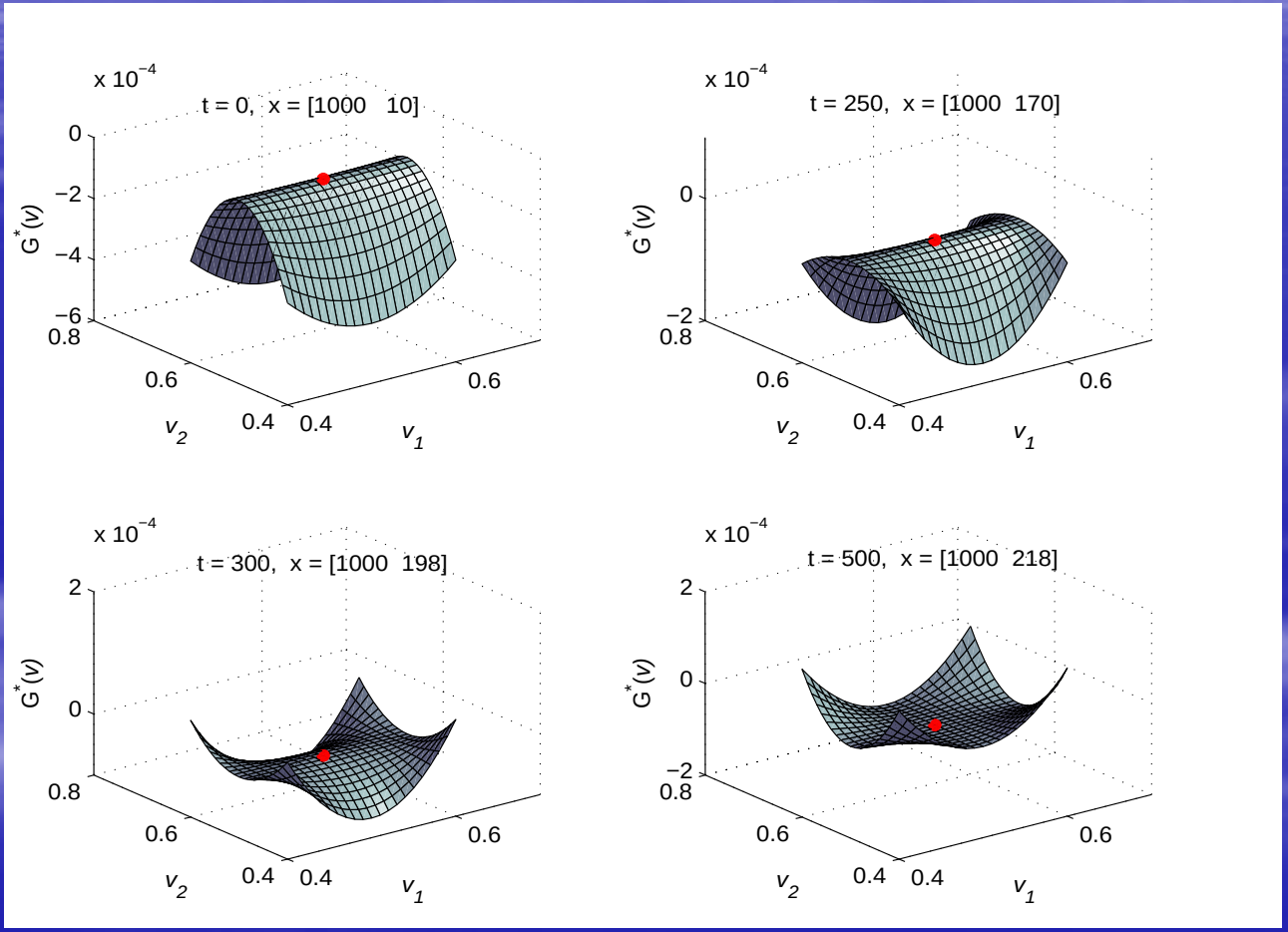
σ_j = Variance in K distributions

σ_E = Variance in E distributions

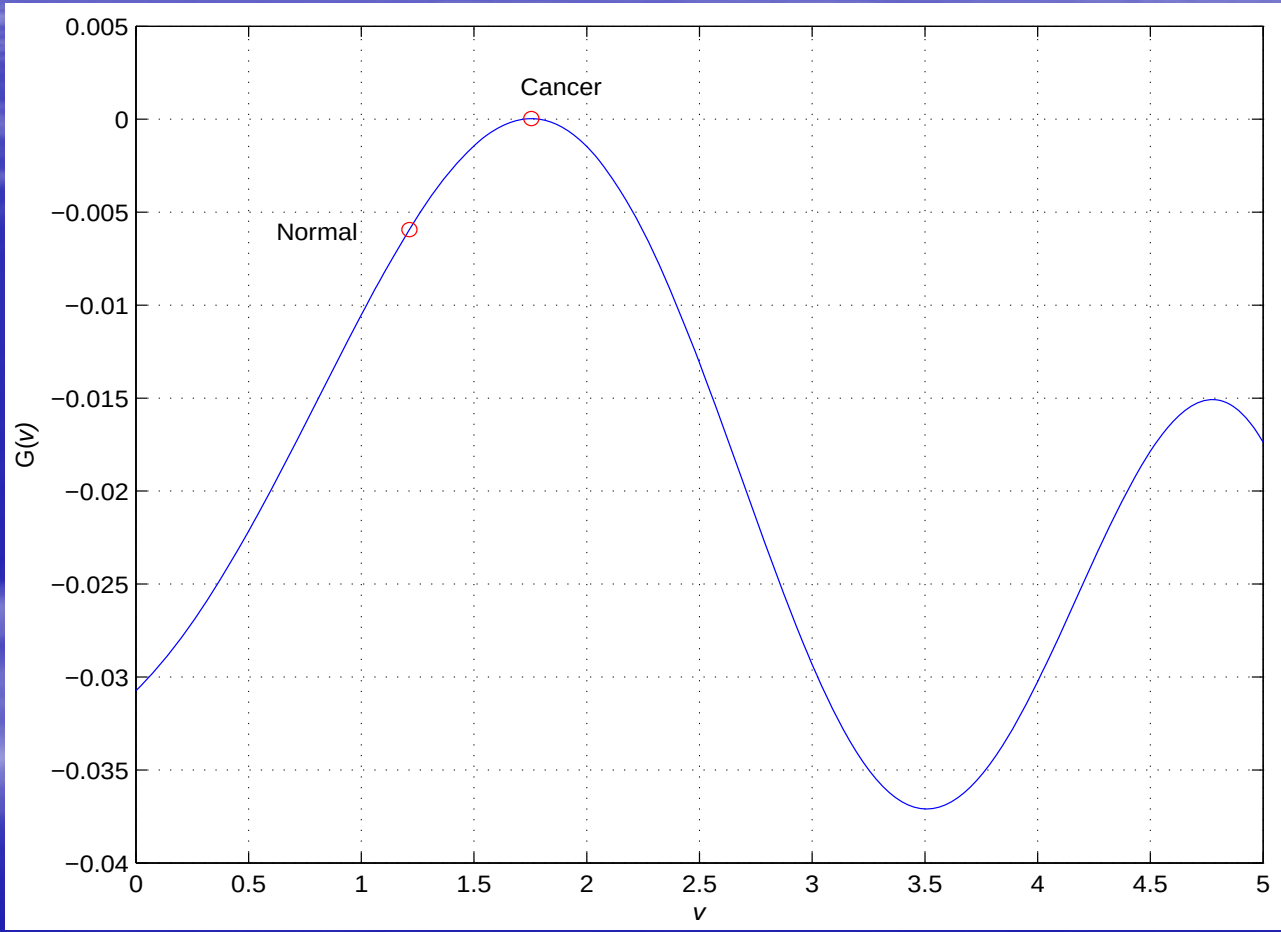
Fitness landscape with only normal cells. The ridge-like maximum allows non-competitive coexistence of multiple phenotypes – a state necessary for formation of functioning, multicellular tissue. Problem – this is not a proper maximum and is subject to invasion by fitter phenotypes.



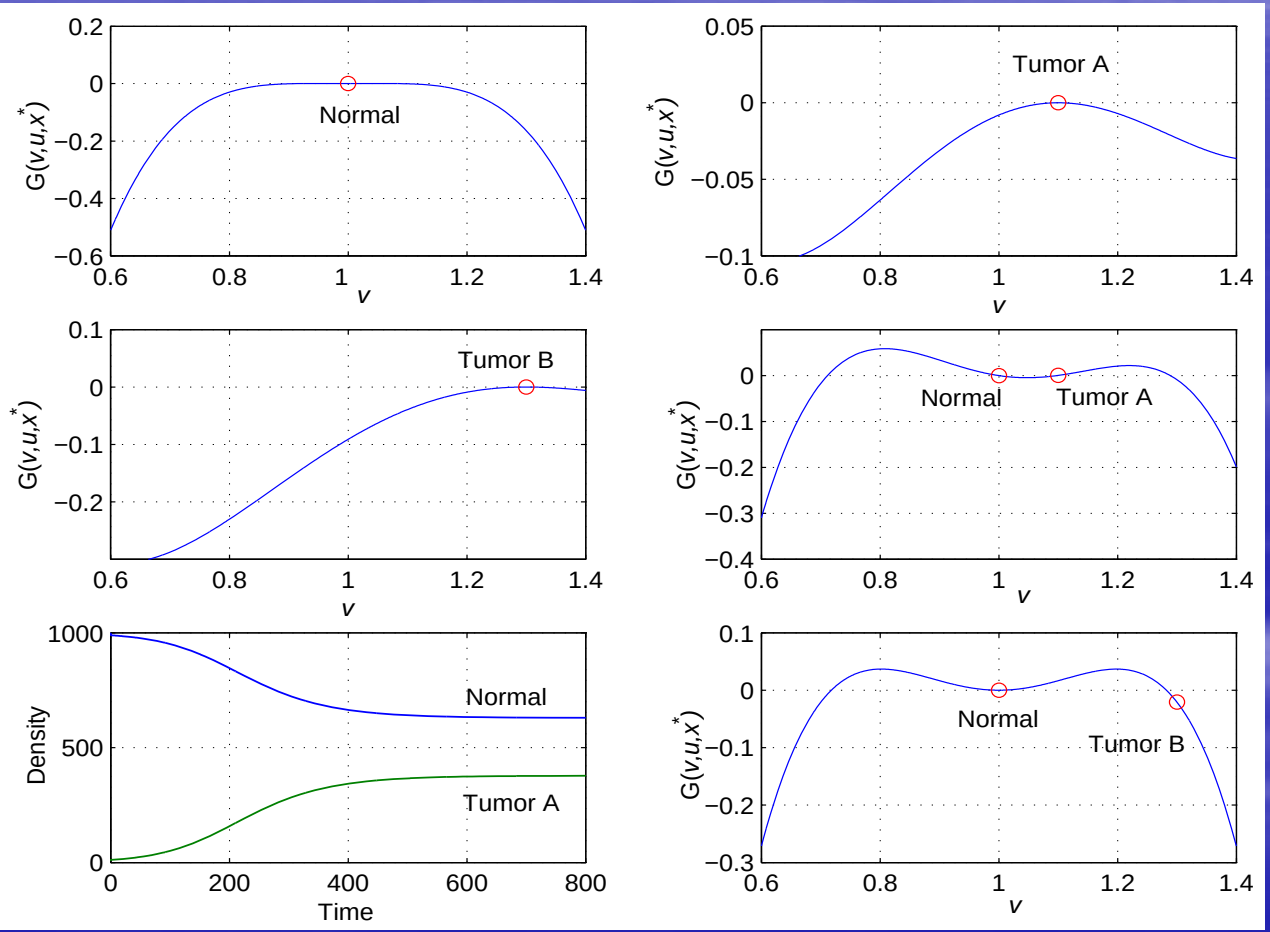
The introduction of a mutant population with a higher fitness warps the fitness landscape so that the extant they come to occupy a minimum adjacent to unoccupied maxima.



Tumor populations reach a maximum only by altering the dynamics of substrate uptake by acquiring the angiogenic and glycolytic phenotypes during a second phase of carcinogenesis dominated by subtstrate competition.



The critical role of cellular mutations in evolution of the malignant phenotype is well accepted. The equally critical role of the environment is less well known but consistent with multiple studies by Bissell et al and observed clinically in Neuroblastoma IVs and probably in metastases.



Conclusions:

- Application of information theory to carcinogenesis demonstrates the important role of Darwinian competition among mutant clones in constraining the effects of accumulating genomic mutations.
- The constraints also produce variations in the observed mutation rate of different gene segments and add a caveat to interpretation of experiments designed to measure the mutation rate and understand the role of specific mutations in tumor biology. Specifically: 1. using the observed mutation rate in any gene to infer the global genomic mutation is possible only with precise knowledge of the contribution of that gene to cellular fitness. 2. tumor cells in-vitro are subject to environmental selection pressures different from those in-vivo. Mutations observed in cell lines may be irrelevant to the same tumor cells when they are in-situ
- During carcinogenesis, cellular information asymptotically approaches a minimum. This state of minimum complexity can be used to accurately predict tumor growth dynamics and suggests fundamental limitations in tumor screening strategies.

Conclusions (cont)

- Evolutionary game theory can be used to define the interactions of the phenotypic properties generated by accumulating mutations and environmental selection properties.
- Growth constraints in normal tissues favor initial mutations that alter cellular reception or processing of growth promoter and inhibitory signals such as mutations in oncogenes and tumor suppressor genes.
- As these mutations accumulate, the populations, although unconstrained by local growth factors, exhibit only self-limited growth due to substrate limitations. This results in transition to a previously unknown phase of carcinogenesis dominated by competition for substrate and provides the evolutionary dynamics for development of the angiogenic and glycolytic phenotypes.
- The cellular properties of invasive cancer populations represent the summation of both stages of evolution.

Conclusions (cont)

- In general, the evolutionary models demonstrate malignant phenotypes will inevitably emerge from the fitness landscape necessary for maintenance of multiple phenotypes in a cooperative, non-competitive environment. That is, tumors are the price of the environment necessary to maintain functioning multicellular organisms. This is evident in the number of benign lesions such as colon polyps or skin nevi that increase monotonically with age. These mutant populations, although benign, have the potential to form cancers if they can evolve to a local fitness maximum.
- The development of a clinical cancer from a premalignant lesion is dependent on the speed of evolution. If a tumor population approaches a fitness maximum within the lifetime of the host, he/she develops cancer. Otherwise, the tumor is insignificant.
- The evolutionary rate is determined by the mutation rate and the clonal selectivity of the environment. This combines the cell-centric approach and the environmental approach into a single conceptual model of carcinogenesis.
- Alterations in the environment may substantially alter tumor growth even in the presence of a stable genome. Consider strategies that treat normal cells and alter the adaptive landscape rather than treating just tumors.