Evidence Based Hypothesis: ALPHA

(*Acidic Lactate sequentially induces Lymphogenesis,Phlebogenesis,Arteriogenesis*)

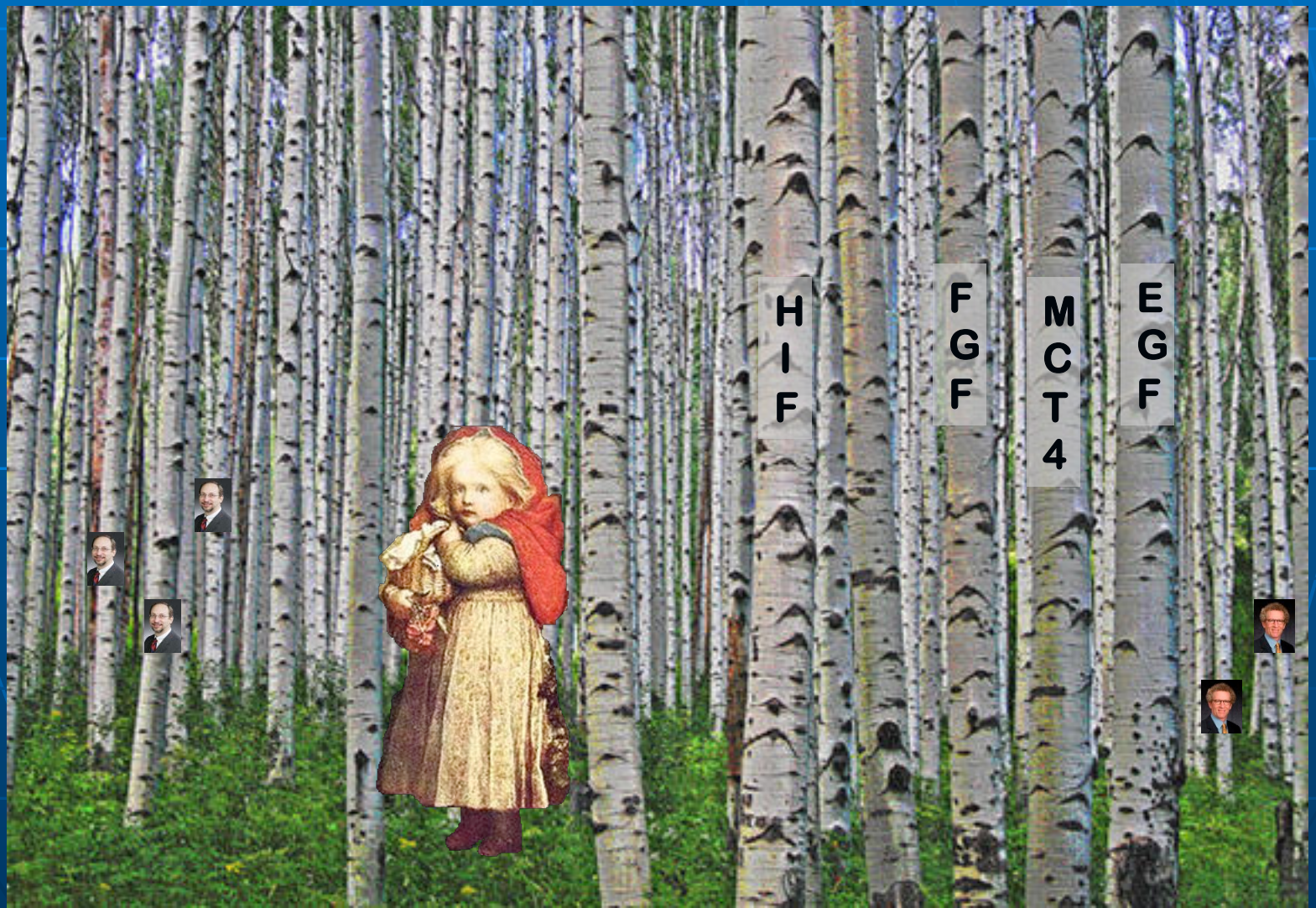
a new Vasculogenesis concept for Glycolysis# which Better explains imaging perfusion measurements

#Haaga JR,Haaga RE, ALPHA Glycolytic Vasculogenesis, Surgery, September, 2013

- John Haaga MD, FACR,etc, Tenured Professor, Chairman Emeritus

Case Western Reserve University

Thousands of vasculogenesis reports like trees in a forest. Confused/lost because there are so many! ALPHA is a new "map"



DISCUSSION

- 5000 references in high impact journals provide evidence basis for theory
- Brief Review Traditional and ALPHA vasculogenesis
- Inconsistencies of Traditional Theory
- Biochemical and molecular basis of ALPHA
- Modern imaging perfusion more consistent with ALPHA
- Preliminary proof of ALPHA
- “Real” biomarkers correlate with ALPHA

Traditional Theory: 1) Cancer uses aerobic requiring oxygen. 2) Becomes hypoxic when grows larger than 1-2 mm 3) Hypoxia slows growth & causes dormancy. 4) Hypoxia induces HIF which induces VEGF 5) VEGF stimulates arteries to restore normoxia and tumor growth

ALPHA Theory: 1) Cancer uses aerobic AND glycolysis provides many pro-cancer advantages. 2)Excessive lactate impairs glycolysis which precludes advantages. 3) Lymph/venous drainage needed to manage lactate 4) Lactate increases HIF by 3 mechanisms and VEGF,FGF by other paths

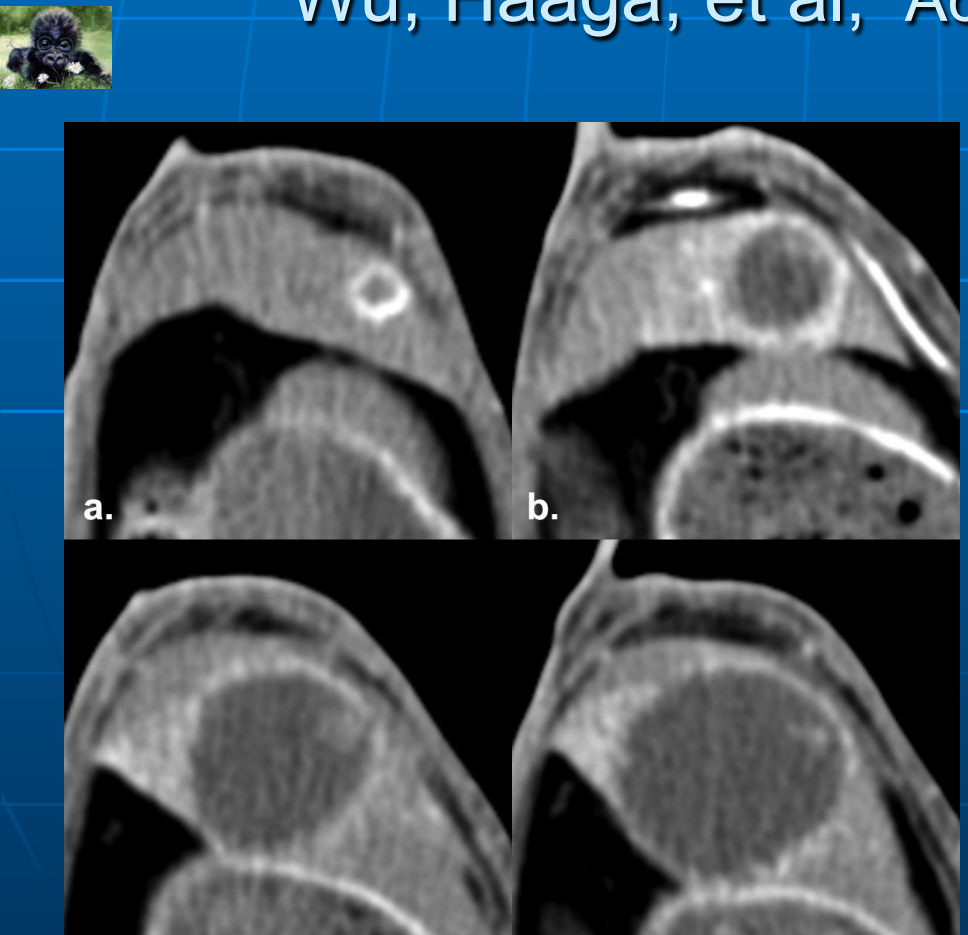
5) Vessels develop sequentially as first lymphatics, next veins, then arteries

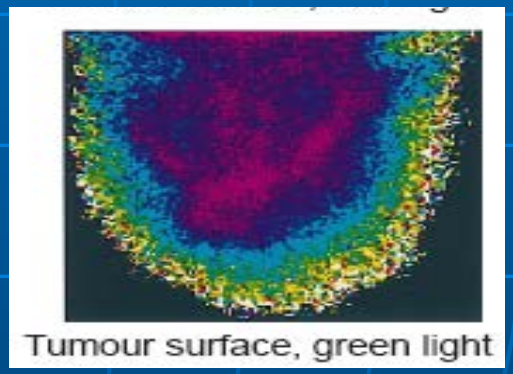
Inconsistencies of Traditional Theory

- With growth or loss of arterial flow cancers become glycolytic which helps cancers. After tumors grow>2mm they become hypoxic/glycolytic and more aggressive
- Rx with anti-VEGF drugs or embolization improve tumors temporarily but usually recur in more aggressive form
- Imaging perfusion measurements are not consistent with arterial theory. Arterial peaks and flow not useful, more consistent with ALPHA, i.e. lymph, veins

With growth Tumors become glycolytic "Evolutionary Changes in Blood flow in VX2 tumor over 28days".

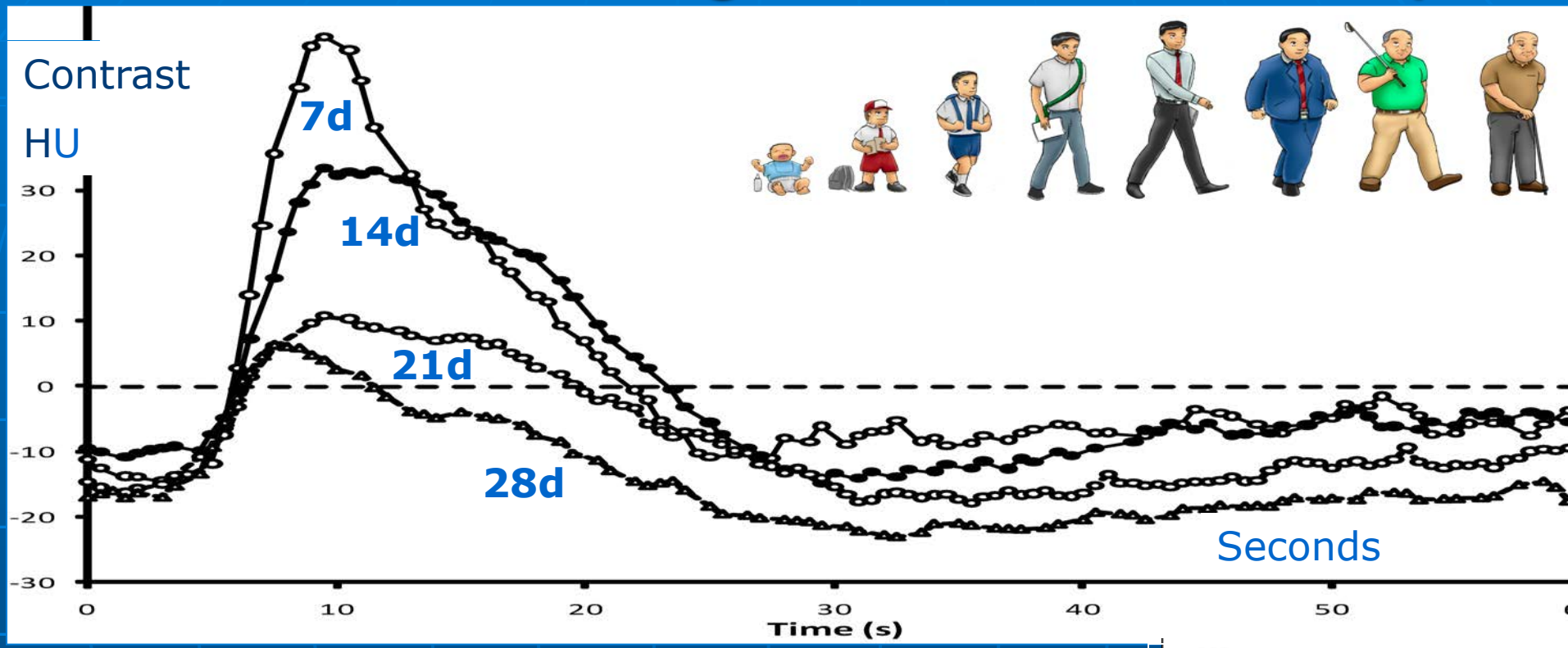
Wu, Haaga, et al, Acad Rad 2009:16;p1483



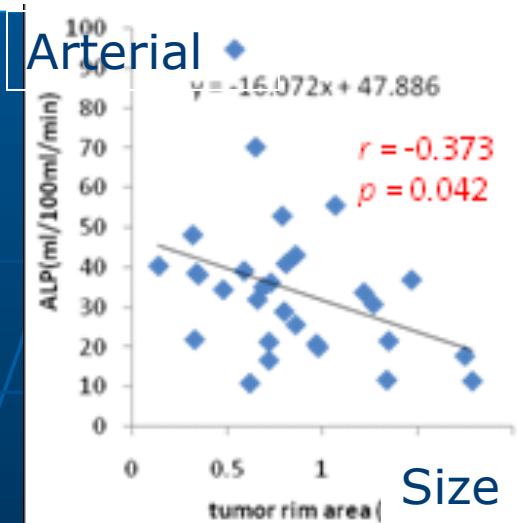


- When tumors outgrow arterial supply,O2 drops but tumors continue growth by glycolysis

Over Time, changes occur flow drops



R-correlation between tumor growth and arterial perfusion is negative. Glycolysis supports further growth



Scans confirm glycolytic tumors grow

Literature confirms hypoxia and glycolysis help cancer growth/invasion/metastases

EDITORIALS

Tumor Hypoxia: Chicken, Egg, or a Piece of the Farm?

C. Norman Coleman, James B. Mitchell, Kevin Camphausen

Anti-VEGF treatment reduces blood supply and increases tumor cell invasion in glioblastoma

Olivier Keunen[a,b], Mikael Johansson[a,c], Anaïs Oudin[a], Morgane Sanzey[a], Siti A. Abdul Rahim[a], Fred Fack[a], Frits Thorsen[b], Torfinn Taxt[b,d], Michal Bartos[e], Radovan Jirik[e,f], Hrvoje Miletic[b,g], Jian Wang[b], Daniel Stieb
Linda Stuhr[b], Ingrid Moen[b], Cecilie Brekke Rygh[b], Rolf Bjerkvig[a,b,1], and Simone P. Niclou[a,1,2]

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- After occlusion of veins and arteries, tumor becomes hypoxic and glycolytic
- Cell type changed from pleomorphic cells to spindle shape with fewer mitochondria, and cellular motility with invasive traits.
- Increased local invasion- cell motility (lactate >hyaluronan>motility-Maxwell
- Increased alternate angiogenic factors

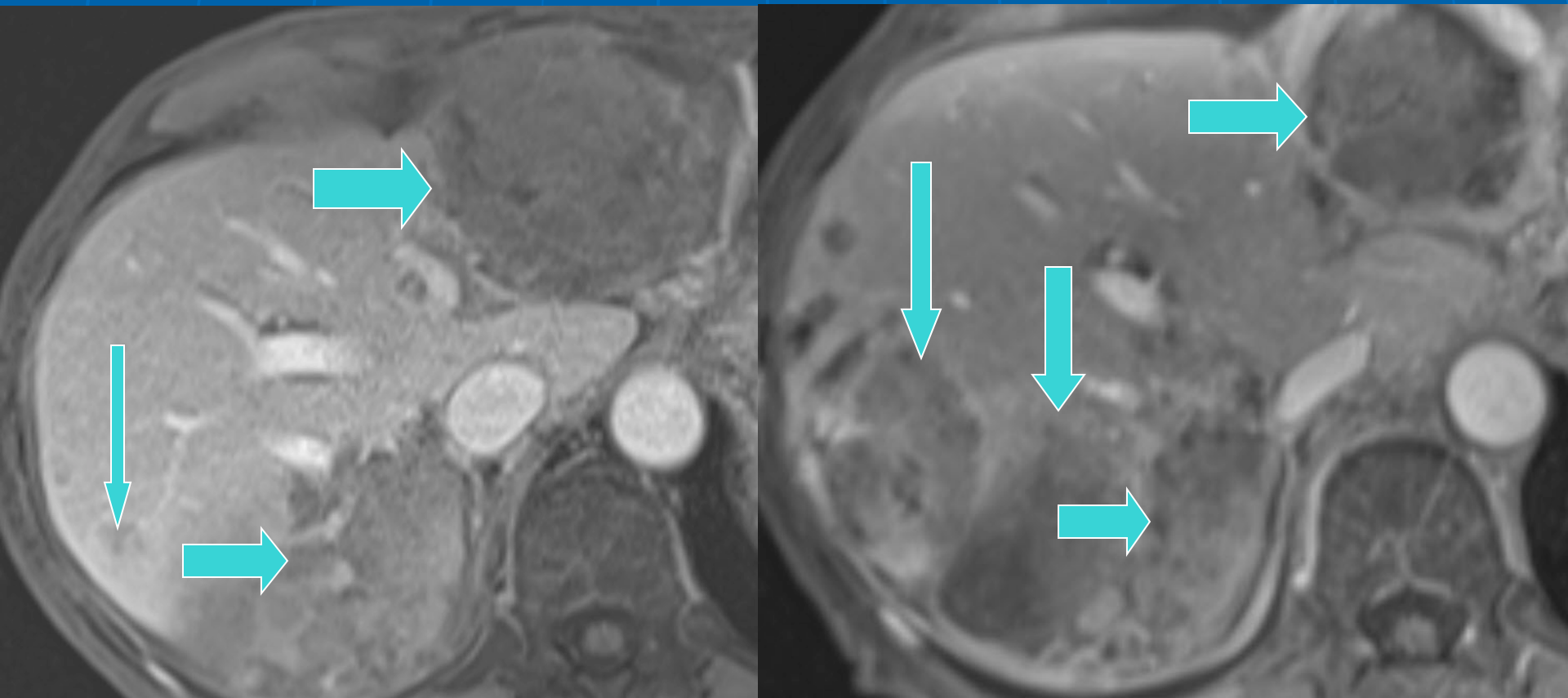
Mechanism: lactate modulates invasion Lactate>CD44>Hyaluronan>RHAMM>causes motility and invasion of cells

Keunen O et al. PNAS 2011;108:3749-3754

A:aerobic tumor with no invasion

B: glycolytic tumor with motility and invasion, arrow

Tumor Arterial Embolization (particles, chemo, or radioactive Ytrium) temporarily reduces cancers but recur with aggressive glycolytic phenotype



Aerobic/Glycolysis need different vessels

- Aerobic metabolism using 1 glucose and 1 O2 efficiently makes 38 ATP's and 2 CO2. ARTERIES NEEDED
- Glycolysis using 1 glucose and NO oxygen makes 2 ATP's and 2 Lactate. For 38 ATP also 38 Lactate. 100X FASTER because feed forward & fewer reactions

NEED LYMPH & VEINS to manage lactate:

Moderate lactate levels support many pro-cancer advantages.

High lactate slows glycolysis by feedback impairing cancer advantages

Glycolysis/moderate lactate aids cancer

ATP production 100x faster than aerobic, few reactions & feed forward mechanism

Van Heiden,Science 2009, Weinberg, MIT, 2009

Mother cell proliferation depends on growth substrates (proteins,DNA,etc) which come from side reactions of glycolysis

Moderate level of Lactate is a powerful pro cancer modulator: increases HIF, motility, invasion, mutation, immune escape ,anti-apoptotic,

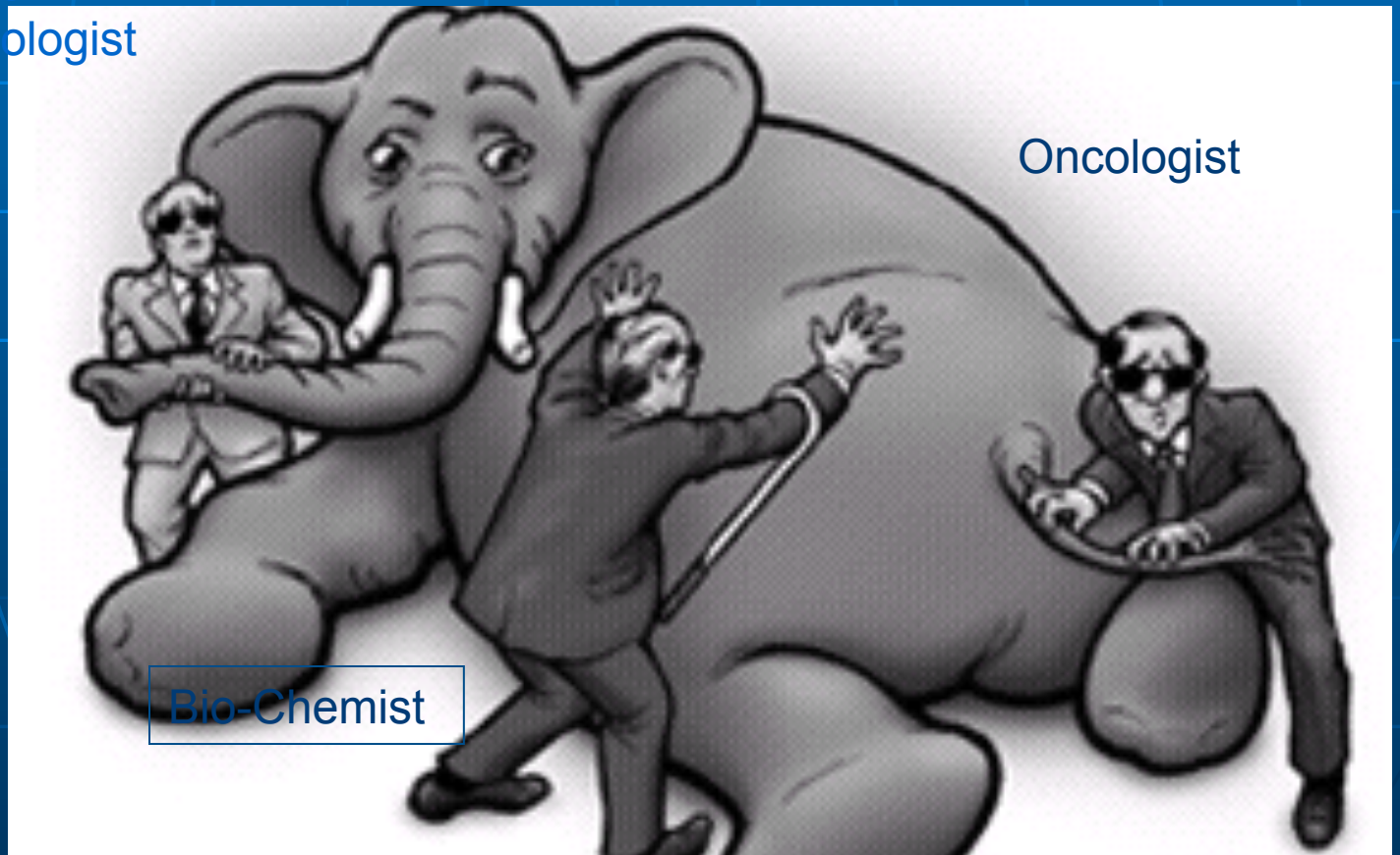
HIGH LACTATE STOPS GLYCOLYSIS IMPAIRING CANCER

High Lactate stops glycolysis by end product inhibition and low pH inhibition of phosphofructose Kinase

- High lactate decreases ATP and substrate supply (lipid, protein, nucleotide supply) so no growth
- If mother cells can't grow to double biomass for daughter cells they do not divide/proliferate but lapse into G0 (Van Heiden "Science" 09, Weinberg 09).

- TO AVOID EXCESS LACTATE GLYCOLYTIC TISSUE MUST DEVELOP LYMPHATICS AND VEINS; LACTATE VASCULOGENESIS

Science is too specialized, isolated in information “stovepipes”. Like blind men feeling elephant. ALPHA integrates data explaining glycolytic vasculogenesis



ALPHA Preliminary PROOF

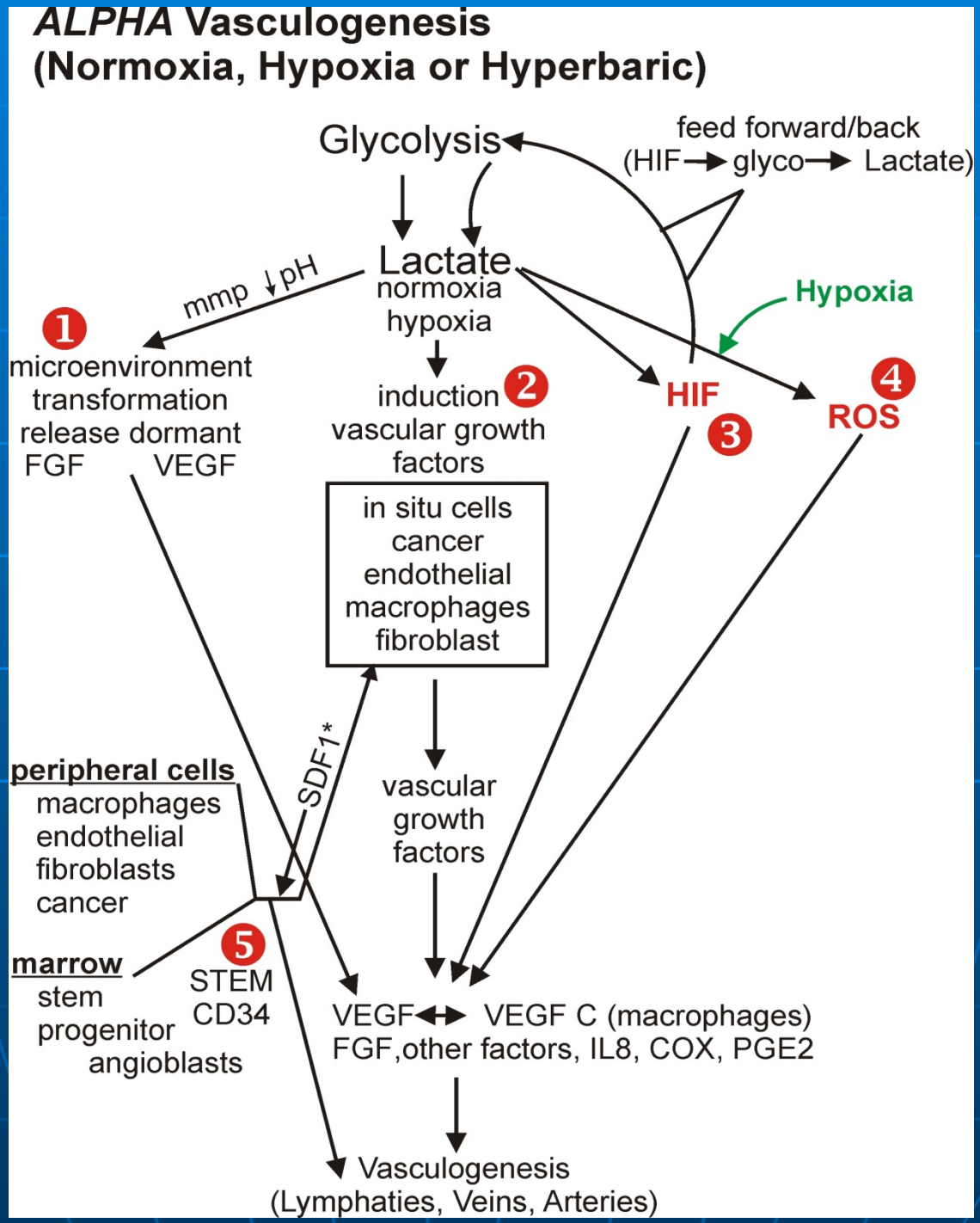
- Lactate stimulates vasculogeneis by vascular growth factors, HIF, ROS, and attraction of stem angioblasts.
- Sequence of vessel development is lymphatics first, veins then arteries
- JRH animal experiment (adapted from animal model of Indraccola et al "Interruption of tumor dormancy by angiogenic burst from microenvironment.", PNAS,2006)

Example reports of how Lactate/pH induces Vascular Growth Factors

D'Arcangelo D et al. Acidosis ….Induces Basic FGF and VEGF Expression. *Circul Res* 2000;86:312.

Hunt et al,.. "Aerobically derived lactate induces VEGF, TGFb (transforming growth factor),IL-1, and HIF, Antioxid. Redox.Signal, 2007;9(8),p102

Vegran F,Boidot R, Michiels C, "Lactate Influx … drives angiogeneis." Increase FGFx2, IL8x8 fold,NFkB Cancer Research 2011;71,p2550-


ALPHA Vasculogenesis
(Normoxia, Hypoxia or Hyperbaric)
Glycolysis
feed forward/back
(HIF → glyco → Lactate)
Lactate
normoxia
hypoxia
mmp ↓pH
1
microenvironment
transformation
release dormant
FGF VEGF
induction 2
vascular growth
factors
Hypoxia
HIF
3
4
ROS
in situ cells
cancer
endothelial
macrophages
fibroblast
SDF1*
vascular
growth
factors
peripheral cells
macrophages
endothelial
fibroblasts
cancer
marrow
stem
progenitor
angioblasts
5
STEM
CD34
VEGF ↔ VEGF C (macrophages)
FGF,other factors, IL8, COX, PGE2
Vasculogenesis
(Lymphaties, Veins, Arteries)

Sequence of Vessel Development: lymphatics first, veins then arteries

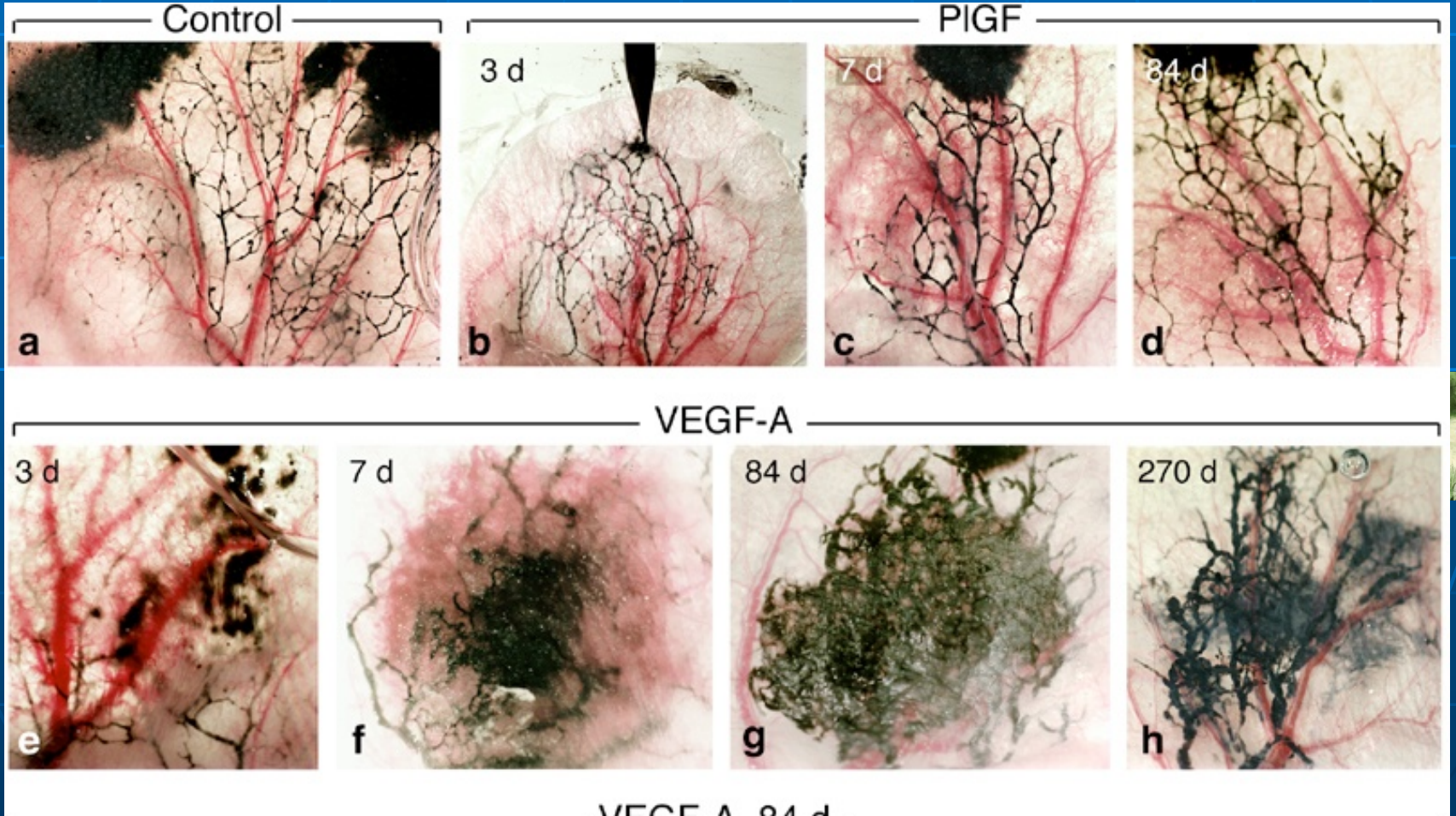
1)Transfected VEGF gene-Lab. Invest (Nature Group) Impact 3.6

2)Growth factor pellet implant in Cornea Proc Nat Acad Science Impact 9.7

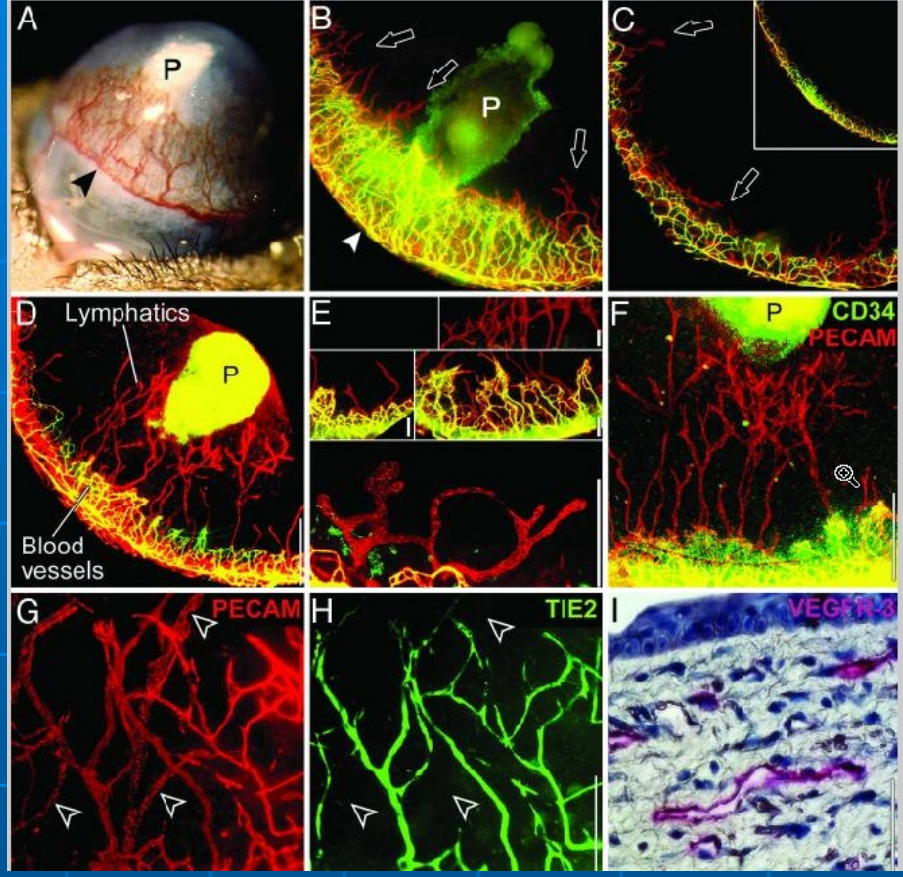
3)Denovo skin cancer model-Cancer Research, Impact 7.8

4)Xenograft implant in mouse-Circulation Research, Impact 9.5

“Vascular permeability factor/vascular endothelial growth factor induces lymphangiogenesis as well as angiogenesis” Nagy JA, Vasile E, Feng D, et al.. J Exp Med 2002;196:1497–506



FGF2 induces VEGFC at lowest levels and VEGFA at higher levels, Impact 9.6

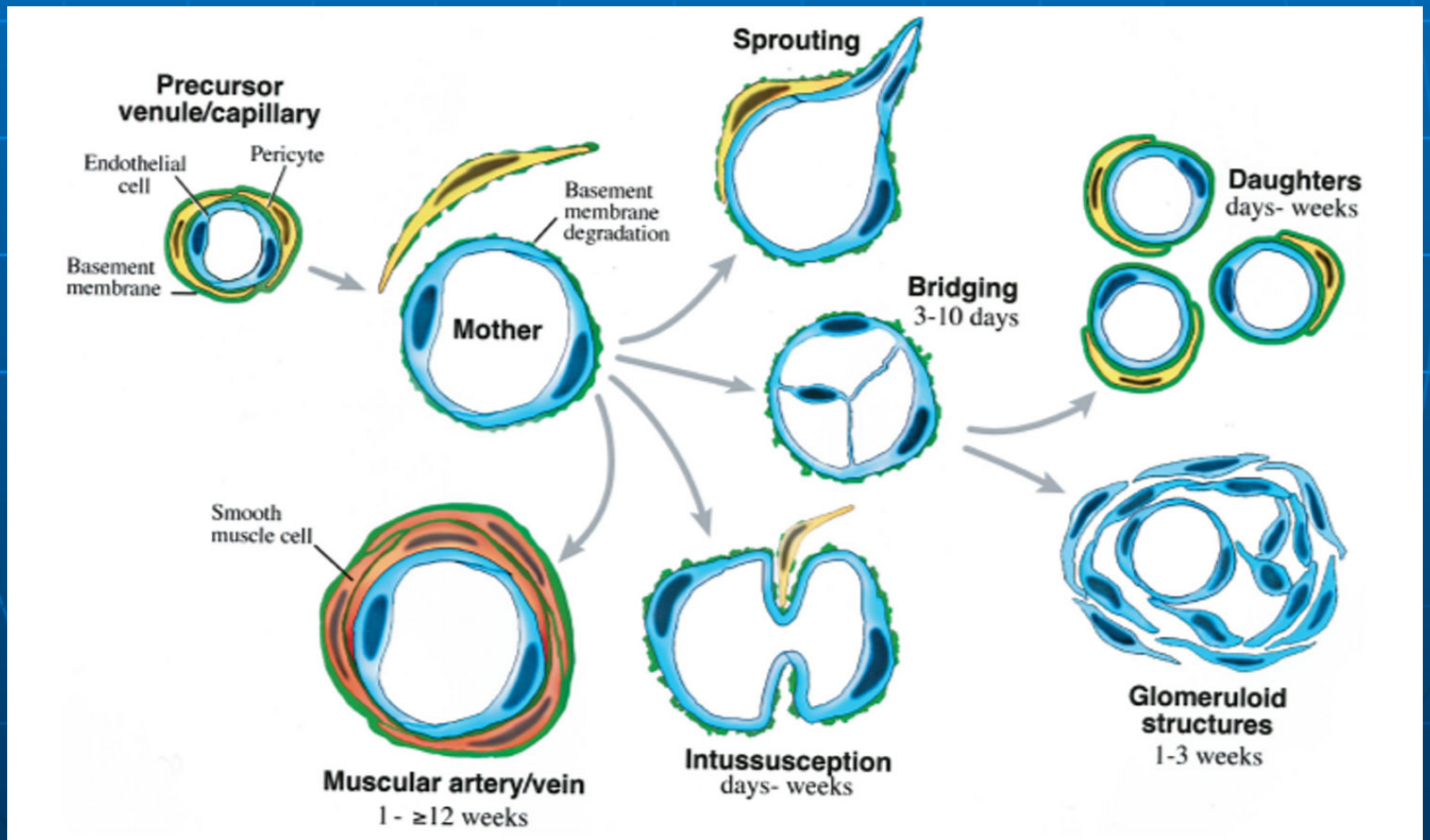


"Low-Dose FGF-2 Selectively Stimulates Lymphangiogenesis", Dose-dependent response of FGF-2 for Lymphangiogenesis.

Chang etal *PNAS*, 2004, 101(32) senior author Kaipainen,Med Nobel Institute, Stockholm,

First Blood vessels formed are veins "cannot have flow in without flow out"

Pettersson A, Nagy JA, Brown LF, Heterogeneity of the Angiogenic Response Induced in Different Normal Adult Tissues by Vascular Permeability Factor/Vascular Endothelial Growth Factor, Lab Invest 2000, 80:99–115

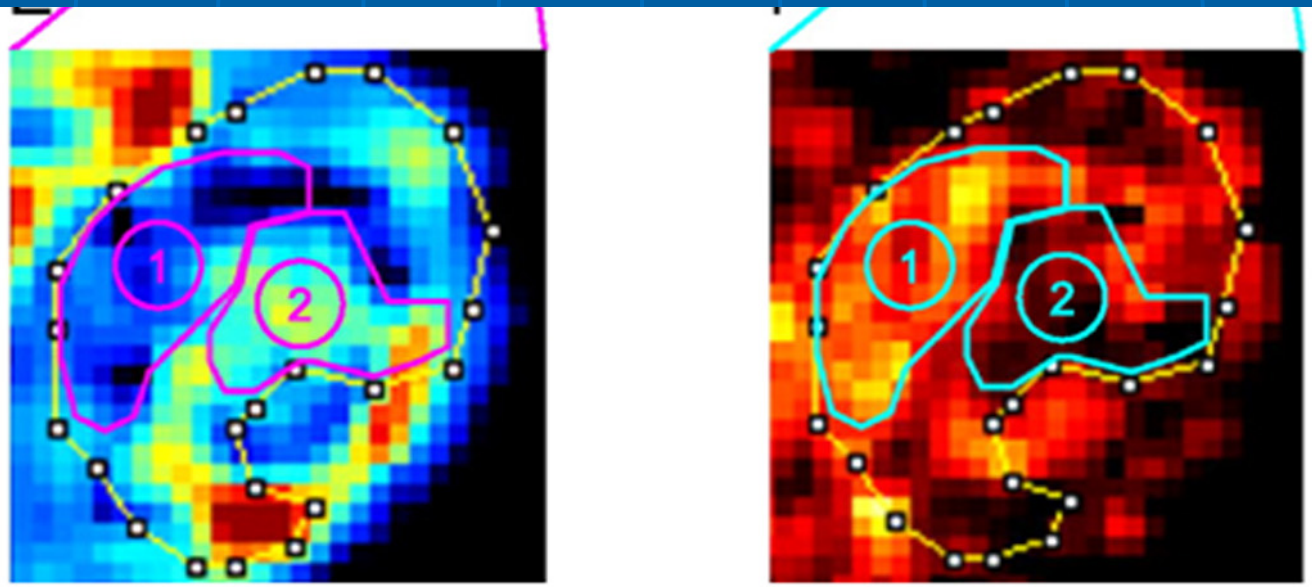


Why should a radiologist care?

Because vascular perfusion parameters from MRI, CT, PET, and ultrasound more consistent with ALPHA

1) Diffusion permeability- Ktrans, Kep, Patlack- Depends on Veins
2) Contrast Washout and kinetic curves depend on increased veins
3) Blood volume-predominantly veins

Simultaneous measurement of oxygenation and Ktrans permeability, Matsumoto et al, PNAS, 2009 vol. 106 no. 42, 17898-17903. NO correlation between oxygen and permeability

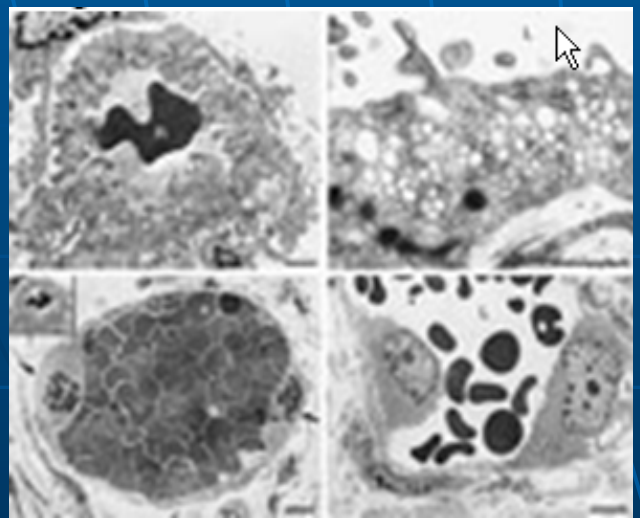


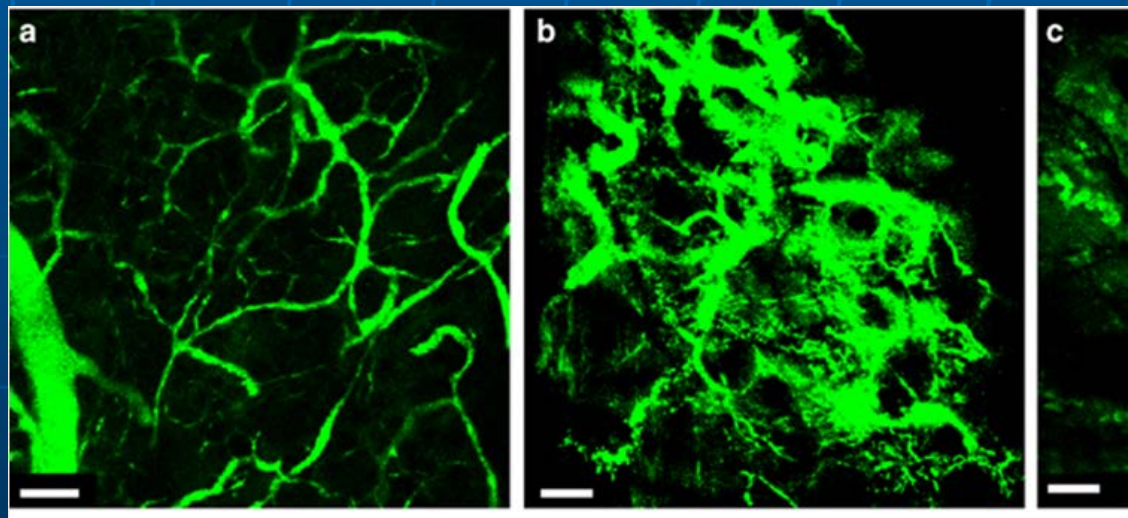
Matsumoto S et al. PNAS 2009;106:17898-17903

Permeability occurs thru veins-Ktrans

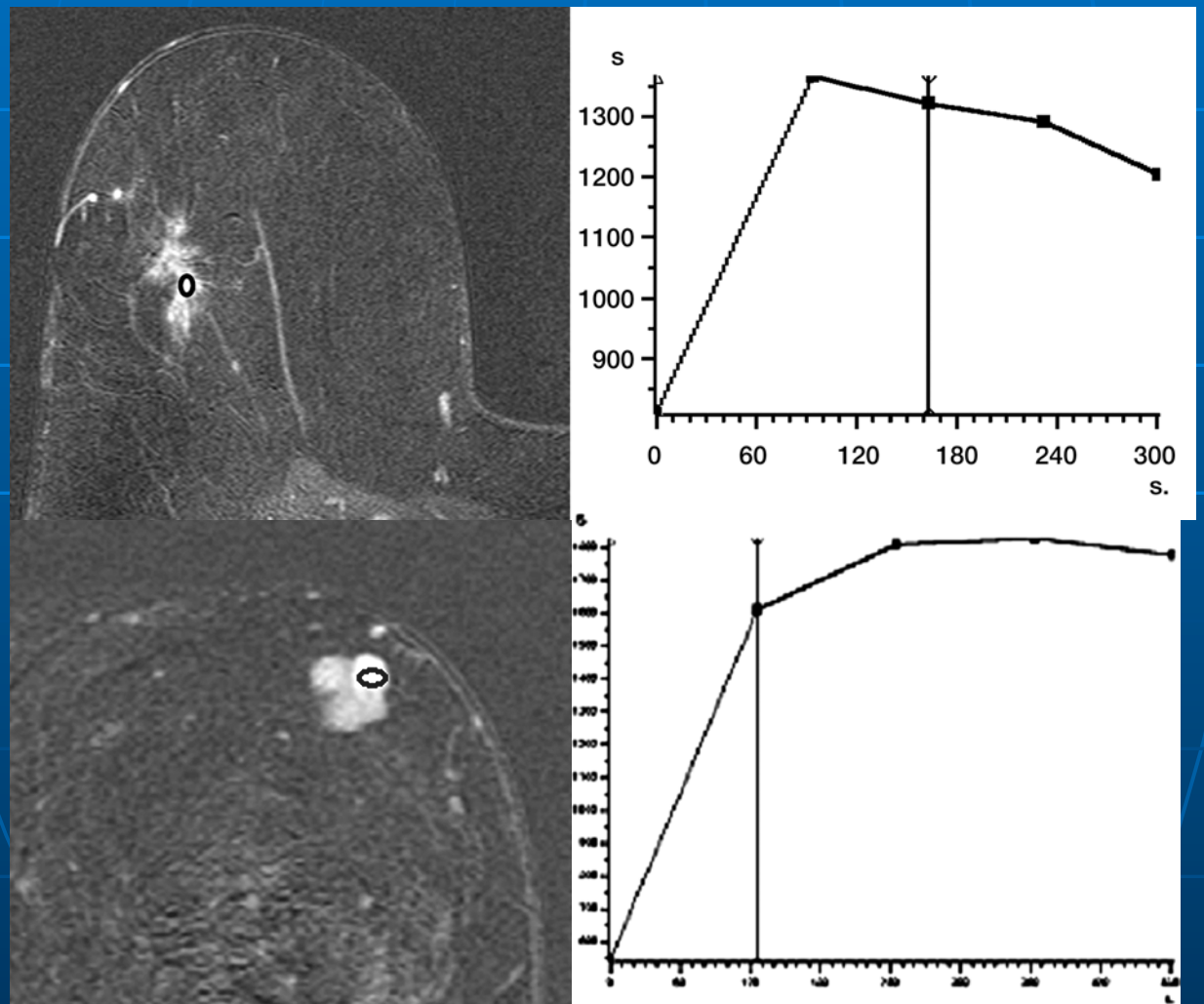
"All tracers leaked primarily from venules and small veins at the tumor-host interface, for the most part vessels lined by a continuous endothelium. The predominant pathway by which all four tracers exited venules in all three tumors "

Kohn et al **Lab Invest. 1992 Nov;67(5):596**

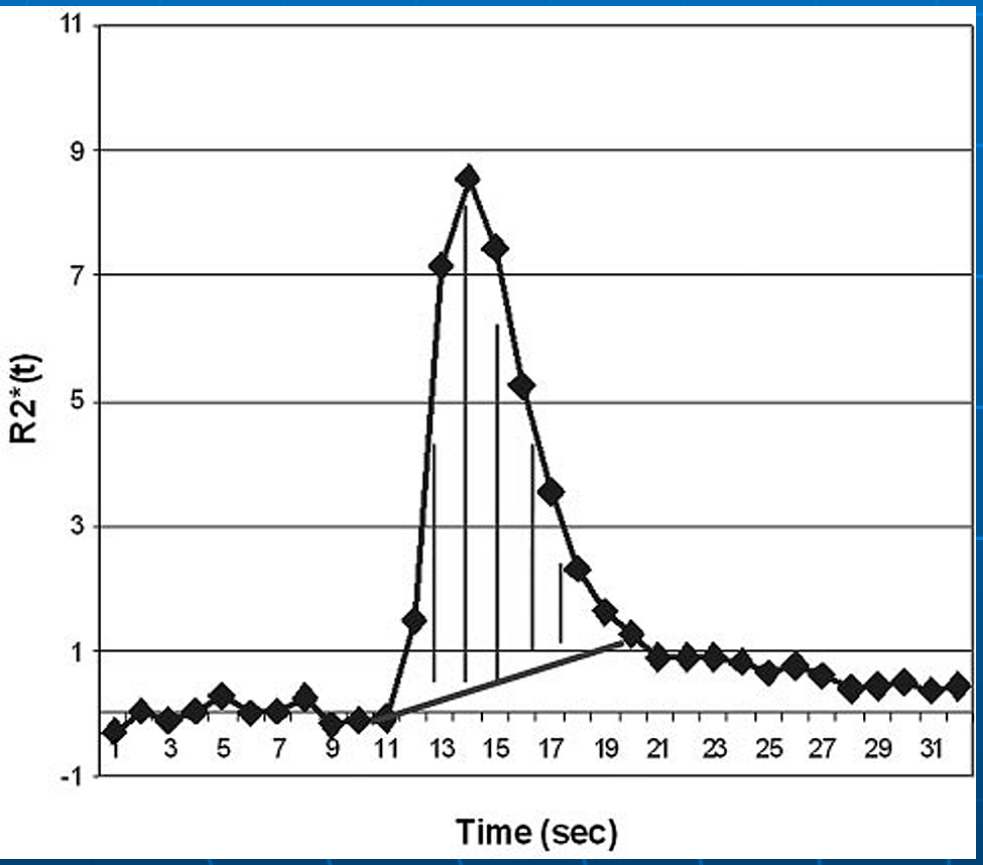




Kinetic curves for MRI DCE breast depend on venous outflow, Kuhl et al, Radiology, 1999;211:101-110



Blood volume and permeability values on MRI or CT useful for differentiating tumors

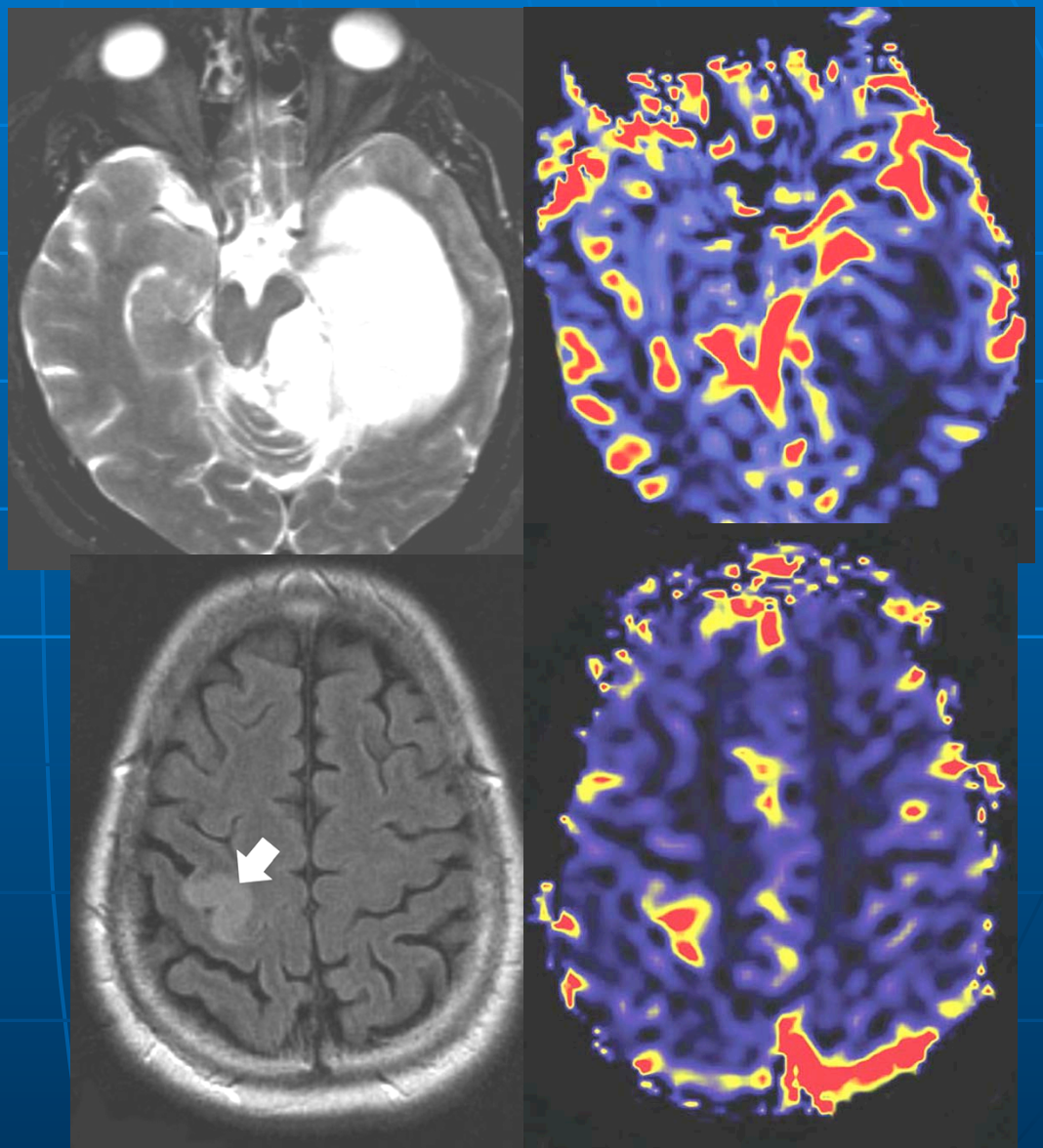


- Duong et al calculated components of blood volume to be 29% arterial and 71% venous. Hence, mostly reflects venous system

Spaminato et al (Radiology) reported degree of differentiation predicted by Blood Volume

Low blood volume map indicates low blood volume low grade oligoastro

Relative cerebral blood volume map shows elevated vascularization- anaplastic oligoden



Blood volume is increased,via vein: vascular biomarkers *Ephrin B2 artery, Ephrin B4 vein*

Noren et al, PNAS, 2004

B4 increases vessel size, blood volume and tumor growth. (CONSISTENT with ALPHA)

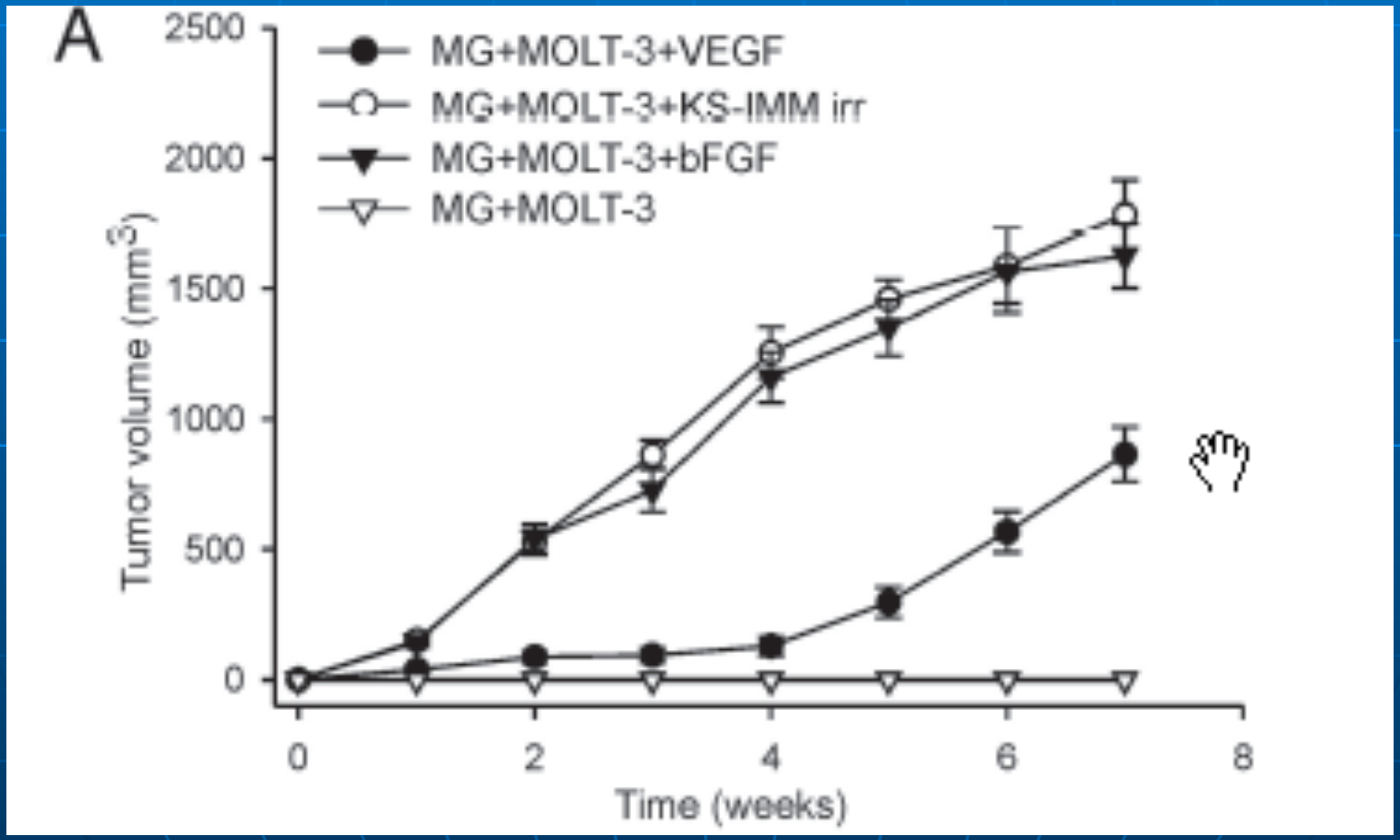
Overexpression B2 suppresses tumor Growth (CONTRARY TO OLD THEORY)

Same results in 10 additional reports

Preliminary Proof of ALPHA

- We duplicated experiment by Indraccola et al (PNAS), which converted non angiogenic tumor MOLT3 to growing vascular tumor.
- MOLT3 alone didn't grow, MOLT3 with VEGF or FGF grew, and MOLT3 with irradiated sarcoma cells grew.
- They concluded dormancy interrupted by angiogenic burst from microenvironment.
- Haaga et al, studied the same "dormant", non-growing, non-angiogenic cell line MOLT3 using lactate.WHICH GREW

“Interruption of tumor dormancy (MOLT3) by a transient angiogenic burst within the microenvironment” Indracolla et al PNAS,2006

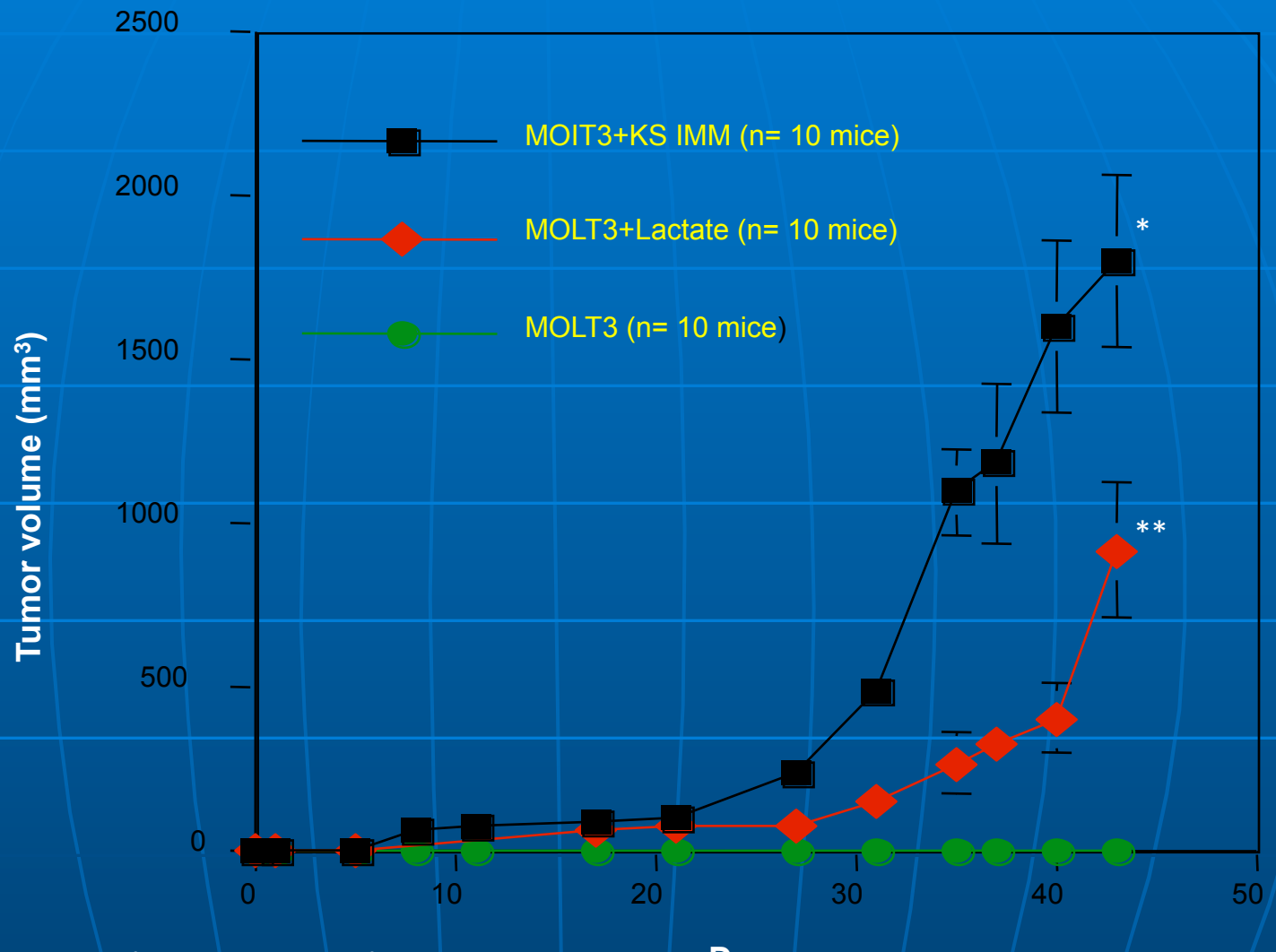


Haaga Experiment
Repeated MOLT3 model
Using same cell line cohorts in SCID mice except included group with MOLT3 and Lactate.

Molt3 cells
Lactate (30mg/ml)

Molt3 cells only
No Tumor

Xenograft tumors grown in NOD SCID Mice

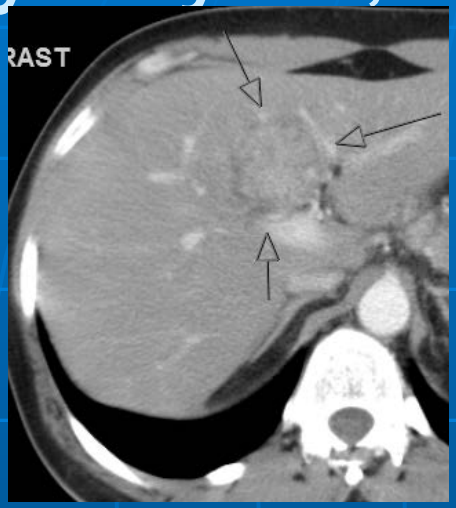


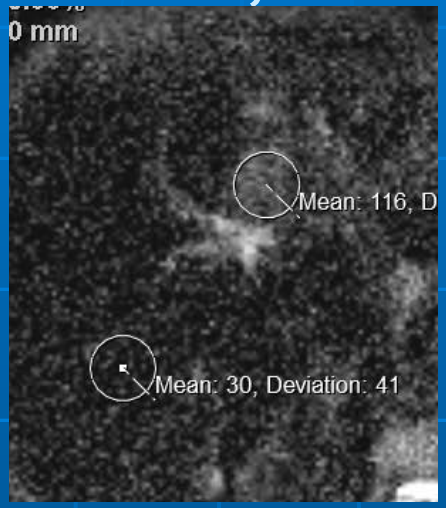
- Molt3 cells ($5x10^6$) + KS cells ($5x10^6$, irradiated 45 Gy before s.c. injection into the flanks of NOD SCID mice. *P*= 0.0546 (between groups of molt3+KS) and molt3+lactate
- Molt3 cells ($5x10^6$) + extracellular matrix gel + Lactate (30 mg/ml). *P*< 0.05 (between groups of molt3+lactate and molt3 only
- Molt3 cells ($5x10^6$) + extracellular matrix gel

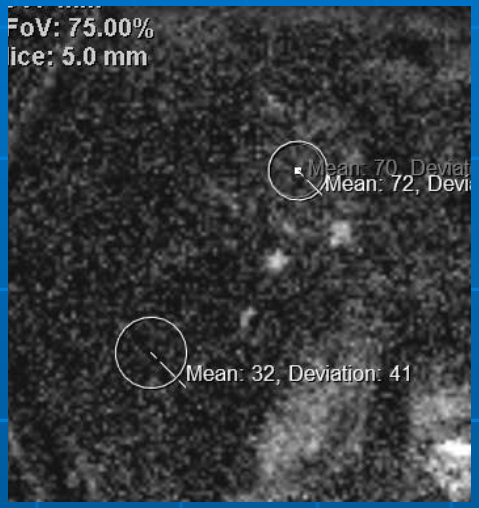
Future Applications

- New Imaging methods related to DWI and ADC. Relates to lactate production and interstitial edema
- Improved treatment: instead of treating only the aerobic metabolism should treat both aerobic and glycolytic

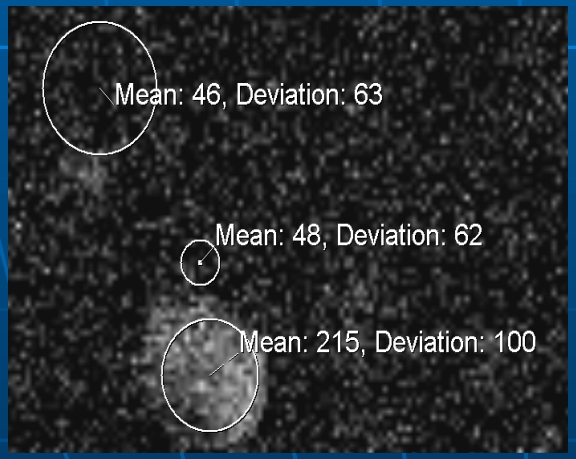
Blocking waste enzymes changes glycolysis,lactate,interstitial fluid & ADC

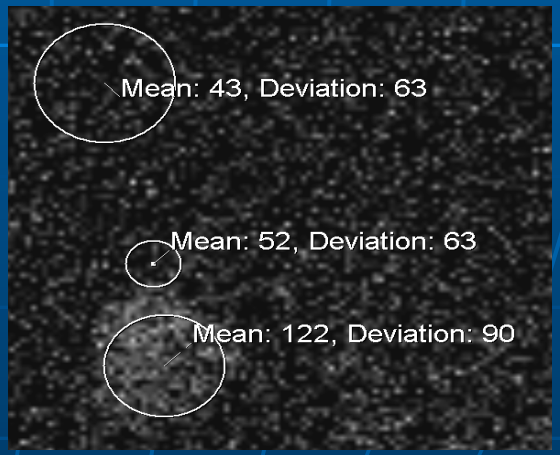






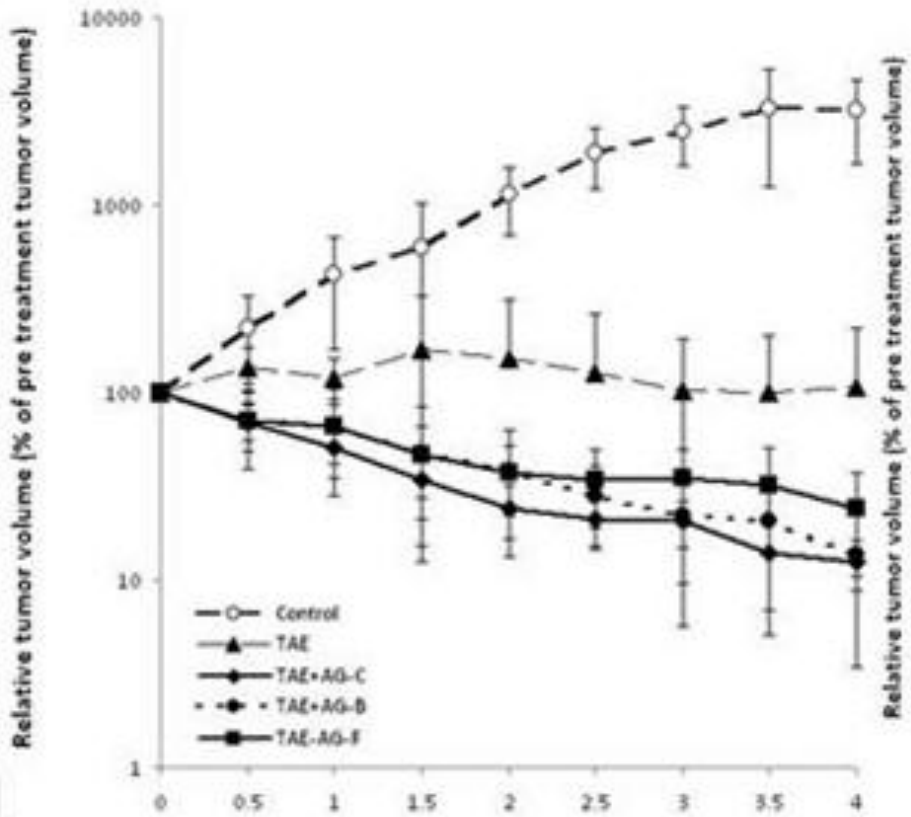
Liposarcoma pre and post drug-116 to 72





Metastatic breast to kidney-215 to 122

Rx of liver cancer is most effective if both aerobic and glycolytic treated simultaneously



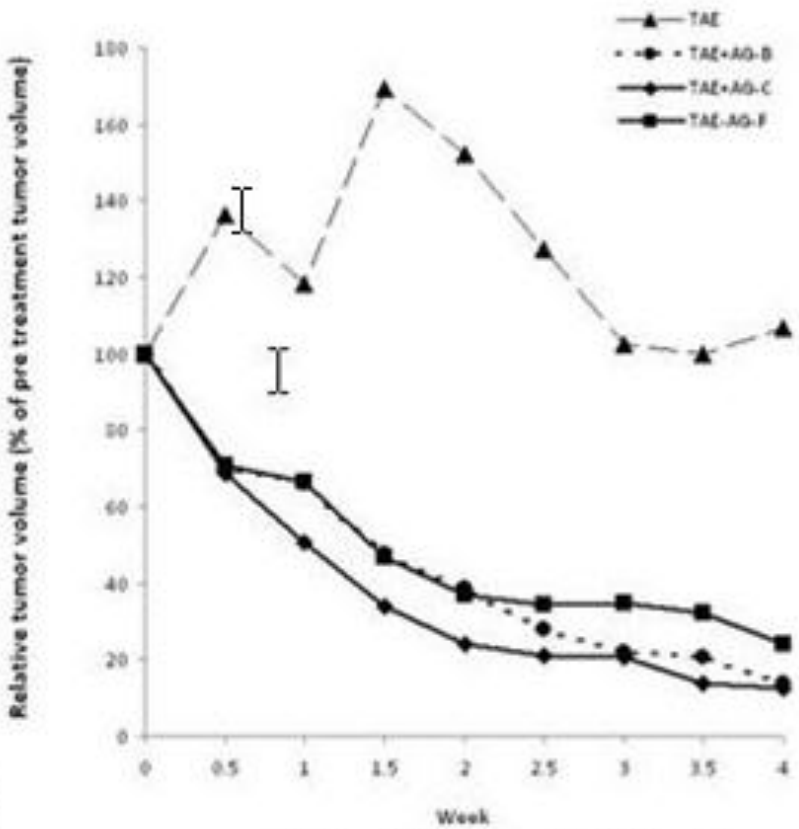


Fig a Control group and Rx group Fig b. Treatment groups:TAE-AG-B and TAE-C best

Folkman was a friend to many BUT Original report has ??? Statistics

National Cancer Institute
National Institutes of Health
6130 Executive Boulevard, MSC 7412
Bethesda, MD 20892

Ladies/Gentlemen:

This is in regard to the grant entitled, "Use of vasoactive agents to enhance specificity of CT/MRI imaging"; application number 1 R21 CA11558-01A1. I have reviewed the preliminary data, submitted for the grant by Dr. Haaga and his team. I find this principle and concept very exciting and it has great possibilities for the future.

It is my belief that Dr. Haaga's research will impact the diagnosis of tumor chemotherapy assessment and treatment, and even translation to the area of biomarkers.

If this grant is approved, I would be happy to be a consultant to Dr. Haaga as he pursues the development and refinement of this principle.

Sincerely yours,

Judah Folkman

Judah Folkman, MD

- Graph of single animal used for conclusions
- Arrow shows onset of arterial flow via injection of fluorescein
- Growth precedes flow in this single animal. Other data from 10 animals re-evaluated by Tien and Chankung

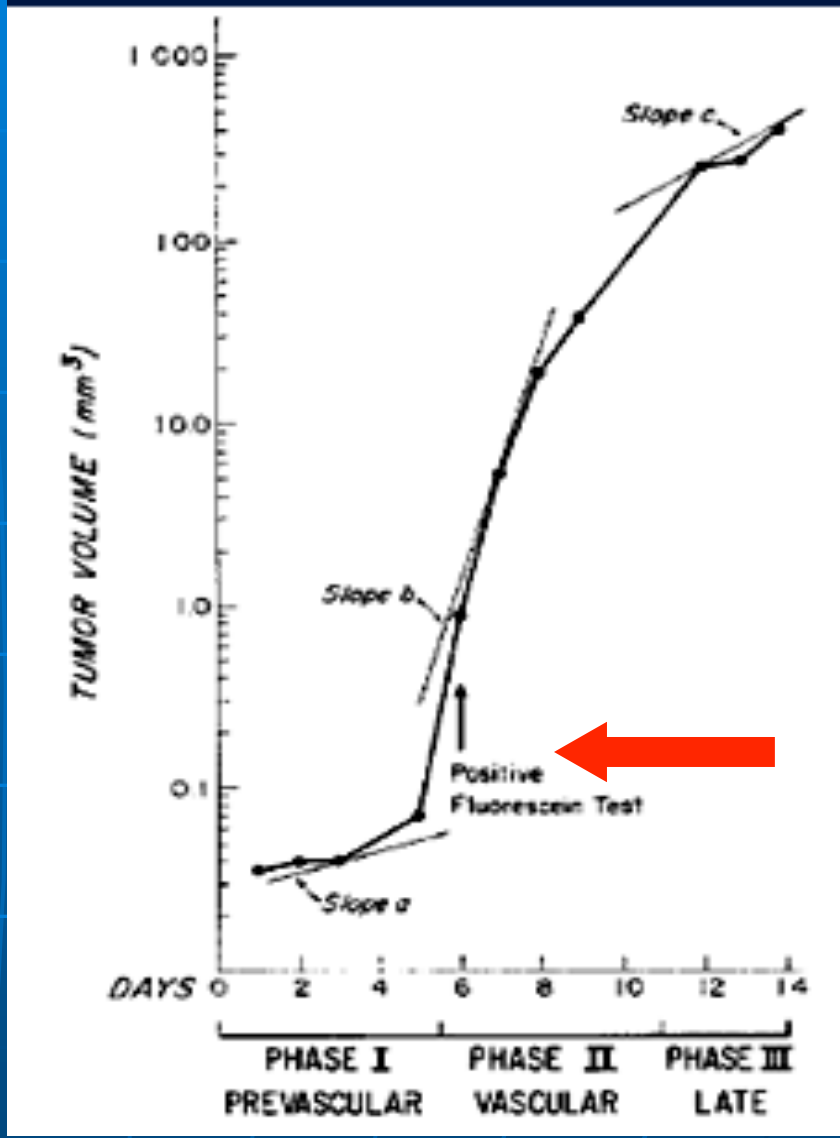


FIG. 5. The characteristic growth curve of an iris implant (BP No. 29R) plotted on a semi-logarithmic scale. Positive fluorescein test on day 6 represents earliest evidence of perfusion of the tumor, and coincides with the beginning of exponential volume increase. Slopes "*a*," "*b*," and "*c*," corresponding to prevascular, vascular, and late phases of growth, are indicated.

10 animals studied, but their conclusions drawn from one animal/graph not 10.

TABLE I

Rates of Growth and Time of Vascularization of 10 Iris Tumors

Experiment No.	Growth curve slopes* (Prevascular) *a*	(Vascular) *b*	(Late) *c*	Primary inflection (*a* to *b*)	Vascularization‡
				day	*day*
1	0.04	0.49	0.23	5–7	7
2	0.05	0.68	0.14	5–6	6
3	0.08	0.58	—	5–6	6
4	0.03	0.96	0.14	6–7	7
5	0.04	0.87	0.25	6–7	7
6	0.16	0.54	0.13	4–6	6
7	0.08	0.51	0.21	6–7	7
8	0.19	0.70	0.15	5–6	6
9	0.06	0.64	0.12	5–6	6
10	0.12	0.56	0.12	5–7	7
Mean ± SD =	0.085 ± 0.055	0.65 ± 0.155	0.16 ± 0.051		

* Estimated from semilogarithmic plots (see text).

‡ "Positive" fluorescein test or gross appearance of tumor vessels.

Analysis of Gimbrone data: N. Tien PhD, Dean Engineering. V.Chankung,PhD

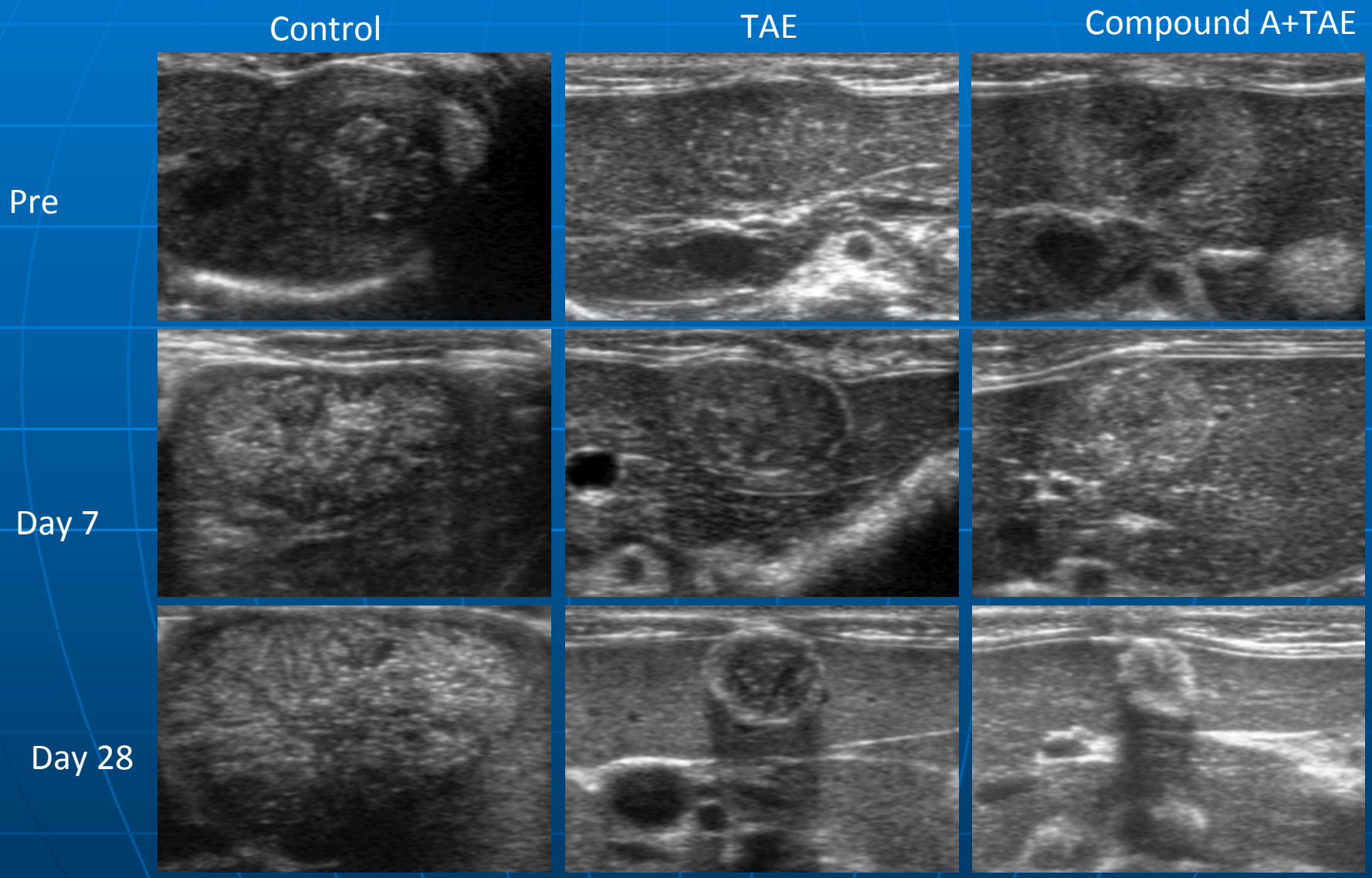
"Using a statistical t-test at 95% confidence level, it confirms that arterial vascularization occurs approximately 1 day after the exponential growth begins. ..In conclusion, based on the data in Grimbrone et, al (1972), the tumors appear to BEGIN its rapid exponential growth about 1 day prior to arterial vascularization. If this is true, then there must be a mechanism other than arterial vascularization that serves as a primary factor to stimulate the abrupt growth."

Research supported by John and Ellen Haaga thru Elizabeth Haaga Memorial Fund CWRU

?? Know any foundations which fund “out of the box” concepts….NIH doesn’t

Improved RX of Hepatocellular Carcinoma in Rat with bimodal Rx of aerobic and glycolytic metabolism.

Tumor growth



Collating many “overlooked” data from high impact journals support ALPHA “Lactate” is the 800 Lb Gorilla “overlooked” in the angiogenesis room .

"ALPHA Glycolytic Vasculogenesis" published in journal "Surgery", Sept. 2013

F

For PDF, please email John.Haaga@uhhospitals.org

Please view computer lecture which has more details. Original report by Gimbrone and Folkman has serious statistical issues.

Traditional Theory based on: "Tumor Dormancy in Vivo by Prevention of Neovascularization",

Gimbrone Folkman et al, J.Exp Med 1972

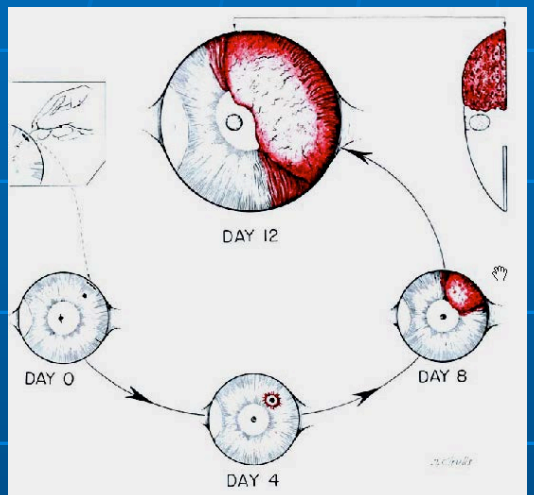
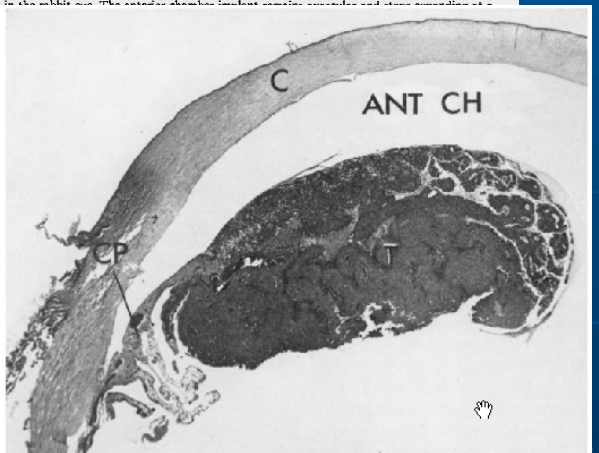


FIG. 1. The patterns of development of two simultaneous implants of Brown-Pearce tumor



- Small tumor implants inserted into two sites, anterior chamber and ciliary body with vessels
- Tumor size monitored with ophthalmic slit lamp
- Onset of Arterial flow detected by intravenous fluorescein .

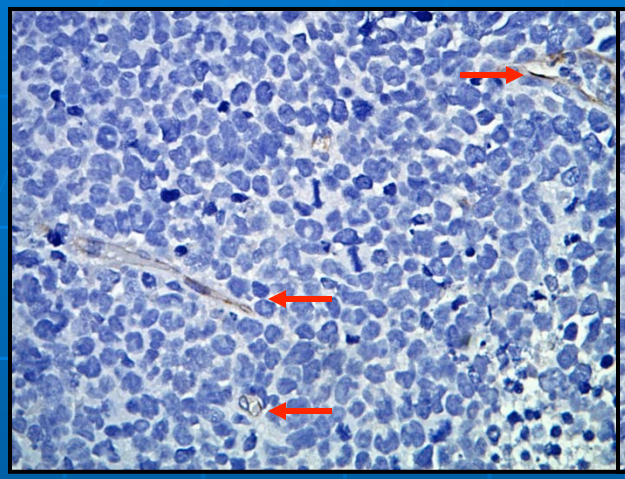
Conclusion

Cancer prefers and requires glycolysis because it and lactate provide pro cancer beneficial processes.

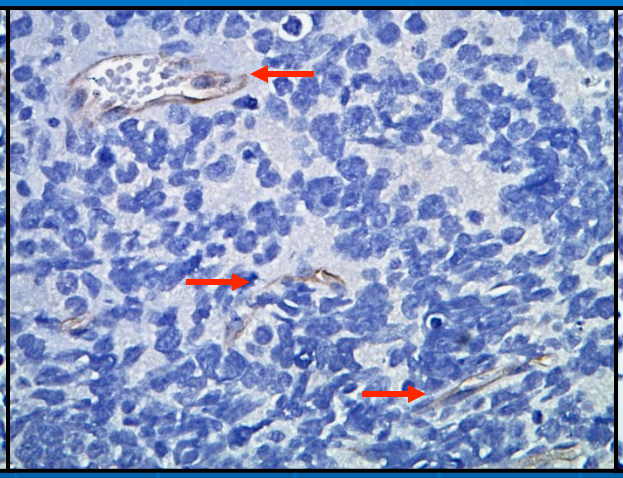
ALPHA Vasculogenesis complements the traditional vasculogenesis concept because it explains the role and mechanism for glycolytic vasculogenesis

To avoid cellular arrest caused by excess lactate, vascular growth factors develop lymphatics first, veins, and then arteries

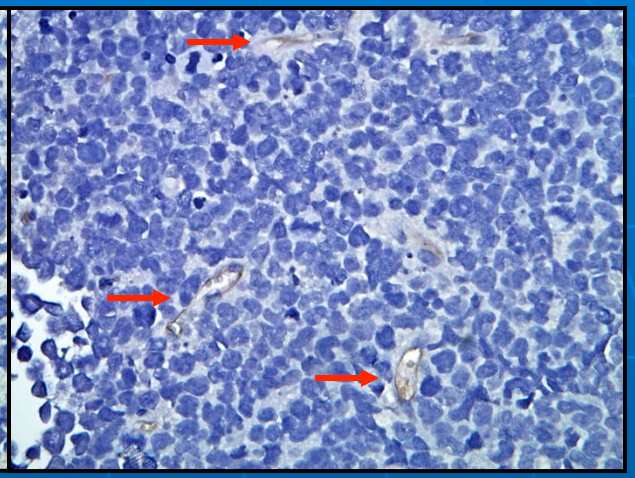
Kaposi and MOLT3Lac MVD by CD31 same



MOLT3+KS d40 (A)



MOLT3+KS d40 (B)



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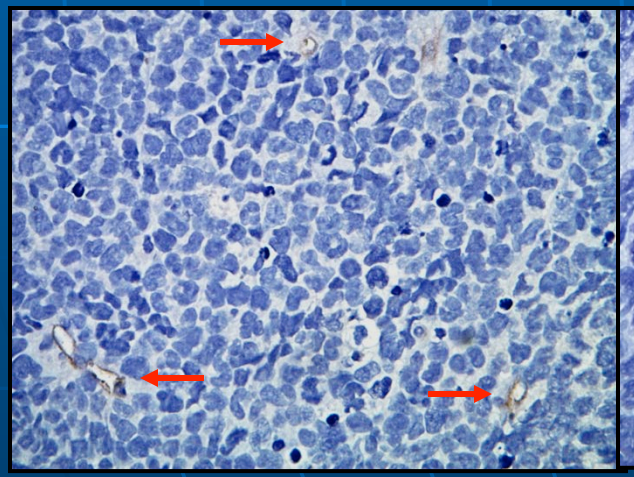
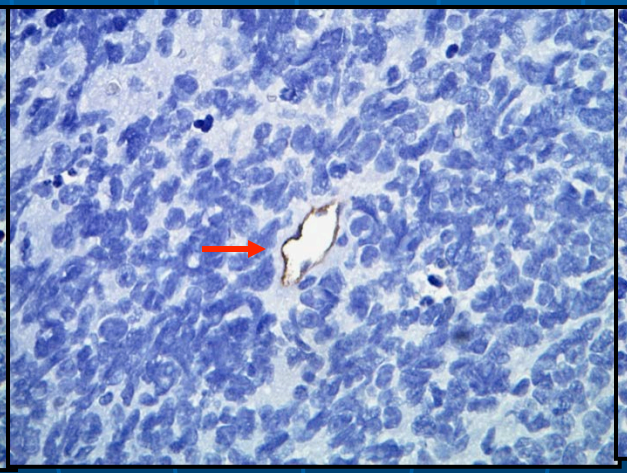
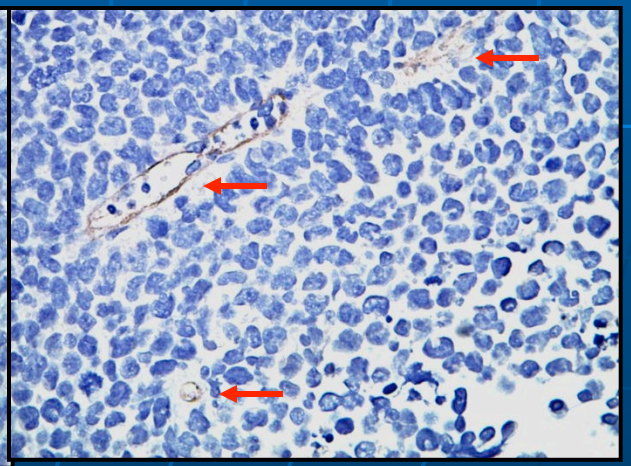


MOLT3+Lac d40 (B)

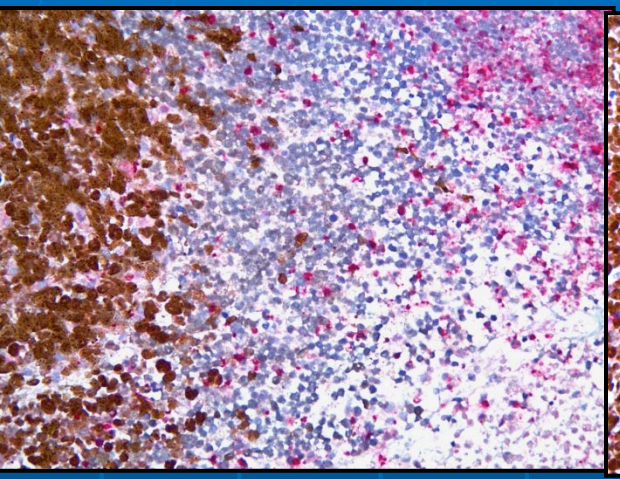


MOLT3+Lac d40 (C)

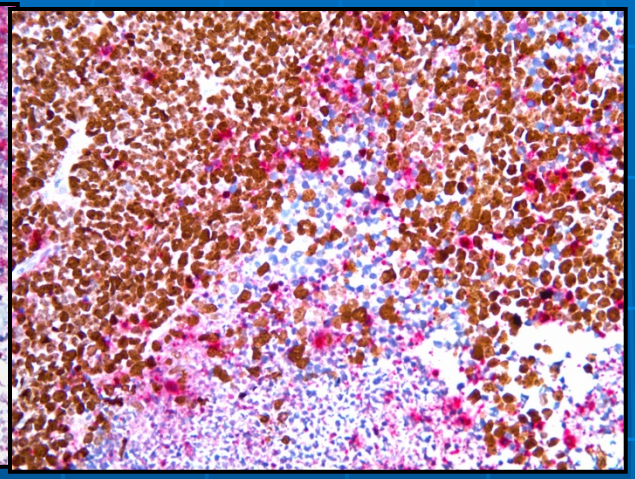


MOLT3+Lac d40 (D)

ALPHA – Ki-67 + Cleaved Casp3 IHC Results, differs from Indraccola et al , same vessels but remarkable proliferation and apoptosis



MOLT3+KS d40 (A)



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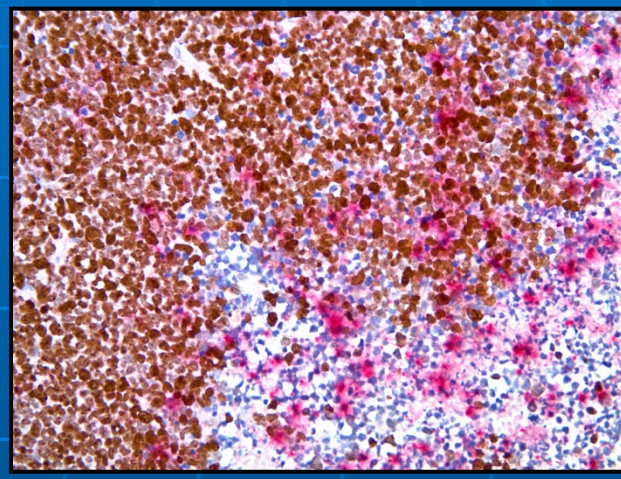
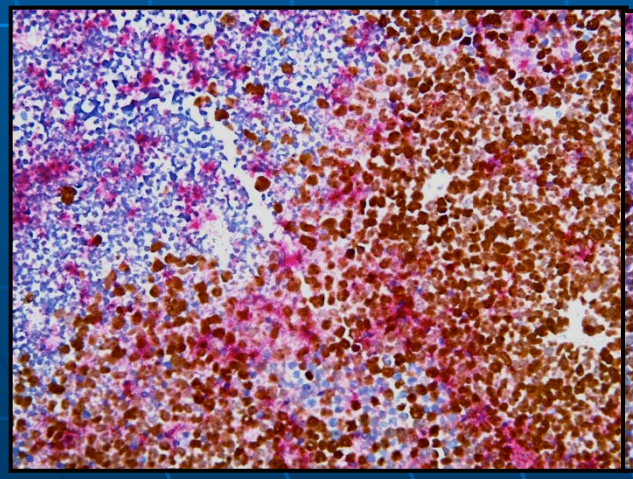


MOLT3+Lac d40 (A)



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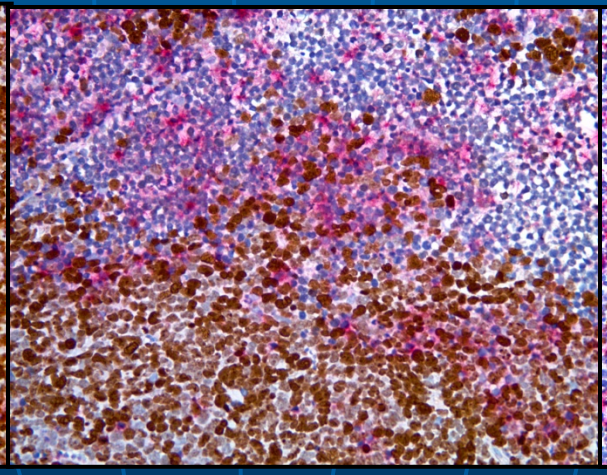
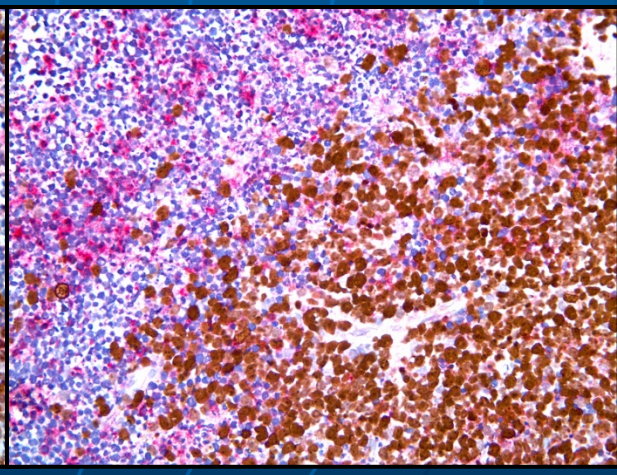


MOLT3+Lac d40 (C)



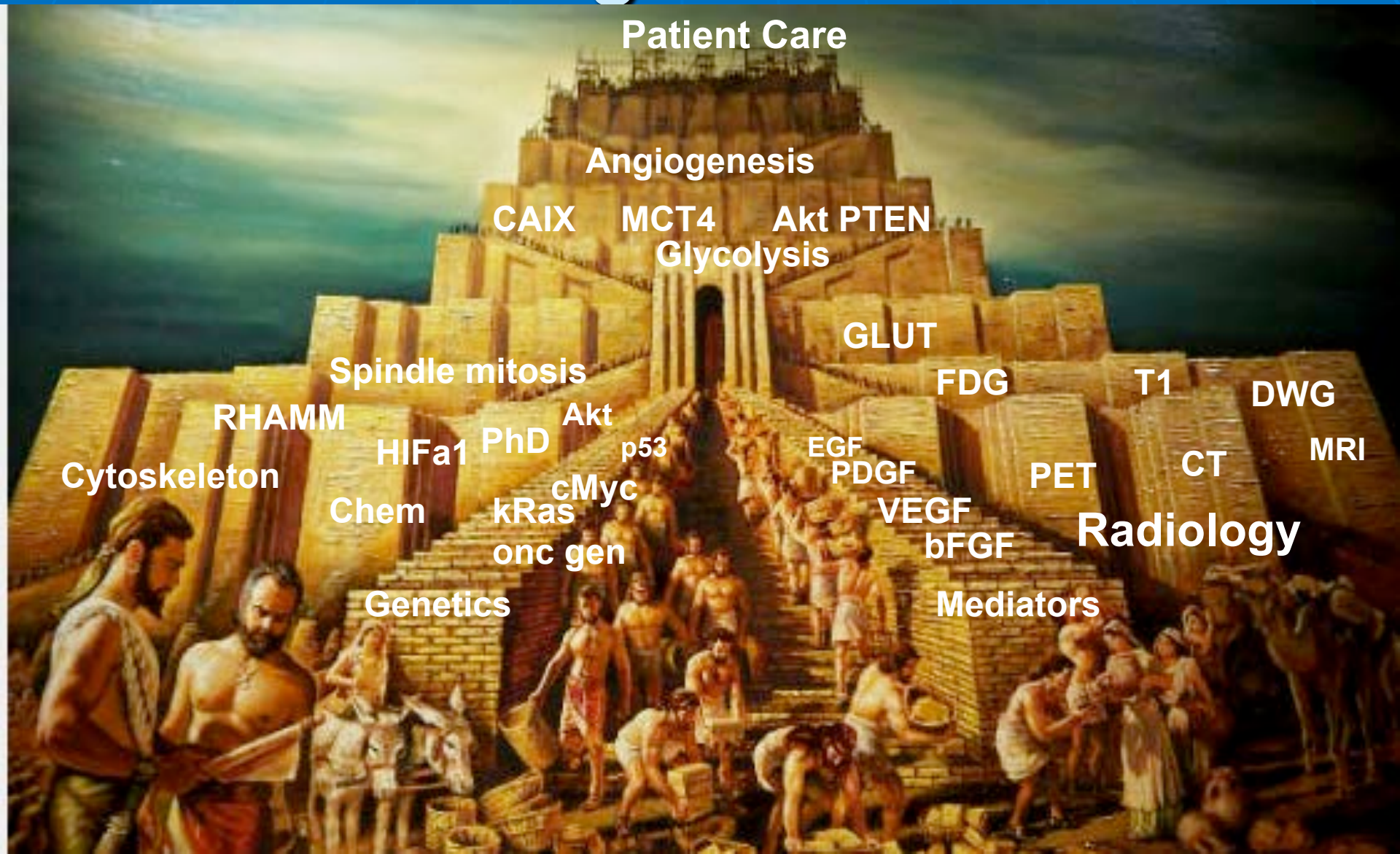
MOLT3+Lac d40 (D)

THE END

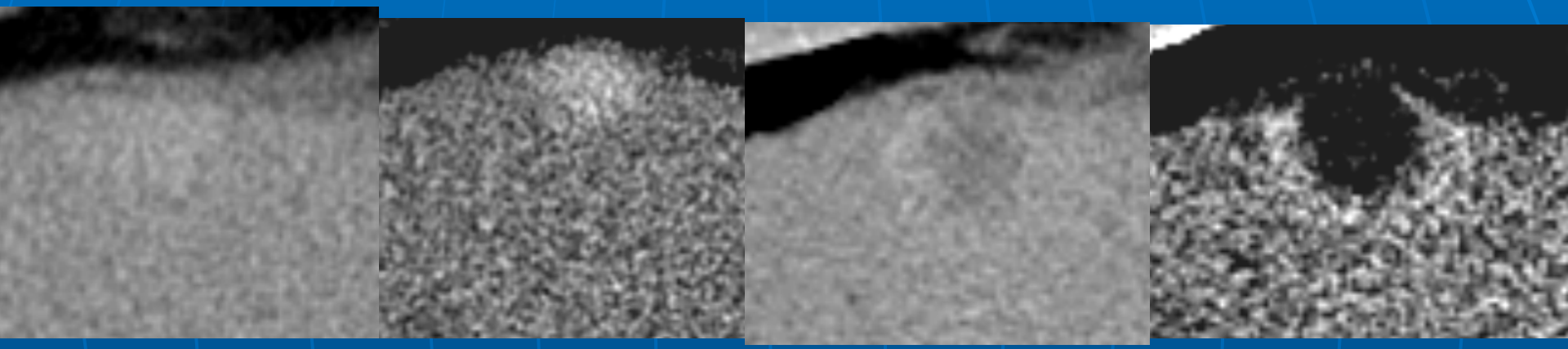
Analysis of Gibrone data: N. Tien PhD, Dean Engineering. V.Chankung,PhD

"Using a statistical t-test at 95% confidence level, it confirms that arterial vascularization occurs approximately 1 day after the exponential growth begins. ..In conclusion, based on the data in Grimbrone et, al (1972), the tumors appear to BEGIN its rapid exponential growth about 1 day prior to arterial vascularization. If this is true, then there must be a mechanism other than arterial vascularization that serves as a primary factor to stimulate the abrupt growth."

Too much data, alphabet jargon: Little integration of data



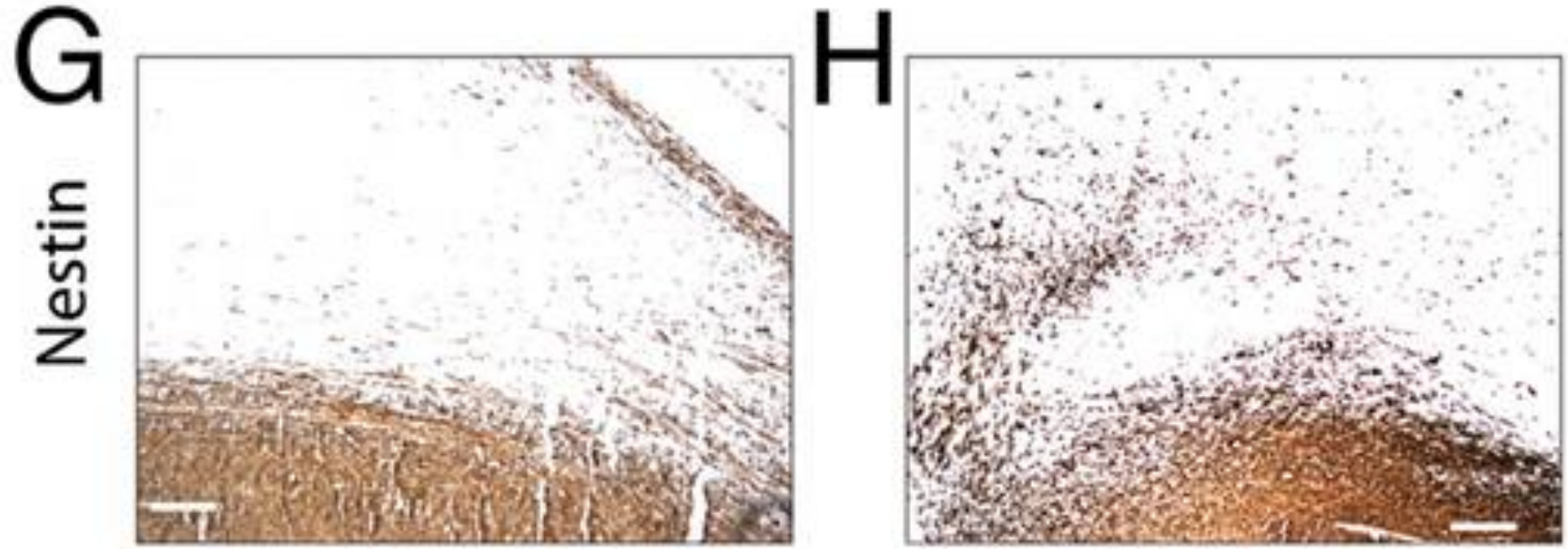
“Traditional Mantra” is tumors become hypoxic when they grow larger than 2mm



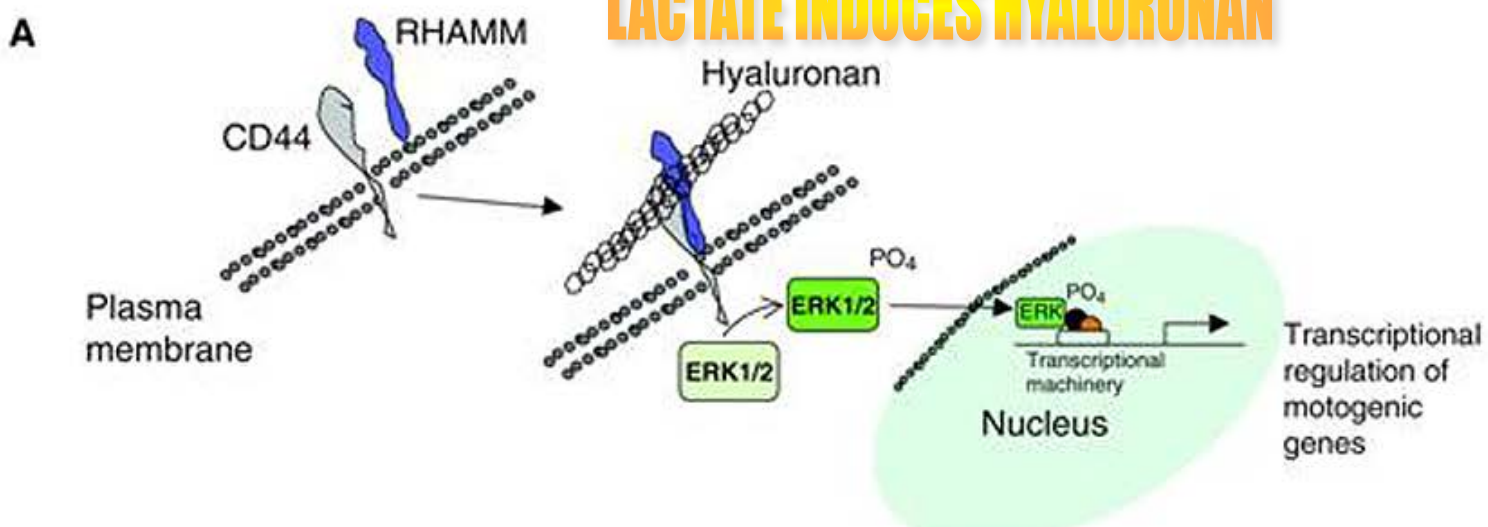
Cancer uses glycolysis in normoxia or hypoxia, because of pro cancer advantages

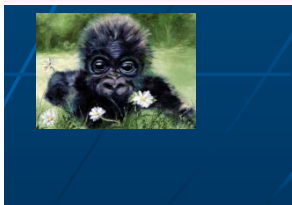
Anti-VEGF treatment reduces blood supply and increases tumor cell invasion in glioblastoma

Olivier Keunen[a,b], Mikael Johansson[a,c], Anaïs Oudin[a], Morgane Sanzey[a], Siti A. Abdul Rahim[a], Fred Fack[a], Frits Thorsen[b], Torfinn Taxt[b,d], Michal Bartos[e], Radovan Jirik[e,f], Hrvoje Miletic[b,g], Jian Wang[b], Daniel Stieb



LACTATE INDUCES HYALURONAN





Is Traditional Vasculogenesis the "law" or other principles needed?

Many inconsistencies of the traditional theory exist in both RX and imaging

Scandinavian Contributions

- Vascular endothelial growth factor C induces angiogenesis in vivo Cao, Yihai, Alitalo,K, PNAS November 24, 1998 vol. 95 no. 24 14389-14394
- Anna Pettersson et al, Heterogeneity of the Angiogenic Response Induced in Different Normal Adult Tissues by Vascular Permeability Factor/ Vascular Endothelial Growth Factor Lab Invest 2000, 80:99−115
- Lynn K. Chang et al, Arja Kaipainen, Dose-dependent response of FGF-2 for lymphangiogenesis PNAS p 11658−11663 August 10, 2004 vol. 101 no. 32