Microenvironmental influence on angiogenesis and tumor cell survival

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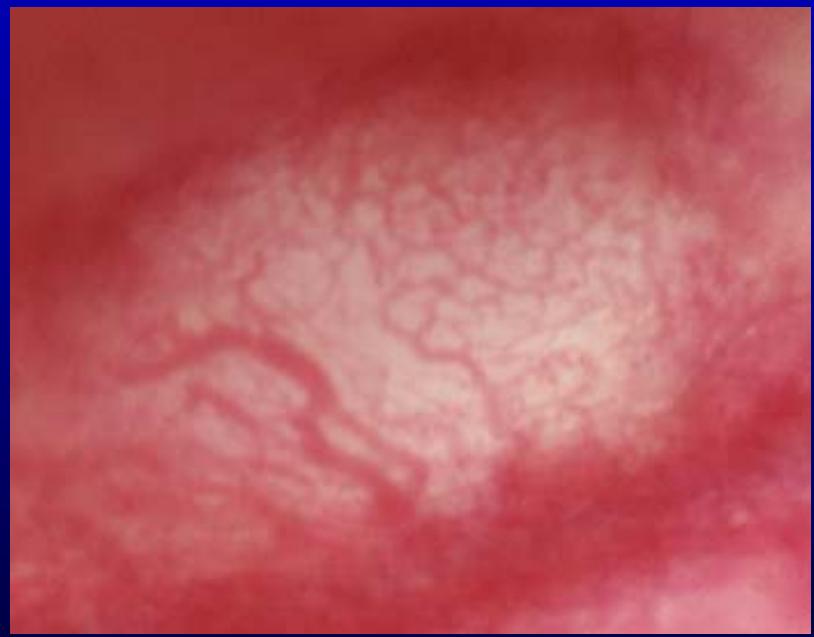
Lecture Outline

- Angiogenic Switch
- Tumor-host cell interactions
  - Endothelial cell
  - Macrophage
- Effects of Rx and microenvironment on angiogenesis



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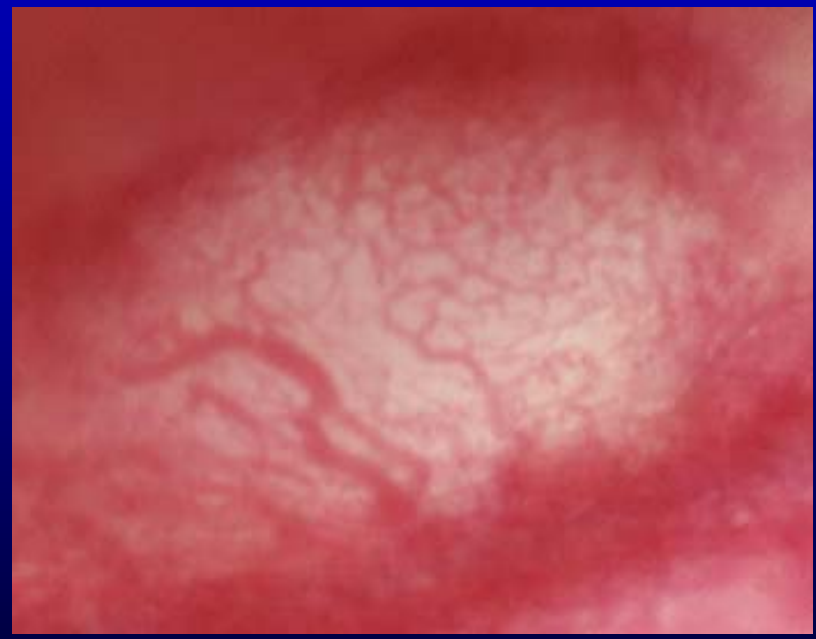
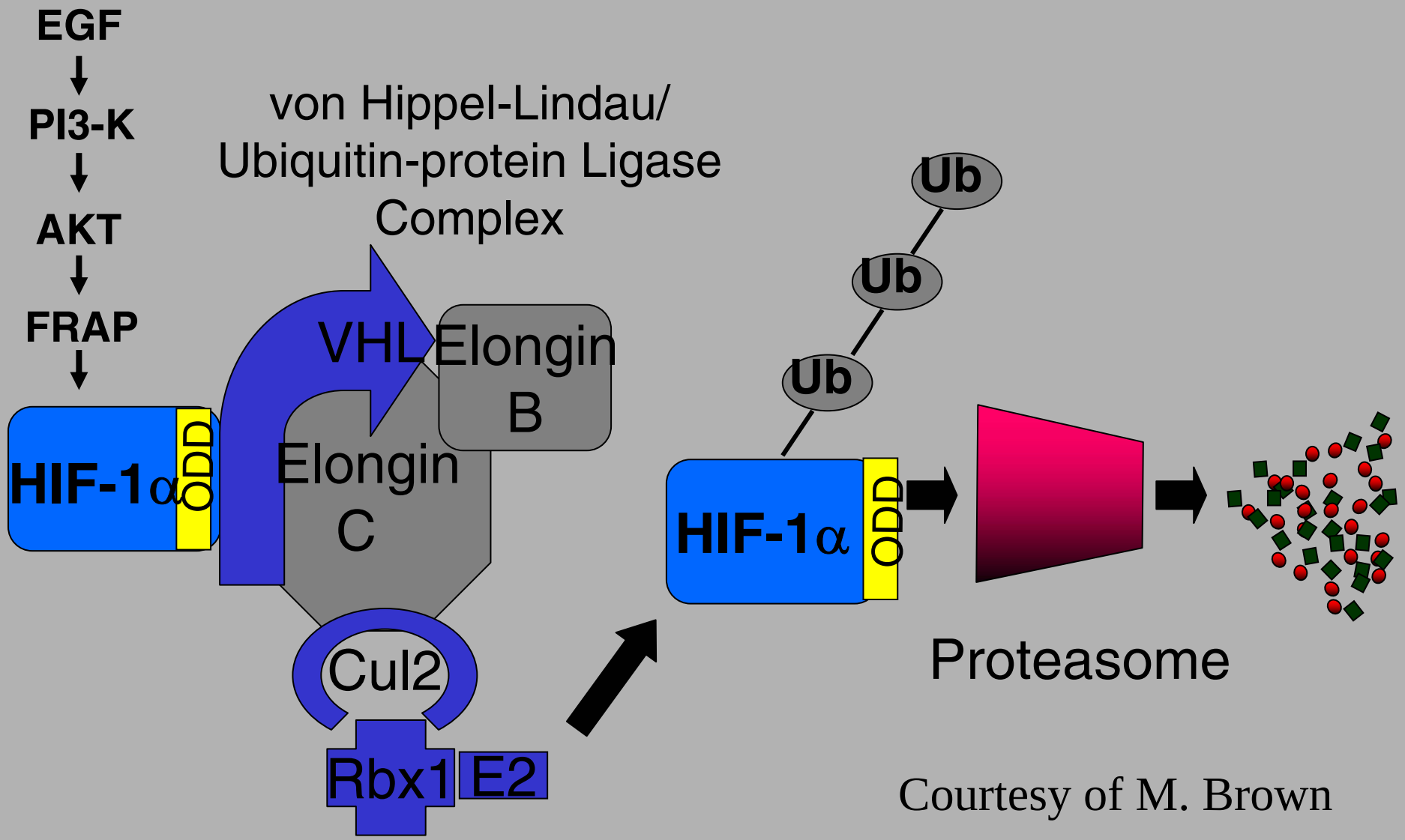


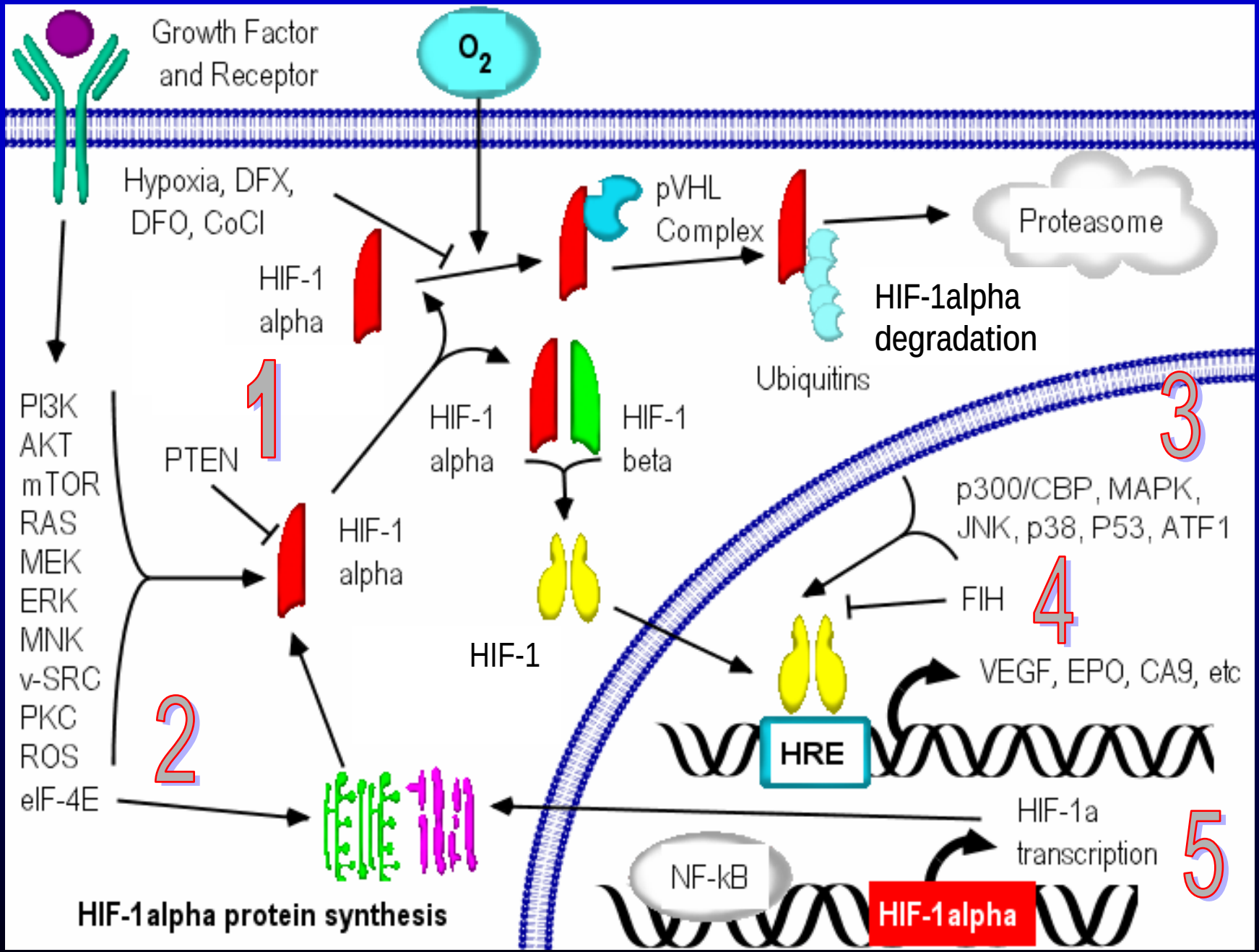
What causes the angiogenic switch in tumors?

- Hypoxia
- Oncogene mutations or upregulation
  - Ras, myc, epidermal growth factor, Her2 upregulation
    - Kerbel et al., Mol Med 4:286, 1998
- Loss of suppressor gene function
  - PTEN via PI3K
    - Brader and Eccles, Tumori 90:2, 2004
  - P53
    - Bardos and Ashkroft, Bioessays 26:262, 2004

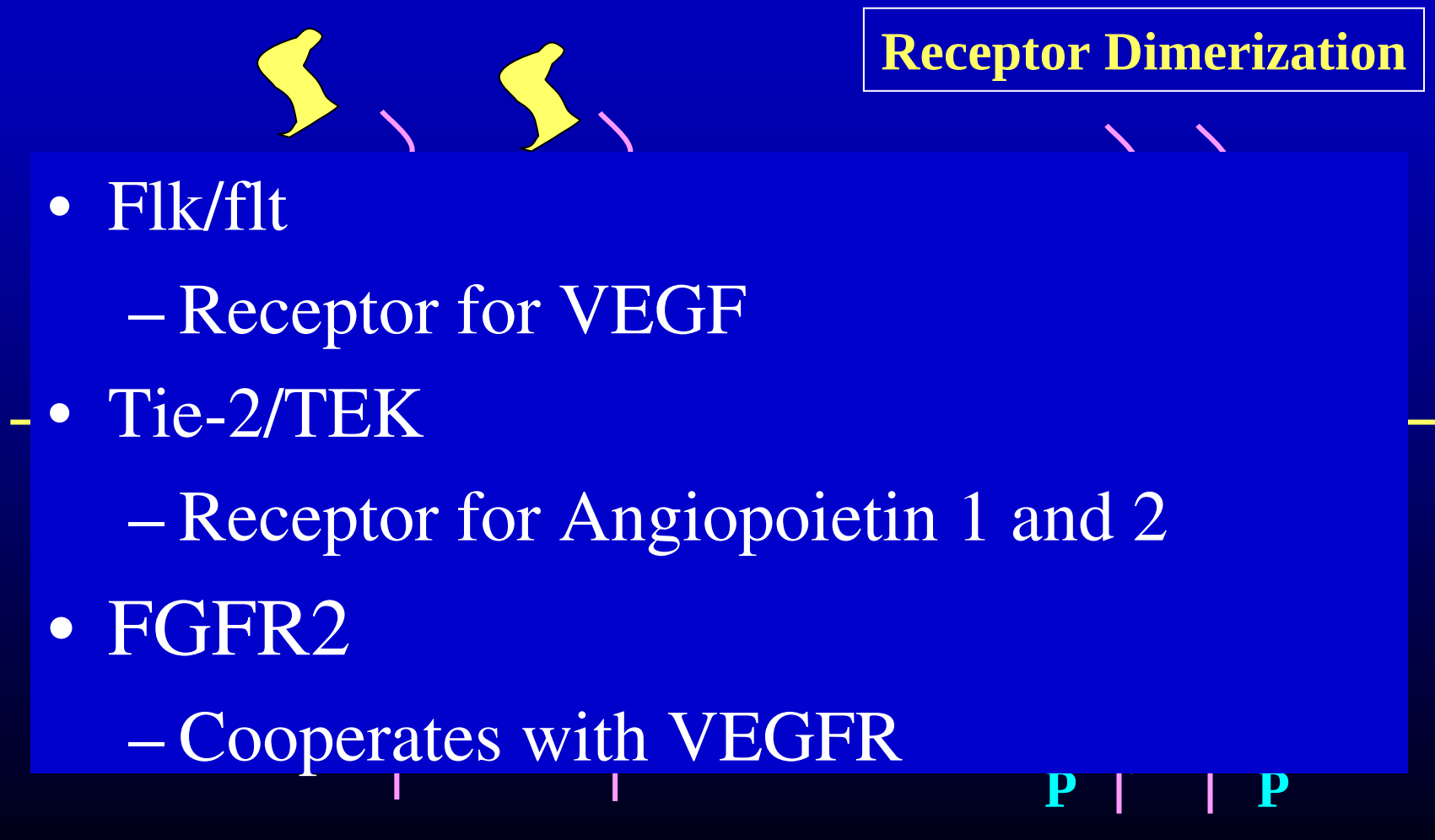
HIF-1α Protein Stability is Regulated by O_2 and by VHL Tumor Suppressor



Courtesy of M. Brown


Growth Factor
and Receptor
O_2
Hypoxia, DFX,
DFO, CoCl
HIF-1
alpha
pVHL
Complex
Proteasome
HIF-1alpha
degradation
Ubiquitins
PI3K
AKT
mTOR
RAS
MEK
ERK
MNK
v-SRC
PKC
ROS
eIF-4E
1
PTEN
HIF-1
alpha
HIF-1
alpha
HIF-1
beta
3
p300/CBP, MAPK,
JNK, p38, P53, ATF1
FIH
4
HIF-1
VEGF, EPO, CA9, etc
HRE
2
HIF-1a
transcription
5
NF-kB
HIF-1alpha
HIF-1alpha protein synthesis

Key tyrosine kinase receptors involved in angiogenesis regulation



- Flk/flt
  - Receptor for VEGF
- Tie-2/TEK
  - Receptor for Angiopoietin 1 and 2
- FGFR2
  - Cooperates with VEGFR

Functions of VEGF and Tie2 Receptors

- VEGF binding
  - Hyperpermeability
  - Endothelial cell
    - Proliferation, migration, survival
- Ang 1 to Tie2
  - Maintain vessel maturity
- Ang 2 to Tie2
  - Endothelial cell de-differentiation
  - Loss of pericyte, SMC association with vessels
  - Increased receptivity to VEGF

Ang-1 contributes to vascular maturity

- Pericytes, smooth muscle cells associate with endothelial cell
  - Arrest of endothelial proliferation
  - Endothelial cell survival
  - Vasoreactivity

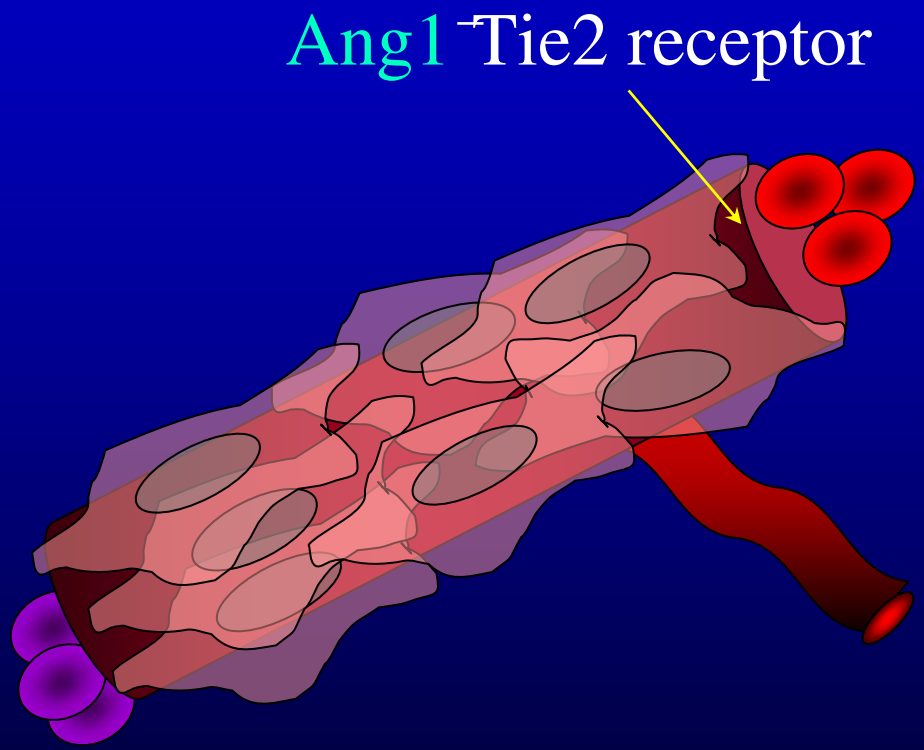
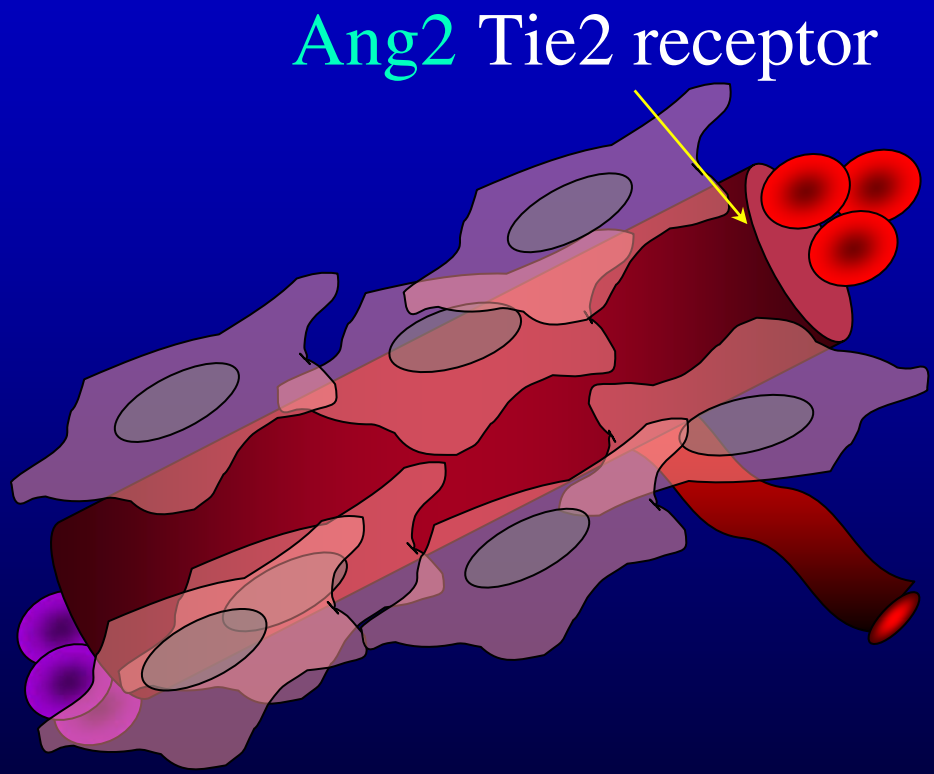


Figure courtesy of M. Neeman

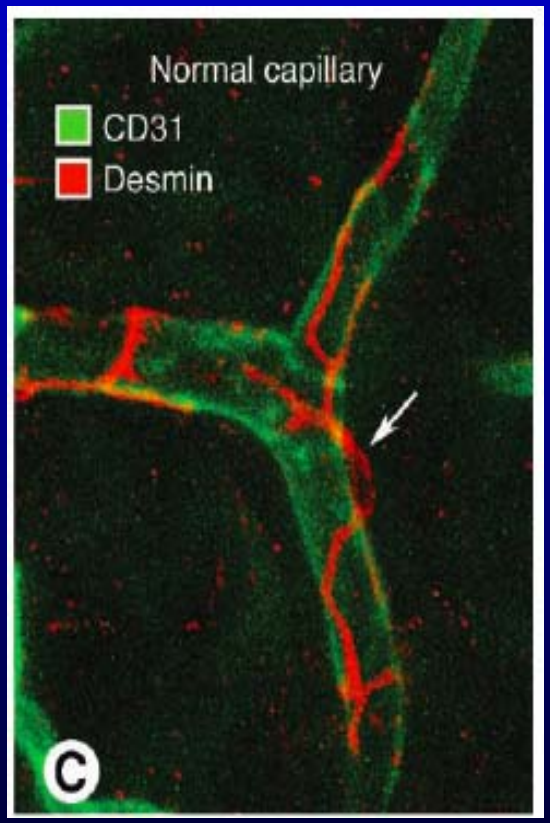
Ang 2 contributing to vessel immaturity

- Pericytes, smooth muscle cells disassociate with endothelial cell
  - Facilitates reactivity to VEGF
  - Increases permeability
  - Loss of vasoreactivity

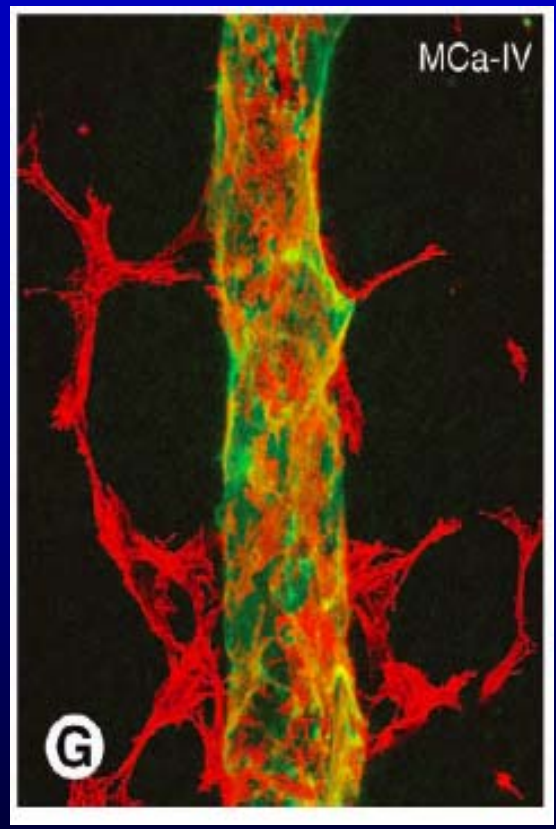


VEGF now becomes survival factor

Pericyte Structure: Normal vs. Tumor Microvessels



Ang / Tie2

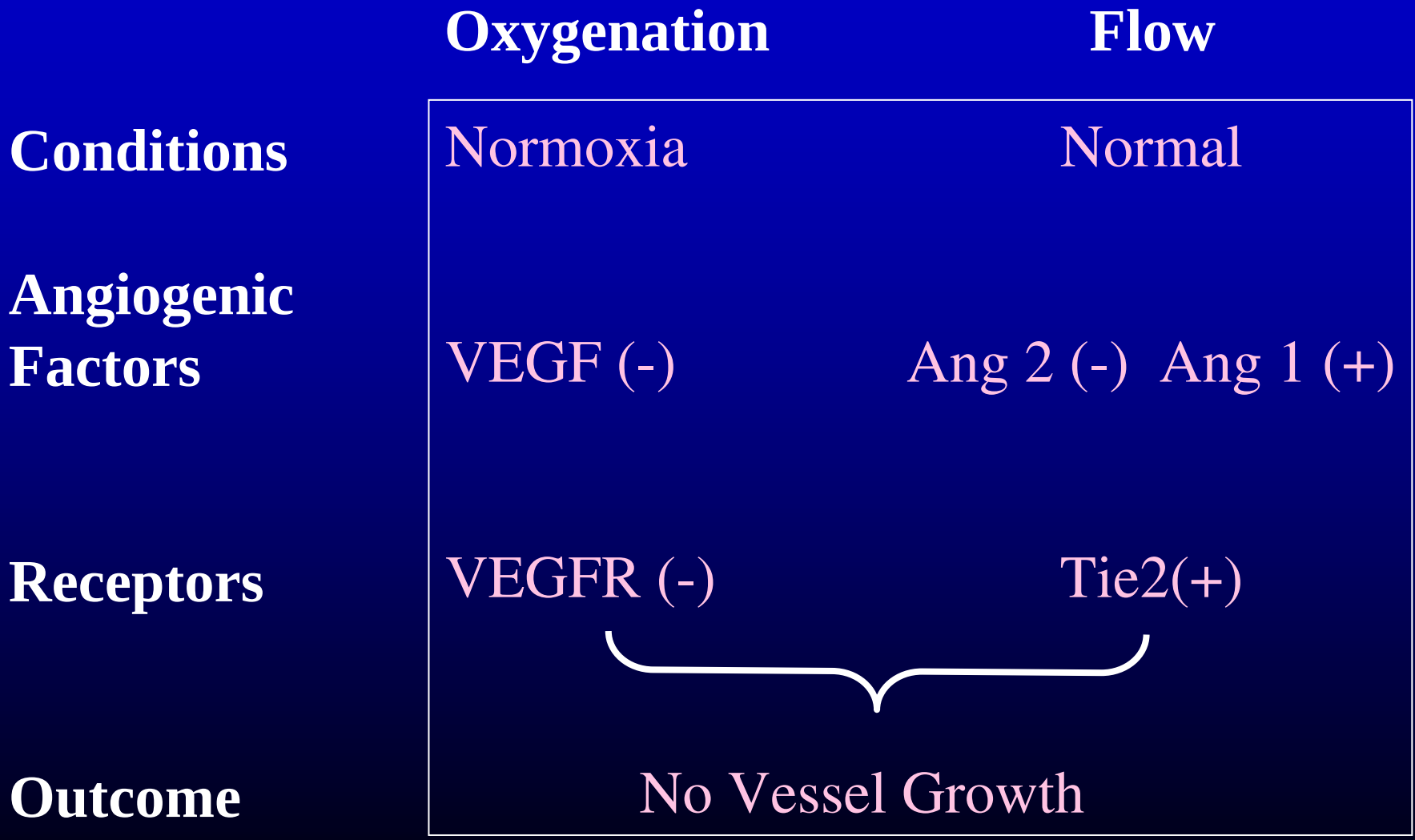


Ang 1(+)

Ang 2(+)

Morikawa et al., Am J Path 160: 985, 2002

VEGF vs. Tie 2 signaling for vessel growth / maturation



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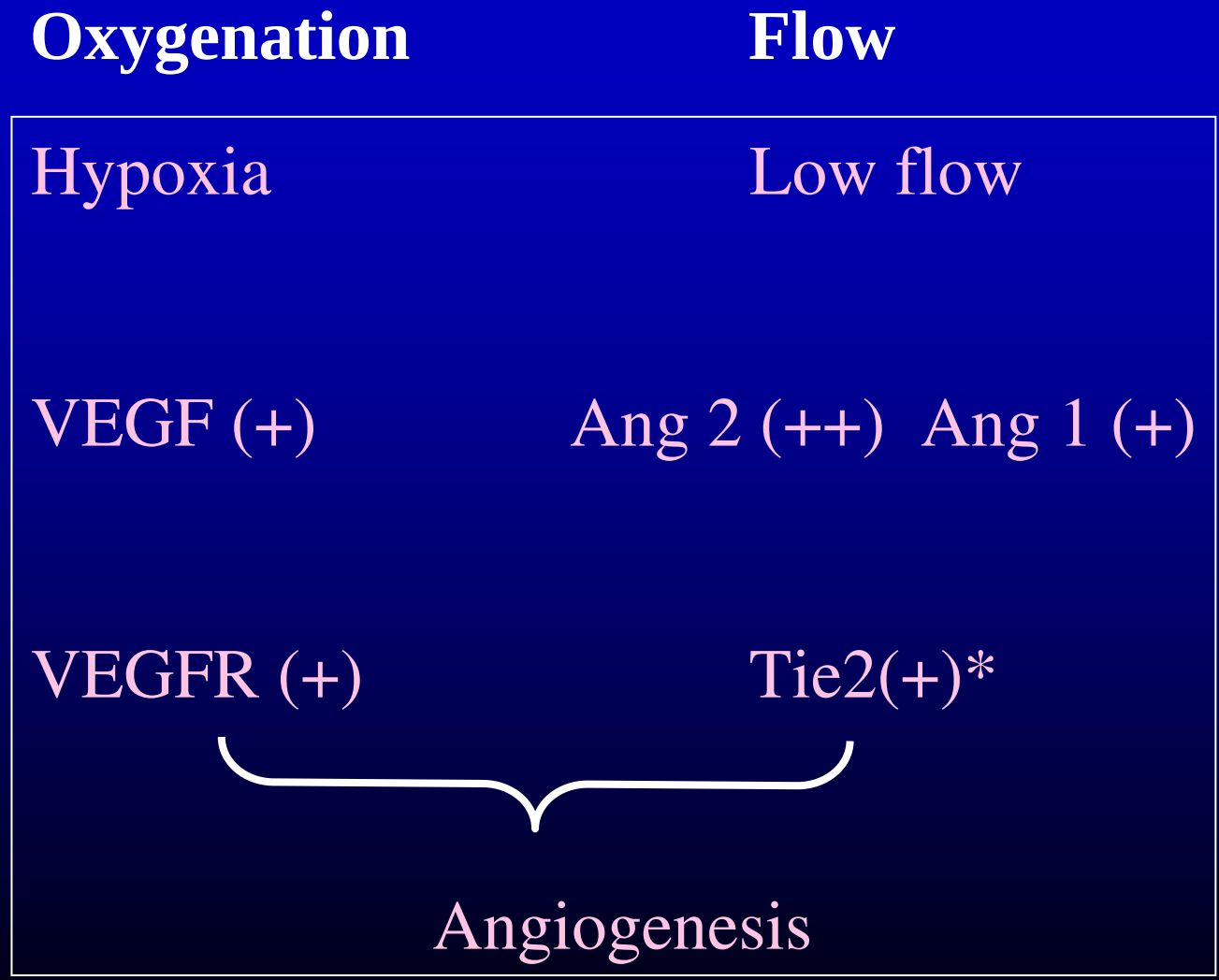
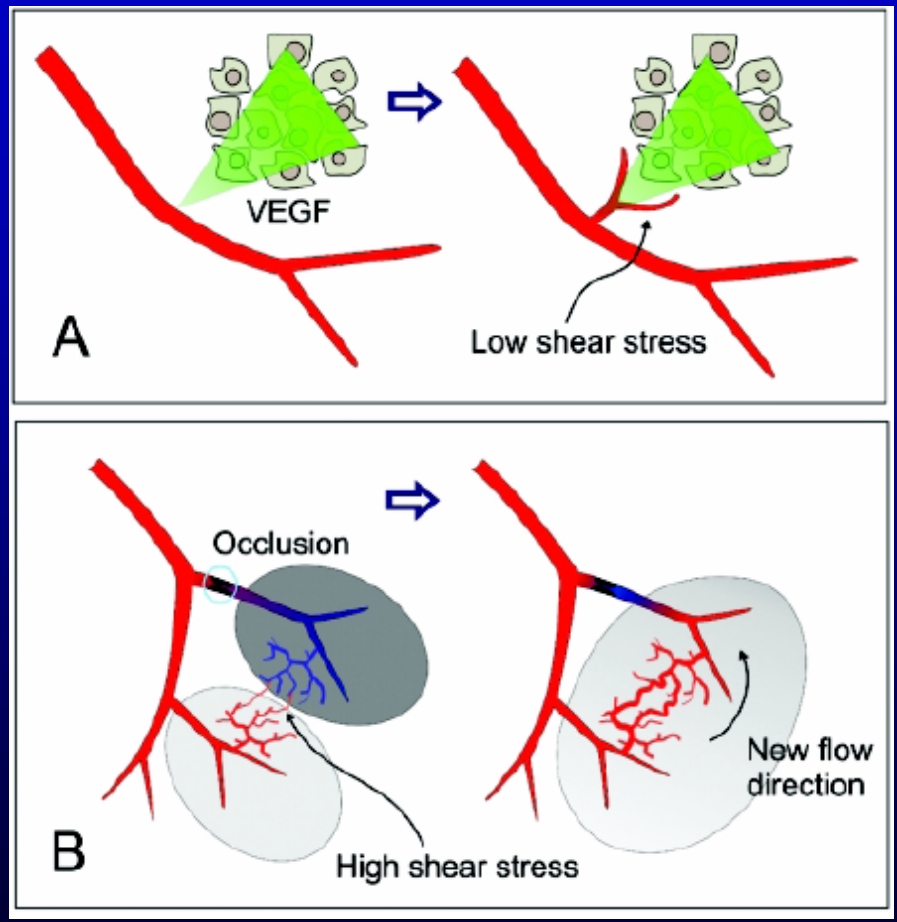
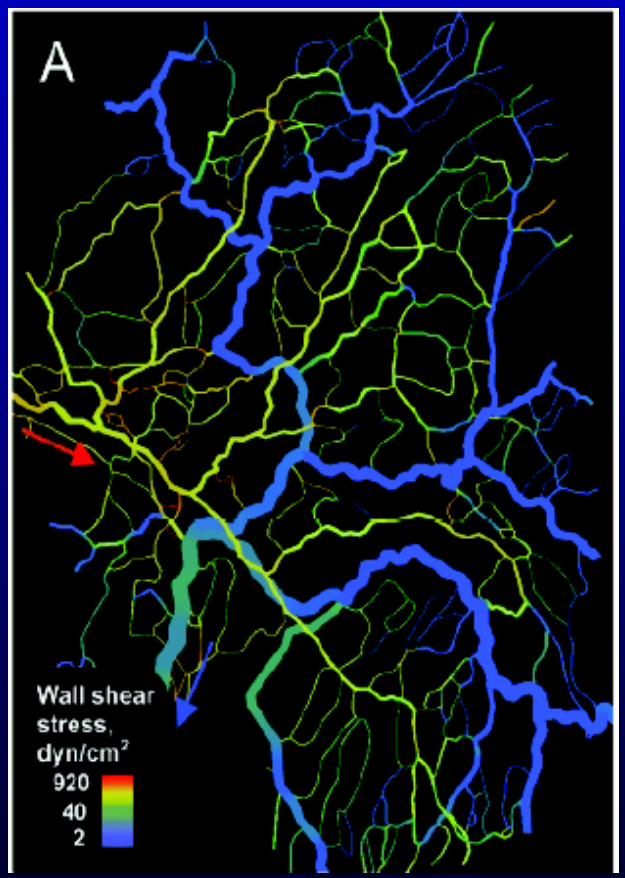


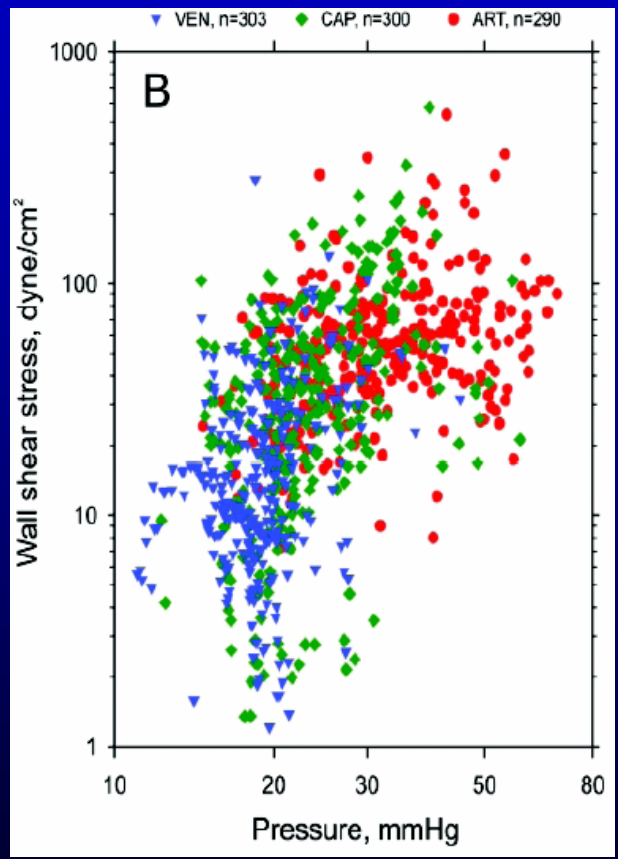
Vascular adaptation in response to changes in shear stresss



From: Zakrzewicz, Secomb, Pries, News Phys Sci, 2002

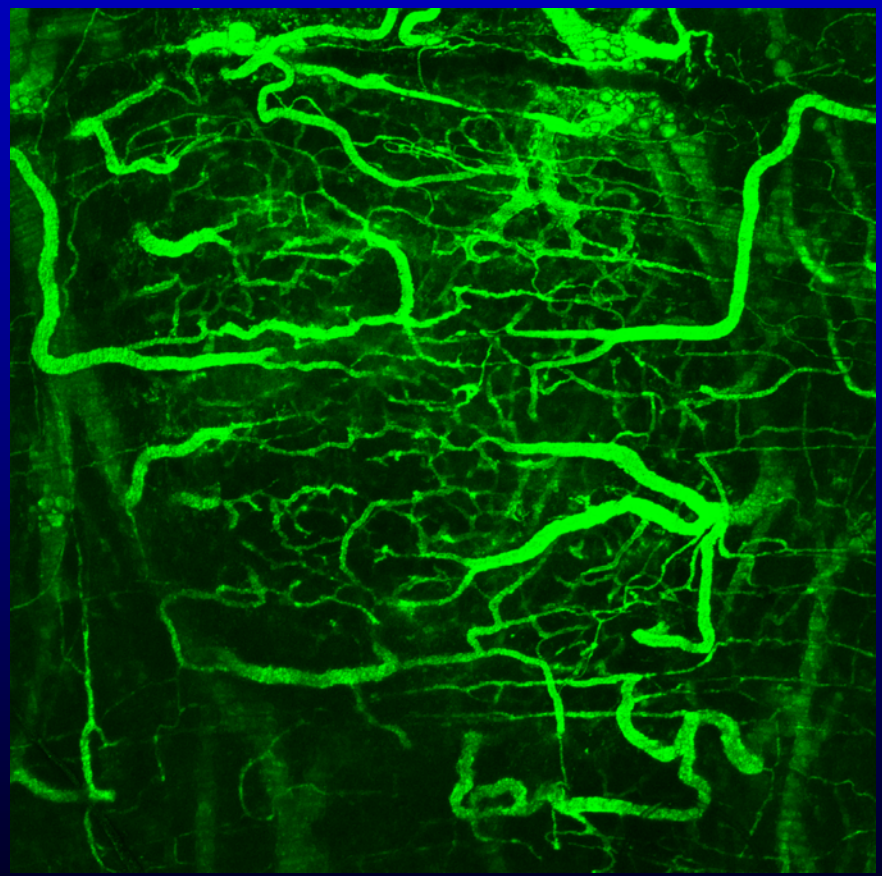
Variation in shear stress in mesenteric vascular network

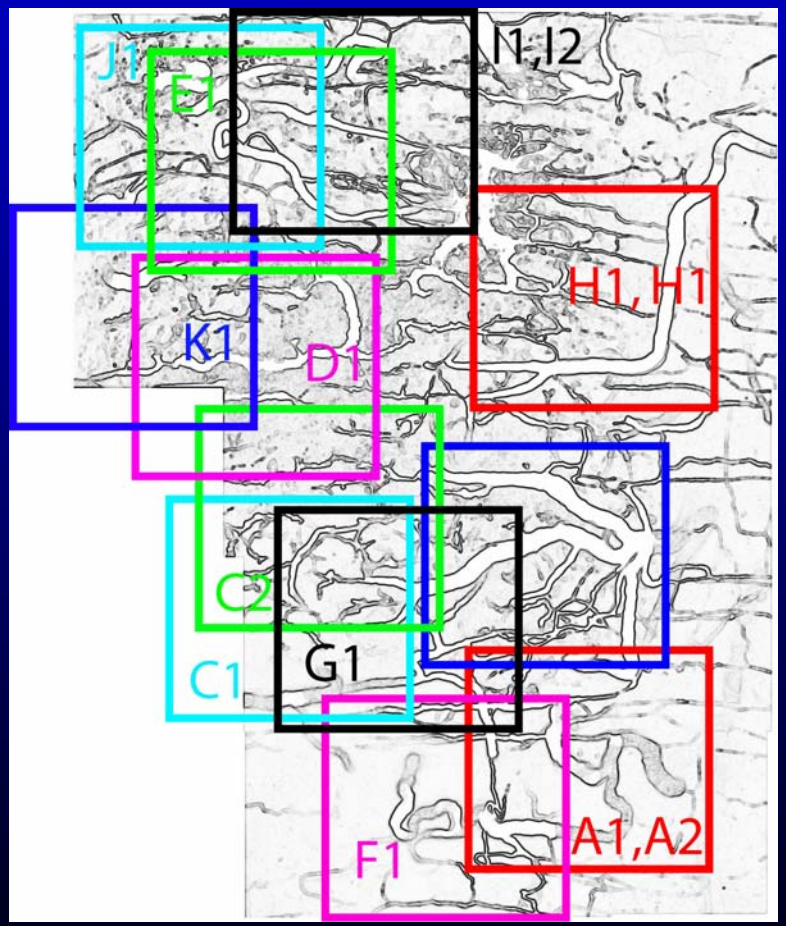




From: Zakrzewicz, Secomb, Pries, News Phys Sci, 2002

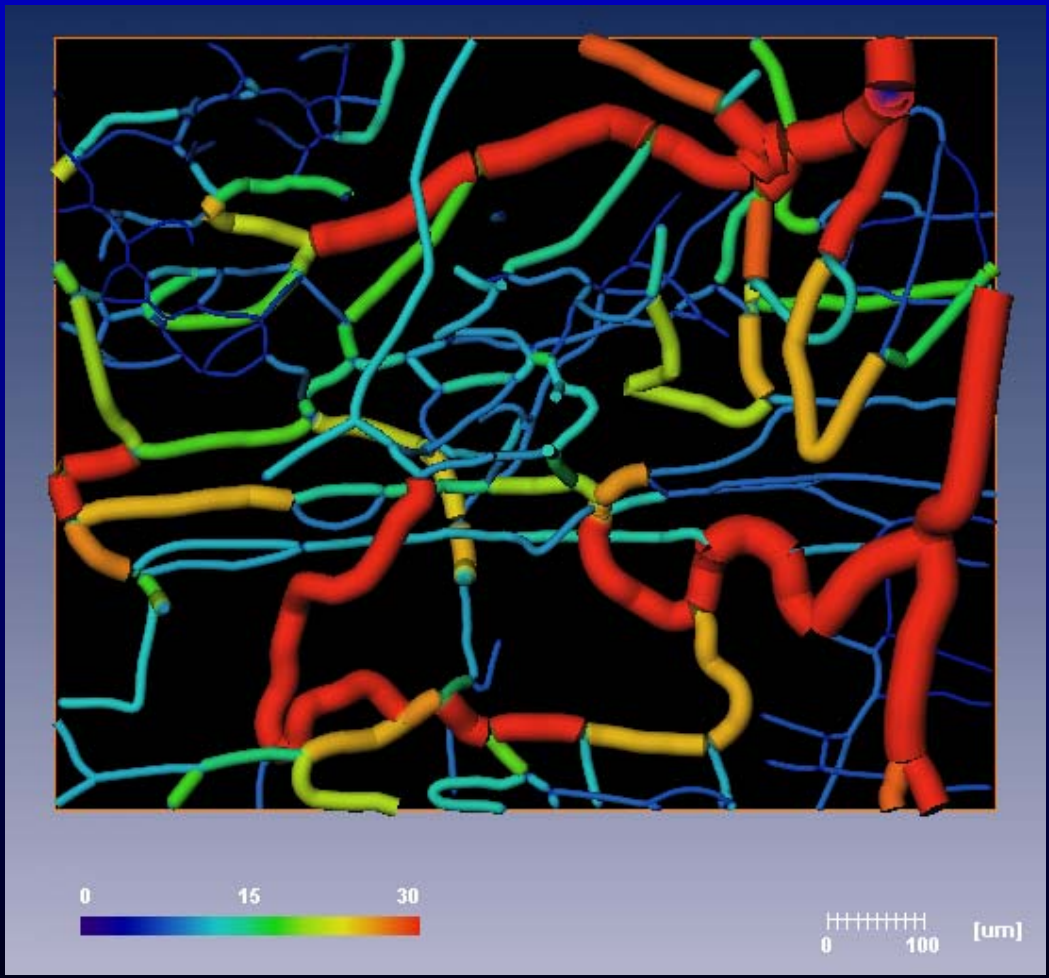
Studying vascular adaptation in tumor microvasculature





Dreher, Dewhirst, unpublished

Segmented model for vascular adaptation simulations



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  - Macrophage, fibroblast
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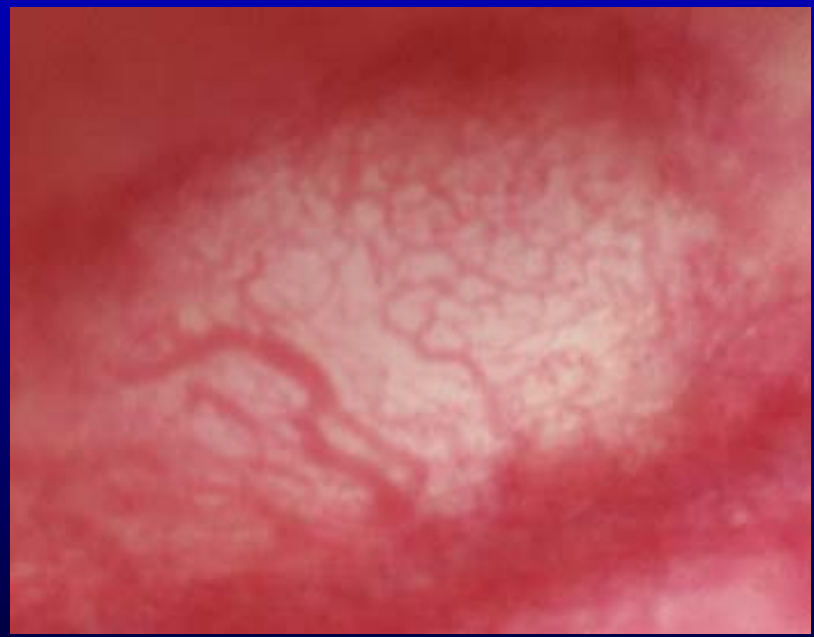
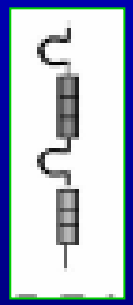
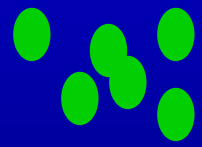


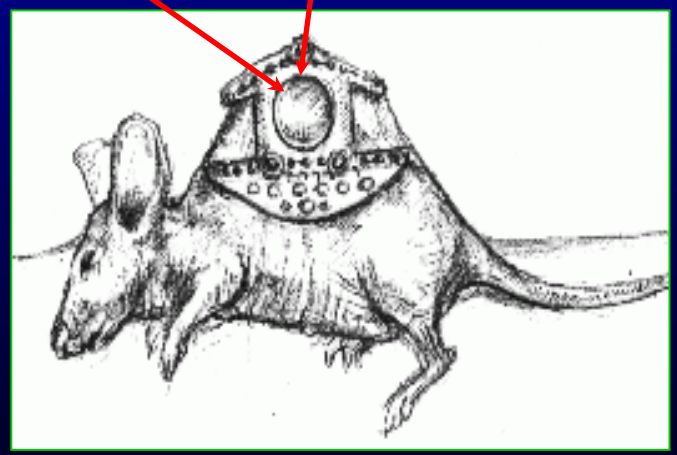
Truncated receptor proteins added to window tissue at time of surgery and tumor cell transplant

Receptor body



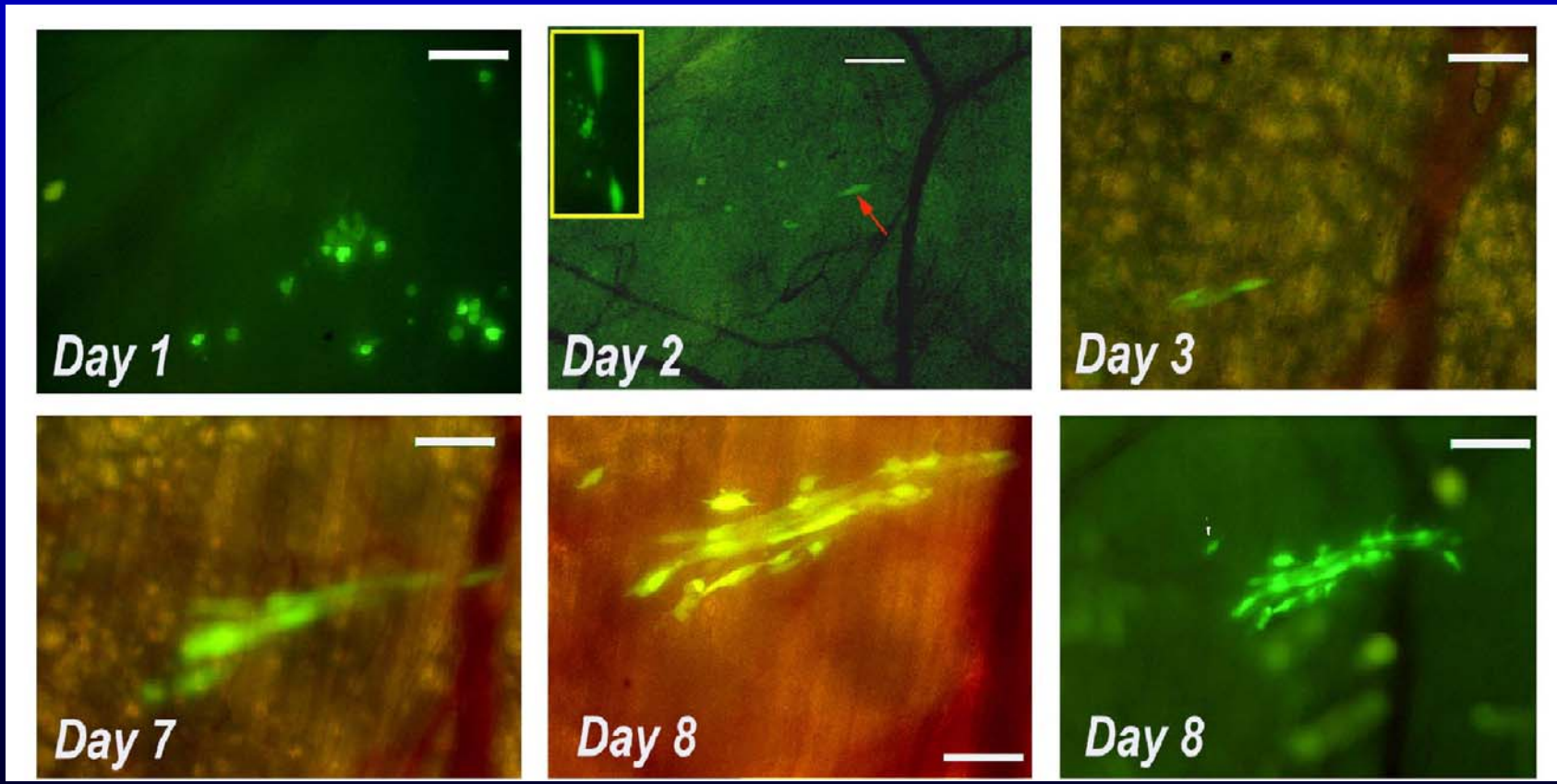


GFP_{cmv} reporter cells



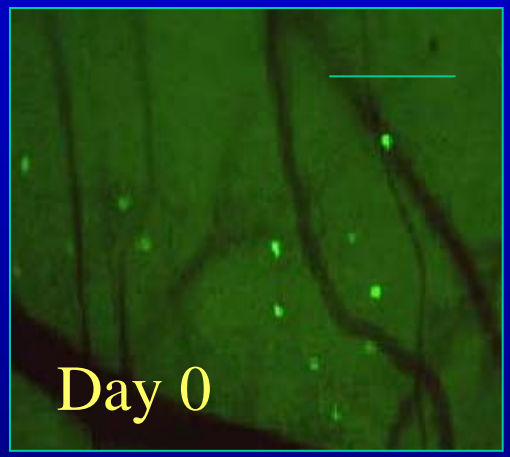
Serially monitor daily post transplant

Chemotactic behavior of 4T1 tumor cells toward host vessels

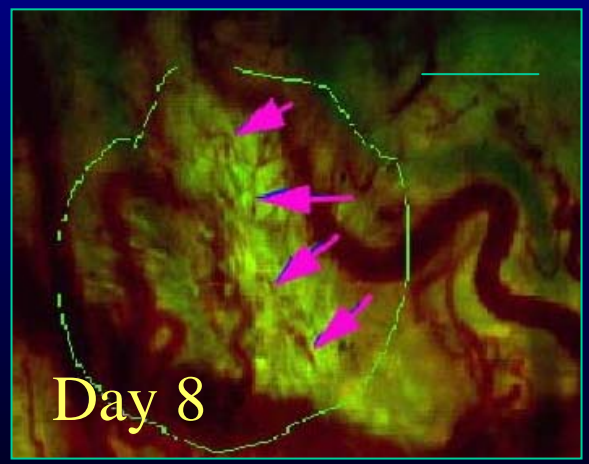


CY Li et al, JNCI, 2000

Pre-angiogenic tumor and vessel behavior



Proliferation / Chemotaxis

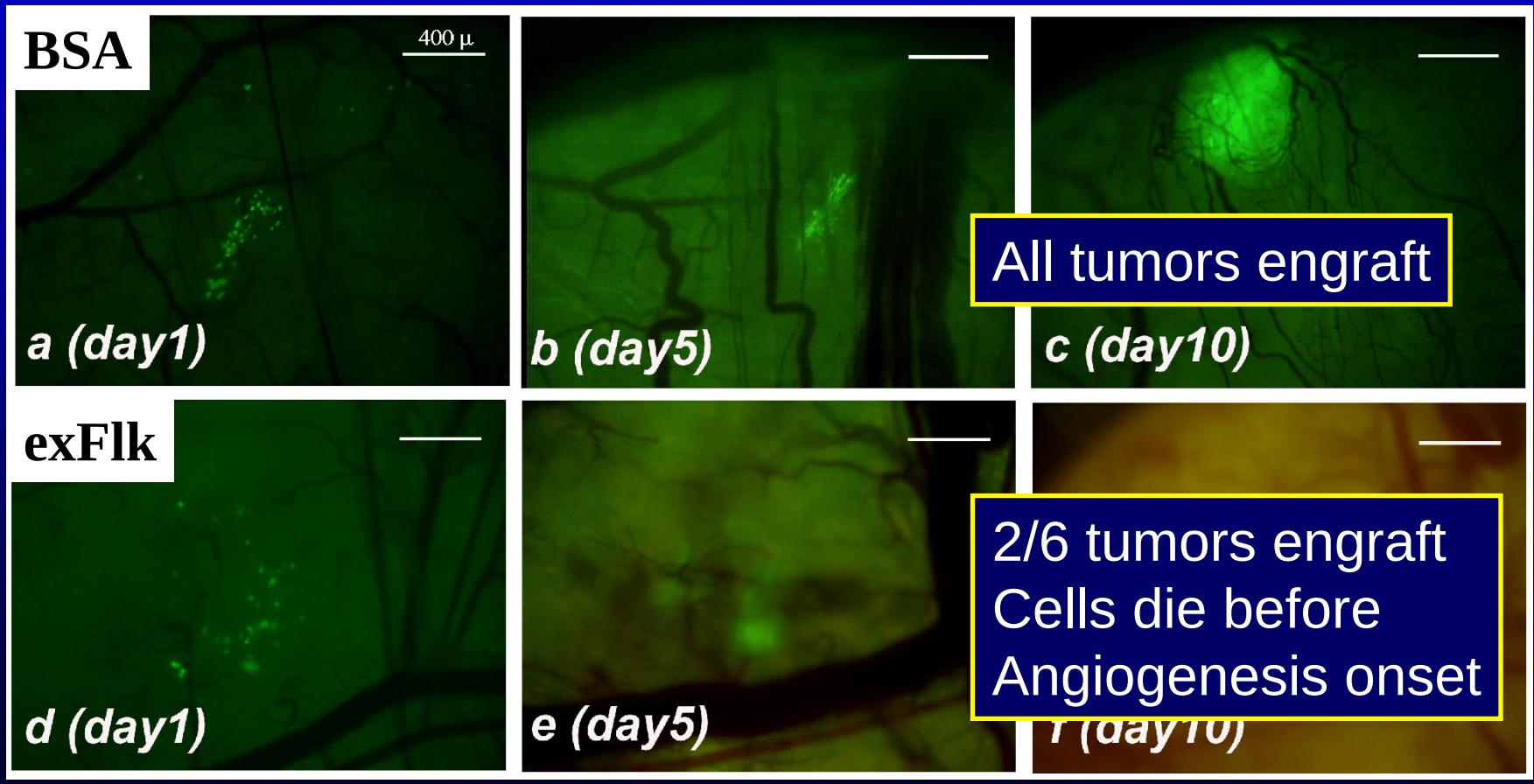


Angiogenesis

Bar = 300μm

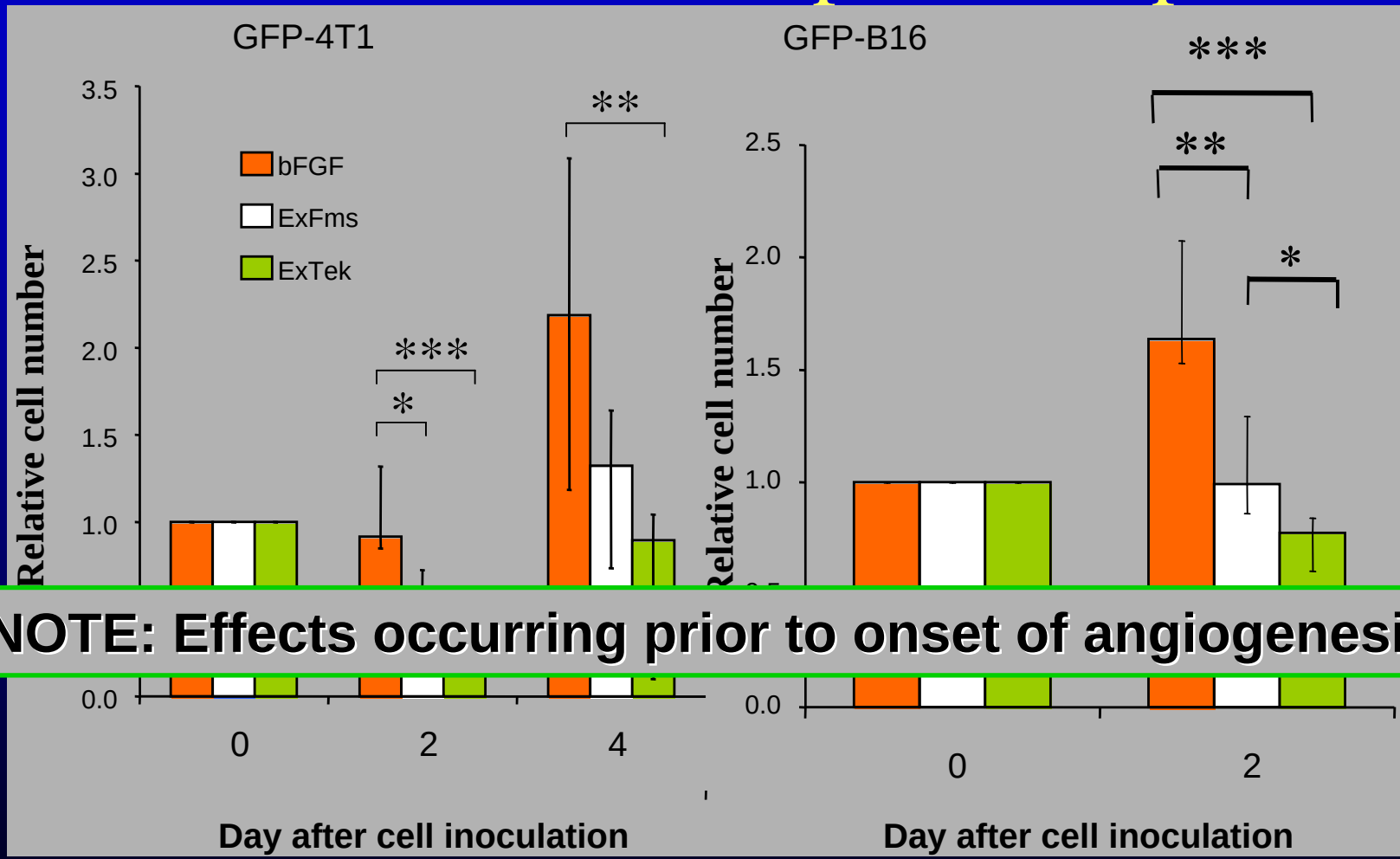
C.Y. Li et al., JNCI, 2000

exFlk (VEGF blockade) inhibits proliferation/migration toward host vasculature



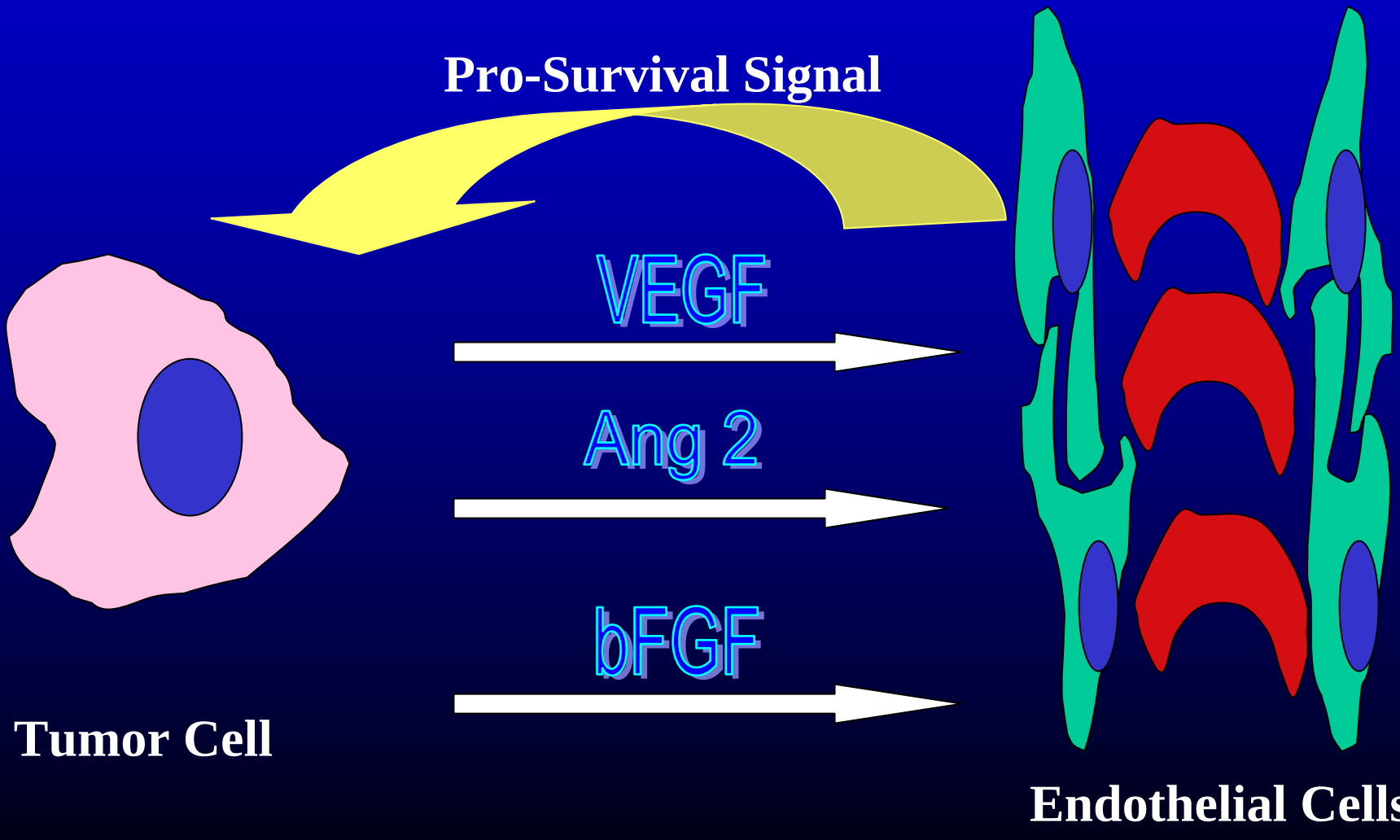
C.Y. Li et al., JNCI, 2000

Effects of Tie2 blockade vs bFGF on tumor cell survival post transplant

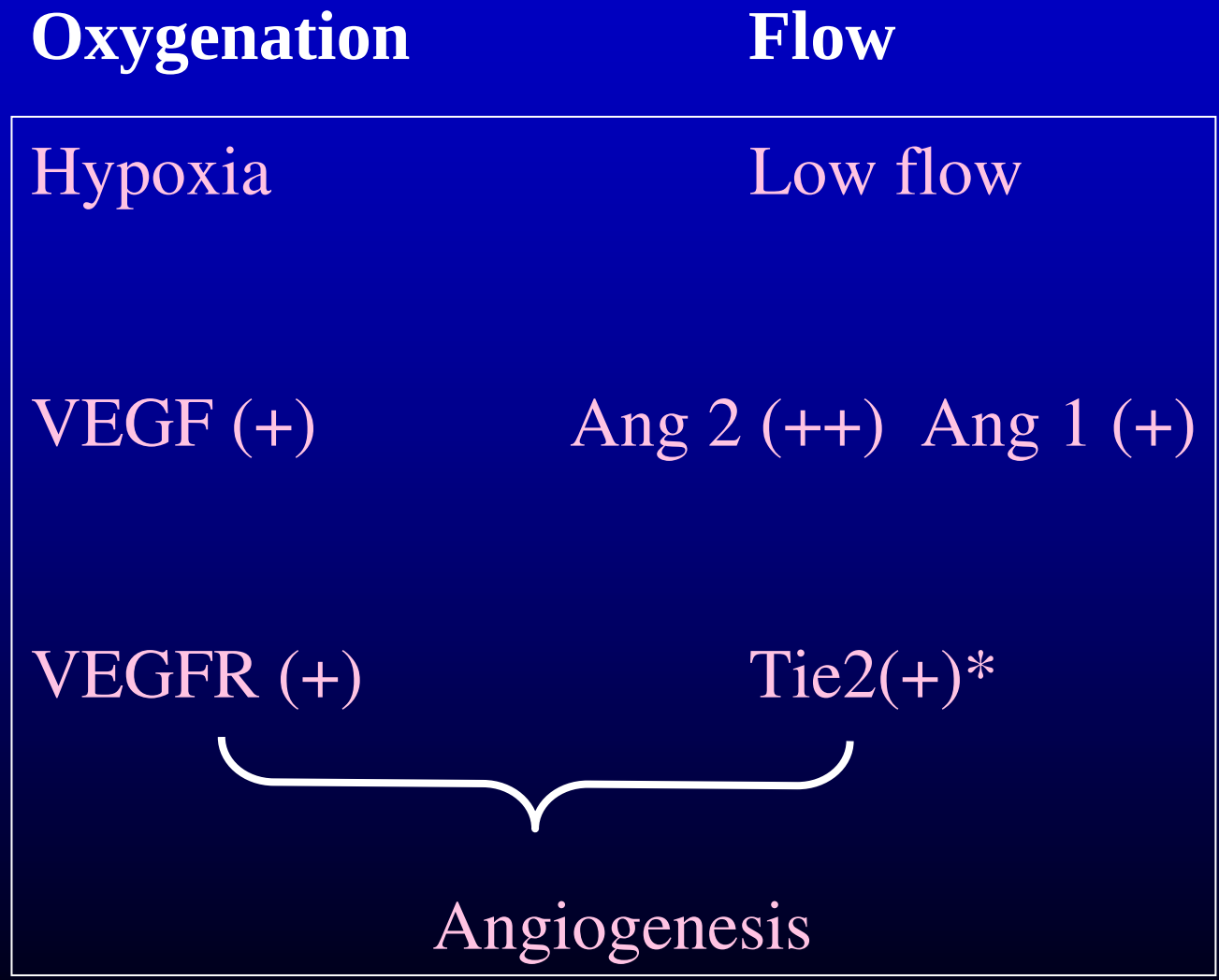


From: Shan et al, FASEB J 18:326, 2004

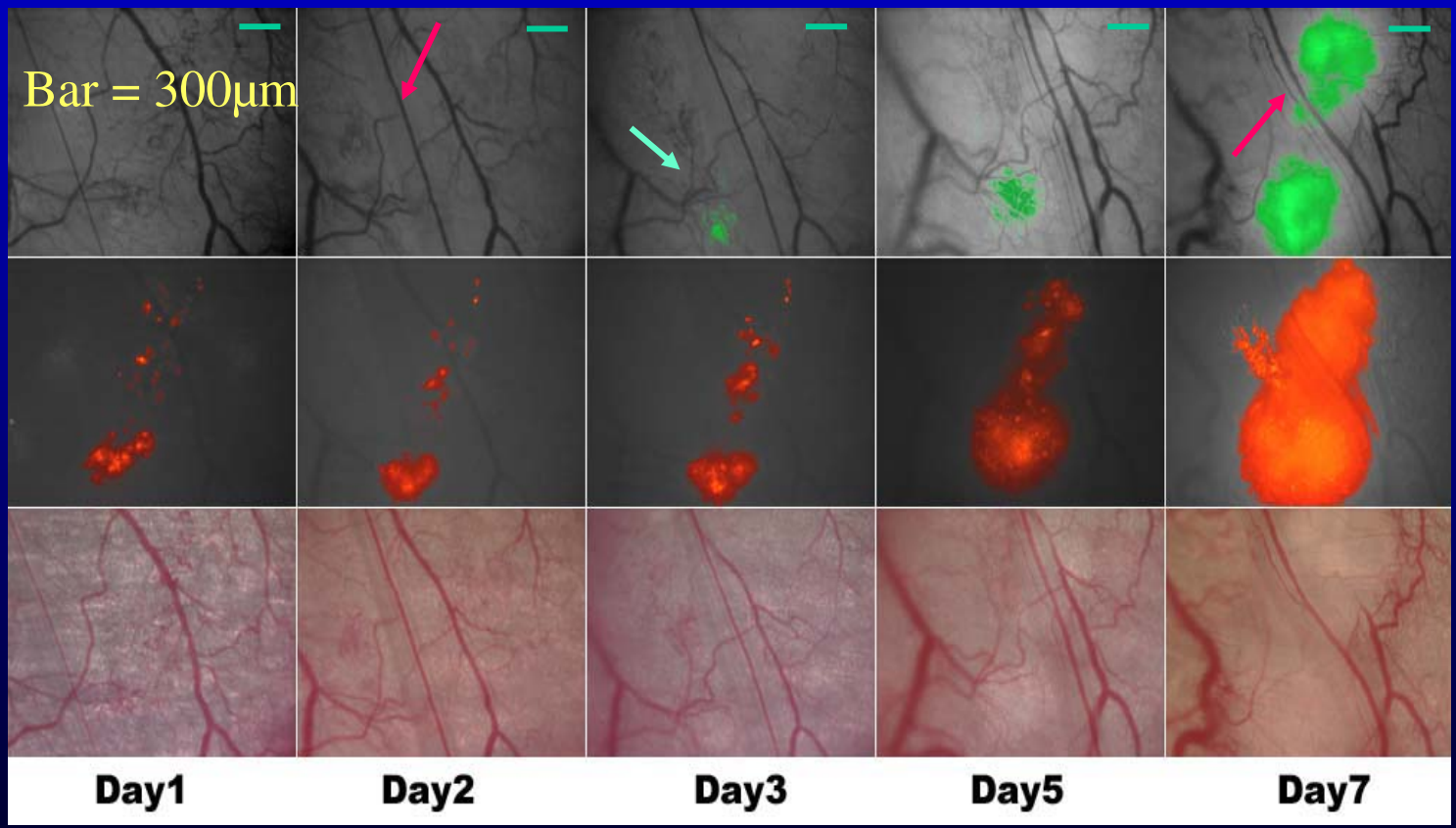
Working model for paracrine survival signaling



VEGF vs. Tie 2 signaling for vessel growth / maturation



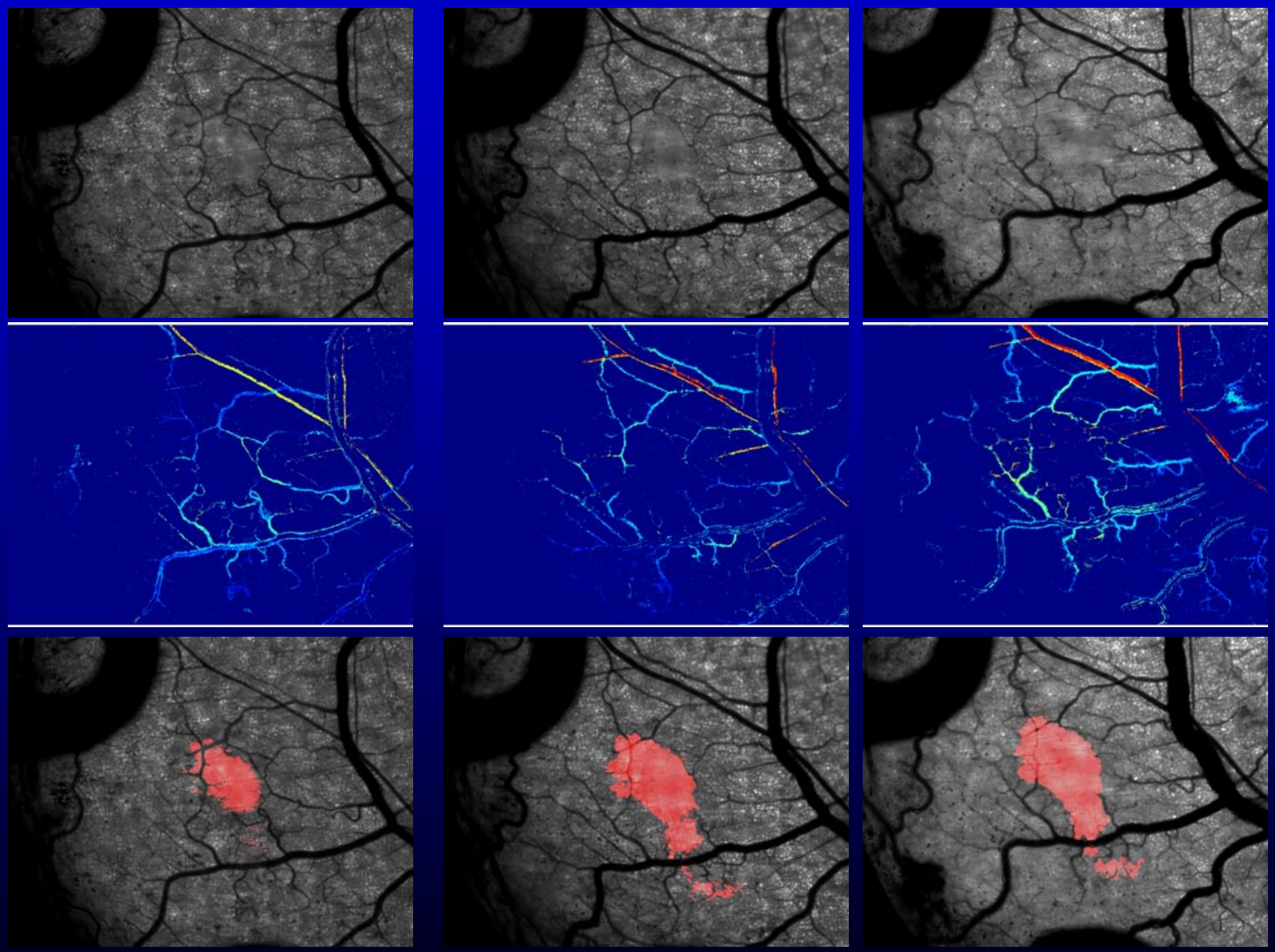
At what point during tumor growth does hypoxia influence angiogenesis?



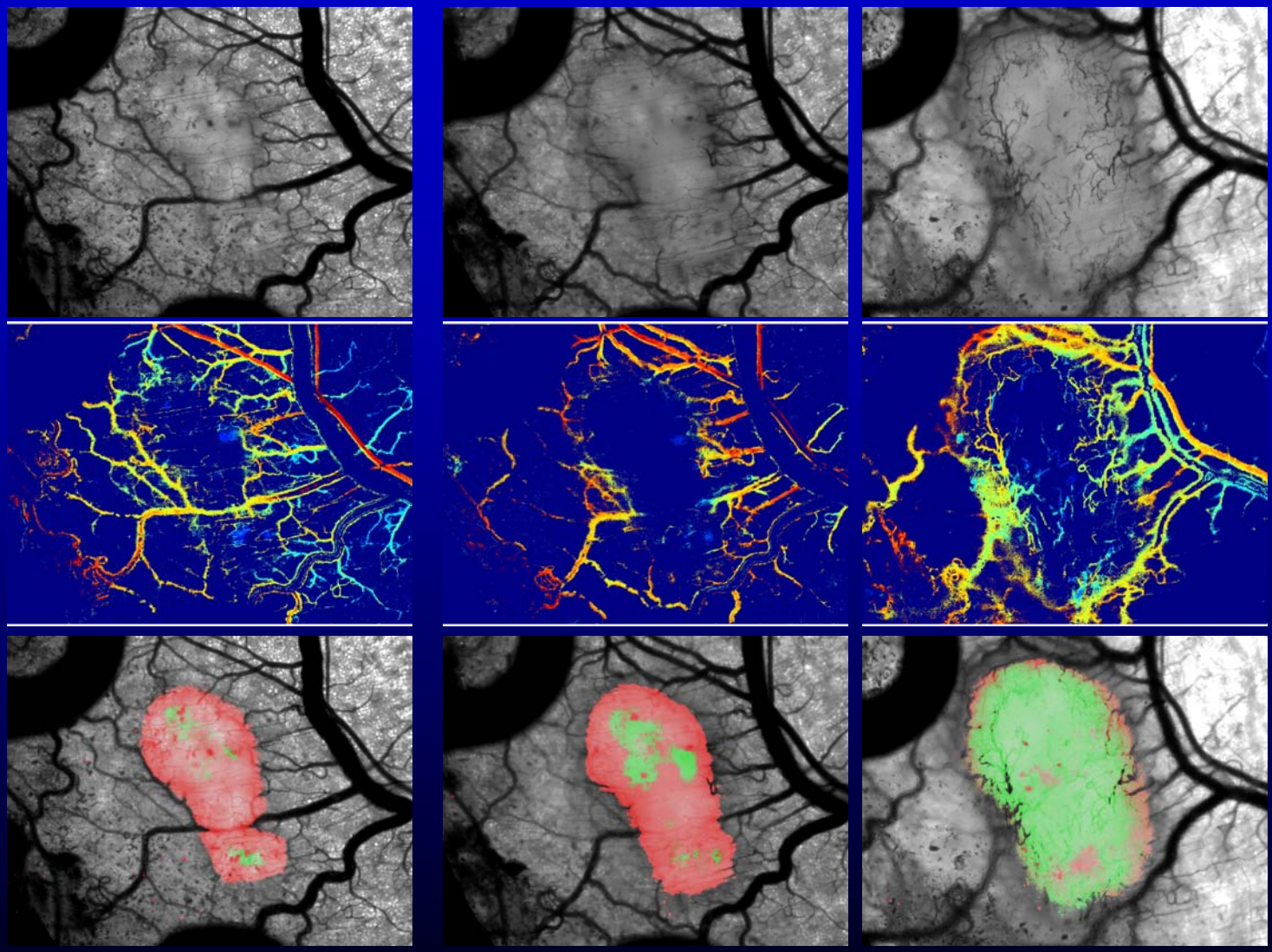
Dewhirst, Cao and Li, unpublished

4T1 mammary CA

4T1 Mouse Mammary Carcinoma

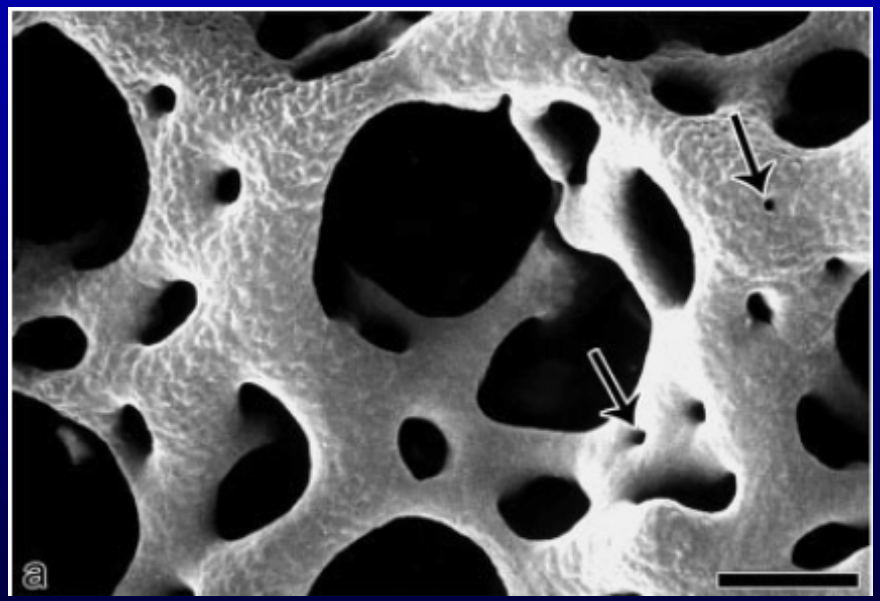


4T1 Mouse Mammary Carcinoma



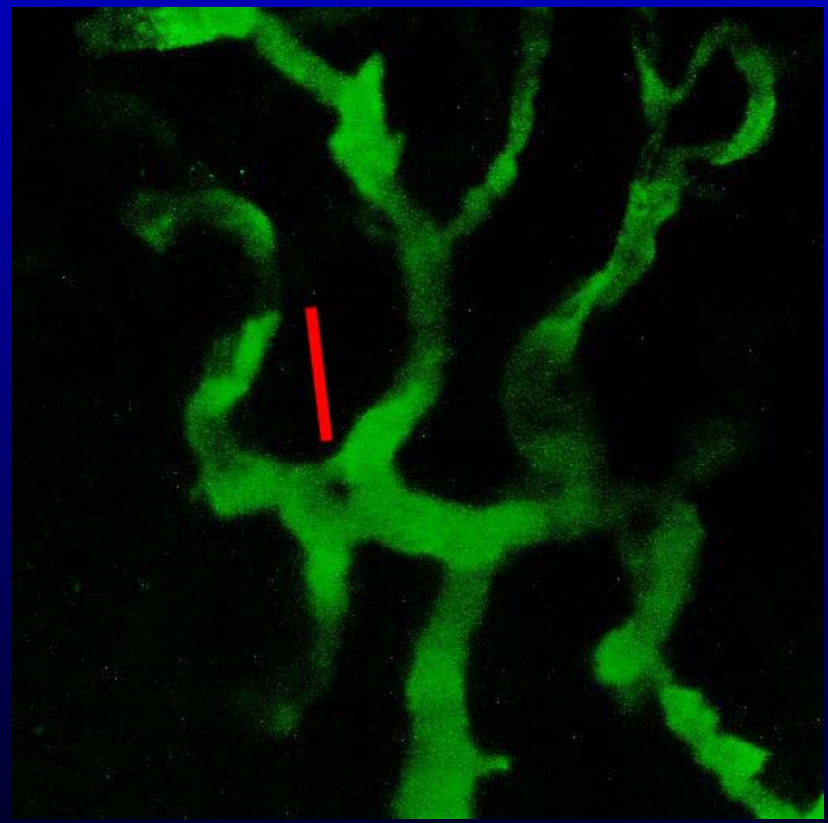
Intussceptive angiogenesis examples

Observation in CAM



Burri et al, Dev Dyn, 2004

FaDu xenograft



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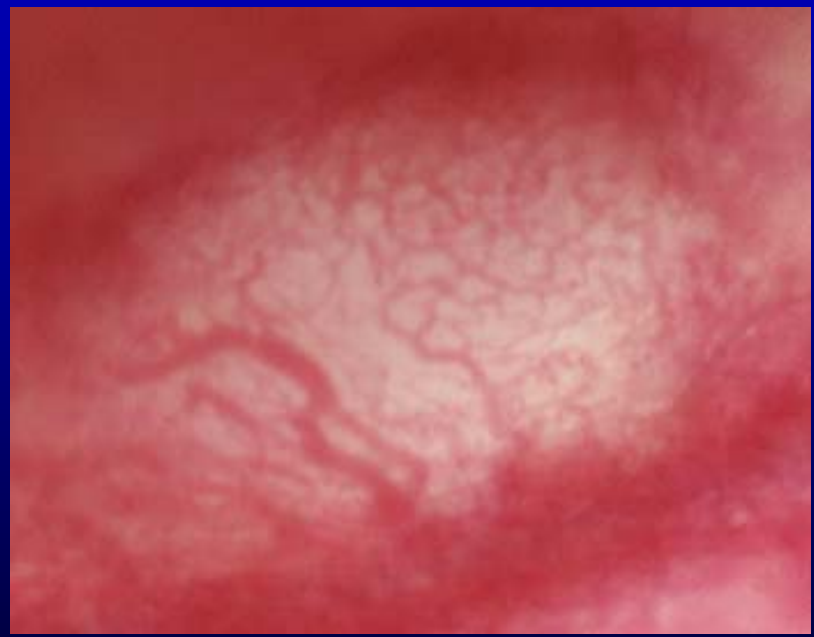
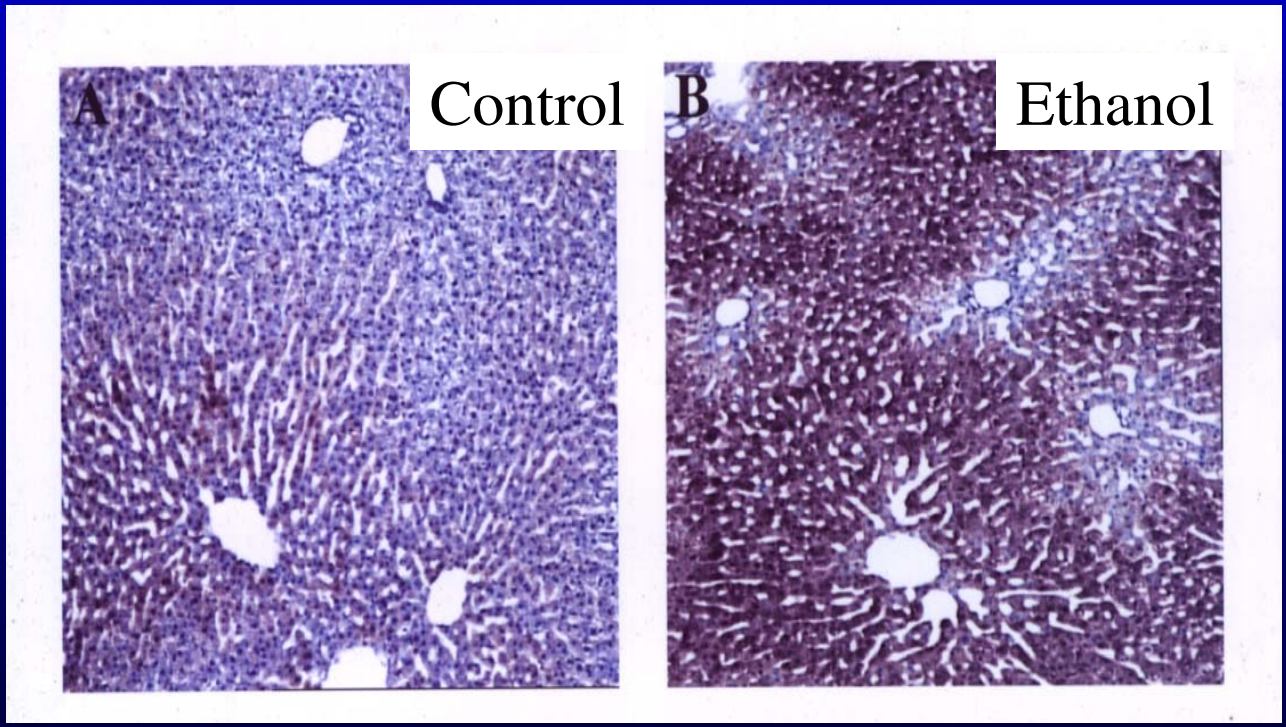
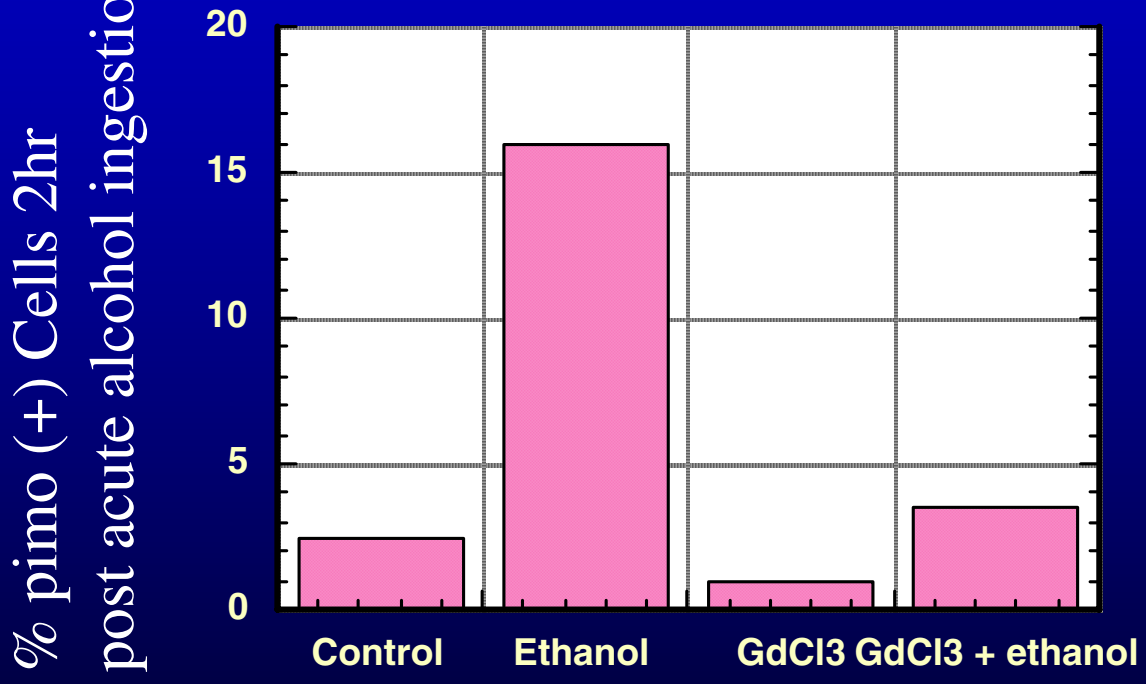


Liver hypoxia caused by acute alcohol (2 h post ethanol administration)



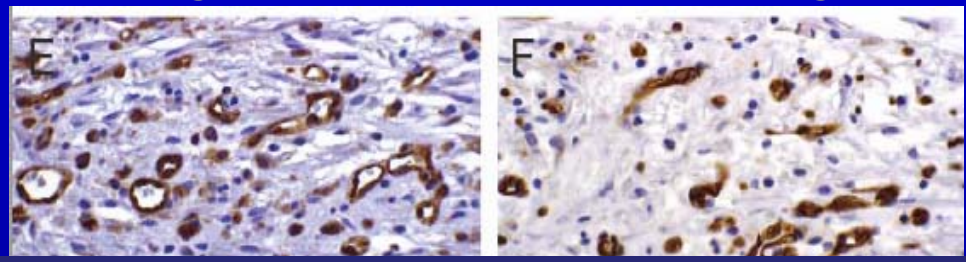
Arteel *et al.*, *Am.J.Physiol.* 271, G494-G500, 1996

Blockade of Kupffer cell activation reduces hepatic hypoxia post ethanol ingestion

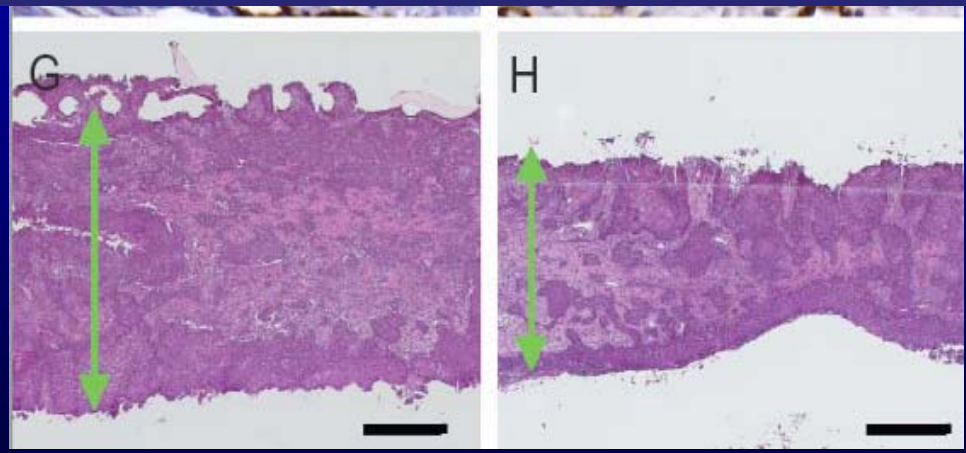


Arteel et al, AJP **271, G494-G500**

Dietary glycine reduces angiogenesis and tumor growth - fibrin gel chamber



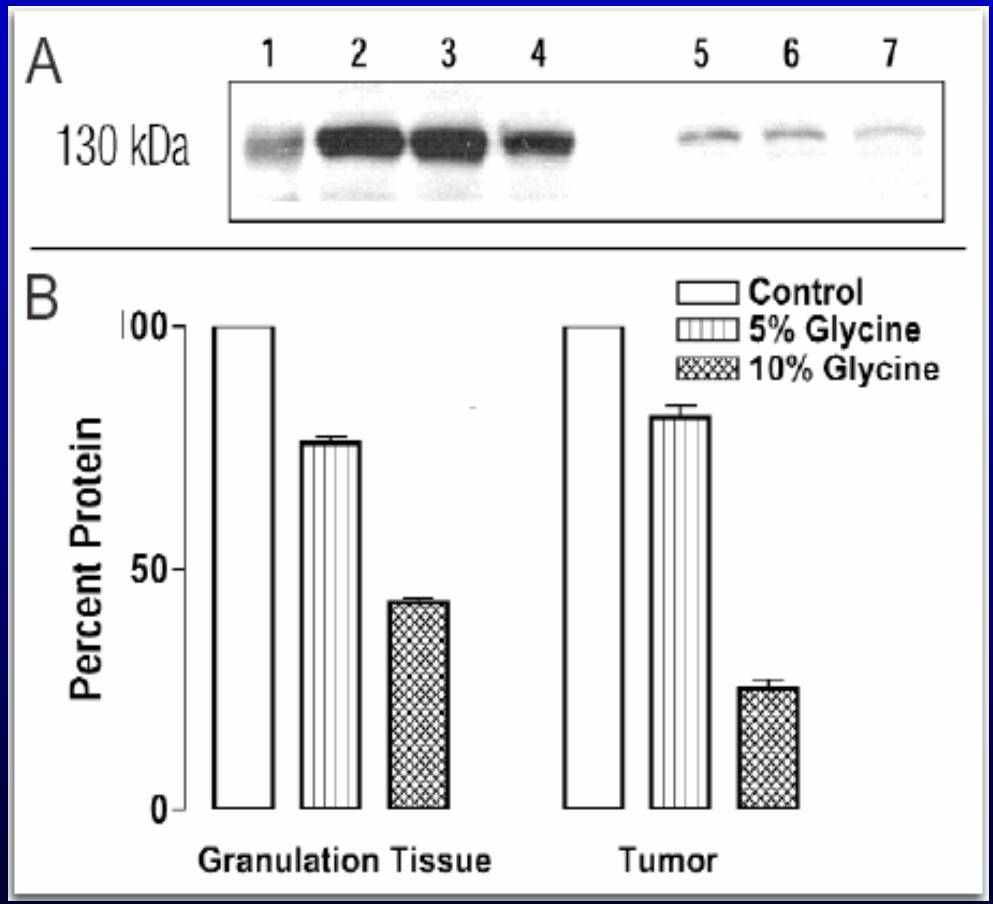
Glycine blocks Chloride channels in macrophages; prevents macrophage activation



Control 10% Glycine in diet

Amin et al, Can Biol Ther2; 173, 2003

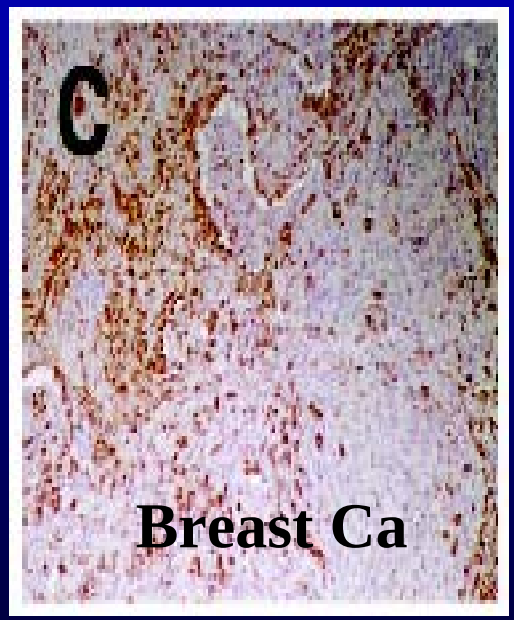
10% dietary glycine reduces tissue iNOS levels

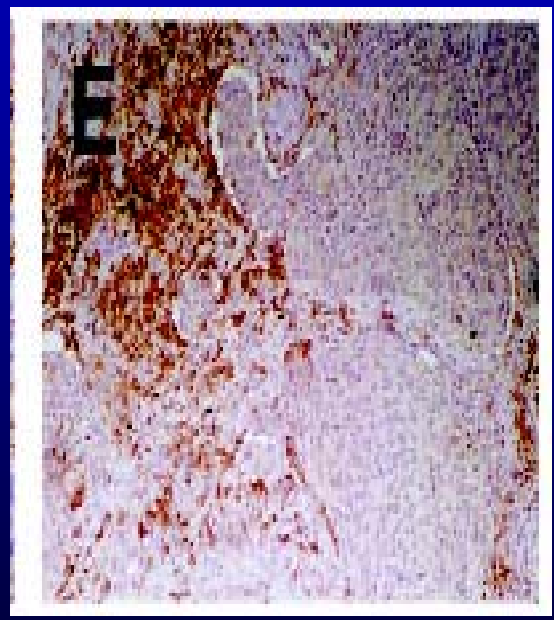


Amin et al, Can Biol Ther2; 173, 2003

Hif-2a and macrophages colocalize in human breast cancer

CD68 **HIF-2α**

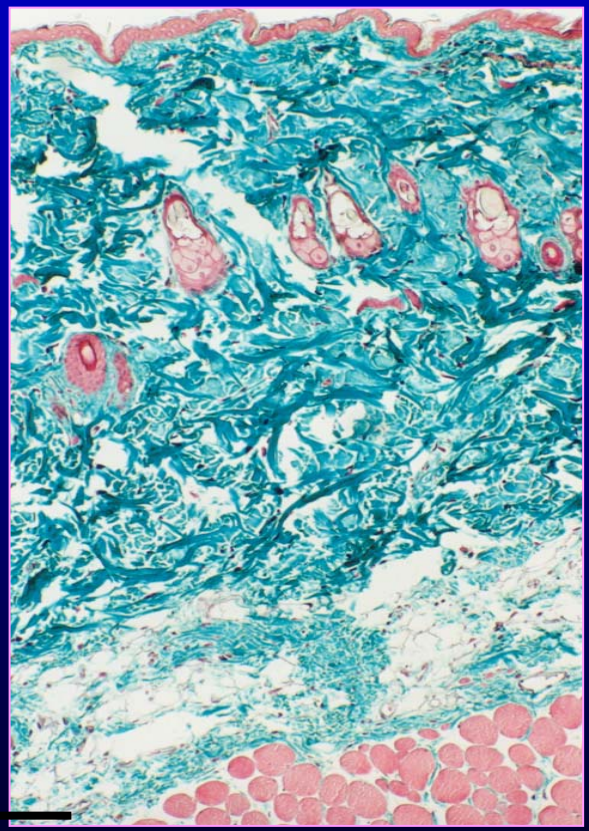




Talks et al., Am. J. Pathol 157:411-421, 2000

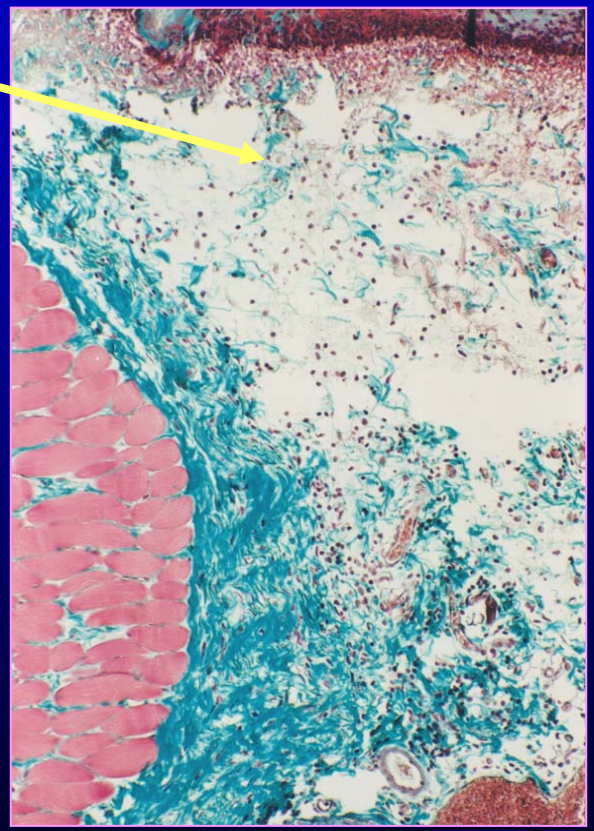
Histology of Wound Healing Reaction

Normal Rat Skin (25X)



Prov. Fibrin Matrix

Day 1 (25X)

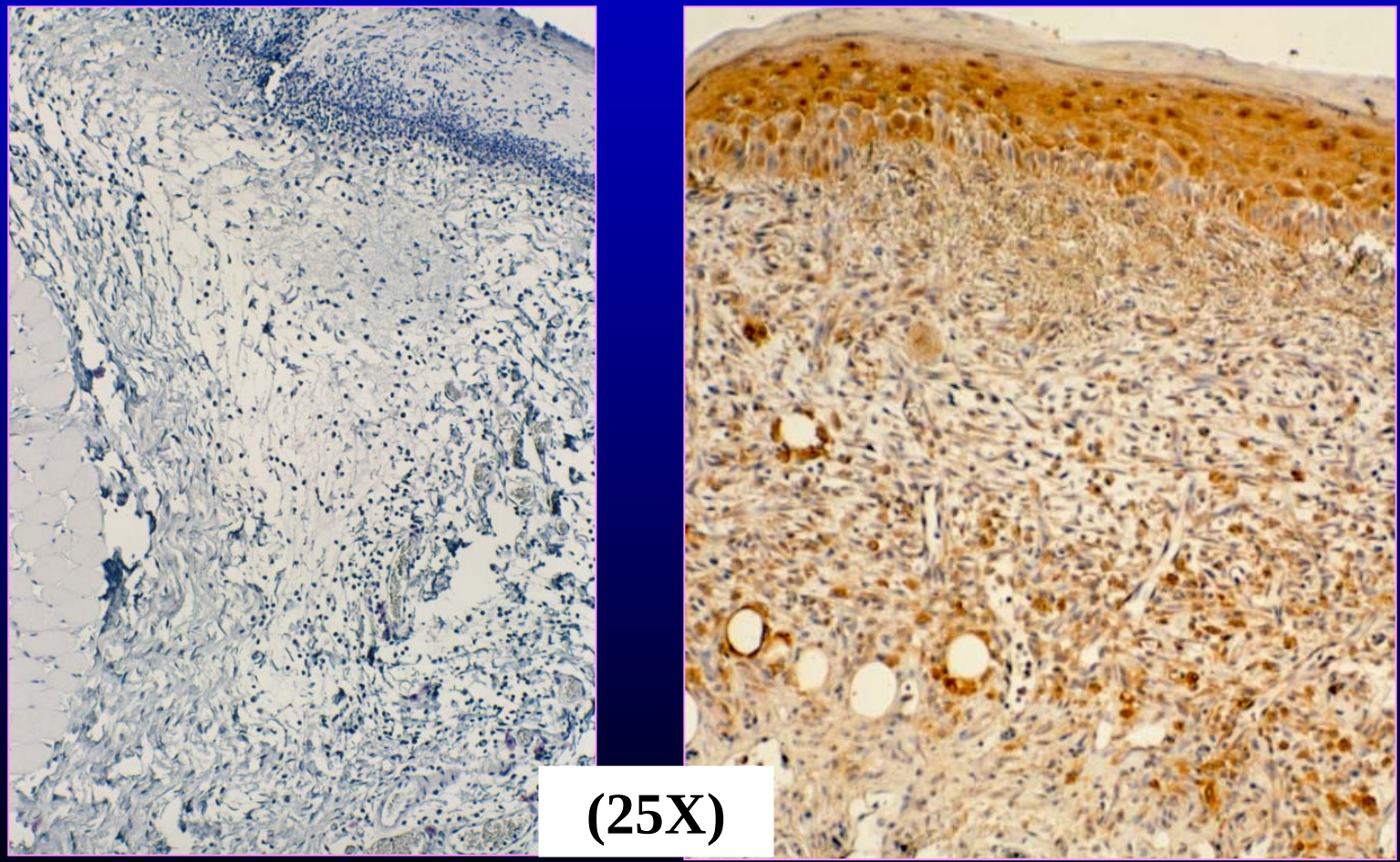


Z. Haroon, Ann Surg 231: 137, 2000

Hypoxia in Provisional Fibrin Matrix on day 1 (-) vs. at day 4 (++++)

Day 1

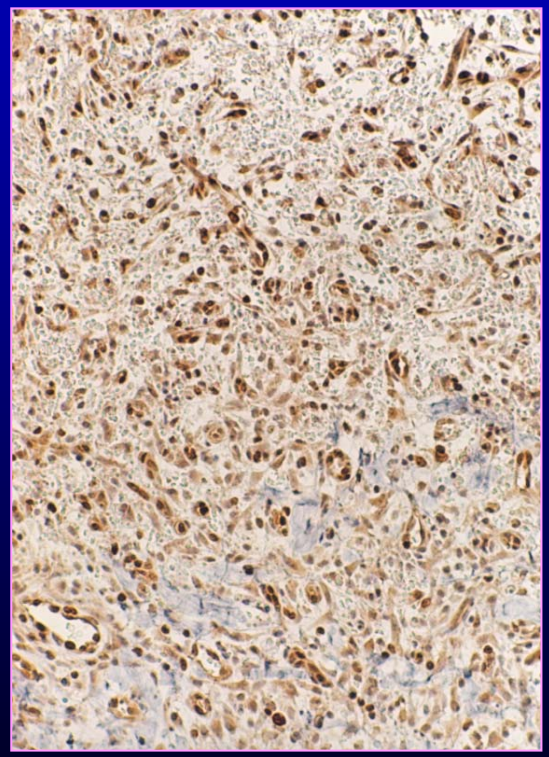
Day 4



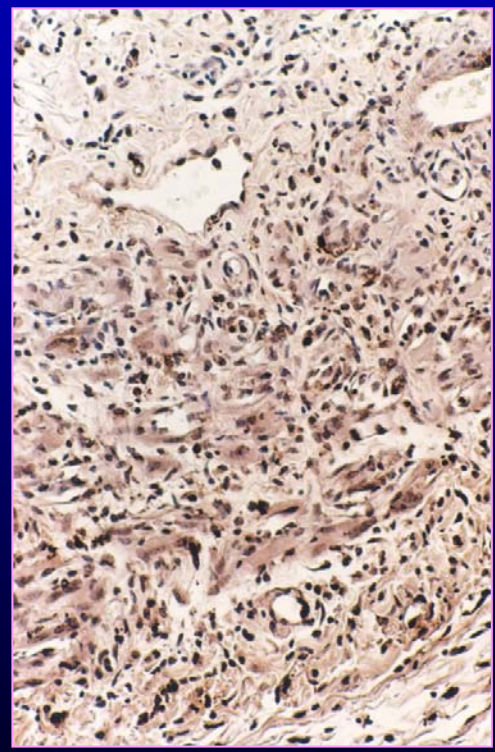
(25X)

Proliferation and Apoptosis are Maximum at Day 4

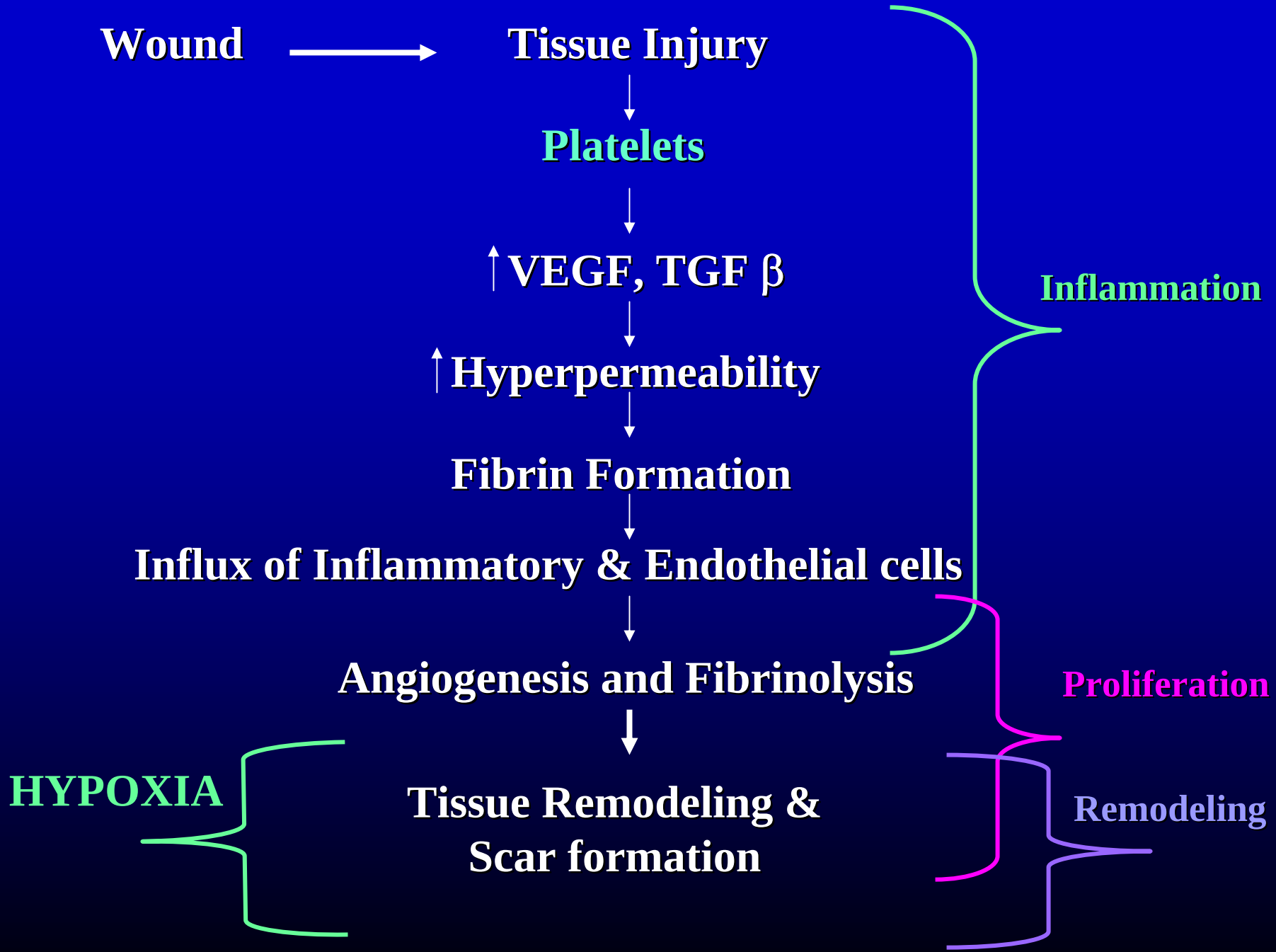
Ki67



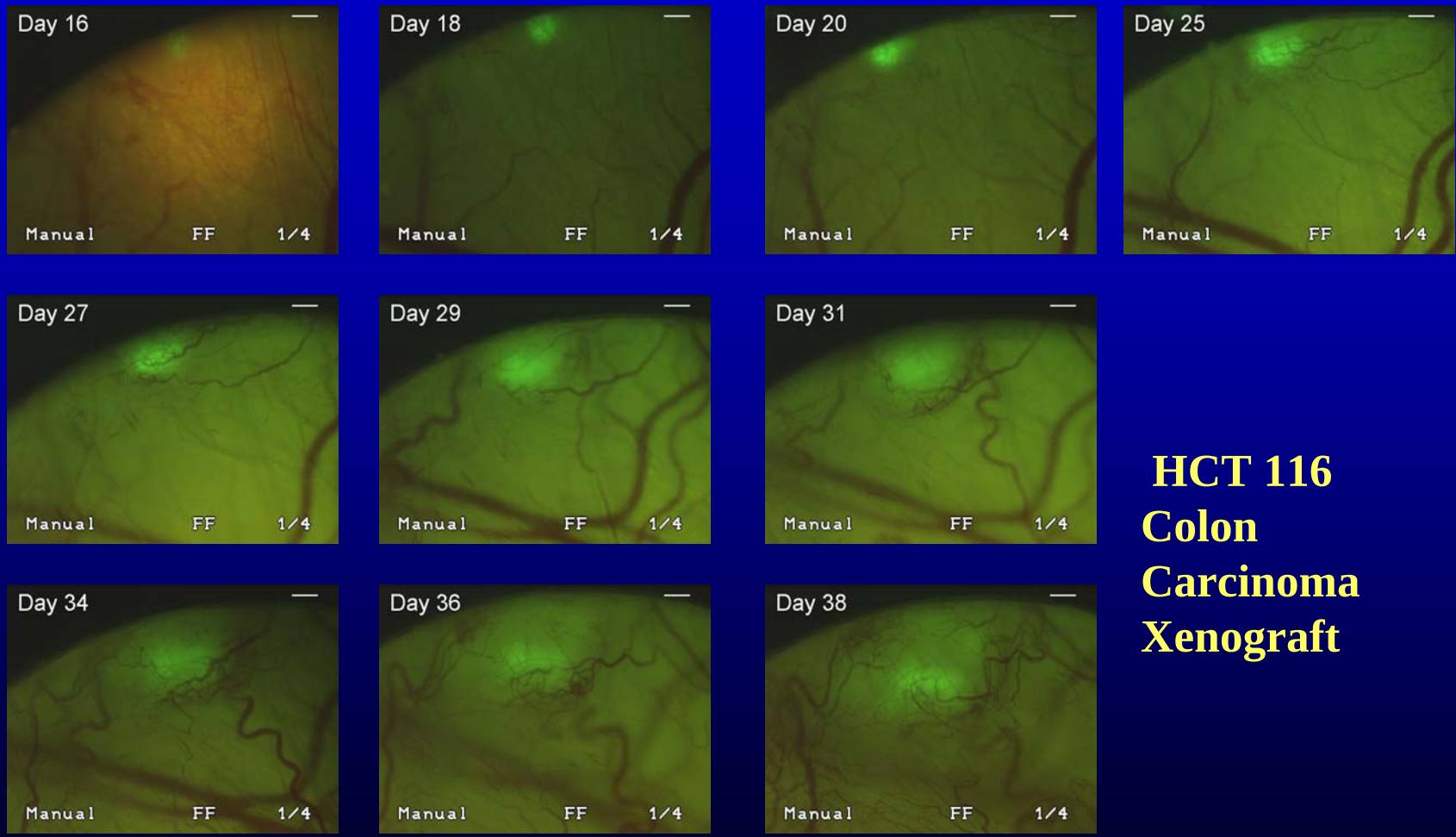
TUNEL



Hypoxic Induction of P53 / Apoptosis?


Wound
Tissue Injury
Platelets
VEGF, TGF β
Hyperpermeability
Fibrin Formation
Influx of Inflammatory & Endothelial cells
Angiogenesis and Fibrinolysis
Tissue Remodeling &
Scar formation
HYPOXIA
Inflammation
Proliferation
Remodeling

Examples of vascular remodeling and regression or collapse



HCT 116 Colon Carcinoma Xenograft

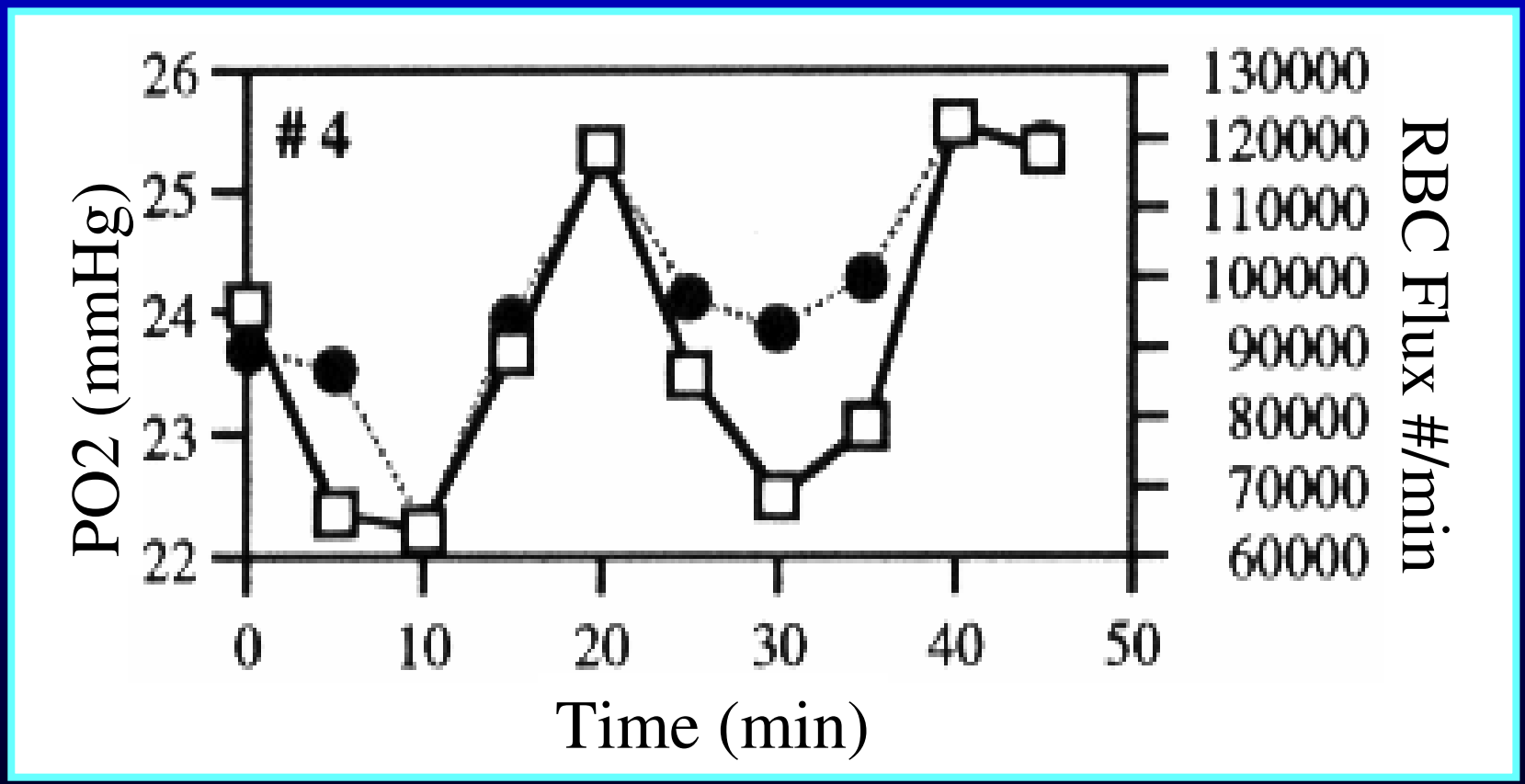
Bar: 200 μm

Dewhirst et al, Sem in Hematol Oncol, in press, 2004

Demonstration of Static Flow

QuickTime™ and a YUV420 codec decompressor are needed to see this picture

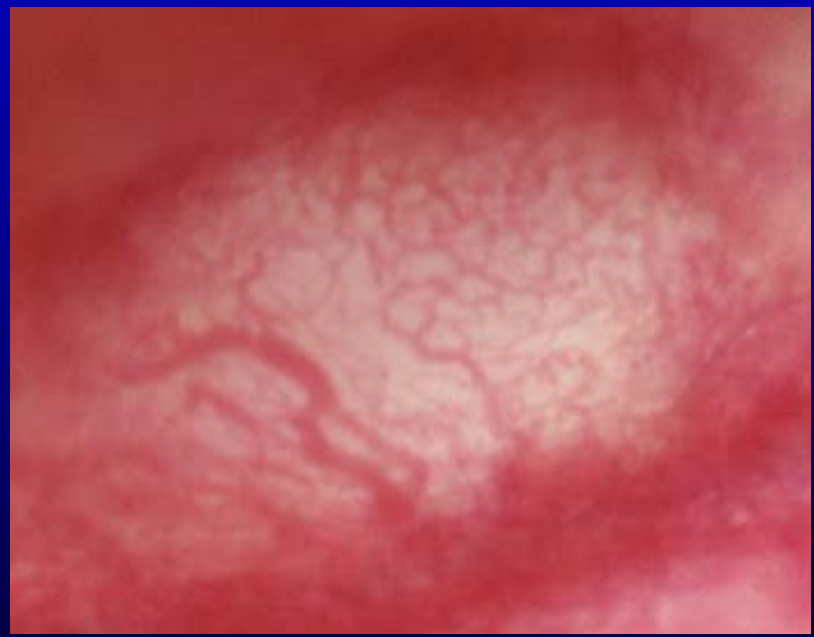
Red cell flux relates to perivascular pO2



From Kimura et al., 1996

Lecture Outline

- Angiogenic Switch
- Tumor-host cell interactions
  - Endothelial cell
  - Macrophage, fibroblast
- Effects of Rx and microenvironment on angiogenesis

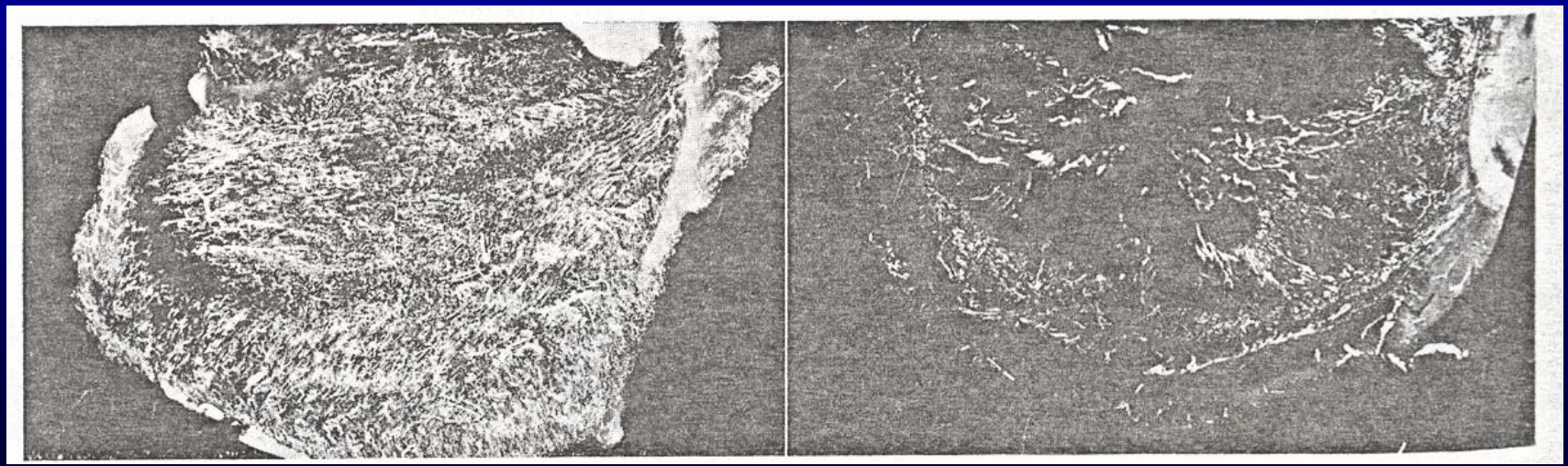


Supervascularized state of irradiated tumors

- First described by Rubin and Casarett, Clin Radiol, 17:346-355, 1966
- Qualitative assessment - done using microangiography

Irradiated

Sham Irradiated



5Gy x 3, daily Fx, Walker carcinosarcoma

Paradoxical HIF-1 Signaling During Tumor Reoxygenation: The Role of Free Radicals and Stress Granules

Mark W. Dewhirst
Ben Moeller
Yiting Cao
Chuan Li

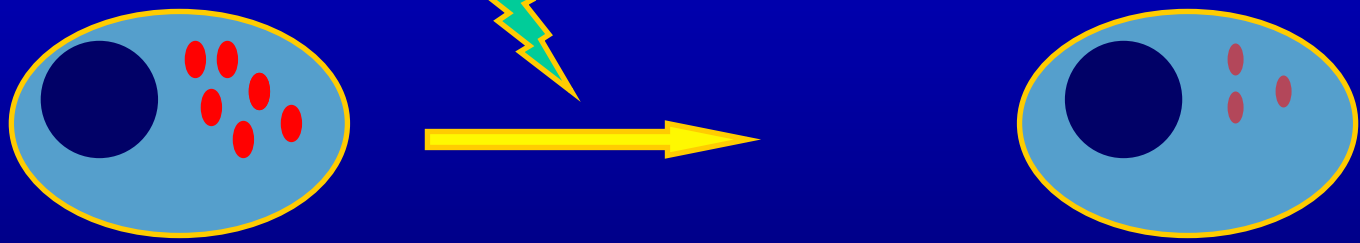
Moeller et al., Cancer Cell, May 2004, page 429

Two HIF-1 - mechanisms protect against endothelial death post RT

Hypoxic tumor cell

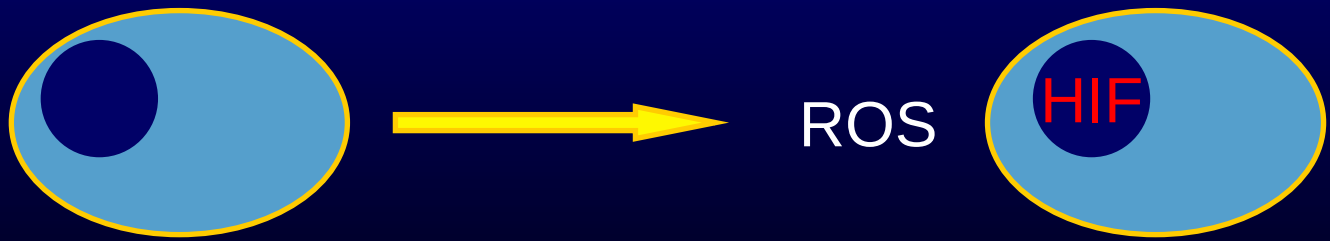
RT

Reoxygenation



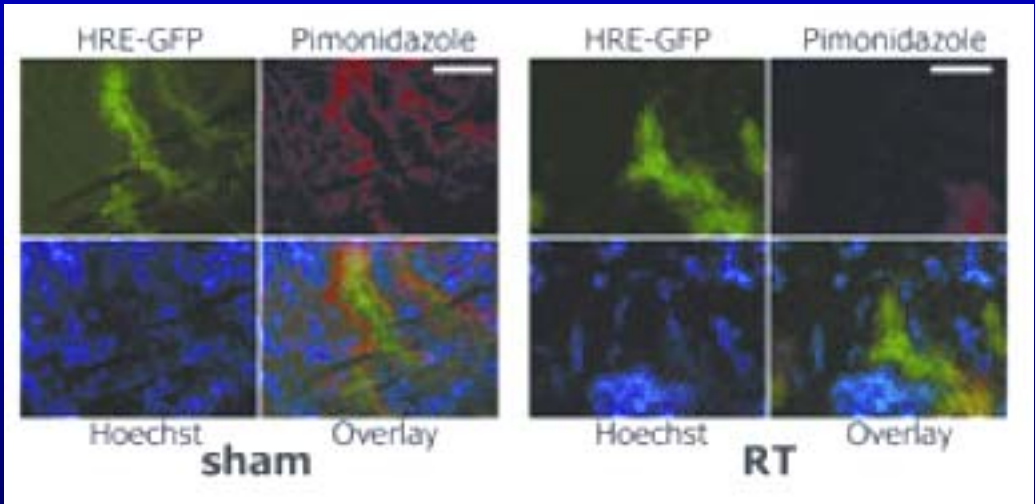
- Stress Granules
  Protect HIF-1 transcripts

Stress Granules Depolymerize
Releasing HIF-1 regulated mRNAs



HIF-1α
Stabilized
Enters nucleus

Overlap between Hypoxia Marker & HRE-GFP pre/48hr post RT



Sham = 92% overlap HRE-GFP with Pimo

Irradiated = 18% Overlap

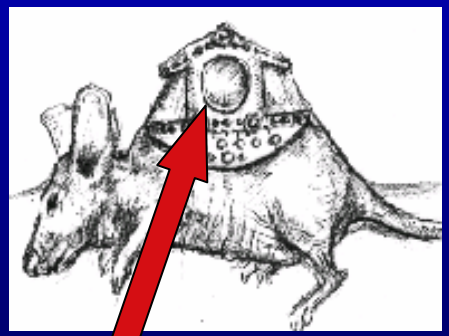
Red = Pimonidazole - hypoxia marker drug
Blue = Hoechst - perfusion marker
Green = HRE-GFP reporter gene

HRE-GFP expressed in Aerobic cells post RT

Moeller et al. *Cancer Cell*. 5: 429, 2004

Methods to test whether RT induces reoxygenation → free radicals → HIF-1

4T1



4T1 HRE-GFP

HIF-1 mediated GFP Tumor cells

radiation (reoxygenation) vs. sham-RT

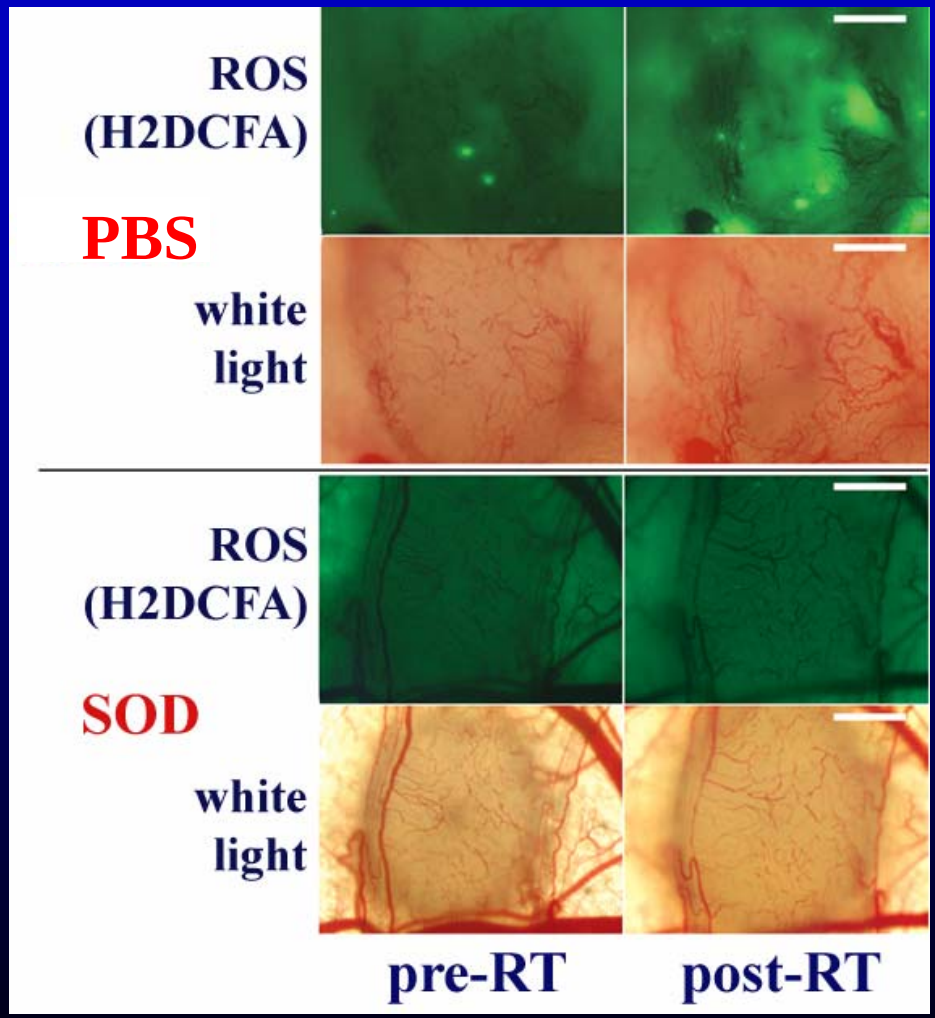
antioxidant vs. PBS

H_2DCFA or DCFA

microscopy (change ROS)

microscopy (change HIF-1 GFP)

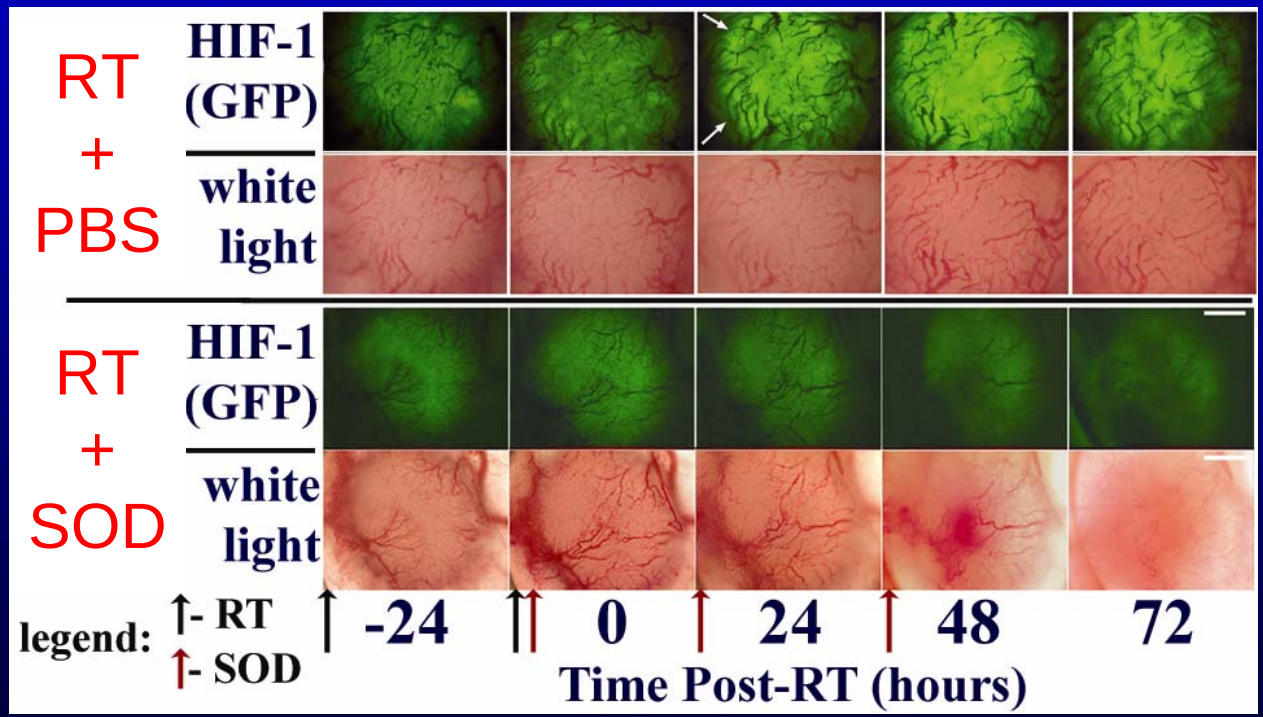
Reoxygenation post RT increases free radicals



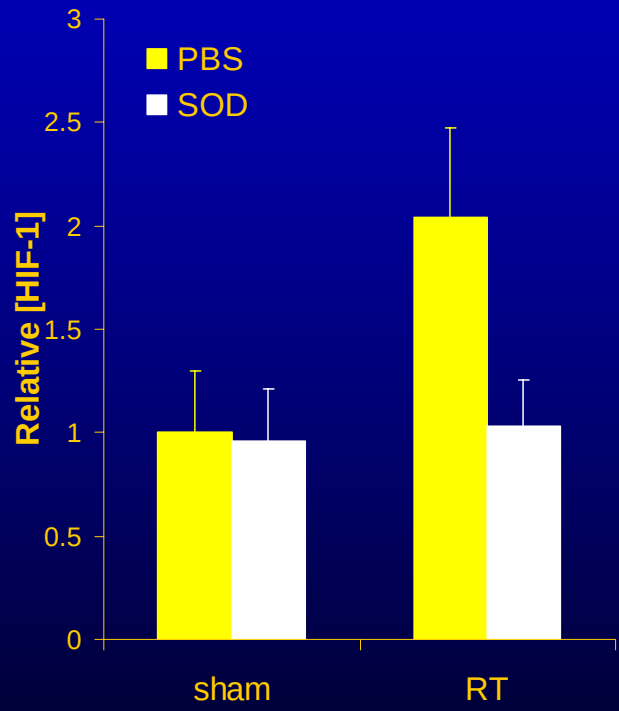
2 x 5Gy
24hr after 2nd RT dose

bar = 300μm

Free radicals post RT increase HIF-1α levels



HIF-1 ELISA

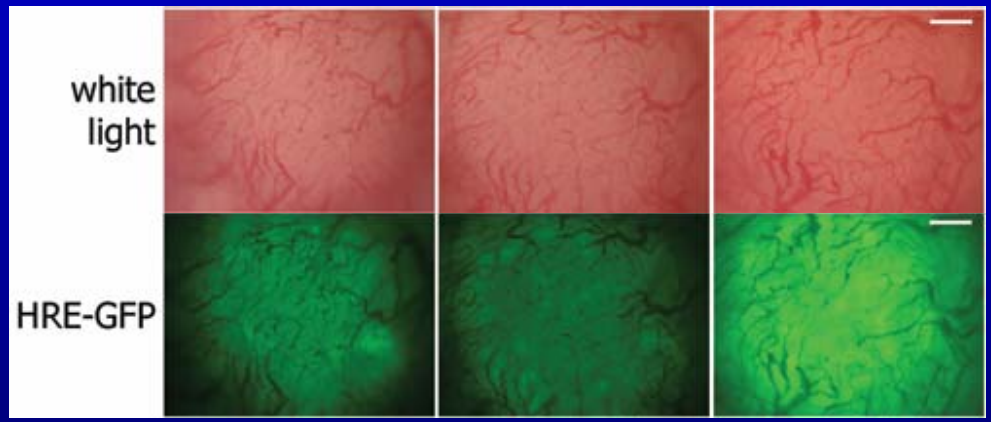


bar = 300μm

RT = 5Gy x 2

Significance of Reoxygenation-Mediated HIF-1 Activation

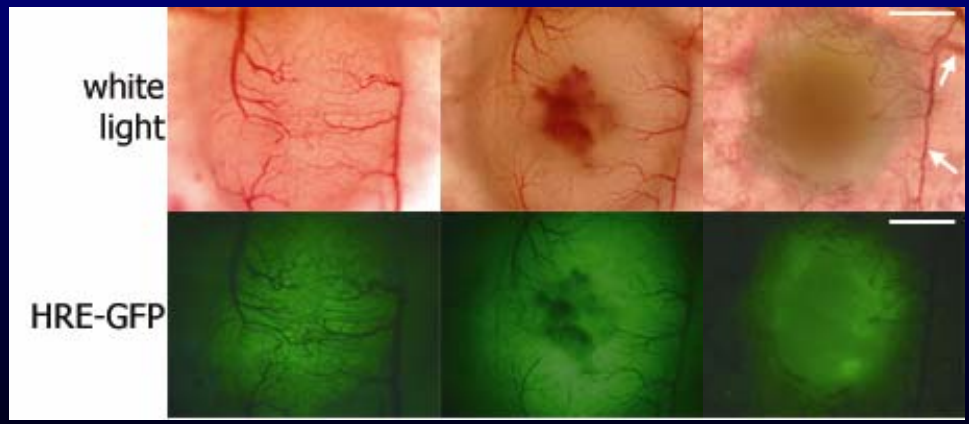
RT
(reoxygenation)



bar = 300μm

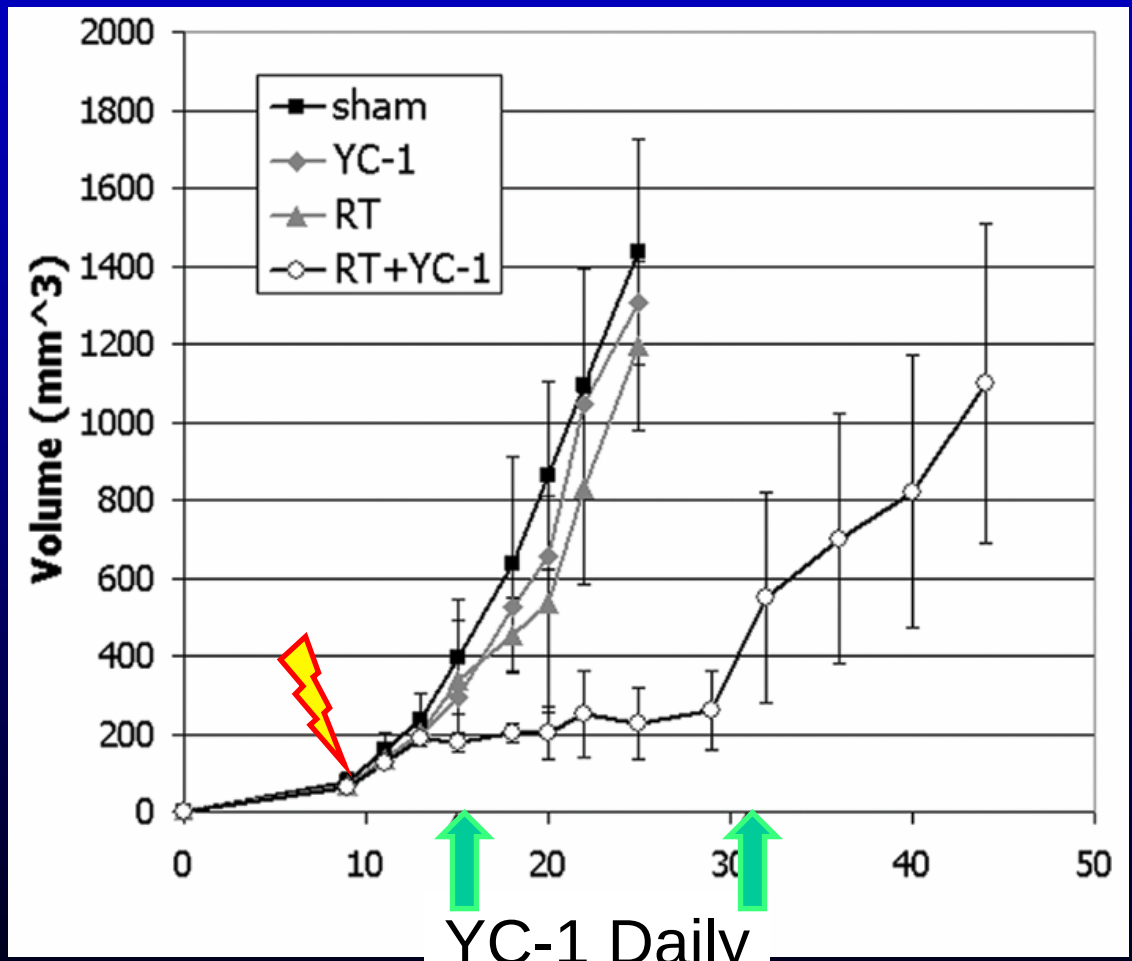
0 hours 24 hours 48 hours

RT
+
YC-1
(HIF-1 inhibitor)



bar = 500μm

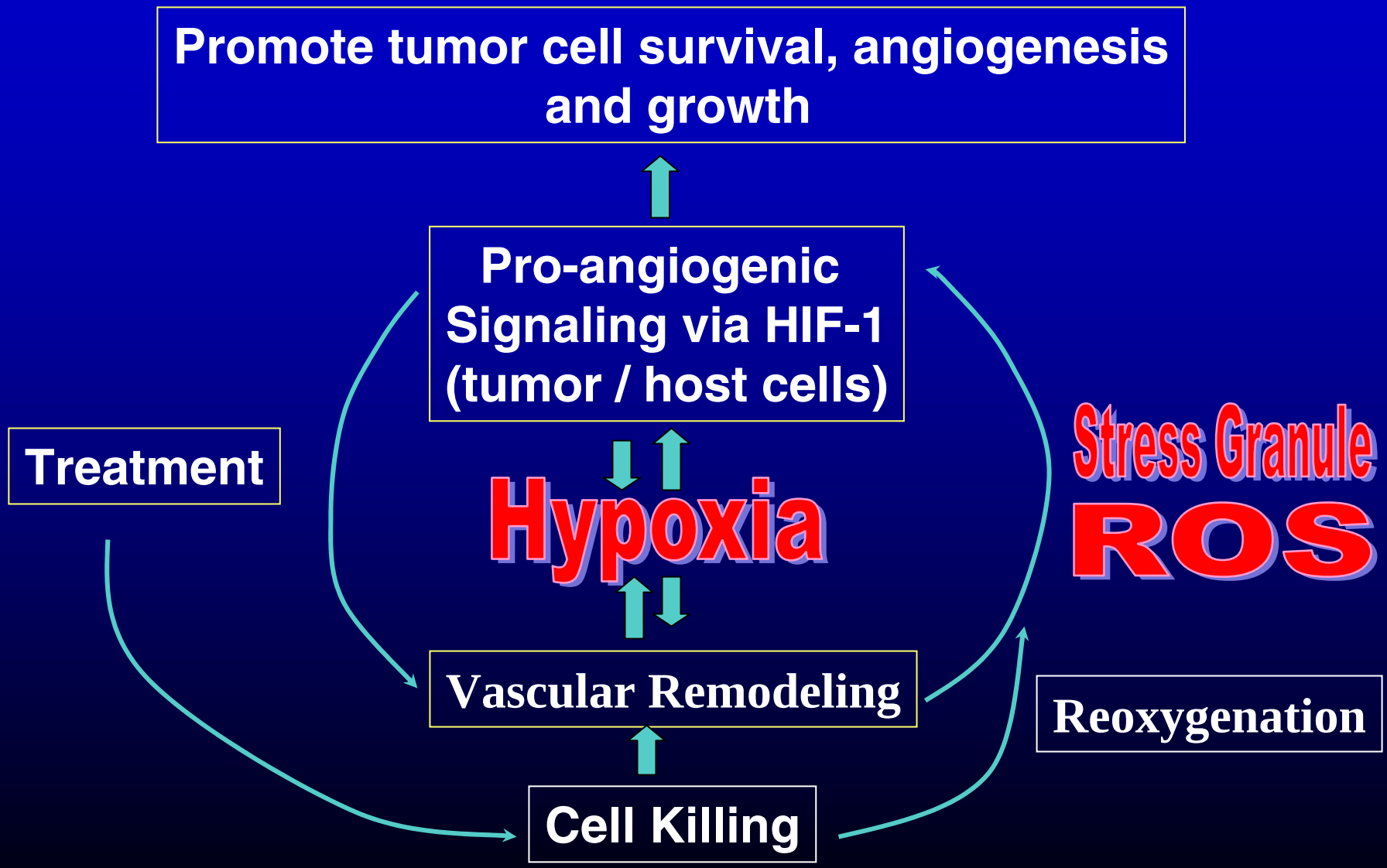
HIF-1 Activation blockade increases RT response



4T1
10 Gy x 1

YC-1
5mg/kg

Tying the pieces together



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