Insights on Angiogenesis For Hyperbaric Medicine

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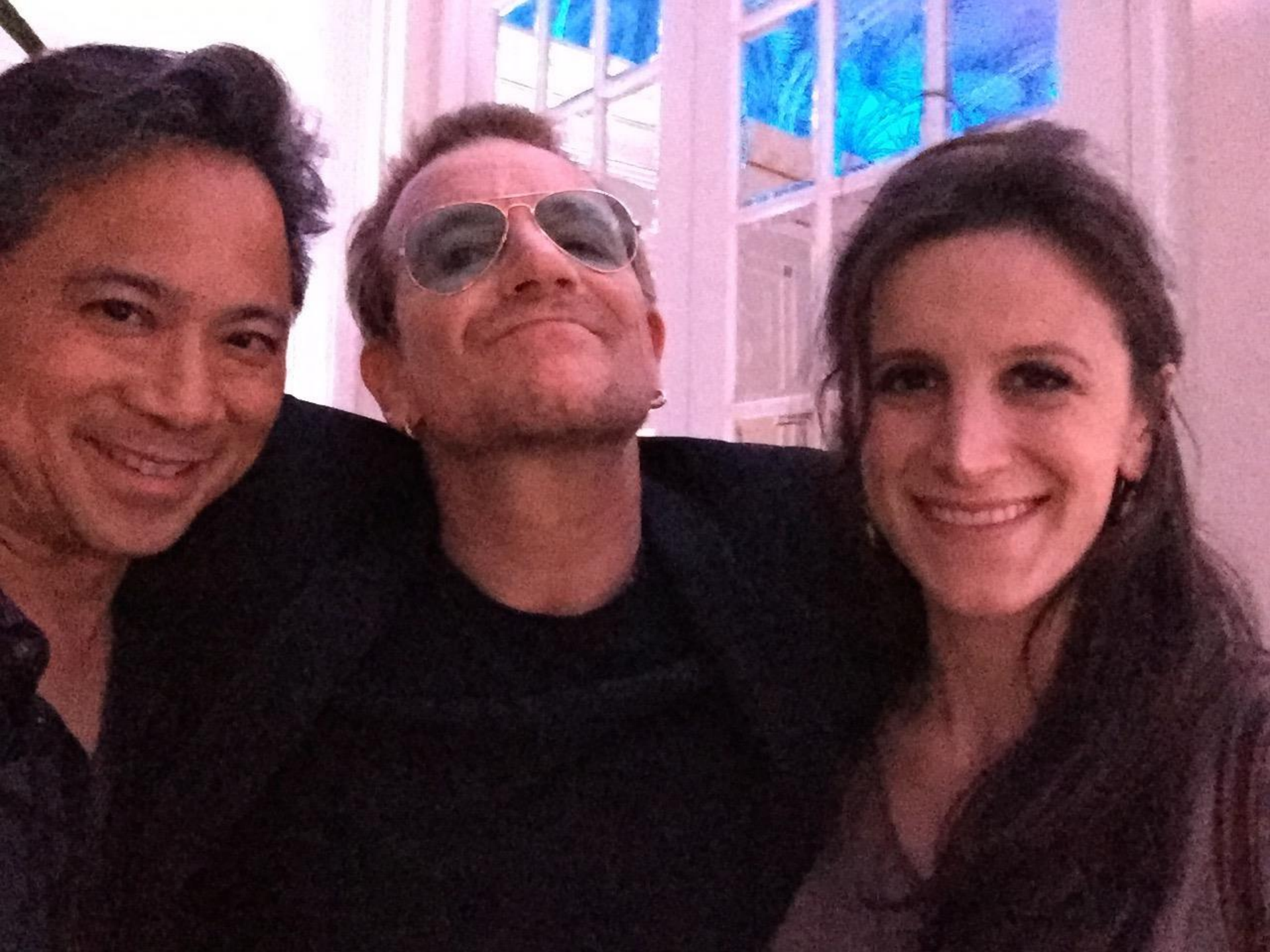
The Angiogenesis Foundation

June 18, 2015
Annual Scientific Meeting
Undersea & Hyperbaric Medical Society
Montreal, Quebec

The **Angiogenesis** Foundation



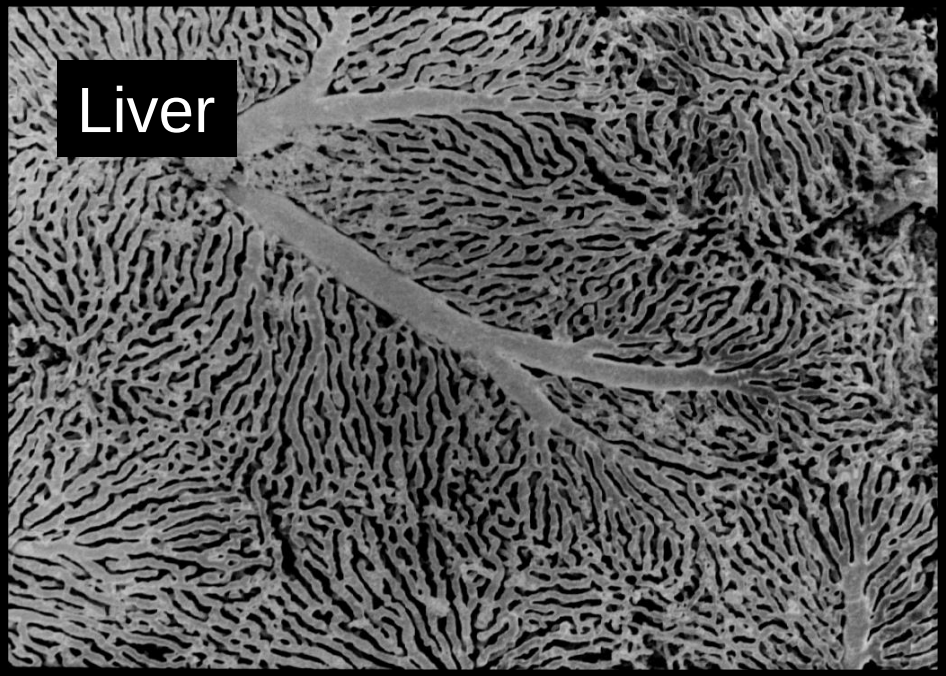
3 TAKE HOME POINTS:

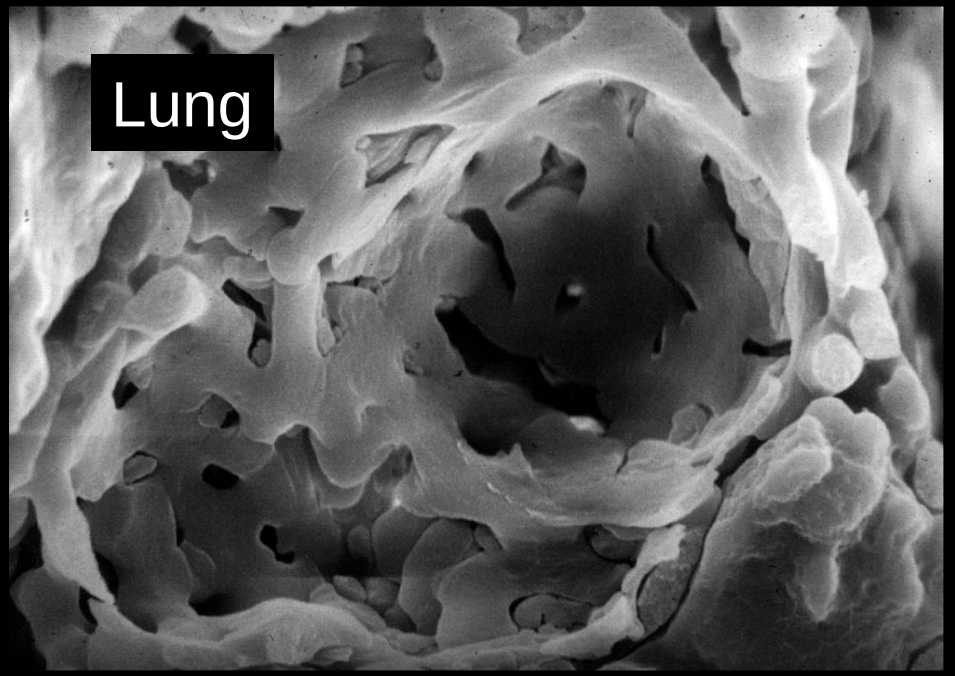
1. Angiogenic responses are much more complex process than previously assumed (not just “on” or “off”.

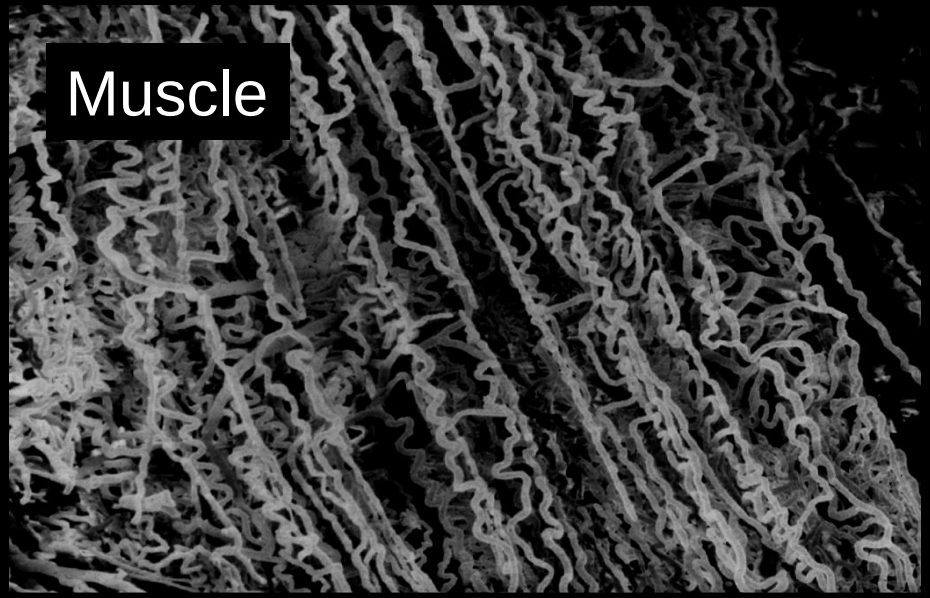
2. Oxygen sensing (hypoxia / hyperoxia) in angiogenesis defends microvascular homeostasis.

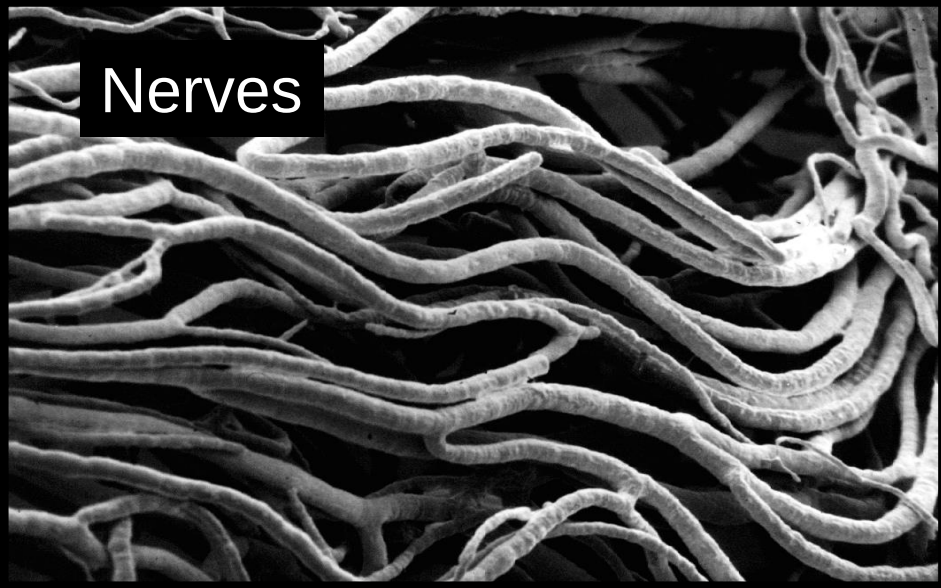
3. Regenerative changes can be promoted by hyperbaric interventions.


10 µm

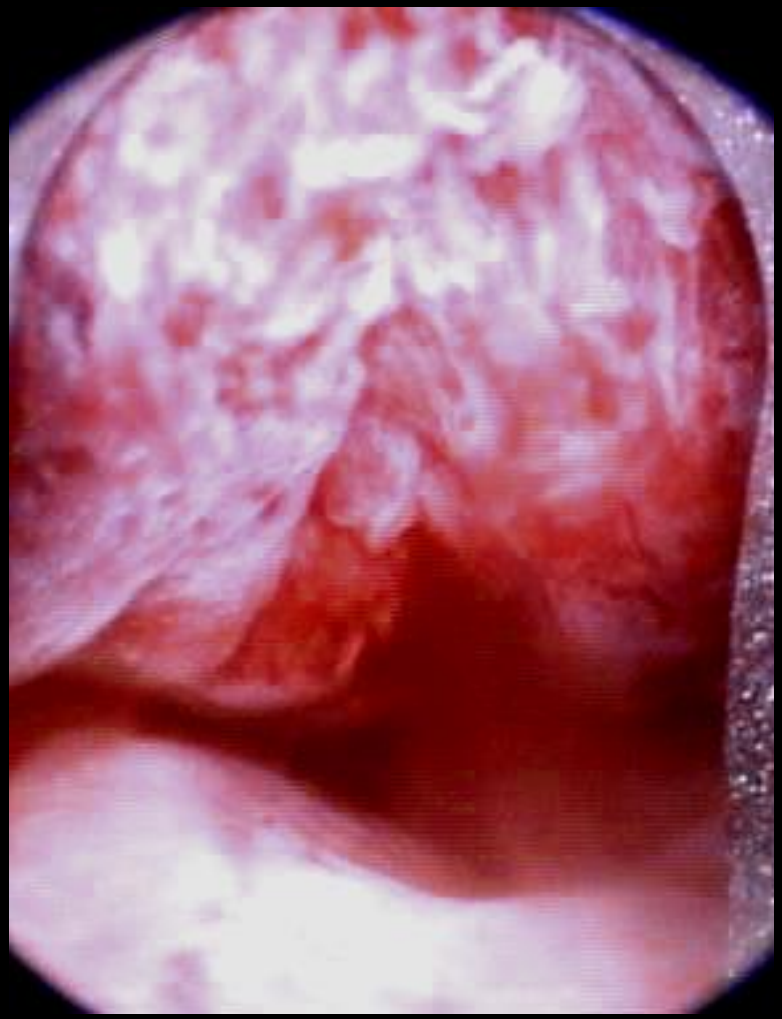

Liver


Lung

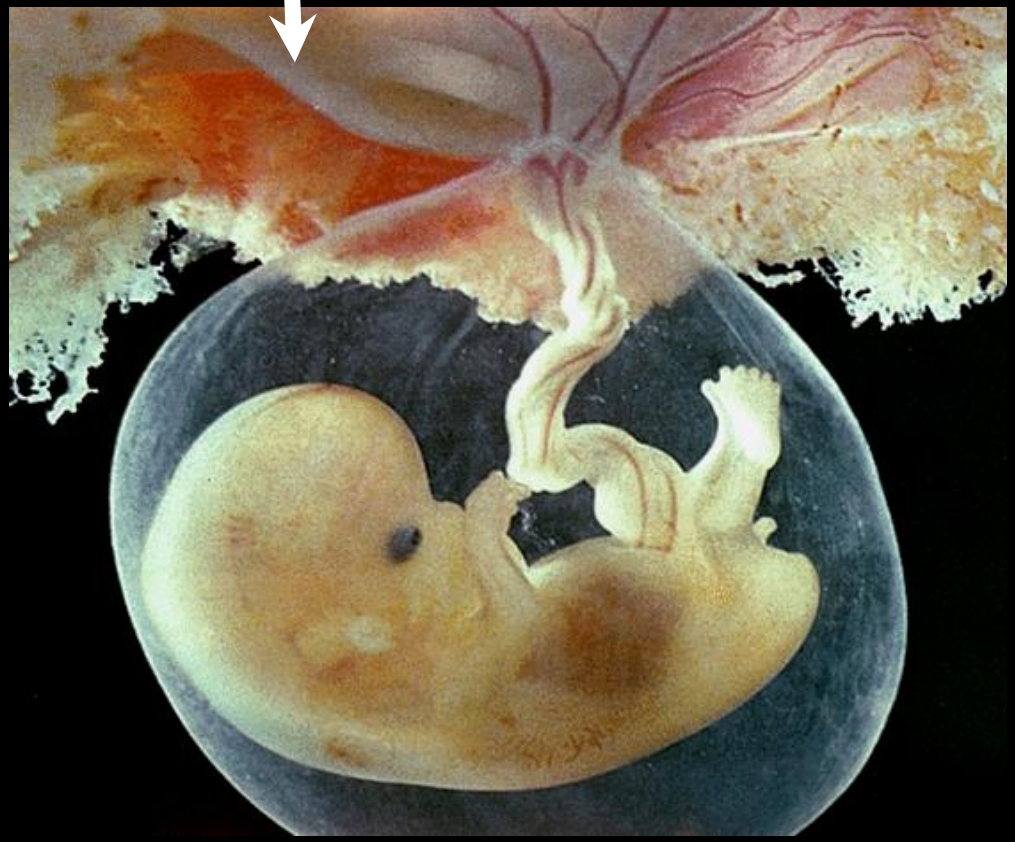

Muscle

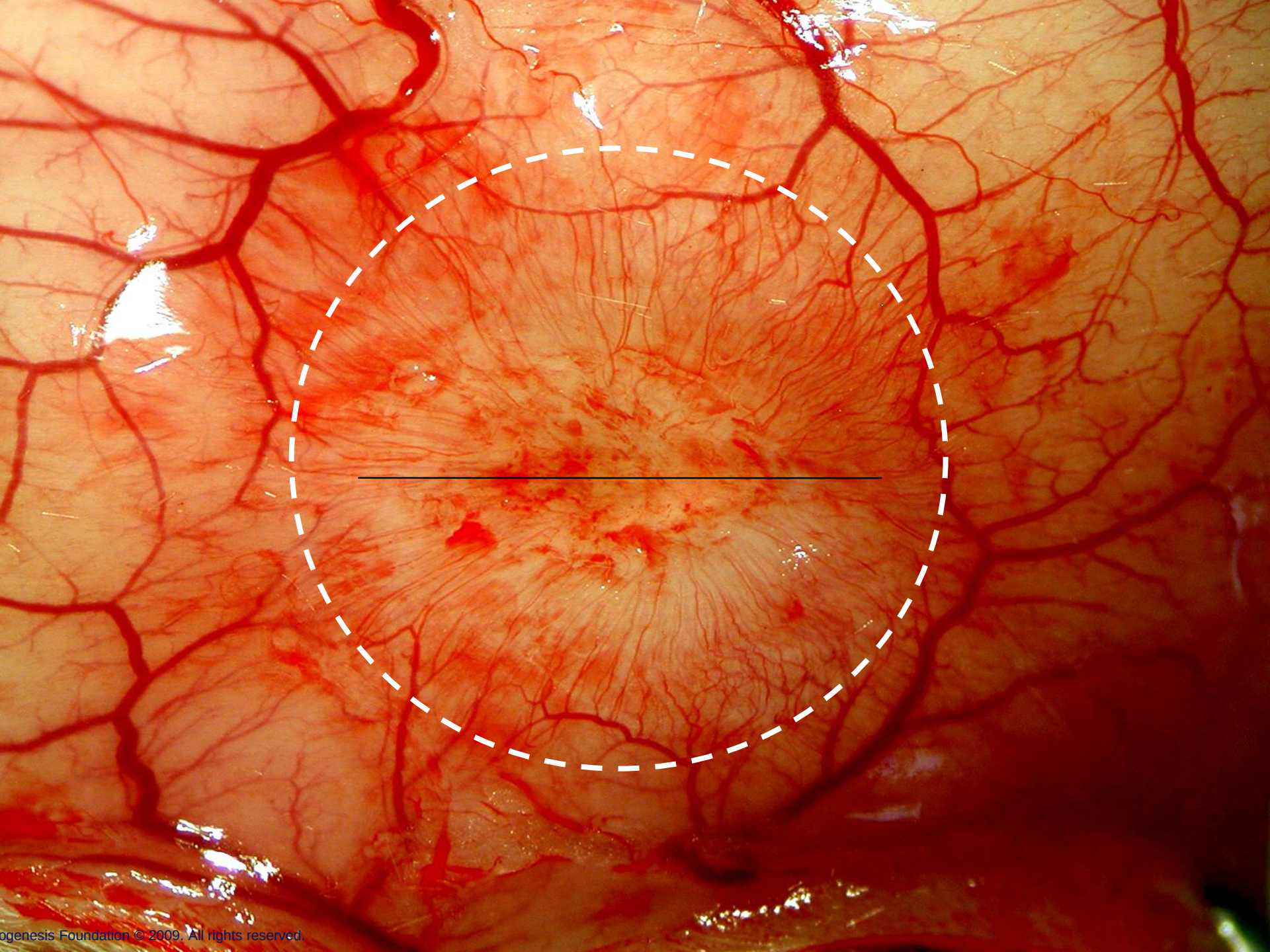

Nerves

Uterus

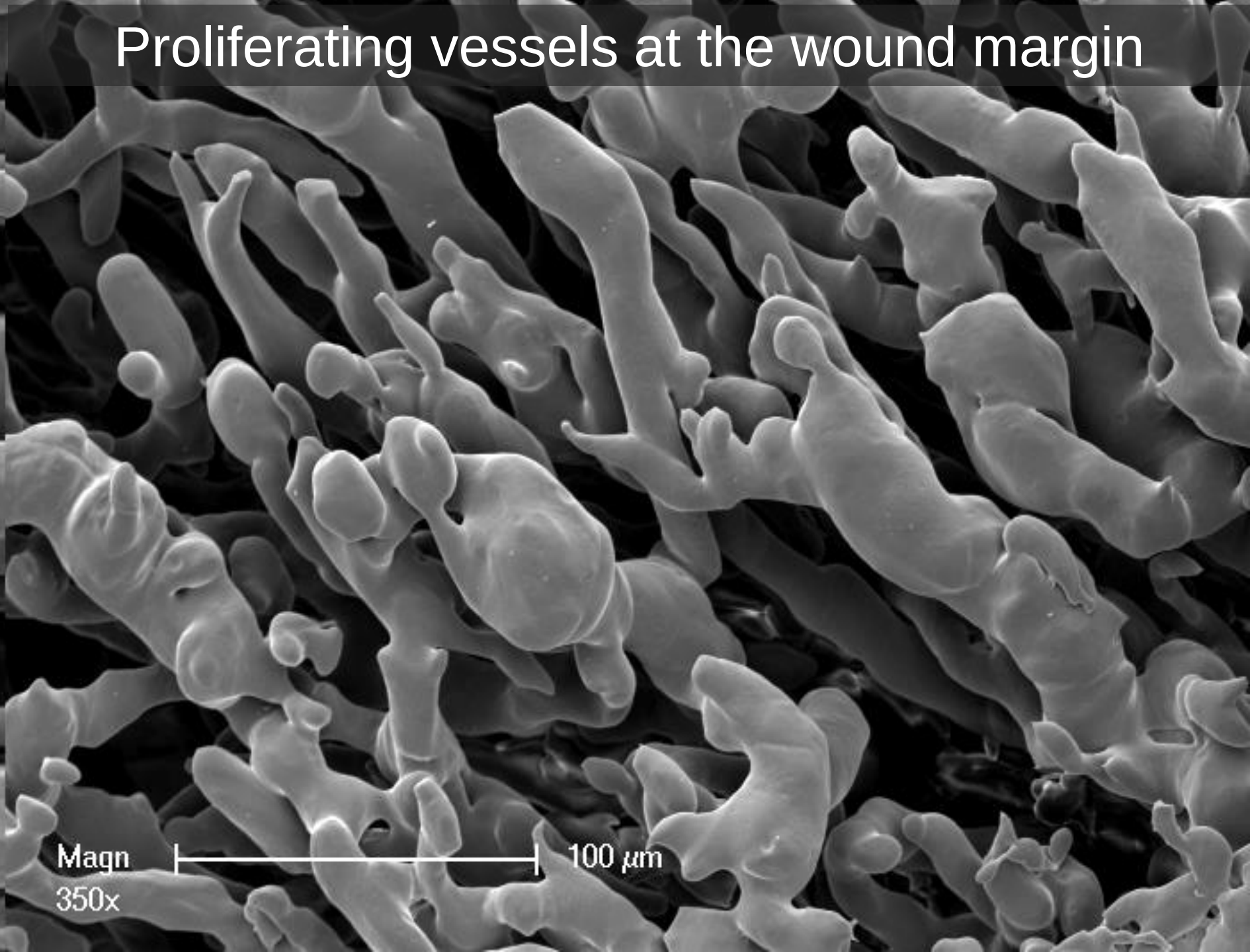


Placenta





Proliferating vessels at the wound margin



INHIBITORS

STIMULATORS

DISEASE

INHIBITORS

STIMULATORS

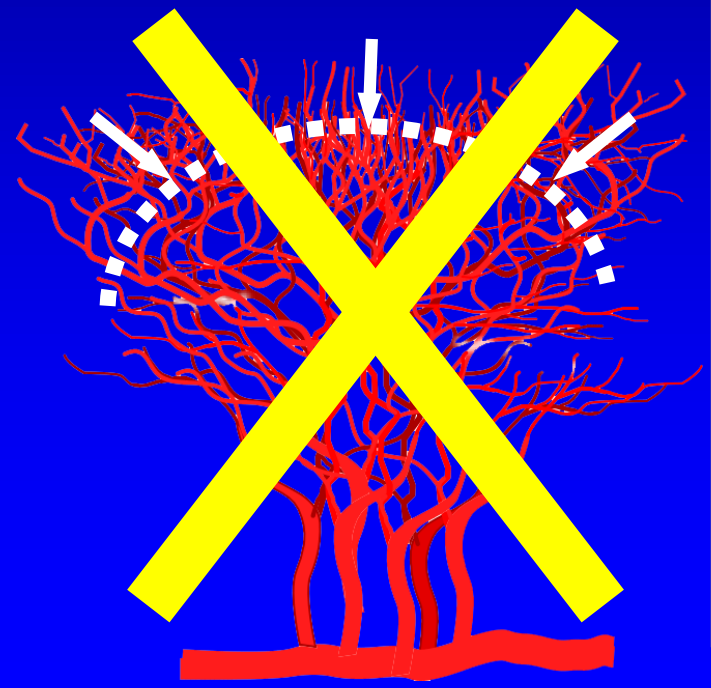
HYPERTROPHIC SCAR

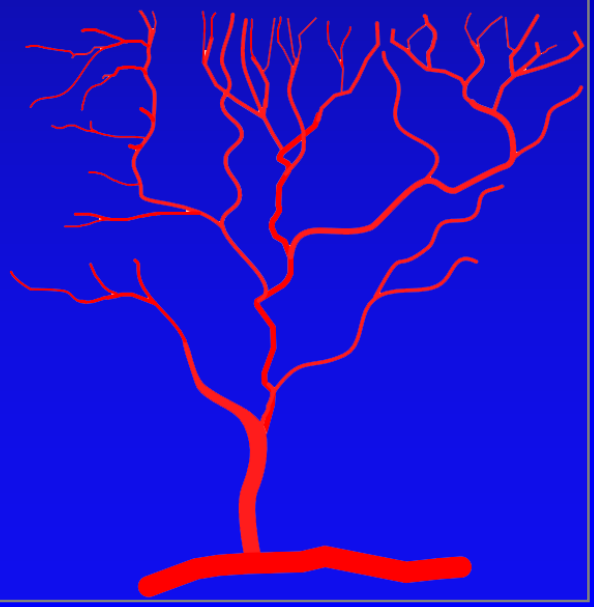
CHRONIC WOUND

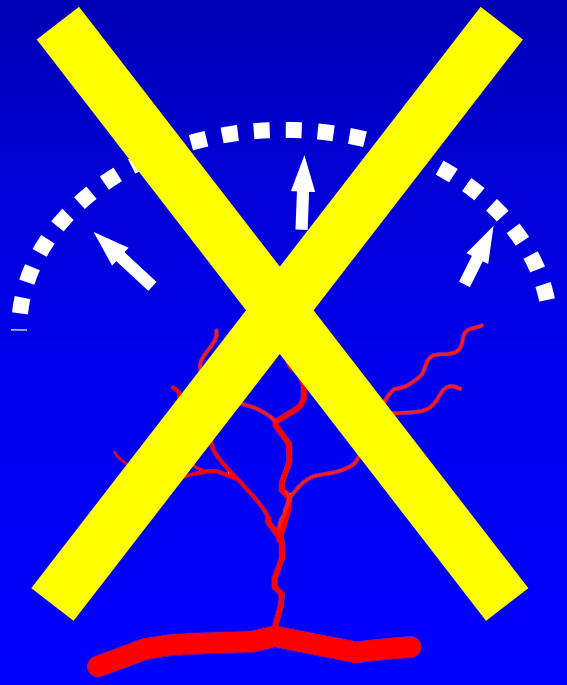
Pruning to baseline

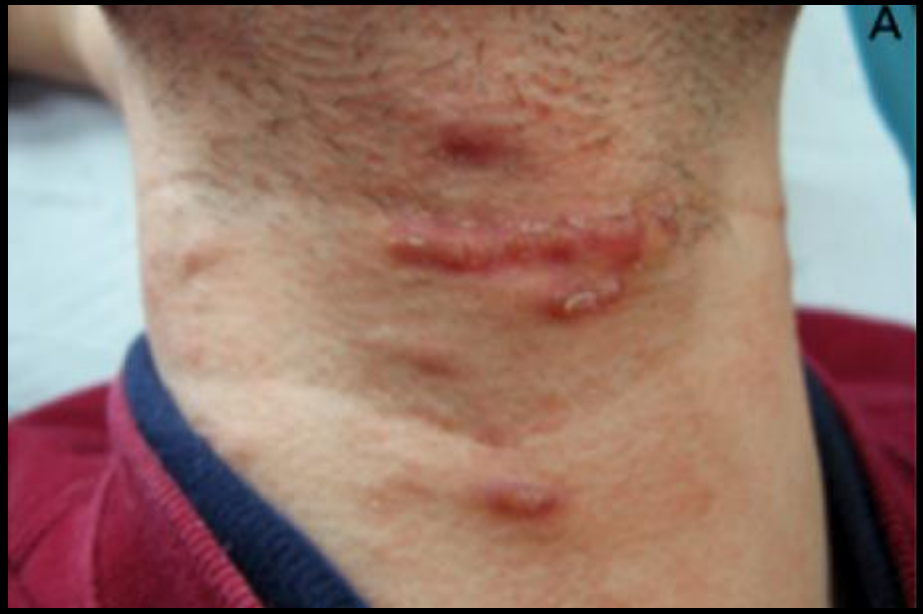
Normal Balance

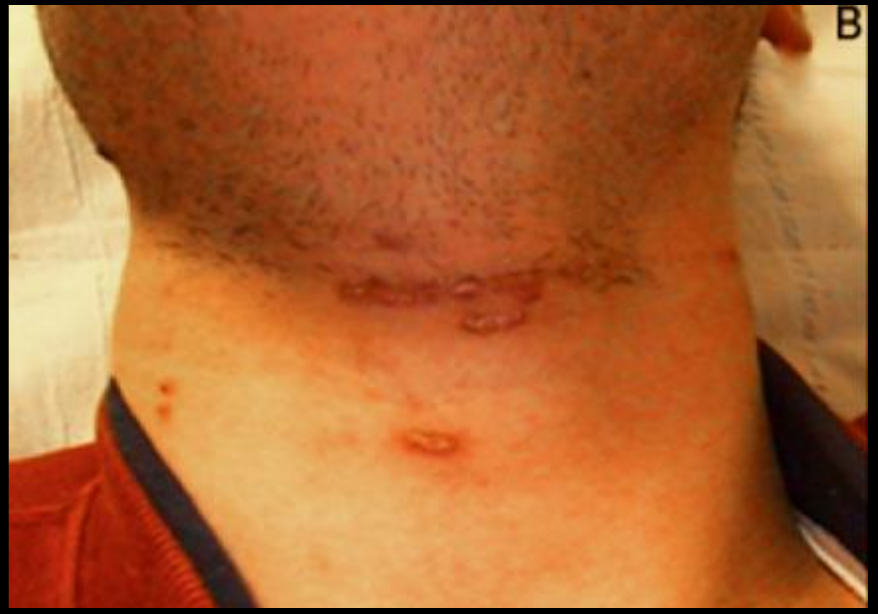
Restoring to baseline

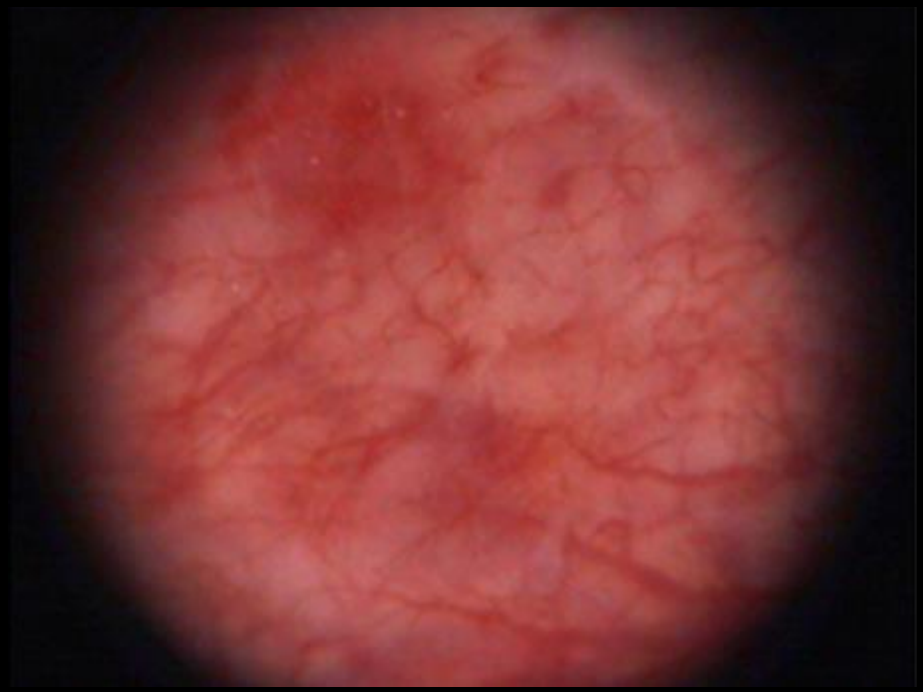


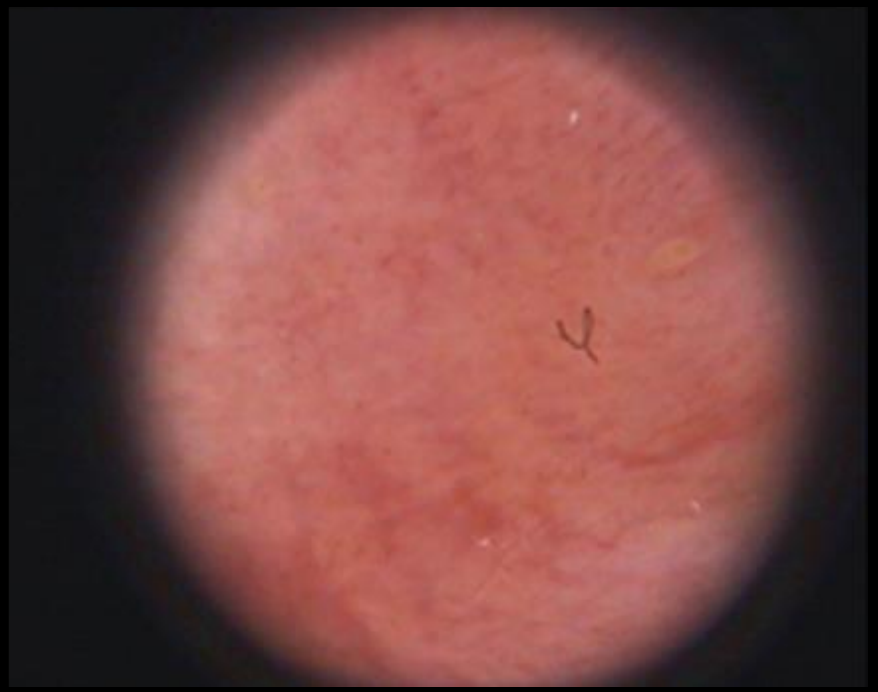






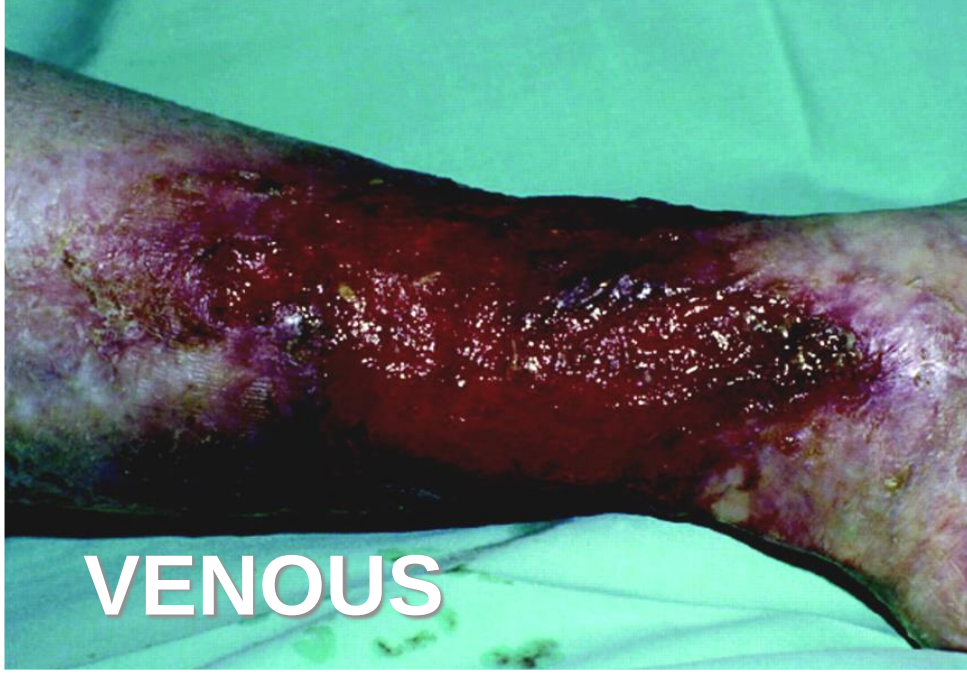


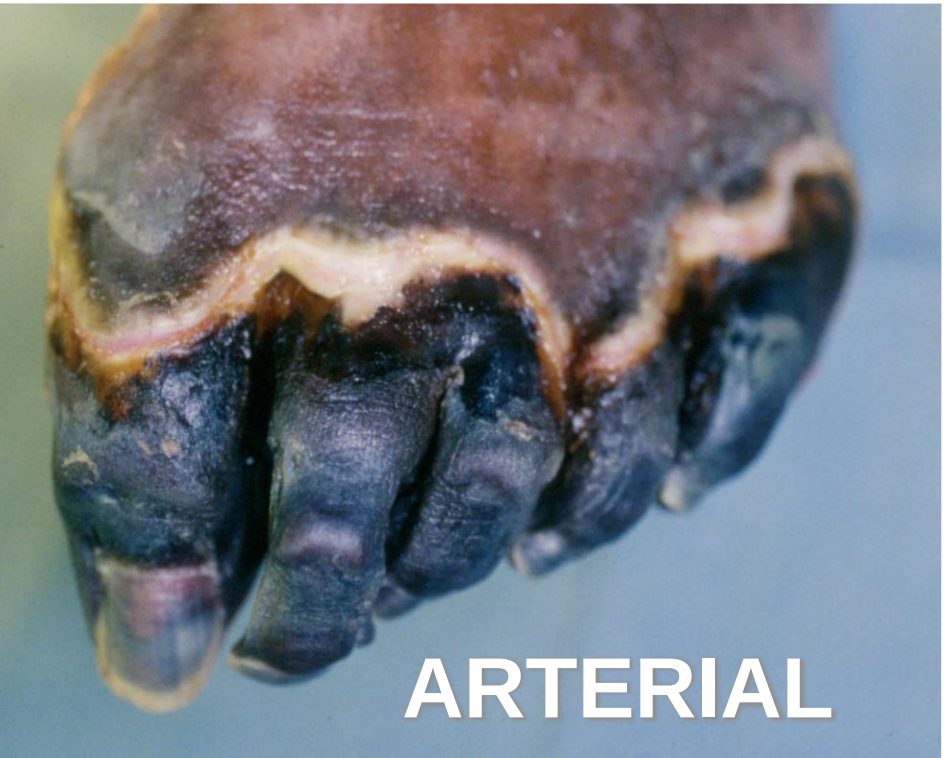


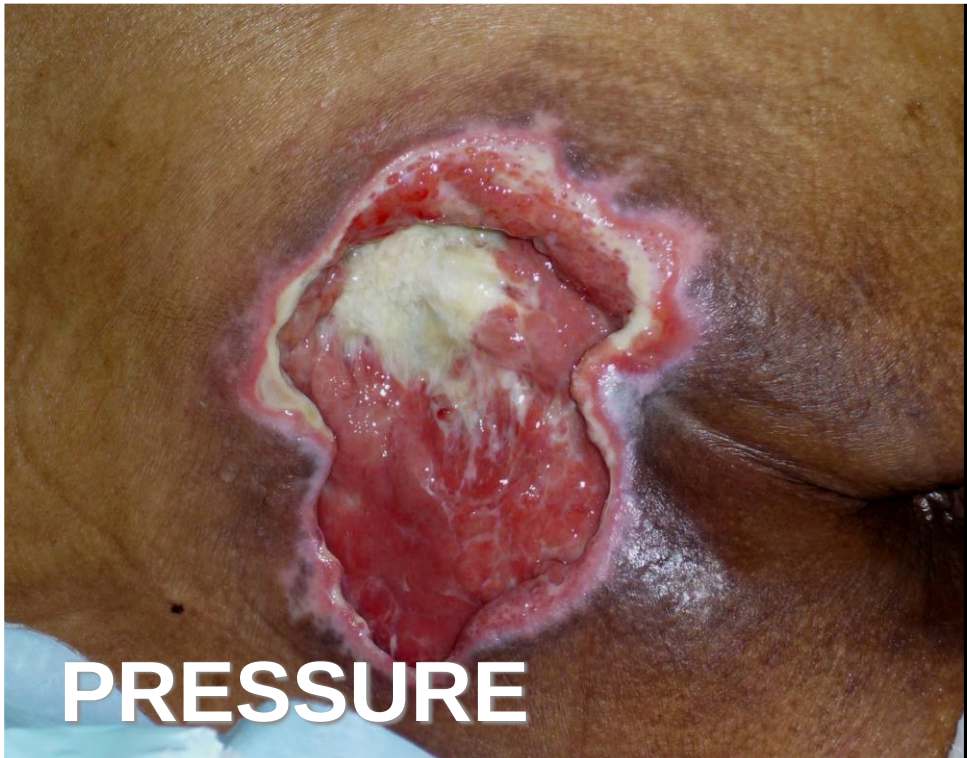


— Dermatol Surg 2010;36:1439-44.


DIABETIC


VENOUS

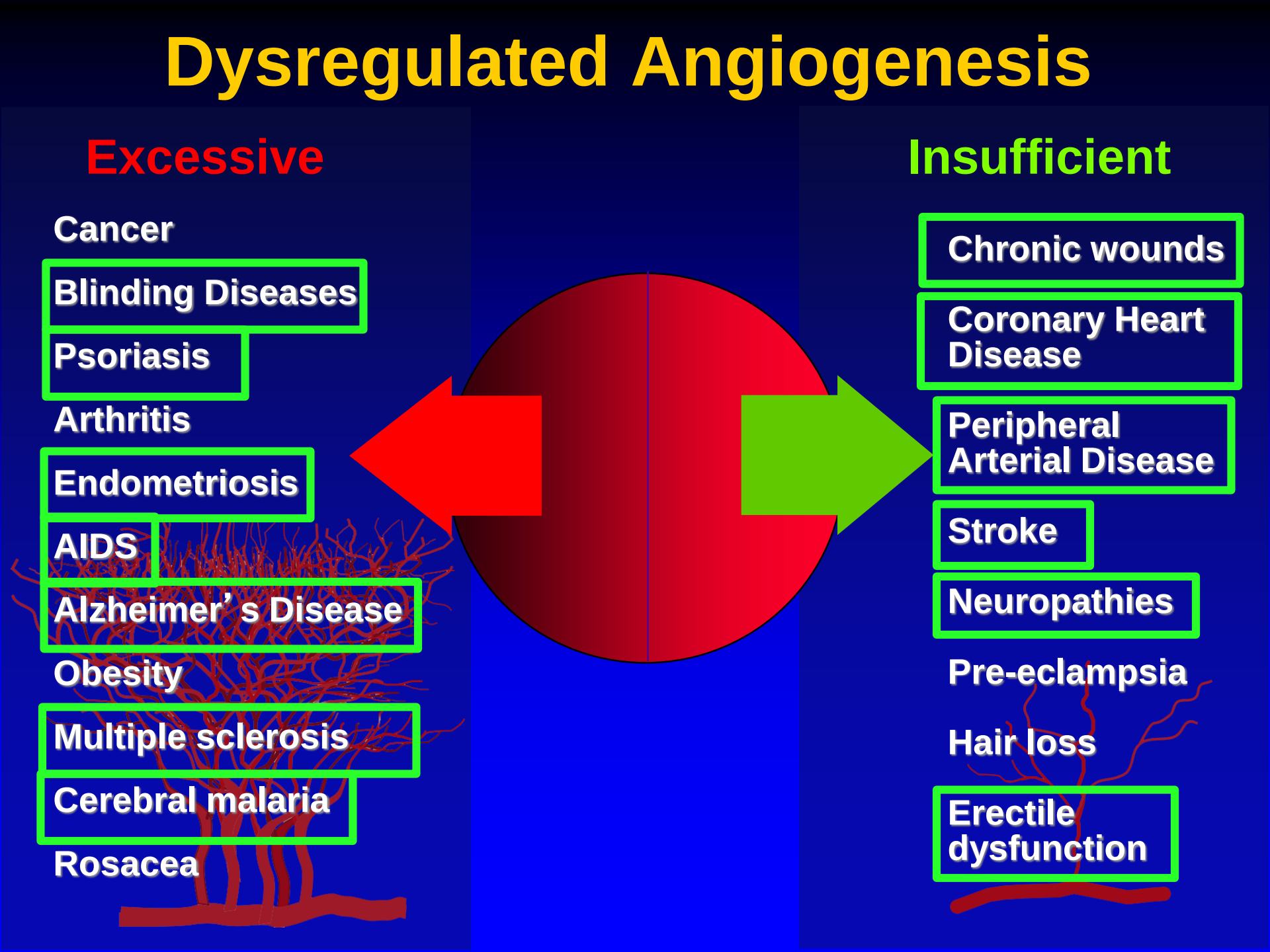

ARTERIAL


PRESSURE

Dysregulated Angiogenesis

Excessive

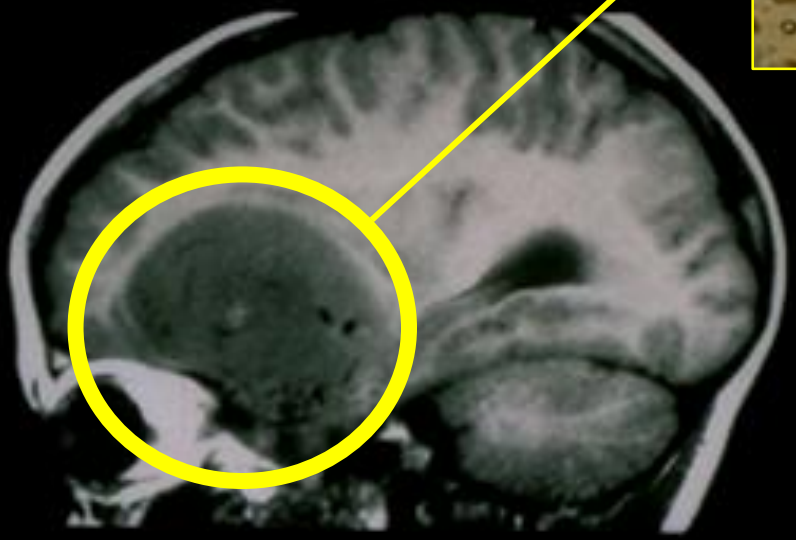
- Cancer
- Blinding Diseases
- Psoriasis
- Arthritis
- Endometriosis
- AIDS
- Alzheimer's Disease
- Obesity
- Multiple sclerosis
- Cerebral malaria
- Rosacea

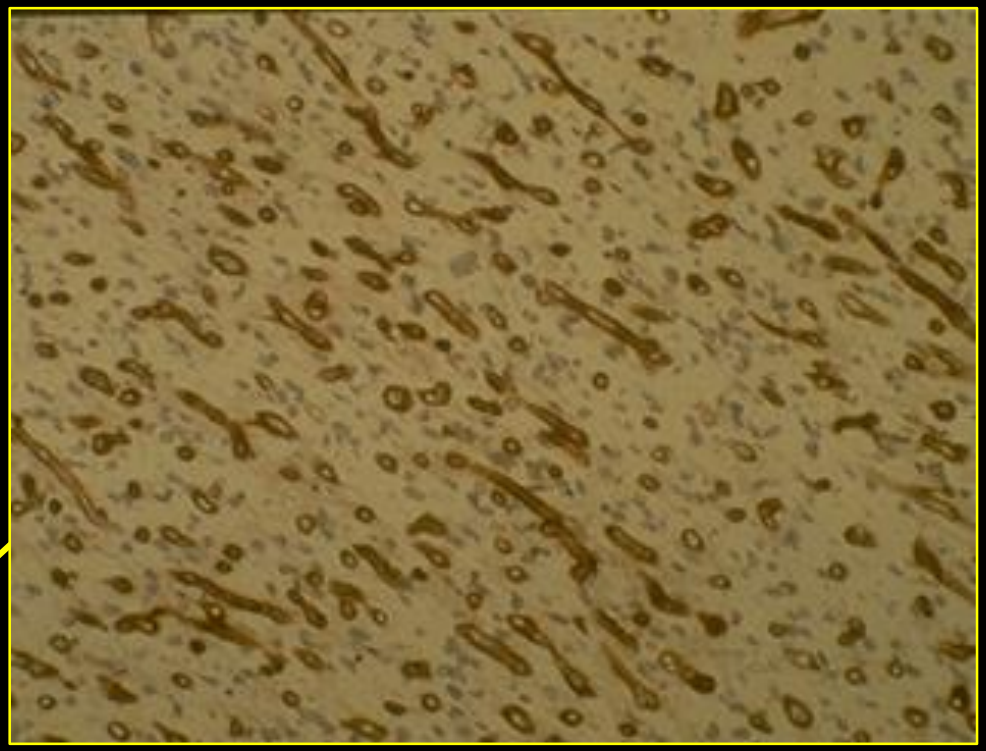


Insufficient

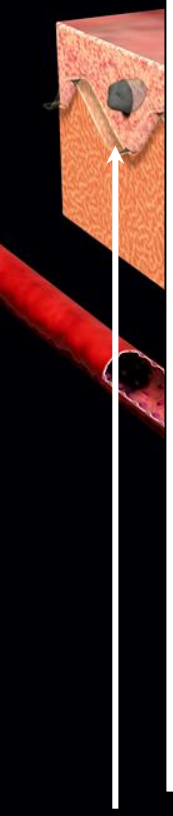
- Chronic wounds
- Coronary Heart Disease
- Peripheral Arterial Disease
- Stroke
- Neuropathies
- Pre-eclampsia
- Hair loss
- Erectile dysfunction

Cancer





NATURE 2004



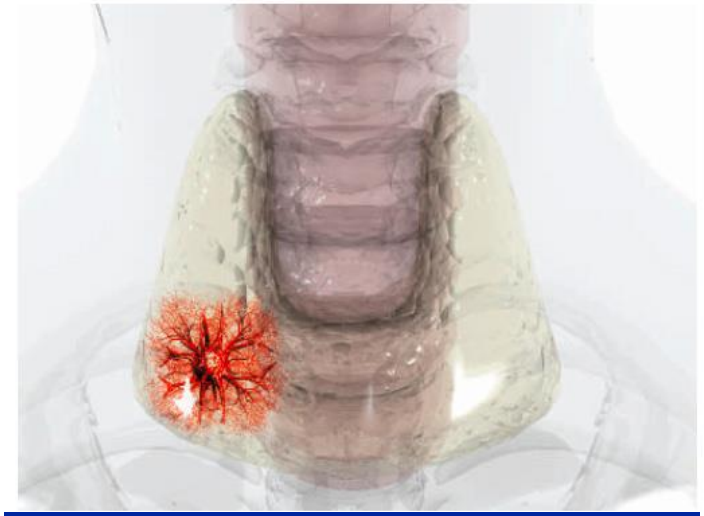
essay concepts

Cancer without disease

Do inhibitors of blood-vessel growth found naturally in our bodies defend most of us against progression of cancer to a lethal stage?

Judah Folkman and Raghu Kalluri

Many of us may have tiny tumours without knowing it. In fact, autopsies of individuals who died of trauma often reveal microscopic colonies of cancer cells, also known as *in situ* tumours. It has been estimated that more than one-third of women aged 40 to 50, who did not have cancer-related disease in their life-time, were found at autopsy with *in situ* tumours in their breast. But breast cancer is diagnosed in only 1% of women in this age range. Similar observations are also reported for prostate cancer in men. Virtually all autopsied individuals aged 50 to 70 have *in situ* carcinomas in their thyroid gland, whereas



Cancer caused by angiogenic tumours of the thyroid gland is a rare event, despite many of us carrying *in situ* tumours.

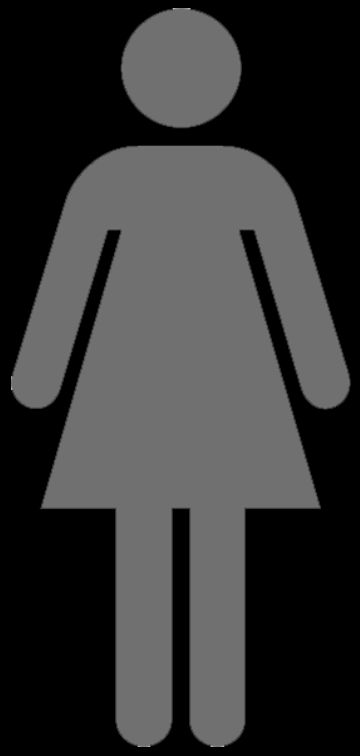
C. DARKIN

generally results in a microscopic tumour where the high rate of tumour cell division is

is a very low incidence of solid tumours in patients with Down Syndrome, who circulate elevated levels of endostatin, an endogenous angiogenesis inhibitor, due to an extra copy of chromosome 21. An increased incidence of prostate cancer in patients with a specific polymorphism in endostatin is also noted. These examples suggest that either an increase or decrease in the angiogenic defence can alter the rate of cancer progression. Therefore, genetic control of physiological levels of endogenous angiogenesis inhibitors, such as thrombospondin-1, tumstatin and endostatin, may provide a last line of defence against the conversion of *in situ* tumours into a malignant phenotype of cancer. Although drugs such as Avastin, which inhibits VEGF (the growth

Microscopic tumor (0.5 mm^3)

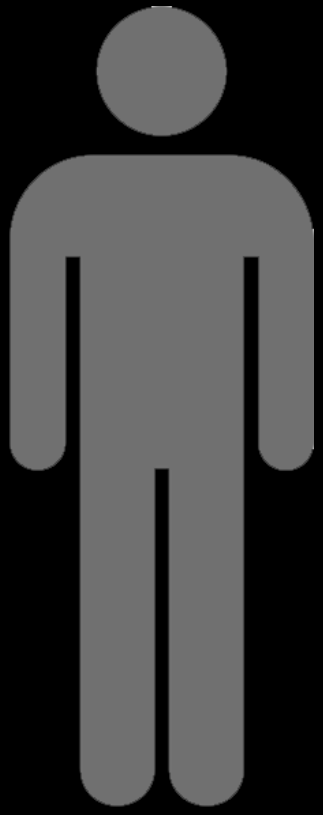
Autopsies of healthy women 40-50 years old who died from trauma



40%

Microscopic breast cancer

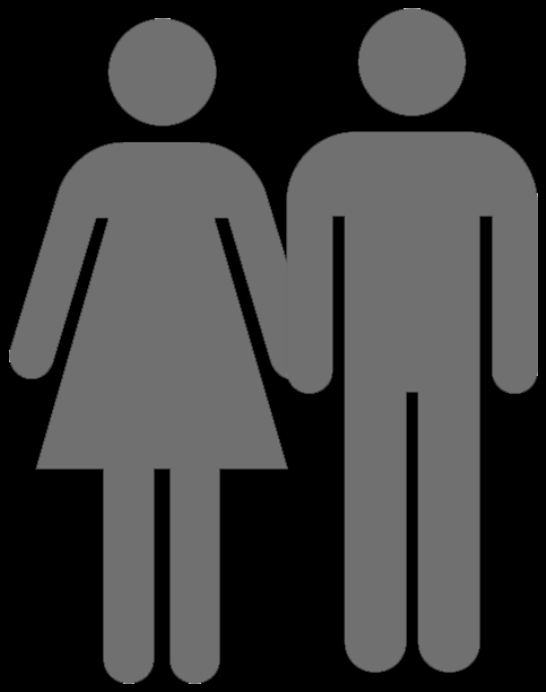
Healthy men 50-60 years old



50%

Microscopic prostate cancer

People in their 70s

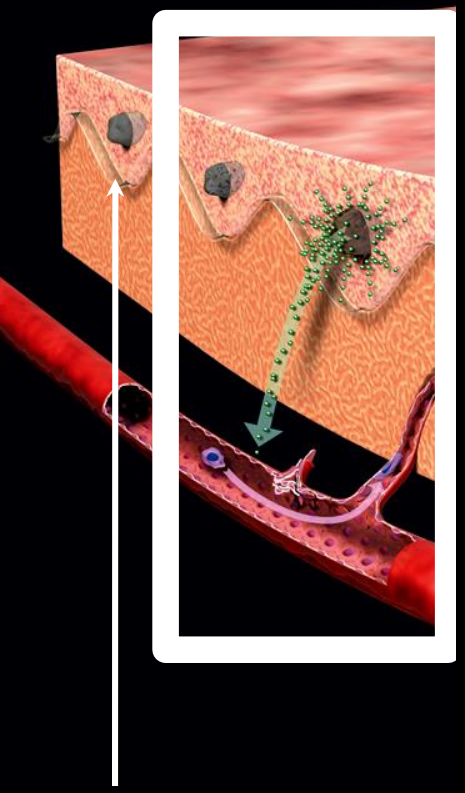


100%

Microscopic thyroid cancer

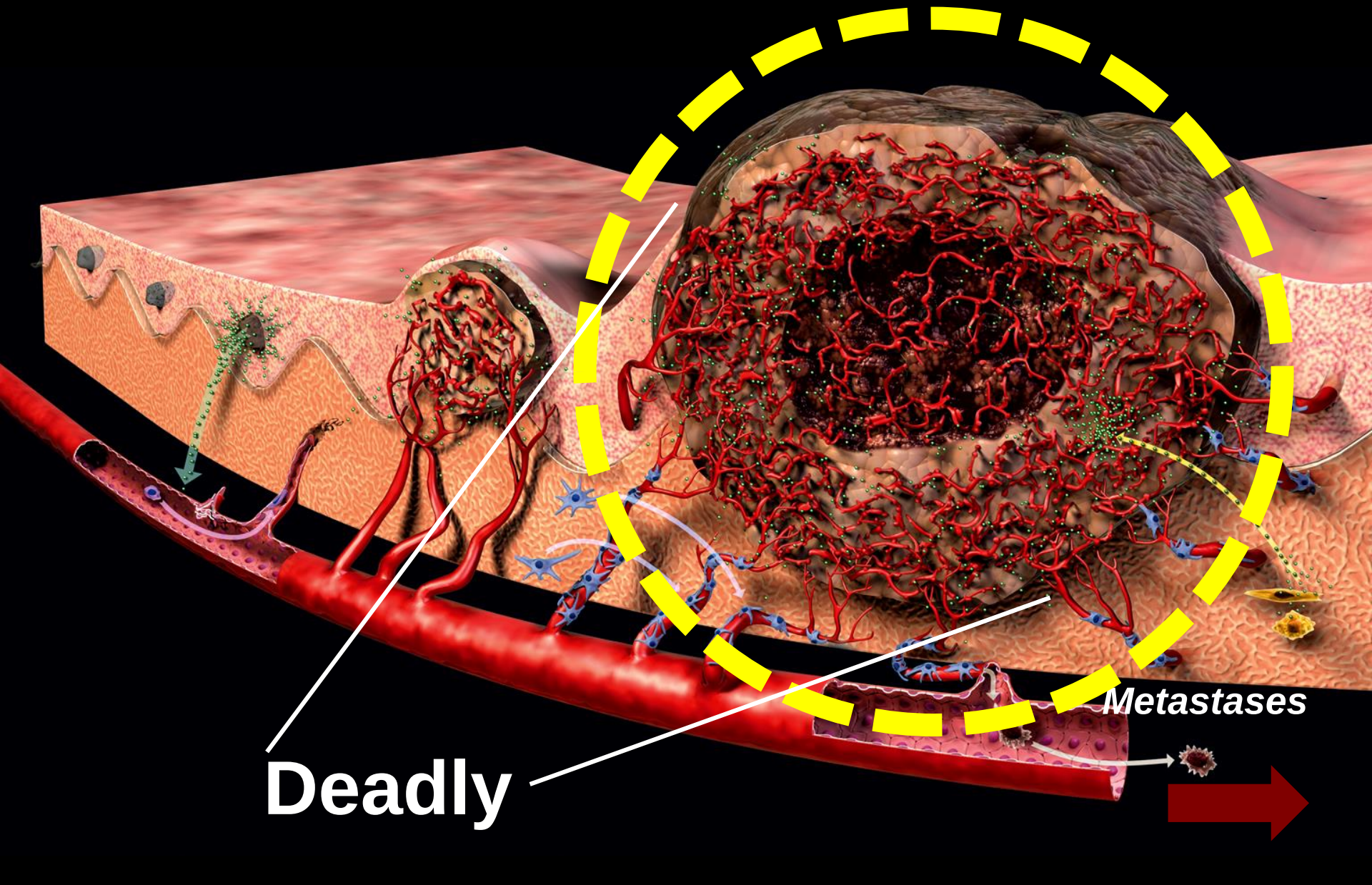
How cancer becomes dangerous

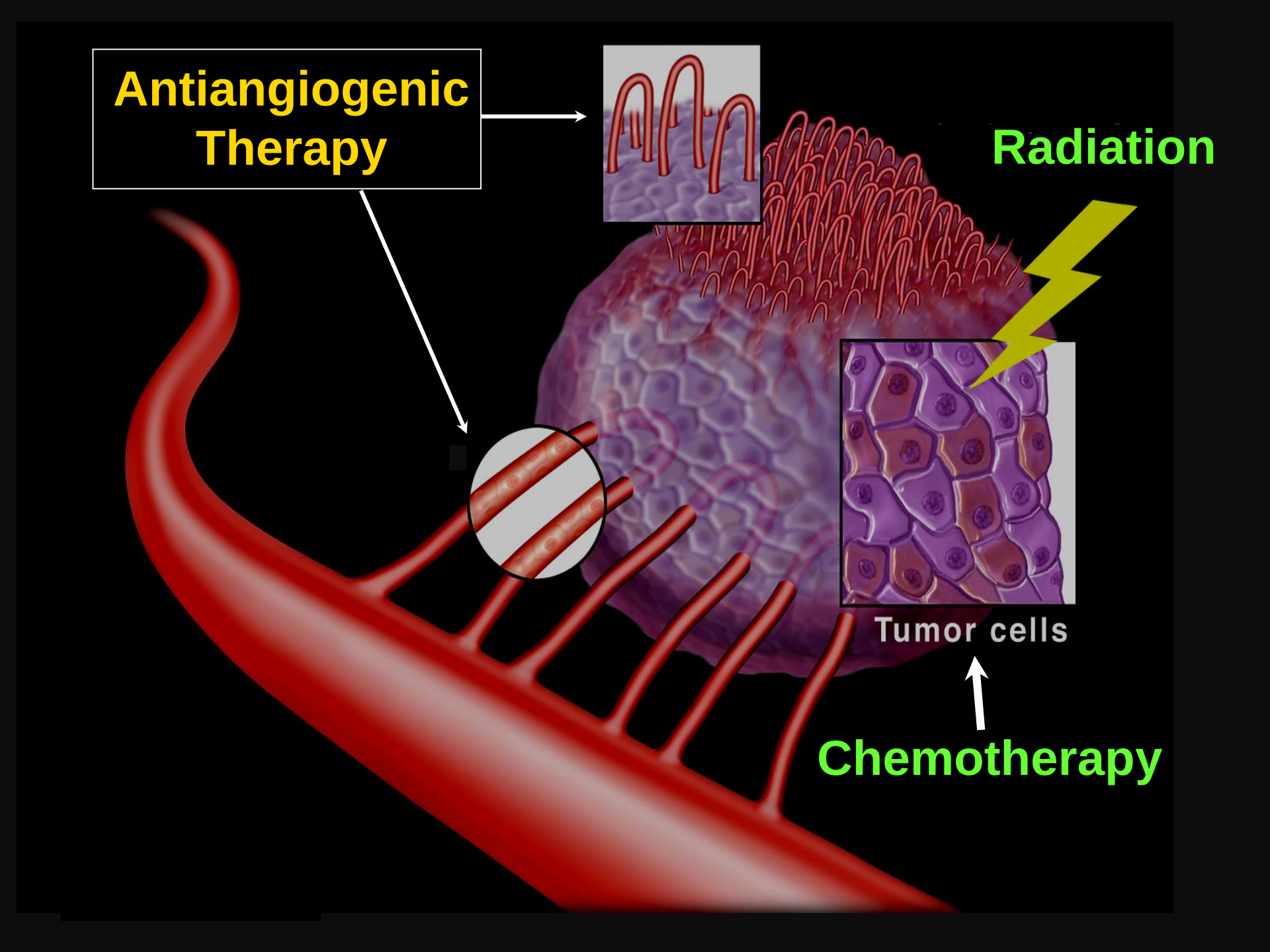
YEARS …



Harmless

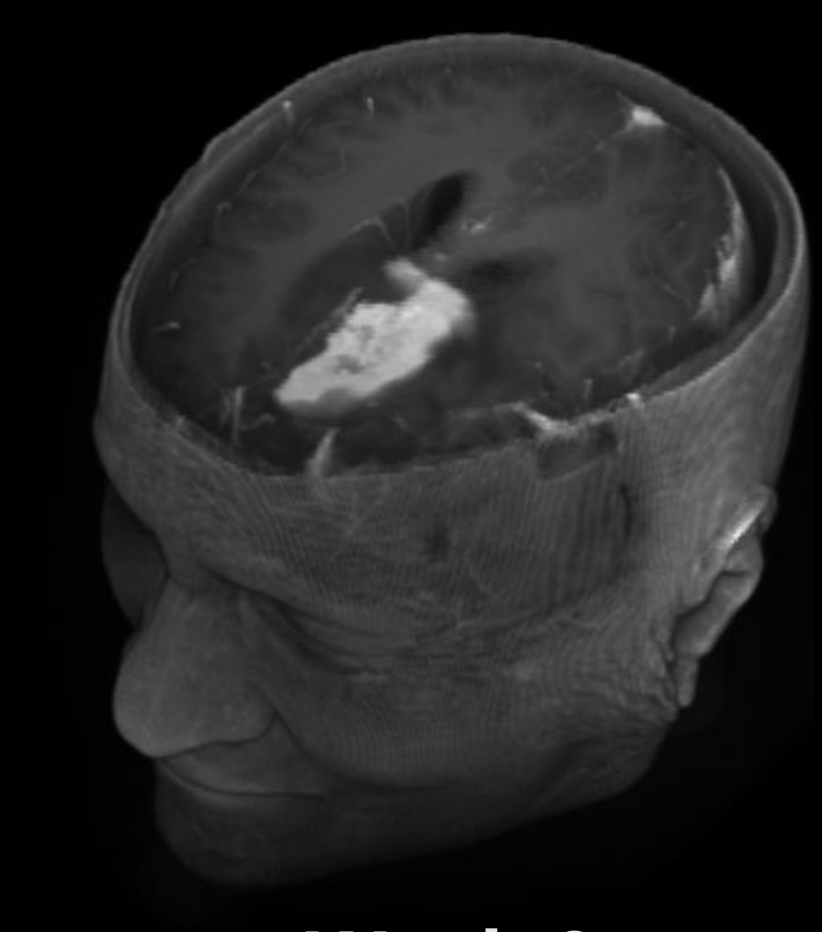
How cancer becomes dangerous



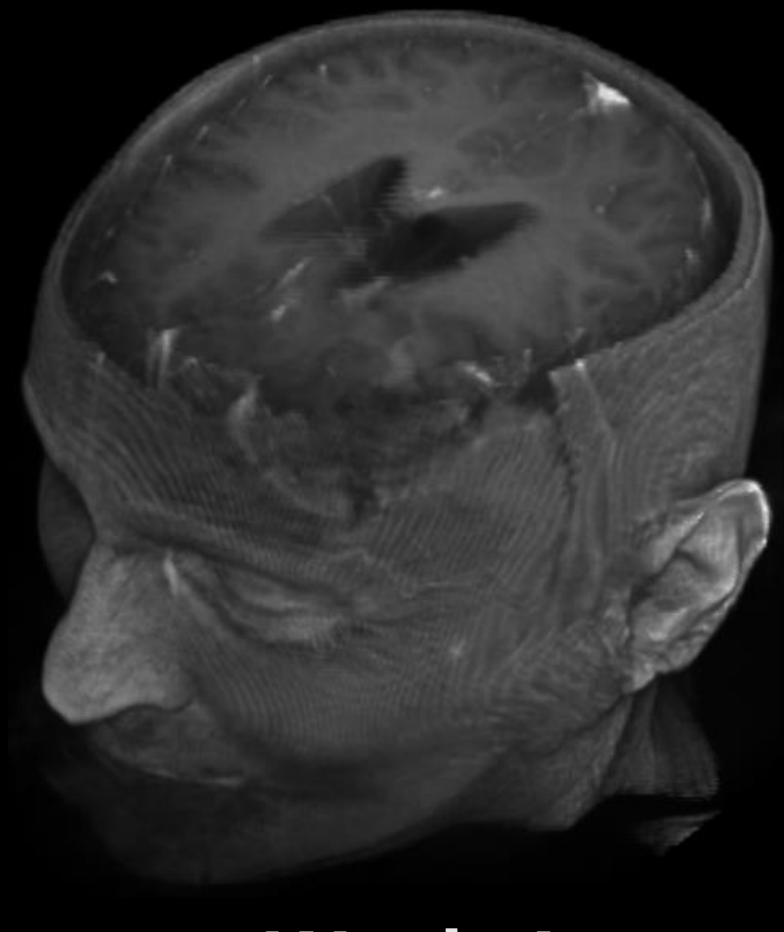

Antiangiogenic Therapy
Radiation
Tumor cells
Chemotherapy

Antiangiogenic Therapy

Brain Tumor



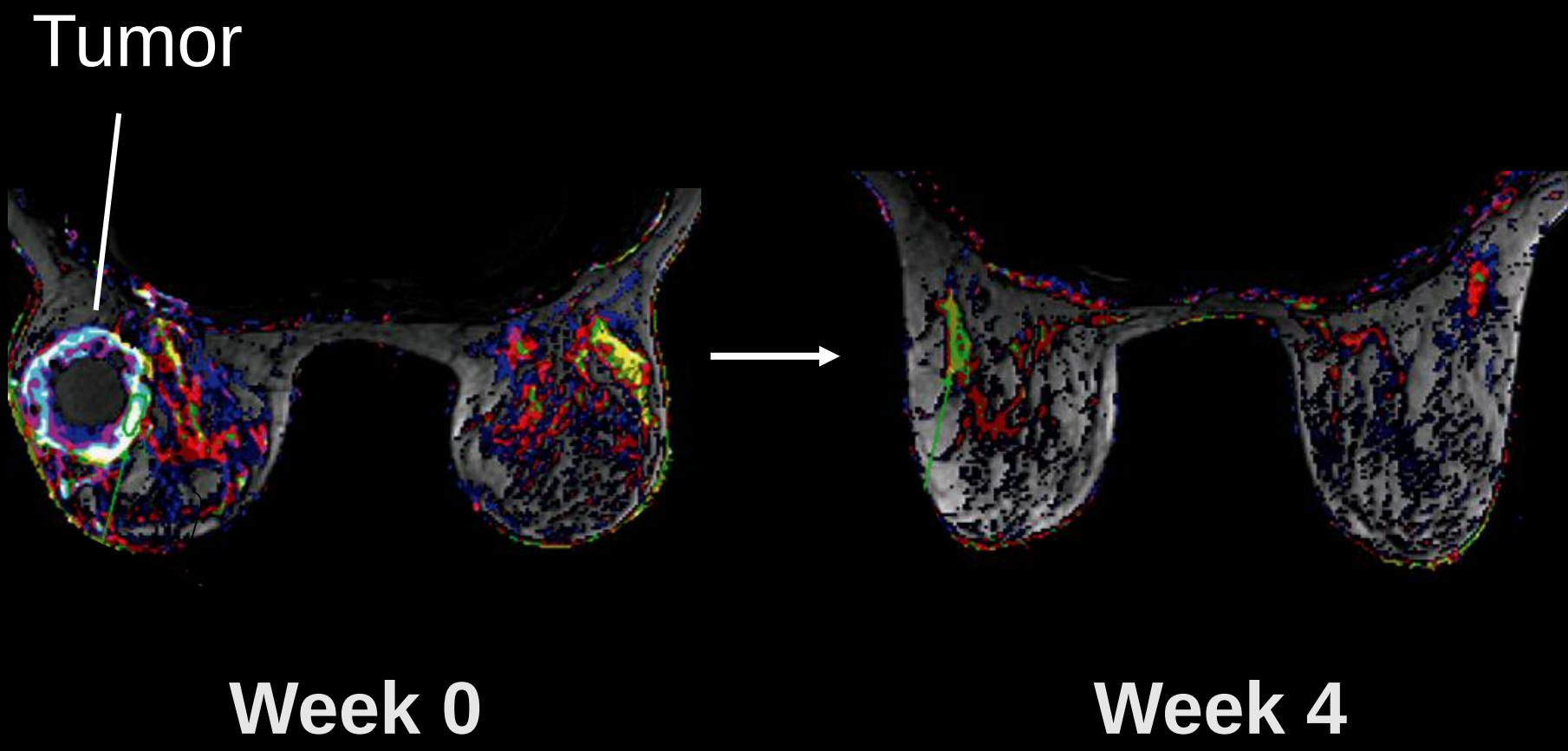
Week 0



Week 4

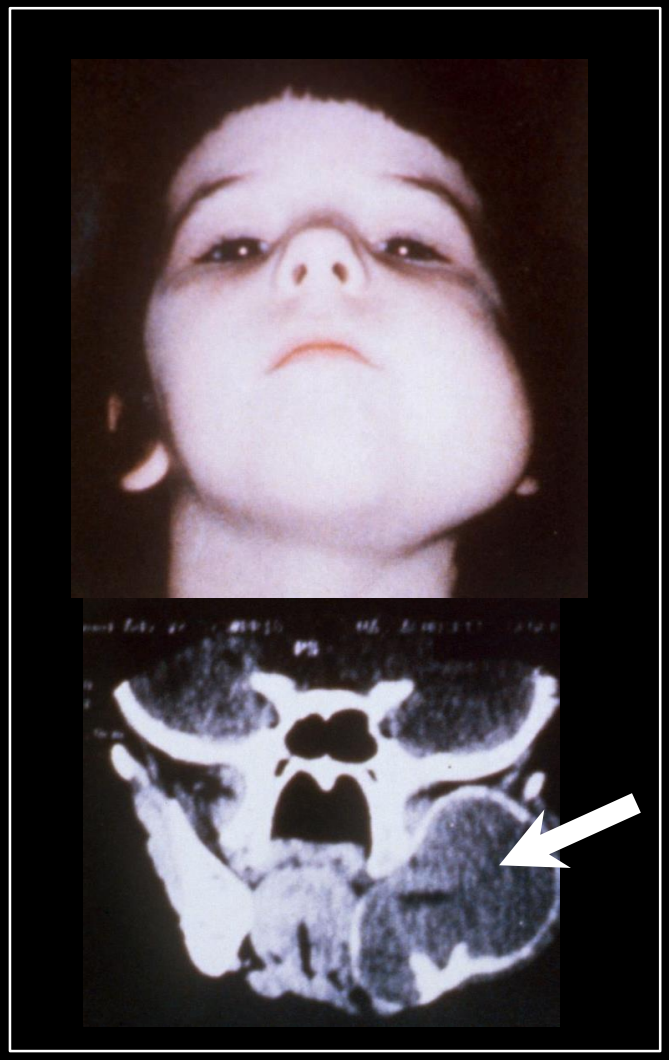
Antiangiogenic Therapy

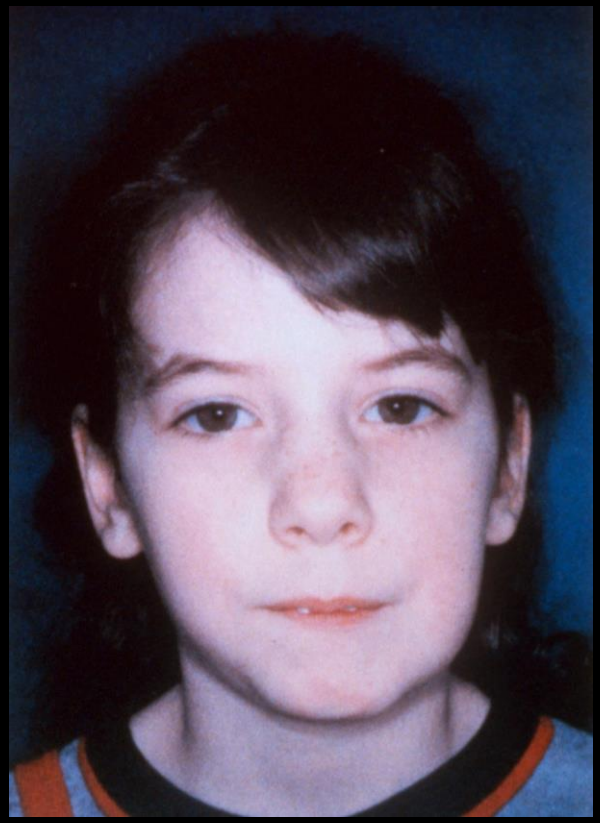
Breast Cancer



Antiangiogenic Therapy

Giant cell tumor

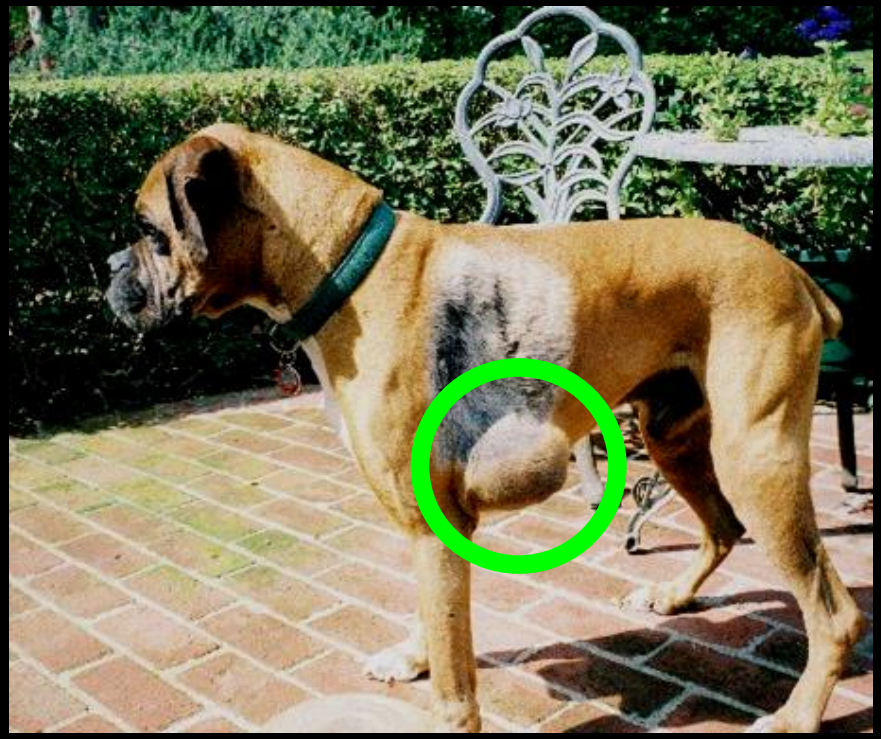




One Year Later

Antiangiogenic Therapy

Malignant Neurofibroma



Week 0

Week 7

Angiogenic Stimulatory Factors

FGF (1-7)

VEGF/VPF

TGF-α

TGF-β

TNF-α

Angiogenin

IL-3

IL-8

PD-ECGF/ *(Thymidine phosphorylase)*

G-CSF

Placental Growth Factor

Scatter Factor/HGF

Pleiotrophin

Proliferin

Progranulin

Follistatin

PDGF-BB

Corticotropin-releasing hormone

Cyr16

VG5Q

Adrenmedullin

BDNF

NGF

Substance P

Neurokinin A

Neuropeptide Y

Secretoneurin

midkine

Many Endogenous Angiogenesis Inhibitors

- Interferon α
- Interferon β
- Interferon γ
- Angiostatin
- Endostatin
- Kringle 5
- TIMPs
- IL-4,-10,-12
- IP-10
- MIG
- Canstatin
- Tumstatin
- Thombospondin-1
- Platelet factor 4
- Prolactin 18KD
- Tetrahydro S
- Vasostatin
- PEDF
- Meth-1,-2
- PAI
- Troponin-1
- 2-Methoxyestradiol
- Fibronectin 20KD
- Maspin
- CTAP III
- PSP94
- PSA
- Vasoinhibin-1
- ARHGAP18
- VEGF-Ax

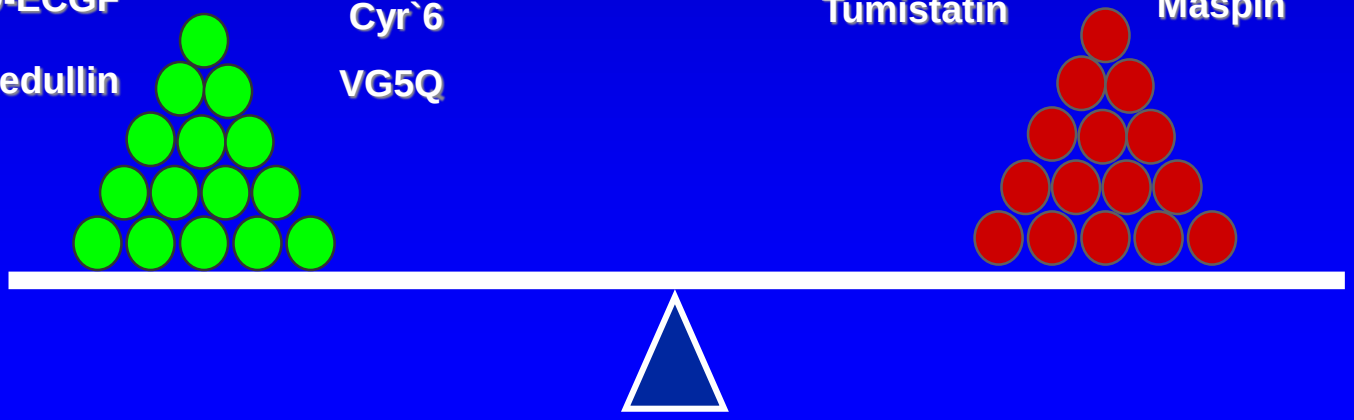
Continuous Homeostatic Balance is KEY

Positive

FGF (1-7)
VEGF/VPF
TGF-α
TGF-β
TNF-α
Angiogenin
IL-3
IL-8
PD-ECGF
Adrenomedullin
PlGF
G-CSF
Scatter Factor
Pleiotrophin
Proliferin
Follistatin
midkine
PDGF-BB
Cyr`6
VG5Q

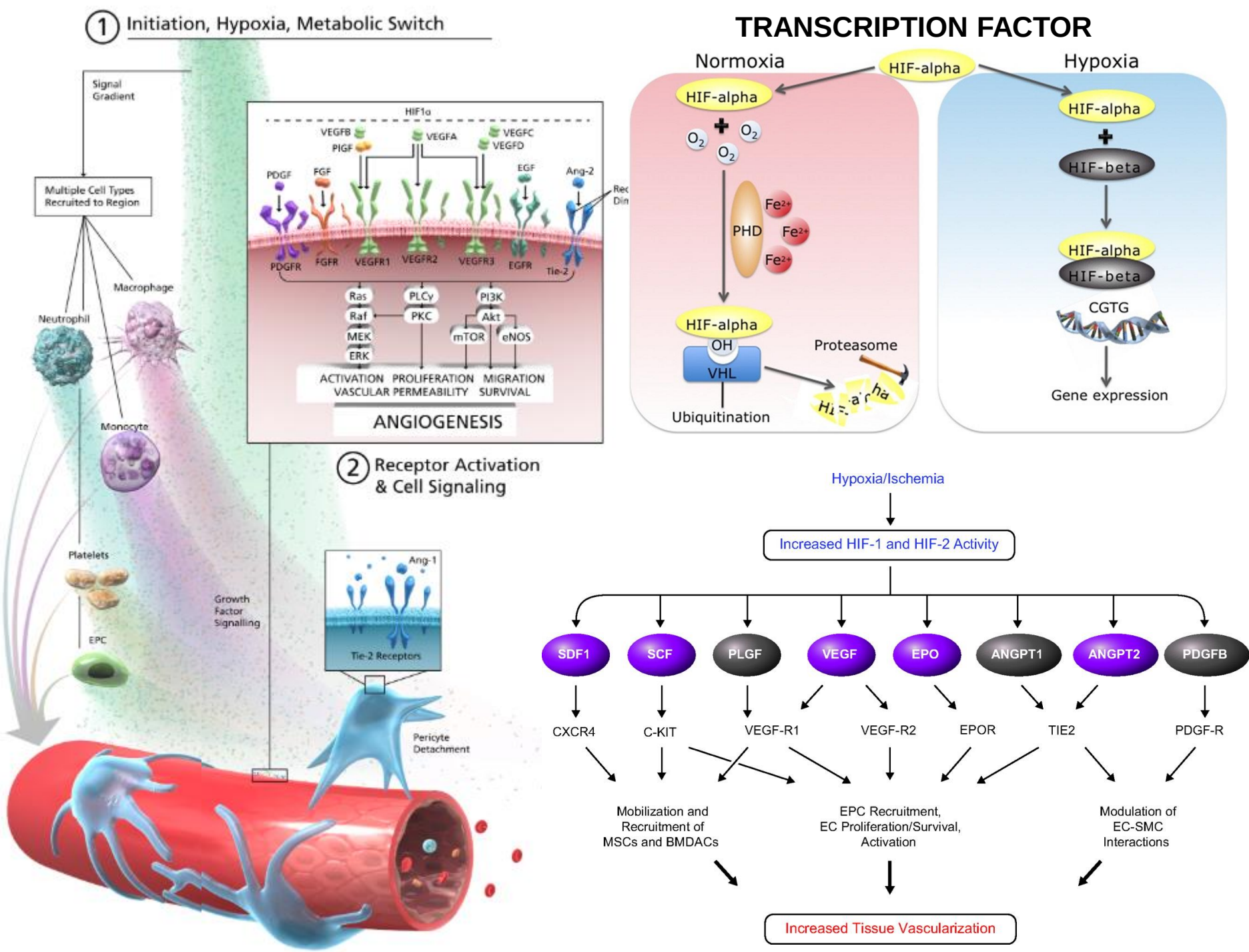
Negative

Interferon- α
Interferon- β
Interferon-γ
Angiostatin
Endostatin
Kringle 5
TIMPs
IL-4, -10, 12
IP-10
MIG
Canstatin
Tumistatin
Thrombospondin-1
Platelet factor-4
Prolactin 16 Kd
Tetrahydro-S
Vasostatin
PEDF
Meth-1, -2
PAI
Troponin-1
2-Methoxyestradiol
Fibronectin 20 Kd
Maspin

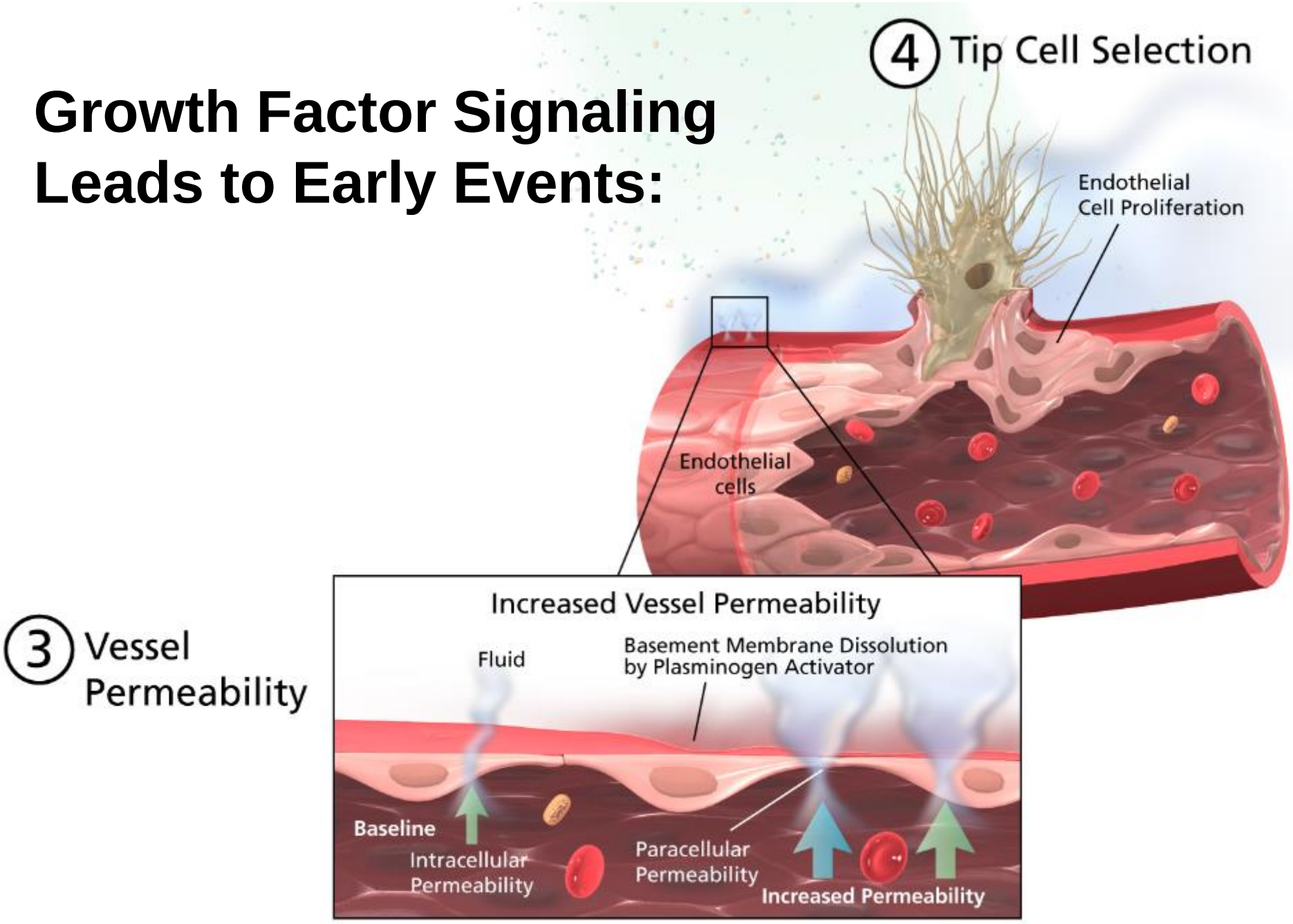


So, how does angiogenesis occur?

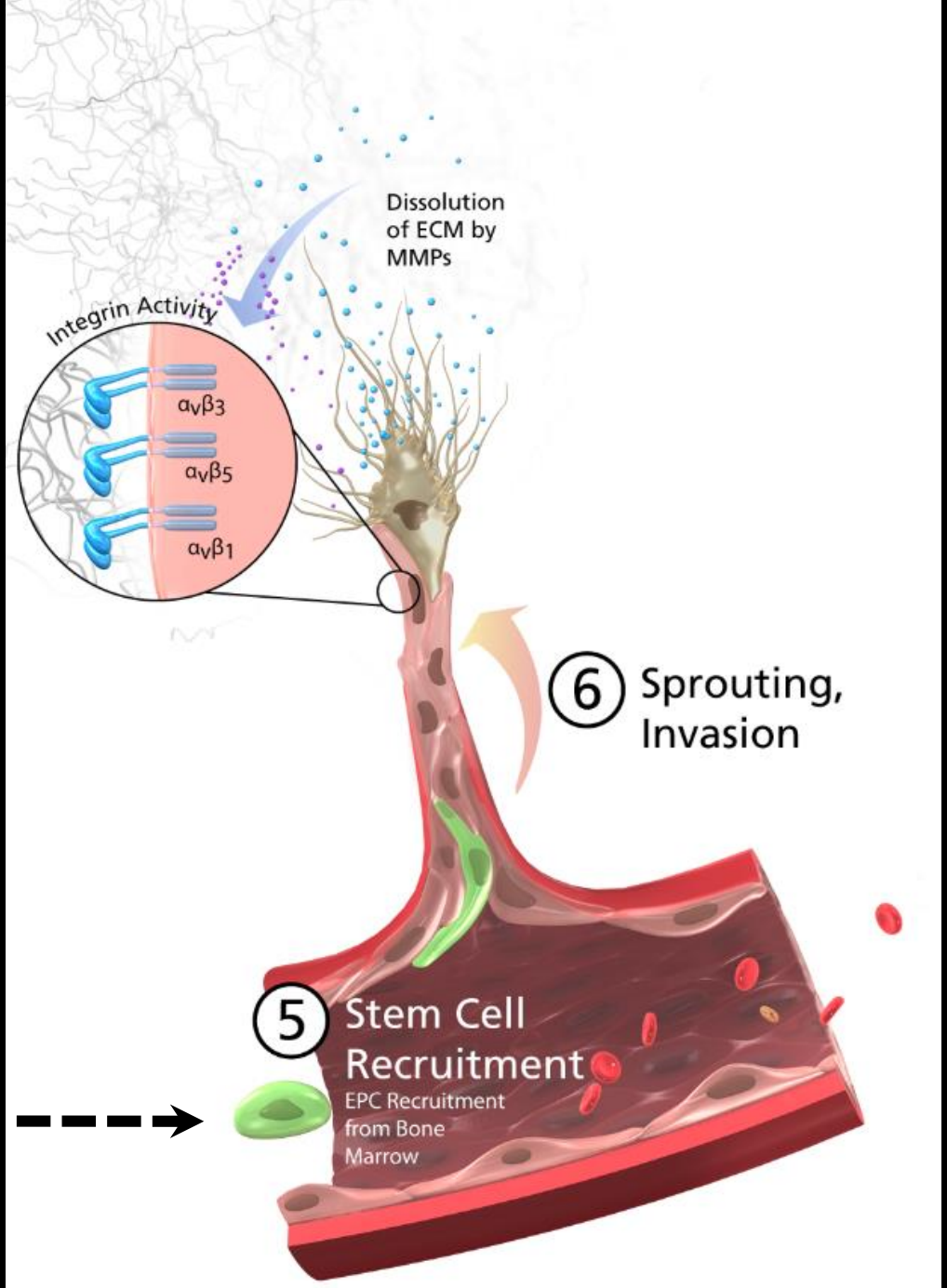
2015 Update


1 Initiation, Hypoxia, Metabolic Switch
Signal Gradient
Multiple Cell Types Recruited to Region
Macrophage
Neutrophil
Monocyte
Platelets
EPC
HIF1α
VEGFB
PIGF
VEGFA
VEGFC
VEGFD
PDGF
FGF
EGF
Ang-2
PDGFR
FGFR
VEGFR1
VEGFR2
VEGFR3
EGFR
Tie-2
Ras
Raf
MEK
ERK
PLCγ
PKC
PI3K
Akt
mTOR
eNOS
ACTIVATION PROLIFERATION MIGRATION
VASCULAR PERMEABILITY SURVIVAL
ANGIOGENESIS
2 Receptor Activation & Cell Signaling
Growth Factor Signalling
Ang-1
Tie-2 Receptors
Pericyte Detachment
TRANSCRIPTION FACTOR
Normoxia
HIF-alpha
O2
PHD
Fe2+
HIF-alpha
OH
VHL
Proteasome
Ubiquitination
Hypoxia
HIF-alpha
HIF-beta
HIF-alpha
HIF-beta
CGTG
Gene expression
Hypoxia/Ischemia
Increased HIF-1 and HIF-2 Activity
SDF1
SCF
PLGF
VEGF
EPO
ANGPT1
ANGPT2
PDGFB
CXCR4
C-KIT
VEGF-R1
VEGF-R2
EPOR
TIE2
PDGF-R
Mobilization and Recruitment of MSCs and BMDACs
EPC Recruitment, EC Proliferation/Survival, Activation
Modulation of EC-SMC Interactions
Increased Tissue Vascularization

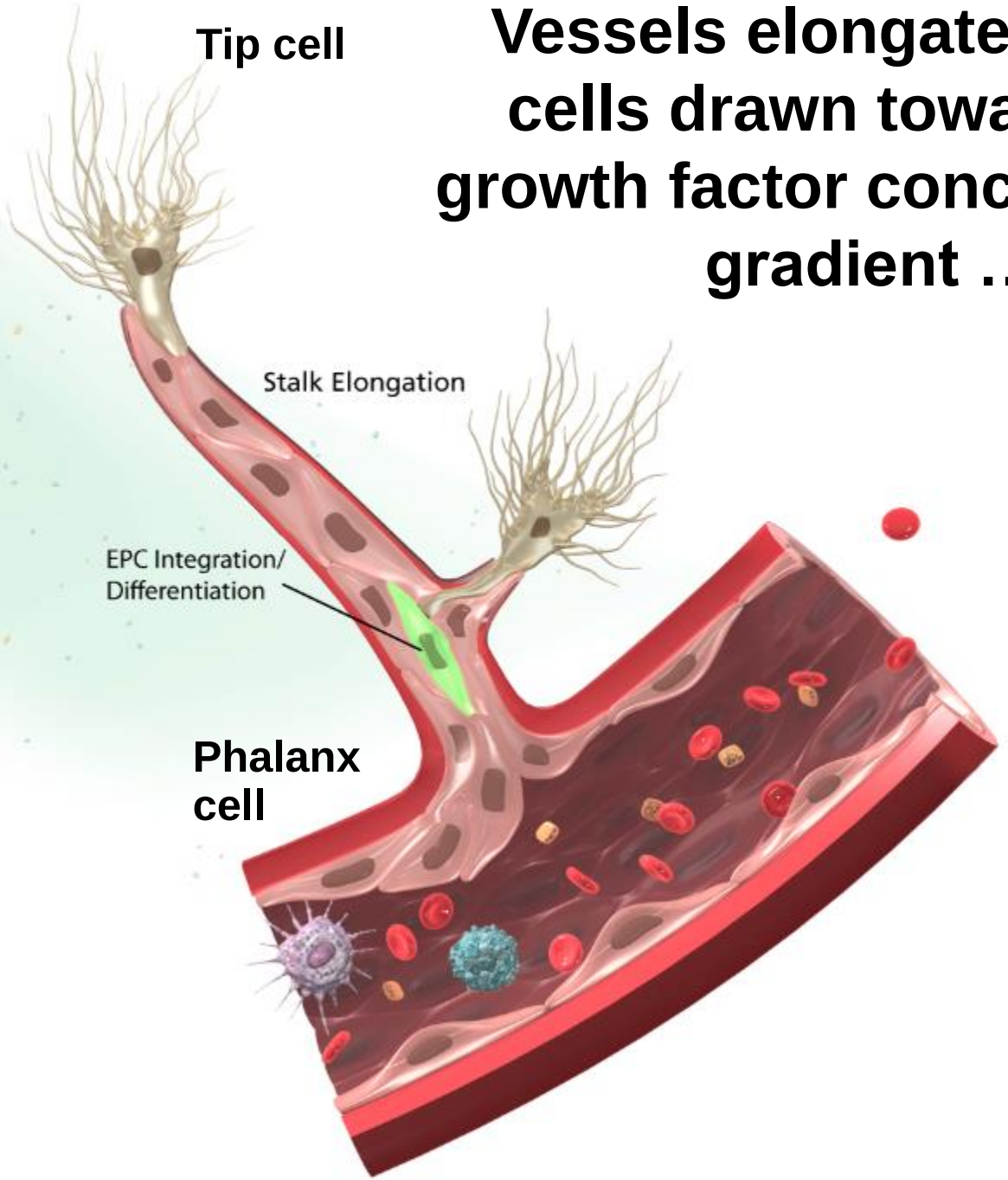
Growth Factor Signaling Leads to Early Events:



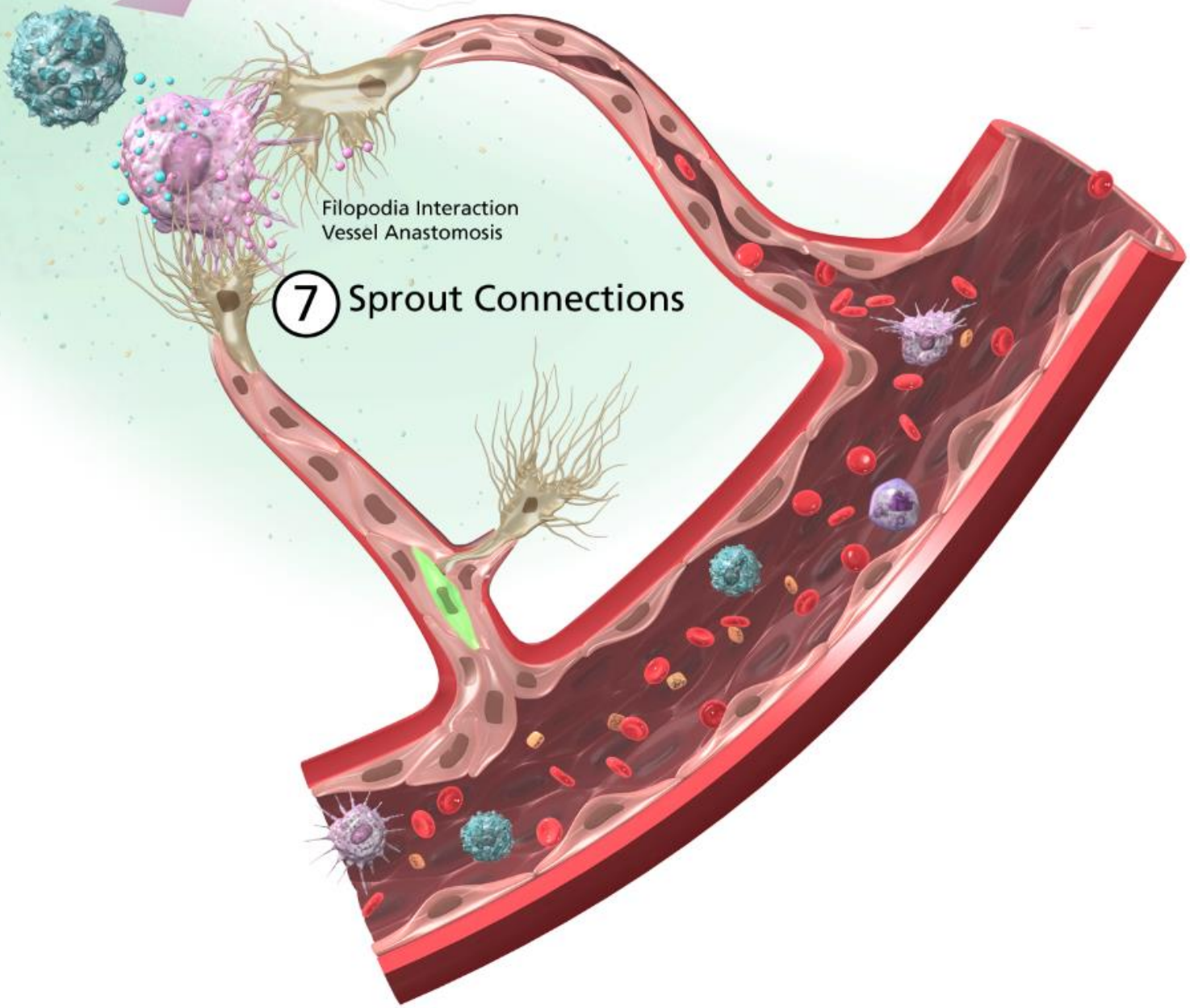
Soluble Growth Factor signals also mobilize stem cells …



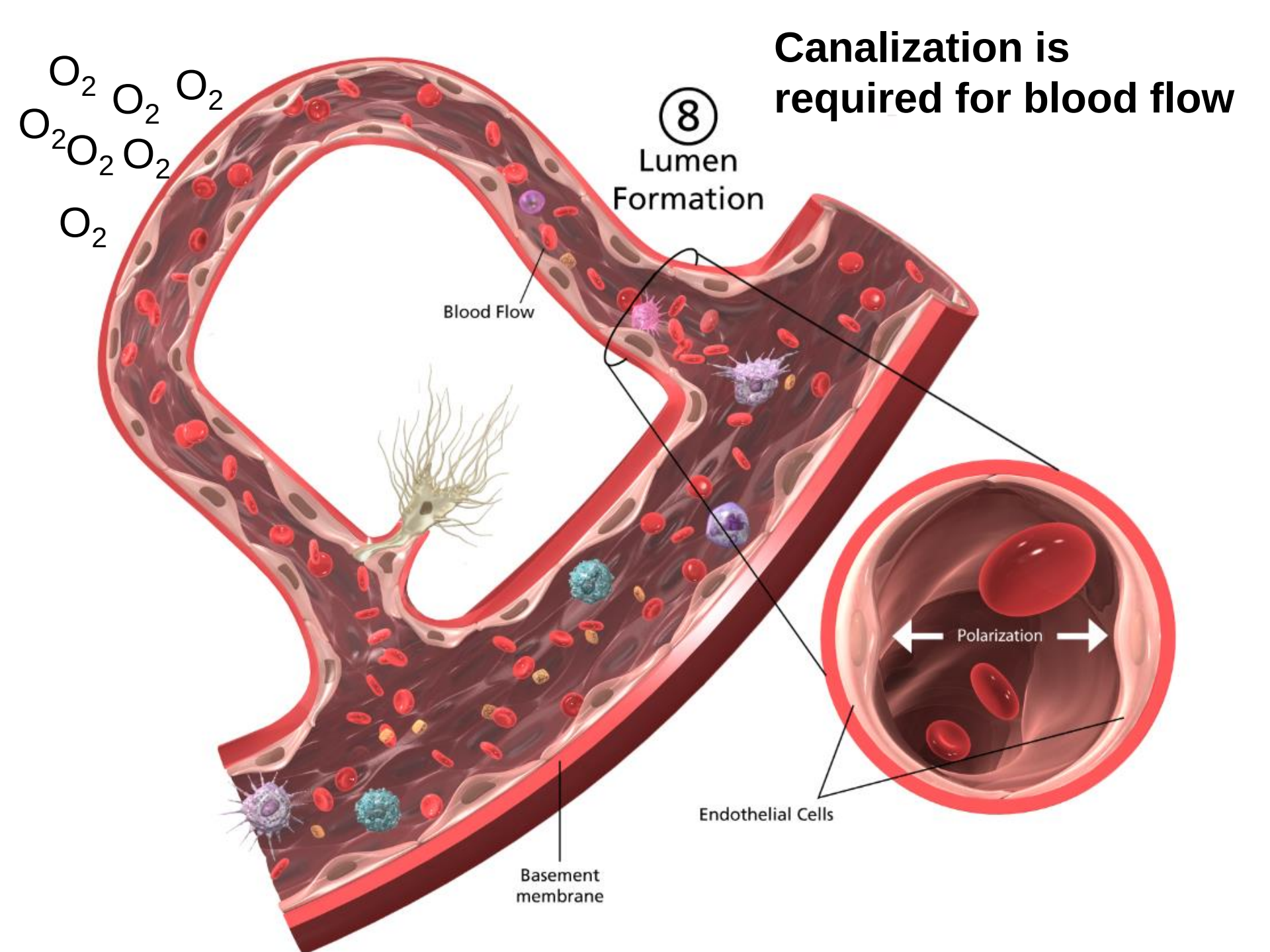
Vessels elongate with tip cells drawn towards the growth factor concentration gradient …



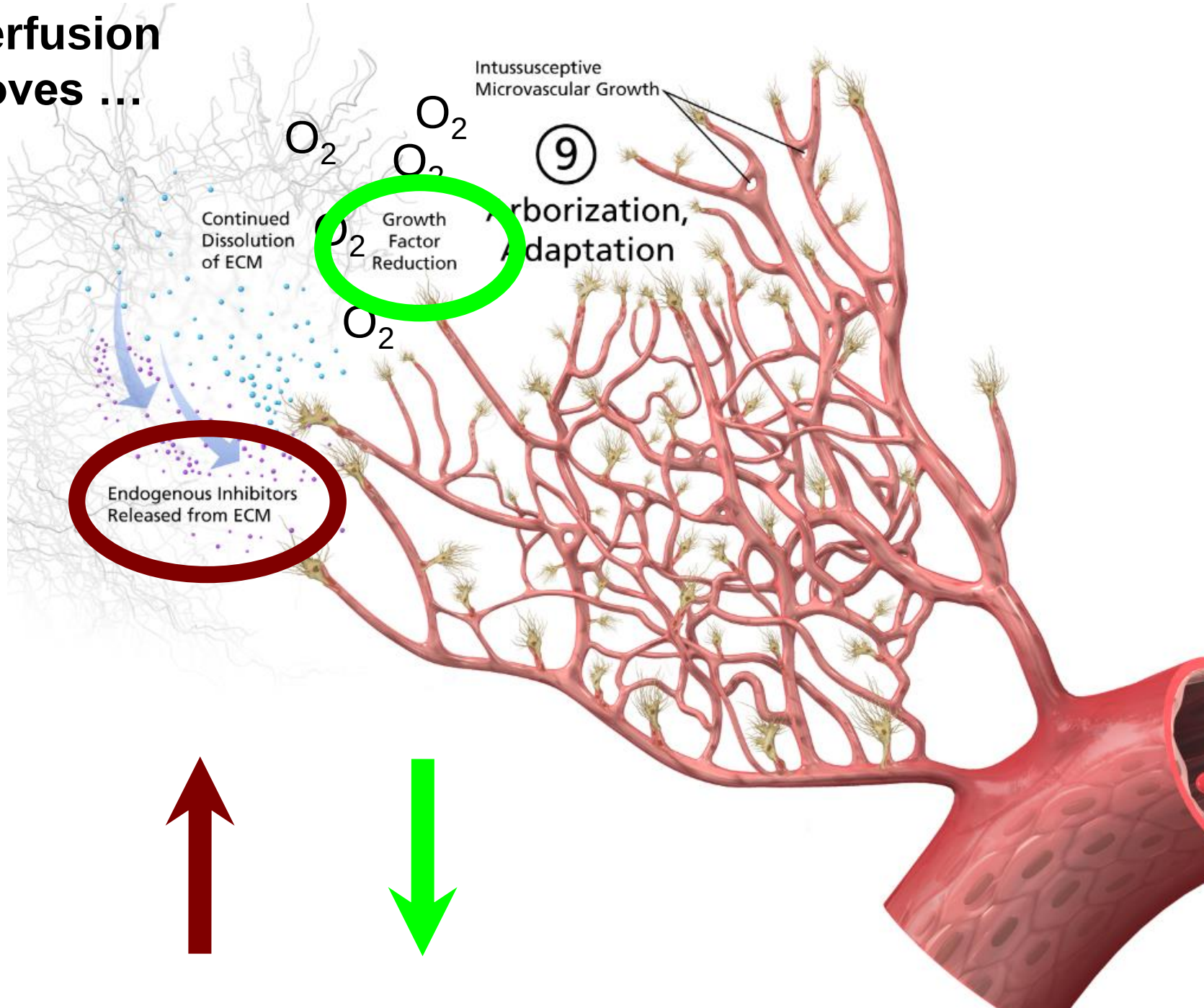
Inflammatory cells ‘broker’ loop formation

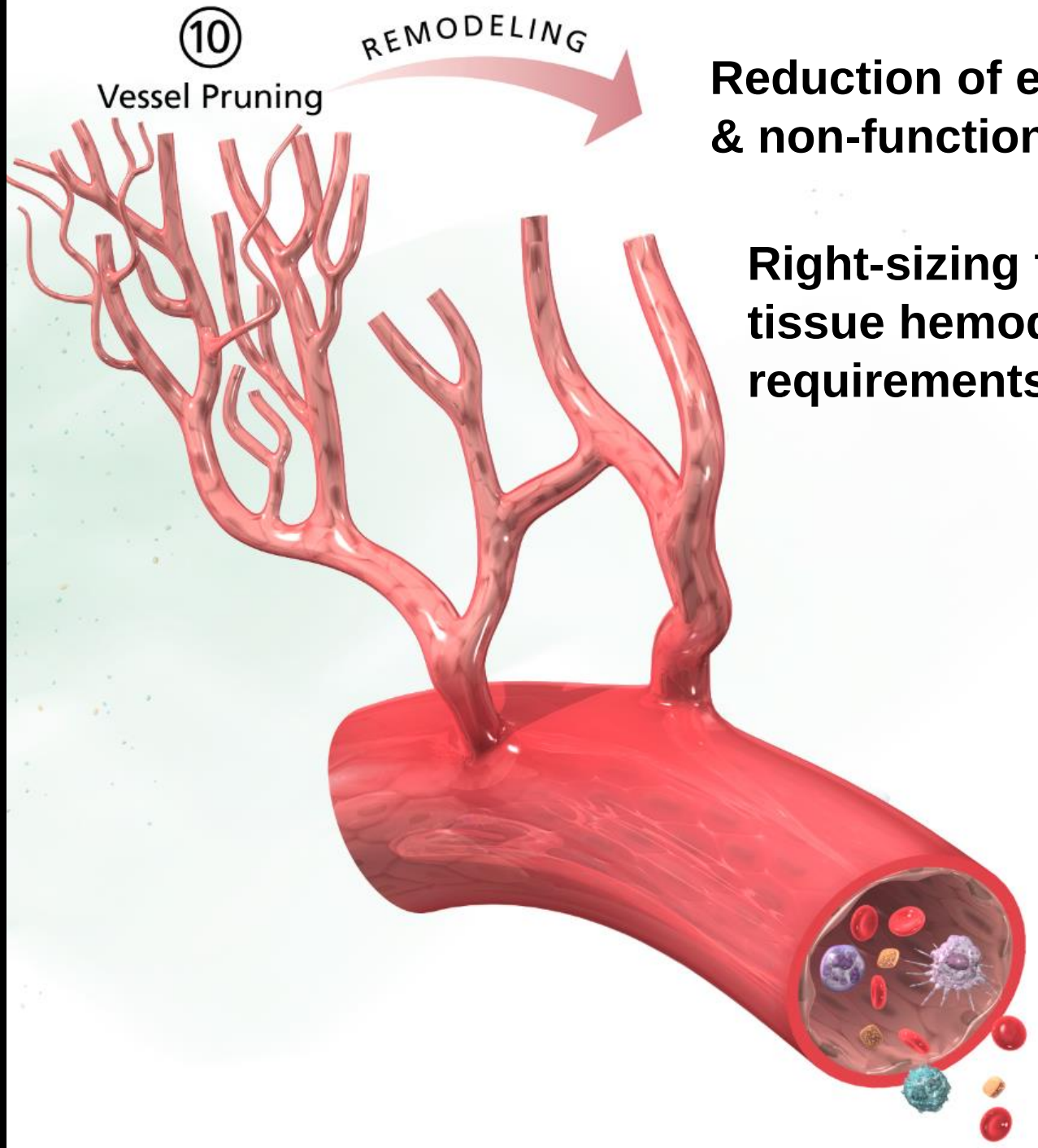


Canalization is required for blood flow



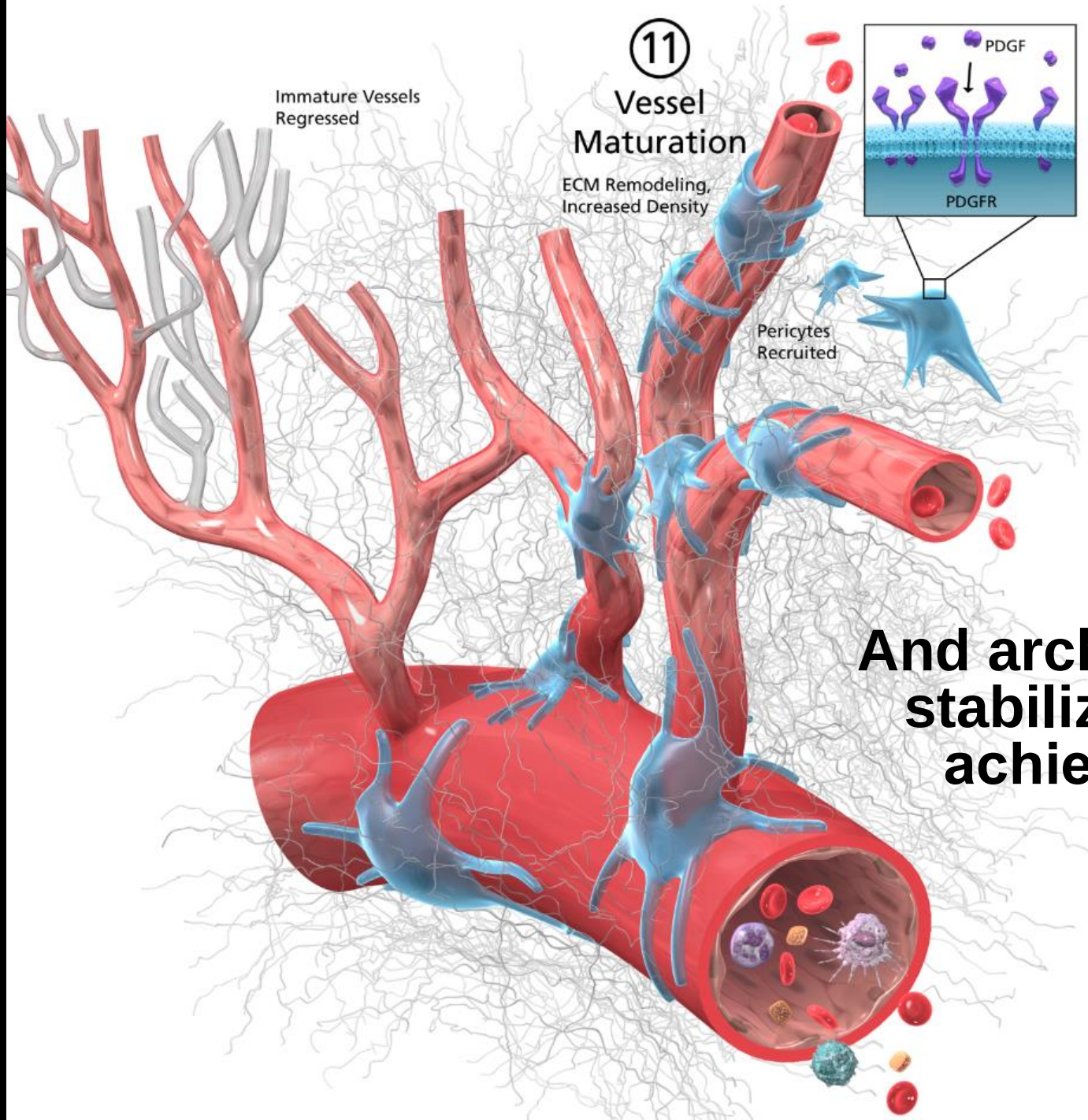
As perfusion improves …



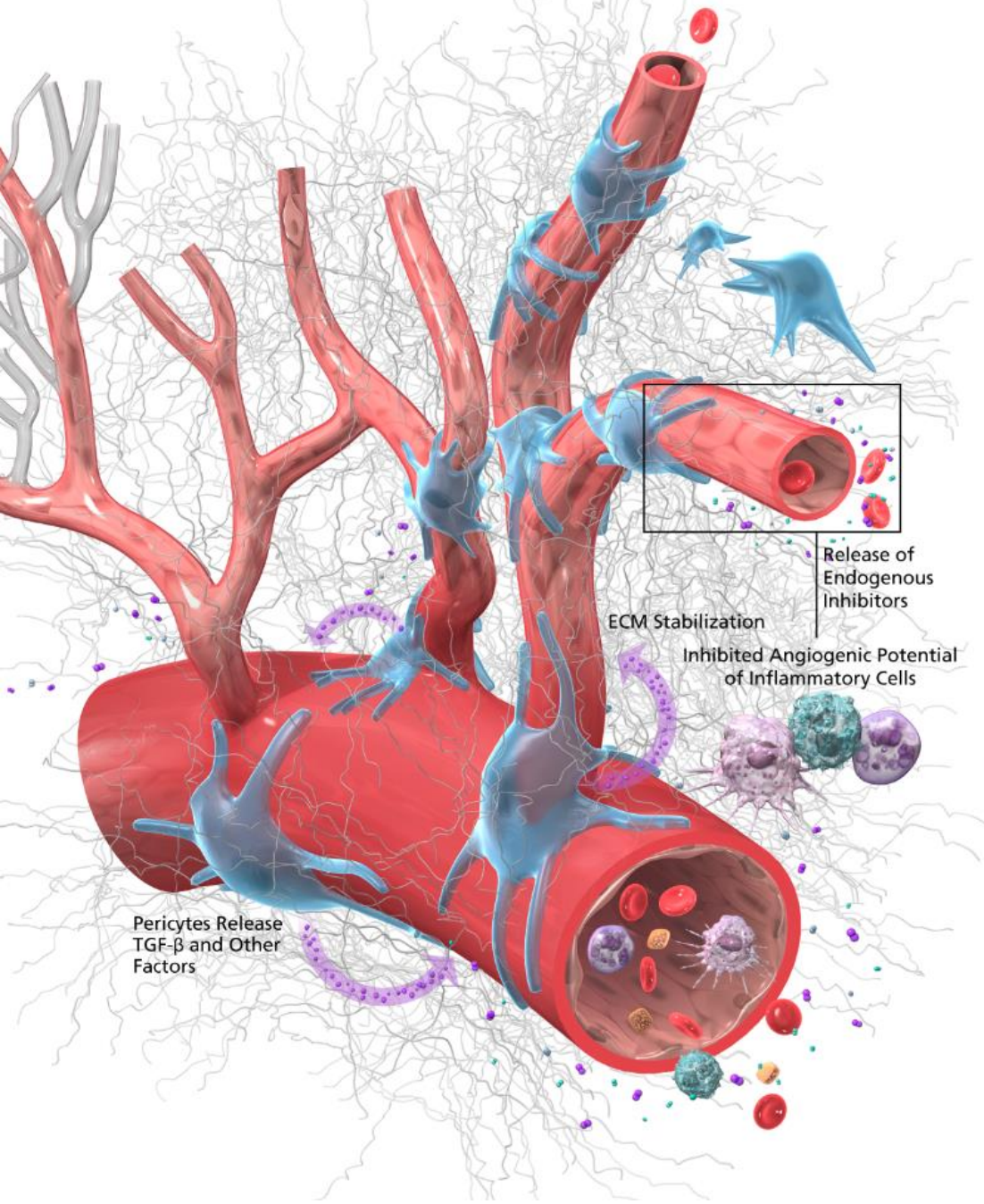


Reduction of excessive & non-functional vessels

Right-sizing for optimal tissue hemodynamic requirements



And architectural stabilization is achieved …

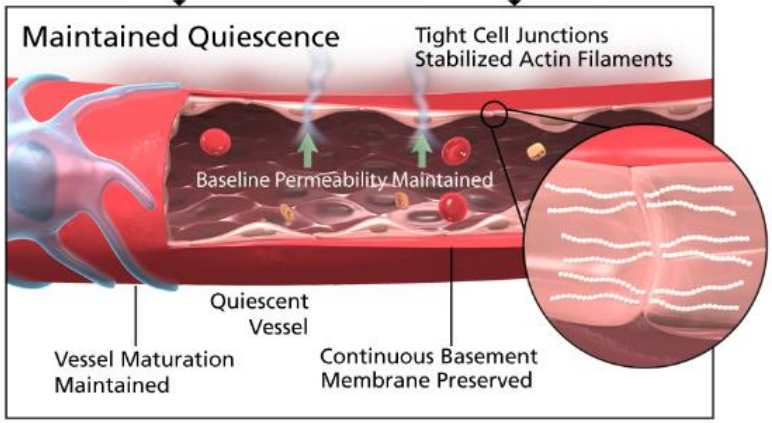


⑫ Return to Quiescence

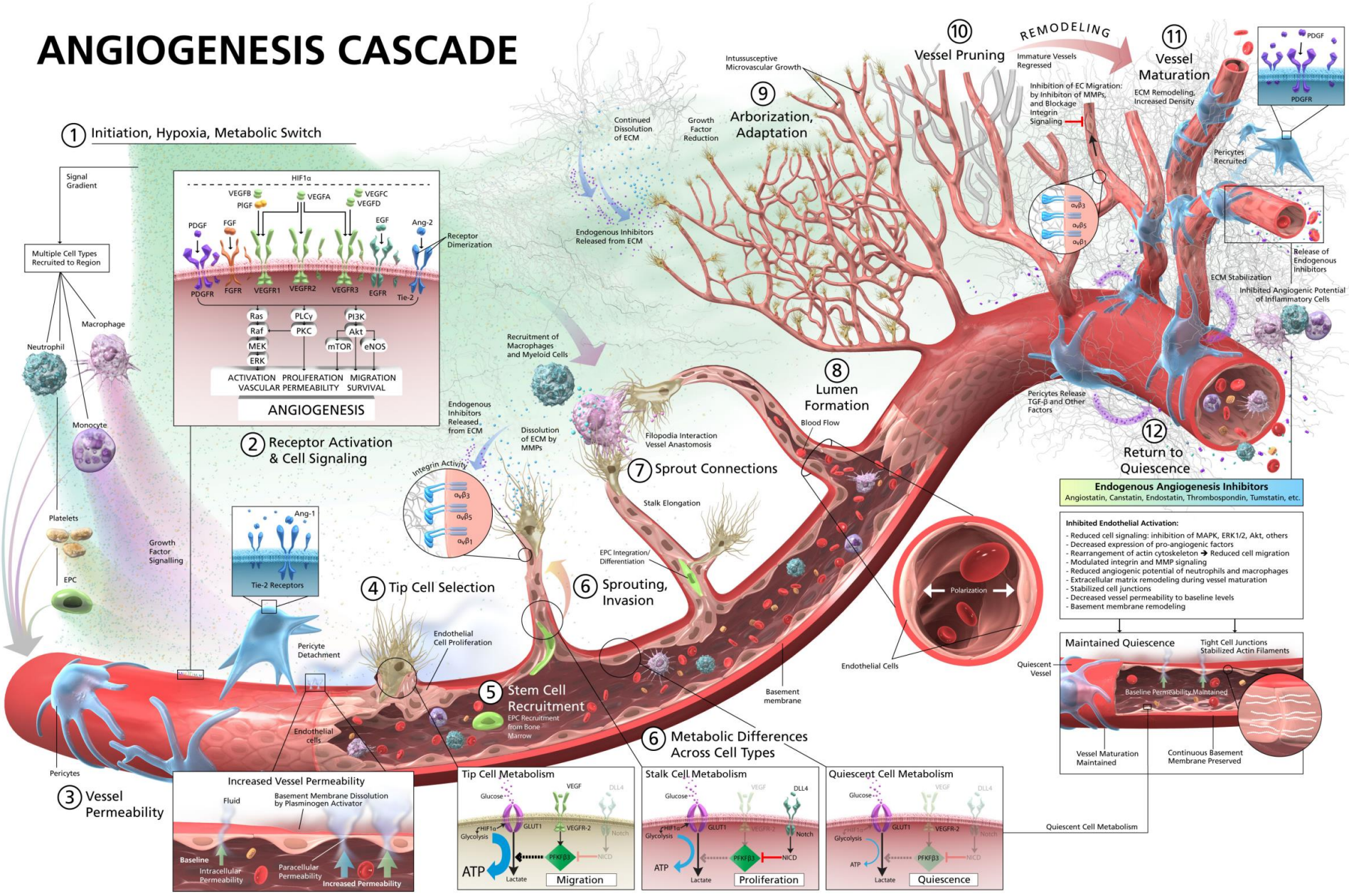
Endogenous Angiogenesis Inhibitors
Angiostatin, Canstatin, Endostatin, Thrombospondin, Tumstatin, etc.

Inhibited Endothelial Activation:

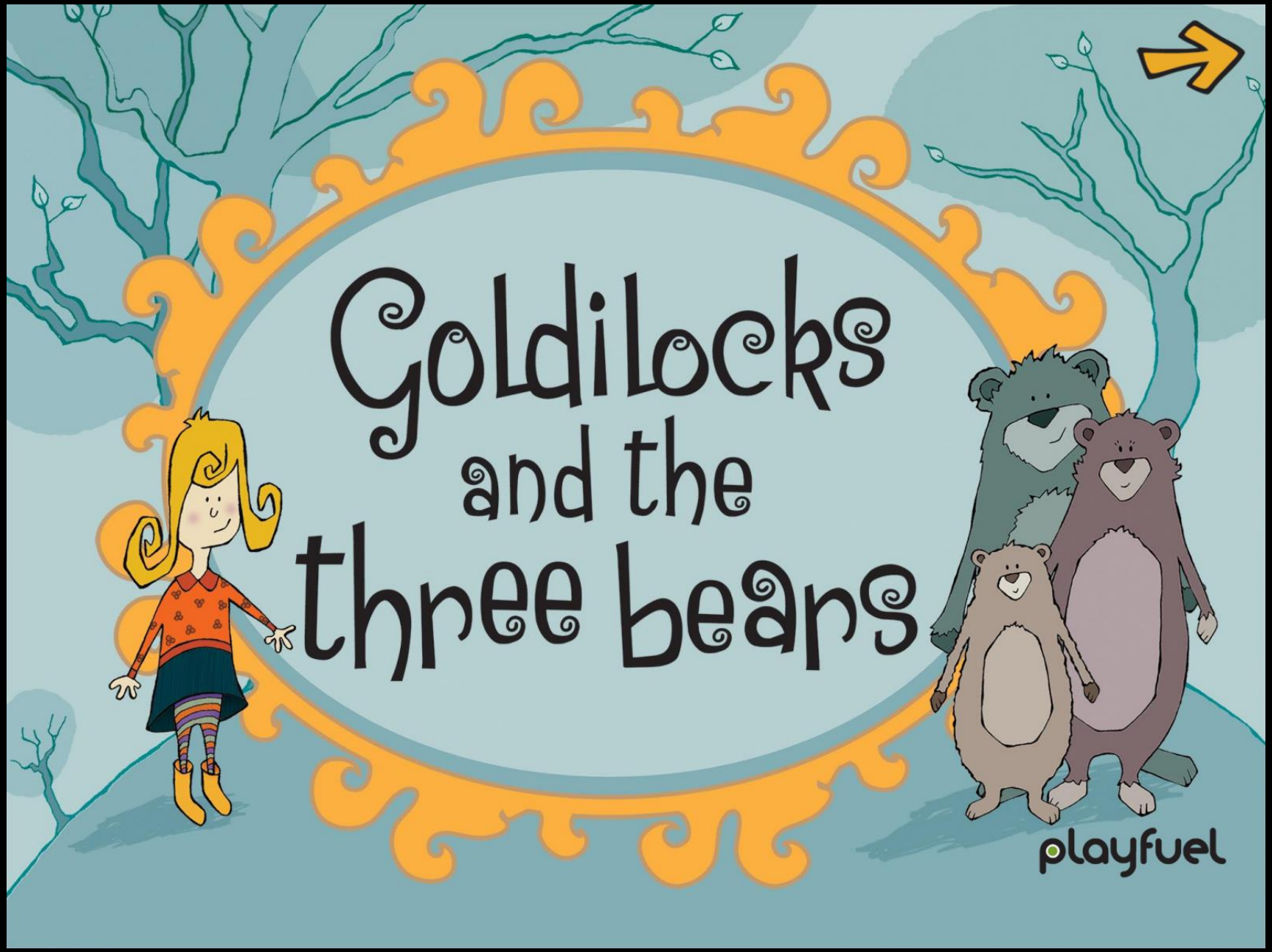
- Reduced cell signaling: inhibition of MAPK, ERK1/2, Akt, others
- Decreased expression of pro-angiogenic factors
- Rearrangement of actin cytoskeleton ➔ Reduced cell migration
- Modulated integrin and MMP signaling
- Reduced angiogenic potential of neutrophils and macrophages
- Extracellular matrix remodeling during vessel maturation
- Stabilized cell junctions
- Decreased vessel permeability to baseline levels
- Basement membrane remodeling

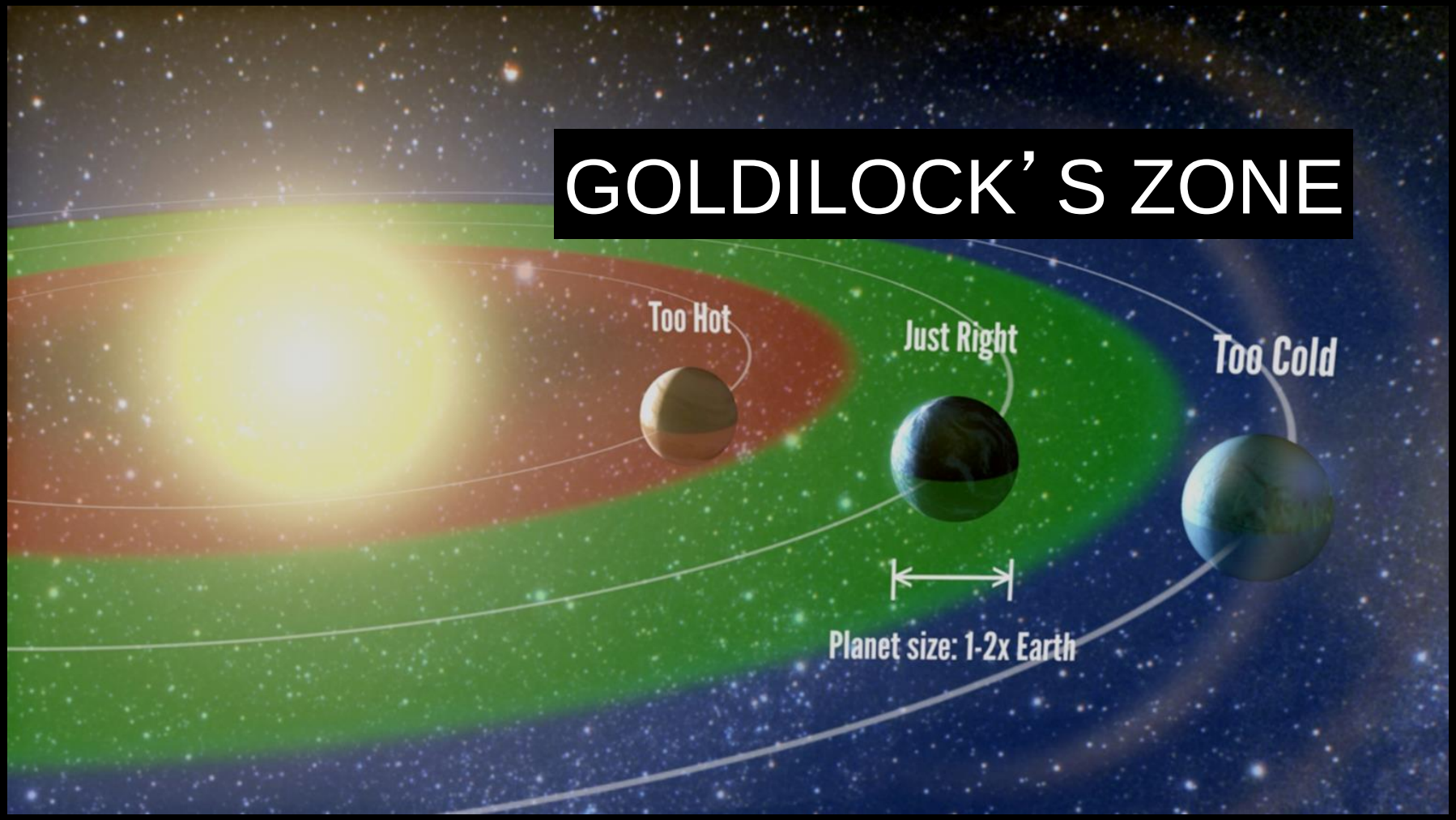


Back to Baseline .



NEW: Blood vessels normally exist in a "Goldilock's Zone"


Goldilocks
and the
three bears
playfuel


GOLDILOCK'S ZONE
Too Hot
Just Right
Too Cold
Planet size: 1-2x Earth

Physiological Homeostasis

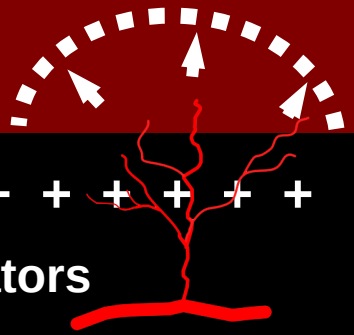
NOT TOO MUCH

Endogenous Inhibitors

— — — — — — — — — — — — — —

"GOLDILOCK'S ZONE"

JUST THE RIGHT NUMBER OF VESSELS



NOT TOO LITTLE

+ + + + + + + + + + + + + + + +

Angiogenesis Stimulators

KEY QUESTIONS:

If HYPOXIA induces angiogenesis, then why does hyperoxia also promote it?

If HYPEROXIA induces angiogenesis, then why doesn't it promote dangerous neovascularization, i.e., tumor growth?

Impact of HYPOXIA on Angiogenesis

- **Increases pro-angiogenic factors, Hypoxia-Induced Factor (HIF1α), VEGF, MMP-2, and MMP-9.**
  - *Cell Mol Bio Res 1994;40(1):35*
  - *Oncol Rep 2014;31(4):1947*

- **Increases angiogenesis inhibitors: Thrombospondin-1 (TSP-1) and Angiopoietin-like 4.**
  - *Fibrogenesis Tissue Rep 2011;4:!3*
  - *Proc Natl Acad Sci 2013;110(36):E3245*
  - *Oncogene 2014;33(17):2273*

- **Downregulates angiogenesis inhibitors Endostatin and Angiostatin.**
  - *Biochem Biophys Res Commun 2001;288:1149*
  - *Proc West Pharmacol Soc 2007;50:47*

Angiogenesis Response

INHIBITORS ↑ TSP-1, ANGPT4

HYPOXIA

"GOLDILOCK'S ZONE"

JUST RIGHT

HYPOXIA ↑ HIF, VEGF

STIMULATORS ↑ MMP-2, MMP-9

INHIBITORS ↓ Endostatin, Angiostatin, TSP1, PEDF, TSP2

Impact of HYPEROXIA on Angiogenesis

• Decreases VEGF expression but increases basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), Ang-2, TNFα, and MMP-9 in vivo.

— *Circ J 2007;71:405*

— *BBRC 2002;296:710*

• Increases mobilization of endothelial progenitor cells (EPCs) from bone marrow into circulation.

— *Vascular 2006;14(6):328*

• Increases production of endogenous angiogenesis inhibitors: Pigment Epithelium-derived Factor (PEDF), endostatin, and TIMP-1.

— *Am J Resp Cell Mol Biol 2015;52:295*

— *Sichuan Da Xue Xue Bao Yi Xue Ban 2006;37:614*

— *Wound Repair Regen 2009; 17:179*

Angiogenesis Response

INHIBITORS ↑ PEDF, Endostatin, TIMP-1

HYPEROXIA

"GOLDILOCK'S ZONE"

JUST RIGHT

HYPEROXIA

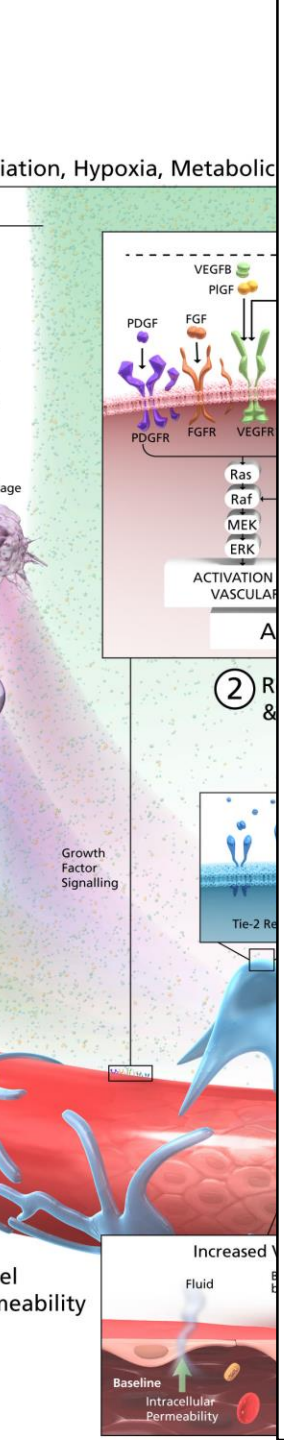
STIMULATORS ↑ bFGF, HGF, Ang-2,
↑ TNFα, MMP-9

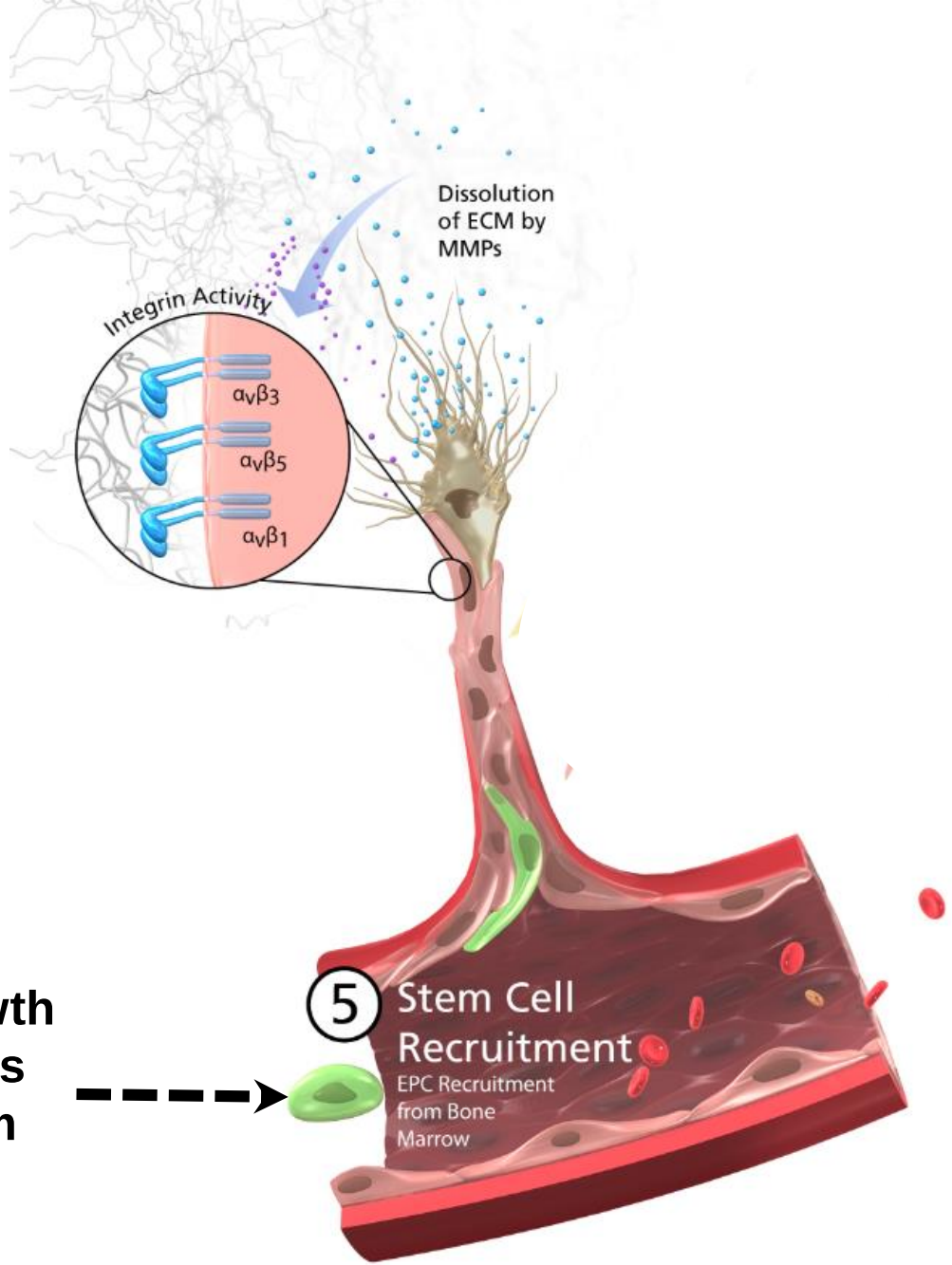
TAKE HOME

Angiogenesis oxygen sensing is an evolutionary mechanism designed to meet the functional needs of tissue and protect the microcirculatory ‘status quo’

BEYOND THE STATUS QUO …

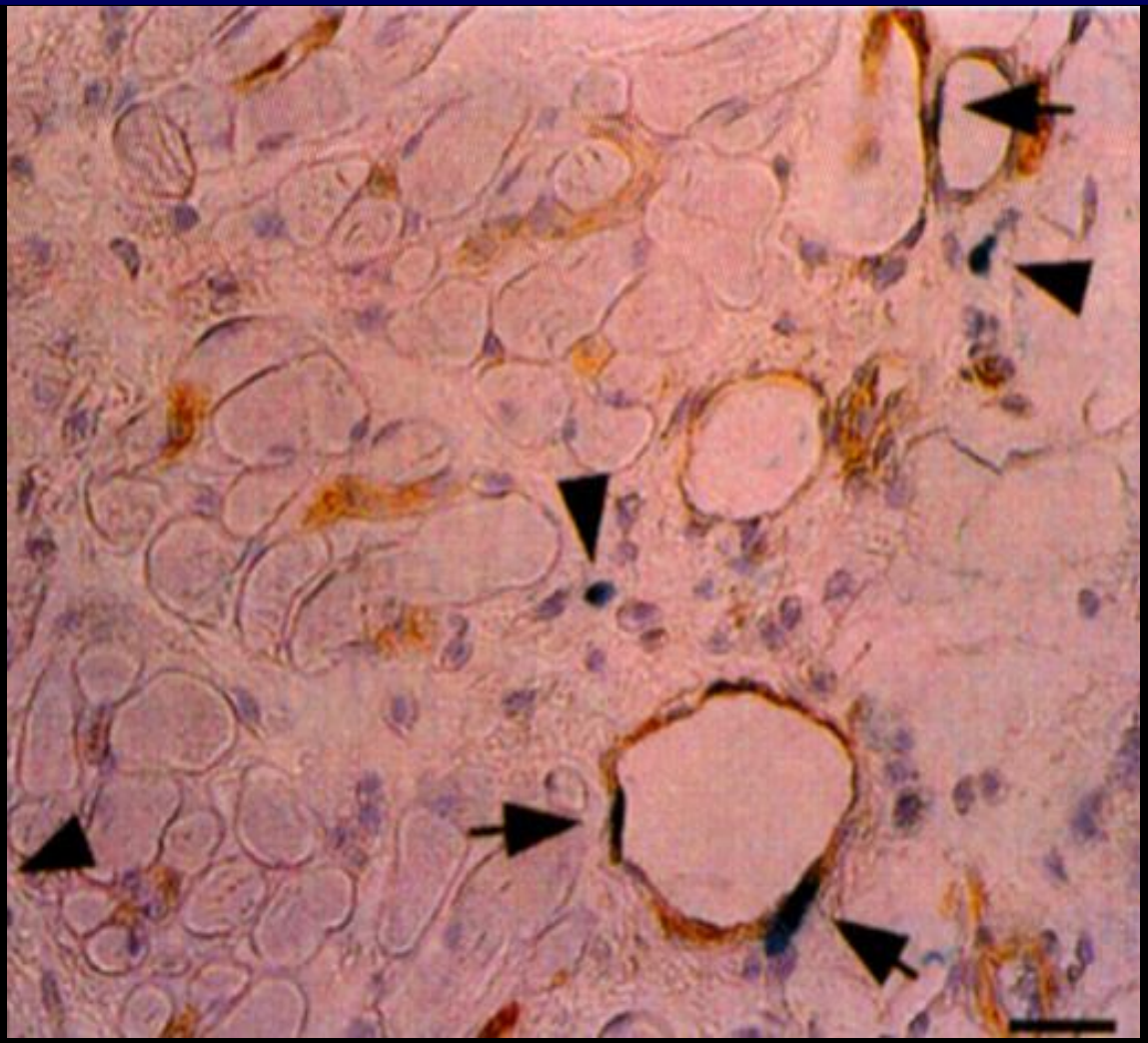
HYPEROXIA
Can Stimulate Vascular Stem Cell Mobilization and Recruitment





Soluble Growth Factor signals mobilize stem cells …

Physiology: Wounding Rapidly Mobilizes Endothelial Progenitor Cells (EPC)

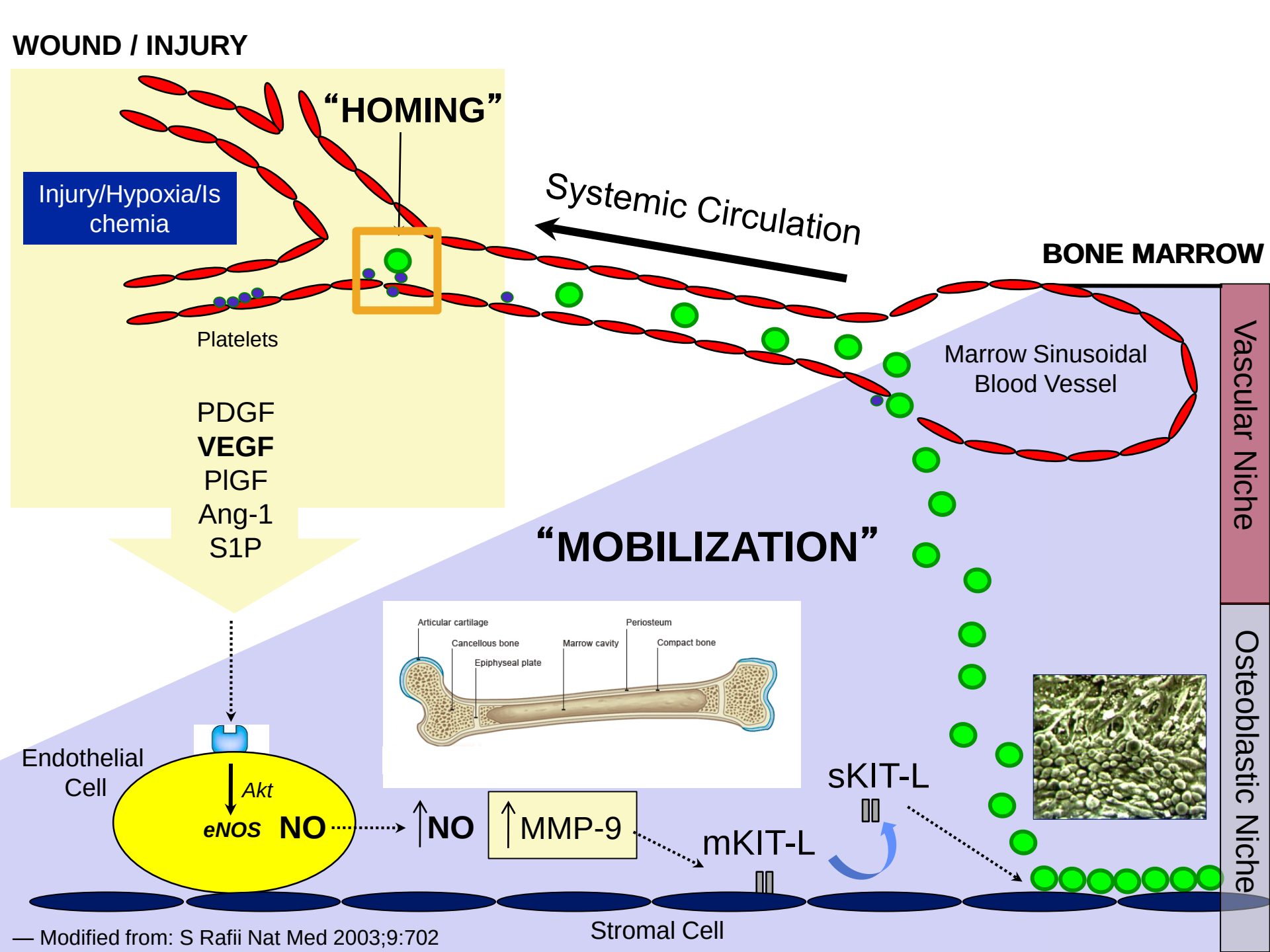


In Granulation Tissue:

5% - 26% of endothelial cells originate from the bone marrow

JR Crosby et. al. (DR Bowen-Pope), Circ Res. 2000;87:728.
T Murayama, et. al. (T Asahaa). Exp Hemato.l 2002;30:967.

EPCs contribute to post-natal vasculogenesis and are thought to promote regenerative repair through the release of paracrine factors


WOUND / INJURY
"HOMING"
Injury/Hypoxia/Ischemia
Systemic Circulation
BONE MARROW
Platelets
Marrow Sinusoidal Blood Vessel
PDGF
VEGF
PlGF
Ang-1
S1P
"MOBILIZATION"
Vascular Niche
Osteoblastic Niche
Articular cartilage
Periosteum
Cancellous bone
Marrow cavity
Compact bone
Epiphyseal plate
Endothelial Cell
Akt
eNOS
NO
NO
MMP-9
mKIT-L
sKIT-L
Stromal Cell
— Modified from: S Rafii Nat Med 2003;9:702

DEFECTS in DIABETES

WOUND / INJURY

Injury/Hypoxia/Ischemia

"HOMING"

Systemic Circulation

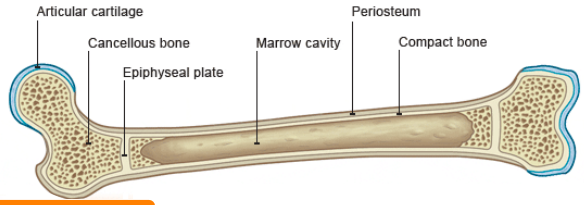
BONE MARROW

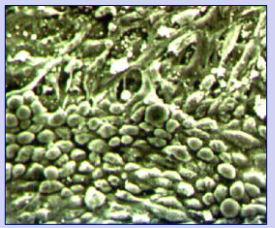
Platelets

Marrow Sinusoidal Blood Vessel

PDGF
VEGF
PlGF
Ang-1
S1P

"MOBILIZATION"





Endothelial Cell

Akt
eNOS
NO
X
NO

MMP-9

mKIT-L

sKIT-L

Stromal Cell

Vascular Niche

Osteoblastic Niche

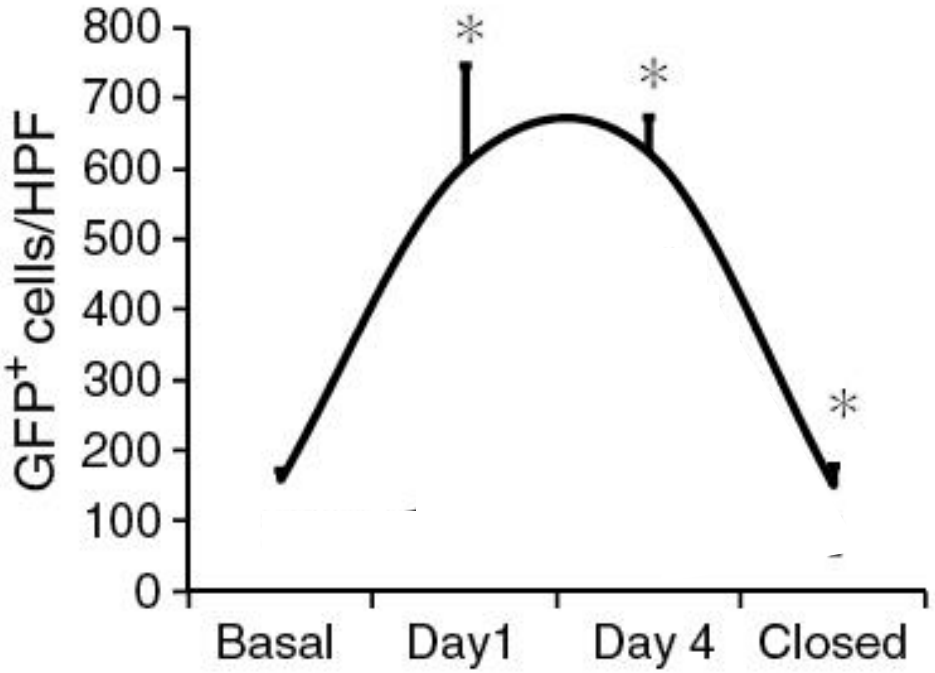
— Modified from: S Rafii Nat Med 2003;9:702

Number of Bone Marrow-derived Stem Cells in Wound Tissue During Healing

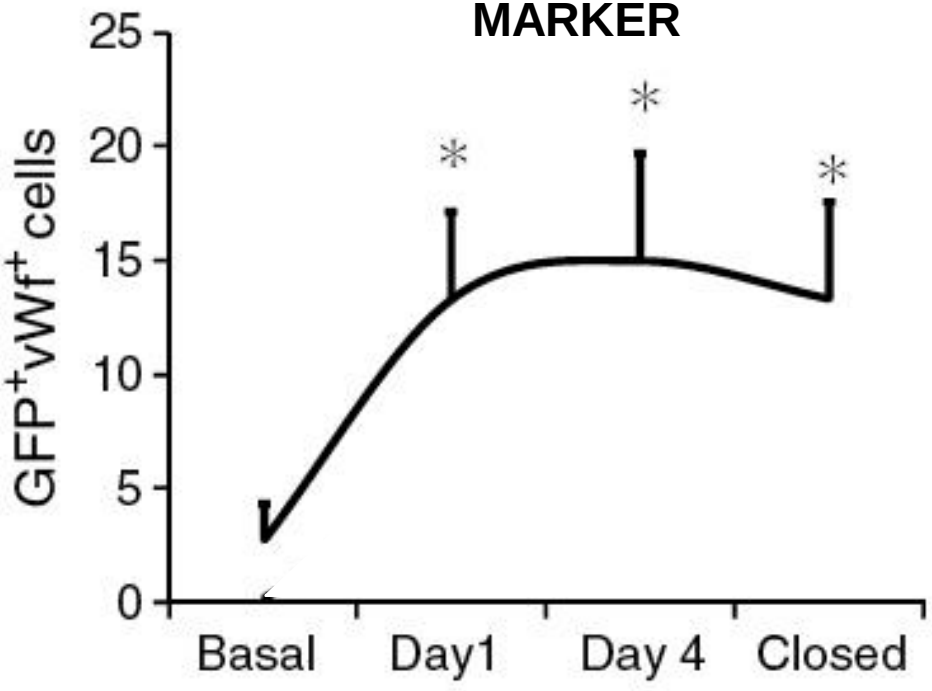
Non-diabetic

Diabetic

TOTAL BM CELLS

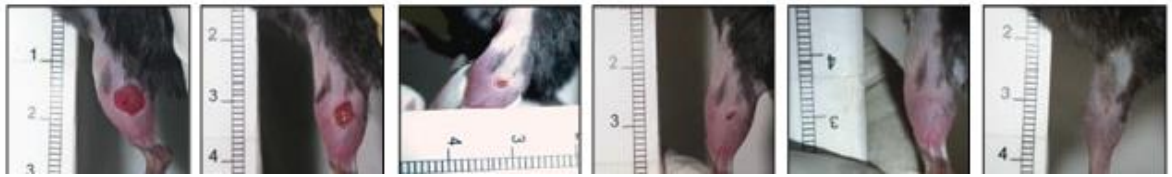


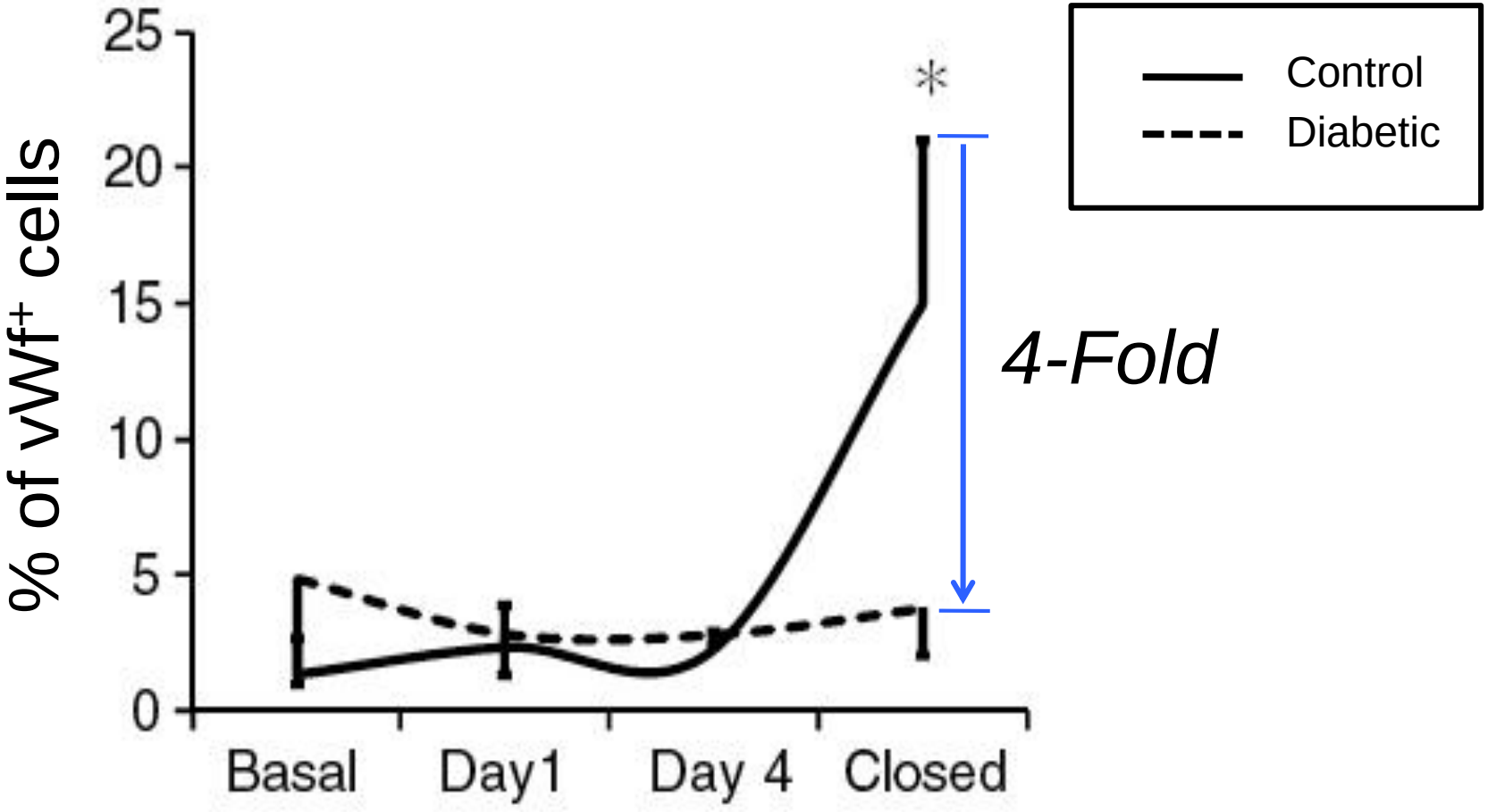
BM CELLS W/ VASCULAR MARKER



— Albiero, et. al. (Gianpaolo Fadini, Univ. Padova) Diabetologica 2011;54:945-53

Contribution of Bone Marrow-derived Stem Cells to Total Wound Vasculature



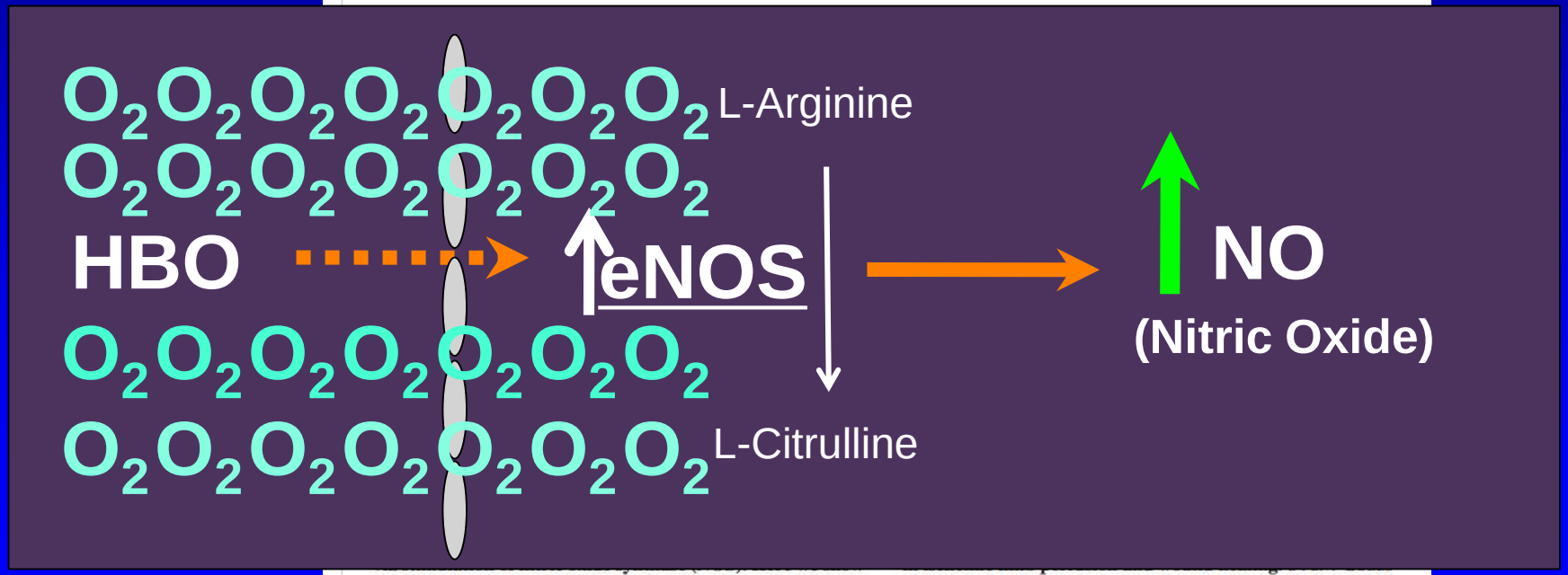


— Albiero, et. al. (Gianpaolo Fadini, Univ. Padova) Diabetologica 2011;54:945-53

Hyperbaric oxygen (HBO) treatment increases NO production in bone marrow

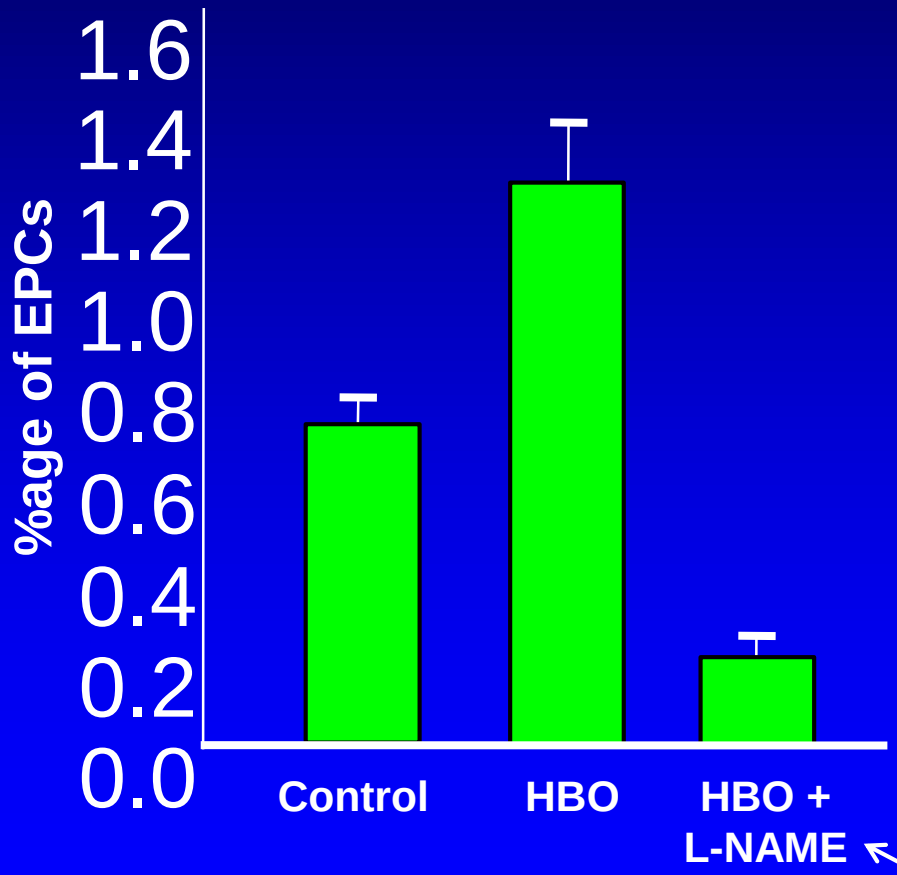
STEM CELLS
TRANSLATIONAL AND CLINICAL RESEARCH

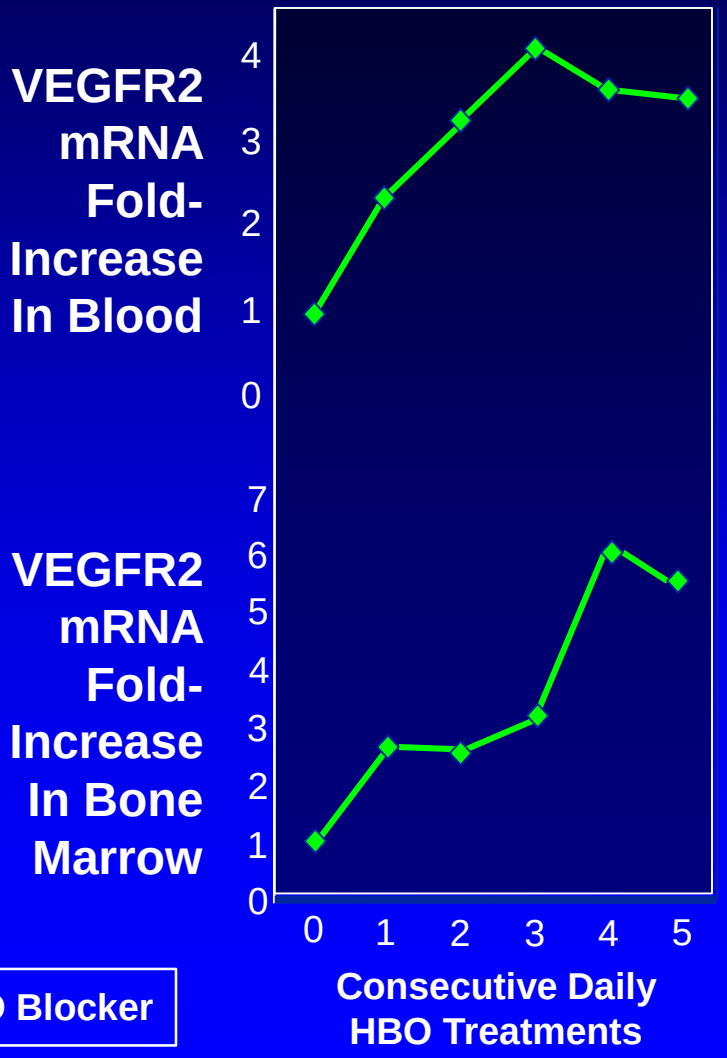
Endothelial Progenitor Cell Release into Circulation Is Triggered by Hyperoxia-Induced Increases in Bone Marrow Nitric Oxide



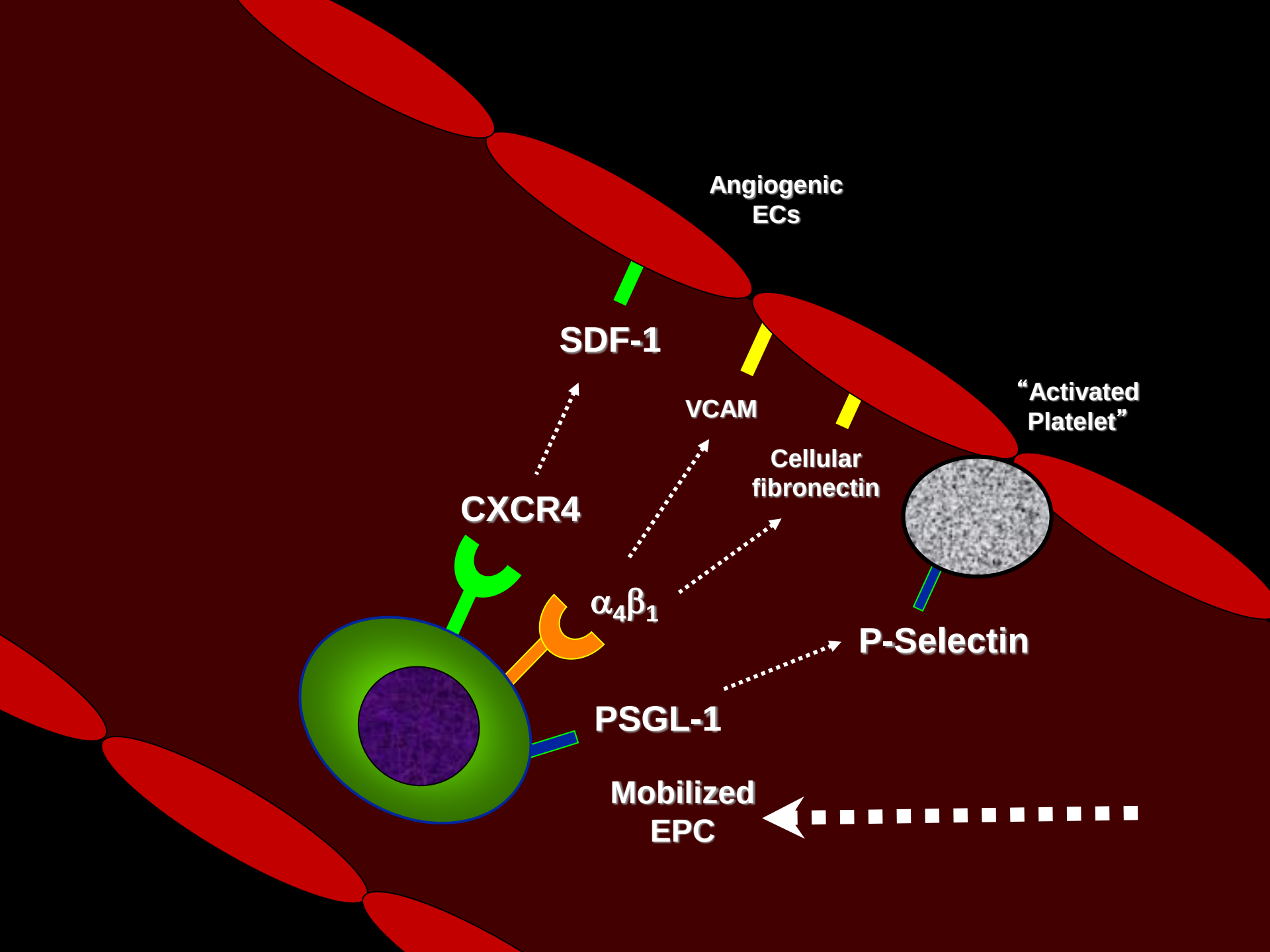
that HBO increases bone marrow NO in vivo thereby in- 2006;24:2309–2318

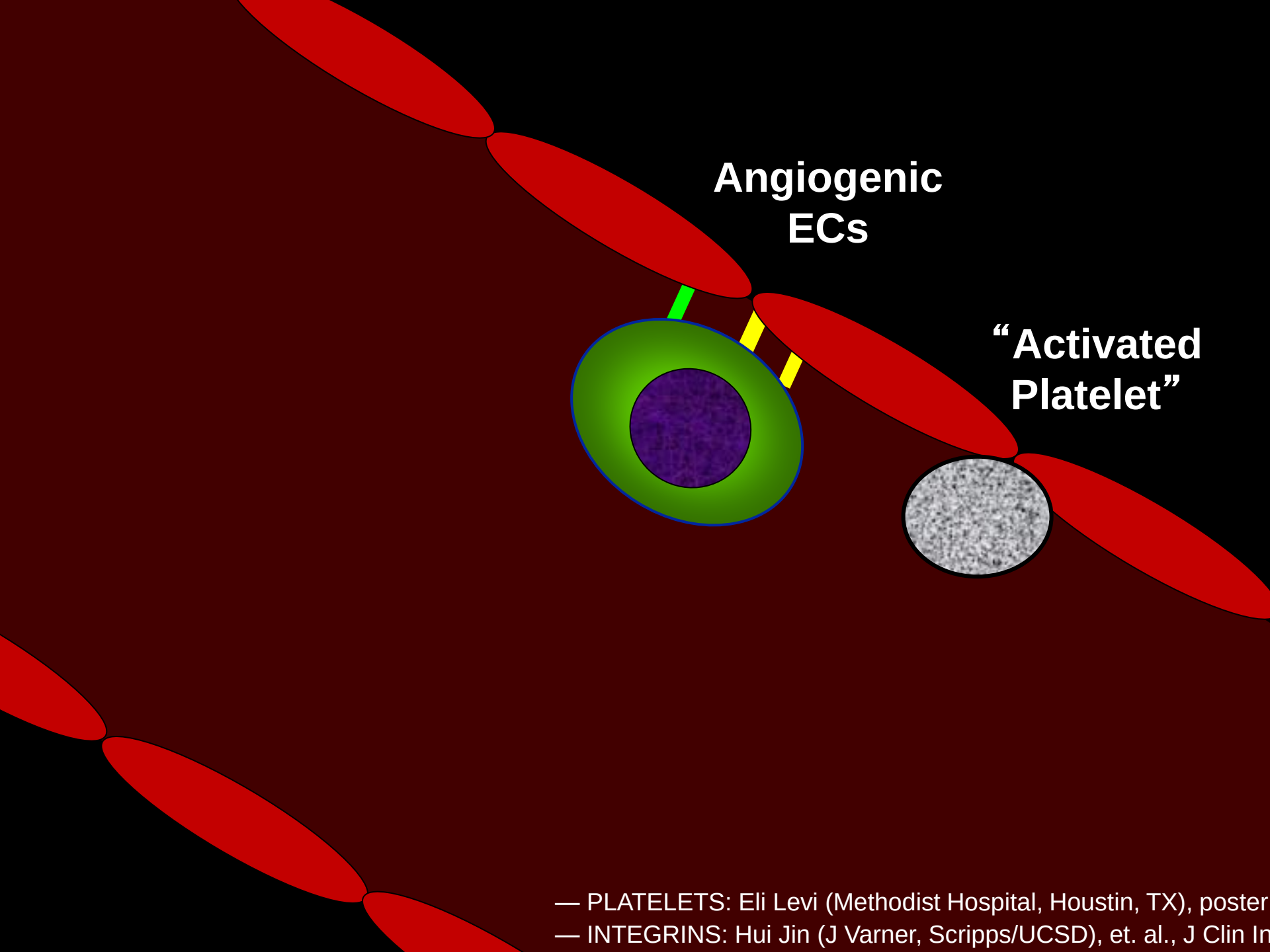
HBO therapy improves EPC mobilization in vivo





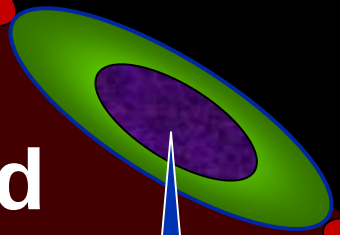
— L Goldstein, et. al. Stem Cells 2006;24:2309


Angiogenic
ECs
SDF-1
VCAM
"Activated
Platelet"
Cellular
fibronectin
CXCR4
$\alpha_4\beta_1$
P-Selectin
PSGL-1
Mobilized
EPC



— PLATELETS: Eli Levi (Methodist Hospital, Houstin, TX), poster
— INTEGRINS: Hui Jin (J Varner, Scripps/UCSD), et. al., J Clin In

Incorporated EPC



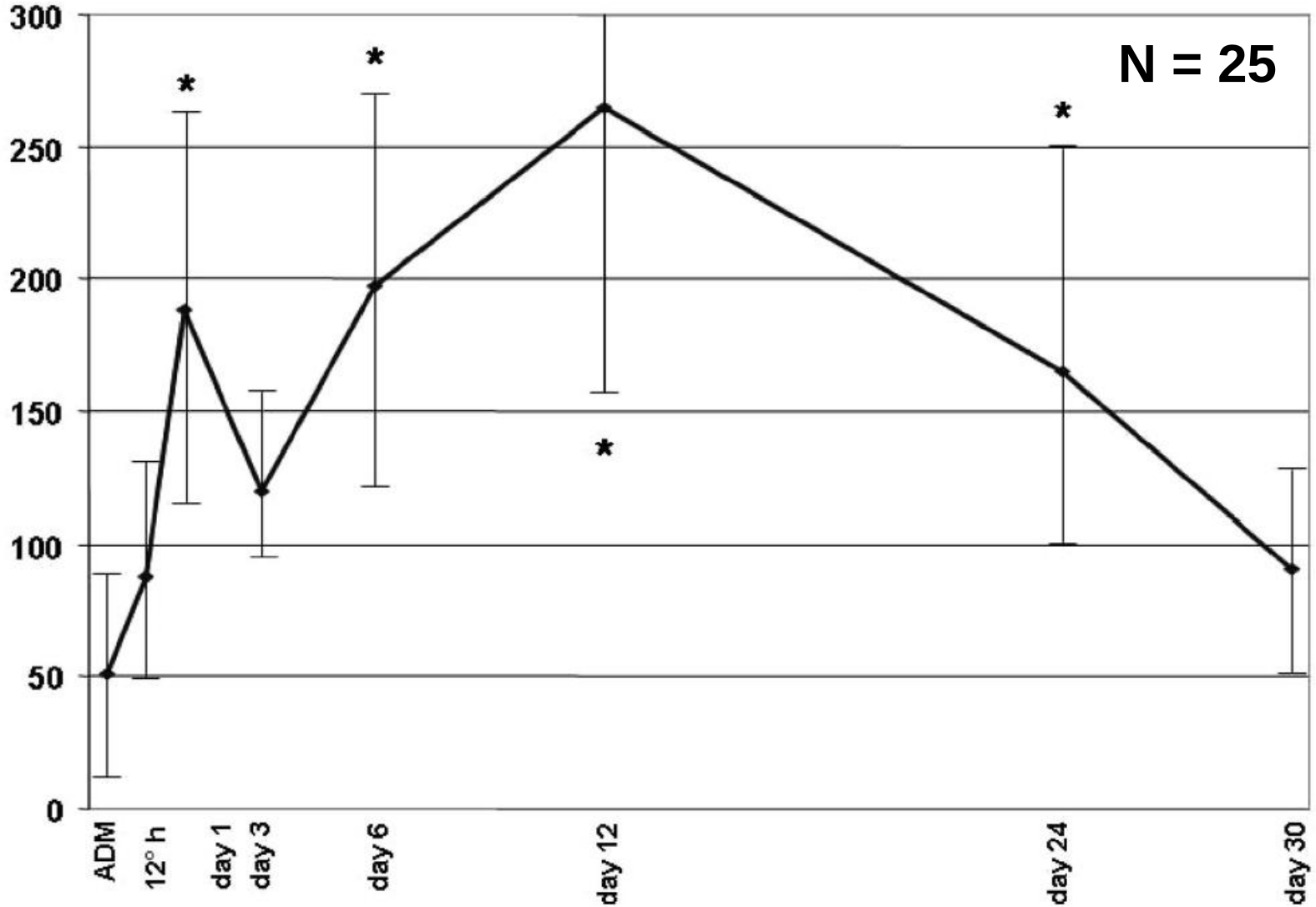
PARACRINE FACTORS:

- Adrenomedullin
- Angio-associated Migratory Protein
- Angiogenin
- Angiopoietin-1
- Bone Morphogenic Protein-2, -6
- Connective Tissue Growth Factor
- Endothelin-1
- Fibroblast growth factor-2, -7
- Hepatocyte Growth factor
- Insulin-like Growth Factor-1
- Interleukin-1, -6, -11
- Kit Ligand
- MMP-1, -2, -9
- Monocyte chemoattractant protein-1
- Placental growth factor
- Platelet-derived growth factor
- Pleiotrophin
- Frizzled-related protein-1, -2
- Thrombospondin-1
- Thymosin β4
- TIMP-1, -2
- Transforming Growth Factor-β
- Tumor Necrosis Factor-α
- Vascular Endothelial Growth Factor

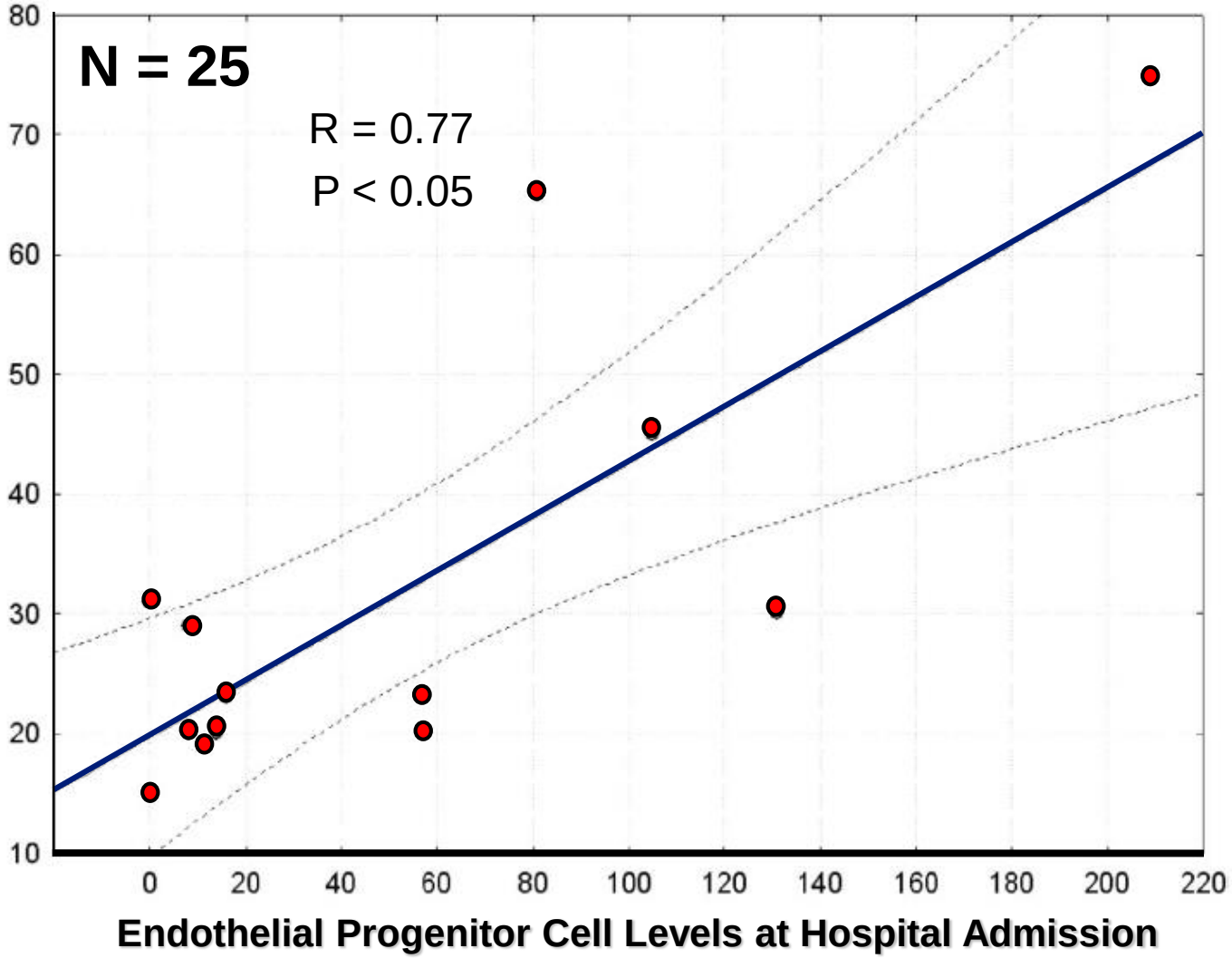
Clinical Correlates

Vascular Stem Cells Are Mobilized in Patients Following Burn Injury

— Carlo Foresta (Univ. Padova), J Trauma 2011;70(2):459-465

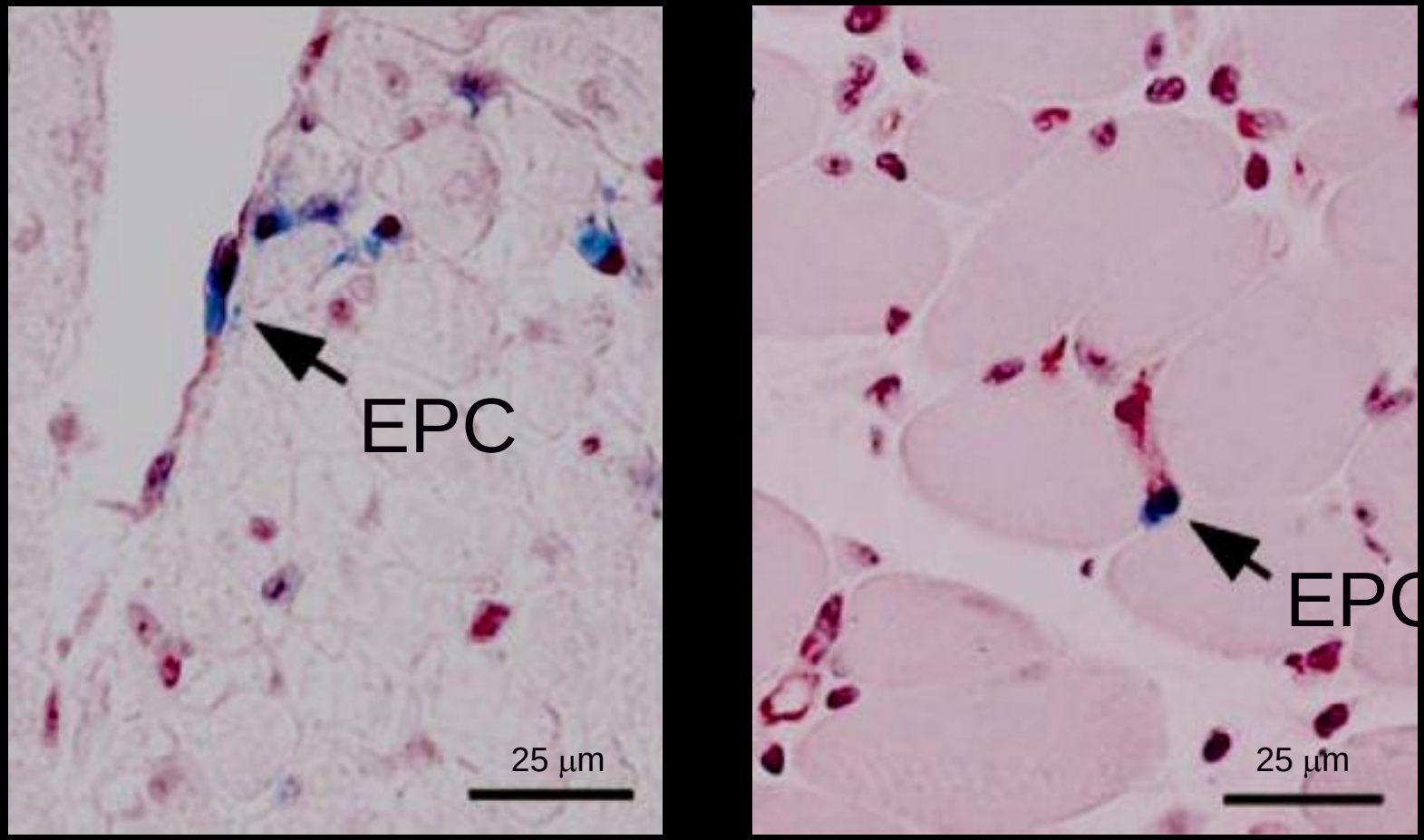


The Greater the Injury, the More Vascular Stem Cells are Mobilized in Response



— Modified from: C Foresta (Univ. Padova), J Trauma 2011;70(2):459-465

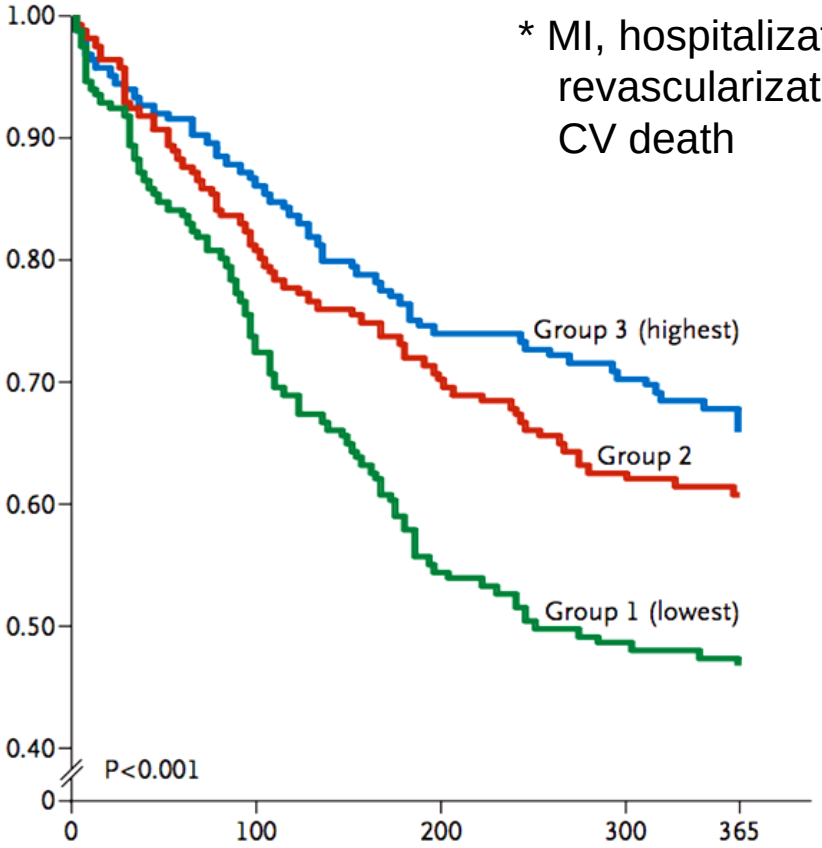
Endothelial Progenitor Cells Recruited to Ischemic Zones After Myocardial Infarction



Asahara et al. *Circ Res.* 1999;85:221.

Higher Circulating EPC Levels Correlate With *Better* Survival of Major Cardiovascular Events* at 12 Months

Cumulative Event-free Survival

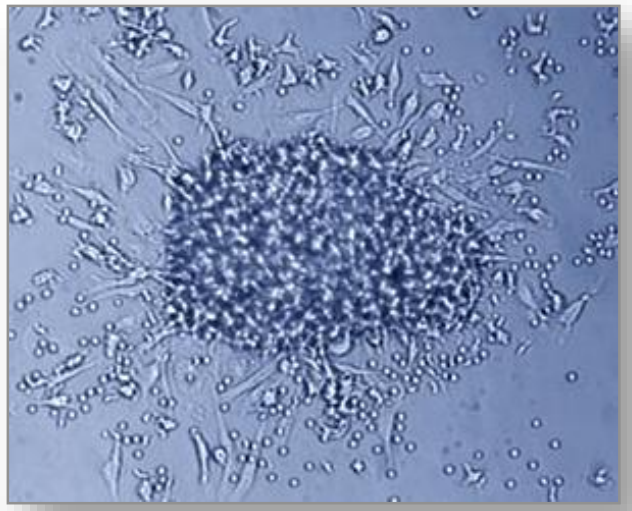


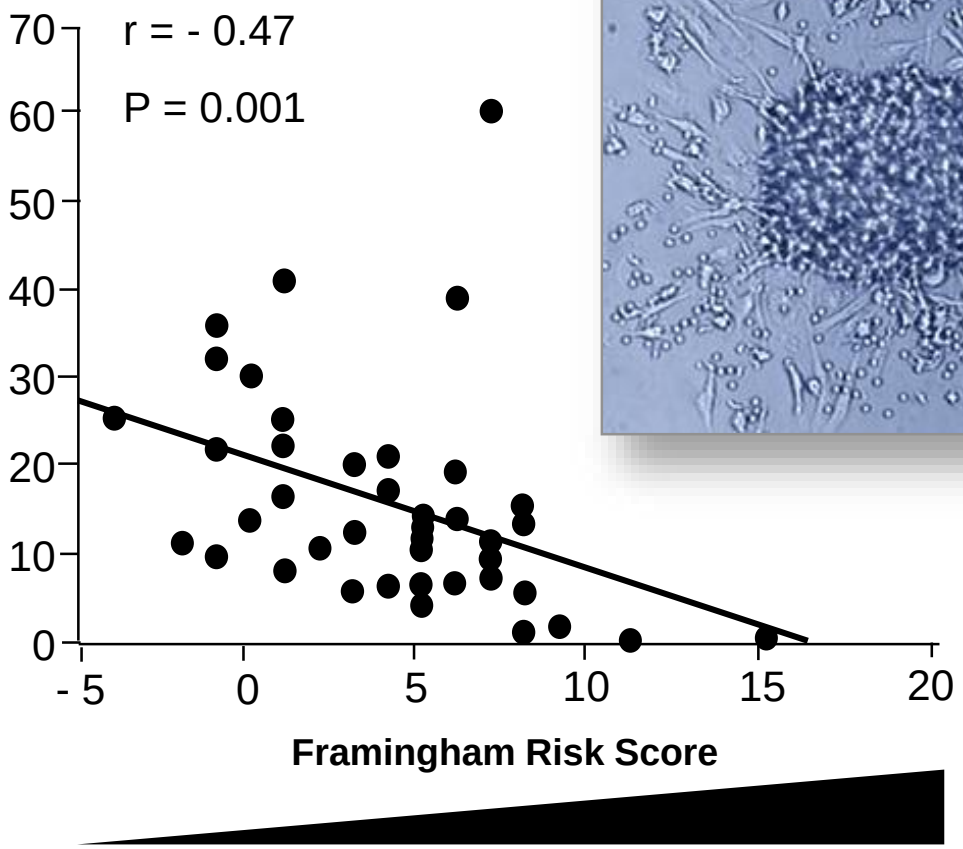
* MI, hospitalization, revascularization, CV death

N = **519 men** with CAD undergoing angiography (arterial blood sample)

Nikos Werner et. al. (Homburg-Sar) *NEJM*. 2005;999.

Low Circulating EPC Levels Correlate With *Increased* Cardiovascular Risk



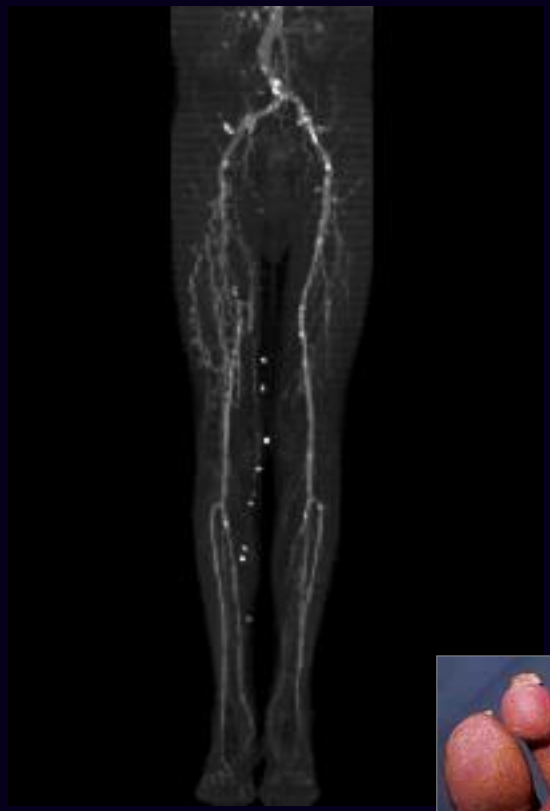


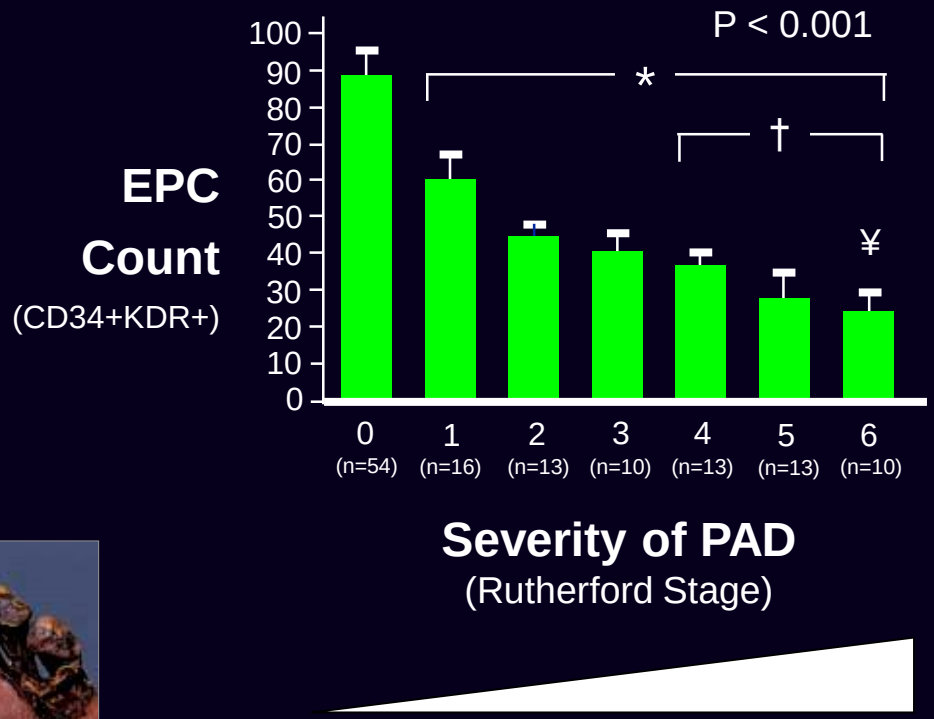
N = 45 men
(20 mL venous blood sample)

Hill et. al. (NHLBI) *NEJM* 2003;348:593.

Low Circulating EPC Levels Are Associated With Severity of Vasculopathy in PAD

— Fasini et. al. (Univ. Padova) Arterioscler Thromb Vasc Biol 2006;;26:2140

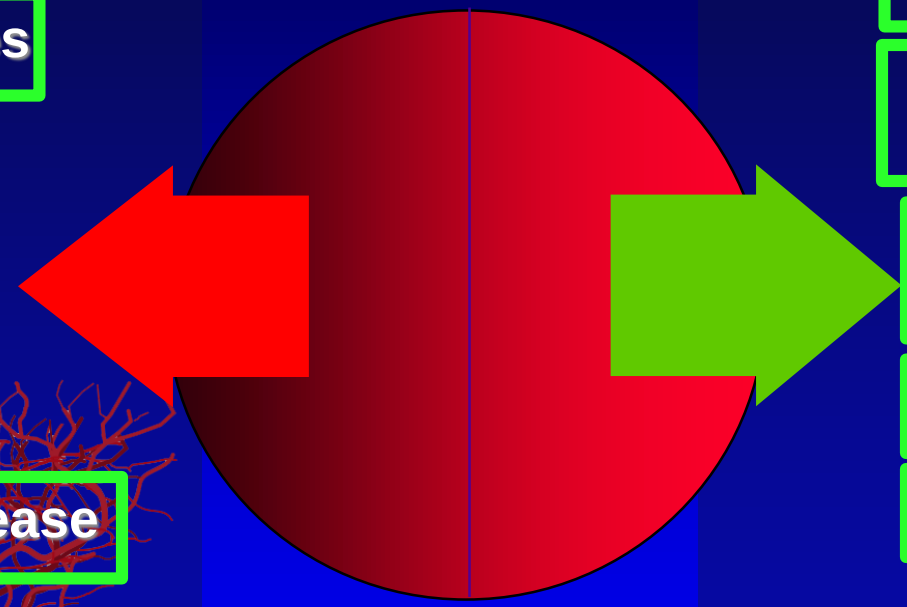




Angiogenesis Out of Balance

Excessive

- Cancer
- Blinding Diseases
- Psoriasis
- Arthritis
- Endometriosis
- AIDS
- Alzheimer's Disease
- Obesity
- Multiple sclerosis
- Cerebral malaria
- Rosacea



Insufficient

- Chronic wounds
- Coronary Heart Disease
- Peripheral Arterial Disease
- Stroke
- Neuropathies
- Pre-eclampsia
- Hair loss
- Erectile dysfunction

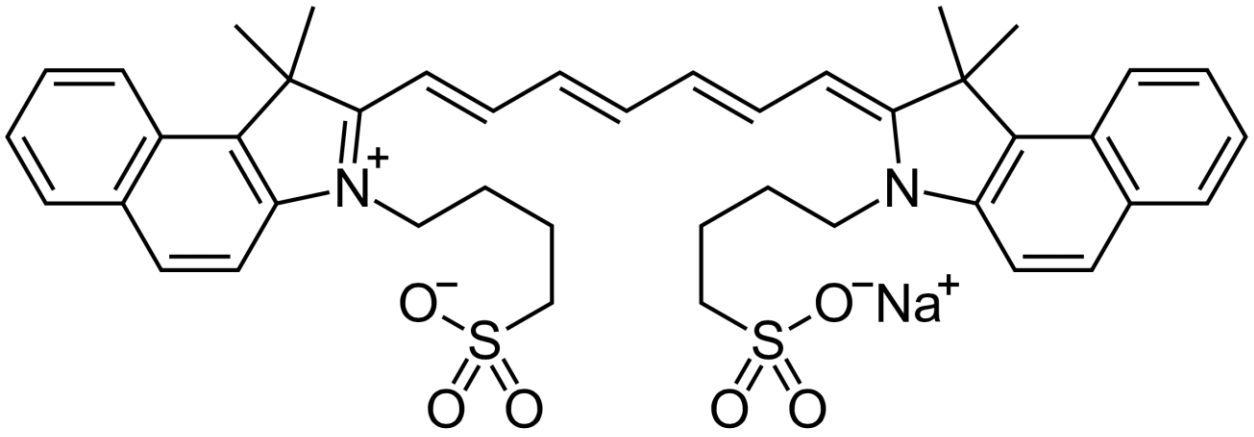
ANGIOGENESIS IMAGING: Biomarkers for microcirculatory change following HBOT

ANGIOGENESIS IMAGING METHODOLOGIES

Angiogenesis Foundation - NIH initiative

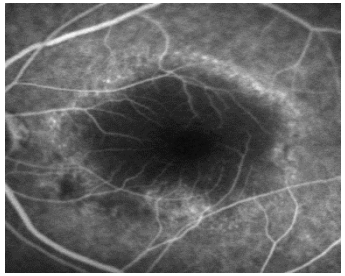
Indocyanine Green Dye

- Developed by Kodak (1950s)
- Widely used in medical applications since the 1970's:
  - Retinal angiography
  - Liver function and cardiac output tests
- Clinically safe
- Excreted by liver, so no renal contraindications
- 3-5 minute half-life
- Only contraindication – patients with sensitivity to iodides

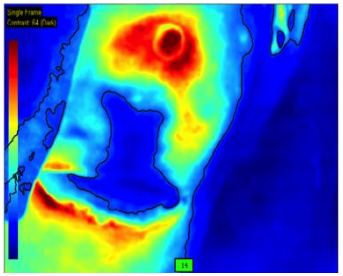


History of perfusion assessment with Indocyanine Green (ICG)

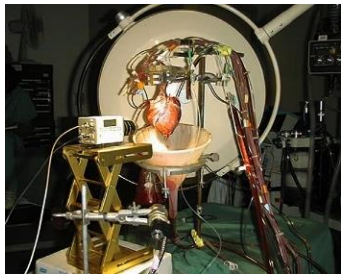
First used in the **1970s** during retinal angiography



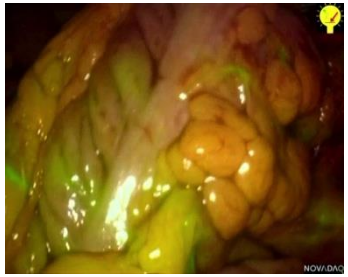
SPY used to assess skin perfusion in **plastic surgery** in 2007



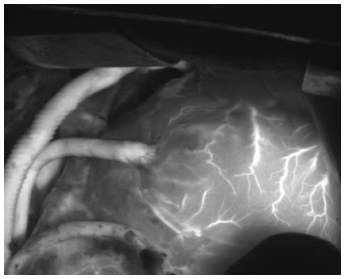
SPY fluorescence technology developed in **1999**



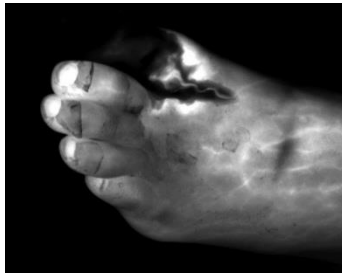
SPY FDA cleared for **organ transplant** and **GI procedures**



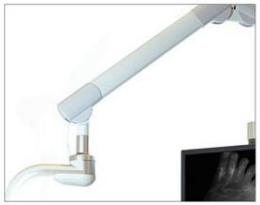
SPY introduced to US market for **cardiac surgery** applications in 2005



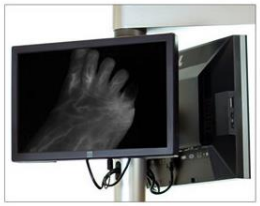
LUNA developed and introduced into **wound care** procedures in **2013**



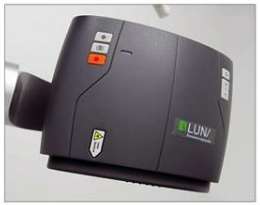
Perfusion Assessment Tools



Multi-directional imaging arm

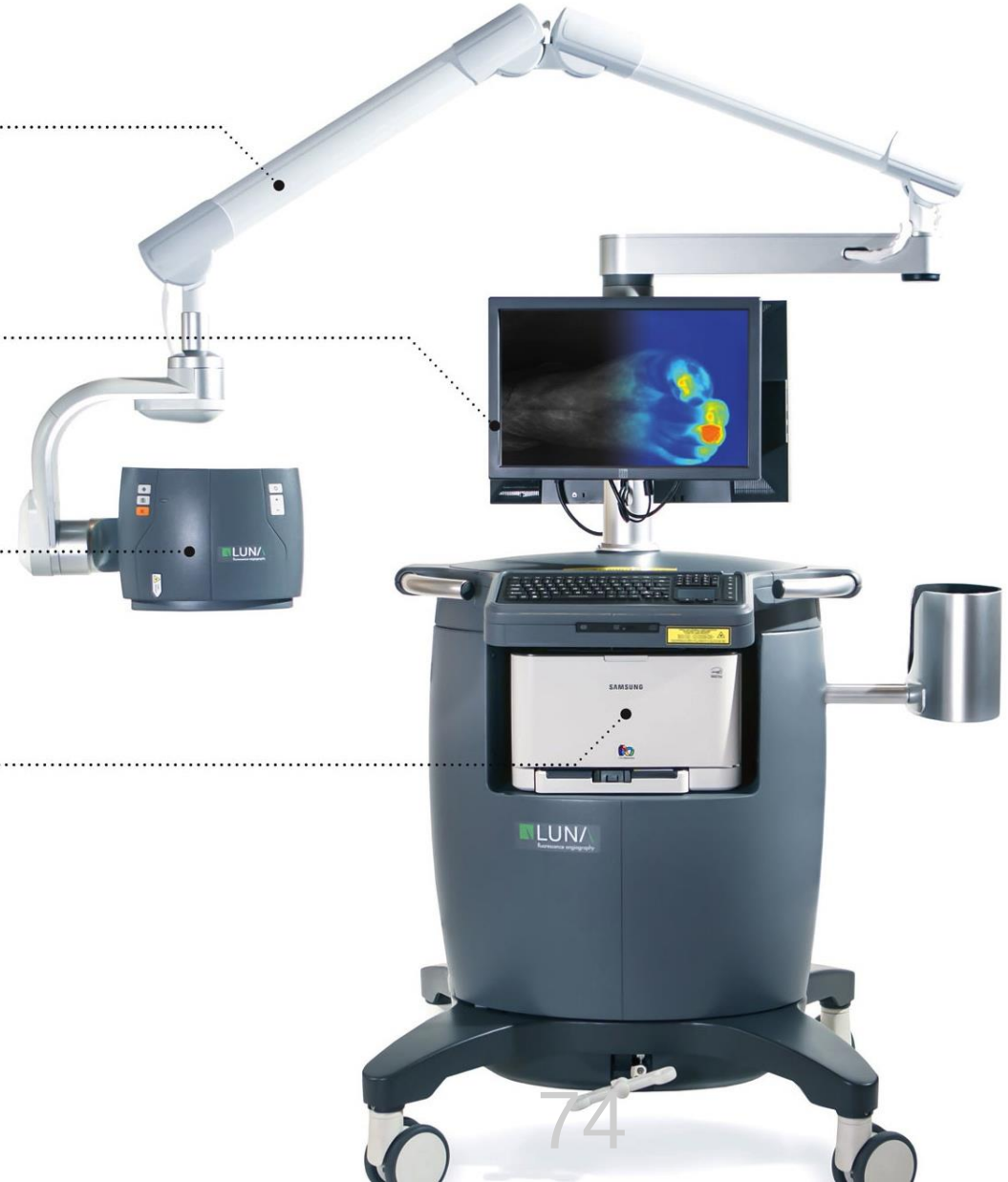


26” dual LCD monitors



SPY Fluorescence imaging head

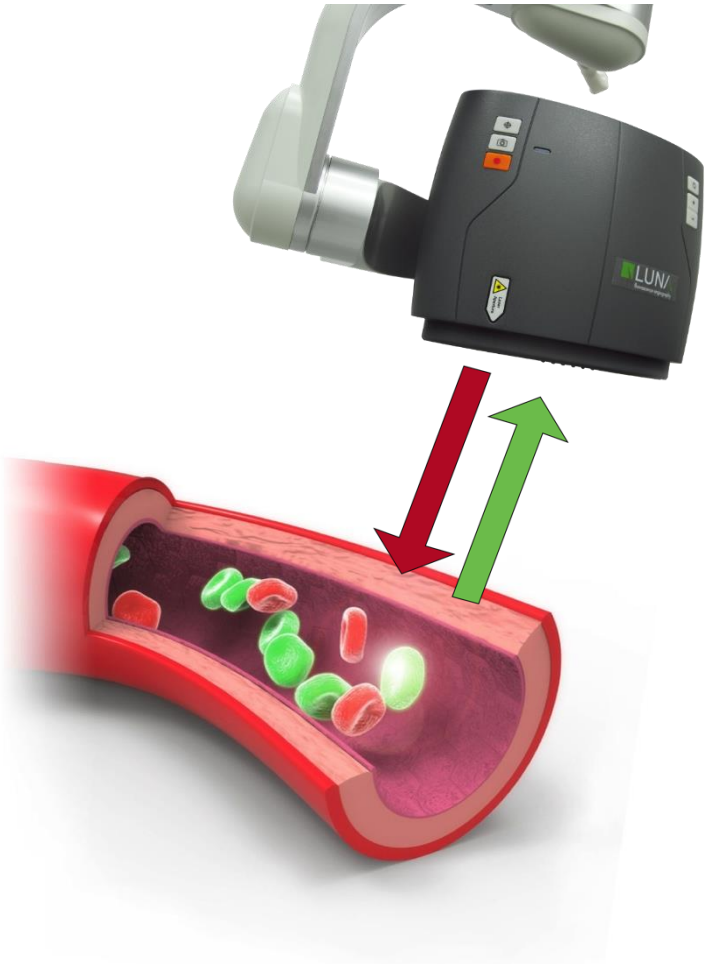
High-definition color printer

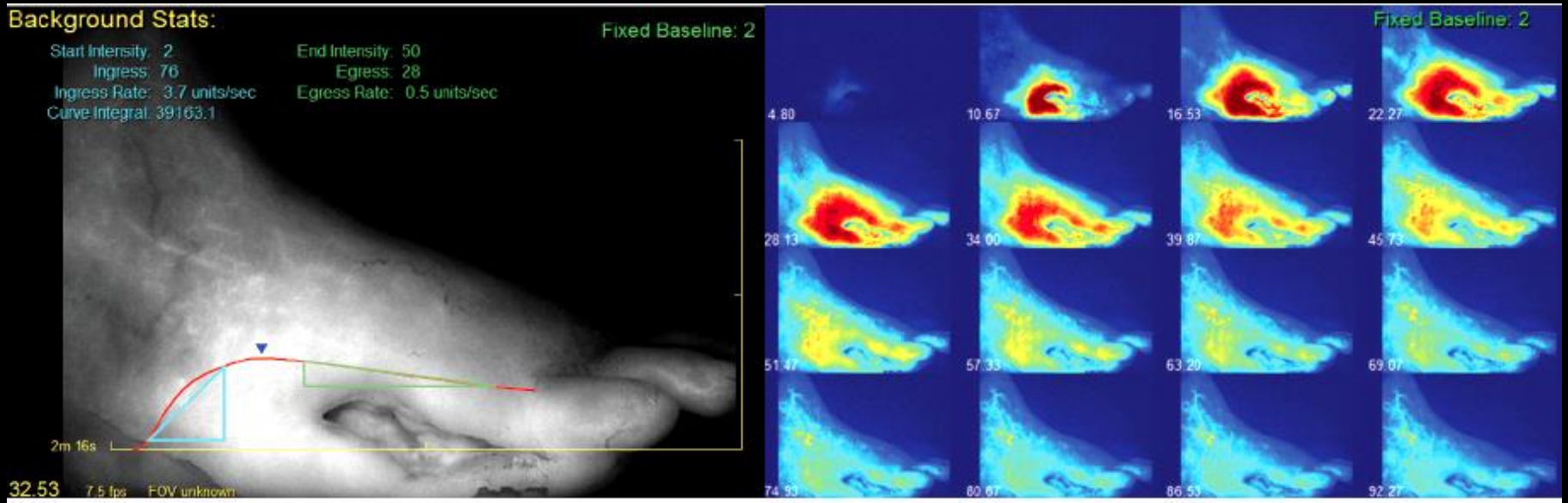


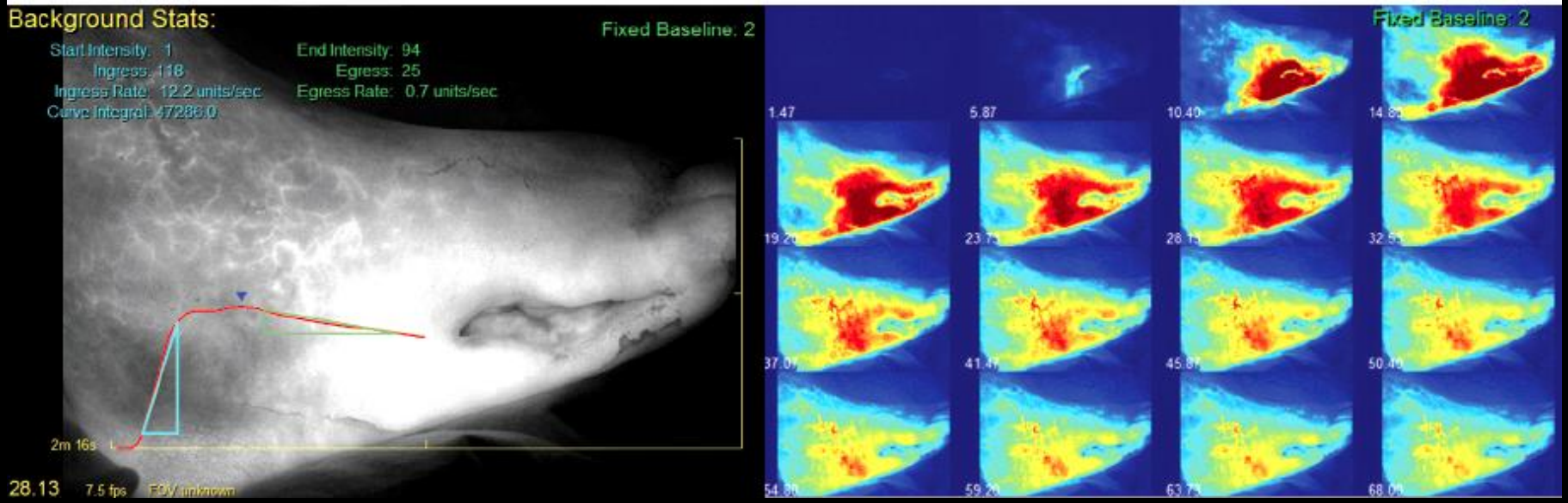
Wound Fluorescence Microangiography

1. Indocyanine Green (3 cc) injected intravenously with a 10 cc saline flush

2. Low-level light source excites ICG, fluorescence captured in real-time and displayed on monitor




Background Stats:
Fixed Baseline: 2
Start Intensity: 2
End Intensity: 50
Ingress: 76
Egress: 28
Ingress Rate: 3.7 units/sec
Egress Rate: 0.5 units/sec
Curve Integral: 39163.1
2m 16s
32.53
7.5 fps
FOV unknown
Fixed Baseline: 2
4.80
10.67
16.53
22.27
28.13
34.00
39.87
45.73
51.47
57.33
63.20
69.07
74.93
80.67
86.53
92.27


Background Stats:
Fixed Baseline: 2
Start Intensity: 1
End Intensity: 94
Ingress: 118
Egress: 25
Ingress Rate: 12.2 units/sec
Egress Rate: 0.7 units/sec
Curve Integral: 47286.0
2m 16s
28.13
7.5 fps
FOV unknown
Fixed Baseline: 2
1.47
5.87
10.40
19.20
23.73
28.13
37.07
41.47
45.87
50.40
54.80
59.20
63.73
68.00

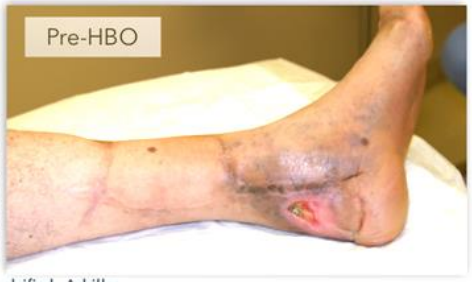
Indocyanine Green Imaging(LUNA) Assessment of Hyperbaric Oxygen Therapy

Stephen D. Guthrie, MD, PhD and Barbara R. Guthrie, MD
Designed Altobaric Research Foundation; Livonia Michigan

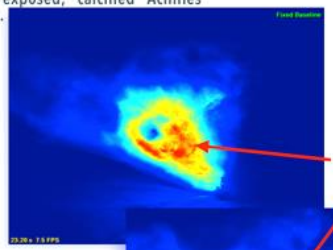
Seventeen patients at The Institute for Wound Care and Hyperbaric Medicine(IWCHM) receiving hyperbaric oxygen treatments (HBO_2Rx) for Limb Salvage underwent indocyanine green imaging (LUNA^{tm}) of the threatening wound. These LUNA^{tm} images provided guidance in:

- Assessing candidacy for HBO_2Rx
- Establishing the HBO_2Rx depth, duration and frequency
- Confirming that the wound had regained the capacity for unassisted secondary healing (CUSH).

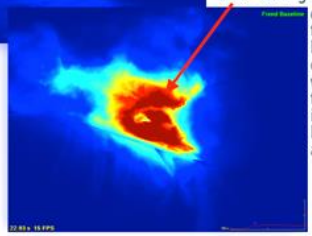
A case is presented here where LUNA^{tm} images were used to establish that CUSH had been reached after a relatively short (20 sessions) HBO_2Rx course of treatment.

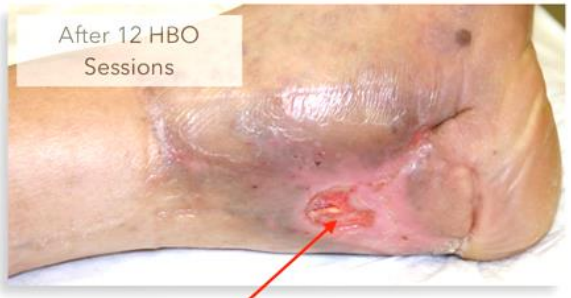


Mr. A.A. is an otherwise healthy 48 y.o. gentleman who incurred an open tibio-fibular fracture thirty five years ago that required a musculo-cutaneous flap for stable coverage. This remained closed until sixteen months ago when this distal breakdown occurred with exposed, calcified Achilles tendon insertion.

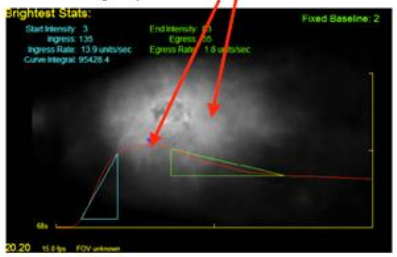


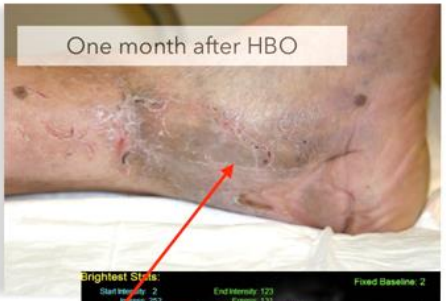
The initial LUNA^{tm} image before hyperbaric chamber exposure and immediately after exiting the chamber demonstrate the typical luminescence of the chronic wound and the expected increase in luminescence after HBO_2Rx.



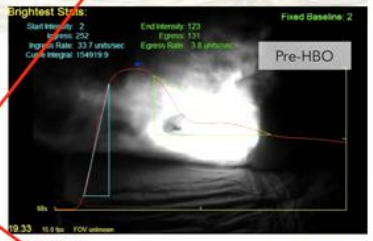


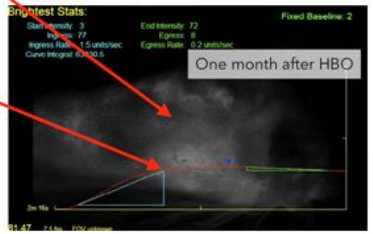
As the wound improves clinically, the maximum luminescence at the peak of the LUNA^{tm} sequence subsides and the AutoView summary of the whole image capture softens





When the wound is fully healed, there is little luminescence remaining at the site and the AutoView summary of the entire sequence has the appearance of a non-wounded area.

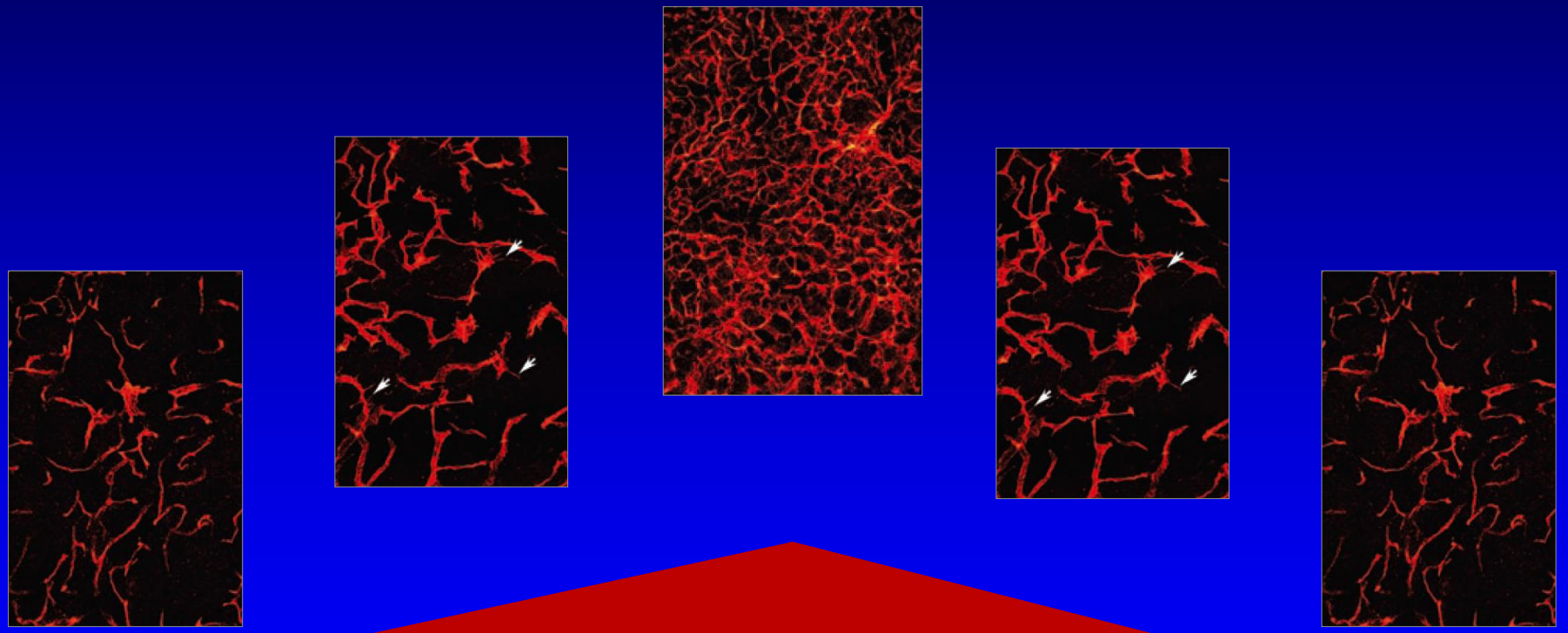




Normal Wound Healing

ANGIOGENESIS “ON”

VESSEL NORMALIZATION



BASELINE VASCULARITY

MAXIMAL ANGIOGENESIS

BASELINE VASCULARITY

ACUTE INJURY

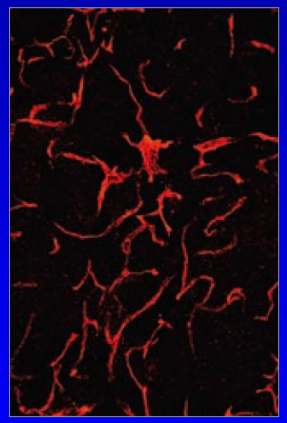
HEALED

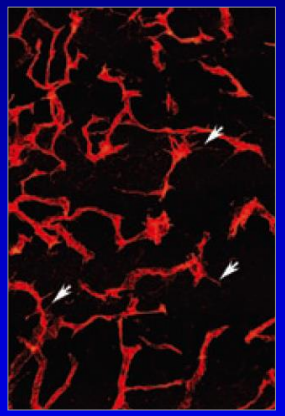
Chronic Wound Healing

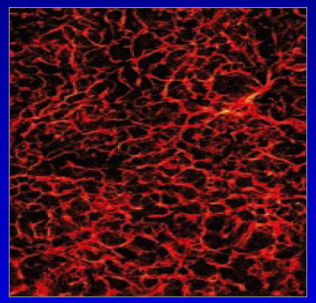
ANGIOGENESIS “ON”

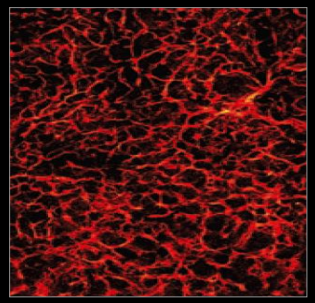
PERSISTS ABNORMALLY “STUCK”

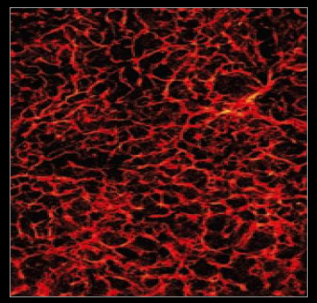
“Hyperfluorescenc











CHRONIC INFLAMMATION

BASELINE VASCULARITY

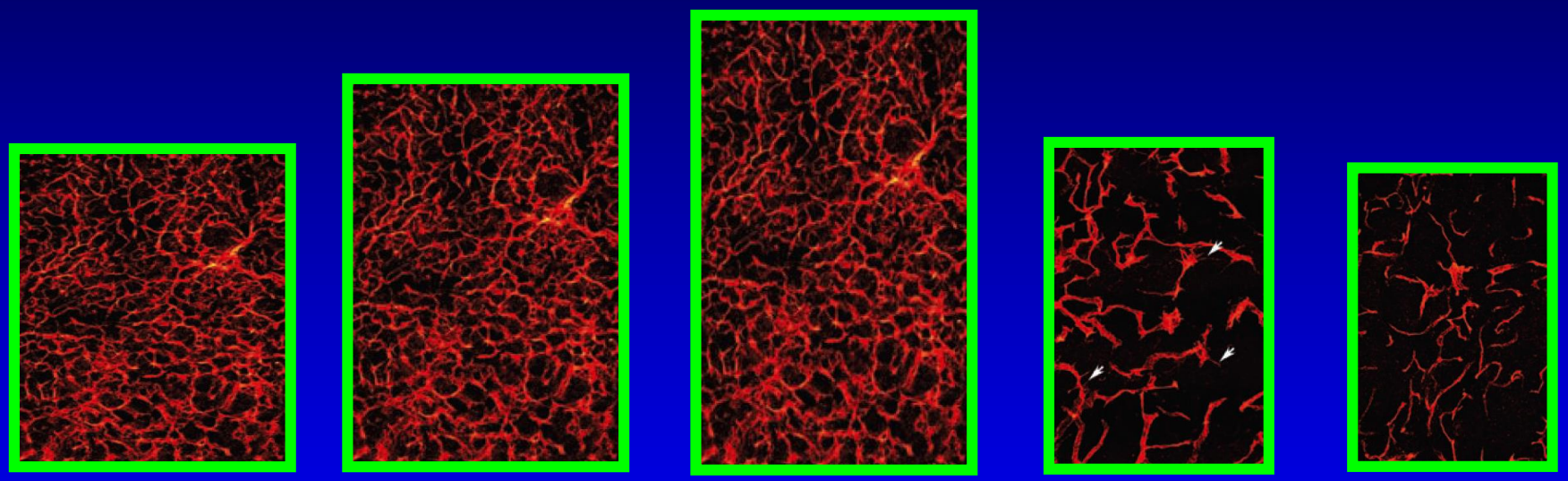
ACUTE INJURY

PERIMETER ANGIOGENESIS

NOT HEALED

Wound Microcirculation Monitoring

HBOT

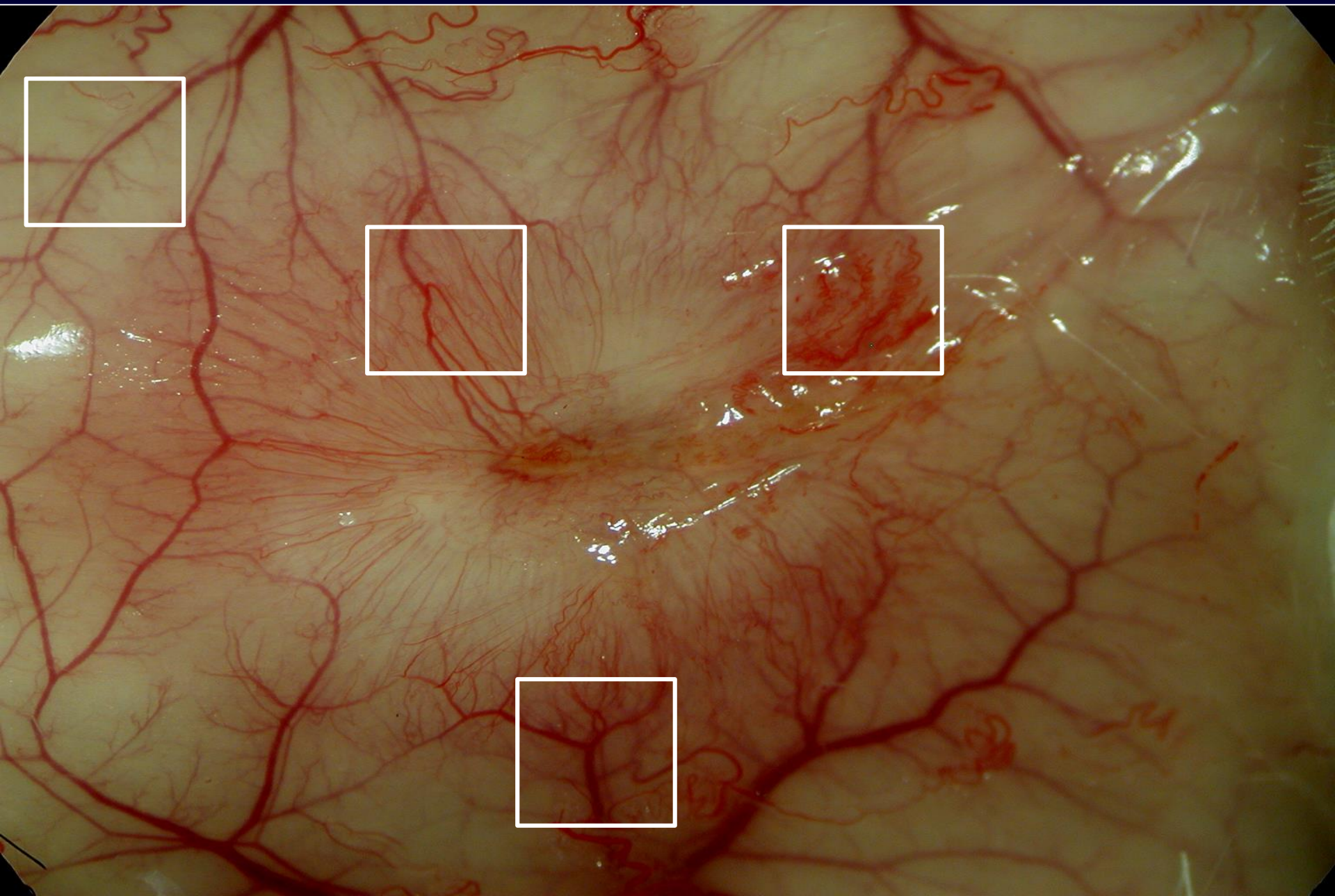


"Hyperflurescence"

"Treat to normal" vascularity

ANGIOGENESIS INCREASES IN HEALING, THEN IS PRUNED TO PHYSIOLOGICAL BASELINE

Angiogenesis Near End of Healing



3 TAKE HOME POINTS:

1. Angiogenic responses are much more complex process than previously assumed (not just “on” or “off”).

2. Oxygen sensing (hypoxia / hyperoxia) in angiogenesis defends microvascular homeostasis.

3. Regenerative changes can be promoted by hyperbaric interventions.

FUTURE DIRECTIONS

1. Can the Goldilock's mechanisms of angiogenesis be over-ridden by dose or frequency of HBOT?

2. Is the quality of new vessels induced by HBOT different than that induced simply by hypoxia – can we create higher quality vessels (more stable, larger calibre, healthier endothelium, etc.)

3. Can we use angiogenesis imaging to determine the Optimal Biological Dose of HBOT?

“Scientific knowledge is in perpetual evolution; it finds itself changed from one day to the next.”

— Jean Piaget (1896-1980)