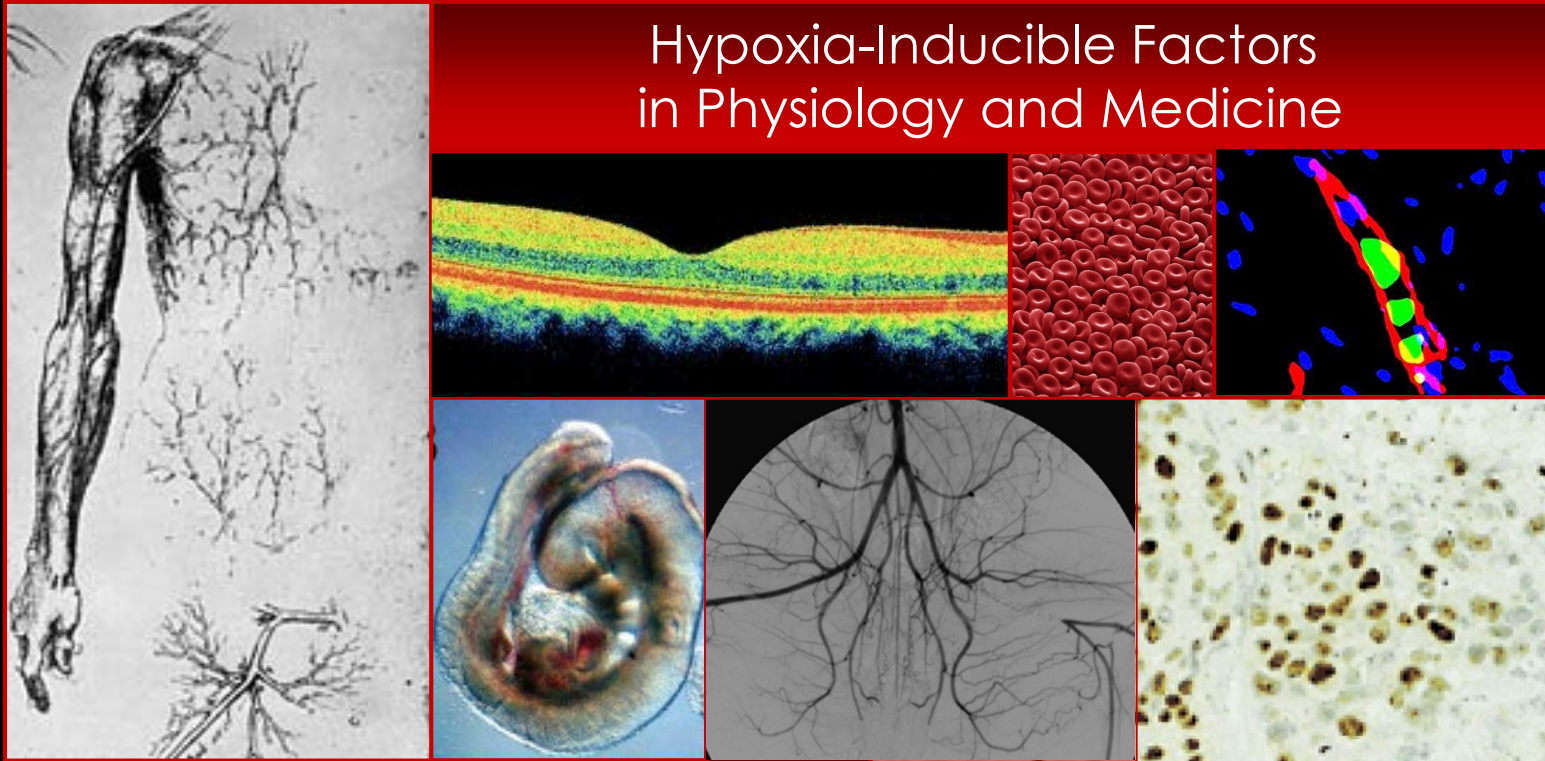
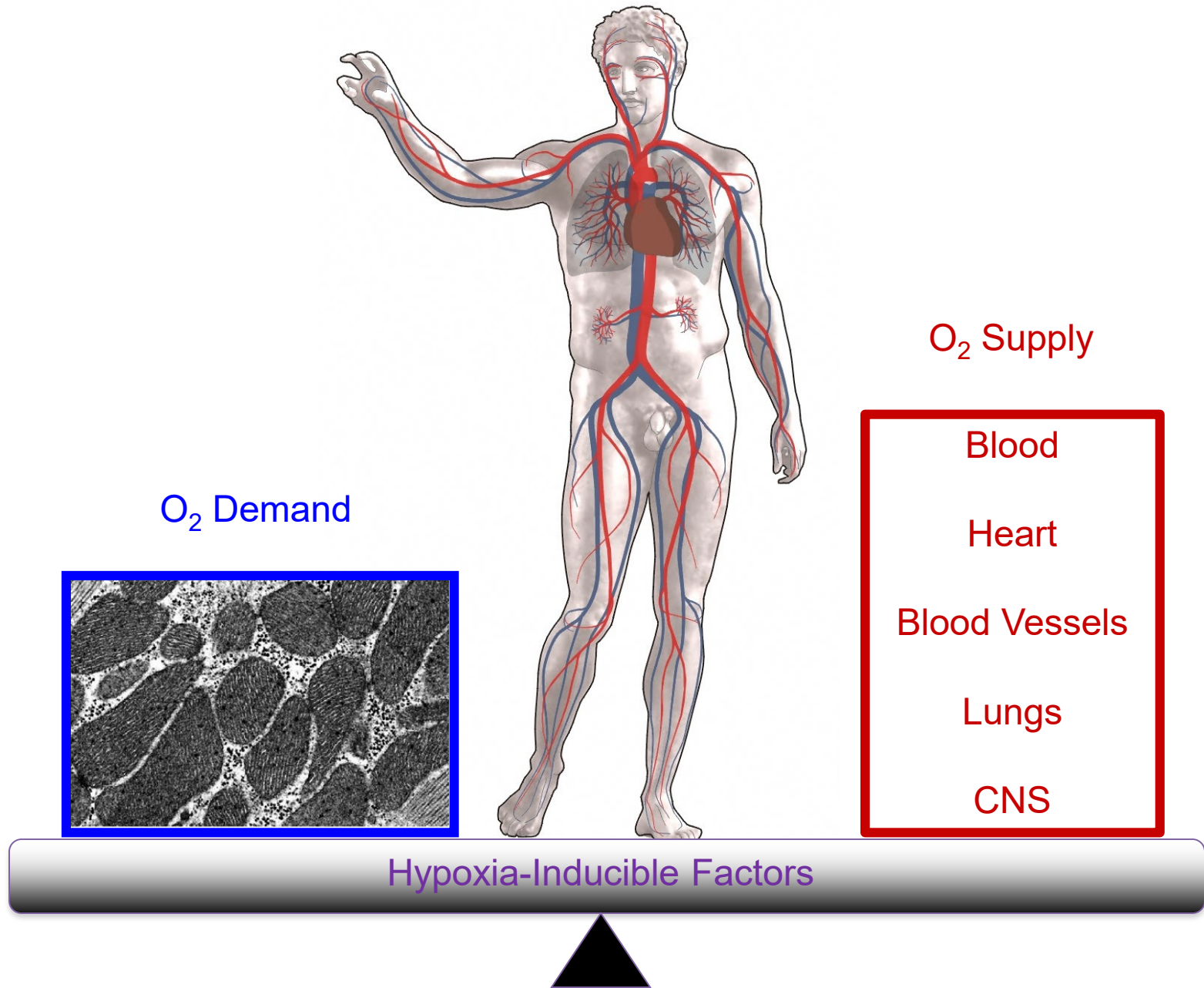


Hypoxia-Inducible Factors in Physiology and Medicine

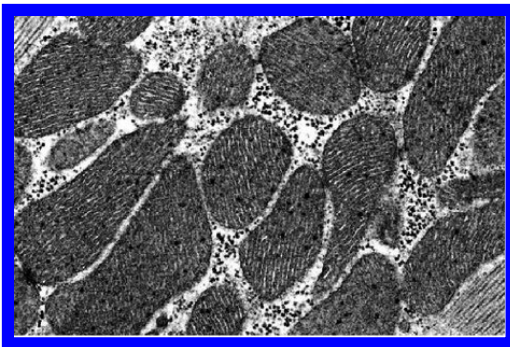


Gregg L. Semenza, M.D., Ph.D.
Vascular Program, Institute for Cell Engineering;
Departments of Genetic Medicine, Pediatrics, Oncology,
Medicine, Radiation Oncology, and Biological Chemistry;
Johns Hopkins University School of Medicine
Baltimore, Maryland USA

Oxygen Homeostasis: A Balancing Act



O₂ Demand



O₂ Supply

Blood

Heart

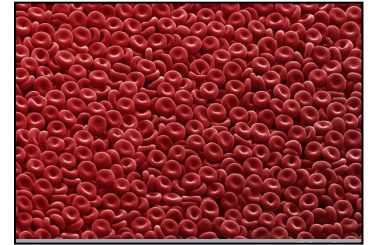
Blood Vessels

Lungs

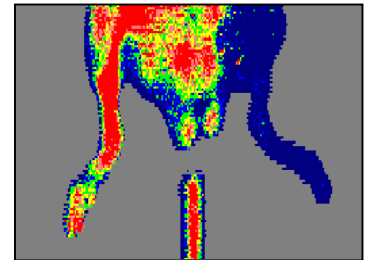
CNS

Hypoxia-Inducible Factors

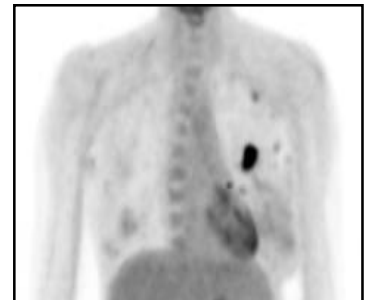
Control of Red Blood Cell Production



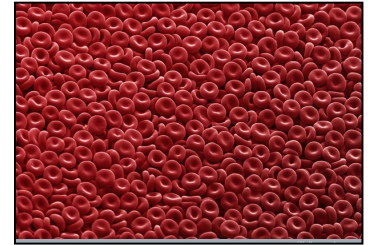
Cardiovascular Disease



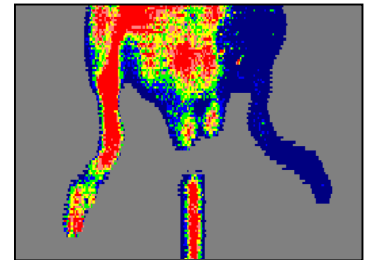
Cancer



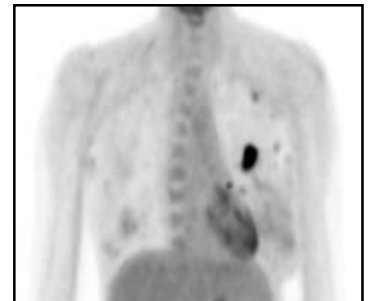
Control of Red Blood Cell Production



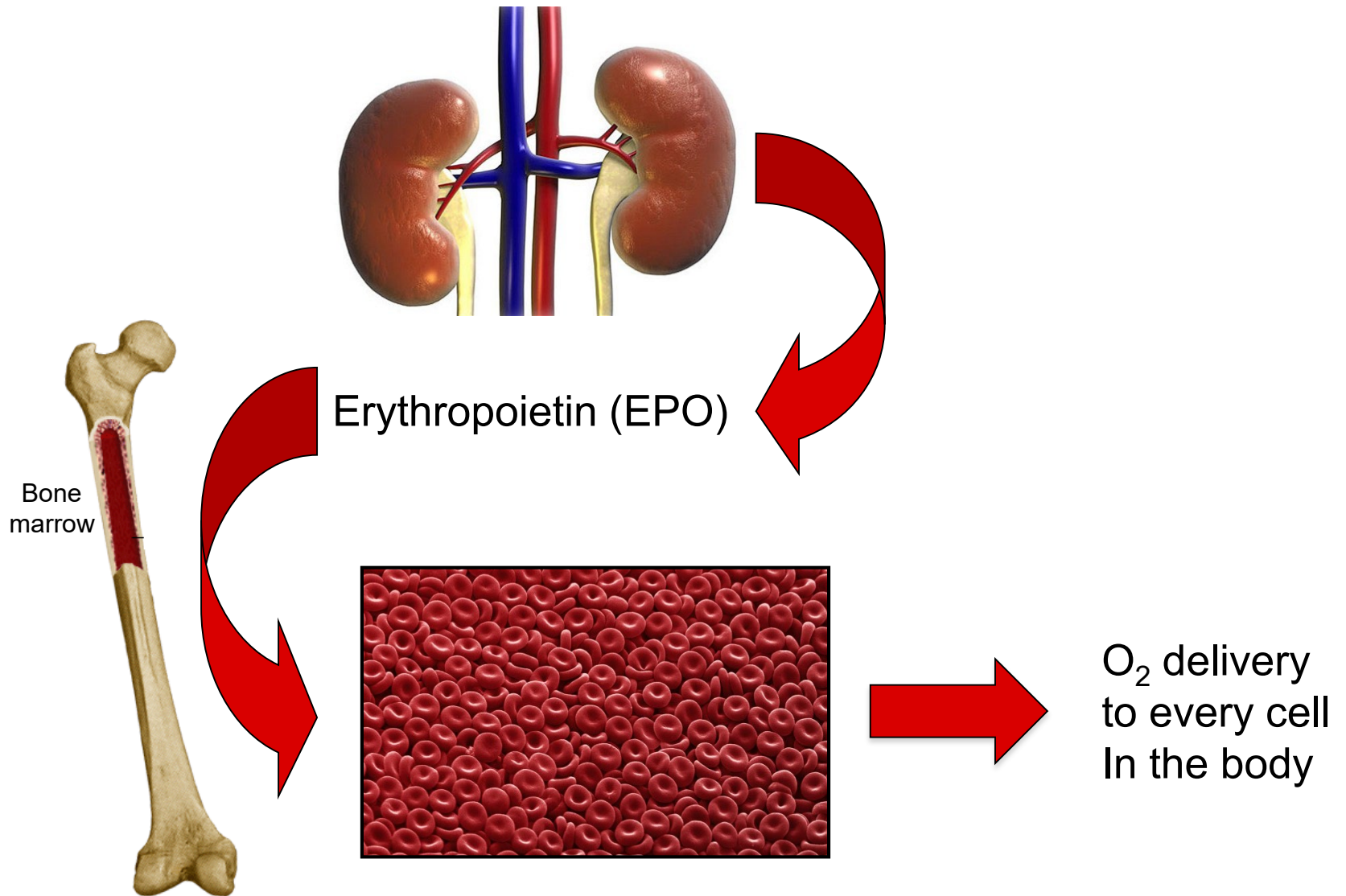
Cardiovascular Disease



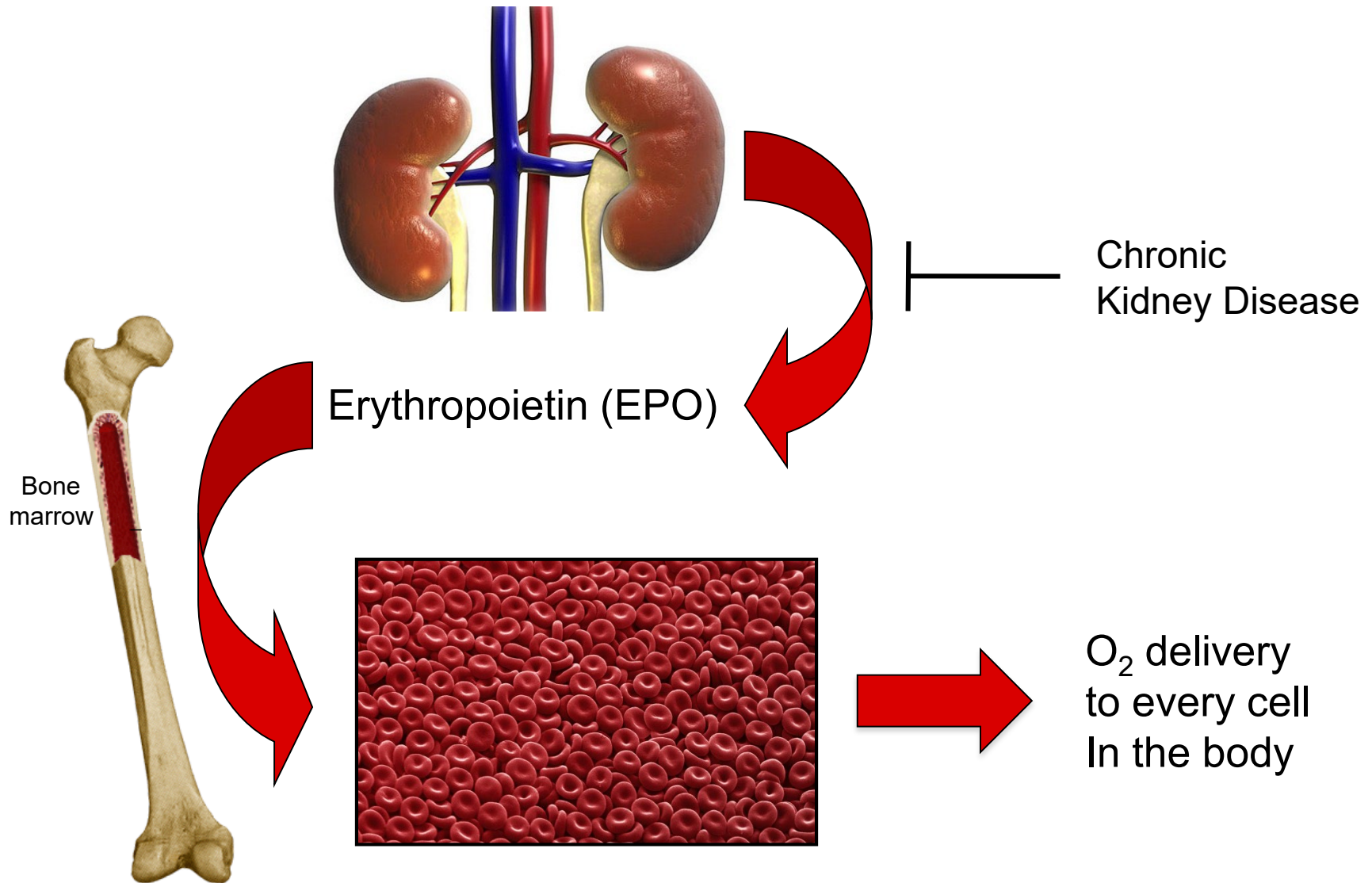
Cancer



Erythropoietin Controls Red Blood Cell Production

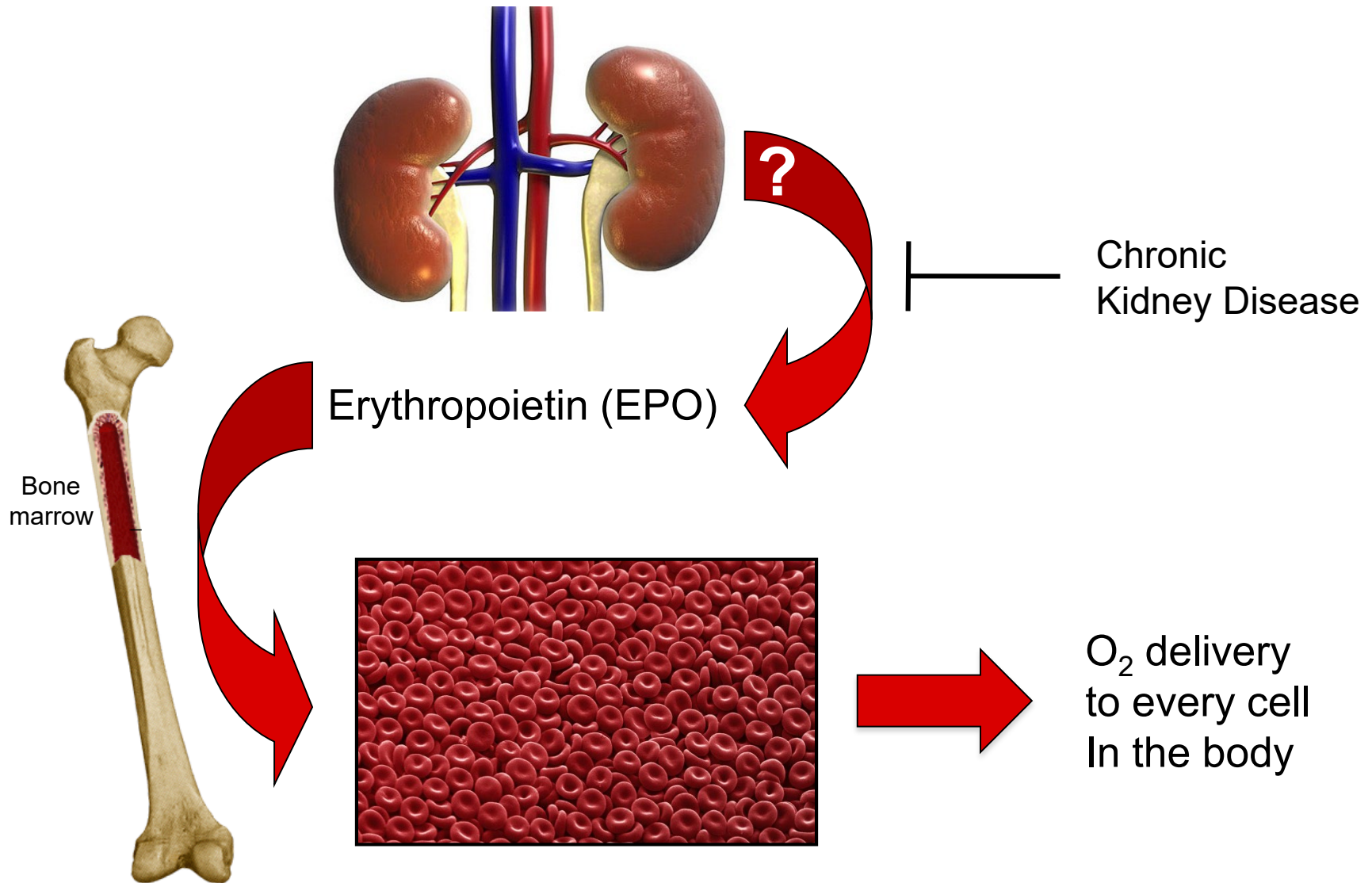


Erythropoietin Controls Red Blood Cell Production

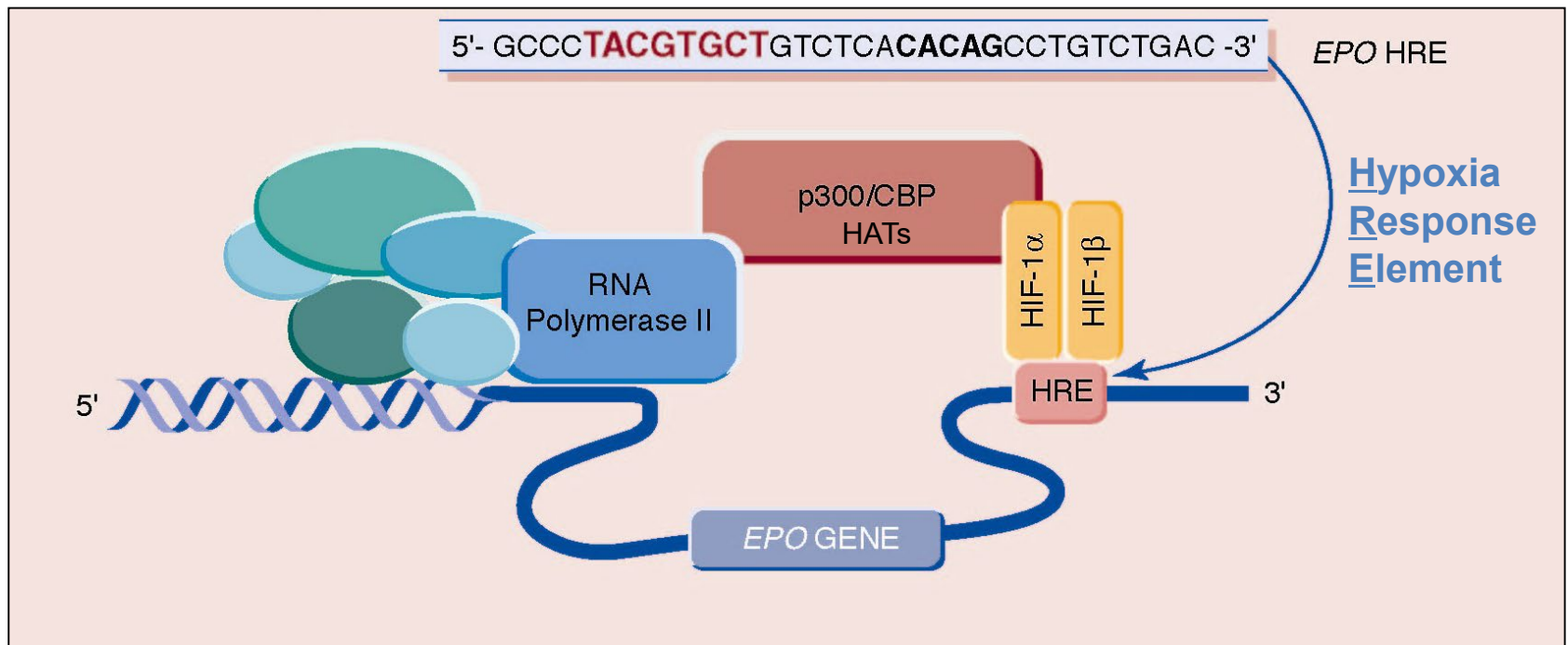


Erythropoietin Controls Red Blood Cell Production

What Controls Erythropoietin Production?

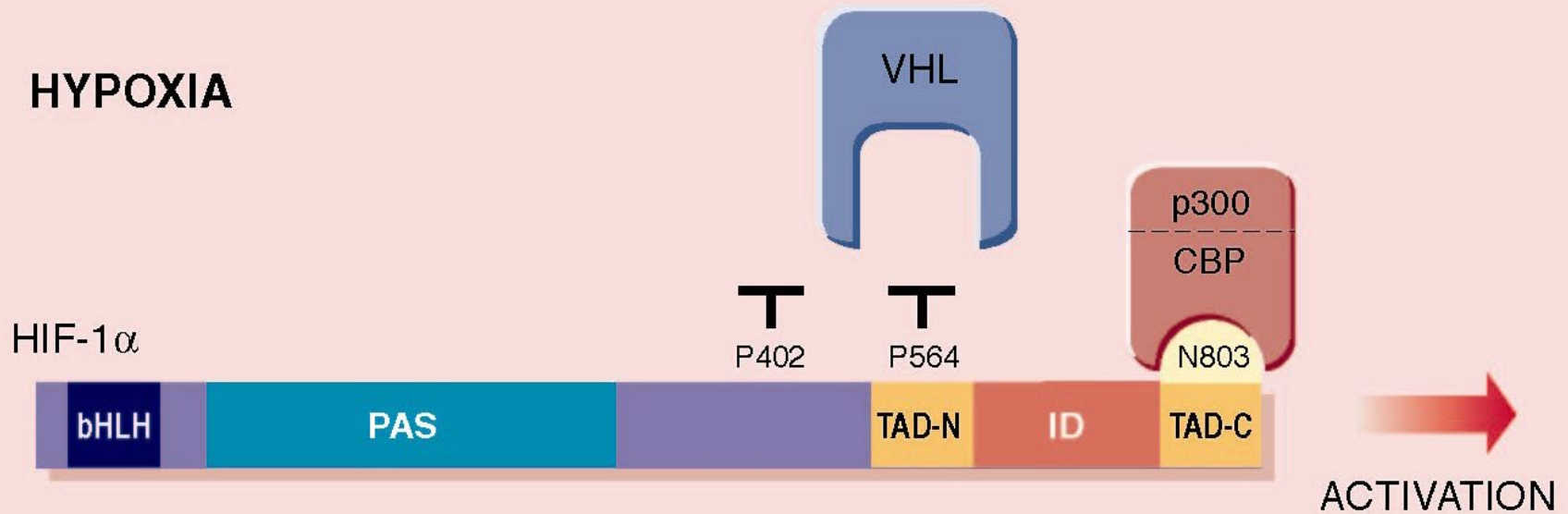


Hypoxia-Inducible Factor 1 (HIF-1) Binds to the *EPO* Gene and Activates Transcription



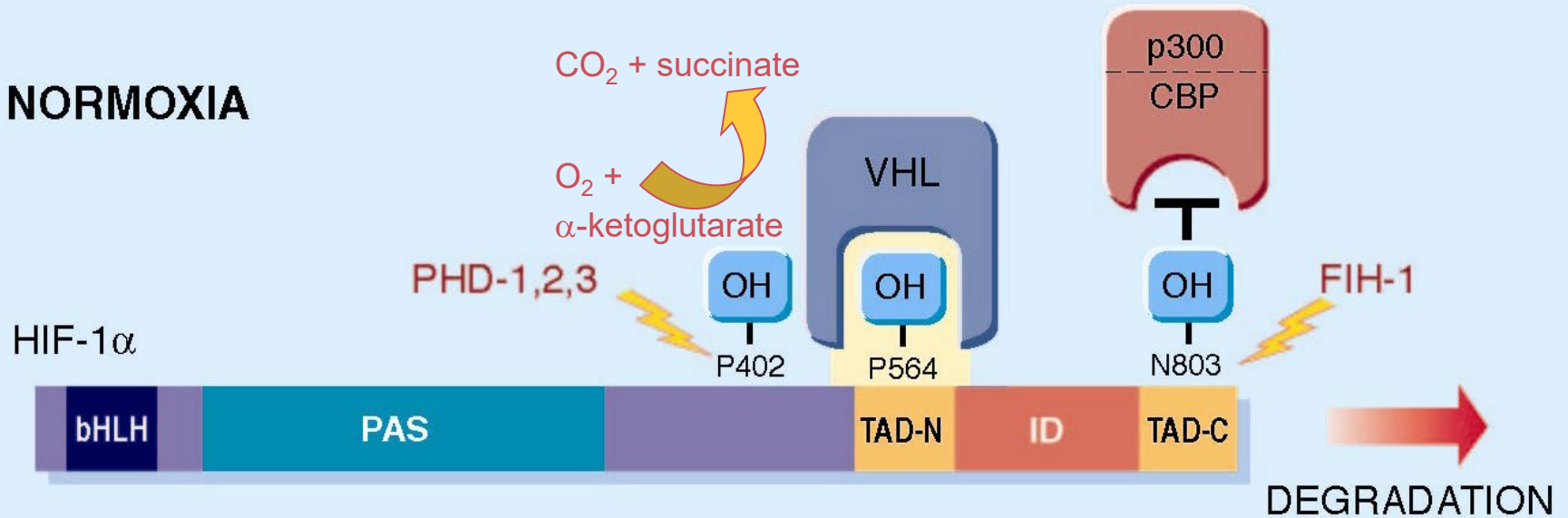
G. L. Semenza and G. L. Wang, *Mol. Cell. Biol.* 12: 5447, 1992
G. L. Wang and G. L. Semenza, *J. Biol. Chem.* 270: 1230, 1995
G. L. Wang et al. *Proc. Natl. Acad. Sci. USA* 92: 5510, 1995

HYPOXIA



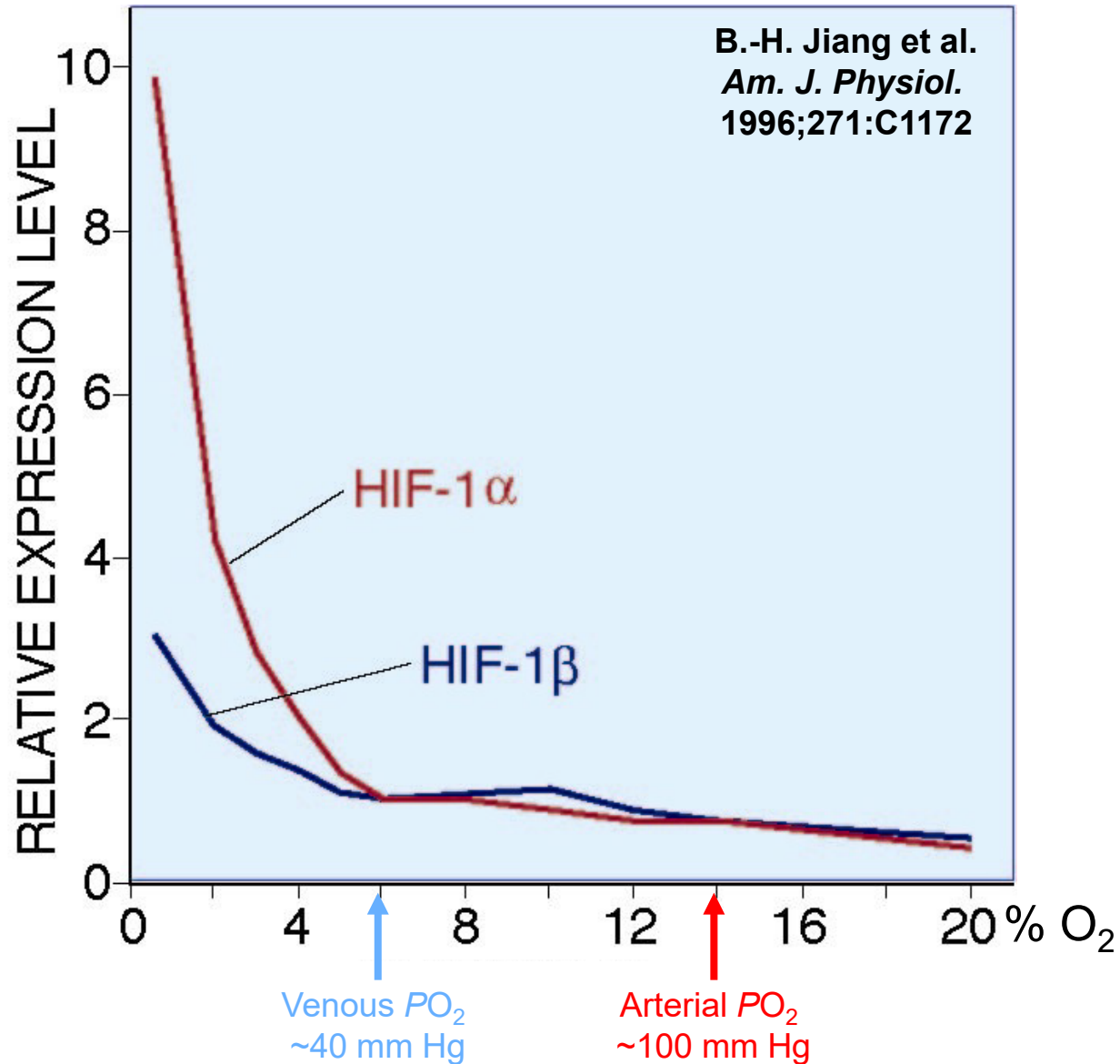
HIF-1 α is Regulated by Oxygen-dependent Hydroxylation

NORMOXIA

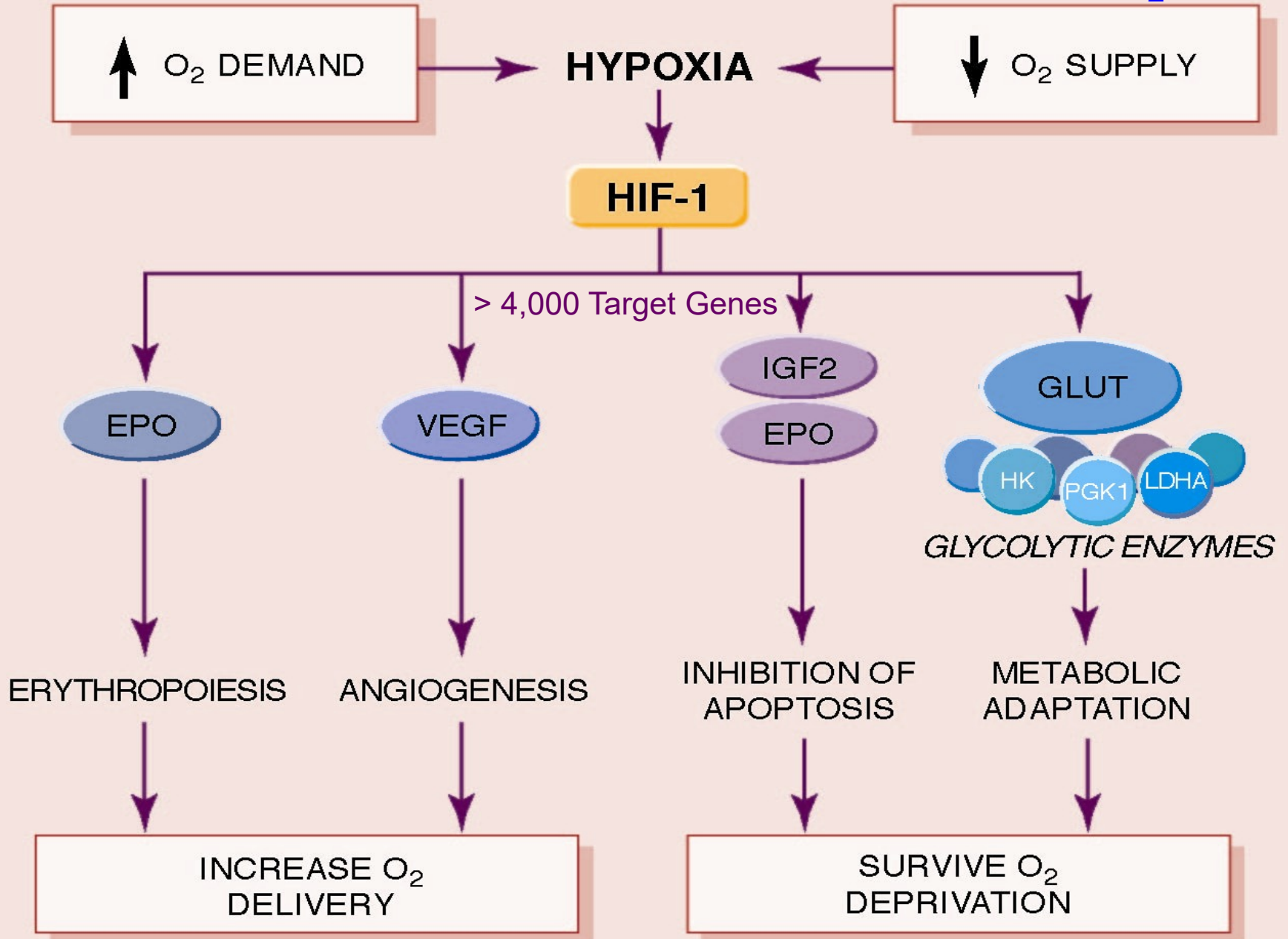


PHDs = Prolyl Hydroxylase Domain proteins target HIF-1 α for destruction when O_2 is available.

HIF-1 α Protein Accumulates in Response to Hypoxia Leading to Increased Transcription of HIF-1 Target Genes

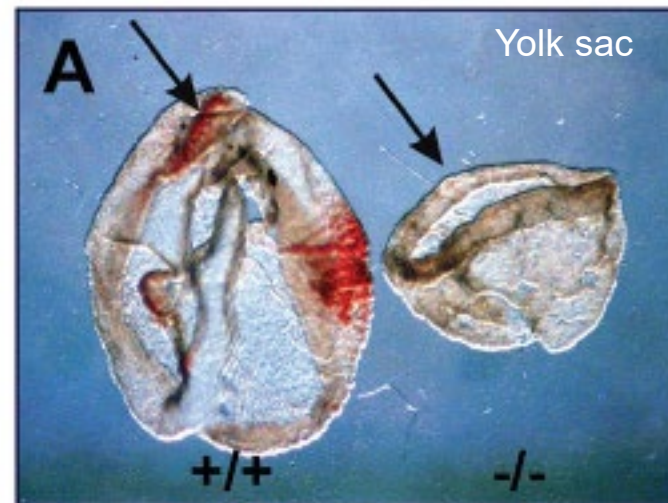
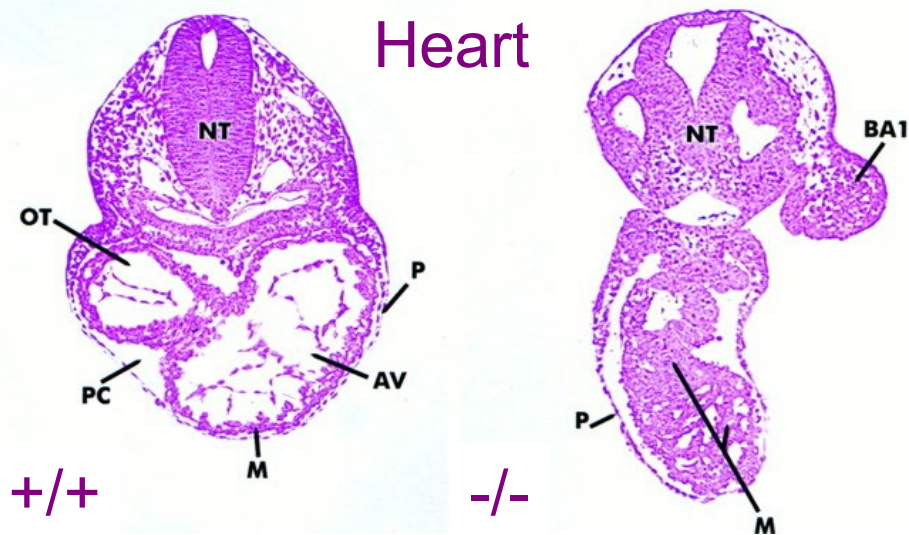


HIF-1 Mediates Homeostatic Responses to Reduced O₂ Levels



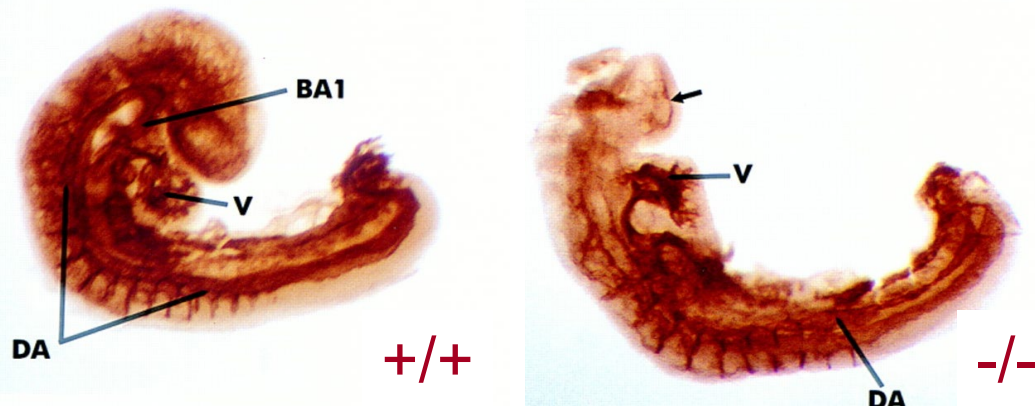
HIF-1 α is Required for Development of the Circulatory System

Heart



Blood

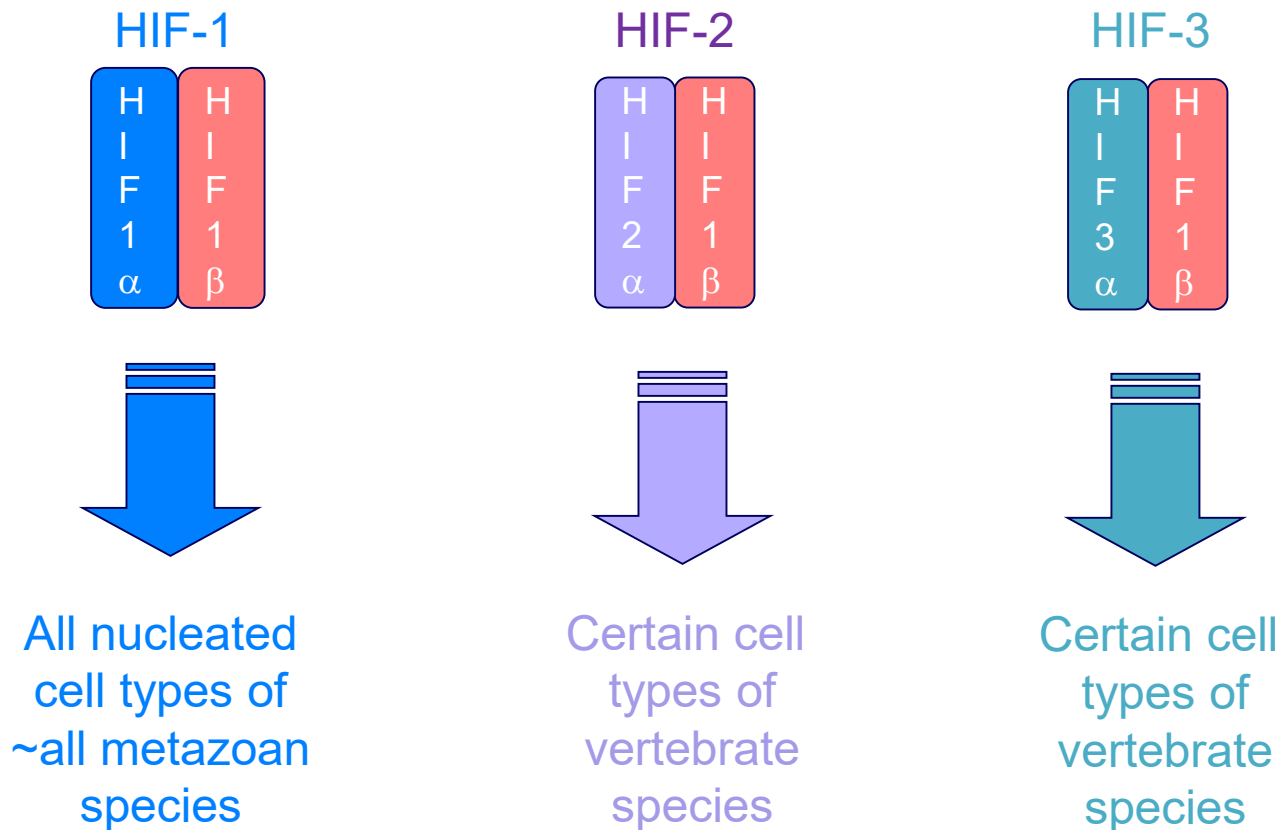
Blood Vessels



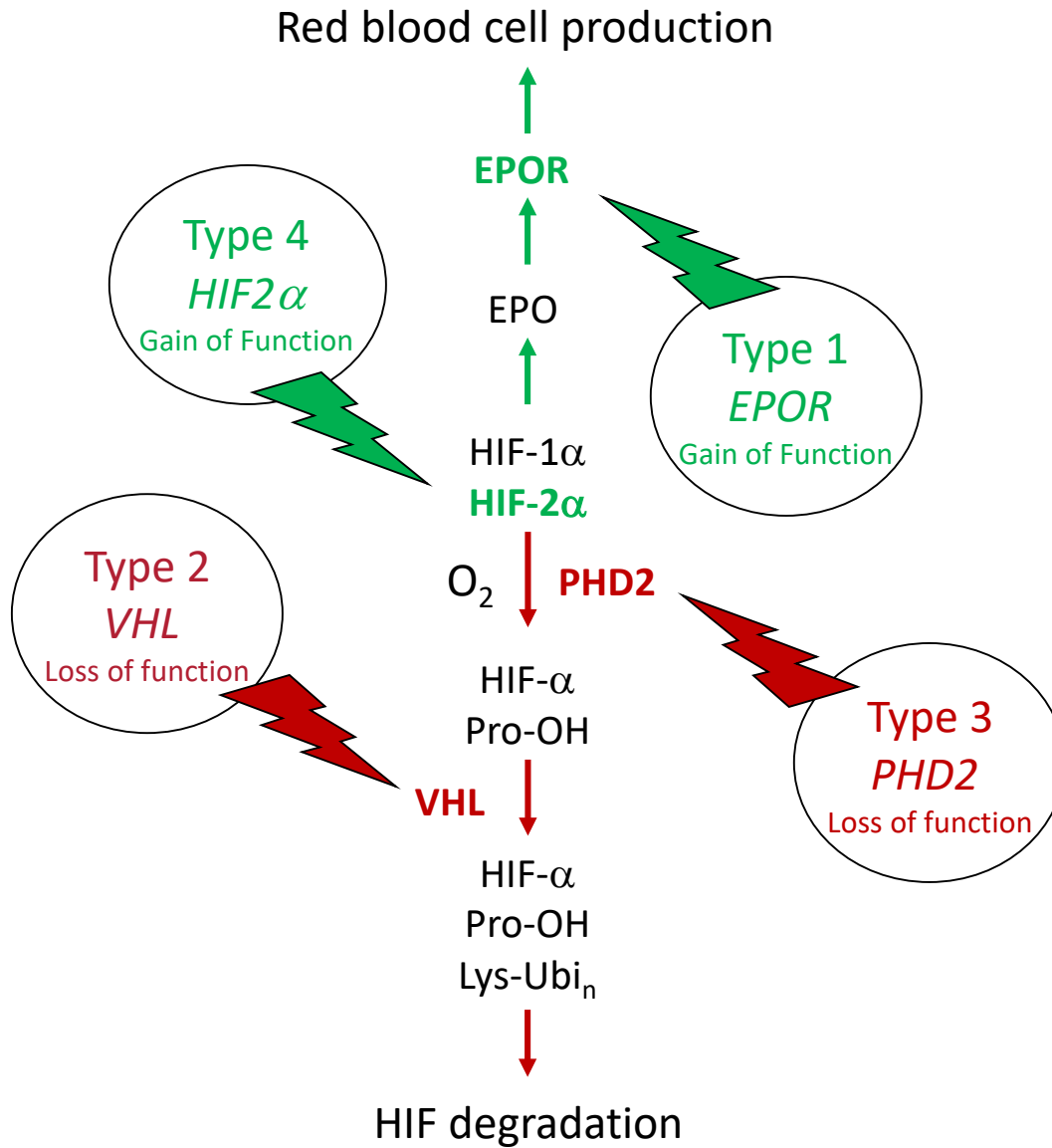
N. V. Iyer et al., *Genes Dev.* 12: 149, 1998

D. Yoon et al., *J. Biol. Chem.* 281:25703, 2006

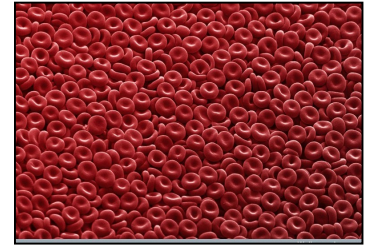
HIF-1 α , HIF-2 α and HIF-3 α Heterodimerize with HIF-1 β and Activate Gene Transcription



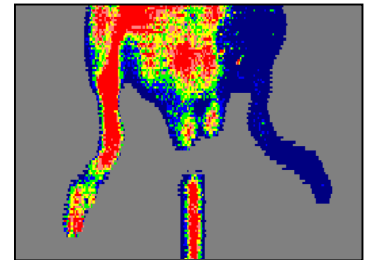
Congenital Polycythemia (Too Many Red Cells) is Caused by Mutations in the HIF Pathway



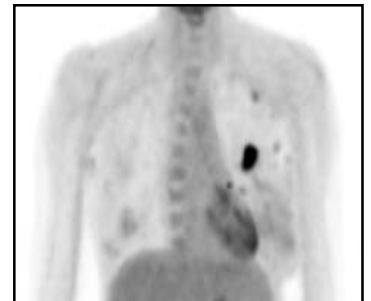
Control of Red Blood Cell Production



Cardiovascular Disease



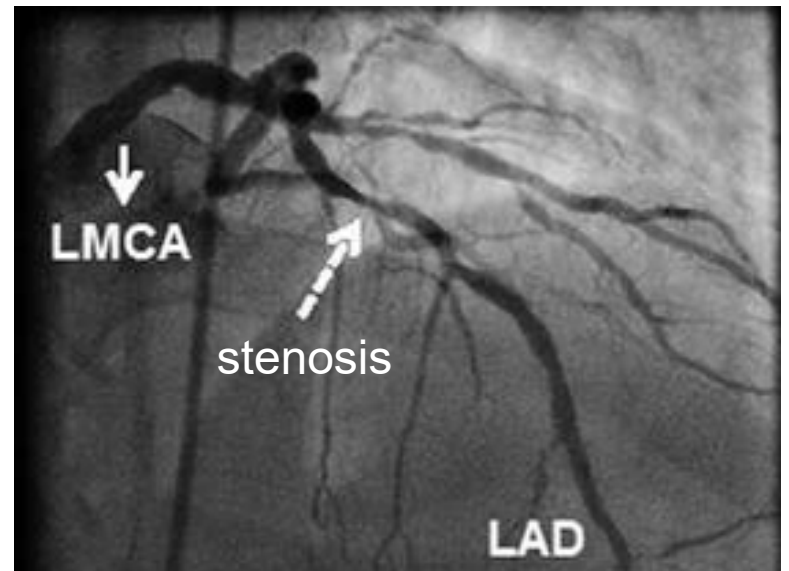
Cancer



Cardiovascular Disease is the Leading Cause of Death In the Industrialized World

CORONARY ARTERY → CHEST PAIN
HEART ATTACK

PERIPHERAL ARTERY → LEG PAIN
AMPUTATION



Cardiovascular Disease is the Leading Cause of Death In the Industrialized World

CORONARY ARTERY → CHEST PAIN
HEART ATTACK

PERIPHERAL ARTERY → LEG PAIN
AMPUTATION



Critical Limb Ischemia: End-Stage Peripheral Arterial Disease

Critical Limb Ischemia

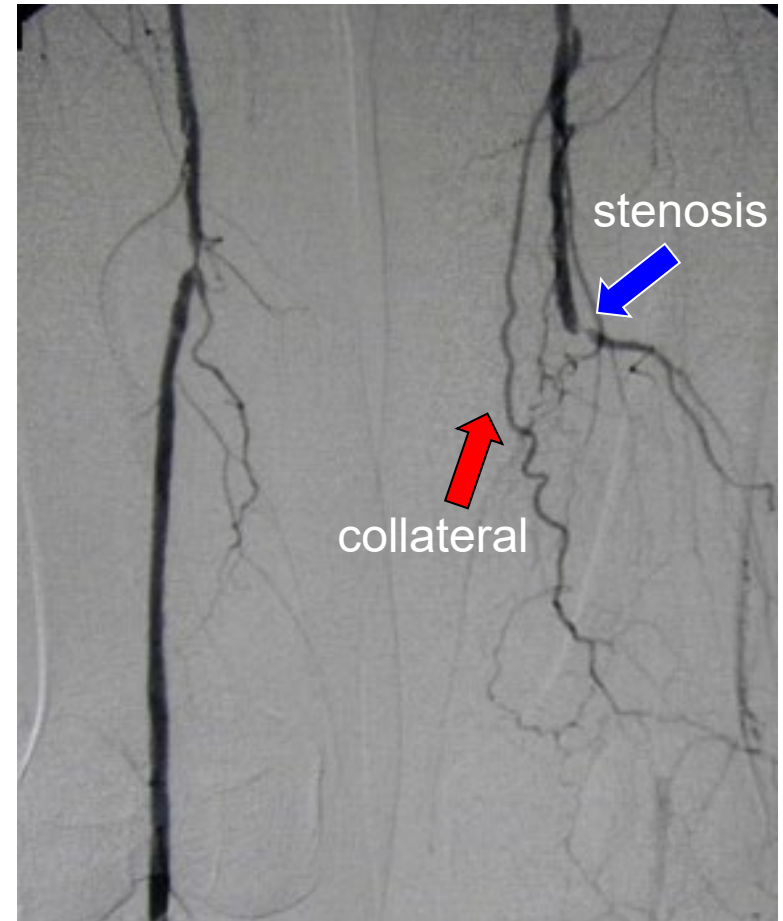
Perfusion is not sufficient to maintain tissue viability, leading to:

Ischemic pain at rest

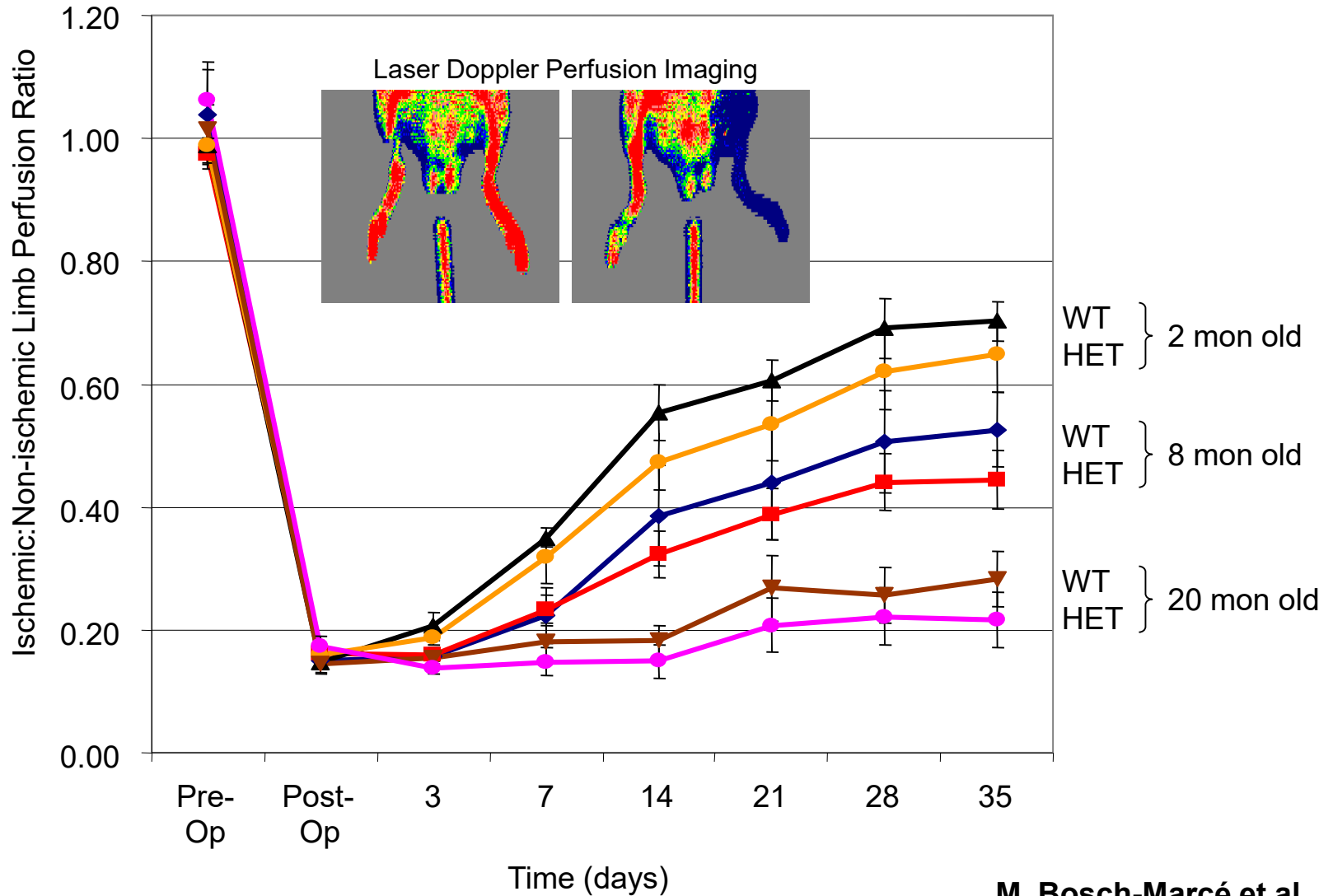
Ischemic ulcers

Gangrene

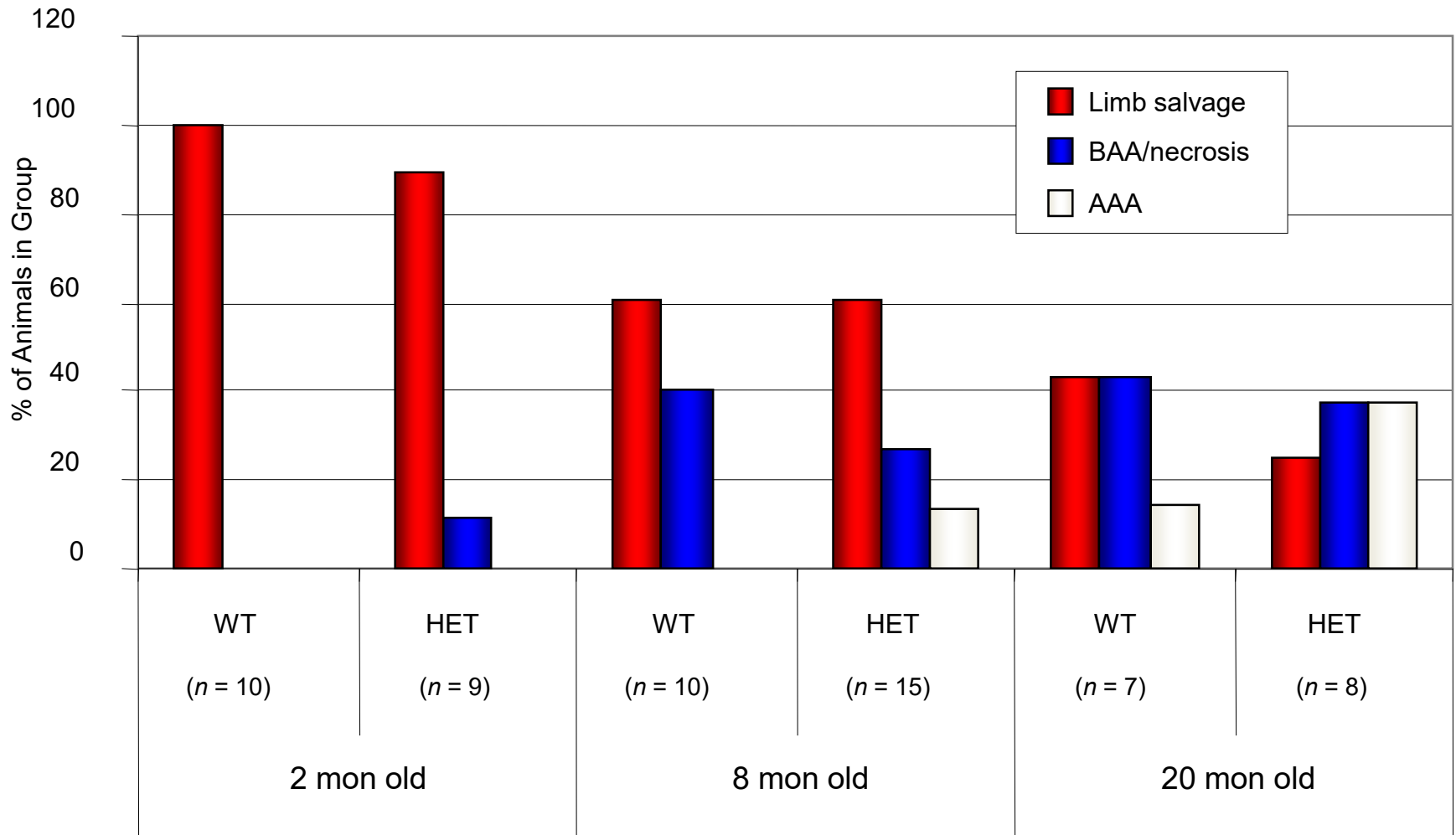
Limb amputation



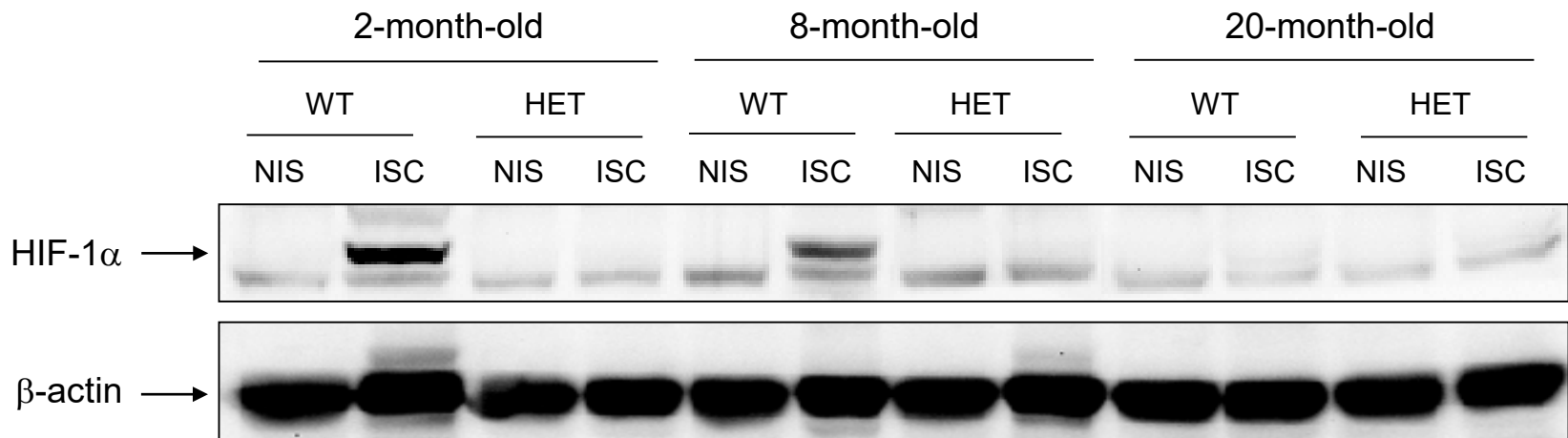
Analysis of Vascularization after Femoral Artery Ligation in Wild-type (WT) and Heterozygous HIF-1 α -Null (HET) Mice



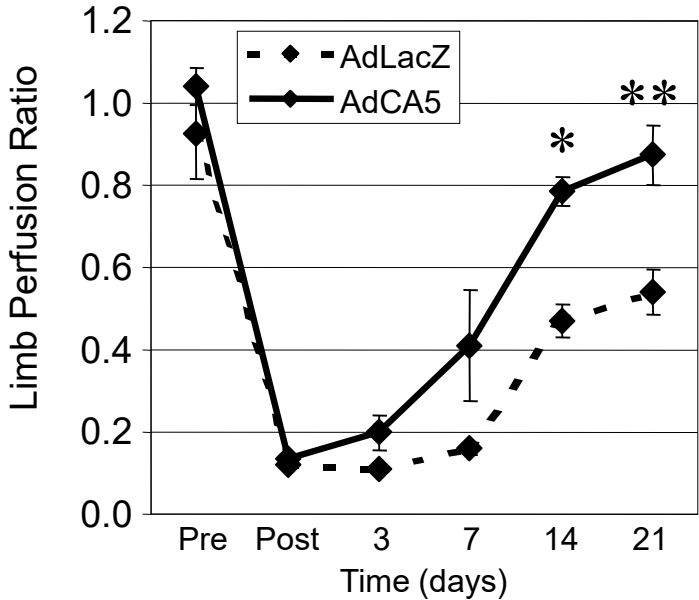
Additive Effects of Aging and *Hif1a* Genotype on Limb Salvage



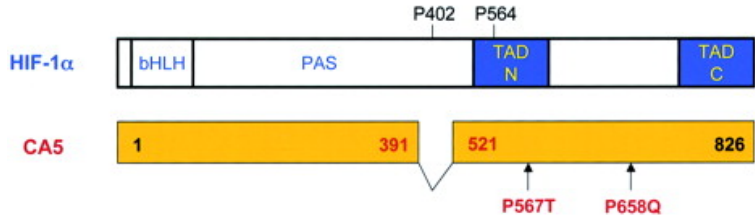
Effects of Aging and *Hif1a* Genotype on Ischemia-induced HIF-1 α Protein Levels



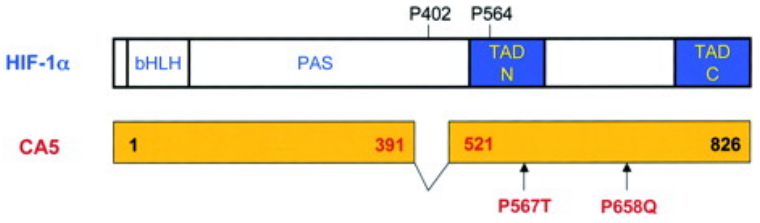
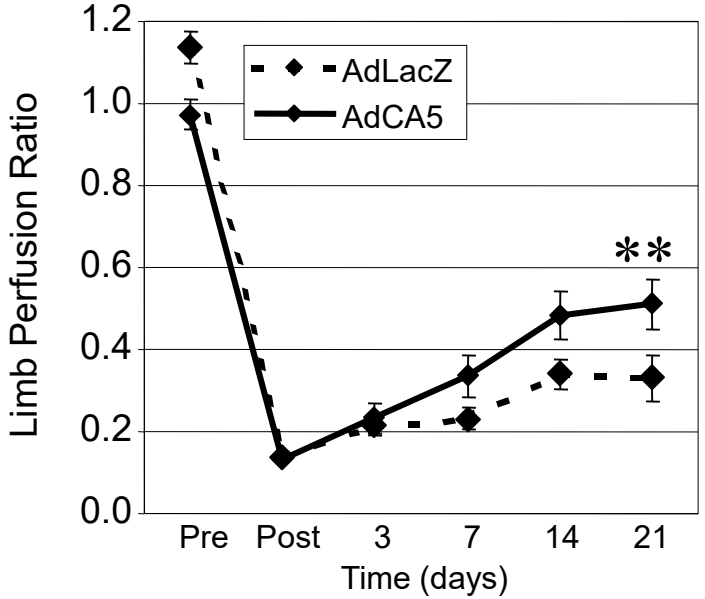
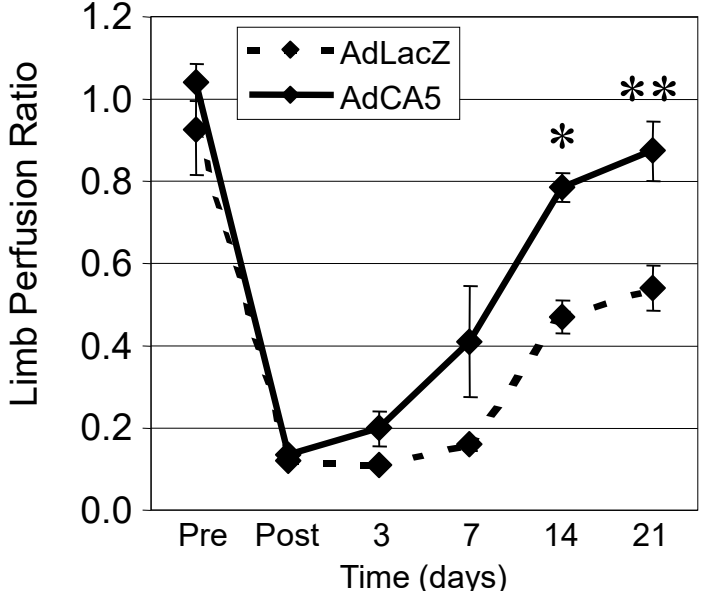
Improved Recovery of Perfusion in Mice by HIF-1 α Gene Therapy



2-month-old
C57BL/6J mice
Ad: 6×10^8 pfu

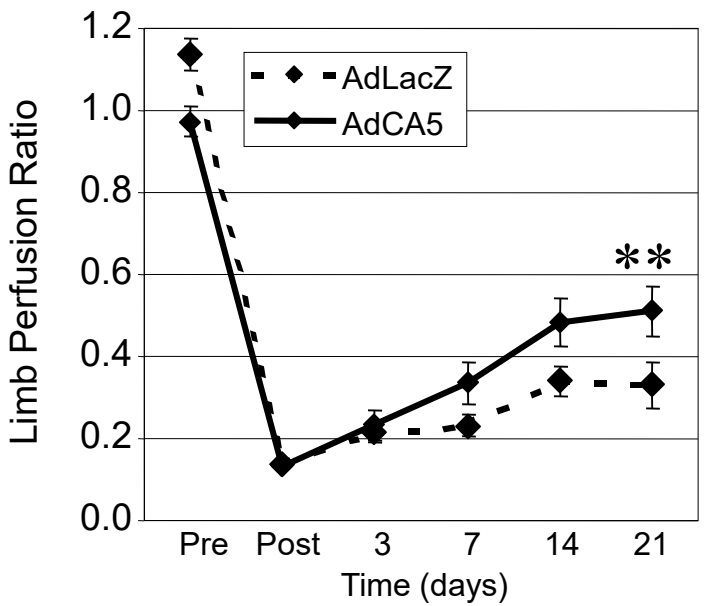
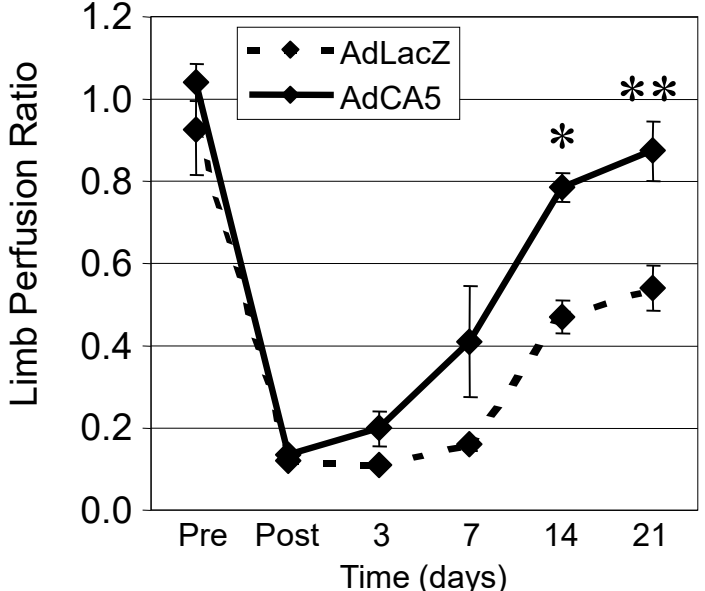


Improved Recovery of Perfusion in Mice by HIF-1 α Gene Therapy

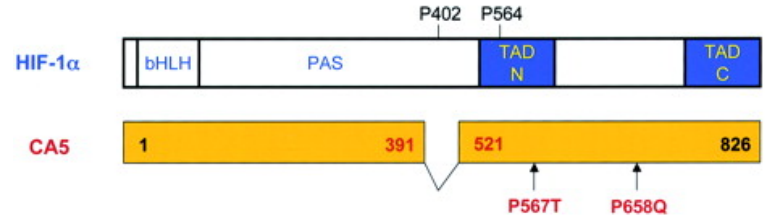


8-month-old C57BL/6J mice
Ad: 2×10^8 pfu

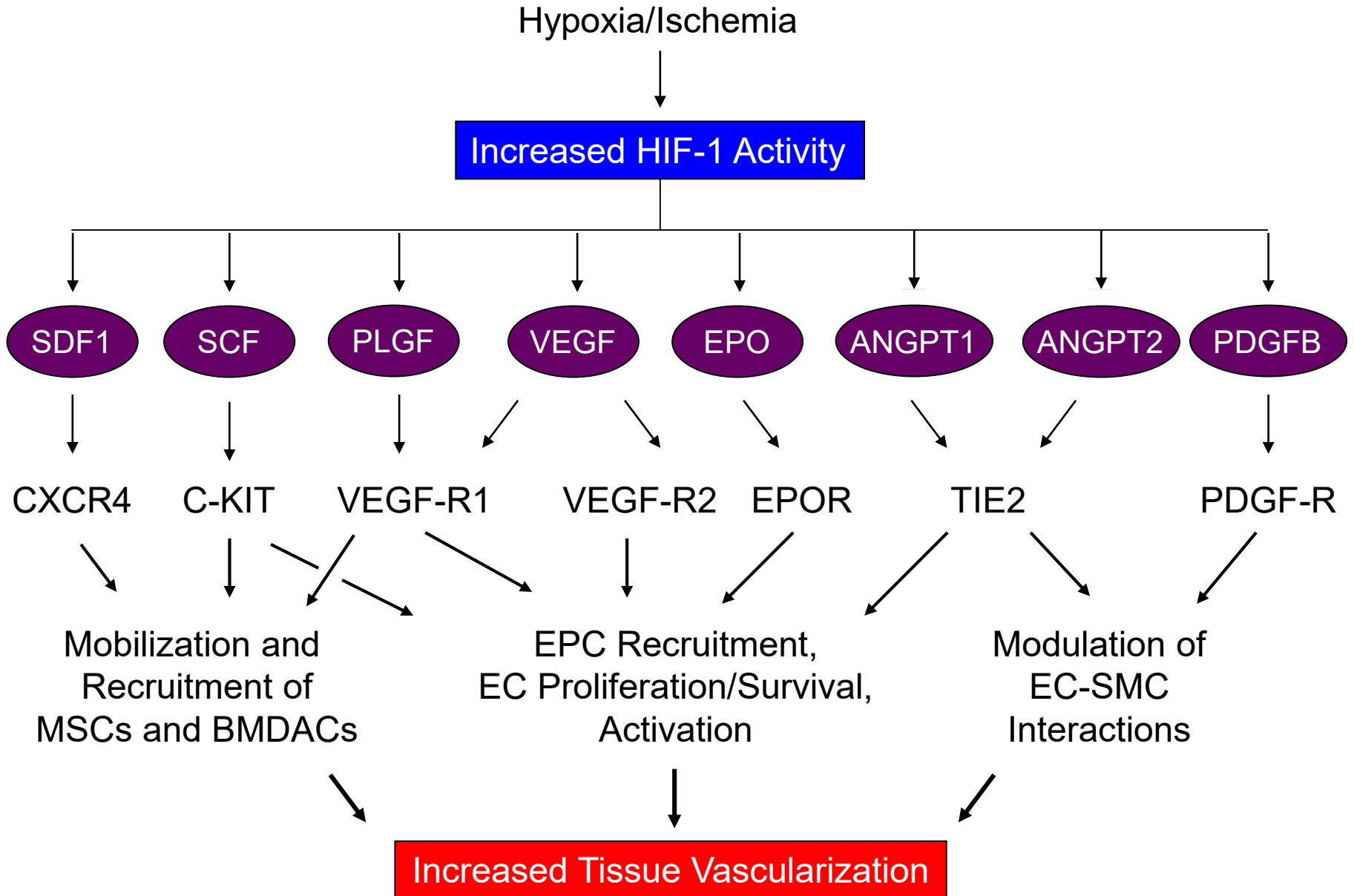
Improved Recovery of Perfusion in Mice by HIF-1 α Gene Therapy



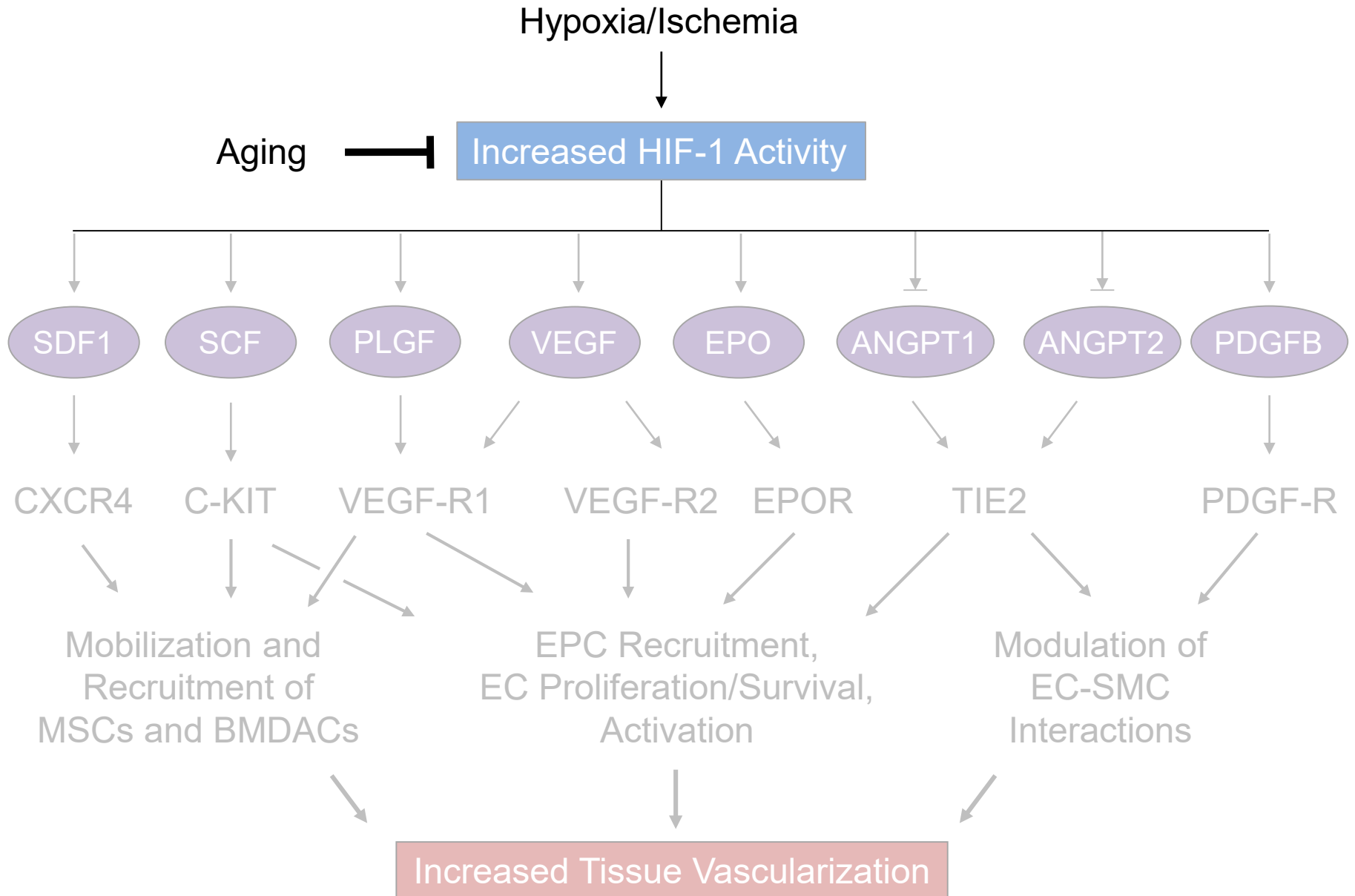
AdCA5 gene therapy corrects age-related impairment of vascularization



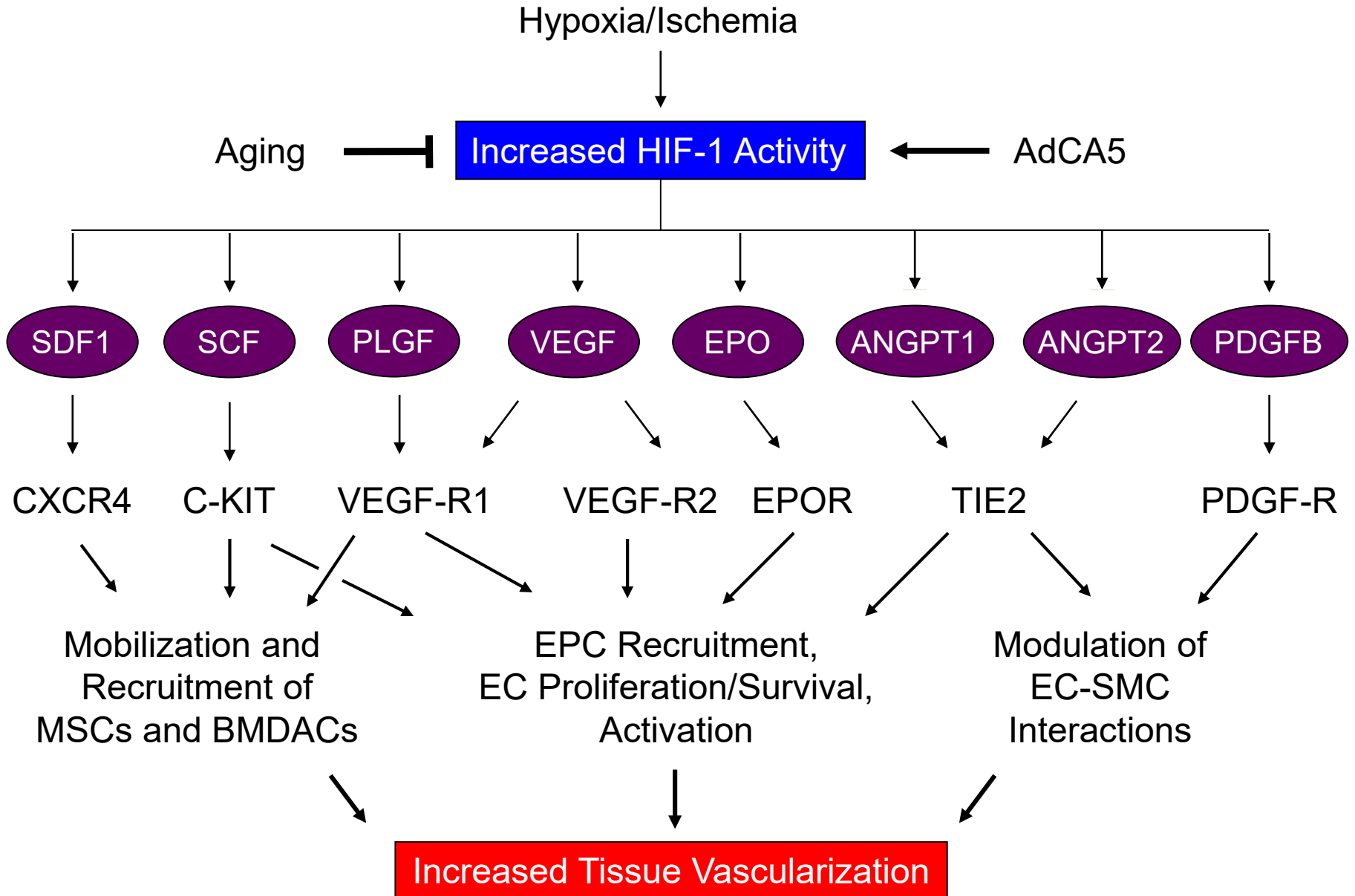
HIF-1 Regulates the Expression of Angiogenic Growth Factors



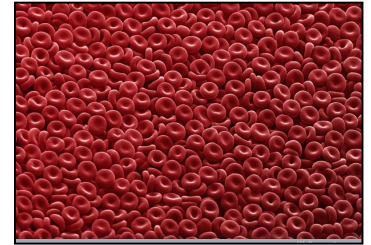
HIF-1 Regulates the Expression of Angiogenic Growth Factors



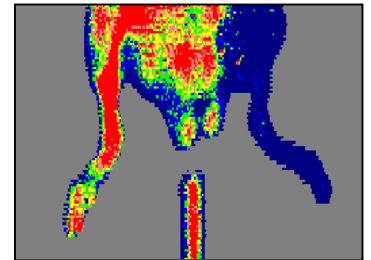
HIF-1 Regulates the Expression of Angiogenic Growth Factors



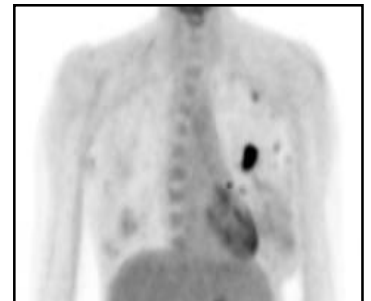
Control of Red Blood Cell Production



Cardiovascular Disease

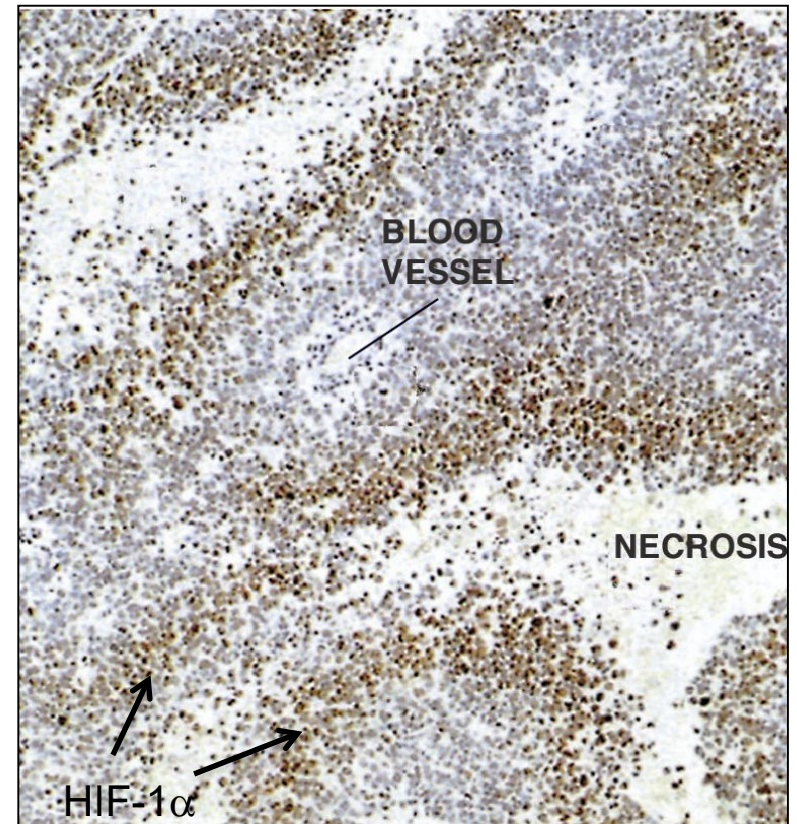


Cancer



Advanced Human Cancers Commonly Contain Regions of Intratumoral Hypoxia

Direct measurements of O_2 concentration in human tumors have demonstrated that $PO_2 < 10$ mm Hg is associated with a significantly increased risk of invasion, metastasis, and patient mortality.



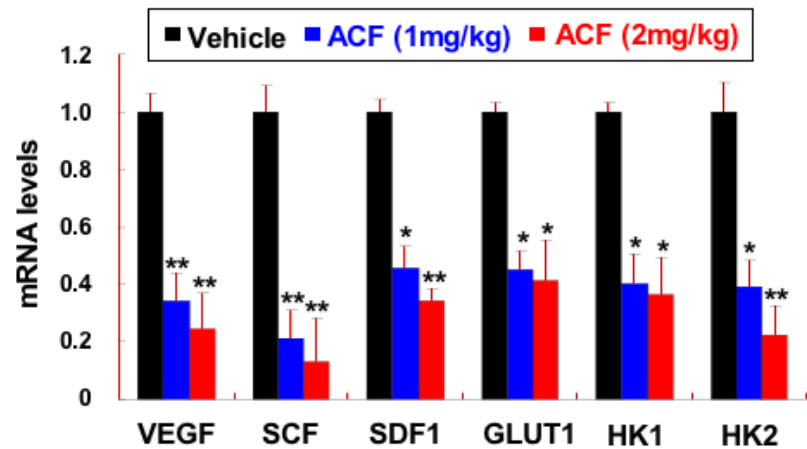
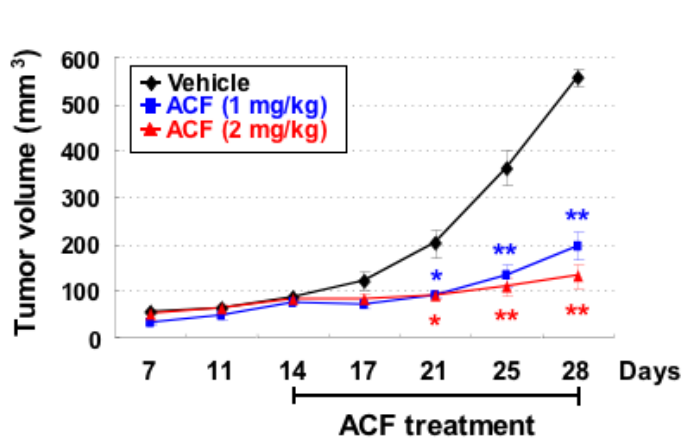
expression by hypoxic cancer cells

HIF-1 α Overexpression is Associated with Patient Mortality

Bladder, transitional cell
Bladder, superficial urothelial, p53 mutant
Upper urinary tract, transitional cell
Brain, astrocytoma, diffuse
Brain, oligodendroglioma
Breast, LN-positive
Breast, LN-negative
Breast, HER2-positive
Breast, unselected
Breast, ER-positive
Cervical, early-stage
Cervical, S/P RTX
Cervical, IB-IIIB, S/P RTX
Endometrial, stage I
Ovarian, p53 mutant
Ovarian, serous
Colorectal
Colorectal (Dukes B, s/p resection)
Esophageal
Gastric
GI stromal tumor, stomach
Hepatocellular
Pancreatic
Pancreatic
Prostate
Lung, NSCLC
Laryngeal
Oropharyngeal
Soft tissue sarcoma

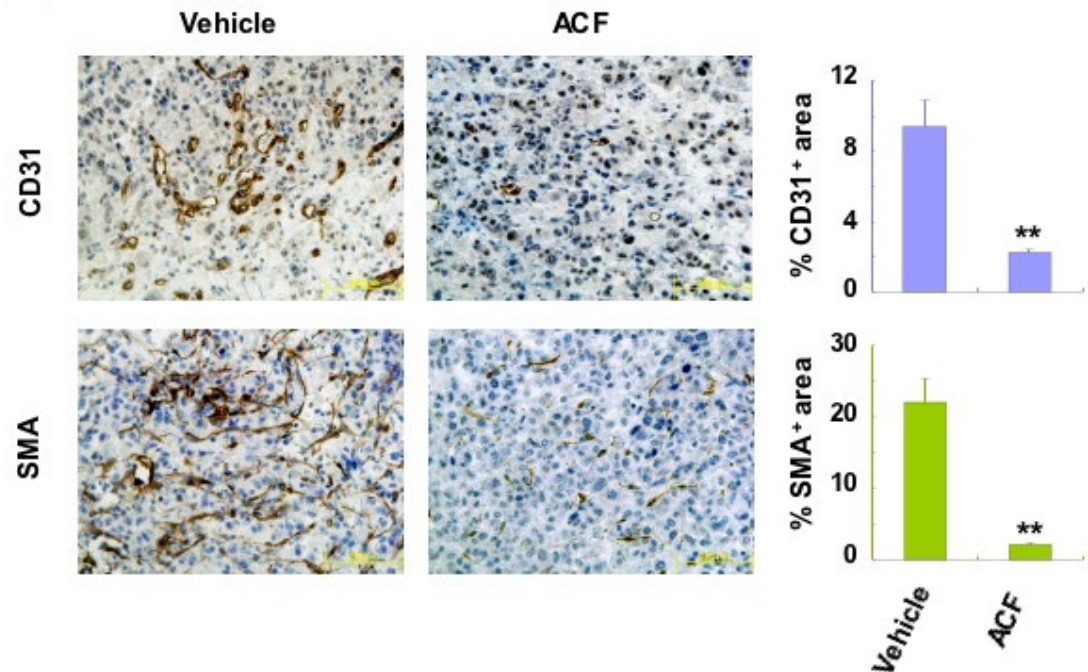
Theodoropoulos et al., *Eur. Urol.* 46:200, 2004
Theodoropoulos et al., *BJU Int.* 95:425, 2005
Nakanishi et al., *Clin. Cancer Res.* 11:2583, 2005
Korkolopoulou et al., *Neuropathol. Appl. Neurobiol.* 30:267, 2004
Birner et al., *Cancer* 92:165, 2001
Schindl et al., *Clin. Cancer Res.* 8:1831, 2002
Bos et al., *Cancer* 97:1573, 2003
Giatromanolaki et al., *Clin. Cancer Res.* 10:79772, 2004
Vleugel et al., *J. Clin. Pathol.* 58:172, 2005
Generali et al., *Clin. Cancer Res.* 12:4562, 2006
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Burri et al., *Int. J. Radiat. Oncol. Biol. Phys.* 56:494, 2003
Bachtiary et al., *Clin. Cancer Res.* 9:2234, 2003
Sivridis et al., *Cancer* 95:1055, 2002
Birner et al., *Clin. Cancer Res.* 7:1661, 2001
Daponte et al., *BMC Cancer* 8:335, 2008
Schmitz et al., *Int. J. Colorectal Dis.* 24:1287, 2009
Rajaganeshan et al., *Int. J. Colorectal Dis.* 23:1049, 2008
Tzao et al., *Dis. Markers* 25:141, 2008
Griffiths et al., *Br. J. Cancer* 96:95, 2007
Takahashi et al., *Oncol. Rep.* 10:797, 2003
Xie et al., *Dig Dis Sci* 53:3225, 2008
Shibaji et al., *Anticancer Res.* 23:4721, 2003
Sun et al., *Int. J. Oncol.* 30:1359, 2007
Nanni et al., *J. Clin. Invest.* 119:1093, 2009
Giatromanolaki et al., *Br. J. Cancer* 85:881, 2001
Schrijvers et al., *Int. J. Radiat. Oncol. Biol. Phys.* 72:161, 2008
Aebersold et al., *Cancer Res.* 61:2911, 2001
Shintani et al., *Virchows Arch.* 449:673, 2006

HIF Inhibitor Acriflavine Inhibits Tumor Growth and Vascularization in a Mouse Model of Prostate Cancer

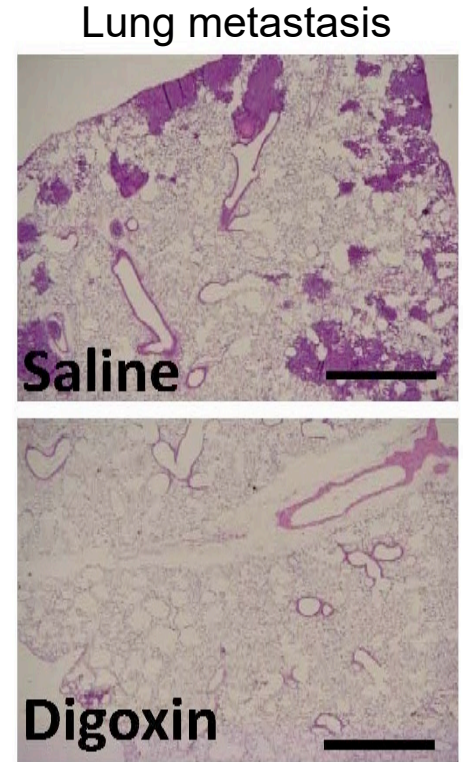
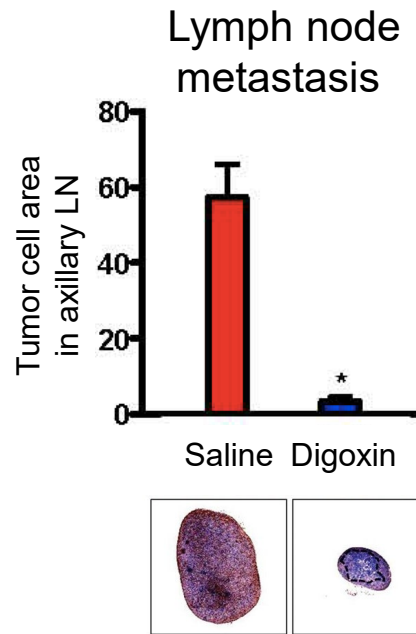
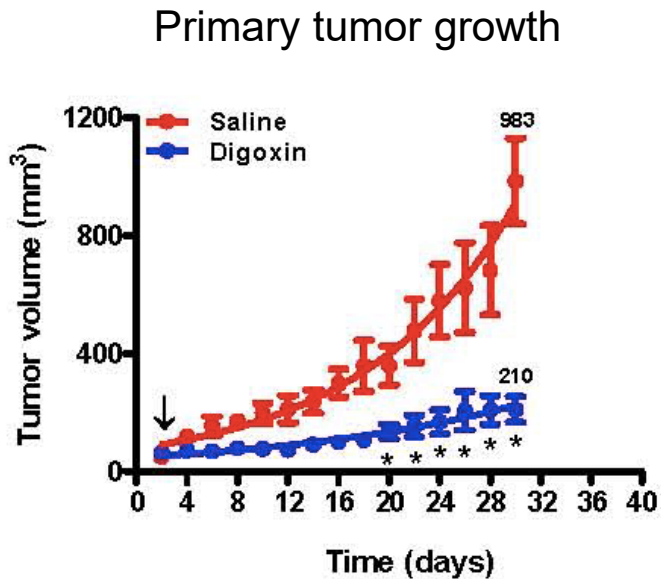


Acriflavine (ACF) inhibits dimerization of HIF- α and HIF-1 β subunits

K. Lee et al.
Proc. Natl. Acad. Sci. USA
2009;106:2353

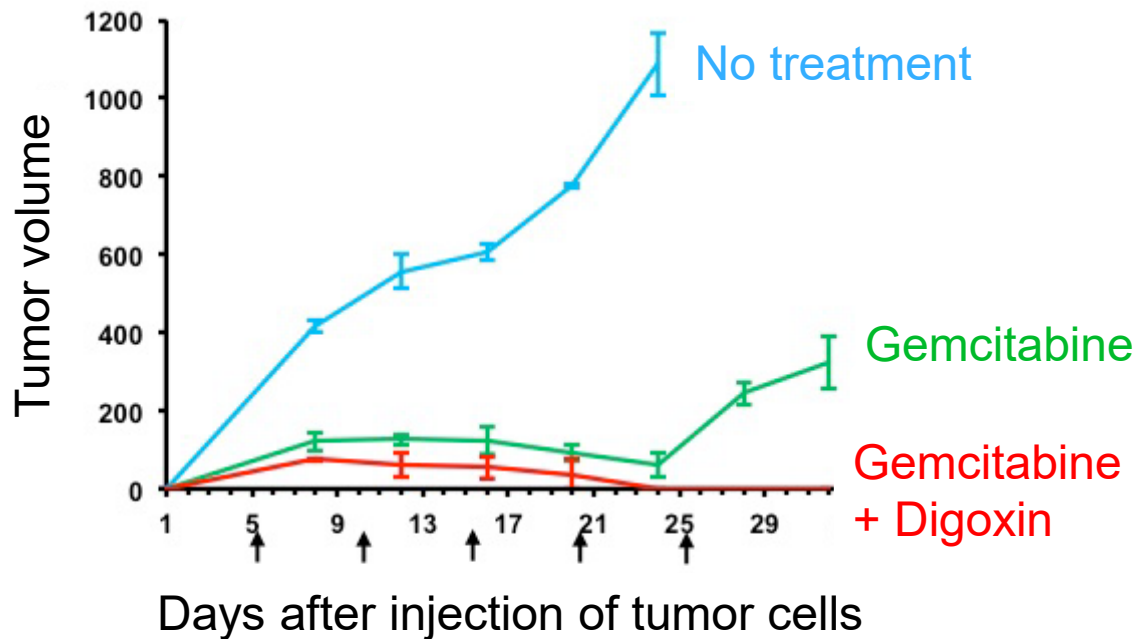


HIF Inhibitor Digoxin Decreases Primary Tumor Growth and Metastasis in a Mouse Model of Breast Cancer

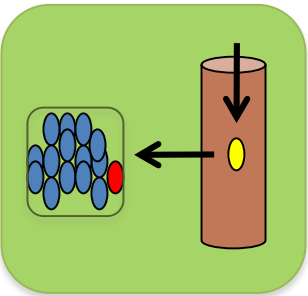


H. Zhang et al. *Oncogene* 2012;31:1757
L. Schito et al. *Proc. Natl. Acad. Sci. USA* 2012;109:E2707
C.C. Wong et al. *J. Mol. Med.* 2012;90:803

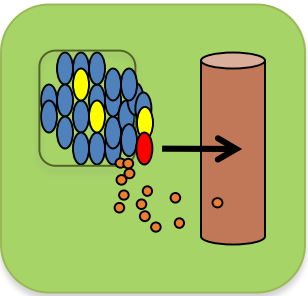
Treatment with Gemcitabine + HIF Inhibitor Digoxin Causes Tumor Eradication



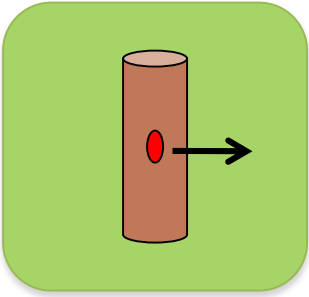
Deconvoluting the Role of HIFs in Breast Cancer Metastasis



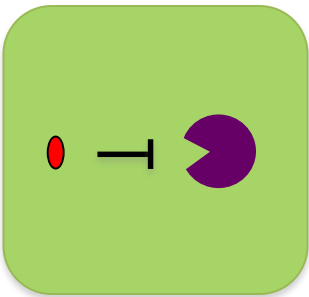
MSC/Mφ Cooptation
CXCR3, CCR5
CXCL16, PGF, CSF1



Migration/Invasion
Microvesicle Formation
RHOA, ROCK1
P4HA1, P4HA2, PLOD2
RAB22A



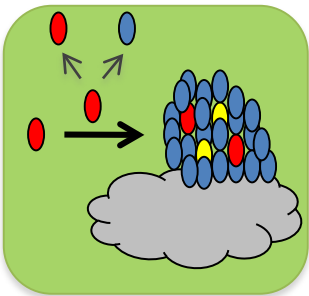
Margination/Extravasation
L1CAM,
ANGPTL4



Immune Evasion
CD47, CD73, PDL1



Premetastatic Niche Formation
LOX, LOXL2, LOXL4

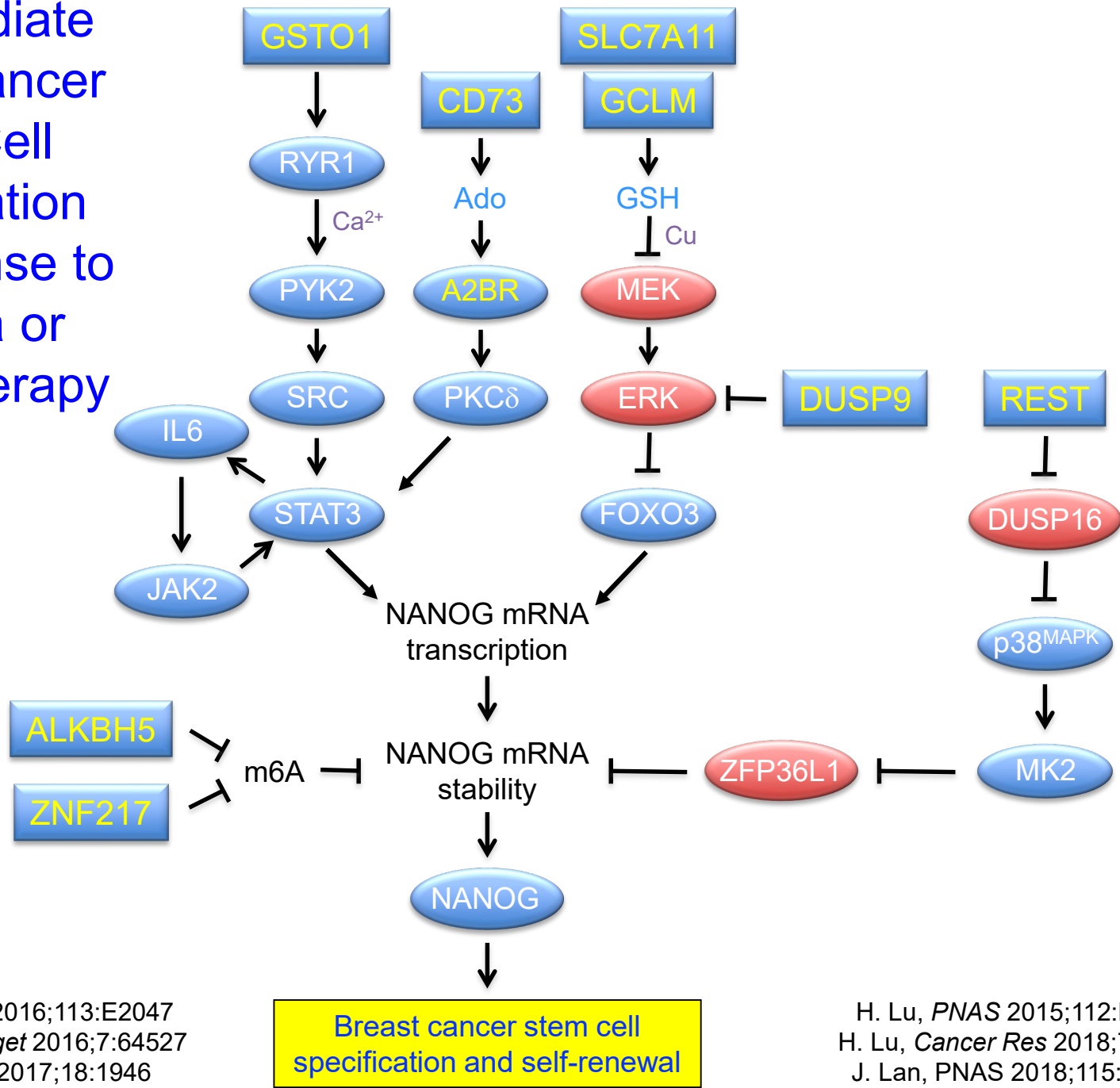


Cancer Stem Cell Specification
WWTR1, SIAH1
IL6, IL8, MDR1, CD47
SLC7A11, GCLM
ALKBH5, ZNF217,
PHGDH, GSTO1



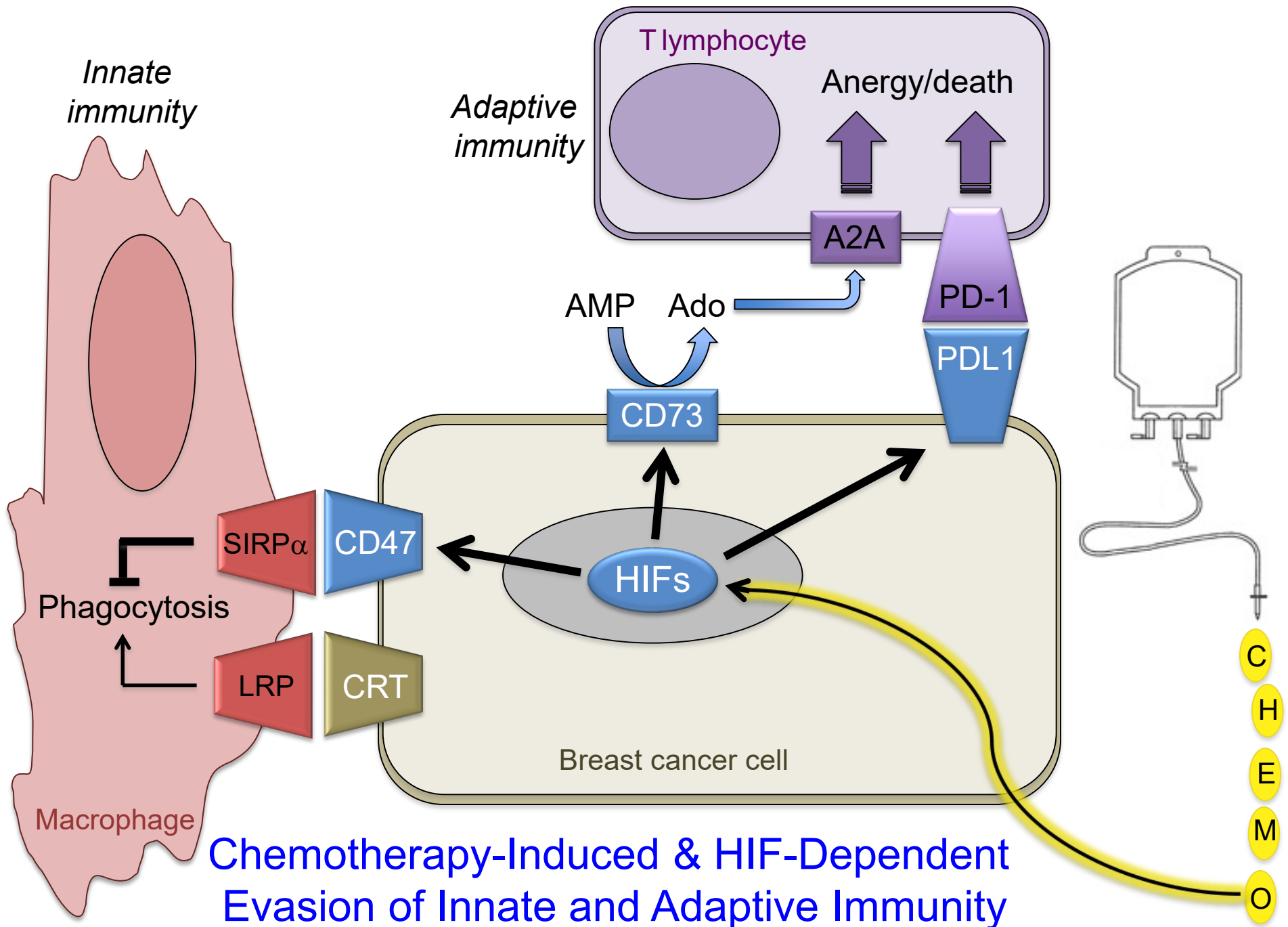
D Samanta et al. *PNAS* 2018;115:E1239
 H Lu et al. *Cell Rep* 2017;18:1946
 D Samanta et al. *Cancer Res* 2016;76:4430
 C Zhang et al. *PNAS* 2016;113:E2047
 C Zhang et al. *Oncotarget* 2016;7:64527
 H Zhang et al. *PNAS* 2015;112:E6215
 H Lu et al. *PNAS* 2015;112:E4600
 D Samanta et al. *PNAS* 2014;111:E5429
 L Xiang et al. *Oncotarget* 2014;5:12509
 T Wang et al. *PNAS* 2014;111:E3234
 DM Gilkes et al. *PNAS* 2014;111:E384
 L Xiang et al. *J Mol Med* 2014;92:151
 P Chaturvedi et al. *PNAS* 2014;111:E2120
 DM Gilkes et al. *Cancer Res* 2013;73:3285
 DM Gilkes et al. *Mol Cancer Res* 2013;11:456
 P Chaturvedi et al. *J Clin Invest* 2013;123:189
 L Schito et al. *PNAS* 2012;109:E2707
 CC Wong et al. *J Mol Med* 2012;90: 803
 H Zhang et al. *Oncogene* 2012;31:1757
 CC Wong et al. *PNAS* 2011;108:16369

HIFs Mediate Breast Cancer Stem Cell Specification In Response to Hypoxia or Chemotherapy



C. Zhang, *PNAS* 2016;113:E2047
 C. Zhang, *Oncotarget* 2016;7:64527
 H. Lu, *Cell Rep* 2017;18:1946

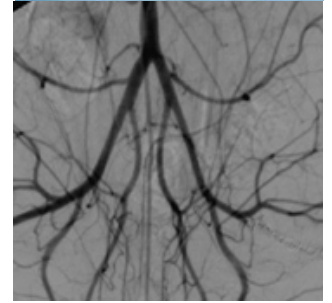
H. Lu, *PNAS* 2015;112:E4600
 H. Lu, *Cancer Res* 2018;78:4191
 J. Lan, *PNAS* 2018;115:E9640



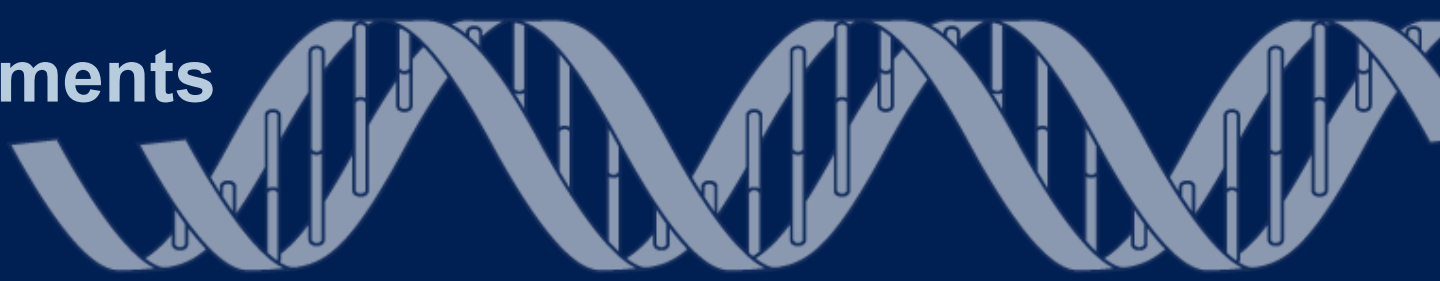
Chemotherapy-Induced & HIF-Dependent Evasion of Innate and Adaptive Immunity

HIF Pathway in Biology and Medicine

1. **Development:** HIF-1 α , HIF-1 β , HIF-2 α , PHD2, and VHL are all required for normal mammalian embryonic development.
2. **Physiology:** Even modest gain or loss of function of pathway components interferes with normal postnatal physiological responses to hypoxia (congenital polycythemia).
3. **Medicine:** HIFs play key roles in cancer and cardiovascular disease, the major causes of mortality in the U.S. population. Clinical trials are planned or underway to evaluate novel therapies that target HIFs for inhibition (cancer, retinopathy) or activation (anemia, cardiovascular disease).
4. **Evolution:** HIF-1 α , HIF-1 β , PHD, and VHL homologs are found in all metazoan species. Genetic evidence implicates the HIF pathway as the critical genetic target for successful adaptation to high altitude in Tibetan and Andean populations.



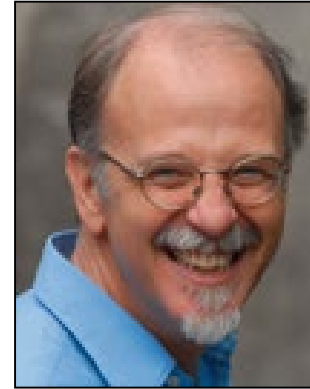
Acknowledgments



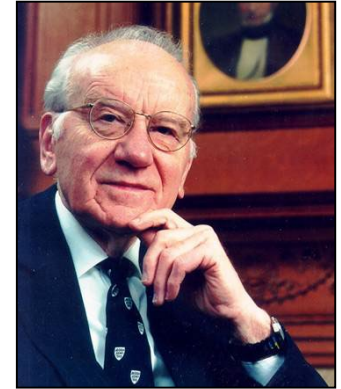
Rose Nelson



Haig Kazazian



Stylianos Antonarakis



Victor McKusick



Larissa Shimoda



Akrit Sodhi



Nanduri Prabhakar



John Gearhart



Thomas Kelly



Guang Wang



Chi Dang

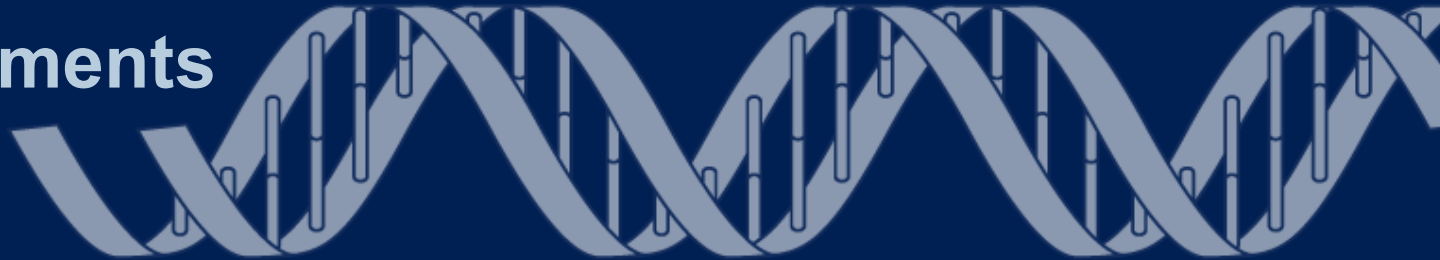


Josef Prchal



Mike Armstrong

Acknowledgments



Guang Wang
Raquel Dureza
Bing-Hua Jiang
Narayan Iyer
Lori Kotch
Aimee Yu
Faton Agani
Erik Laughner
Sandra Leung
Brian Kelly
Kiichi Hirota
Hiroaki Okuyama
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Xiaofei Wei
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