Angiogenesis

Dr Anna M. Randi

Reader

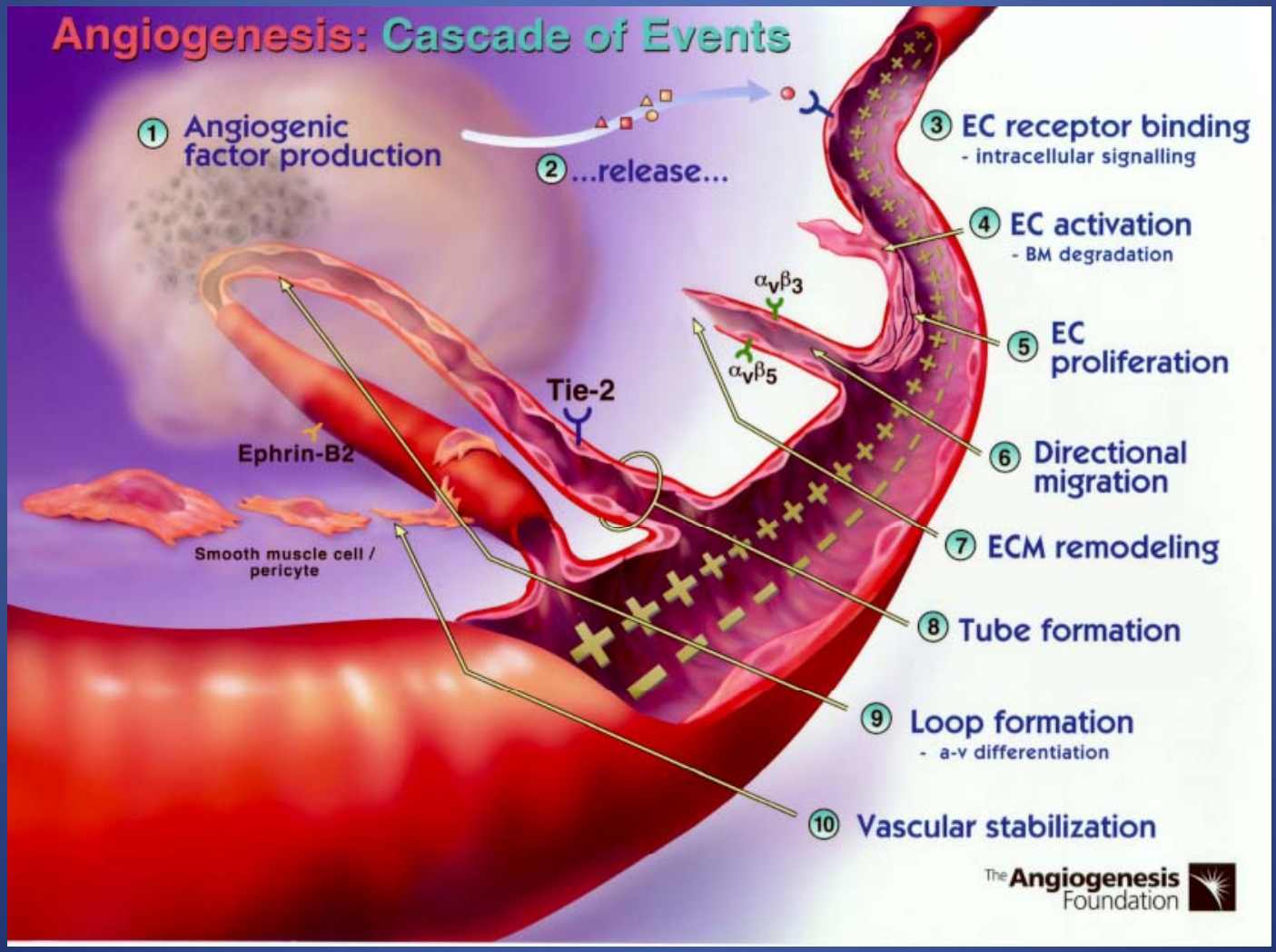
NHLI Cardiovascular Sciences

Angiogenesis

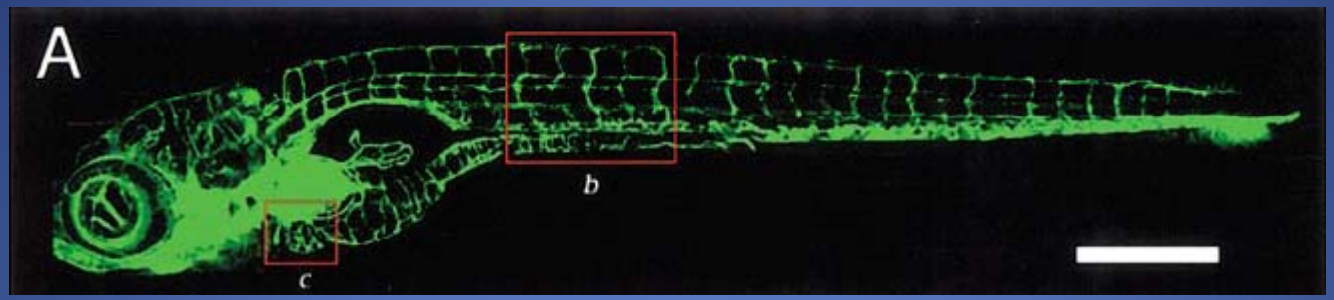
1. Basic concepts of angiogenesis
2. Molecular and cellular mechanisms
3. Angiogenesis in disease
4. Angiogenesis as a therapeutic target in cancer
5. Therapeutic Angiogenesis
6. Methods to investigate angiogenesis

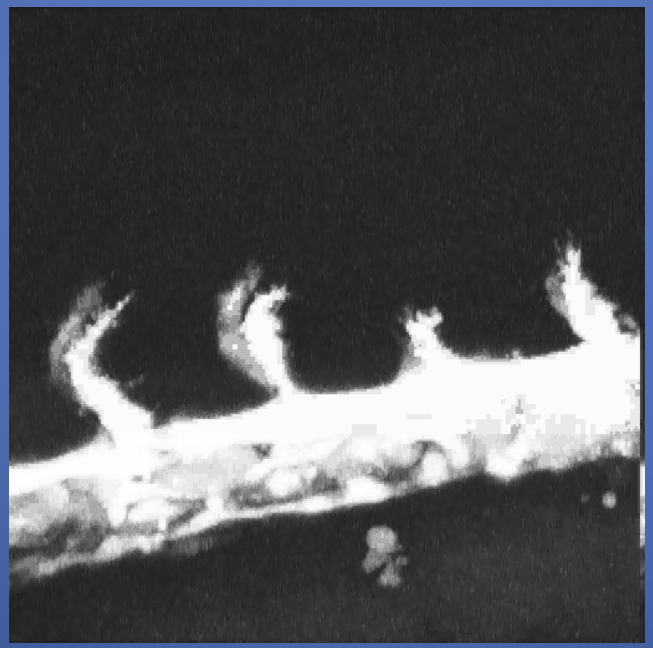
Angiogenesis

- The formation of neo-vessels from pre-existing blood vessels



Angiogenesis live

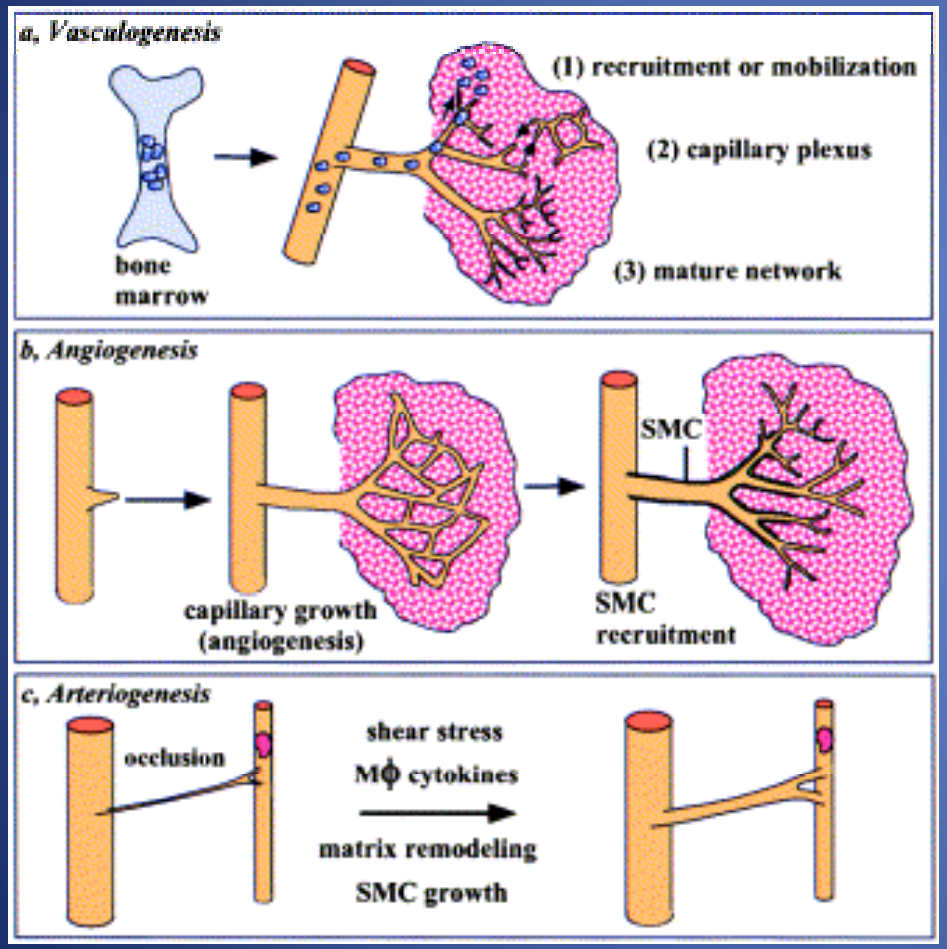




http://blip.tv/weizmann-institute-of-science/vessel-formation-in-zebrafish-4795807

Lawson & Weinstein, Developmental Biology 248, 307–318 (2002)

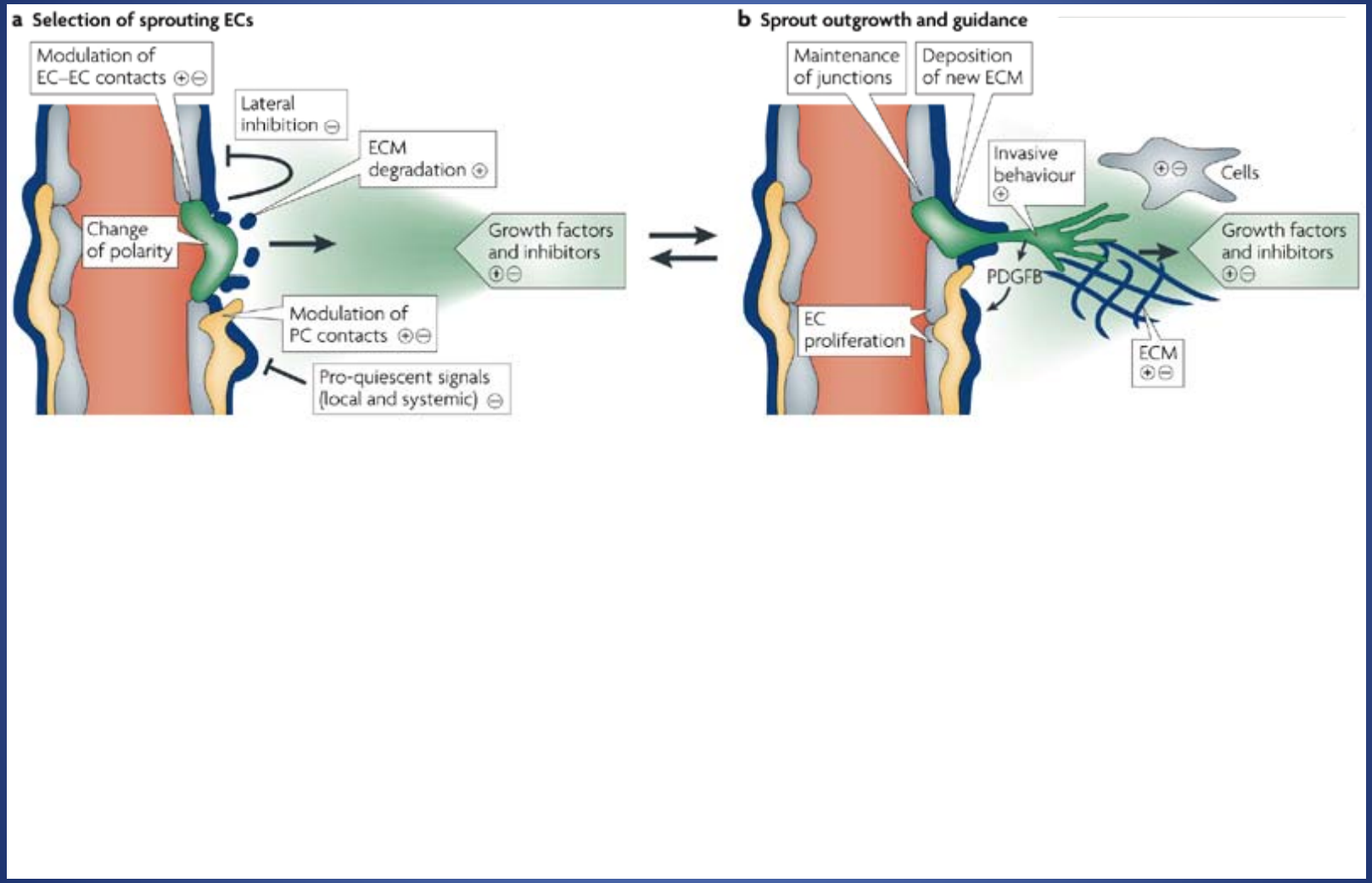
How to make a blood vessel



Carmeliet Nature Medicine 6, 389 - 395 (2000)

- Vasculogenesis
  (endothelial progenitor cell mobilization)
- Angiogenesis
  (sprouting)
- Arteriogenesis
  (collateral growth)

Model of Sprouting Angiogenesis



Adams & Alitalo, Nat Rev Mol Cell Biol. 2007 Jun;8(6):464-78

Initial stimulus for angiogenesis: *hypoxia*

Hypoxia: deficiency in the amount of oxygen reaching the tissues

Endothelial cells contain:

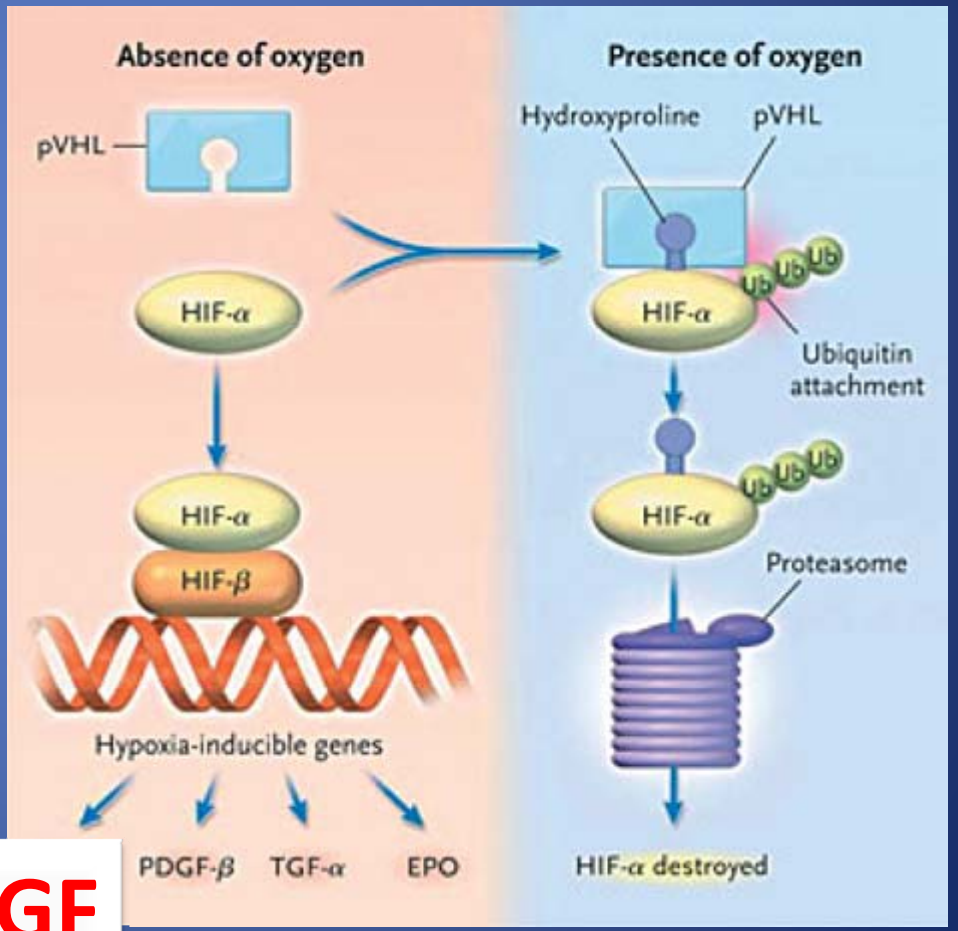
1. oxygen sensors (such as prolyl hydroxylase domain 2 – PHD2)
2. hypoxia inducible factors (such as hypoxia-inducible factor – HIF)

Initial stimulus for angiogenesis: *hypoxia*

Hypoxia: deficiency in the amount of oxygen reaching the tissues

HIF: hypoxia-inducible transcription factor, controls regulation of gene expression by oxygen

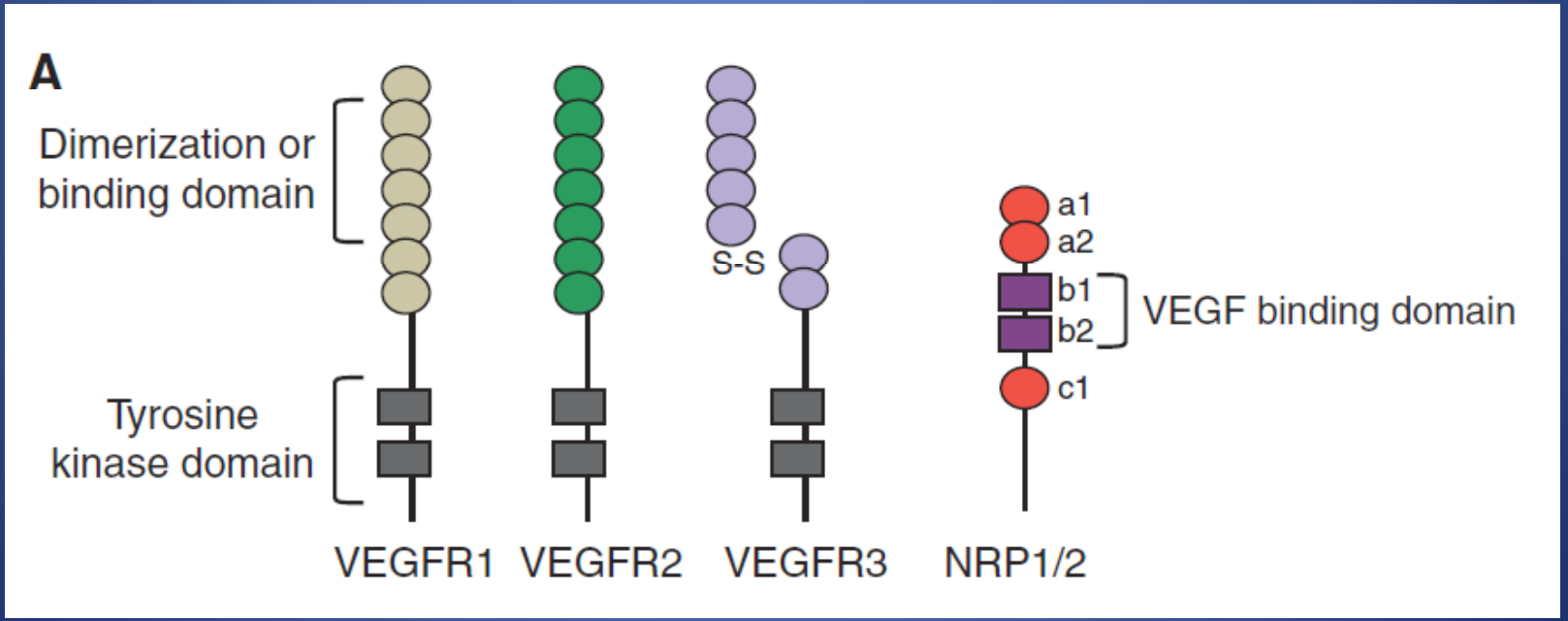
pVHL: Von Hippel–Lindau tumor suppressor gene, controls levels of HIF



VEGF

Vascular Endothelial Growth Factor (VEGF)

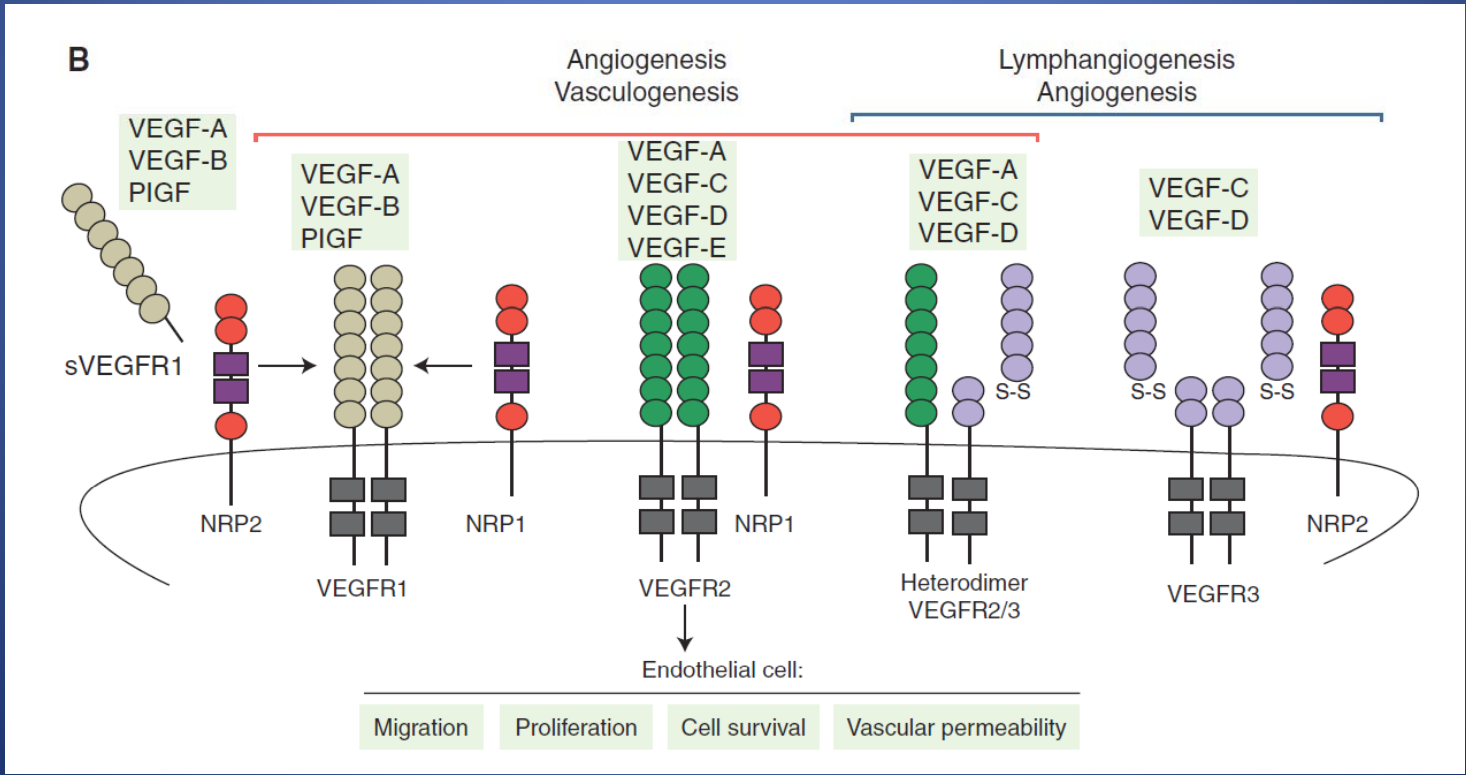
- Family of 5 members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor(PlGF)
- Three tyrosine kinase receptors: VEGF receptor (VEGFR)-1, VEGFR-2, and VEGFR-3 and co-receptors Nrp1 and Nrp2



R. Blanco and H. Gerhardt

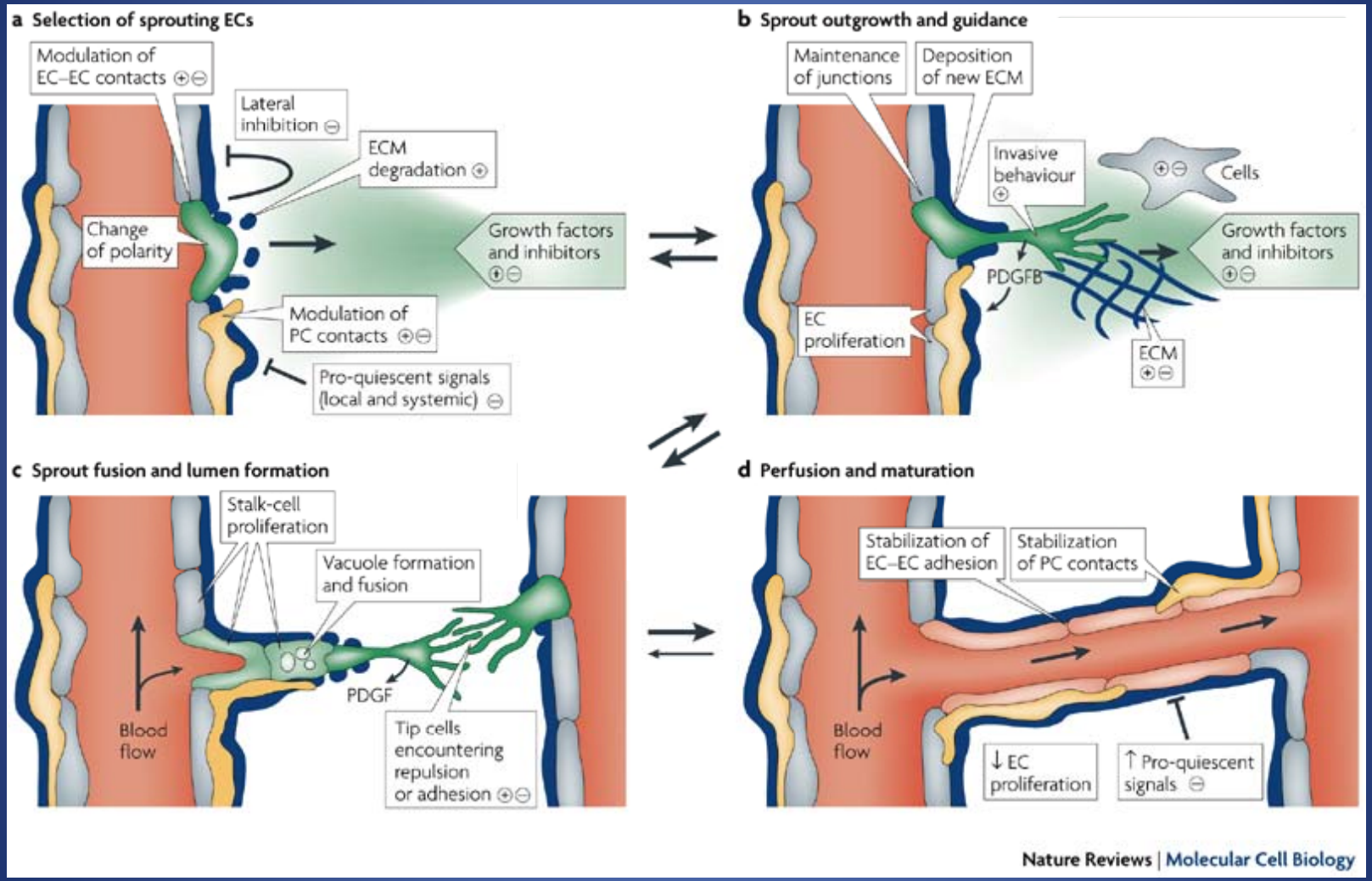
Vascular Endothelial Growth Factor (VEGF)

- VEGFR-2 is the major mediator of VEGF-dependent sprouting angiogenesis, activating a variety of downstream signaling pathways that regulate endothelial cell migration, survival, proliferation, and tube formation.
- In mice, loss of even a single VEGF allele results in embryonic lethality at days 11 -12, due to vascular defects



R. Blanco and H. Gerhardt

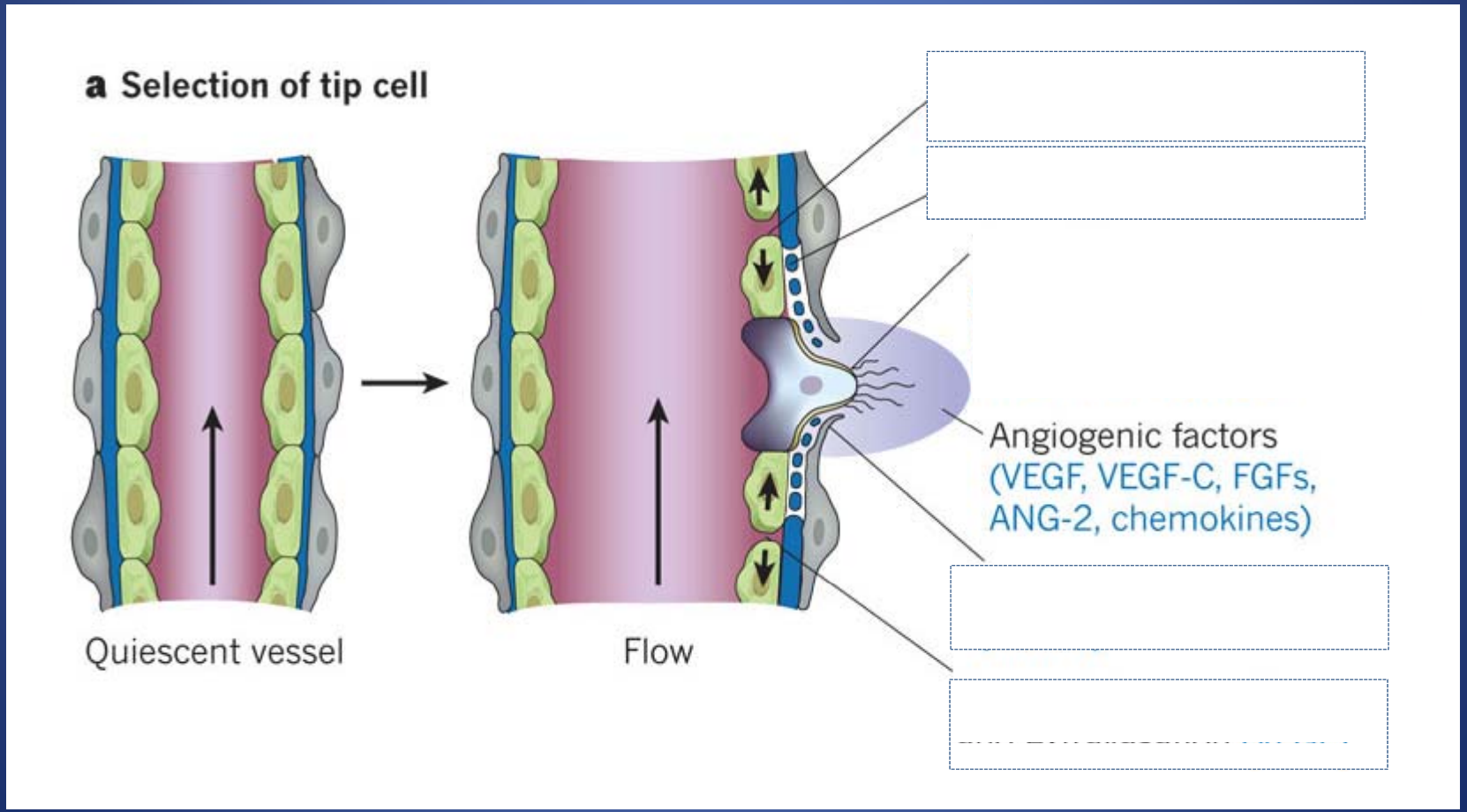
Model of Sprouting Angiogenesis



Adams & Alitalo, Nat Rev Mol Cell Biol. 2007 Jun;8(6):464-78

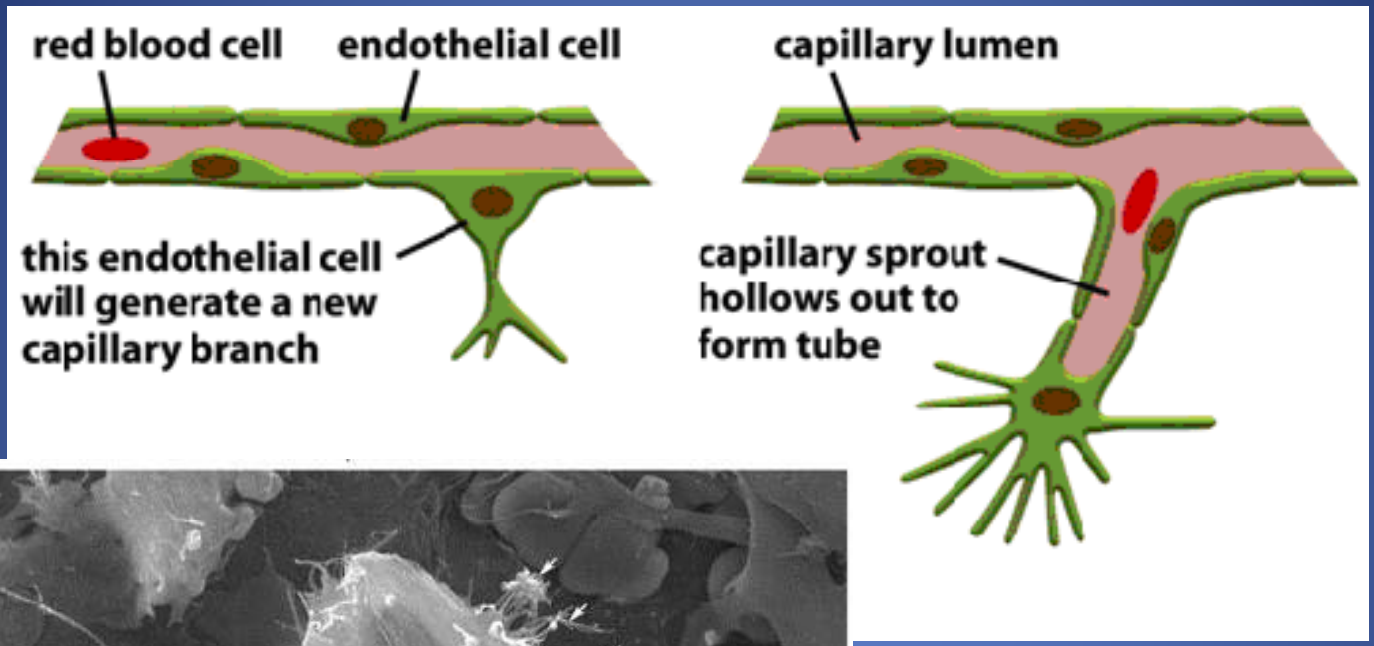
Tip cells and sprouting angiogenesis

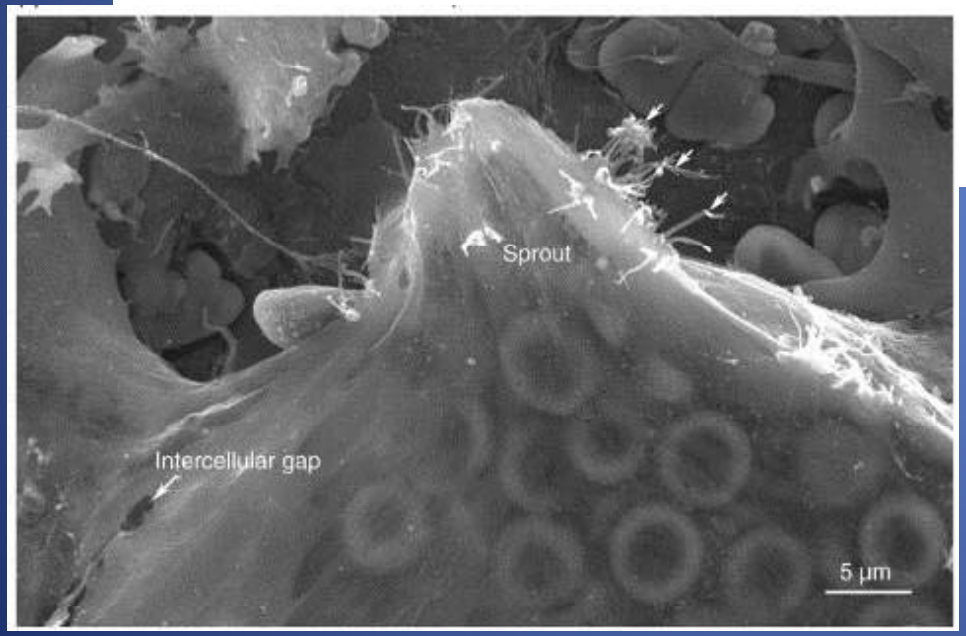
In sprouting angiogenesis, specialised endothelial tip cells lead the outgrowth of blood-vessel sprouts towards gradients of VEGF



Carmeliet & Jain (2011) Nature 473, 298–307

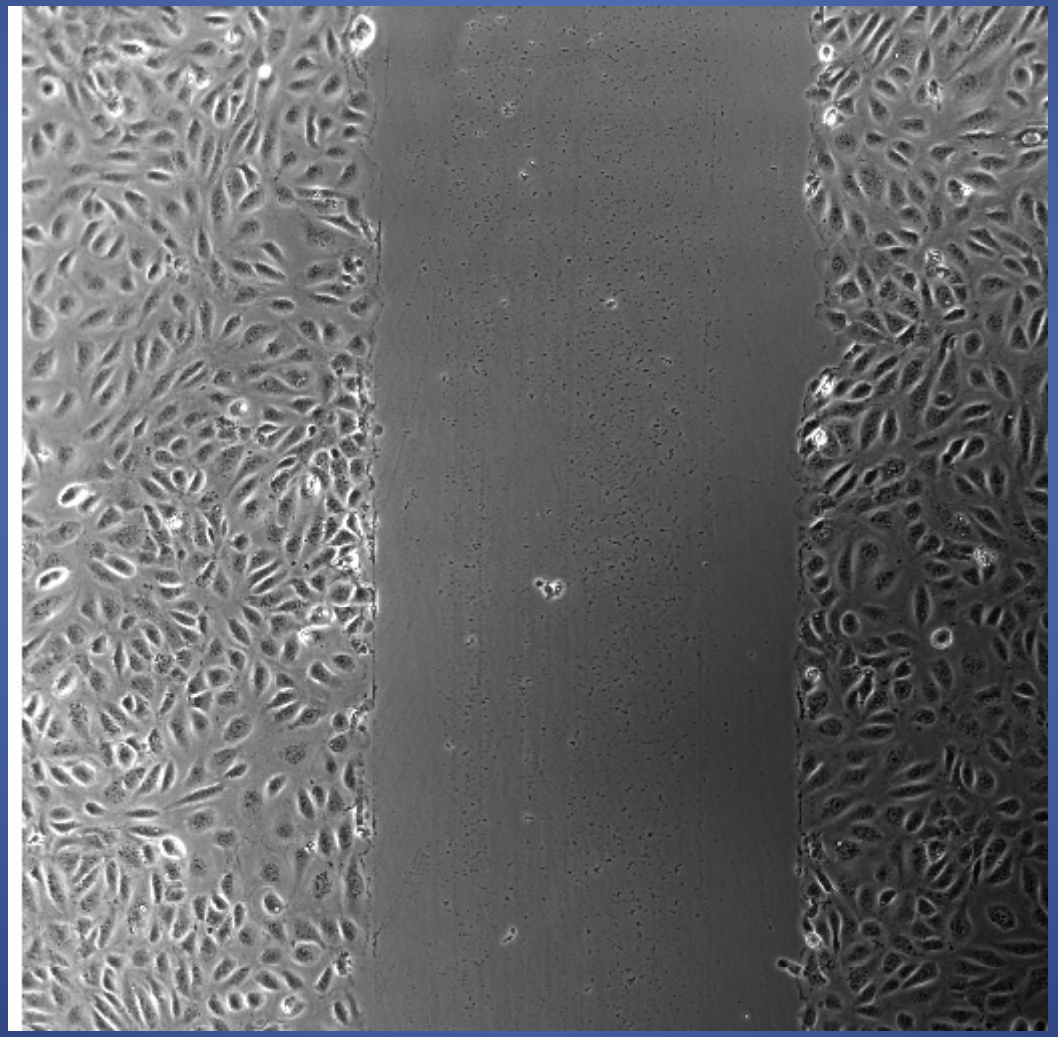
Endothelial Cell Sprouts Initiate Angiogenesis



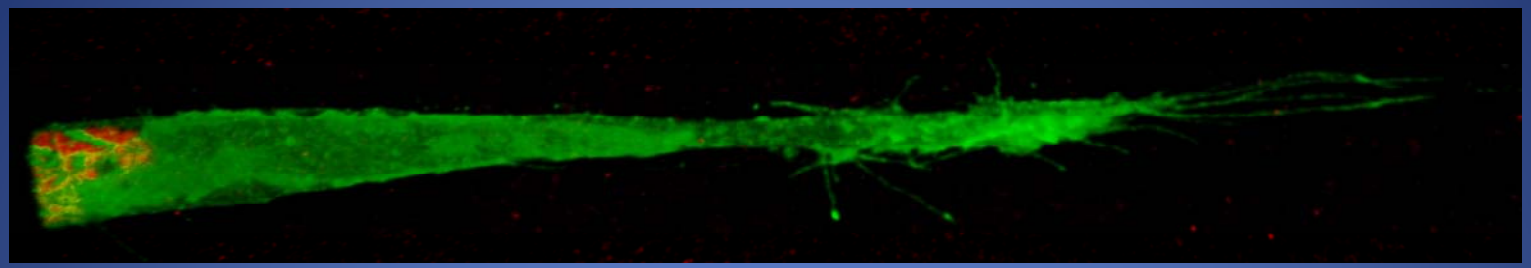


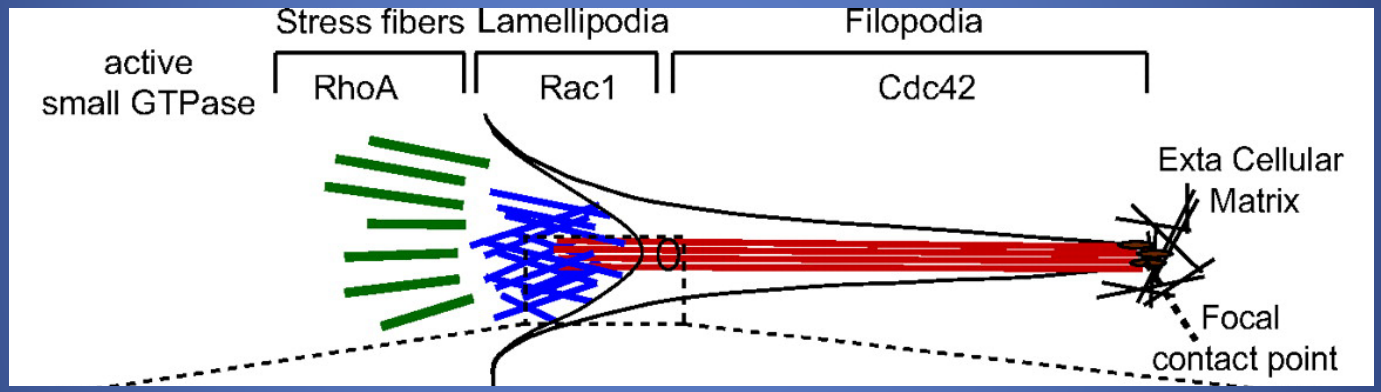
D. McDonald Current Opinion in Genetics & Development ***Vol 15*** *(2005) Pg 102*

Endothelial cell migration



Cytoskeletal rearrangements during tip cell migration

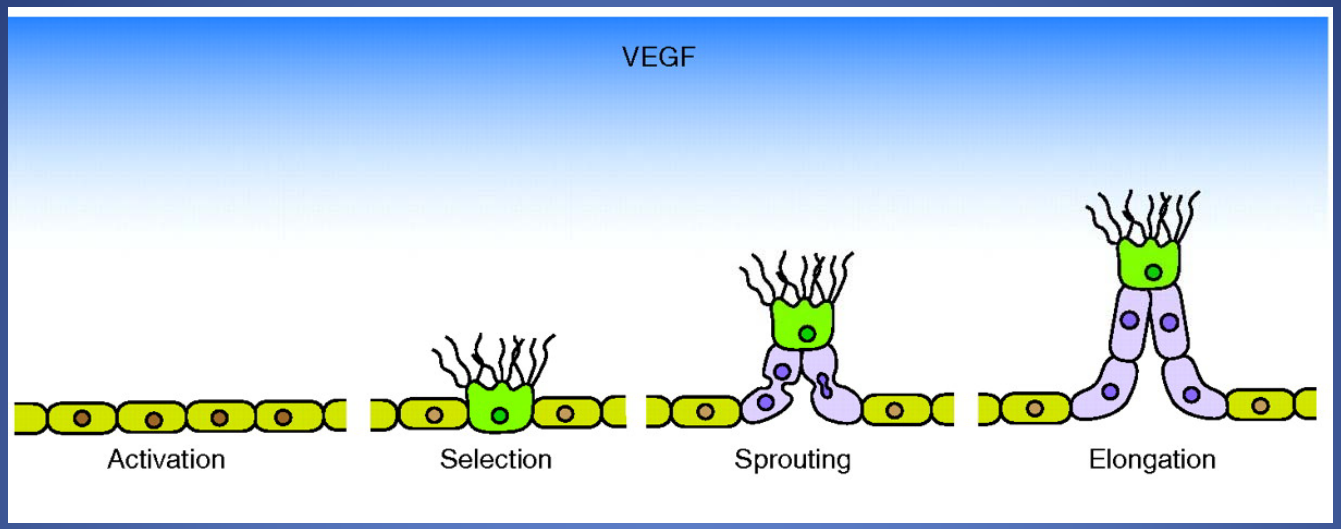




De Smet, F. et al. Arterioscler Thromb Vasc Biol 2009;29:639-649

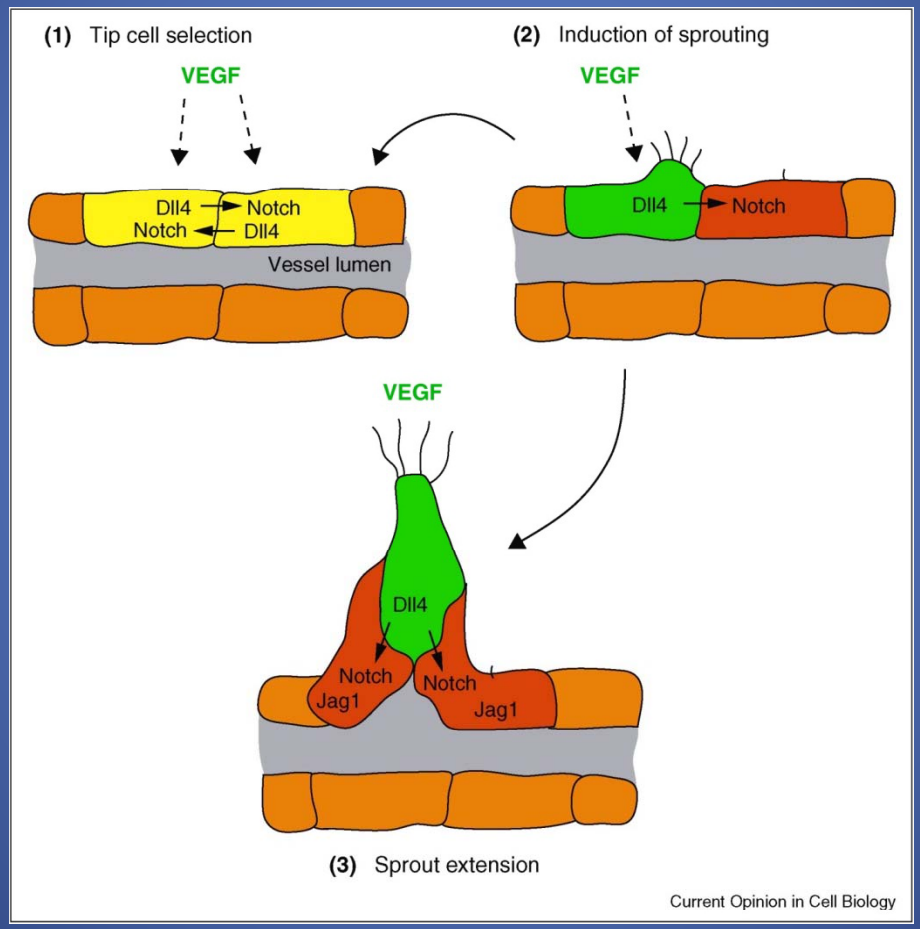
- Cell migration is a complex process, requiring the coordinated activity of the actin and microtubule cytoskeleton and the adhesion system.
- Migrating cells have a polarized morphology and form distinctive structures, such as lamellipodia and filopodia that are required for directional migration.

The initiation of blood vessel formation

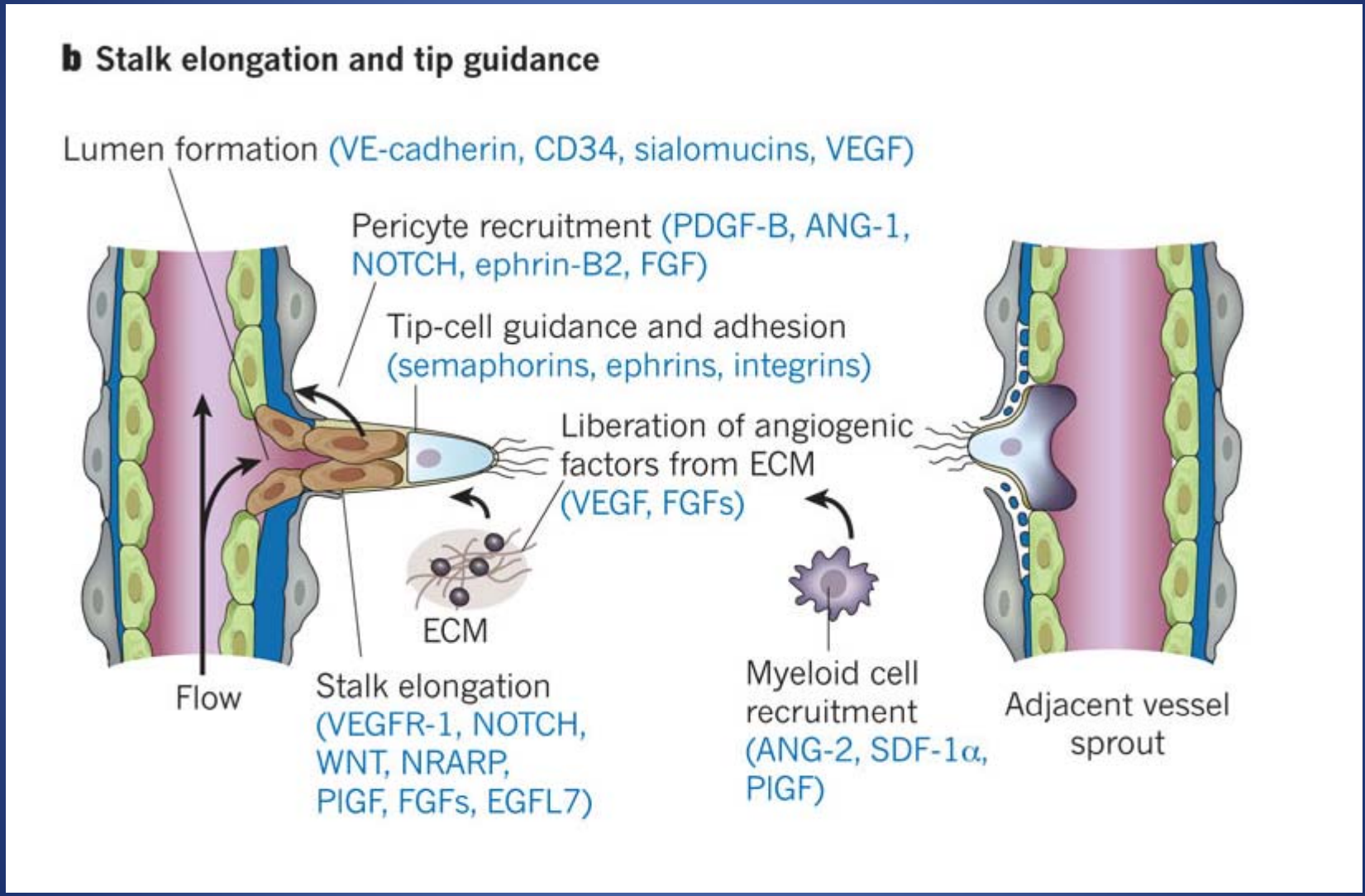


1. The presence of VEGF (blue gradient) activates the endothelium (yellow cells) of existing blood vessels.
2. Tip cells sprout towards the VEGF gradient, and the adjacent stalk cells follow the guiding tip cell and proliferate to support sprout elongation.
3. A VEGF/notch-dependent regulatory mechanism ensures the selection of a limited number of tip cells (green) by blocking tip cell formation in the immediate neighbours (via lateral inhibition).

Tip cells are selected by VEGF/Notch signalling

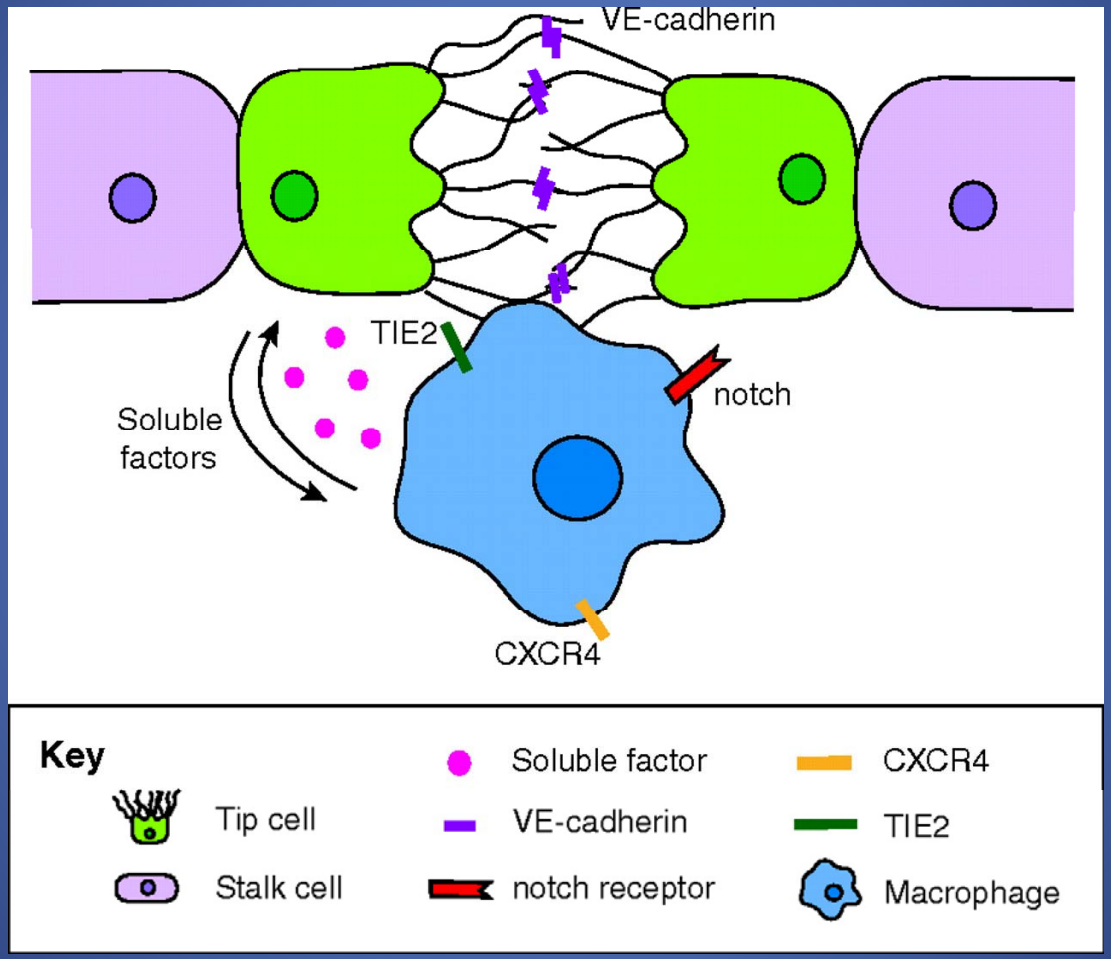


Sprout outgrowth and guidance

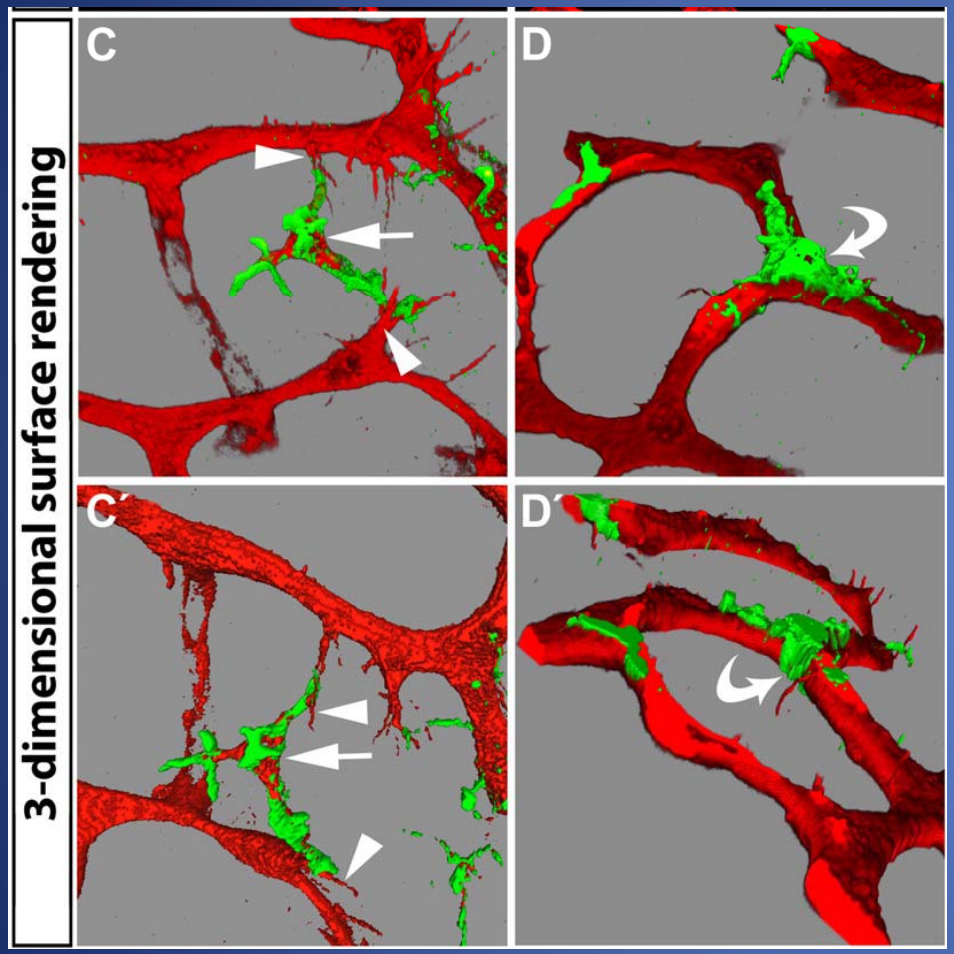


Carmeliet & Jain (2011) Nature 473, 298–307

Schematic illustration of tip cell fusion



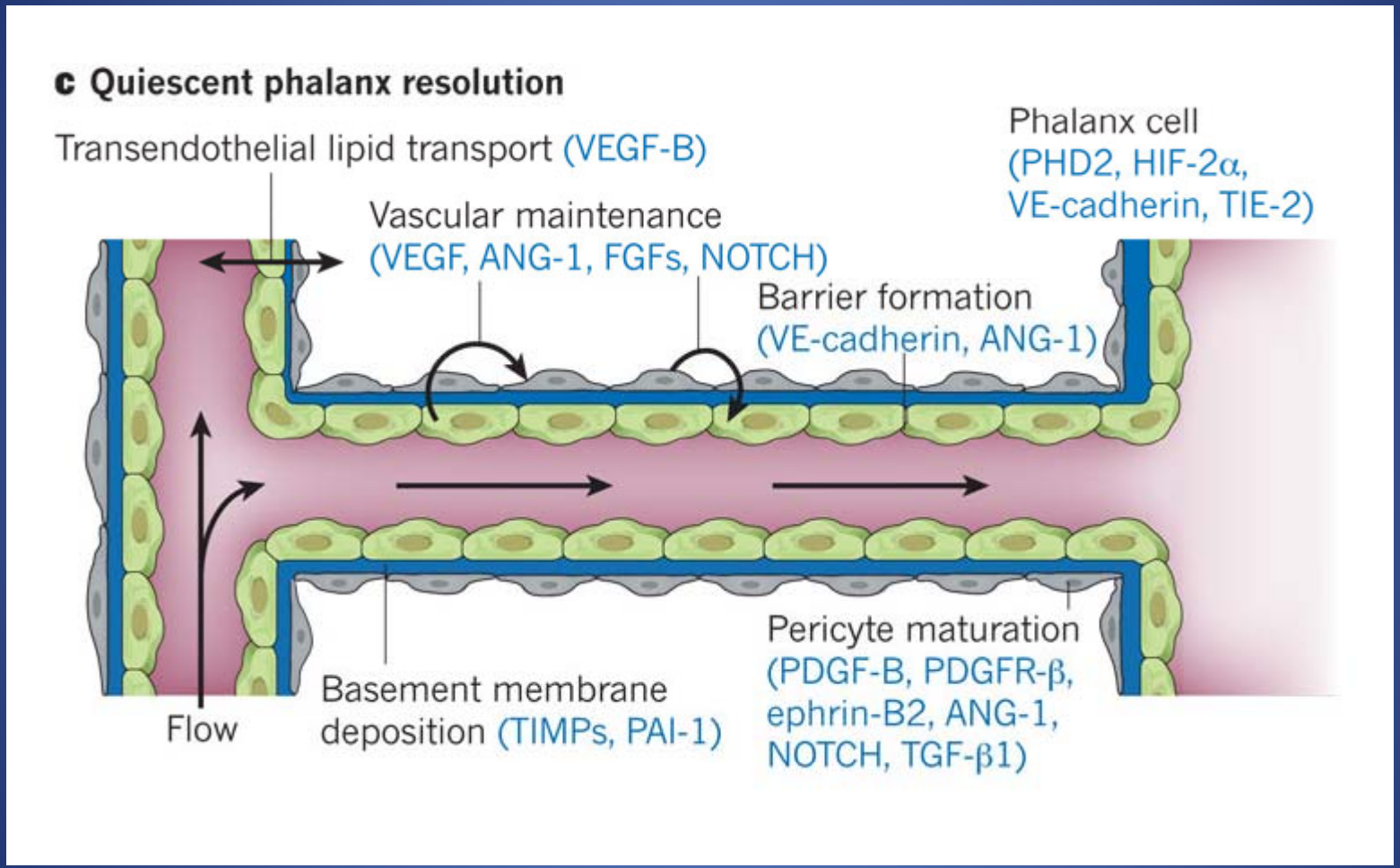
Macrophages participate in vessel anastomosis



Fantin et al (2010) Blood 16:829–840

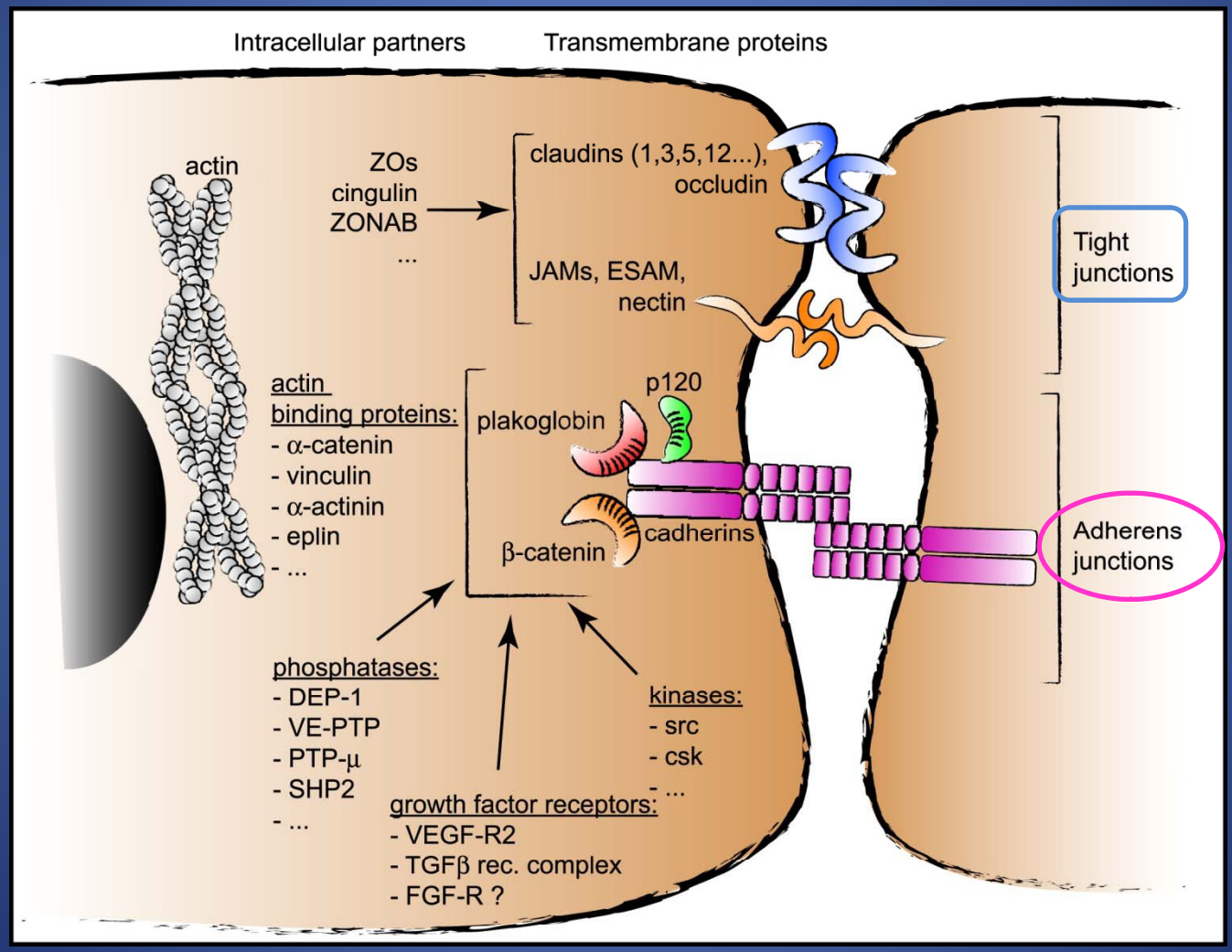
- Macrophages play a significant role in both physiological and pathological angiogenesis
- Macrophages carve out tunnels in the extra cellular matrix (ECM), providing avenues for capillary infiltration.
- Tissue-resident macrophages are associated with angiogenic tip cells during anastomosis

Stabilisation and quiescence of new vessels

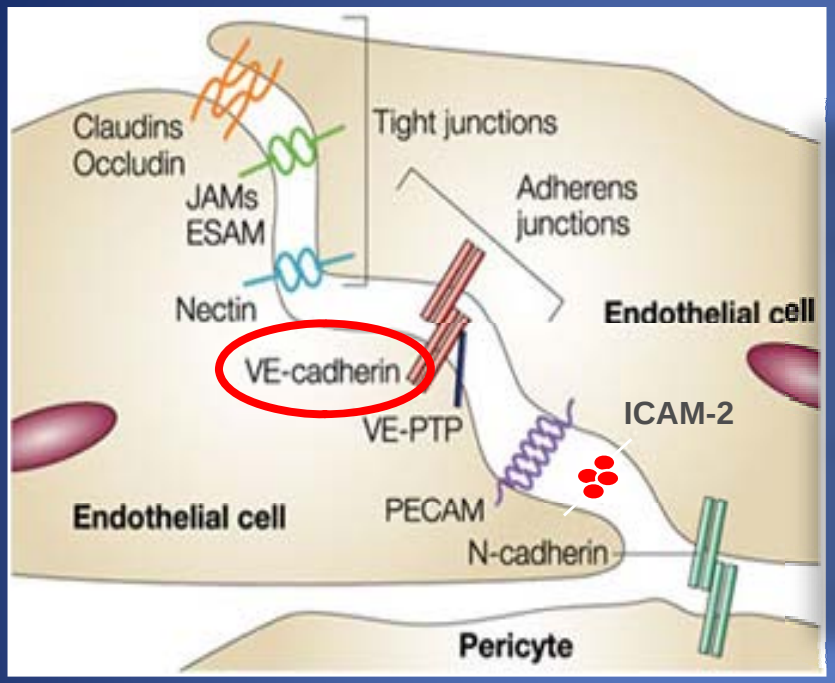


Carmeliet & Jain (2011) Nature 473, 298–307

Cell-cell Junctions in Endothelial Cells



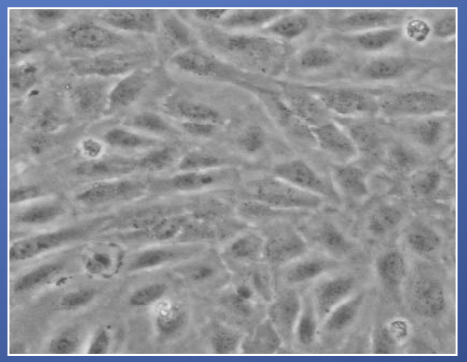
Endothelial adhesion molecule VE-Cadherin



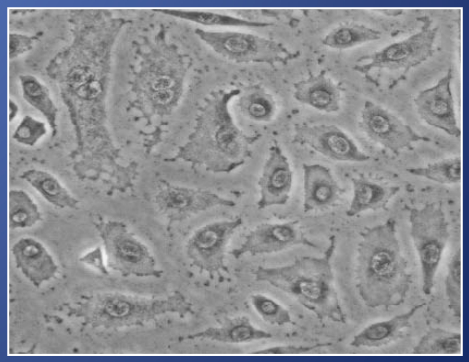
Modified from: *Dejana, Nature Rev Mol Cell Bio 2004*

- Constitutively expressed at junctions
- Homophilic interaction mediates adhesion between endothelial cells and intracellular signalling
- Controls contact inhibition of cell growth
- Promotes survival of EC

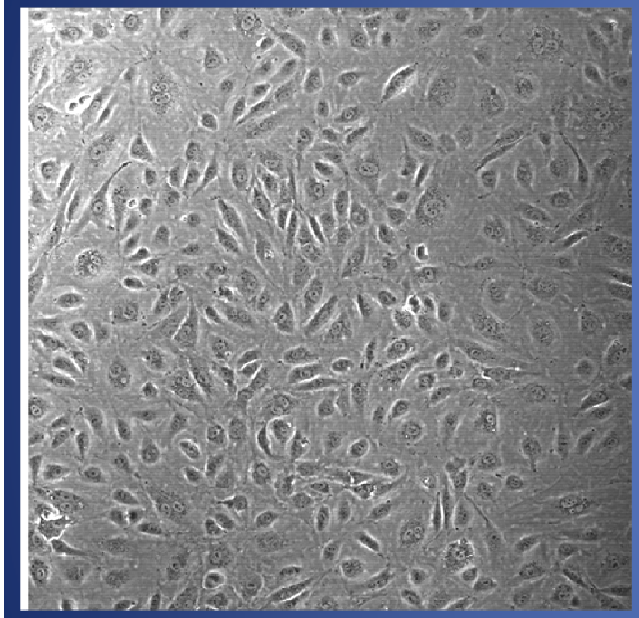
Control cells

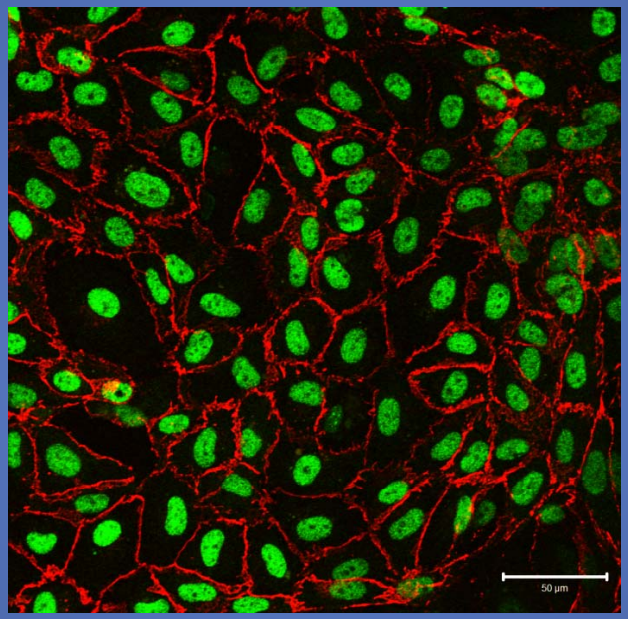


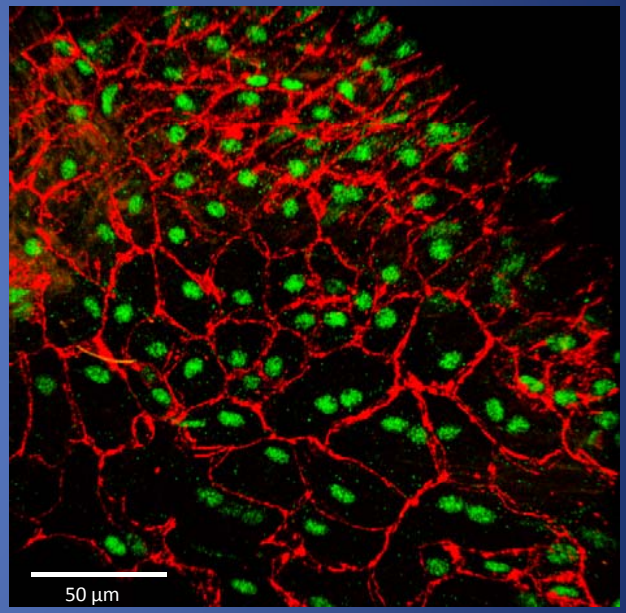
VE-cadherin inhibited



VE-cadherin is essential for vessel stabilisation and quiescence



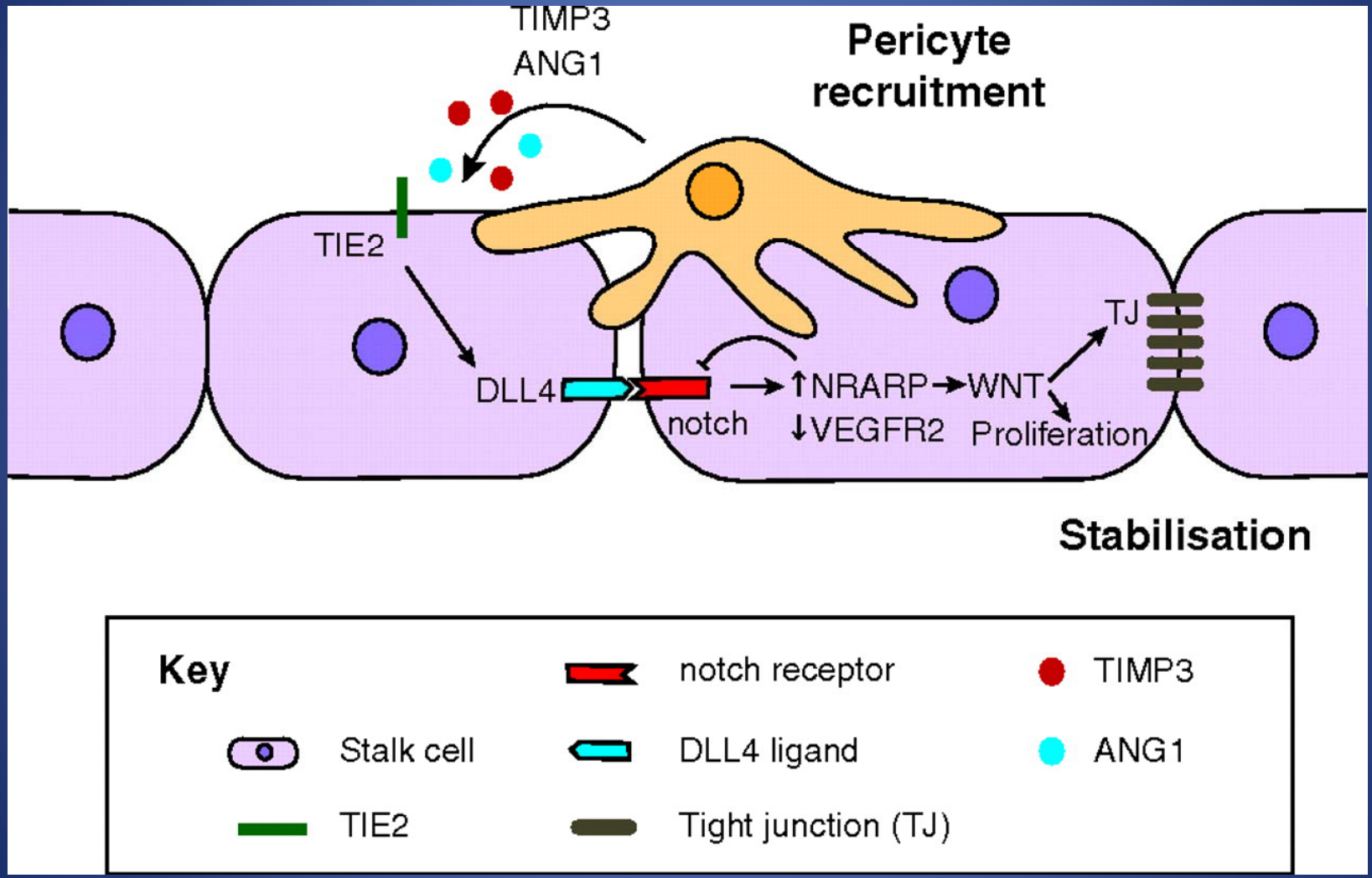




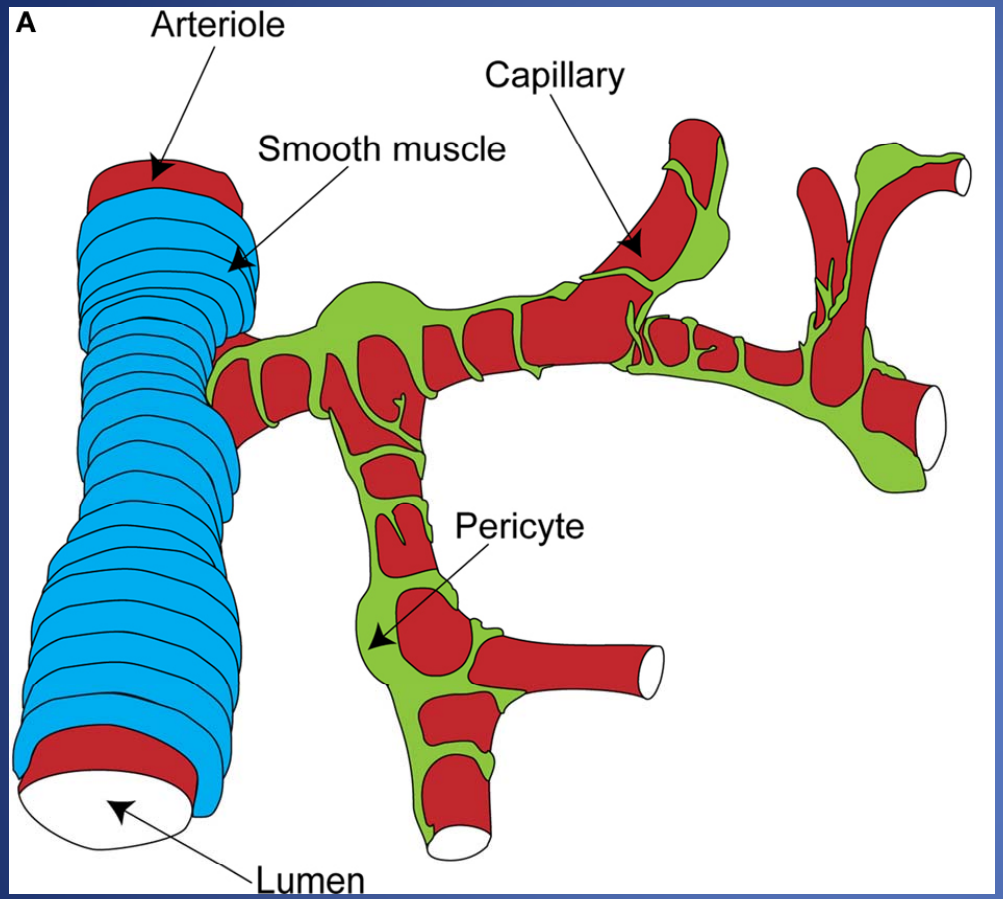
Human umbilical vein endothelial cells (HUVEC)

Human saphenous vein endothelium

Vessel stabilisation – mural cell attachment



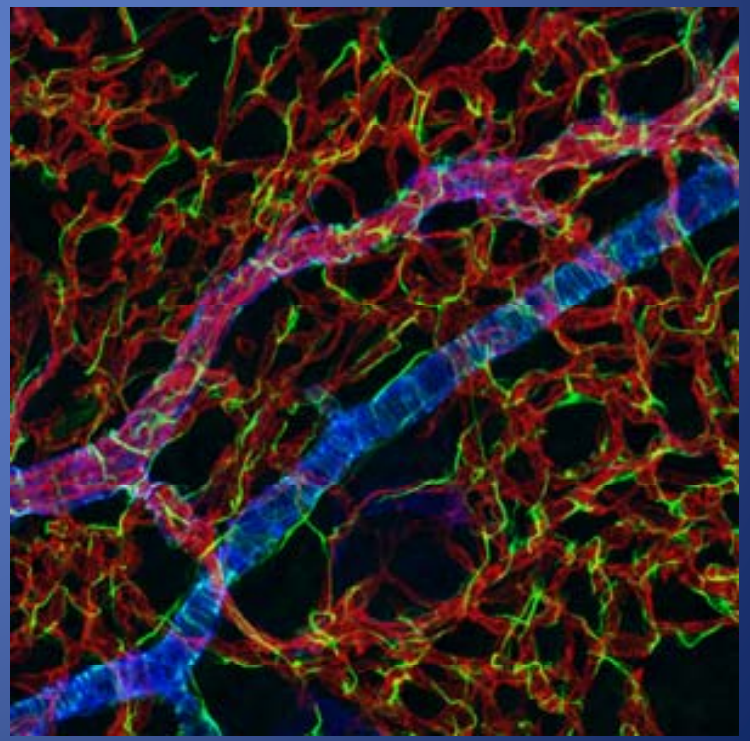
Mural cells help to stabilise the neovessels



Hamilton et al (2010) Front Neuroenerg.

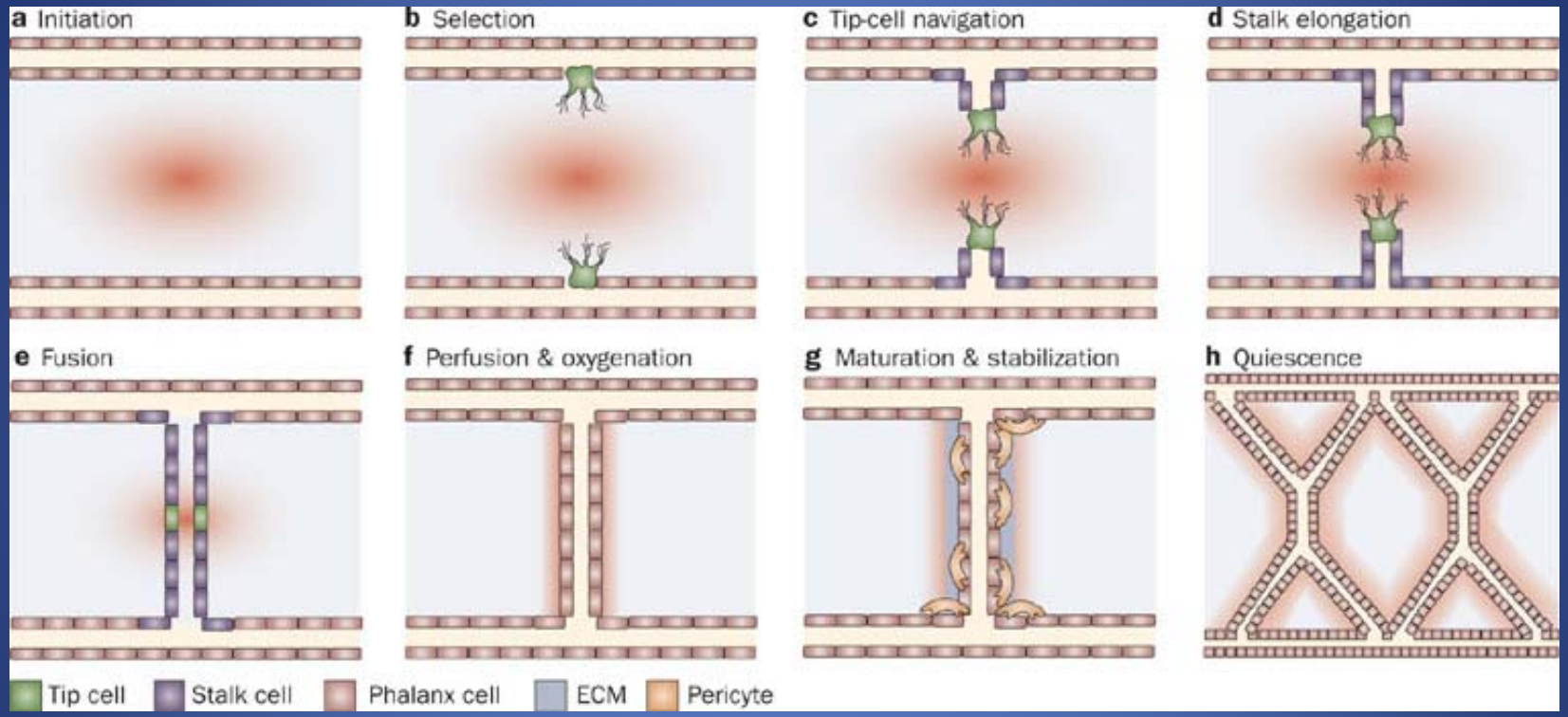
Blood vessels in embryonic skin

Red: Endothelial cells
Green: pericytes
Blue: vascular smooth muscle cells



Ralf Adams, Germany

Sprouting angiogenesis - summary

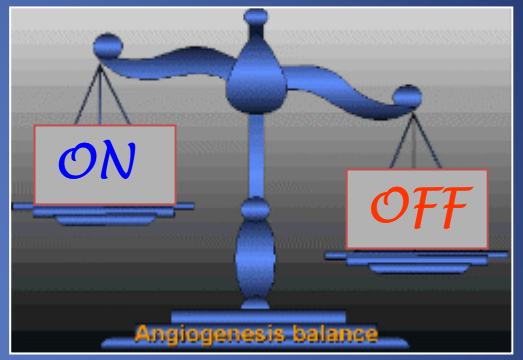


Carmeliet P *et al.* (2009) Branching morphogenesis and antiangiogenesis candidates: tip cells lead the way *Nat Rev Clin Oncol* doi:10.1038/nrclinonc.2009.64

What controls angiogenesis?

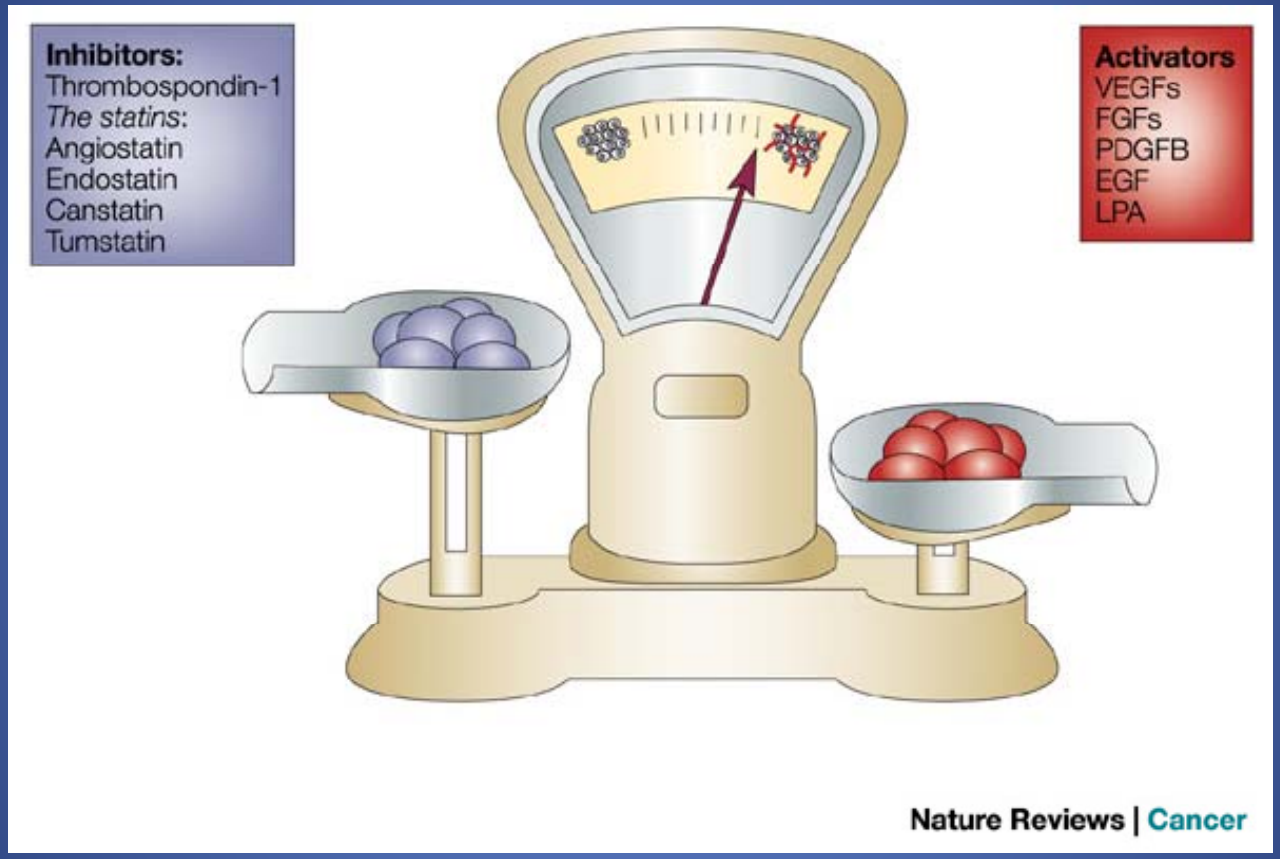
- Control of angiogenesis in the healthy body occurs through a series of switches:

Stimulating factors (*ON* switches)
Inhibitors (*OFF* switches)



- When stimulating factors are in excess of inhibitors, the balance favours blood vessel **growth**
- When inhibitors are present in excess of stimulators, angiogenesis is **stopped**
- The normal, healthy body maintains a balance of angiogenesis modulators, and generally angiogenesis is *OFF*

Angiogenesis : a balance



ANGIOGENESIS

Health

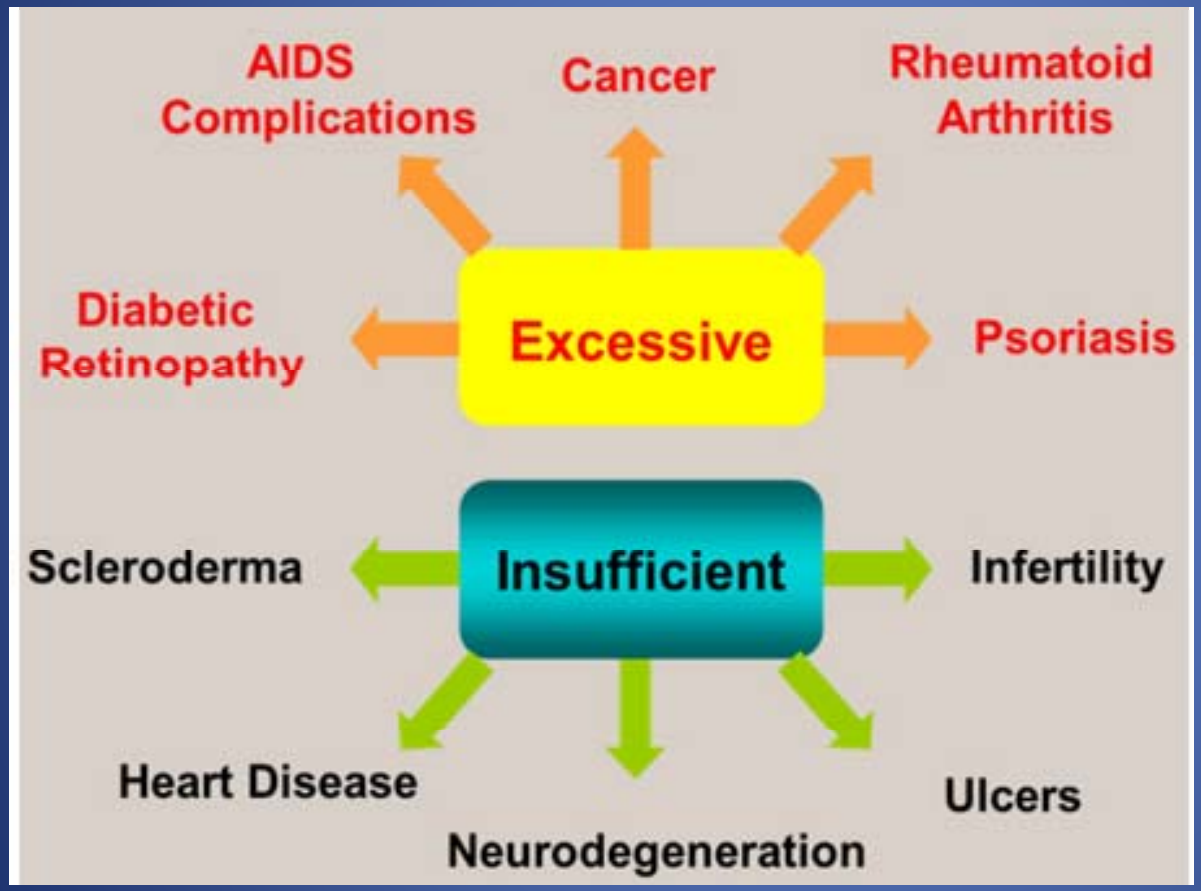
- Embryonic development
- Menstrual cycle
- Wound healing

Disease

- Cancer
- Chronic inflammatory diseases (RA, IBD, kidney…)
- Diabetic retinopathy
- Cardiovascular disease
- Psoriasis
- Vascular malformations (angiodysplasia, haemangioma)
- ……

Modified from P. Carmeliet - Nature. 2005;438:932-6

Too much or too little angiogenesis....



Therapeutic intervention

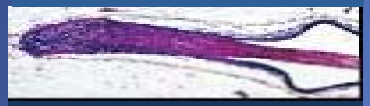
Inhibitors

Activators

ANGIOGENESIS AND DISEASE

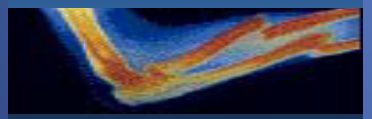
Insufficient

Baldness



MI - Ischemia

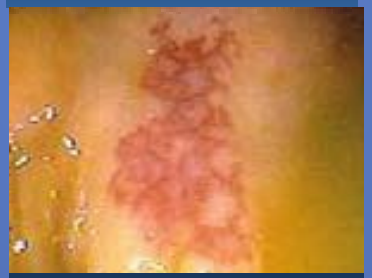
Limb Fractures



Thrombosis

Vascular Malformations

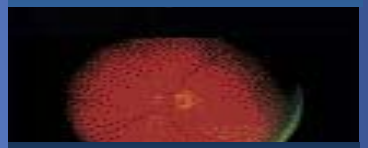
Angiodysplasia



HHT

Excessive

Retinal Disease



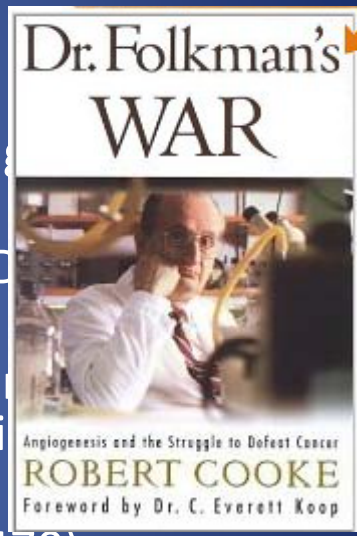
Cancers

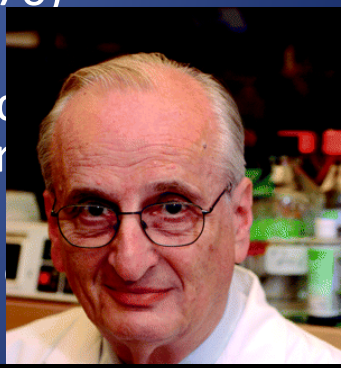
Atheroscerosis

Obesity

Angiogenesis and cancer: history

- 1787 - British surgeon Dr. John Hunter first uses the term 'angiogenesis' (new blood vessel growth) to describe blood vessels growing in the reindeer antler
- ……
- 1971 - Surgeon Dr. Judah Folkman hypothesizes that tumor g is dependent upon angiogenesis.
- 1983 - Vascular Permeability Factor (VPF), is discovered by D Harold Dvorak
- 1989 - Vascular endothelial growth factor (VEGF), is discover Napoleone Ferrara and Jean Plouet. It turns out to be identi VPF
- 1992: The first clinical trial of an antiangiogenic drug (TNP-470) begins in cancer patients
- 1999: Dr. Richard Klausner, Director of the U.S. National Canc Institute, designates the development of antiangiogenic ther for cancer as a national priority.
- 2004: Avastin (Bevacizumab) is FDA approved for the treatm advanced colorectal cancer.

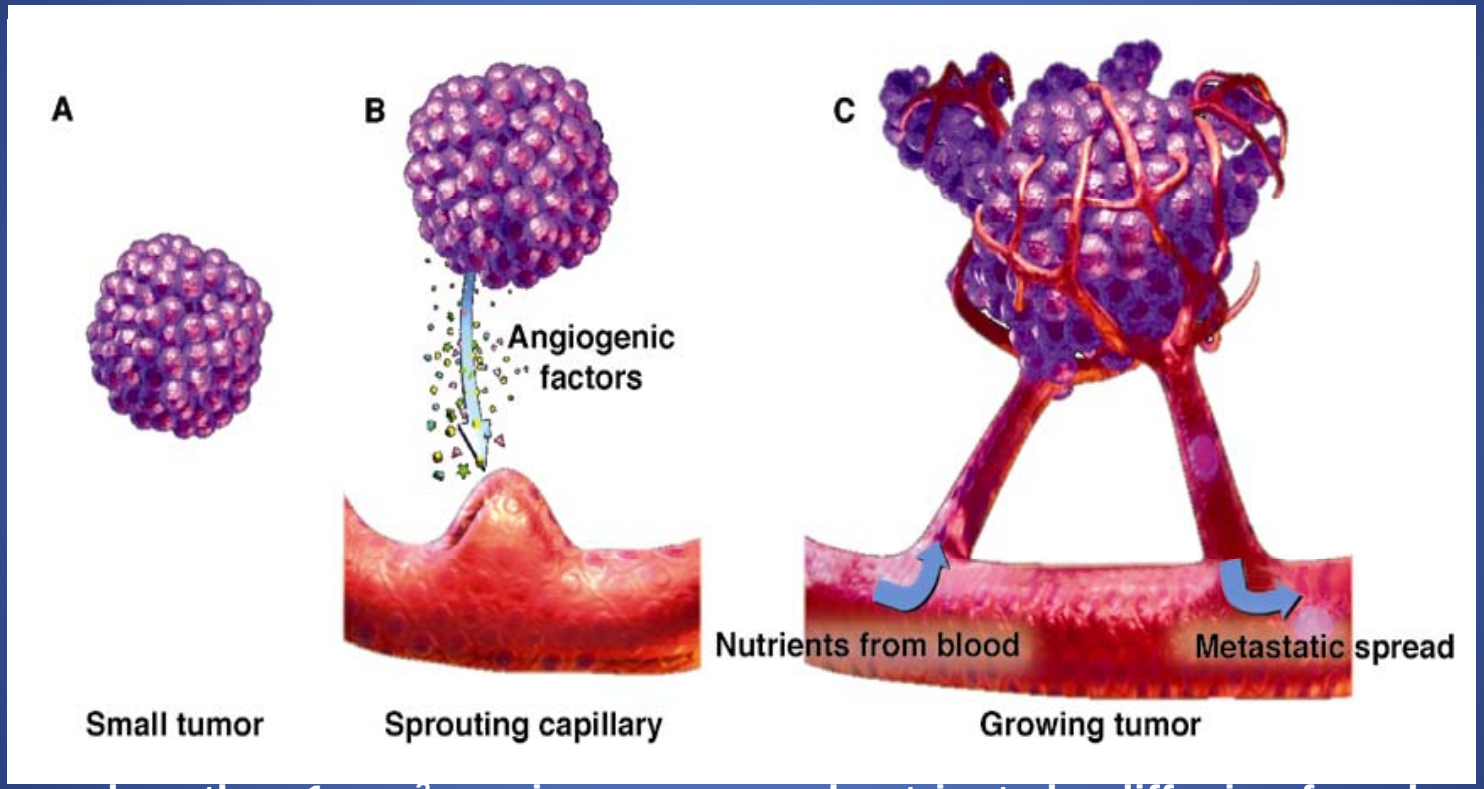




Moses Judah Folkman: 1933 - 2008

www.angio.org

Tumor Angiogenesis and Neovasculature



A, Tumors less than 1 mm³ receive oxygen and nutrients by diffusion from host vasculature. B, Larger tumors require new vessel network. Tumor secretes angiogenic factors that stimulate migration, proliferation, and neovessel formation by endothelial cells in adjacent established vessels. C, Newly vascularized tumor no longer relies solely on diffusion from host vasculature, facilitating progressive growth.

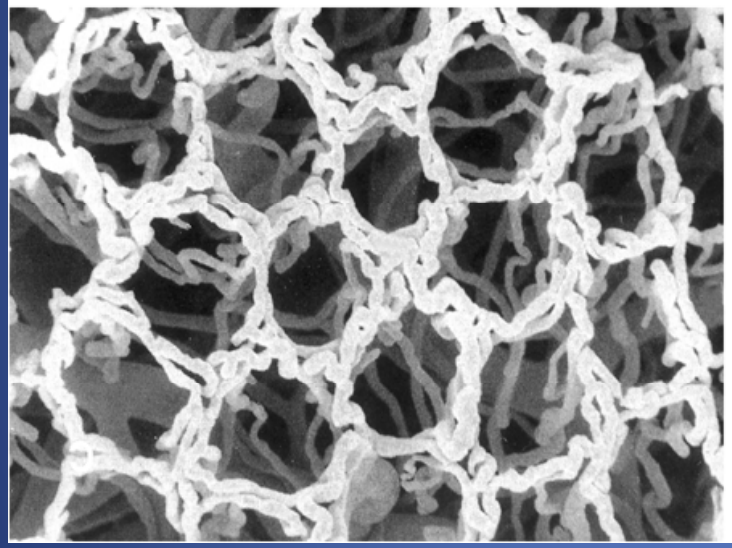
Tumour Blood vessels

Tumour blood vessels are architecturally different from normal vessels:

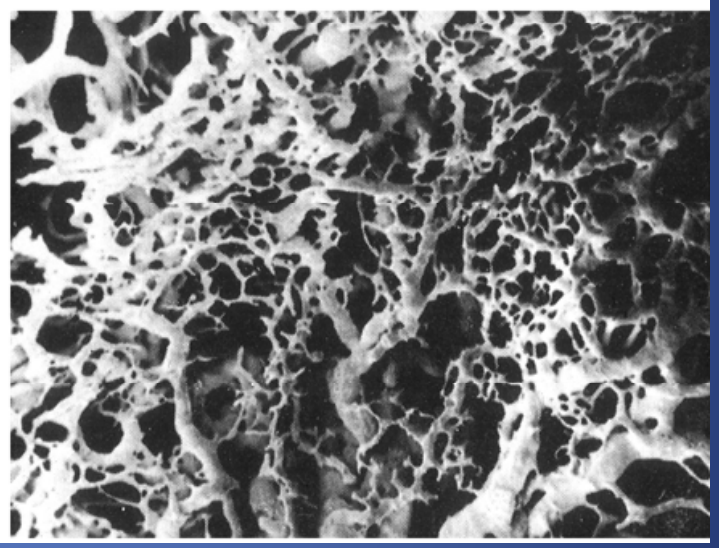
- irregularly shaped, dilated, tortuous and can have dead ends.
- not organized into definitive venules, arterioles and capillaries
- tumours' vascular network is often leaky and haemorrhagic, partly due to the overproduction of VEGF (induced by ischemia)
- perivascular cells, usually in close contact with the endothelium, often become more loosely associated
- some tumours may recruit endothelial progenitor cells from the bone marrow

Tumor Neovasculature: Comparative Tortuosity and Disorganization

Normal colorectal mucosa

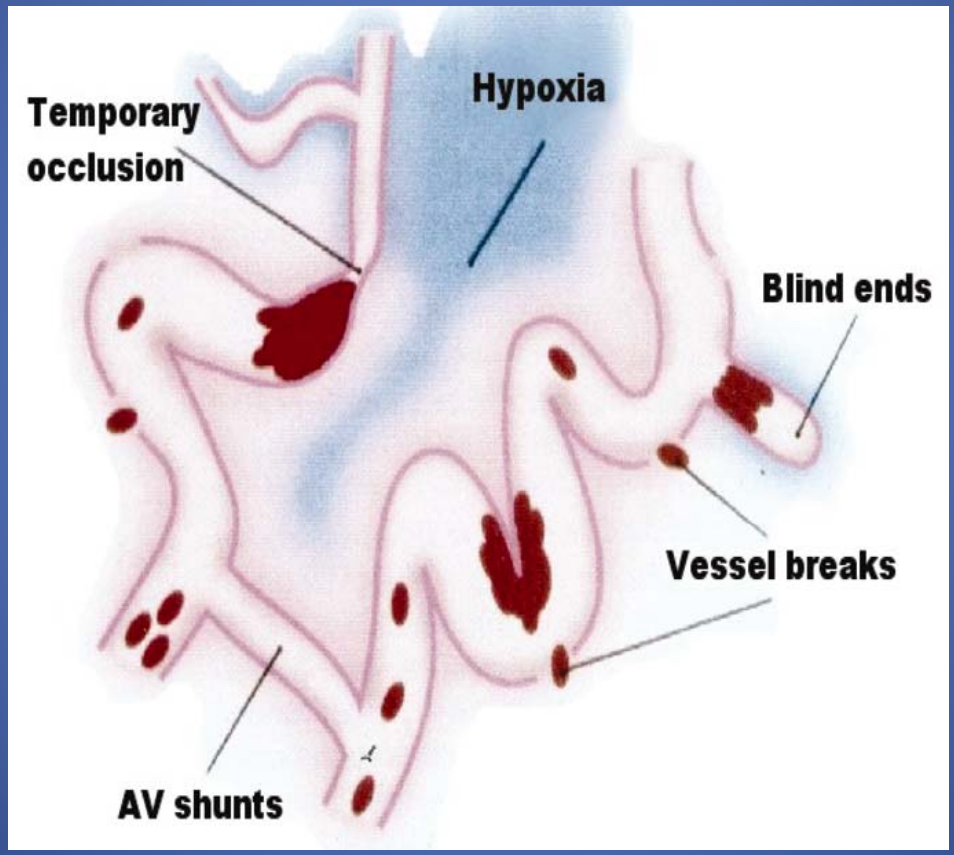


Nearby colorectal cancer



From Konerding et al. In Molls and Vaupel, eds. *Blood Perfusion and Microenvironment of Human Tumors*, 2002.

Abnormal Structural Features of Tumor Microvasculature



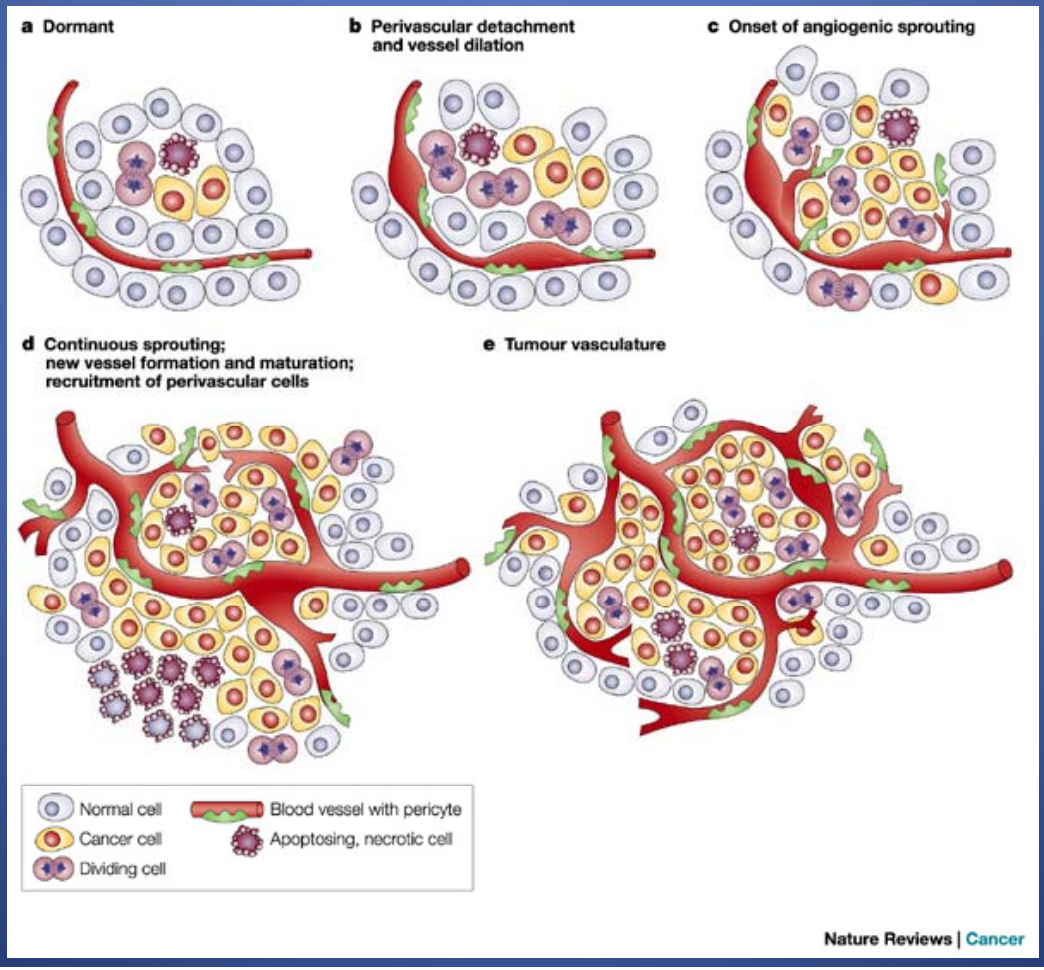
AV = arteriovenous.

From Brown and Giaccia. *Cancer Res*. 1998;58:1408-1416.

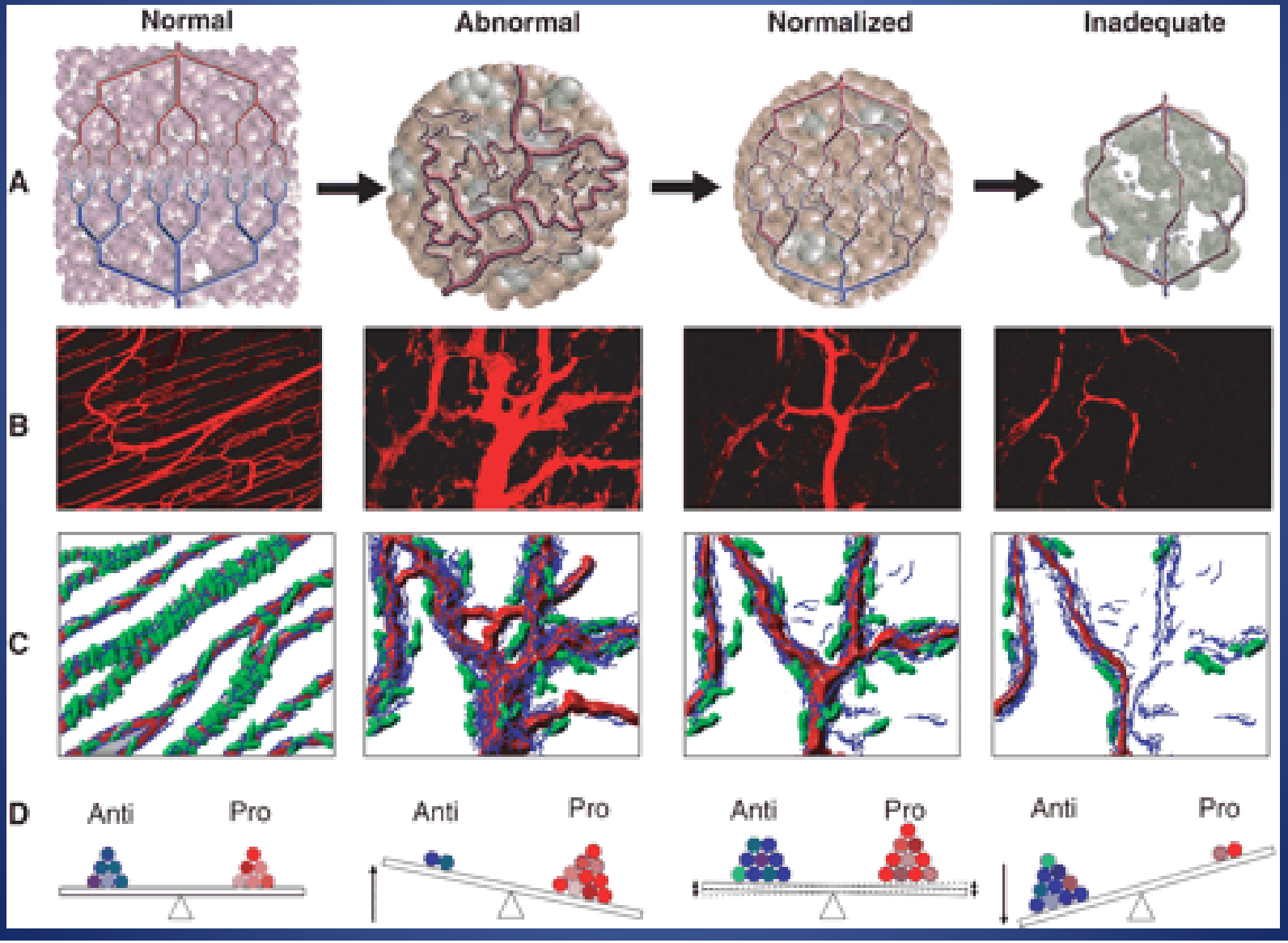
Tumor angiogenesis

The angiogenic switch

The angiogenic switch is a discrete step in tumour development that can occur at different stages in the tumour-progression pathway, depending on the nature of the tumour and its microenvironment

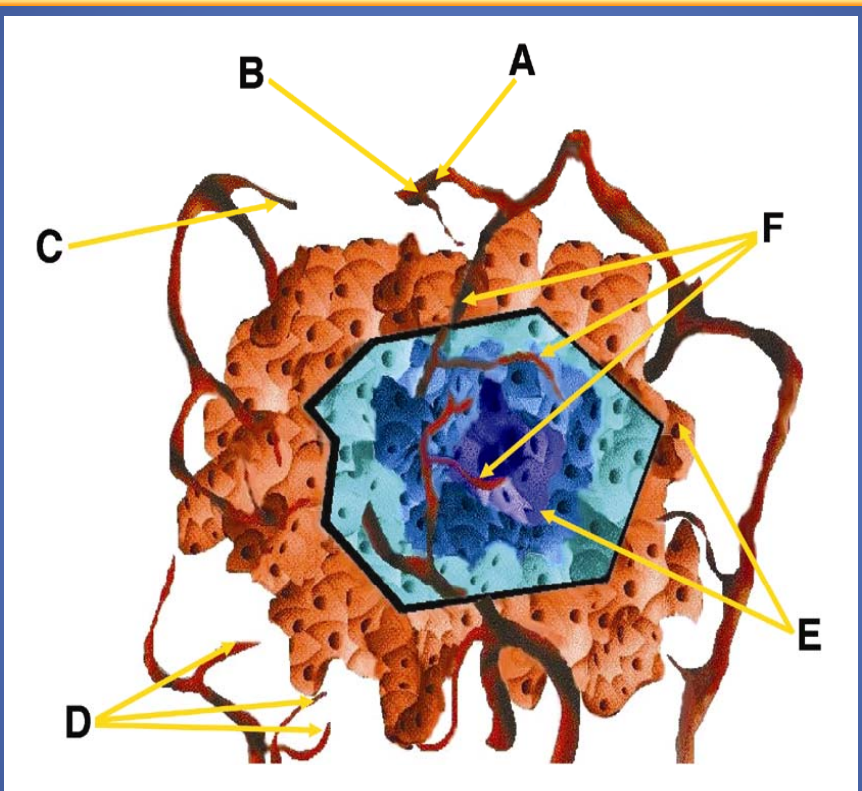


Anti-angiogenic treatment: normalization of tumor blood vessels



Jain R

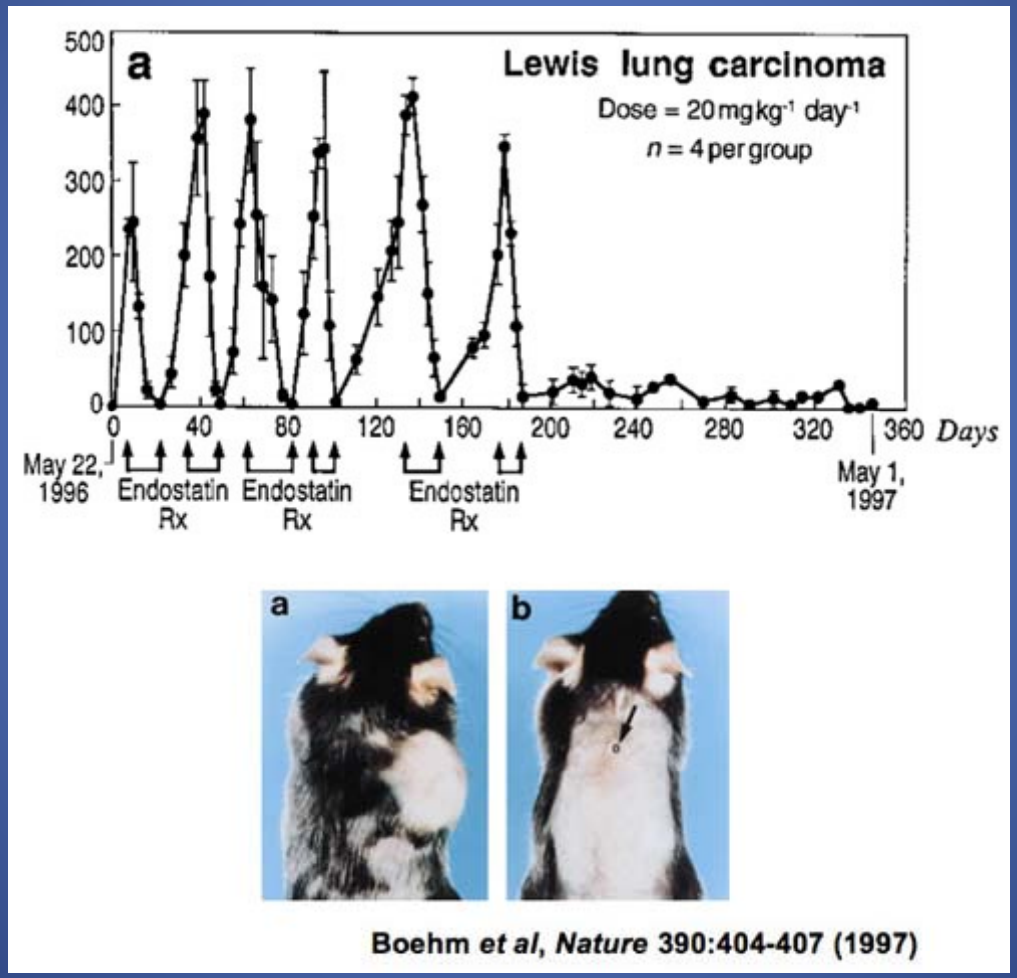
Approaches to Compromising Tumor Neovasculature



A, Matrix breakdown inhibition; B, signal transduction inhibition; C, receptor antagonism; D, inhibition of endothelial cell function (eg, proliferation, migration, and tube formation); E, blockade of activators of angiogenesis; F, compromise existing tumor vasculature.

From Siemann et al. Radiother Oncol. 2000;57:5-12.

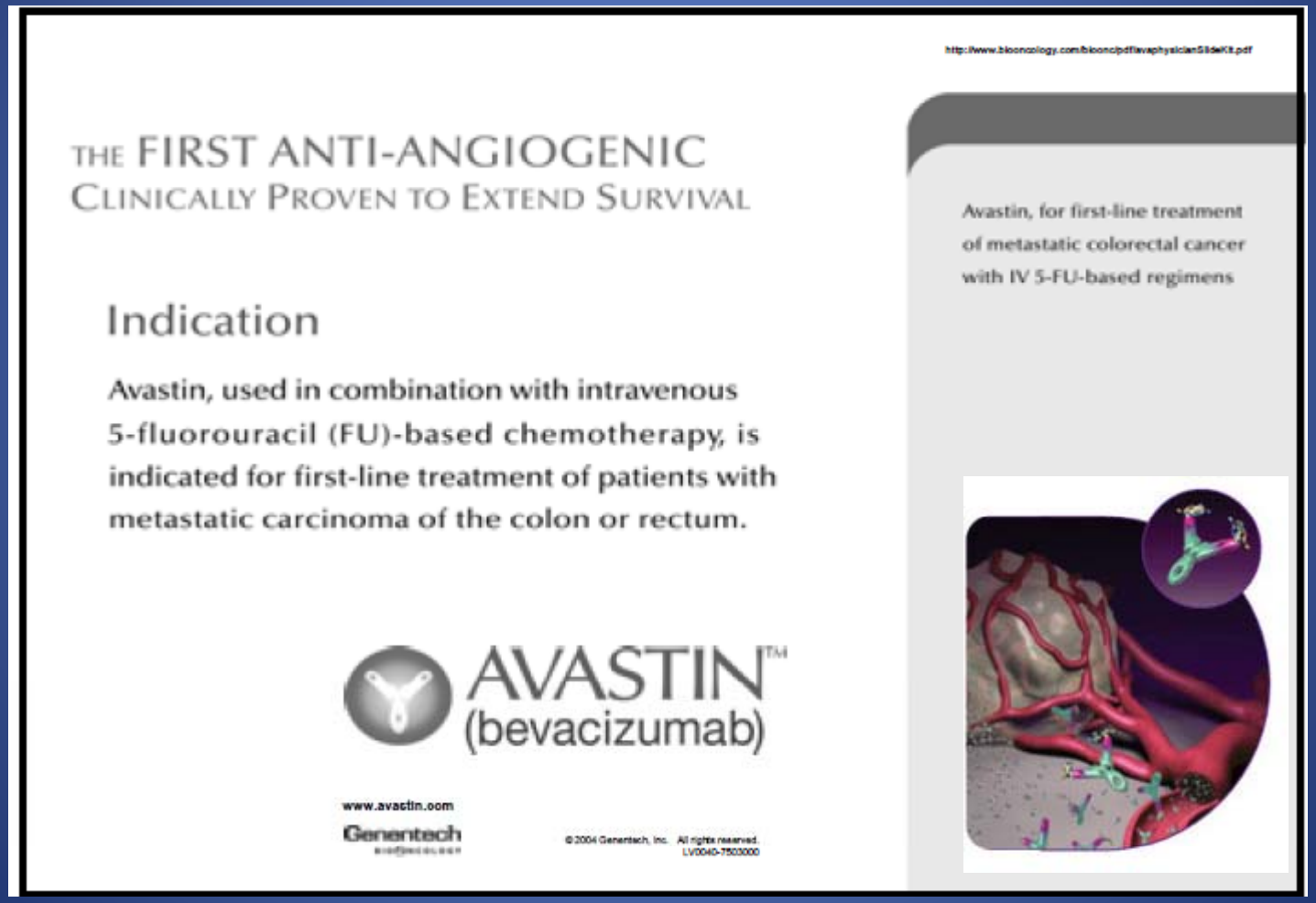
In vivo proof of concept studies: Endostatin inhibits solid tumor growth



Boehm *et al*, *Nature* 390:404-407 (1997)

Endostatin: endogenous anti-angiogenesis peptide

Anti-VEGF humanised MAb: Avastin



www.avastin.com

Avastin and cancer: hope and disappointment

- Avastin first approved in 2004 for treatment of advanced colon cancer (combination therapies)
- Approved since for advanced lung (2006), kidney and brain (glioblastoma) cancers (2009).
- Avastin approved for metastatic breast cancer in 2008 under the accelerated approval program
- *but……..*
- **FDA approval withdrawn for metastatic breast cancer 2010**

- Avastin side effects
  - GI perforation
  - Hypertension
  - Proteinuria
  - Venous thrombosis
  - Haemorrage
  - Wound healing complication
- no overall survival advantage over chemo alone
- no quality-of-life or survival advantage

FDA website http://www.fda.gov/Drugs
The Avastin Story N Engl J Med. 2011;365:1454-5

http://www.nice.org.uk/newsroom/pressreleases/AvastinBevacizumabNotRecommended.jsp

The New York Times
Thursday, November 4, 2010 Last Update: 6:09 AM ET

Avastin Falls Short in Test as Colon Cancer Medicine

By ANDREW POLLACK
Published: April 22, 2009

In results from a widely watched clinical trial, the drug Avastin failed to show a significant effect on preventing the recurrence of colon cancer, the drug's maker, Genentech, said early Wednesday.

Although Avastin is already a best-selling cancer treatment, success in this trial could have paved the way to a new use of the drug, potentially increasing sales by billions of dollars a year.

Now those efforts will be set back. And it appears that the S
giant Roche may have paid more than necessary when it a
March to buy, for $46.8 billion, the portion of Genentech it

Roche shares were down more than 10 percent on Wednes

Genentech and Roche said they would continue to try to de
stage cancer.

BBC NEWS
HEALTH

24 August 2010 Last updated at 07:36

Critics condemn bowel cancer drug rejection

By Helen Briggs
Health reporter, BBC News

Campaigners have condemned a decision to turn down a bowel cancer drug for use on the NHS in England and Wales.

The health watchdog NICE says the cost of avastin - at about £21,000 per patient - does not justify its benefits.

Anti-angiogenic therapy in cancer – does it work?

- In some cases benefits are transitory, and followed by a restoration of tumour growth and progression
- In other cases there is no objective benefit
- Two modes of unconventional resistance:
  - evasive resistance, an adaptation to circumvent the specific angiogenic blockade
  - intrinsic or pre-existing indifference

Types of Cancer in Active Phase III Treatment Clinical Trials of Angiogenesis Inhibitors

- Breast cancer
- Esophageal cancer
- Gastrointestinal Stromal Tumors (GIST)
- Kidney (renal cell) cancer
- Leukemia
- Liver (adult primary) cancer
- Lymphoma
- Melanoma
- Multiple myeloma
- Non-small cell lung cancer (NSCLC)
- Ovarian epithelial cancer
- Pancreatic cancer
- Prostate cancer
- Stomach (gastric) cancer

National Cancer Institute
www.cancer.gov

The future of therapies targeting angiogenesis

- Combination therapy : Anti-angiogenic therapy in combination with other anti-cancer therapies
- Resistance: combinatorial strategies targeting resistance mechanisms
- Novel targets = novel molecular mechanism
- Anti-angiogenic therapy in other diseases:
  - Rheumathoid Arthritis
  - Retina vascularization (diabetic retinopathy)
- Pro-angiogenic therapy in ischemia

The future of therapies targeting angiogenesis

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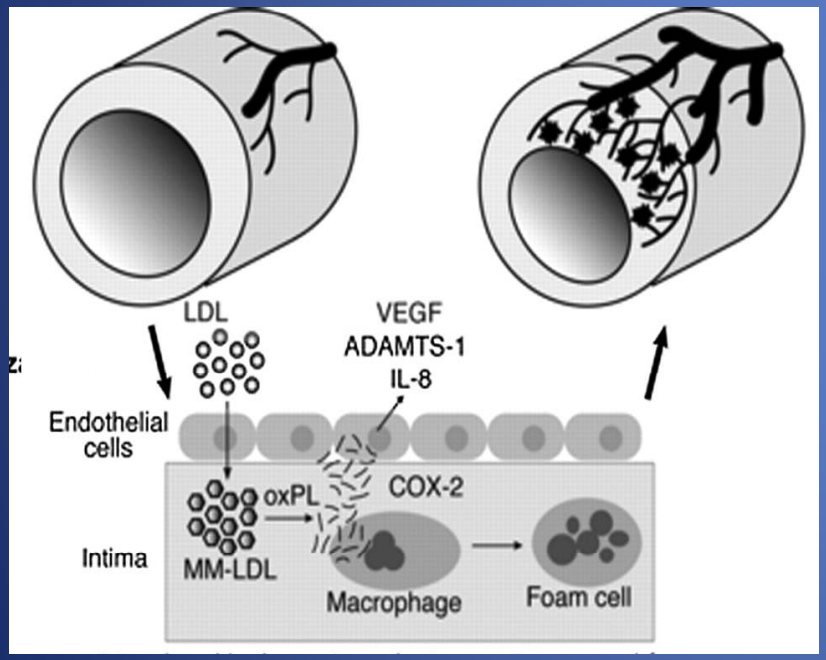
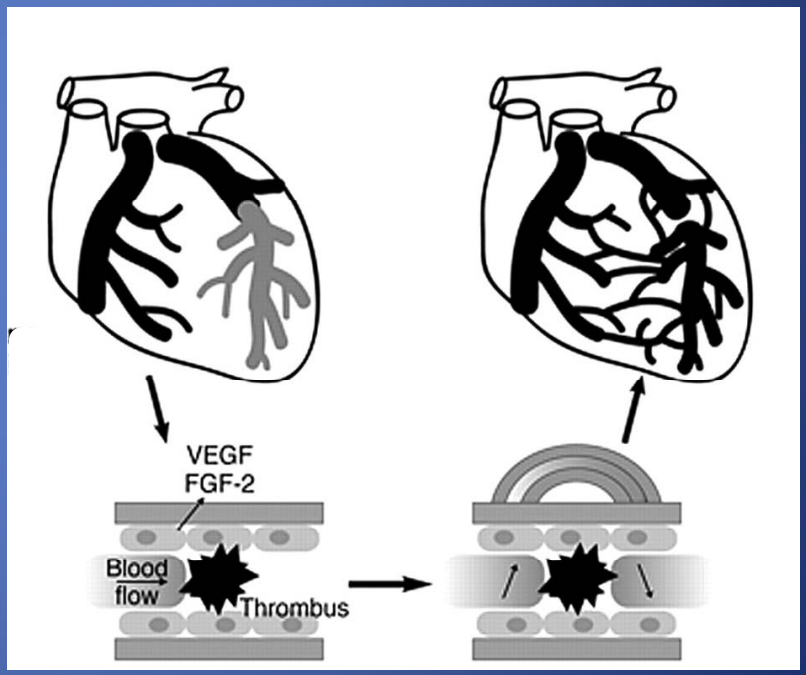
Angiogenesis in cardiovascular disease

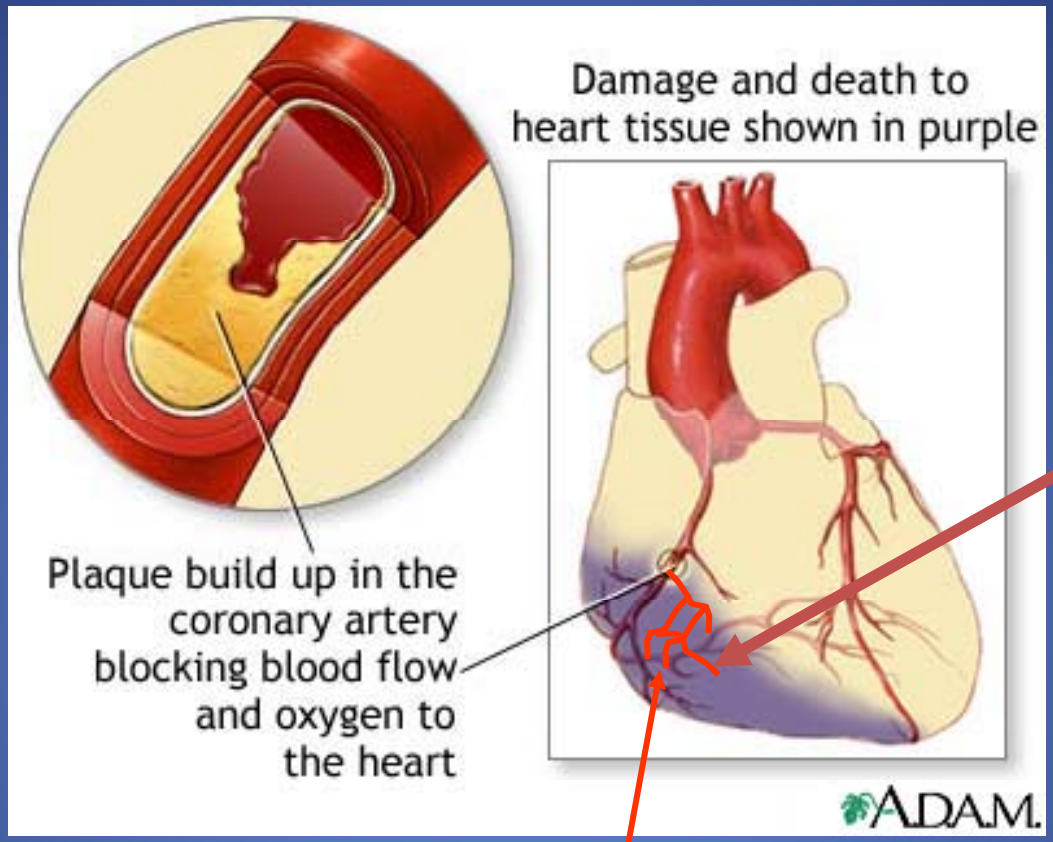
Plaque angiogenesis

Compensatory angiogenesis





from Chen, C.-H. et al. Circ Res 2006



Reduced blood flow: ischemia

Therapeutic Angiogenesis for Coronary Artery Disease:

deliver growth factors to the ischemic heart muscle to induce new vessel growth

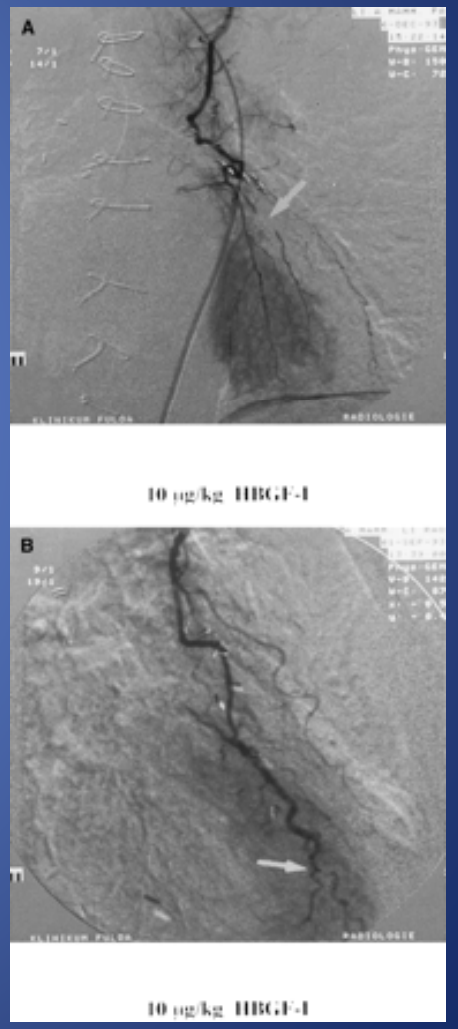
first therapeutic angiogenesis for myocardial ischemia

Schumacher B, et al.

Induction of neo-angiogenesis in ischemic myocardium by human growth factors: first clinical results of a new treatment of coronary heart disease.

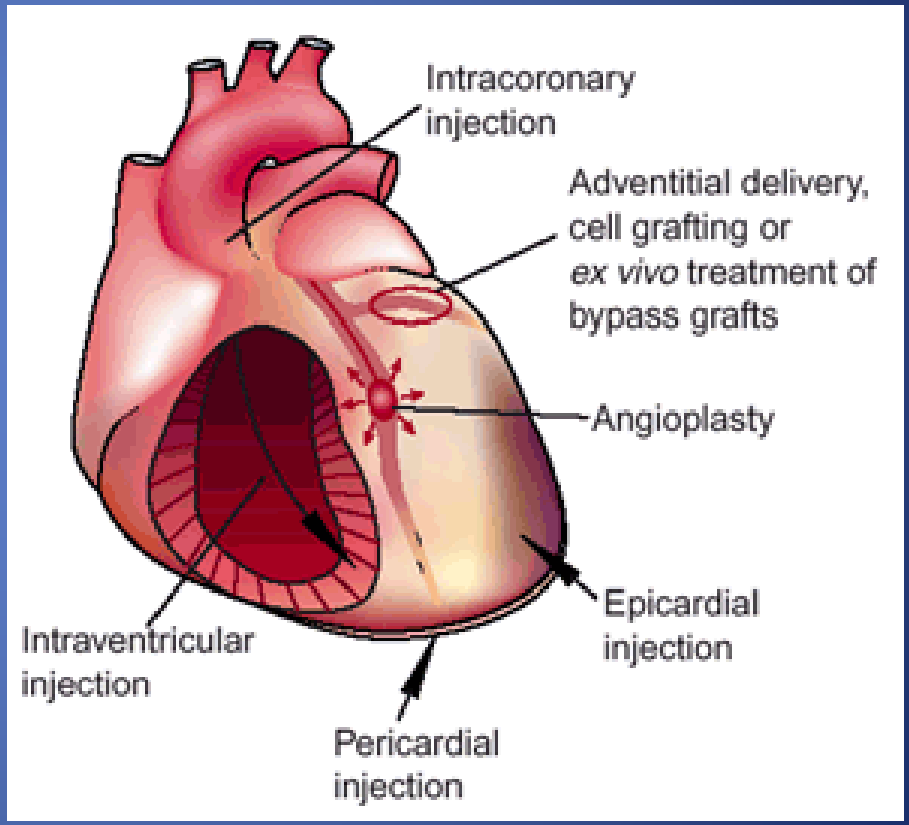
Circulation 1998;97(7):645-650.

- 20 patients with three-vessel coronary disease undergoing coronary bypass
- FGF-I injected close to the vessels after bypass
- Twelve weeks later, formation of capillaries in all cases around the site of injection demonstrated by imaging



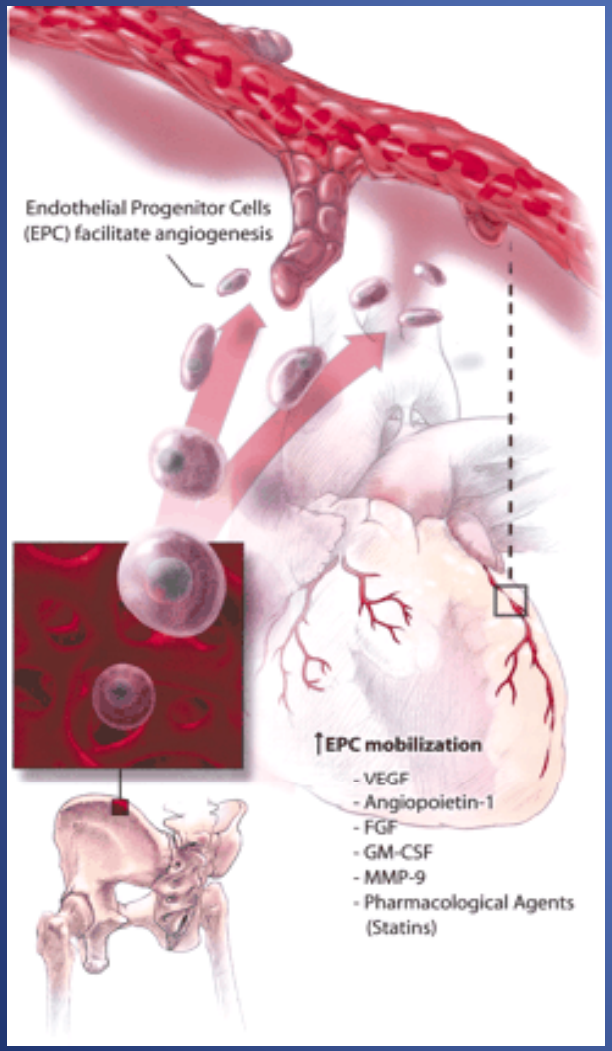
approaches to therapeutic angiogenesis

- Protein therapy
- Gene therapy
- Cell Therapy



Nat Med 2003;9:694-701

Bone marrow-derived Endothelial Progenitor Cells



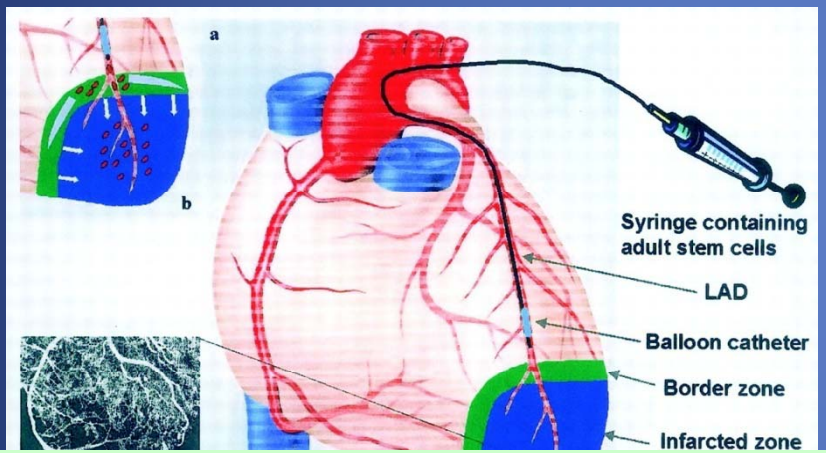
Szmitko et al, Circulation. 2003;107:3093

- Bone marrow-derived CD34+ hematopoietic progenitor cells from adults can differentiate ex vivo to an endothelial phenotype.
- Circulating EPCs facilitate angiogenesis and re-endothelialization at sites of vascular damage (*Ashara, Science, 1997*)
- Mobilization of EPCs from BM is enhanced by growth factors, statins, erythropoietin,…
- Two types of "EPC":
  - Progenitors of the endothelial lineage, that can be expanded in culture and form blood vessels in vivo
  - Pro-angiogenic cells (PAC), cells of the myeloid lineage that can release pro-angiogenic factors

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 21, 2006 VOL. 355 NO. 12

Intracoronary Injection of Mononuclear Bone Marrow Cells in Acute Myocardial Infarction



REPAIR-AMI TRIAL

Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans

- 204 patients with acute MI (and placebo group)
- Improvement in ejection fraction in post-MI patients following infusion of BM-derived stem cells

Stem cell treatments for the heart: Update 2012

Thirty-three randomised clinical trials (1765 pt)

Short-term functional improvement

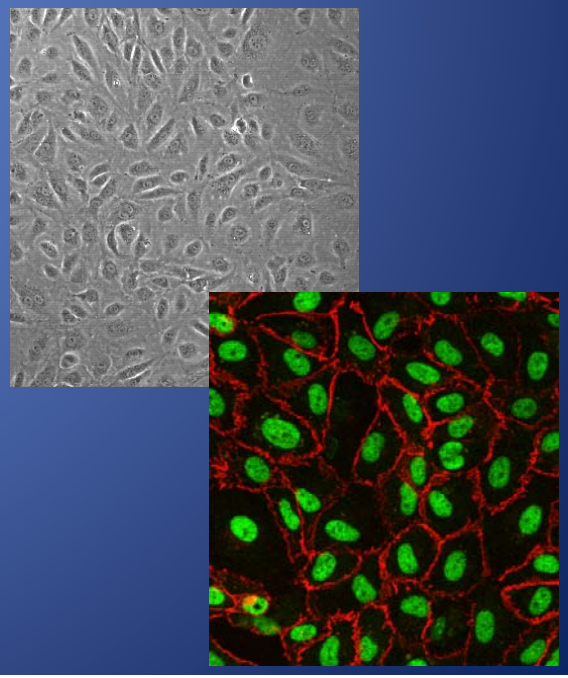
No long term improvement in mortality

>More studies, more patients, better standardization of methods

How to study angiogenesis in the lab

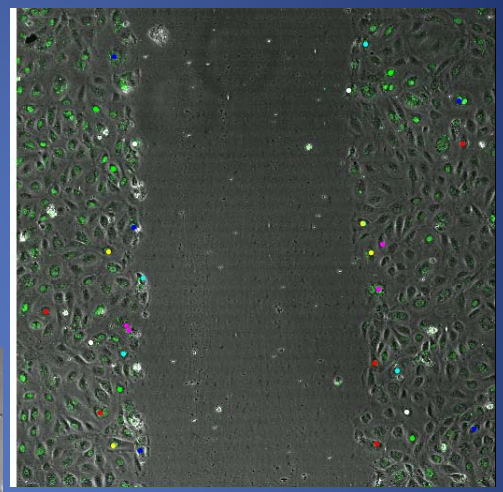
Primary endothelial cells from:

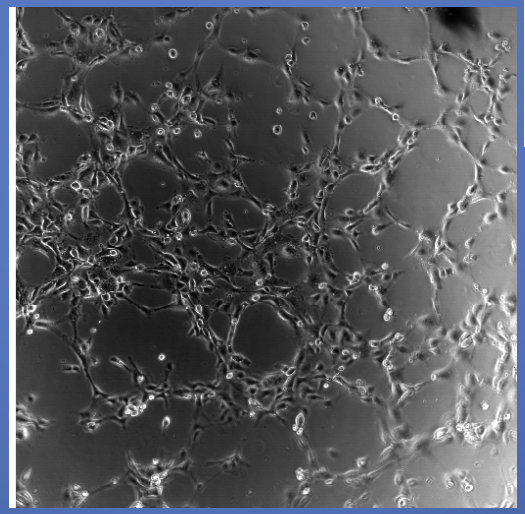
- Human
  - Umbilical cords
  - Surplus tissue from surgery
  - Blood outgrowth endothelial cells
- Animal
  - Blood vessels e.g. bovine aorta
  - Organs e.g. heart or lung

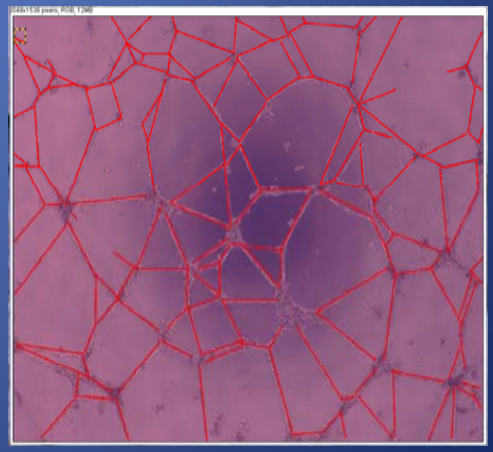


How to study angiogenesis in the lab

- *In vitro* assays:
  - Proliferation
  - Migration
  - Matrigel
- *In vivo* assays:
  - Matrigel plug
  - Aortic ring
  - Hindlimb Ischemia



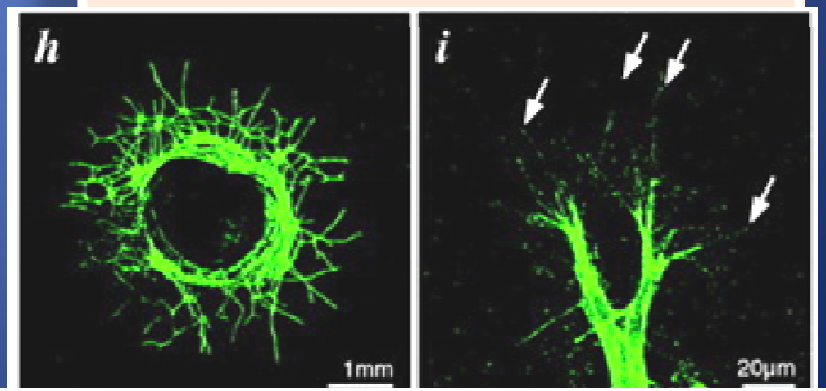




How to study angiogenesis in the lab

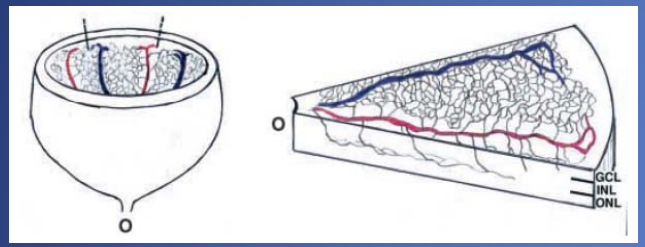
- *In vitro* assays:
  - Proliferation
  - Migration
  - Matrigel
- *In vivo* assays (mouse):
  - Matrigel plug
  - Aortic ring
  - Retina angiogenesis
- *In vivo* assays (Zebrafish)

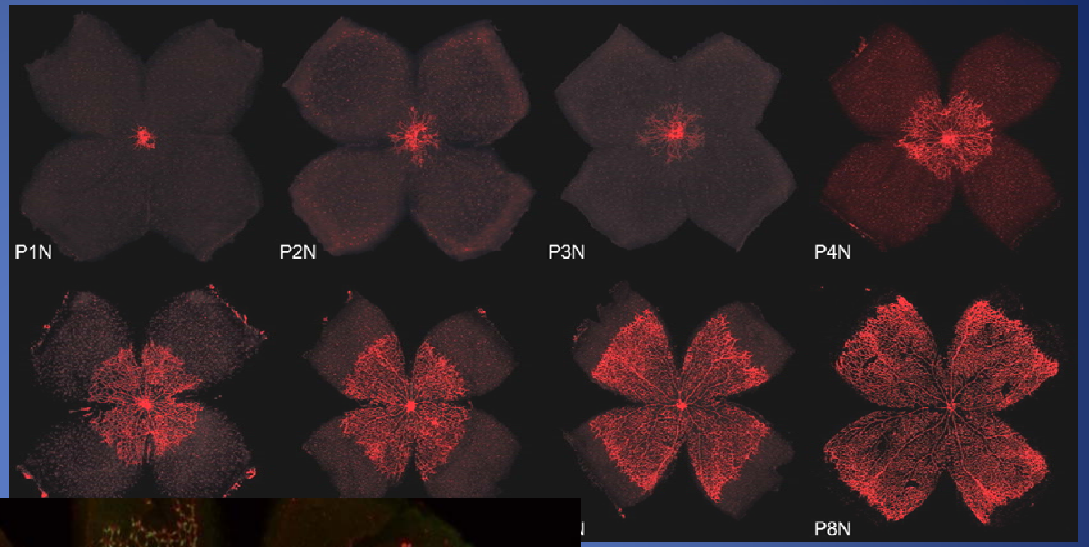
- Inject Matrigel subcutaneously into abdomen
- Matrigel will form a solid plug
- Leave mice for 7 days
- Remove plug from mouse, section and stain to look for new blood vessels

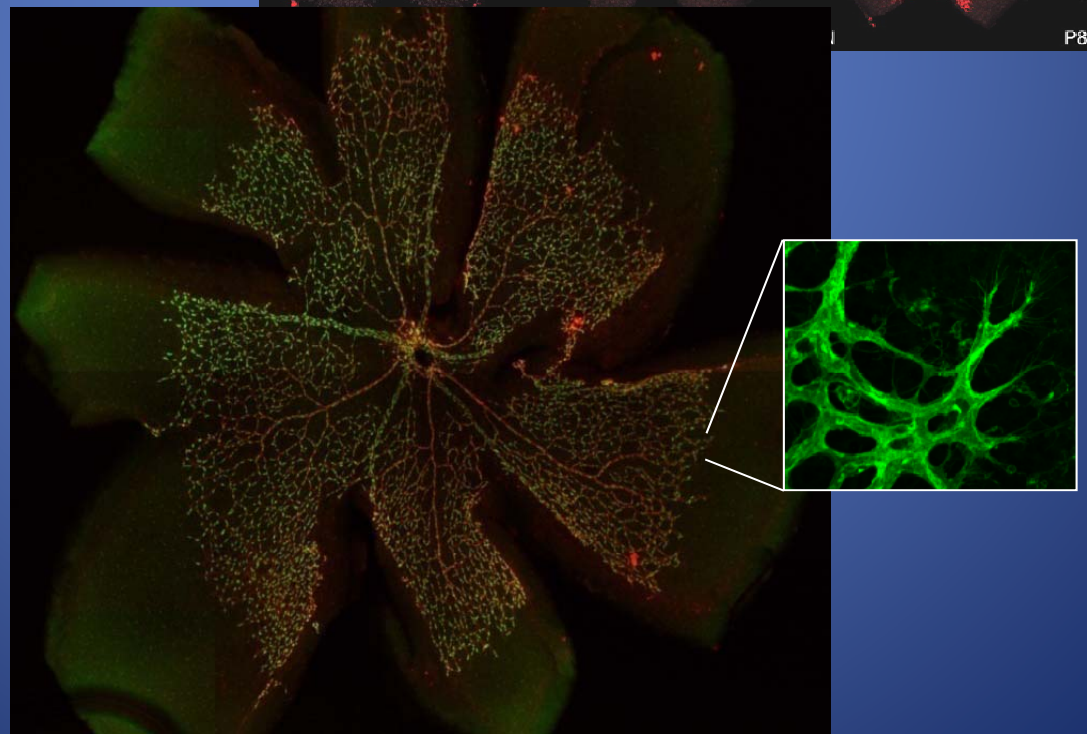


Angiogenesis in the mouse retina

Retinal angiogenesis :
occurs post-partum in mouse
between day 0-14







Angiogenesis - Further Reading

- Carmeliet P & Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. *Nature* 473:298-307
- Eilken H & Adams RH (2010) Dynamics of endothelial cell behaviour in sprouting angiogenesis. *Current Opinion in Cell Biology* 22:617-625
- Geudens I & Gerhardt H (2011) Coordinating cell behaviour during blood vessel formation. *Development* 138:4569-4583
- Potente M, Gerhardt H & Carmeliet P (2011) Basic and therapeutic aspects of angiogenesis. *Cell* 146:873-887