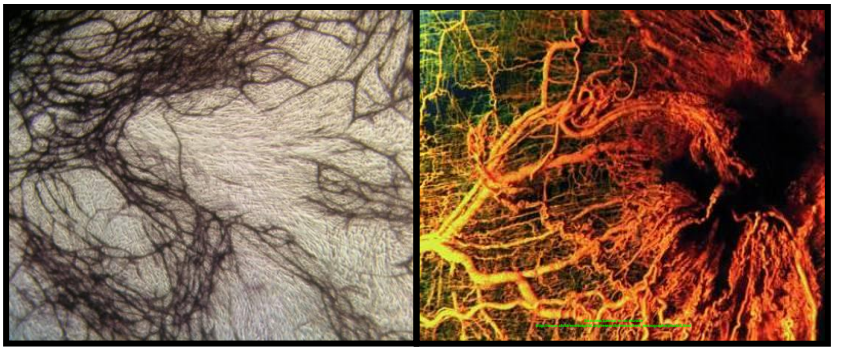
Angiogenesis



UNIVERSITY OF OXFORD

Imperial College London

Dr Ewa Paleolog

Kennedy Institute of Rheumatology

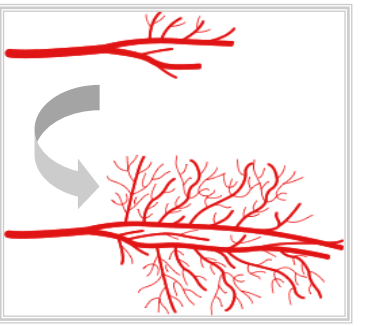
Learning Objectives

1. Consider the basic principles underlying angiogenesis
2. Understand processes and key regulatory molecules involved in angiogenesis
3. Recognise the role played by angiogenesis in disease
4. Examine the potential of angiogenesis as a therapeutic target, particularly in ischemic disease
5. Review the use of angiogenesis inhibitors in disease, particularly solid cancers

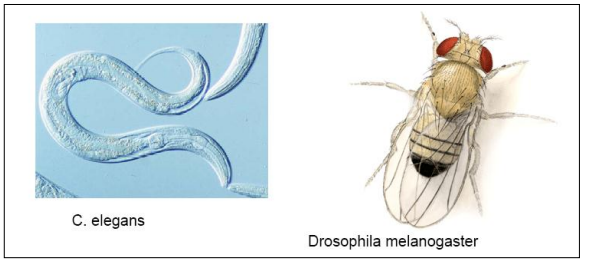
What?

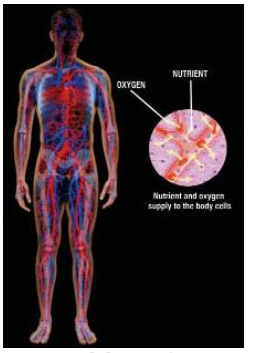
angiogenesis { "angeion" - vessel; "genesis" - production

Formation of new blood vessels by a process of sprouting from pre-existing vessels



Why?

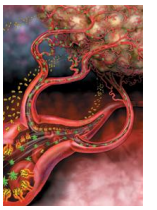




- In primitive/small animals, oxygen is capable of diffusing throughout their small body to all cells
- In other species, which develop later and grow to a larger size, a vascular network distributes oxygen and nutrients in the blood to distant cells

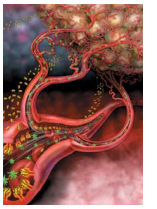
Why?

- More than 10^{12} (1,000,000,000,000) endothelial cells line the inside of blood vessels
- Area covered equivalent to an area of 1000 m^2 in a 70kg adult
- In the adult, the proliferation rate of vascular endothelial cells is very low
- Turnover time can exceed 1000 days



Why?

- Mammalian cells require oxygen and nutrients for their survival
- Diffusion limit for oxygen is 100-200μm
- For multi-cellular organisms to grow beyond this size, recruitment of new vessels is critical
- In many pathological situations, a similar principle applies



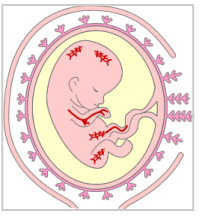
Where?

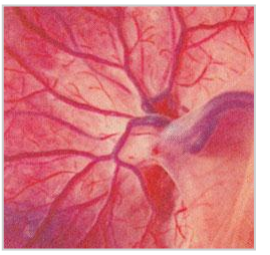
Physiological	Pathological
Embryo formation	Cancer, Rheumatoid Arthritis, AIDS complications, Blindness, Psoriasis, Excessive, ANGIOGENESIS, Stroke, Infertility, Insufficient, Heart Disease, Ulcers, Scleroderma
Skeletal development	
Female menstrual cycle	
Wound healing	
Fracture healing	

Where?

Physiological angiogenesis:

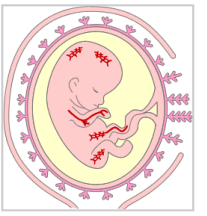
- In the developing **embryo**, 'blood islands' in the yolk sac grow & fuse to form capillary network
- Endothelial cell precursors (angioblasts) migrate & fuse to form vessels by **vasculogenesis**
- This primitive vasculature extends by angiogenesis



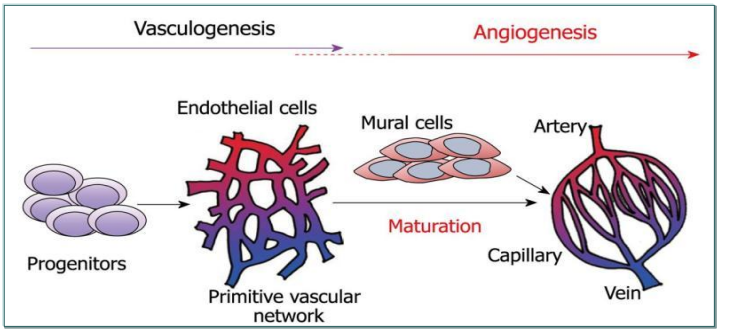


Where?

Physiological angiogenesis:



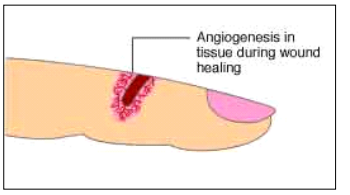
- In the developing **embryo**, 'blood islands' in the yolk sac grow & fuse to form capillary network



Where?

Physiological angiogenesis:

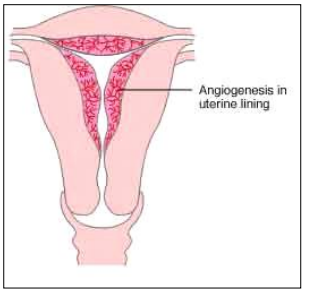
- Key role in **wound healing**
- New blood vessels are necessary in the area of the wound for the dissemination of growth factors
- The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialisation, and wound contraction



Where?

Physiological angiogenesis:

- Angiogenesis occurs as part of **female menstrual cycle** - each cycle, endometrial tissue regenerates, differentiates and regresses
- Important during the development of follicles, corpus luteum formation and (if appropriate) embryo implantation



Learning Objectives

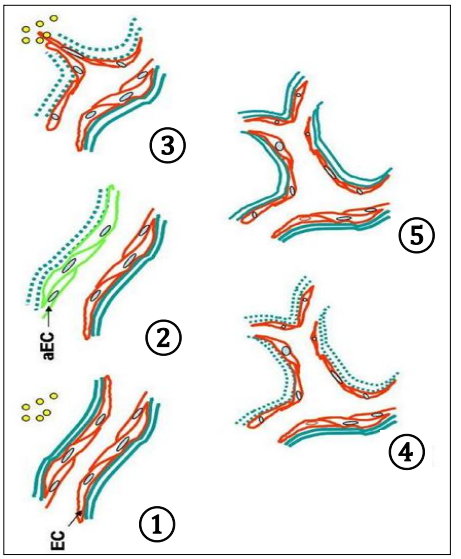
1. **Consider the basic principles underlying angiogenesis**
   - **What?**
   - **Why?**
   - **Where?**

➢ **TO MAINTAIN SUPPLY OF OXYGEN AND NUTRIENTS TO TISSUES**

Learning Objectives

1. Consider the basic principles underlying angiogenesis
2. Understand processes and key regulatory molecules involved in angiogenesis
   - The angiogenic balance
   - Vascular endothelial growth factor

Processes underlying angiogenesis



1. STIMULUS
2. ENDOTHELIAL ACTIVATION
3. DEGRADATION OF BASEMENT MEMBRANE
4. MIGRATION
5. FORMATION OF MATURE NEW VESSEL

The angiogenic balance

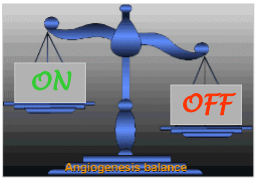
HYPOTHESIS

- **Angiogenesis is tightly controlled by the balance of two sets of counteracting factors – angiogenic activators and inhibitors**
- **The stability of the 'angiogenic switch' determines the time of initiation of the subsequent angiogenic process**

The angiogenic balance

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

  Stimulating factors (ON switches)
  Inhibitors (OFF switches)



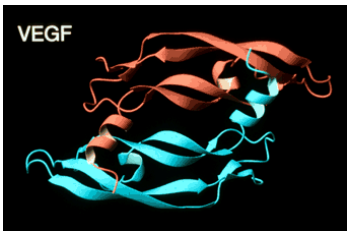
- **When growth factors are in excess of inhibitors, the balance favours blood vessel growth**
- **When inhibitors are present in excess of stimulators, angiogenesis is stopped**
- **The normal, healthy body maintains a balance of angiogenesis modulators, and generally angiogenesis is OFF**

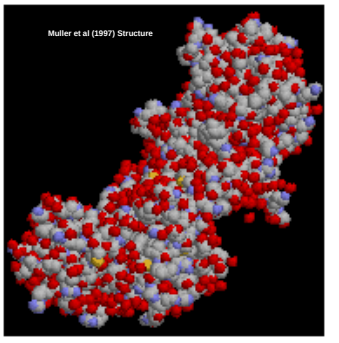
The angiogenic balance

ON	OFF
VEGF (VEGF-A)	TSP-1
VEGF-B, -C, -D, -E	Interferon γ
PlGF	TGFβ
FGF-1, FGF-2	TNFα
PDGF	IP-10
HGF	Groα
TGFβ	Angiostatin
TNFα	Endostatin
Angiogenin	
Angiopoietins	

Vascular endothelial growth factor

- Most potent angiogenic stimulus characterized
- Stimulates endothelial proliferation & migration
- Secreted by various cell types, including fibroblasts, osteoblasts, macrophages, platelets & lymphocytes





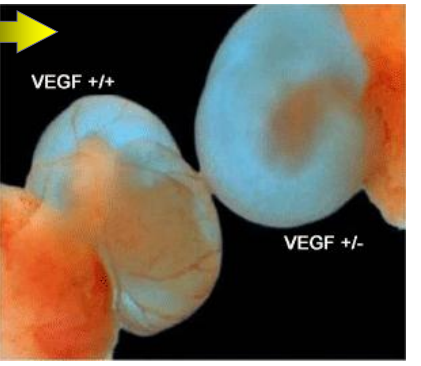
Vascular endothelial growth factor

Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene
Nature 1996, Ferrara et al

Loss of a single VEGF allele is lethal in the mouse embryo. Angiogenesis and blood-island formation are impaired, resulting in developmental anomalies

Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele
Nature 1996, Carmeliet et al

Formation of blood vessels is abnormal in heterozygous VEGF-deficient embryos, resulting in death at mid-gestation



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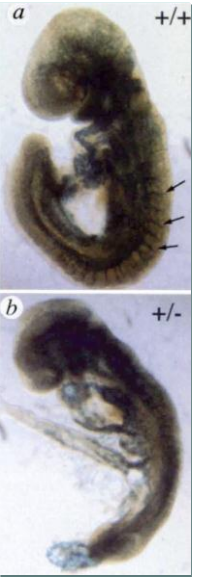
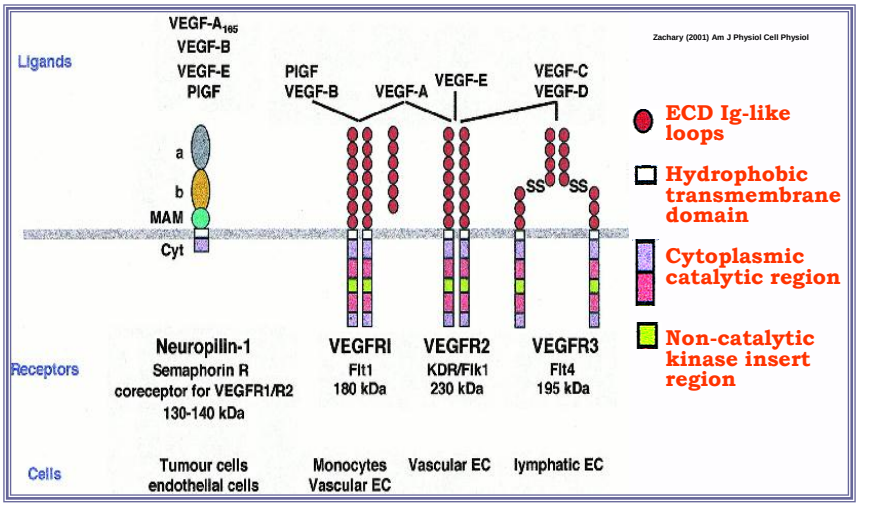


Vascular endothelial growth factor



Learning Objectives

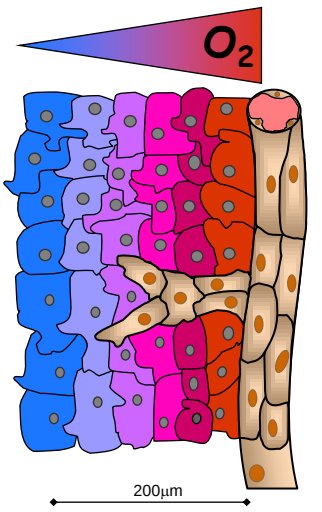
1. Consider the basic principles underlying angiogenesis
2. Understand processes and key regulatory molecules involved in angiogenesis

Learning Objectives

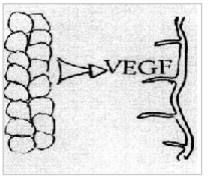
1. Consider the basic principles underlying angiogenesis
2. Understand processes and key regulatory molecules involved in angiogenesis
3. Recognise the role played by angiogenesis in disease
    - Relationship between oxygen and VEGF

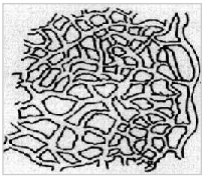
Links between blood supply and oxygen

- Oxygen tension is highest close to blood vessels
- Limit for O_2 diffusion from pre-existing vessels 100-200μm
- Low oxygen induces VEGF
- Low oxygen is a feature of many diseased tissues



Links between blood supply and oxygen





VEGF

normoxia

O_2

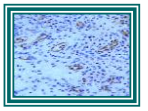
O_2

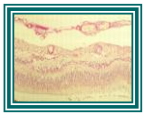
Tissue distant from nearest blood vessels and relatively hypoxic

Hypoxia-driven neovascularisation is mediated by high levels of VEGF

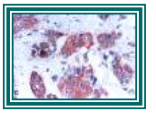
VEGF and disease:

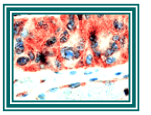
Arthritis
Psoriasis
Fractures
AIDS
Atherosclerosis
Endometriosis
Infections
Diabetes
Crohn's disease
Ulcerative colitis

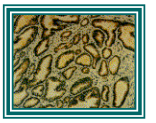




Tumours:

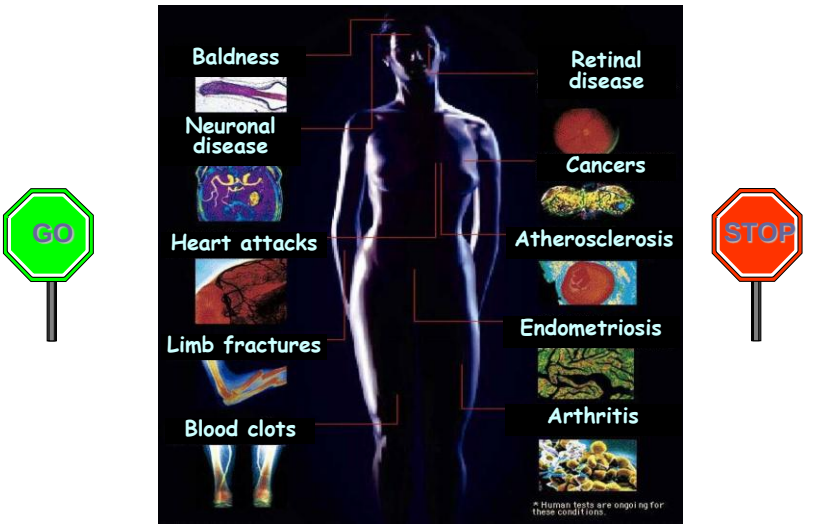






Bladder
Breast
Lung
Myeloma
Skin
Brain
Ovarian
Prostate
Lymphoma

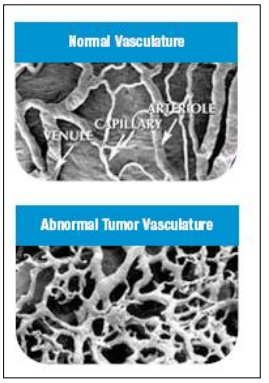
Angiogenesis and disease



Why is angiogenesis so important in cancer?

Growth

- Malignant tumour cells promote angiogenesis by sending signals, in the form of growth factors, to nearby blood vessels
- Because tumours produce large amounts of angiogenesis-activating factors, they overwhelm the natural inhibitors that keep blood vessel growth in check
- Sustained angiogenesis is the result

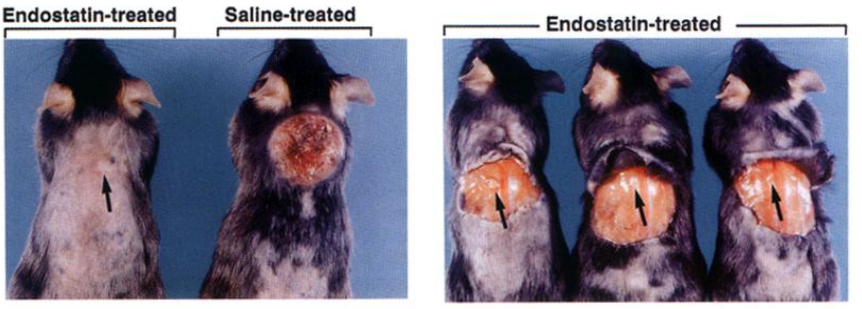


Why is angiogenesis so important in cancer?

Metastasis

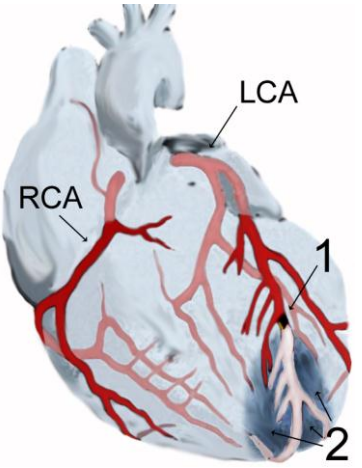
- Some of the most devastating and deadly effects of cancer arise from its ability to spread, or *metastasize*, from one location to many throughout the body
- In order to metastasize, tumour cells must be able to enter the circulation
- Angiogenesis not only feeds tumour growth but also connects tumours to the bloodstream, allowing tumour cells to travel to other parts of the body and invade tissues and organs

Treatment with angiogenesis inhibitor can suppress growth of tumors in mice



O'Reilly et al., Cell, 1997

Angiogenesis & ischaemic diseases



- Occlusion (1) of a branch of the left coronary artery (LCA)
- Myocardial infarction of the tip of the anterior wall of the heart (2)
- The resulting ischemia or oxygen shortage causes damage and potential death of heart tissue

Learning Objectives

1. Consider the basic principles underlying angiogenesis
2. Understand processes and key regulatory molecules involved in angiogenesis
3. Recognise the role played by angiogenesis in disease

- **ANY DISEASE INVOLVING CHANGES TO BLOOD SUPPLY AND/OR TISSUE MASS IS "ANGIOGENESIS-DEPENDENT"**

Learning Objectives

1. Consider the basic principles underlying angiogenesis
2. Understand processes and key regulatory molecules involved in angiogenesis
3. Recognise the role played by angiogenesis in disease
4. Examine the potential of angiogenesis as a therapeutic target, particularly in ischemic disease
    - Promoting angiogenesis

Promoting angiogenesis in ischemic disease

Angiogenesis & ischaemic diseases

- In ischaemic diseases, supply of blood is impaired due to narrowed or blocked arteries, that starve tissues of nutrients & oxygen
- Coronary atherosclerosis-induced myocardial infarction is one of the leading causes of mortality in developed countries
- Ischaemic disease also affects the lower extremities

Angiogenesis & ischaemic diseases

- Considerable advances in both surgical bypassing and percutaneous revascularisation techniques have been reached
- Many patients cannot benefit from these therapies because of the extension of arterial occlusion and/or microcirculation impairment
- There is a compelling need for alternative therapeutic strategies

Stimulation of angiogenesis in patients with MI

Randomised, single-blind, placebo-controlled pilot study of catheter-based myocardial gene transfer for therapeutic angiogenesis in patients with chronic myocardial ischemia

Circulation 2001 May 1;103(17):2138-43

Vale, Losordo, Milliken, McDonald, Gravelin, Curry, Esakof, Maysky, Symes & Isner

Stimulation of angiogenesis in patients with MI

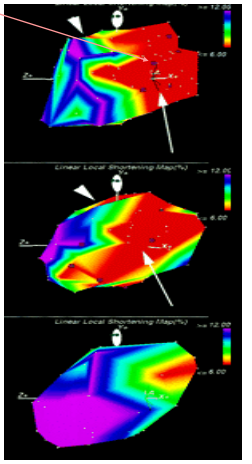
- Catheter advanced percutaneously to left ventricular myocardium of patients with chronic myocardial ischemia
- Patients randomised to receive VEGF plasmid or placebo
- Patients initially randomised to placebo eligible for VEGF if no clinical improvement after 90 days
- VEGF-transfected patients experienced reduced angina and nitroglycerin consumption for up to 360 days when compared with controls

Stimulation of angiogenesis in patients with MI

Catheter site

Electromechanical mapping of ischemia

Ischemia:



Pre-treatment

Post-treatment: placebo

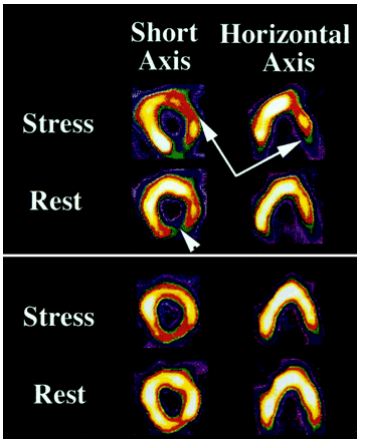
Post-treatment: VEGF

Stimulation of angiogenesis in patients with MI

Myocardial perfusion

(SPECT-sestamibi scans)

Perfusion:



Pre-treatment

Post-treatment: VEGF

Stimulation of angiogenesis in patients with MI

One-year follow-up of direct myocardial gene transfer of vascular endothelial growth factor-2 using naked plasmid deoxyribonucleic acid by way of thoracotomy in no-option patients

Am J Cardiol 2003 Aug 15;92(4):436-9

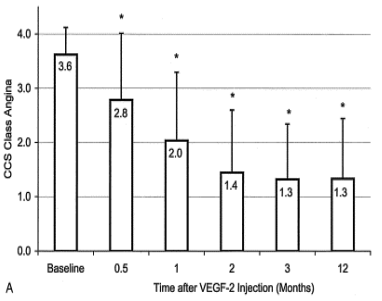
Fortuin, Vale, Losordo, Symes, DeLaria, Tyner, Schaer, March, Snell, Henry, Van Camp, Lopez, Richenbacher, Isner & Schatz

Stimulation of angiogenesis in patients with MI

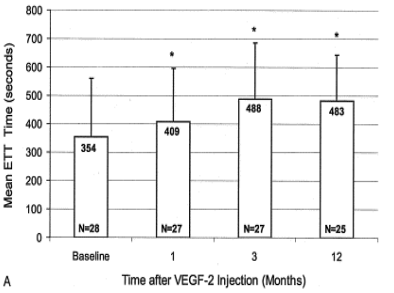
- Patients received naked plasmid DNA encoding VEGF-C
- Direct myocardial injection via thoracotomy
- Few major adverse events at 1 year
- Procedure associated with clinical improvement
- Reduced number of angina episodes and nitroglycerin consumption
- But no angiographic evidence of angiogenesis

Stimulation of angiogenesis in patients with MI

Angina



Treadmill time



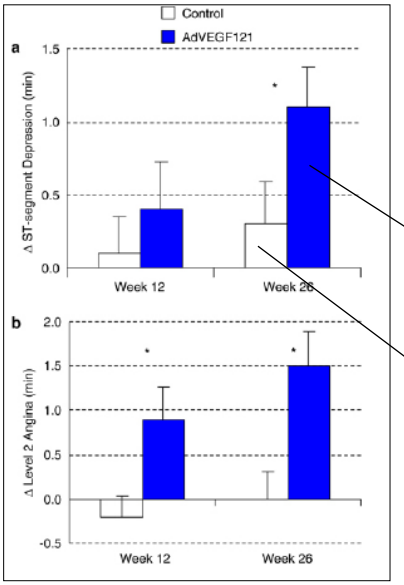
Stimulation of angiogenesis in patients with MI

Angiogenic gene therapy in patients with nonrevascularizable ischemic heart disease: a phase 2 randomized, controlled trial of AdVEGF121 (AdVEGF121) versus maximum medical treatment

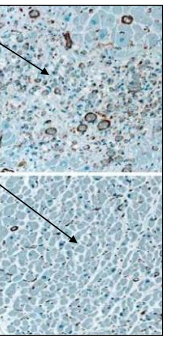
Gene Therapy (2006) 13, 1503–1511

Stewart, Hilton, Arnold, Gregoire, Rivard, Archer, Charbonneau, Cohen, Curtis, Buller, Mendelsohn, Dib, Page, Ducas, Plante, Sullivan, Macko, Rasmussen, Kessler and Rasmussen on behalf of the REVASC Investigators

Stimulation of angiogenesis in patients with MI



- Patients with severe angina due to coronary artery disease and no conventional options for revascularization
- Cardiac gene transfer by direct intramyocardial delivery of a replication-deficient adenovirus-containing VEGF121



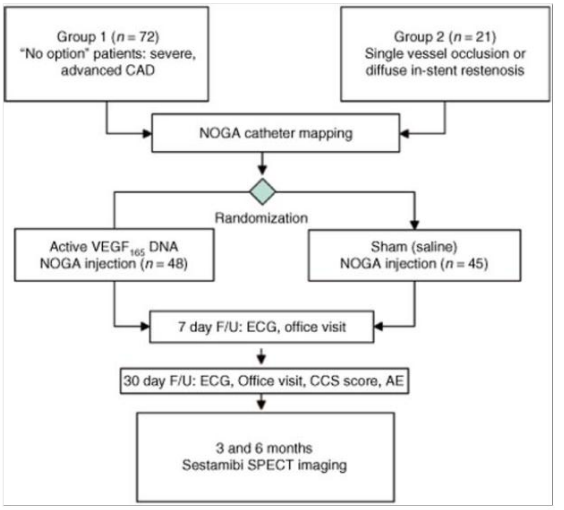
Stimulation of angiogenesis in patients with MI

VEGF gene therapy fails to improve perfusion of ischemic myocardium in patients with advanced coronary disease: results of the NORTHERN trial

Mol Ther. 2009 Jun;17(6):1109-15

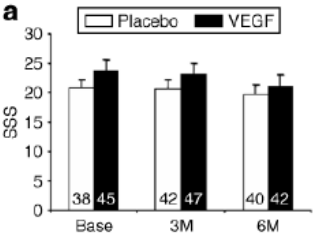
Stewart, Kutryk, Fitchett, Freeman, Camack, Su, Della Siega, Bilodeau, Burton, Proulx, Radhakrishnan; NORTHERN Trial Investigators

Stimulation of angiogenesis in patients with MI

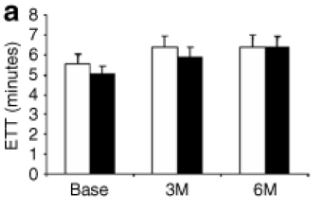


Stimulation of angiogenesis in patients with MI

SPECT sestamibi myocardial perfusion imaging: summed stress score (SSS)



Exercise treadmill time (ETT)



Angiogenesis & ischaemic diseases

Table 1 Clinical phase I/II randomised placebo-controlled therapeutic angiogenesis trials in coronary artery disease: protein therapy

Therapeutic protein	Trial type	n	Route of administration	Results/effects compared with placebo*
VEGF-A_{165}	Phase II	178	IC and IV infusion	Safe. No improvement in ETT, angina, or SPECT compared with controls. High-dose VEGF at 120 days resulted in significant improvement in angina class and non-significant trend in ETT and angina frequency
FGF-2	Phase I/II	24	Epicardial implantation of sustained release Heparin–alginate capsules	Significant improvement in angina frequency and reduction in ischemic area at 3 years
FGF-2	Phase II	337	IC infusion	Safe. No effect on ETT or SPECT; trend towards improvement in angina at 90 days, but not 180 days
GM-CSF	Phase I/II	21	IC injection followed by SC injection over 2 weeks	Significant improvements in collateral flow index and ECG signs of ischaemia

Zachary & Morgan (2011) Heart;97:181-189

Angiogenesis & ischaemic diseases

Table 2 Clinical phase II/III randomised controlled therapeutic angiogenesis trials in coronary and peripheral artery disease: plasmid DNA gene therapy

Trial	Disease	Therapeutic factor	Route of administration	Control treatment	n	Primary end point	Results*
EUROINJECT-ONE	CAD (CCS III–IV)	Naked $VEGF_{165}$ plasmid	Percutaneous intramyocardial injections	Control plasmid	74	Improved myocardial perfusion at 3 months	Negative
GENASIS	CAD (CCS III–IV)	Naked VEGF-2 (VEGF-C) plasmid	Percutaneous intramyocardial injections	Vehicle	295 (404 planned)	ETT at 3 months	Negative at interim analysis, stopped
NORTHERN	CAD (CCS III–IV)	Naked $VEGF_{165}$ plasmid	Percutaneous intramyocardial injections	Vehicle	120 (planned)	Change in myocardial perfusion in stress/rest at 12 weeks	Negative
VIF-CAD	CAD (CCS III–IV)	Naked bicistronic VEGF-A165/FGF-2 plasmid	Percutaneous intramyocardial injections	Control plasmid	?	SPECT at 4 months	Ongoing
DELTA-1	PAD (claudication)	Plasmid-expressing Del-1 formulated with poloxamer 188	Intramuscular injections	Vehicle	157	PWT at 3 months	Negative
Groningen	PAD (CLI)	Naked $VEGF_{165}$ Plasmid	Intramuscular injections	Saline	54	Decrease in amputation rate	Negative (secondary end points positive)
HGF-STAT	PAD (CLI)	Naked HGF plasmid	Intramuscular injections	Saline	104	Wound healing, amputatio rate, rest pain, ABI	Negative
TALISMAN 201	PAD (CLI)	Naked FGF-1 plasmid	Intramuscular injections	Vehicle	125	Ulcer healing at 6 months	Negative (secondary end point of reduced amputation positive)
TAMARIS	PAD (CLI)	Naked FGF-1 plasmid		Vehicle	490 (planned)	Amputation or death	Ongoing

Zachary & Morgan (2011) Heart;97:181-189

Table 3 Clinical phase II/III randomized controlled therapeutic angiogenesis trials in coronary and peripheral artery disease: adenoviral gene therapy

Trial	Disease	Therapeutic factor	Route of administration	Control treatment	n	Primary end point	Results*
KAT	CAD (CCS class II–III)	Ad$VEGF_{165}$ or plasmid/liposome $VEGF_{165}$	Intracoronary injection at the angioplasty	Ringer's lactate	103	Improved myocardial perfusion, 6 months	Positive (AdVEGF group only)
REVASC	CAD (CCS II–IV)	Ad$VEGF_{121}$	Intramyocardial injection via mini-thoracotomy	Best medical care (no placebo)	67	Time to 1 mm ST-segment depression on ETT, 26 weeks	Positive
NOVA	CAD (CCS II–IV)	Ad$VEGF_{121}$	Percutaneous intramyocardial injections	Vehicle	129 (planned)	ETT, 26 weeks	Stopped
AGENT-2	CAD (CCS II–IV)	AdFGF-4	Intracoronary injection	Vehicle	52	SPECT, 8 weeks	Positive
AGENT-3	CAD (CCS II–IV)	AdFGF-4	Intracoronary injection	Vehicle	416	ETT, 12 weeks	Negative (positive for >55 years with CCS III–IV)
AGENT-4	CAD (CCS II–IV)	AdFGF-4	Intracoronary injection	Vehicle	116	ETT, 12 weeks	Negative (significant beneficial effects on ETT, time to angina, and CCS class in women)
AWARE	CAD (CCS III–IV)	AdFGF-4	Intracoronary injection	Vehicle	300 (women)	ETT, 6 months	Ongoing
VEGF peripheral vascular disease trial	PAD (claudication)	Ad$VEGF_{165}$ or plasmid/liposome $VEGF_{165}$	Intra-arterial injection at the angioplasty	Ringer's lactate	54	Increased vascularity i angiography 3 months	Positive (Ad and plasmid groups)
RAVE trial	PAD (claudication)	Ad$VEGF_{121}$	Intramuscular injections	Vehicle (no virus)	105	PWT, 12 weeks	Negative
WALK	PAD (claudication)	AdHIF-1/VP16	Intramuscular injections	Vehicle	300	PWT, 6 months	Ongoing

Angiogenesis & ischaemic diseases

- >2500 patients treated
- No large clinical trial has shown substantial benefit i.e. early promise yet to be translated to clinic
- No indications of long-term effect of VEGF on cardiovascular disease i.e. VEGF does not promote or accelerate atherosclerosis and its clinical consequences (e.g. plaque rupture)
- No indications of any long-term effect on cancer

➢ **Lack of effects due to shortcomings in existing approaches - progress in translation to clinic will follow advances in delivery, expression and biological efficacy?**

Learning Objectives

1. Consider the basic principles underlying angiogenesis
2. Understand processes and key regulatory molecules involved in angiogenesis
3. Recognise the role played by angiogenesis in disease
4. Examine the potential of angiogenesis as a therapeutic target, particularly in ischemic disease

➢ **TO RESTORE VASCULAR SUPPLY AFTER INTERRUPTION**

Learning Objectives

1. Consider the basic principles underlying angiogenesis
2. Understand processes and key regulatory molecules involved in angiogenesis
3. Recognise the role played by angiogenesis in disease
4. Examine the potential of angiogenesis as a therapeutic target, particularly in ischemic disease
5. Review the use of angiogenesis inhibitors in disease, particularly solid cancers
    - **Inhibiting angiogenesis**

Angiogenesis as a therapeutic target in cancer

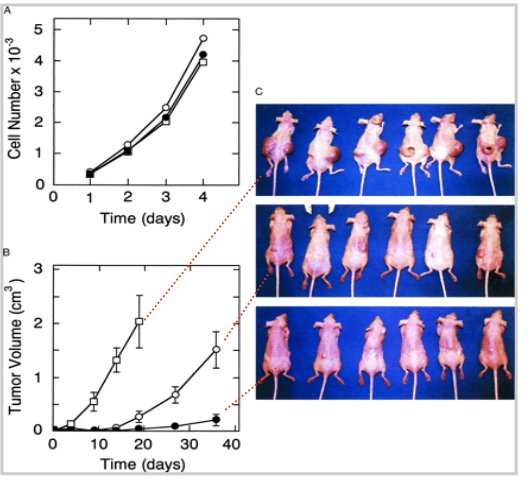
VEGF inhibition: soluble Flt-1

In vitro and *in vivo* growth of HT-1080 clones stably transfected with sFlt-1 cDNA (□<○<●)

Proc Natl Acad Sci U S A 1998 Jul 21;95(15):8795-800

Tumour cell growth

Tumour volume

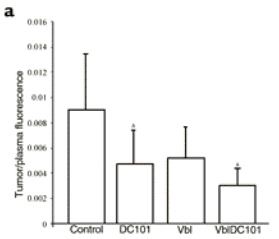


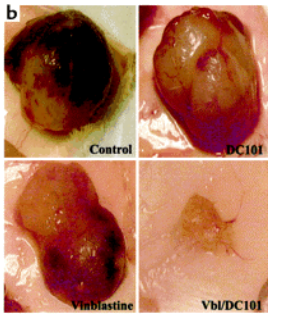
Tumours in situ

VEGF inhibition: anti-Flk-1

Established SK-N-AS neuroblastoma xenografts subjected to a 2-week course of treatment with anti-Flk-1 (DC101) antibody, low-dose vinblastine, or combination. Tumour perfusion measured as intravascular FITC-dextran fluorescence.

J Clin Invest 2000, 105(8):R15-24

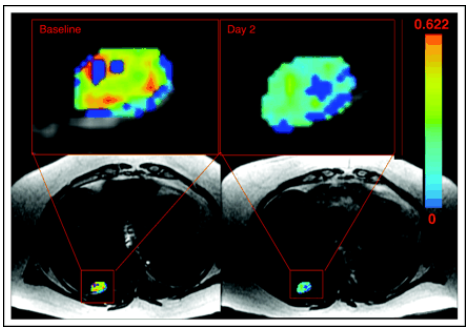




VEGF inhibition: Downstream signalling

- Small molecule inhibitors of downstream tyrosine kinase enzymes
- Broad specificities (VEGF-R, other growth factor receptors)

J Clin Oncol (2005) 23 5464-5473

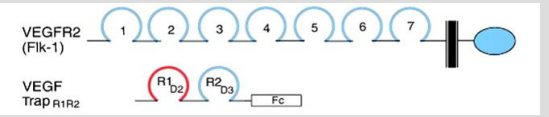


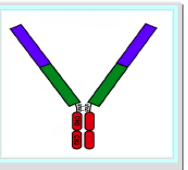
- AG-013736
- Oral TK inhibitor, phase I safety study
- Dynamic contrast-enhanced image
- Reduced perfusion in pulmonary metastasis in RCC patient

VEGF inhibition: VEGF Trap

VEGF Trap

Regeneron Pharmaceuticals



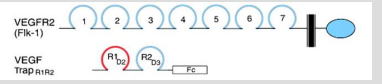


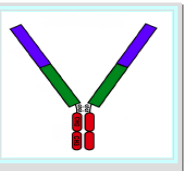
- Composed of two receptor components (VEGF-R1 domain 2 & -R2 D3) fused to Fc antibody portion
- Show increased affinity over that offered by single component reagents
- Latest generation use both components on a single chain, for greater potency & ease of production

VEGF inhibition: VEGF Trap

VEGF Trap

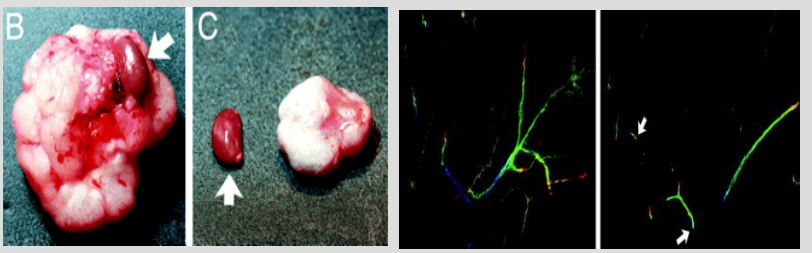
PNAS U S A 2003 Jun 24;100(13):7785-7790





- Established tumours and metastases
- VEGF-Trap abolished vasculature in established kidney xenografts

Control *VEGF-Trap* *Control* *VEGF-Trap*



Anti-VEGF antibody in cancer

THE FIRST ANTI-ANGIOGENIC
CLINICALLY PROVEN TO EXTEND SURVIVAL

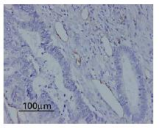


Fig. 2. Vasculature of colorectal carcinoma. Immunohistochemical staining of a colorectal carcinoma with the endothelial marker: anti-CD31.

Indication

Avastin, used in combination with intravenous 5-fluorouracil (FU)-based chemotherapy, is indicated for first-line treatment of patients with metastatic carcinoma of the colon or rectum.

AVASTIN™ (bevacizumab)

www.avastin.com

Genentech BIOONCOLOGY

Avastin, for first-line treatment of metastatic colorectal cancer with IV 5-FU-based regimens

Avastin Phase III study design

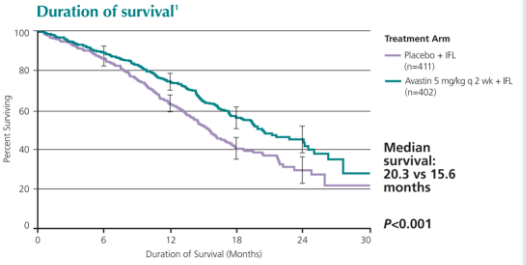
- **Randomized, double-blind, controlled study of 923 patients with previously untreated metastatic colorectal cancer**
- **Primary endpoint: overall survival**
- **Secondary efficacy endpoints: progression-free survival, overall response rate, and duration of response**

AVASTIN™ (bevacizumab)

Avastin, for first-line treatment of metastatic colorectal cancer with IV 5-FU-based regimens

Avastin significantly extended median survival

- **30% increase in median survival in combination with IFL vs IFL alone (N=813)**



Error bars represent 95% confidence intervals.

- **The survival benefit associated with Avastin was observed early in treatment and persisted throughout the course of the trial**

Avastin, for first-line treatment of metastatic colorectal cancer with IV 5-FU-based regimens

Survival benefit observed across all patient subgroups analyzed

Duration of survival by selected baseline patient parameters[2]

Baseline Characteristics	Total N	Duration of Survival Median (mo)
All subjects	813	15.6 / 20.3
Age (years)		
Adults <40	35	15.6 / 22.8
40-64	507	15.8 / 19.6
≥65	271	14.9 / 24.2
Sex		
Female	328	15.7 / 18.7
Male	485	15.4 / 21.2
Race		
White	645	15.3 / 19.6
Others	168	17.5 / n/a*

Placebo + IFL — Avastin 5 mg/kg q 2 wk + IFL

*For race, median survival has not been reached for subjects in the Avastin + IFL "others" subgroup.

AVASTIN™ (bevacizumab)

Avastin, for first-line treatment of metastatic colorectal cancer with IV 5-FU-based regimens

Survival benefit observed across all patient subgroups analyzed

Duration of survival by selected baseline disease parameters[2]

Baseline Characteristics	Total N	Duration of Survival Median (mo)	
No. of metastatic disease sites*			
1	306	17.9	20.5
>1	507	14.6	19.9
Duration of metastatic disease			
<12 months	760	15.7	19.9
≥12 months	53	14.7	24.5

Placebo + IFL — Avastin 5 mg/kg q 2 wk + IFL

*The organs with the most frequent occurrence of metastatic disease were liver (78%), lung (48%), and lymph nodes (25%).[2]

AVASTIN™ (bevacizumab)

Avastin, for first-line treatment of metastatic colorectal cancer with IV 5-FU-based regimens

Anti-VEGF antibody in cancer

Genentech
A Member of the Roche Group

- **Metastatic Colorectal Cancer**
  AVASTIN®, in combination with intravenous 5-fluorouracil-based chemotherapy, is indicated for first- or second-line treatment of patients with metastatic carcinoma of the colon or rectum
- **Non-Small Cell Lung Cancer**
  AVASTIN®, in combination with carboplatin and paclitaxel, is indicated for first line treatment of patients with unresectable, locally advanced, recurrent or metastatic non-squamous, non-small cell lung cancer
- **Metastatic Breast Cancer**
  AVASTIN®, in combination with paclitaxel, is indicated for the treatment of patients who have not received chemotherapy for metastatic HER2-negative breast cancer

Anti-VEGF antibody in cancer

Genentech
A Member of the Roche Group

- **Progressive Glioblastoma**
  Accelerated approval for patients with progressive glioblastoma following prior therapy
- **Metastatic Renal Cell Carcinoma**
  US Food and Drug Administration approved (August 2009) bevacizumab plus interferon alpha for with metastatic renal cell carcinoma, the most common form of kidney cancer, based on data from a Phase III study which showed that increased benefit for patients who received this combination compared to those on interferon alpha alone

Anti-VEGF antibody in cancer

- **Gastrointestinal (GI) perforation:**
  - Treatment with Avastin can result in the development of GI perforation. In clinical trials, these events occurred throughout the course of treatment and in some cases resulted in fatality
- **Wound healing complication:**
  - Treatment with Avastin can lead to slow or incomplete wound healing (for example, when a surgical incision has trouble healing or staying closed). In some cases, this event resulted in fatality. The appropriate waiting time between stopping treatment with Avastin and having surgery has not been determined
- **Hemorrhage:**
  - Some people receiving Avastin with chemotherapy for lung cancer experienced hemoptysis (severe bleeding problem at the site of the tumour). In some cases, this event resulted in fatality

Anti-VEGF antibody in cancer

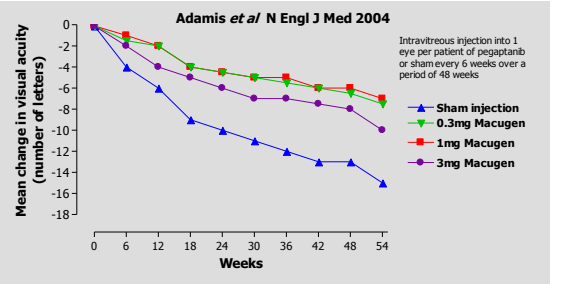
- **Additional serious side effects:**
  - Strokes or heart problems (blood clots), hypertensive crisis (severe hypertension), & congestive heart failure.
- **Most common adverse events:**
  - Weakness, pain, headache, hypertension, diarrhoea, nausea, vomiting, loss of appetite, mouth sores, constipation, upper respiratory infection, nosebleeds, skin irritation, and proteinuria
- **Metastatic breast cancer approval withdrawn 2010/2011:**
  - Ineffective - thought to slow cancer by 6 months, but more recent studies show Avastin only slowed disease by 2-3 months
  - Side-effects, including ulcers in stomach and intestines, as well as blood clots

Angiogenesis as a therapeutic target in neovascular eye disease

Angiogenesis inhibition in eye disease

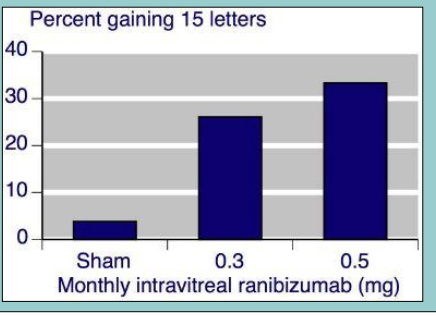
- Age-related macular degeneration (AMD) is the most common cause of vision loss among people over the age of 60
  - Impacts millions of older adults every year
  - Affects central vision and can sometimes make it difficult to read, drive or perform other activities requiring fine, detailed vision
  - Abnormal choroidal blood vessel growth in the choriocapillaris
  - Bleeding, leaking and scarring from vessels cause irreversible damage to photoreceptors and rapid vision loss if left untreated

Angiogenesis inhibition in eye disease



Angiogenesis inhibition in eye disease

- Avastin® is the parent molecule for smaller Lucentis
- Genetech split off Lucentis® fragment from Avastin molecule because Avastin® thought too big to penetrate the retina
- In June 2006, FDA approved LUCENTIS for treatment of neovascular wet AMD after a Priority Review (six-month)



LUCENTIS RANIBIZUMAB INJECTION

Angiogenesis inhibition in eye disease

- 95% treated with LUCENTIS (0.5 mg) maintained vision (loss of less than 15 letters in visual acuity)
- Up to 40% improved (gain of 15 letters or more in visual acuity) vision at 1 year
- Up to 40% achieved vision of 20/40 or better
- Higher incidence of strokes in 0.5mg group compared with 0.3mg group
- Eye- and non–eye-related blood clots (heart attacks, strokes, and death)
- Increased eye pressure within 1 hour of an injection.

LUCENTIS RANIBIZUMAB INJECTION

Angiogenesis inhibition in eye disease

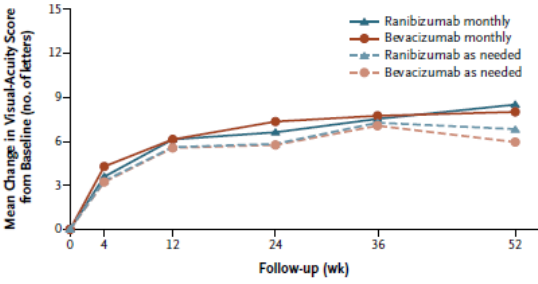
Ranibizumab & bevacizumab for neovascular age-related macular degeneration

N Engl J Med (2011) May 19;364(20):1897-908

Comparison Of Age-related Macular Degeneration Treatment Trial (CATT) Research Group

Angiogenesis inhibition in eye disease

A



Mean (±SE) Change in Visual-Acuity Score from Baseline (no. of letters)					
Ranibizumab monthly	+3.6±0.5	+6.1±0.7	+6.6±0.8	+7.5±0.9	+8.5±0.8
Bevacizumab monthly	+4.3±0.6	+6.1±0.7	+7.3±0.9	+7.7±1.0	+8.0±1.0
Ranibizumab as needed	+3.3±0.6	+5.6±0.7	+5.8±0.7	+7.2±0.7	+6.8±0.8
Bevacizumab as needed	+3.2±0.5	+5.6±0.7	+5.8±0.8	+7.1±0.9	+5.9±1.0

Angiogenesis inhibition in eye disease

KEY FINDINGS

- Based on 1208, primary outcome was mean change in visual acuity between baseline and 1 year: equivalent
- Bevacizumab monthly was equivalent to ranibizumab monthly, with 8.0 and 8.5 letters gained, respectively
- Rates of death, myocardial infarction and stroke were similar
- Proportion of patients with serious adverse events (primarily hospitalization) higher with bevacizumab than ranibizumab (24.1% vs 19%)
- A single dose of ranibizumab costs ($1950) 40 times as much as a single dose of bevacizumab ($50)

Angiogenesis inhibition in eye disease

REGENERON

Regeneron Announces Positive Primary Endpoint Results from a Phase 2 Study of VEGF Trap-Eye in Age-related Macular Degeneration

Data presented at Retina Society Conference in Boston

10/1/2007

Regeneron Announces Positive Primary Endpoint Results from a Phase 2 Study of VEGF Trap-Eye in Age-related Macular Degeneration

Data presented at Retina Society Conference in Boston

Tarrytown, NY (October 1, 2007) – Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) and development partner, Bayer HealthCare AG (NYSE:BAY) of Leverkusen, Germany, today announced positive results from the full analysis of the primary 12-week endpoint of a Phase 2 study evaluating the VEGF Trap-Eye in the neovascular form of age-related macular degeneration (wet AMD). The VEGF Trap-Eye met the primary study endpoint of a statistically significant reduction in retinal thickness, a measure of disease activity, after 12 weeks of treatment compared with baseline (all five dose groups combined, mean decrease of 119 microns, p<0.0001). The mean change from baseline in visual acuity, a key secondary endpoint of the study, also demonstrated statistically significant improvement (all groups combined, increase of 5.7 letters, p<0.0001). Preliminary analyses at 16 weeks showed that the VEGF Trap-Eye, dosed monthly, achieved a mean gain in visual acuity of 9.3 to 10 letters (for the 0.5 and 2 mg dose groups, respectively). In additional exploratory analyses, the VEGF Trap-Eye, dosed monthly, reduced the proportion of patients with vision of 20/200 or worse (a generally accepted definition for legal blindness) from 14.3 percent at baseline to 1.6 percent at week 16; the proportion of patients with vision of 20/40 or better (part of the legal minimum requirement for an unrestricted driver's license in the U.S.) was likewise increased from 19.0 percent at baseline to 49.2 percent at 16 weeks. These findings were presented at the Retina Society Conference in Boston, MA. The data reported at the meeting are available on the Regeneron website (www.regeneron.com on the Events Page, under the Investor Relations heading).

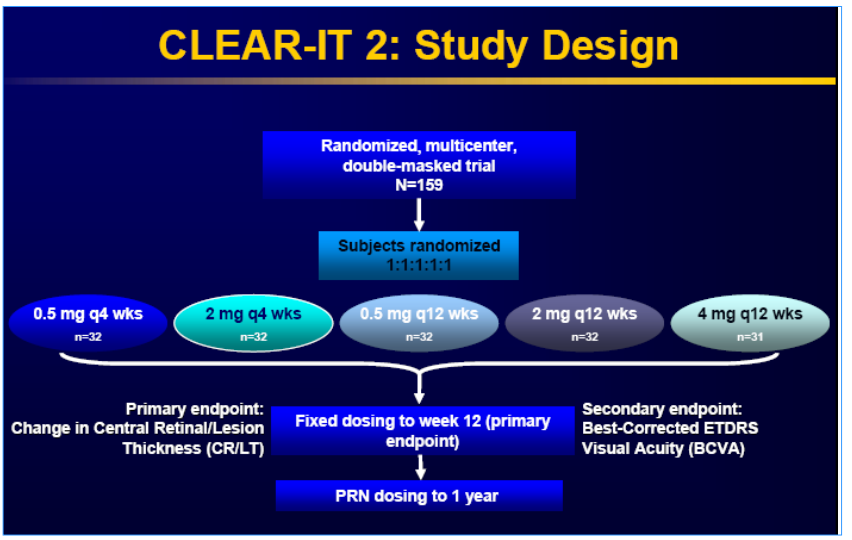
Angiogenesis inhibition in eye disease

The 1-year Results of CLEAR-IT 2, a Phase 2 Study of Vascular Endothelial Growth Factor Trap-Eye Dosed As-needed After 12-week Fixed Dosing

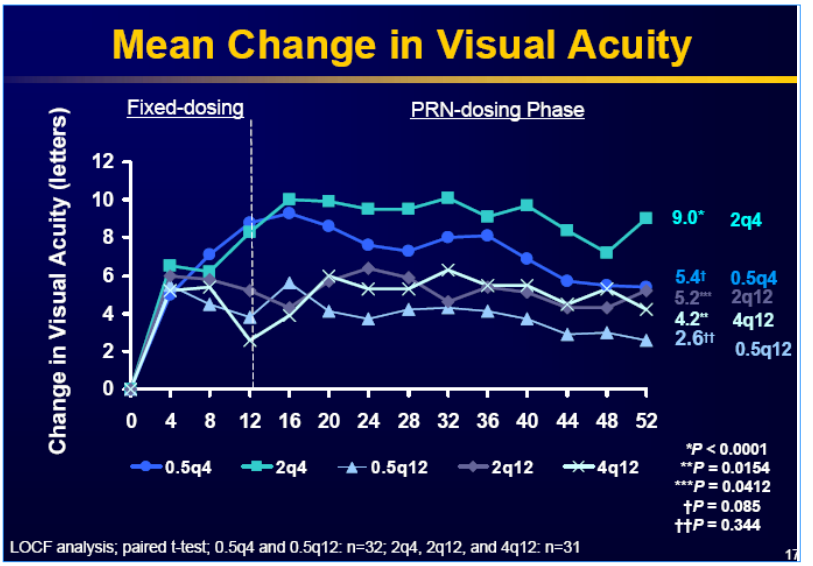
Ophthalmology (2011) Volume 118, Issue 6, Pages 1098-1106

CLEAR-IT 2 Investigators *et al*

Angiogenesis inhibition in eye disease



Angiogenesis inhibition in eye disease



Angiogenesis inhibition in eye disease

- August 2011 VEGF Trap-Eye not approved by the FDA for the treatment wet AMD; decision delayed until 18.11.2011
- Fewer Injections into the Eye: every 2 months instead of monthly (Lucentis)
- Side effects of VEGF Trap-Eye in clinical trials generally mild
- The most frequent side effects were associated with injection procedure, the underlying disease, or aging process: conjunctival hemorrhage, eye pain, retinal haemorrhage, and vitreous floaters

Learning Objectives

1. Consider the basic principles underlying angiogenesis
2. Understand processes and key regulatory molecules involved in angiogenesis
3. Recognise the role played by angiogenesis in disease
4. Examine the potential of angiogenesis as a therapeutic target, particularly in ischemic disease
5. Review the use of angiogenesis inhibitors in disease, particularly solid cancers

➢ **TO REMOVE VESSELS SUPPLYING DISEASE PROCESS**

Status of angiogenesis inhibitors

Bevacizumab (Avastin™)	*Genentech/Hoffmann-La Roche* Anti-VEGF monoclonal antibody	APPROVED: Colorectal, lung, renal, brain cancer USED: AMD
Ranibizumab (Lucentis™)	*Genentech/Hoffmann-La Roche* Anti-VEGF monoclonal antibody	APPROVED: AMD
Pegaptanib sodium (Macugen™)	*Eyetech Pharm/Pfizer* Anti-VEGF aptamer (synthetic nucleic acid ligand)	APPROVED: AMD
VEGF Trap/Eylea (Aflibercept™)	*Regeneron* VEGF-R1 D2 & -R2 D3 fused to Fc	TRIALS: Colorectal, lung, prostate cancer USED: AMD (approval awaited)
PTK787/Vatalanib	*Novartis* VEGF receptor tyrosine kinase inhibitor (R1, R2, R3)	TRIALS: Colorectal cancer
Pazopanib (Votrient™)	*GSK* VEGF receptor tyrosine kinase inhibitor (R1, R2, R3)	APPROVED: Renal cancer TRIALS: Lung, ovarian, bladder cancer
ZD6474/Vandetanib (Caprelsa™)	*AstraZeneca* VEGF receptor tyrosine kinase inhibitor	APPROVED: Thyroid cancer TRIALS: Lung cancer