Angiogenesis in diseases
Inhibitors of angiogenesis

lecture VI
23rd April 2012
Physiological angiogenesis in adults is restricted to placenta, uterus, hair growth, and wound healing.
New capillary formation in response to wounding

Angiogenesis in tissue during wound healing

control 100 μm 60 hours after wounding 100 μm
VEGF is strongly induced after injury
Keratinocytes and macrophages are the major producers
Impaired wound healing in diabetes

Brem & Tomic-Canic, JCI 2007
Inflammation is a component of the wound healing response

- Coagulation
- Inflammation
- Migration/Proliferation
  - Angiogenesis
  - Epithelialization
  - Contraction
  - Fibroplasia
- Remodeling
Tumors resemble wounded tissues that do not heal. Similarities between tumor stroma generation and wound healing.


Wound healing → tumor progression

The ability of the tumor to grow requires continuous remodeling within the tissue, which is very similar to the process of wound healing.
• In 1863, Rudolf Virchow proposed that inflammation is one of the predisposing factors of tumorigenesis.

• “Virchow’s hypothesis has almost been forgotten and ignored for more than a hundred years, but experienced a renaissance in the past 10 years” - Axel Schmidt, In Remembrance of Rudolf Virchow (1821-1902), 2006
Inflammation and Cancer

• Cancer escapes immunosurveillance

• Many studies have found that not only does the immune system fail to eliminate cancer, but that certain aspects of the immune system act to promote the formation and progression of tumors

• Cells known to be involved in inflammation have been linked to tumor promotion

• The role of innate immune cells (i.e. macrophages) in tumor promotion has received the most attention, but there is evidence that cells of the adaptive immune system (i.e. B cells) are also involved
Tumor growth is dependent on angiogenesis
Tumor growth is dependent on the blood vessels
Angiogenesis is necessary for tumor growth.
Angiogenesis facilitates tumor growth and metastasis

Tumor vasculature

Tumor cells
Tumor Angiogenesis: A Balancing Act

Angiogenesis is dependent on the balance between pro- and anti-angiogenic mediators.

**Inhibitors:**
- Thrombospondin-1
- The statins:
  - Angiostatin
  - Endostatin
  - Canstatin
  - Tumstatin

**Activators:**
- VEGFs
- FGFs
- PDGFB
- EGF
- LPA
Mechanisms of angiogenic switch
The Angiogenic Switch is necessary...

for Tumor Growth and Metastasis

Tumor is dormant

Angiogenic switch

Neovascularization:
- Allows rapid tumor growth by providing oxygen, nutrients, and waste removal
- Facilitates metastasis

Somatic mutation
Small avascular tumor
Tumor secretion of angiogenic factors stimulates angiogenesis
Rapid tumor growth and metastasis

The balance hypothesis for the angiogenic switch

Hanahan & Folkman, 1996
• Removes waste from growing tumor

• Provides tumor cells with oxygen and nutrients to grow

• Inhibition of tumor angiogenesis is promising as a cancer treatment
Tumor Angiogenesis

- Tumor Cells
- VEGF
- bFGF
- proteases
- PDGFR
- Extracellular Matrix
- Basement Membrane
- Endothelial Cells
- Supporting cells
- PDGF

Department of Medical Biotechnology
Faculty of Biochemistry, Biophysics and Biotechnology
Blood vessels in tumors are different than in healthy tissue.
Blood vessels in tumors are different than in healthy tissue.

normal tissue
tumor
Blood vessels in tumors are different than in healthy tissue.
Tumor vessels are leaky

no tumor  tumor
Tumors are hypoxic
Hypoxic area in tumors

(a) Blue color (Hoechst dye) – blood vessels in tumors
(b) Red color (nitroimidazol EF5) – hypoxic area in tumor
(c) Merged – hypoxic area close to the blood vessel

Giordano and Johnson 2001
Half-life of endogenous VEGF mRNA is about 65 min. Stability increases ~ 3 times in hypoxia.
Other diseases...

psoriasis
- hyperplastic and inflamed dermal blood vessels

- epidermal thickening (acanthosis) with aberrant keratinocyte proliferation

- inflammatory infiltrates
Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis

Yu-Ping Xia, Baosheng Li, Donna Hyllon, Michael Delmar, George D. Yancopoulos, and John S. Rudge

Blood 2003

Figure 2. Psoriasiform phenotype. Erythematous, scaly, and thickened skin lesions with associated edema develop in homozygote K14-VEGF transgenic mice older than 5 months.
Simultaneous deletion of JunB and c-Jun (DKO*) in the epidermis of adult mice leads to a psoriasis-like phenotype with hyper- and parakeratosis and increased subepidermal vascularization.
A psoriasis-like disease produced in Tie2 transgenic mice

Tie2 – receptor for angiopoietin 1

Voskas D et al., Am J Pathol. 2005
Repression of Tie2 transgene expression with doxycycline reverses the abnormal skin phenotype seen in transgenic mice.
<table>
<thead>
<tr>
<th>Organ</th>
<th>Diseases in mice or humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numerous organs</td>
<td>Cancer (activation of oncogenes; loss of tumor suppressors); infectious diseases (pathogens express angiogenic genes\textsuperscript{112}, induce angiogenic programs\textsuperscript{113} or transform ECs\textsuperscript{114}); autoimmune disorders (activation of mast cells and other leukocytes)</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>Vascular malformations (Tie-2 mutation\textsuperscript{68}); DiGeorge syndrome (low VEGF and neuropilin-1 expression\textsuperscript{33}); HHT (mutations of endoglin or ALK-1 (ref. 69)); cavernous hemangioma (loss of Cx37 and Cx40 (ref. 44)); atherosclerosis; transplant arteriopathy</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>Obesity (angiogenesis induced by fatty diet; weight loss by angiogenesis inhibitors\textsuperscript{115})</td>
</tr>
<tr>
<td>Skin</td>
<td>Psoriasis, warts, allergic dermatitis, scar keloids, pyogenic granulomas, blistering disease, Kaposi sarcoma in AIDS patients\textsuperscript{114}</td>
</tr>
<tr>
<td>Eye</td>
<td>Persistent hyperplastic vitreous syndrome (loss of Ang-2 (refs. 65,116) or VEGF164 (ref. 18)); diabetic retinopathy; retinopathy of prematurity; choroidal neovascularization (TIMP-3 mutation\textsuperscript{51})</td>
</tr>
<tr>
<td>Lung</td>
<td>Primary pulmonary hypertension (germline BMPR-2 mutation; somatic EC mutations\textsuperscript{73,75,76}); asthma; nasal polyps</td>
</tr>
<tr>
<td>Intestines</td>
<td>Inflammatory bowel and periodontal disease, ascites, peritoneal adhesions</td>
</tr>
<tr>
<td>Reproductive system</td>
<td>Endometriosis, uterine bleeding, ovarian cysts, ovarian hyperstimulation\textsuperscript{25}</td>
</tr>
<tr>
<td>Bone, joints</td>
<td>Arthritis, synovitis, osteomyelitis, osteophyte formation\textsuperscript{12}</td>
</tr>
<tr>
<td>Organ</td>
<td>Disease in mice or humans</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------------------------------------------</td>
</tr>
<tr>
<td>Nervous system</td>
<td>Alzheimer disease</td>
</tr>
<tr>
<td></td>
<td>Amyotrophic lateral sclerosis; diabetic neuropathy</td>
</tr>
<tr>
<td></td>
<td>Stroke</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
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<tr>
<td></td>
<td>Diabetes</td>
</tr>
<tr>
<td></td>
<td>Restenosis</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Gastric or oral ulcerations</td>
</tr>
</tbody>
</table>
Treatment of numerous diseases can be improved by pro-angiogenic therapy

Treatment of numerous diseases can be improved by anti-angiogenic therapy
A new form of cancer treatment using drugs called 'angiogenesis inhibitors' that specifically stop new blood vessel growth and starve a tumor by cutting off its blood supply.
The influence of angiogenesis inhibition on the tumor growth

- Tumor without treatment
- Tumor treated with antiangiogenic therapy

Tumor increases and metastases
Tumor decreases
VEGF inhibition dramatically affects vessel development

- Normal vasculature
- Before VEGF inhibition: Mature and stable
- After VEGF inhibition: Disorganised and hyperpermeable

VEGF is over-expressed in a variety of tumors

Stages of angiogenesis

- increase in vessel permeability and thrombin deposition
- loosening of pericyte contact
- proteinase release from endothelial cells
- digestion of basement membrane and extracellular matrix
- migration and proliferation of endothelial cells
- formation of vascular structures
- fusion of new vessels
- initiation of blood flow
  - inhibition of endothelial cell proliferation
  - inhibition of the migration of endothelial cells
- formation of basement membrane
Various strategies to inhibit VEGF signaling

Ferrara and Kerbel, Nature 2005
Different stages of angiogenesis inhibition

Inhibition of VEGF activity

Inhibition of matrix degradation and endothelial cells migration

Inhibition of late stages of angiogenesis

(angiostatin, endostatin)
What is the mechanisms of actions of anti-angiogenic drugs?
Inhibitors of angiogenesis

Synthetic angiogenesis inhibitors (orange keyline) and endogenous angiogenesis inhibitors that were identified in the Folkman laboratory are depicted above the timeline. Examples of additional endogenous angiogenesis inhibitors discovered in other laboratories are depicted below the timeline. The first drugs with anti-angiogenic activity were approved in 2003 (TABLE 2). DBP-MAF, vitamin-D-binding protein–macrophage-activating factor; EFC-XV, endostatin-like fragment from type XV collagen; PEDF, pigment epithelium-derived factor (also known as SERPINF1); PEX, haemopexin C domain autolytic fragment of matrix metalloproteinase 2; sFLT1, soluble fms-related tyrosine kinase 1; TIMPs, tissue inhibitors of matrix metalloproteinase.
Normalisation of blood vessels as the mechanisms of action of anti-angiogenic agents

normal tumor

Jain, Science 2004
Proteins or fragments of proteins that are formed in the body, which subsequently can inhibit the formation of blood vessels by disrupting the angiogenic process.

They are
- present in the circulation
- sequestered in the ECM surrounding cells
<table>
<thead>
<tr>
<th>List of Angiogenesis Inhibitors in the Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiostatin (plasminogen fragment)</td>
</tr>
<tr>
<td>Anti-angiogenic antithrombin III (aaATIII)</td>
</tr>
<tr>
<td>Canstatin</td>
</tr>
<tr>
<td>Cartilage-derived inhibitor (CDI)</td>
</tr>
<tr>
<td>CD59 complement fragment</td>
</tr>
<tr>
<td>Endostatin (collagen XVIII fragment)</td>
</tr>
<tr>
<td>Fibronectin fragment</td>
</tr>
<tr>
<td>Gro-beta</td>
</tr>
<tr>
<td>Heparinases</td>
</tr>
<tr>
<td>Heparin hexasaccharide fragment</td>
</tr>
<tr>
<td>Human chorionic gonadotropin (hCG)</td>
</tr>
<tr>
<td>Interferon alpha/beta/gamma</td>
</tr>
<tr>
<td>Interferon inducible protein (IP-10)</td>
</tr>
<tr>
<td>Interleukin-12 (IL-12)</td>
</tr>
</tbody>
</table>
Angiogenesis inhibitors

**Precursors**
- Fibronectin
- Prolactin
- Plasminogen
- PF-4
- Collagen XVIII
- Plasminogen
- MMP-2

**Antiangiogenic Fragments**
- Heparin-binding fragment
- 16-kD fragment
- Angiostatin (Krinlge 1-3, 4)
- A truncated PF-4
- Endostatin
- Kringle 5

**MMP-2**
- H$_2$N
- PEX
- COOH

**Calreticulin**
- H$_2$N
- Vasostatin
- COOH

**Plasminogen**
- H$_2$N
- Kringle 1-5
- aaA
- COOH

**Antithrombin**
- H$_2$N
- Restin
- COOH

**Collagen XV**
- H$_2$N
- Arresten (NC1 domain)
- COOH

**Collagen IVα1**
- H$_2$N
- Canstatin (NC1 domain)
- COOH

**Collagen IVα2**
- H$_2$N
- Tumstatin (NC1 domain)
- COOH

**Collagen IVα3**
- H$_2$N
Main groups of endogenous inhibitors

- Matrix-derived endogenous inhibitors (endostatin, thrombospondins)
- Fragments of blood coagulation fragments (angiostatin)
- Molecules of the immune system with anti-angiogenic activities (interferons, interleukins)
- Other inhibitors (tissue inhibitors of MMPs – TIMP)
Other angiogenesis inhibitors have been found in nature - in green tea, soy products, fungi, mushrooms, Chinese cabbage, tree bark, shark tissues, snake venom, red wine, and many other substances.

Still other angiogenesis inhibitors have been manufactured synthetically in the laboratory.

Some FDA-approved medicines have also been "re-discovered" to have anti-angiogenic properties.
Currently, a number of clinical trials in progress are combining anti-angiogenic therapy with cytotoxic chemotherapy or radiation, as a way to maximize the anti-tumor treatment in human cancer patients.
<table>
<thead>
<tr>
<th>Anti-Angiogenic Drugs in Clinical Trial for Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A6</td>
</tr>
<tr>
<td>Alpha5Beta1 Integrin Antibody</td>
</tr>
<tr>
<td>ABT-510</td>
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<tr>
<td>Actimid</td>
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<tr>
<td>Angiocol</td>
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<tr>
<td><strong>Angiostatin</strong></td>
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<tr>
<td>Angiozyme</td>
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<tr>
<td>Aplidine</td>
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<tr>
<td>Aptosyn</td>
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<tr>
<td>ATN-161</td>
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<tr>
<td><strong>Avastin (bevacizumab)</strong></td>
</tr>
<tr>
<td>AVE8062A</td>
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<tr>
<td>Benefin</td>
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<tr>
<td>BMS275291</td>
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<tr>
<td>Carboxymidotriazole</td>
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<td>CC4047</td>
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<tr>
<td>CC7085</td>
</tr>
<tr>
<td>CDC801</td>
</tr>
<tr>
<td>Celebrex (Celecoxib)</td>
</tr>
<tr>
<td>CEP-7055</td>
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<tr>
<td>CGP-41251/PKC412</td>
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<tr>
<td>Cilengitide</td>
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<tr>
<td>Combretastatin A4P</td>
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<tr>
<td>CP-547, 632</td>
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<td>CP-564, 959</td>
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<tr>
<td>Dextrazoxane</td>
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<tr>
<td>Didemnin B</td>
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<tr>
<td>DMXAA</td>
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<tr>
<td>EMD 121974</td>
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<tr>
<td><strong>Endostatin</strong></td>
</tr>
<tr>
<td>Flavopiridol</td>
</tr>
<tr>
<td>GBC-100</td>
</tr>
<tr>
<td>Genistein Concentrated Polysaccharide</td>
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<tr>
<td>Green Tea Extract</td>
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<tr>
<td>Interleukin-12</td>
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<tr>
<td>INGN 201</td>
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<tr>
<td>Interferon alfa</td>
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<tr>
<td>Iressa</td>
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<tr>
<td>LY317615</td>
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<tr>
<td>Mab huJ591-DOTA-90 Yttrium (90Y)</td>
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<tr>
<td>Medi-522</td>
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<tr>
<td>Metaret (suramin)</td>
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<tr>
<td>Metastat (Col-3)</td>
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<tr>
<td>Neovastat</td>
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<tr>
<td>NM-3</td>
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<tr>
<td>NPe6</td>
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<tr>
<td>Octreotide</td>
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<tr>
<td>Oltipraz</td>
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<tr>
<td>Paclitaxel</td>
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<tr>
<td>Panzem (2ME2)</td>
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<tr>
<td>Penicillamine</td>
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<tr>
<td>PI-88</td>
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<tr>
<td>PSK</td>
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<tr>
<td>PTK787/ZK222584</td>
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<tr>
<td>Revimid</td>
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<tr>
<td>Ro317453</td>
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<tr>
<td>Squalamine</td>
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<td>SU11248</td>
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<tr>
<td>SU6668</td>
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<tr>
<td>Temptostatin</td>
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<tr>
<td>Tetrathiomol</td>
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<tr>
<td>Thalidomide</td>
</tr>
<tr>
<td>UCN-01</td>
</tr>
<tr>
<td><strong>VEGF Trap</strong></td>
</tr>
<tr>
<td>ZD6126</td>
</tr>
<tr>
<td>ZD647</td>
</tr>
</tbody>
</table>
Specific Angiogenic Inhibitors

- Angiostatin
- Endostatin
- Avastin (Bevacizumab)
- Pegaptanib
Angiostatin

• Angiostatin is a polypeptide of approximately 200 amino acids

• It is produced by the cleavage of plasminogen, a plasma protein that is important for dissolving blood clots

• Angiostatin binds to subunits of ATP synthase exposed at the surface of the cell embedded in the plasma membrane

• It exist as multiple isoforms

• Considerable uncertainty on its mechanism of action, but it seems to involve the inhibition of endothelial cell migration, proliferation and induction of apoptosis
Angiostatin

- Can be cleaved from plasminogen by different metalloproteinases (MMPs), elastase, prostatespecific antigen (PSA), 13 KD serine protease, or 24KD endopeptidase.
Endostatin

- It was first discovered in 1995 in Dr. Folkman’s lab
- Phase I clinical studies began in 1999
- A naturally-occurring 20-kDa C-terminal fragment derived from type XVIII collagen.
- Interfere with the pro-angiogenic action of growth factors such as basic fibroblast growth factor (bFGF/FGF-2) and vascular endothelial growth factor (VEGF)

![Collagen XVIII and Endostatin structure](image)
Endostatin: An Endogenous Inhibitor of Angiogenesis and Tumor Growth

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Boston, Massachusetts 02115
‡Harvard Microchemistry Facility
16 Divinity Avenue
Harvard University
Cambridge, Massachusetts 02138
§Osteoarthritis Sciences, Inc.
Cambridge, Massachusetts 02139
Endostatin treated Lewis Lung Carcinoma

[Bar charts showing proliferation, apoptosis, and angiogenesis comparisons between saline-treated and endostatin-treated groups.]
Inhibition of tumor growth by endostatin
Dose-dependent tumor inhibition

The graph illustrates the relationship between treatment day and tumor volume for saline-treated and endostatin-treated groups. The x-axis represents treatment day, and the y-axis represents tumor volume (mm³). The graph shows a clear dose-dependent inhibition of tumor growth with increasing endostatin treatment concentrations.
Bevacizumab – Avastin

How Avastin Starves a Tumor

Tumors need blood, and they have a devious way to get it:

>>> They secrete a protein called VEGF that docks with receptors in nearby blood vessels, stimulating the growth of new blood vessels.

>>> Genentech foils this plot with Avastin, a drug that binds with VEGF and prevents that protein from attaching to receptors. New blood vessels don’t form, and the tumor starves.
Avastin

- Avastin is a humanized monoclonal antibody (MAb) that targets vascular endothelial growth factor (VEGF).
- Causes regression of tumor vasculature.
- Reduces intra-tumor pressure, thereby improving the delivery of cytotoxic agents to the tumor.
- Also inhibits new tumor blood vessel formation, restricting tumor growth.
- The first anti-angiogenic agent with demonstrated anticancer benefit in phase III trials.
Angiogenesis inhibitors in clinical trials
- Recombinant humanised monoclonal anti-VEGF antibody 93% human, 7% murine
- Recognises all major isoforms of human VEGF
- RhuMAb VEGF binding is restricted to human
- Bevacizumab binds VEGF, preventing interaction with its receptors and activation of downstream signalling pathways
- This ultimately leads to vascular regression, leaving the tumor dormant
Inhibition of experimental human tumor growth by anti-VEGF antibody (precursor of Avastin)

**Fig. 35.3.** Neutralizing antibody to human VEGF inhibits growth of experimental hepatic metastases in the athymic mouse. One day after splenic-portal tumor cell inoculation (2 million HM7 cells), twice-weekly antibody injections were begun, and animals were killed after 4 weeks. Livers of representative animals are shown.
Combined with IFL in previously untreated mCRC
5mg/kg every 2 weeks
(n=813)
Hurwitz H, et al. 2004

Combined with either FOLFOX4 or XELOX in previously untreated mCRC (n=1,920)

**AVANT:** combined with FOLFOX4 or XELOX in stage II/III colon cancer
5mg/kg every 2 weeks or 7.5mg/kg every 3 weeks
(n=3,450)

**AVAIL:** combined with CG in previously untreated stage IIIb, IV or recurrent NSCLC 7.5 or 15mg/kg every 3 weeks (n=830)

**AVOREN:** combined with IFN-α2a in metastatic RCC
10mg/kg every 2 weeks
(n=638)

Combined with Xeloda® in previously treated MBC
15mg/kg every 3 weeks
(n=462)
Miller KD, et al. 2005

Combined with Tarceva™ + gemcitabine in previously untreated PC
5mg/kg every 2 weeks
(n=600)

Combined with gemcitabine in unresectable PC
10mg/kg every 2 weeks
Kindler HL, et al. 2004

Monotherapy in previously treated MBC
3, 10 or 20mg/kg every 2 weeks
(n=75)
Cobleigh MA, et al. 2003

Monotherapy in previously treated RCC
3 or 10mg/kg every 2 weeks
(n=116)
Yang JC, et al. 2003

Combined with CP in previously untreated NSCLC
(n=99)
Johnson DH, et al. 2004

Combined with 5-FU/LV in previously untreated mCRC
5 or 10mg/kg every 2 weeks
(n=209)
Kabbinavar F, et al. 2005

Combined with 5-FU/LV in previously untreated mCRC
5mg/kg every 2 weeks
(n=104)
Kabbinavar F, et al. 2003

Combined with either FOLFOX4 or XELOX in previously treated MBC
15mg/kg every 3 weeks
(n=46)
Cobleigh MA, et al. 2005

Combined with gemcitabine in unresectable PC
10mg/kg every 2 weeks
Kindler HL, et al. 2004

**Phase I**

Dose-escalation trial in solid malignancies. Safety and pharmacokinetics
(n=25)
Gordon MS, et al. 2001

Combined with chemotherapy in metastatic cancers
3mg/kg every week
(n=12)
Margolin K, et al. 2001

5-FU = 5-fluorouracil; LV = leucovorin; mCRC = metastatic colorectal cancer; FOLFOX = 5-FU/LV + oxaliplatin; XELOX = Xeloda + oxaliplatin; IFN = interferon; CP = carboplatin/paclitaxel; CG = cisplatin/gemcitabine; RCC = renal cell cancer; MBC = metastatic breast cancer; IFL = irinotecan/5-fluorouracil/leucovorin; NSCLC = non-small cell lung cancer; PC = pancreatic cancer

**Phase II**

Combined with 5-FU/LV in previously untreated mCRC
5 or 10mg/kg every 2 weeks
(n=104)
Kabbinavar F, et al. 2003

Combined with 5-FU/LV in previously untreated mCRC
5mg/kg every 2 weeks
(n=209)
Kabbinavar F, et al. 2005

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5mg/kg every 2 weeks
(n=600)

**Phase III**

**AVOREN:** combined with IFN-α2a in metastatic RCC
10mg/kg every 2 weeks
(n=638)

Combined with CP in previously untreated NSCLC
(n=99)
Johnson DH, et al. 2004

Combined with gemcitabine in unresectable PC
10mg/kg every 2 weeks
Kindler HL, et al. 2004

Combined with Xeloda® in previously treated MBC
15mg/kg every 3 weeks
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**AVOREN:** combined with IFN-α2a in metastatic RCC
10mg/kg every 2 weeks
(n=638)
Avastin: promising phase II data in renal cancer

Two-sided unadjusted p values:
- High dose versus low-dose: p=0.0821
- Low dose versus placebo: p= 0.0115

Global phase III study starting in H2 2003
Bevacizumab-related events in CRC trials to date: overview

- hypertension (most common event)
- proteinuria
- arterial thrombosis
- bleeding

### Phase II trial of Avastin in metastatic CRC (AVF0780g): adverse events

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>5-FU/LV (n=35)</th>
<th>5mg/kg (n=35)</th>
<th>10mg/kg (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>1 (3)</td>
<td>4 (11)</td>
<td>9 (28)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>4 (11)</td>
<td>8 (23)</td>
<td>9 (28)</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>4 (11)</td>
<td>16 (46)</td>
<td>17 (53)</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>3 (9)</td>
<td>9 (26)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>GI bleeding</td>
<td>0</td>
<td>2 (6)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>29 (83)</td>
<td>32 (91)</td>
<td>24 (75)</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>1 (3)</td>
<td>4 (11)</td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

All events
Not adjusted for treatment duration

A Randomized Trial of Bevacizumab, an Anti–Vascular Endothelial Growth Factor Antibody, for Metastatic Renal Cancer

James C. Yang, M.D., Leah Haworth, B.S.N., Richard M. Sherry, M.D., Patrick Hwu, M.D., Douglas J. Schwartzentruber, M.D., Suzanne L. Topalian, M.D., Seth M. Steinberg, Ph.D., Helen X. Chen, M.D., and Steven A. Rosenberg, M.D., Ph.D.
Increase in survival of patients with renal cell cancer treated with Avastin

Fever, hypertension, proteinuria – adverse effects
The FDA approved Avastin in February 2004 for use in combination with intravenous **5-Fluorouracil** (5-FU)-based chemotherapy as a treatment for patients with first-line metastatic cancer of the **colon or rectum**. In June 2006, the FDA approved Avastin for use in combination with intravenous 5-FU-based chemotherapy for patients with second-line metastatic cancer of the **colon or rectum**. In October 2006, the FDA approved Avastin in combination with **carboplatin and paclitaxel** for the first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic non-squamous, **non-small cell lung cancer**.
# Table 1 | Angiogenesis-dependent diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic retinopathy</td>
<td>Loss of vision</td>
</tr>
<tr>
<td>Rheumatoid arthritis²</td>
<td>Pain and immobility from destroyed cartilage</td>
</tr>
<tr>
<td>Atherosclerotic plaques³</td>
<td>Chest pain, dyspnoea</td>
</tr>
<tr>
<td>Endometriosis⁴⁵</td>
<td>Abdominal pain from intraperitoneal bleeding</td>
</tr>
<tr>
<td>Crohn’s disease⁶</td>
<td>Intestinal bleeding</td>
</tr>
<tr>
<td>Psoriasis⁷</td>
<td>Persistent severe itching</td>
</tr>
<tr>
<td>Uterine fibroids</td>
<td>Vaginal bleeding, abdominal pain</td>
</tr>
<tr>
<td>Benign prostatic hypertrophy</td>
<td>Urinary retention</td>
</tr>
<tr>
<td>Cancer</td>
<td>Bleeding, thrombosis, anaemia, abdominal ascites, bone pain, seizures from cerebral oedema around a tumour and others</td>
</tr>
</tbody>
</table>
Diabetic retinopathy

healthy

diabetic retinopathy
What is AMD?

• Age-related macular degeneration (AMD) is defined as the loss of macular function from the degenerative changes of aging.

• The macula is the most important part of the retina responsible for sharp, central vision.

• AMD is divided specifically into two distinct types: the less severe or “dry” form, and the more severe and debilitating “wet” form.

• The root causes of AMD are still unknown.
Age-related macular degeneration (AMD)
Age-related macular degeneration (AMD)

Normal Macula

Dry AMD: Drusen formation under the Macula

Wet AMD: Macula with abnormal blood vessels
• **Lucentis™ (ranibizumab)** — The FDA approved Lucentis in June 2006 for the treatment of wet AMD.
  - Lucentis (ranibizumab) is a humanized anti-VEGF antibody fragment that inhibits VEGF activity by competitively binding with VEGF.

  - A two-year study showed that **95 percent** of people with wet AMD who received monthly injections of Lucentis experienced no significant loss in visual acuity. Genentech also reported moderate visual *improvement* in 24.8 percent of participants treated with a 0.3 mg dose of Lucentis and 33.8 percent of participants treated with a 0.5 mg dose.
Pegaptanib – Macugen

Aptamer which binds VEGF-A165

VEGF binds to its receptors on the cell surface and stimulates angiogenesis.

Macugen binds to VEGF. This prevents VEGF from binding to the receptors on the cell surface. As a result, angiogenesis is not stimulated.
Pegaptanib sodium


The molecular formula for pegaptanib sodium is

\[ C_{294}H_{342}F_{13}N_{107}Na_{28}O_{188}P_{28} \text{[C}_2\text{H}_4\text{O}]_n \]

(where n is approximately 900)
and the molecular weight is approximately 50 kilodaltons.
Macugen was demonstrated to be effective in prevention of vision loss in two large clinical trials in patients with AMD.
Macugen – anti-angiogenic drug for treatment of AMD

Inhibition of VEGF binding to its receptors
Macugen effects in AMD

Early and Sustained Treatment Benefit

1 Year Data 0.3 mg

Mean vision change (letters)

0 6 12 18 24 30 36 42 48 54

Weeks

-16 -14 -12 -10 -8 -6 -4 -2 0

Macugen
Usual Care

50% Benefit
*P<0.01

DEPARTMENT OF MEDICAL BIOTECHNOLOGY
Faculty of Biochemistry, Biophysics and Biotechnology
Macugen effects in AMD

Macugen Treatment Effect Continues for 2 Years

- 2 Years Treatment (N=133)
- Usual Care (N=107)

Mean change in vision (letters)

Week

Year One

Year Two

P<0.01

45% Benefit

-9.4 letters

-17.0 letters
# Tyrosine kinase inhibitors

## Table 2 - Anti-VEGF therapeutic agents currently in development for breast cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism(s) of action</th>
<th>Molecular target(s)</th>
<th>Stage of clinical development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>Anti-VEGF antibody</td>
<td>VEGF ligand</td>
<td>Approved</td>
</tr>
<tr>
<td>Sorafenib (BAY 43-9006)</td>
<td>Tyrosine kinase inhibitor</td>
<td>Raf-1, VEGF receptors-2 and -3, PDGFR-β, Flt-3, c-Kit</td>
<td>Phase II</td>
</tr>
<tr>
<td>Sunitinib (SU11248)</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1, -2 and -3, Flt-3, PDGFR-α, PDGFR-β, c-Kit</td>
<td>Phase II/III</td>
</tr>
<tr>
<td>Vatalanib (PTK/ZK)</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1, -2 and -3, PDGFR-β, c-Kit, c-Fms</td>
<td>Phase II</td>
</tr>
<tr>
<td>Vandetanib (ZD6474)</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-2 and -3, EGFR</td>
<td>Phase II</td>
</tr>
<tr>
<td>Motesanib (AMG-706)</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1, -2 and -3, PDGFR, c-Kit</td>
<td>Phase II</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1 and -2, PDGFR-β, and c-Kit</td>
<td>Phase II</td>
</tr>
<tr>
<td>Axitinib</td>
<td>Tyrosine kinase inhibitor</td>
<td>VEGF receptors-1, -2 and -3, PDGFR, c-kit</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

VEGF, vascular endothelial growth factor; PDGFR, platelet-derived growth factor receptor.

Marty et al. 2008
Angiogenesis - Basement Membrane Breakdown

Angiogenic Stimulus (VEGF)

Smooth Muscle Cells

Basement Membrane

Endothelium

Matrix metalloproteinases
MMPs are pro-angiogenic

• Degradation of basement membrane and ECM to allow for cell detachment and migration
• Cleavage of VE-cadherin cell-cell adhesion
• Release of active VEGF from ECM stores
• Cleavage of basement membrane to release bFGF and to release and activate TGFβ
MMP-Inhibiting Drugs

- **Marimastat** (left)
  - Binds to zinc ion
  - Very limited success due to toxicity factors and need for cytotoxic combination

- **Batimastat** (right)
  - 1,4 bidentate hydroxamic acid ligand that binds very tightly to the zinc ion in the catalytic (active) site
Future Directions-VEGF-Trap

- Composite decoy receptor based on VEGFR-1 and VEGFR-2 fused to a human Fc segment of IgG1 that binds VEGF

- Decreases free VEGF to bind to receptors and prevent vessel growth

- FDA approved for macular degeneration
VEGF-trap

Twice Weekly Dosing with VEGF-Trap Effectively Inhibits Subcutaneous Growth of a Variety of Tumor Types

Halosh et al. 2002
Take-home messages

• Physiological angiogenesis in adults is restricted, however disturbances of this process is a hallmark of many diseases

• Tumors resemble wounded tissues that do not heal – remodelling, inflammation, angiogenesis...

• One of the strategy to treat tumor/AMD... is the anti-angiogenic therapy

• Angiogenesis inhibitors specifically halt new blood vessel growth and starve a tumor by cutting off its blood supply

• Angiogenesis inhibitors prevent the VEGF from binding with the receptors on the surface of the endothelial cells

• Many anti-angiogenic factors are in the Phase II/III of clinical trails