Biologics for Nephrology

New Treatments

And

New Challenges

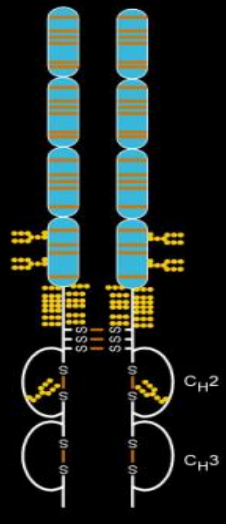
Outline of talk

- What are biologics
- mAB
- VEGF
- Anti-VEGF
- Renal side effect of anti-VEGF

Biologics

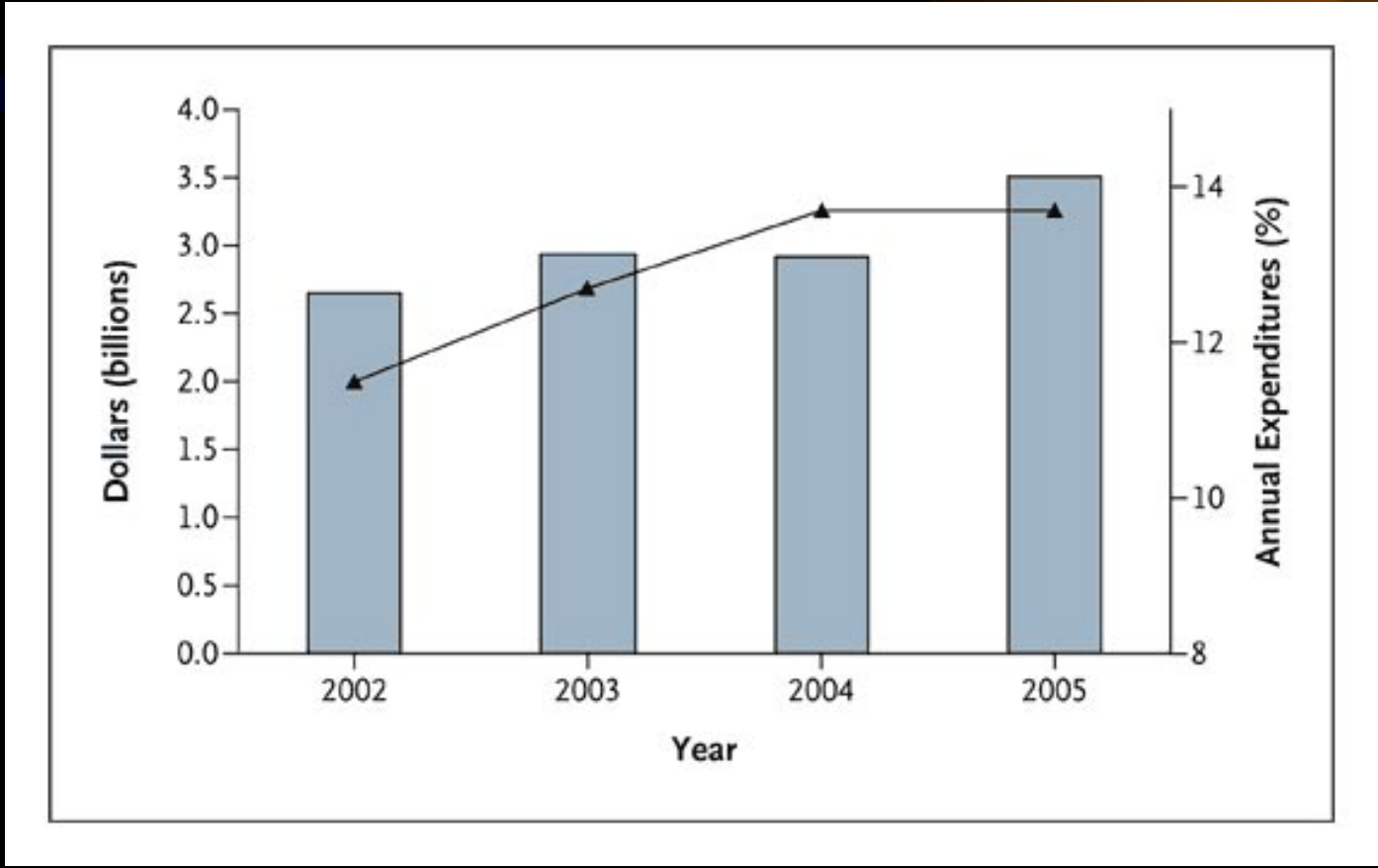
- Substances that are (nearly) identical to the body's own key signalling proteins
- Monoclonal antibodies
- Receptor constructs (fusion proteins)

  Ig-Rec



Enbrel

Total Spending by Nonfederal Hospitals and Percent Annual Expenditures for the Four Most Popular Classes of Protein Therapeutic Products, 2002-2005



Dudzinski D and Kesselheim A. N Engl J Med 2008;358:843-849

The NEW ENGLAND JOURNAL of MEDICINE

Emerging Themes

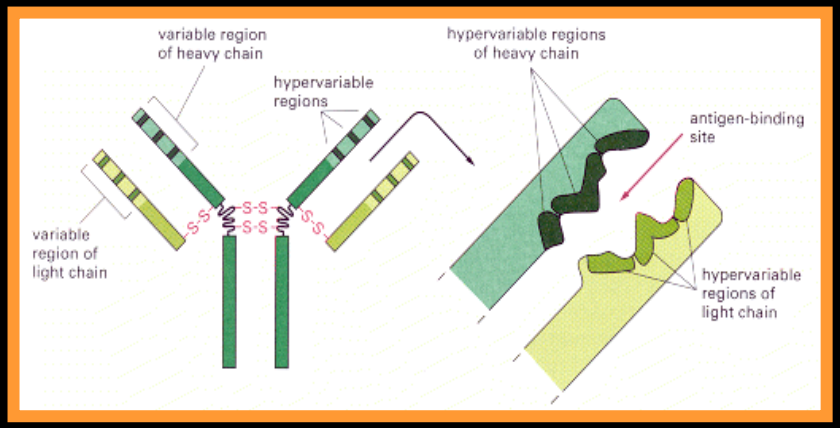
- Antibodies are naturally occurring
- Discovery of their innate properties hinted at great therapeutic potential
  - High-specificity in binding
  - Already present in the body
  - Can activate and couple components of the immune system
- Modification to structure and refinement in production methods have made antibodies a viable modern drug

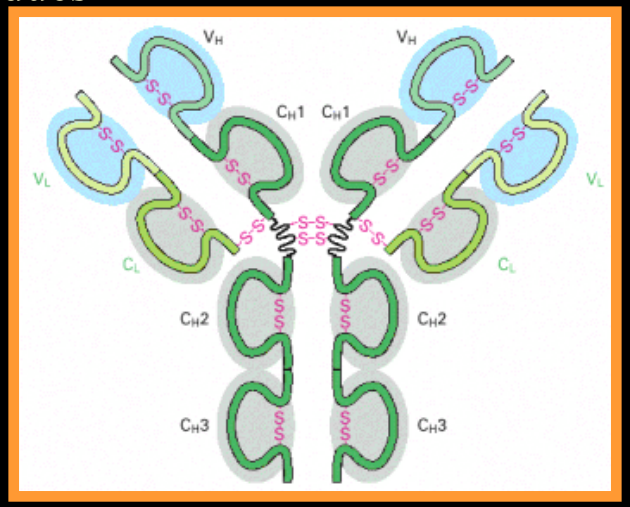
Important Terms

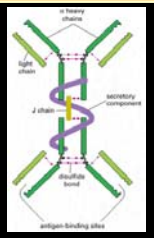
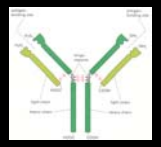
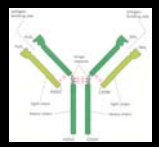
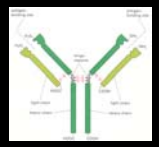
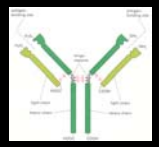
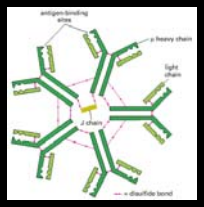
- Antibody – immunoglobulin secreted by B cells
- Antigen (*anti*body *gen*erator) – any substance capable of eliciting an adaptive immune response
- Monoclonal antibodies (mAbs) – antibodies secreted from a single B cell, have identical paratopes
- Epitope – region of the antigen recognized by an antibody
- Paratope – region of the antibody that binds the epitope

The Structure of an Antibody

- 2 identical light chains (~220 amino acids long)
  - Variable domain: V_L
  - Constant domain: C_L
- 2 identical heavy chains (~440 amino acids long)
  - Variable domain: V_H
  - 3 Constant domains: C_H1, C_H2, C_H3
- Covalent, disulfide bonds between cysteine residues
- Flexible "hinge region"

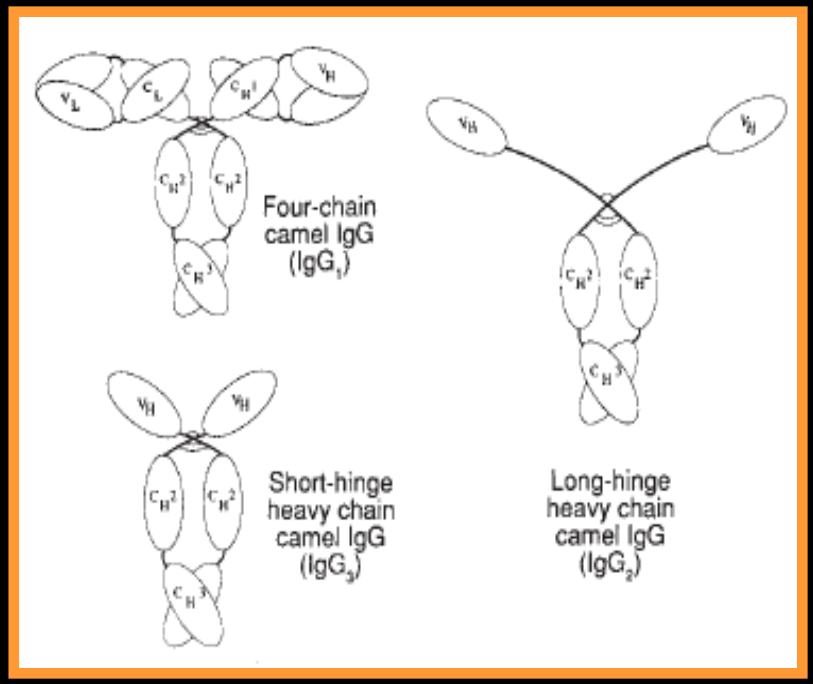




Immunoglobulin (Ig) Class	Heavy Chain	Diagram	Distribution	Biological Activity
IgA	α	 or 	External Secretions	
IgD	δ		B Cell surface receptor	
IgE	ε		Cells that secrete histamines	
IgG	γ		Main antibody in serum Most Stable	Promotes antibody-dependent cellular cytotoxicity (ADCC) Compliment fixation
IgM	μ		First antibody secreted in development	Compliment fixation

Nanobodies

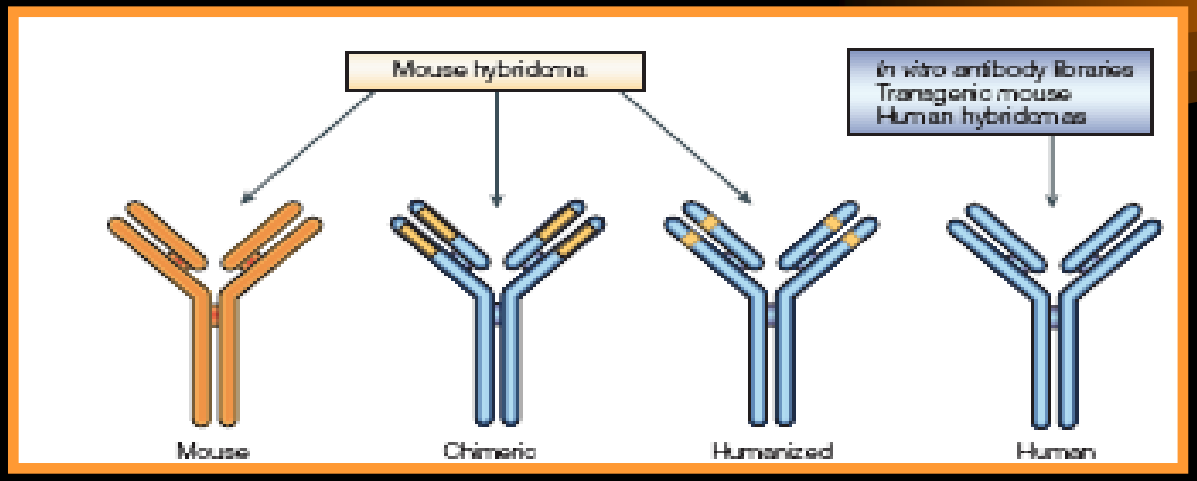
- 1989 - Raymond Hamers
- Discovered in camels
- Completely lack the light chain!
- Same antigen affinity as their four-chain counterparts
- Structure makes them more resistant to heat and pH
  - May lead to development of oral nanobody pills



Mechanisms of Action

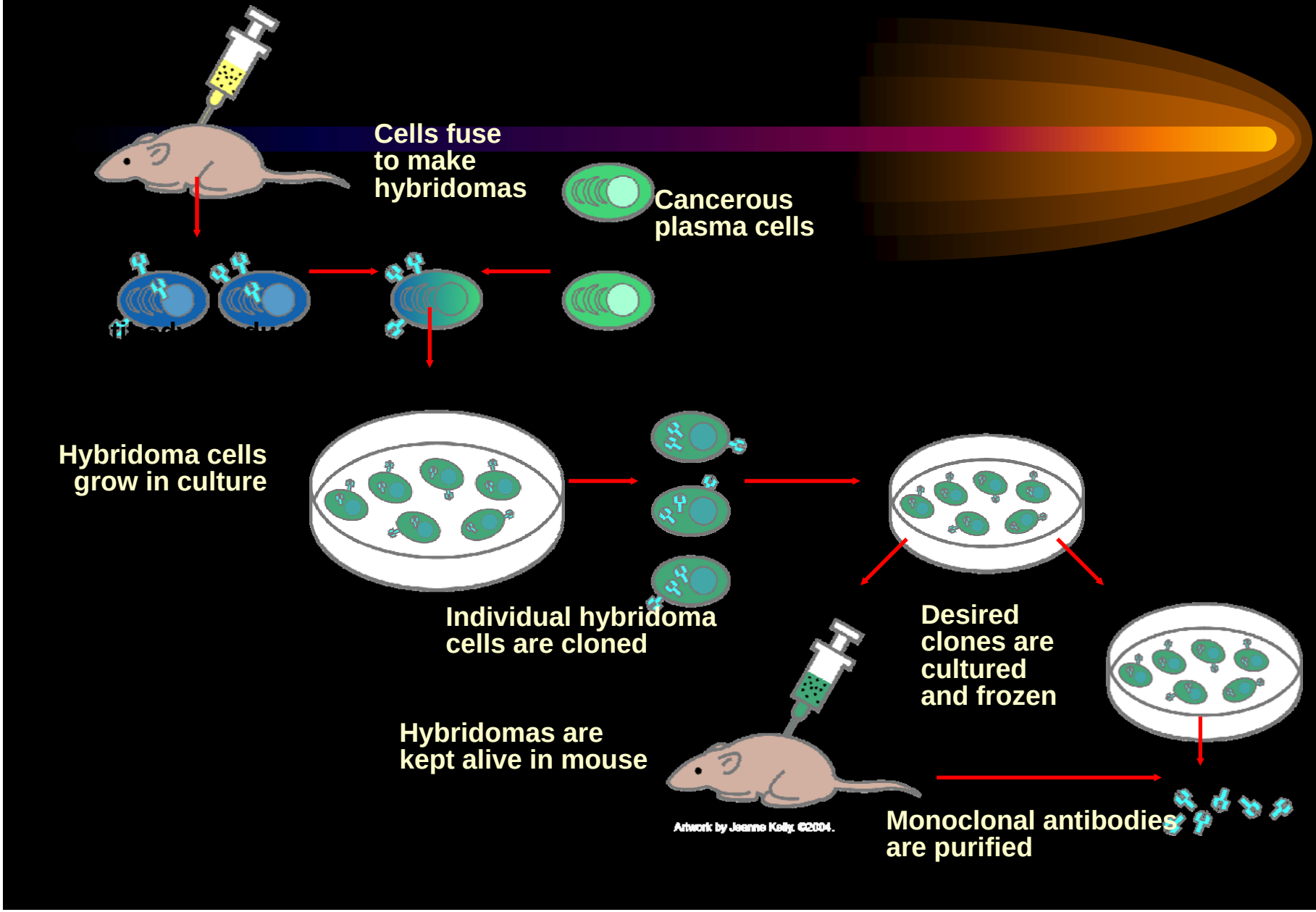
1. Blocking action of molecular targets
   - Can work antagonistically by binding a *receptor* to prevent activation
   - Can also bind the *antigen* and prevent activation
2. "Magic Bullet"
   - Compound with target specificity is coupled with various effector groups
     - Toxins, radionuclei, enzymes, DNA
3. Signal molecules
   - Coupled to mediators of apoptosis, cell division, etc.

"Humanizing" Antibodies



- Chimeric Antibodies
  - Murine Fv + human Fc
  - Human anti-chimeric antibodies (HACA) still observed
- Humanized Antibodies
  - Murine CDRs + human framework and Fc

Hybridoma Technology



Pharmaceutical Antibodies

- The fastest growing segment of the biopharmaceutical market
  - $14 billion in sales for 2005
  - Expected to grow to $30 billion by 2010
- Today, 20 therapeutic mAbs are on the market in the US
- However, an estimated 500 antibody-based therapies are currently under development

Nomenclature of Monoclonal Antibodies

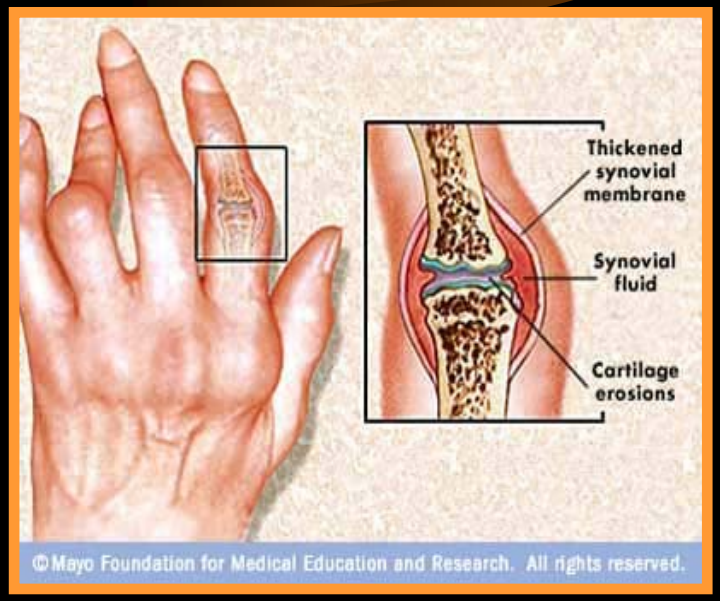
Prefix	Target		Source		Suffix
variable	*-o(s)-*	bone	*-u-*	human	*-mab*
	-vi(r)-	viral	*-o-*	mouse	
	-ba(c)-	bacterial	*-a-*	rat	
	-li(m)-	immune	*-e-*	hamster	
	-le(s)-	infectious lesions	*-i-*	primate	
	-ci(r)-	cardiovascular	*-xi-*	chimeric	
	-mu(l)-	musculoskeletal	*-zu-*	humanized	
	-ki(n)-	interleukin	*-axo-*	rat/murine hybrid	
	-co(l)-	colonic tumor			
	-me(l)-	melanoma			
	-ma(r)-	mammary tumor			
	-go(t)-	testicular tumor			
	-go(v)-	ovarian tumor			
	-pr(o)-	prostate tumor			
	-tu(m)-	miscellaneous tumor			
	-neu(r)-	nervous system			
	-tox(a)-	toxin as target			

Autoimmune Disease

- An immune reaction against self
- Mechanism unknown, arises out of a failure in immune regulation
- Examples:
  - Rheumatoid arthritis
  - Systemic lupus erythematosus
  - Multiple sclerosis (MS)
  - Insulin-dependent diabetes mellitus
  - And the list goes on…

Rheumatoid Arthritis

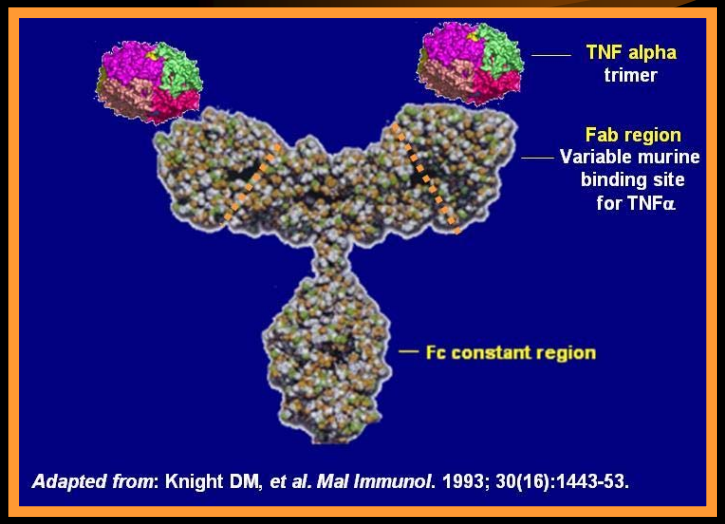
- Chronic, autoimmune disease characterized by:
  - Severe joint inflammation
  - Increased synovial fluid and thickened synovial membrane
  - Destruction of bone and cartilage in several joints
  - Elevated levels of pro-inflammatory cytokines
    - TNF-α, IL-1, IL-6



- Affects 1% of the US population
- Women are 3 times more likely to develop
- If untreated for 2+ more years, irreversible damage occurs

Infliximab

- Remicade® by Johnson & Johnson
- Chimeric mAb
- Anti TNF-α
- Approved by the FDA in 1998
- Administered intravenously
- Designated for use in patients who did not respond to methotrexate
- Proven to slow the clinical and radiological progression of rheumatoid arthritis



Adalimumab

- Humira® by Abbott Laboratories
- Fully human IgG1 mAb
- Anti-TNF-α
- Approved by the FDA in 2002
- Available in 1 mL Humira pens and syringes for convenient use at home

Rituximab

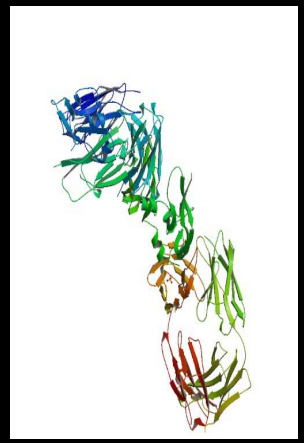
- Rituxan® by Genentech
- Anti-B cell (CD20) antibody
- First approved in 1997 for use in B-cell lymphoma
- Given in combination with Methotrexate
- Directed for patients who do not respond to Anti-TNF treatments
- Indicates the rheumatoid arthritis has a B cell component to its pathology

Vascular endothelial growth factor (VEGF)

- a sub-family of growth factors (platelet-derived growth factor family)
- important signaling proteins involved in
  - vasculogenesis
  - angiogenesis

Vascular endothelial growth factor A

- Angiogenesis
- ↑ Migration of endothelial cells
- ↑ mitosis of endothelial cells
- ↑ MMOP activity
- ↑ αvβ3 activity
- creation of blood vessel lumen
- creates lumen
- creates fenestrations
- Chemotactic for macrophages and granulocytes
- Vasodilation (indirectly by NO release)



- $VEGF_{xxx}$ poor prognosis in breast cancer.
- $VEGF_{xxx}$ is also released in rheumatoid arthritis in response to TNF-α,
- $VEGF_{xxx}$ in diabetic retinopathy (DR).
- $VEGF_{xxx}$ the wet form age-related macular degeneration (AMD)
- VEGF-D elevated in angiosarcoma
- $VEGF_{xxx}$ is a potential target for the treatment of cancer
- pulmonary emphysema decreased levels of VEGF in the pulmonary arteries.

$VEGF_{xxx}$ production

- Hypoxic cells produce HIF, Hypoxia Inducible Factor, a transcription factor.
- HIF stimulates release of $VEGF_{xxx}$, among other functions (including modulation of erythropoeisis).
- Circulating $VEGF_{xxx}$ binds to VEGF Receptors on endothelial cells, triggering a Tyrosine Kinase Pathway leading to angiogenesis.

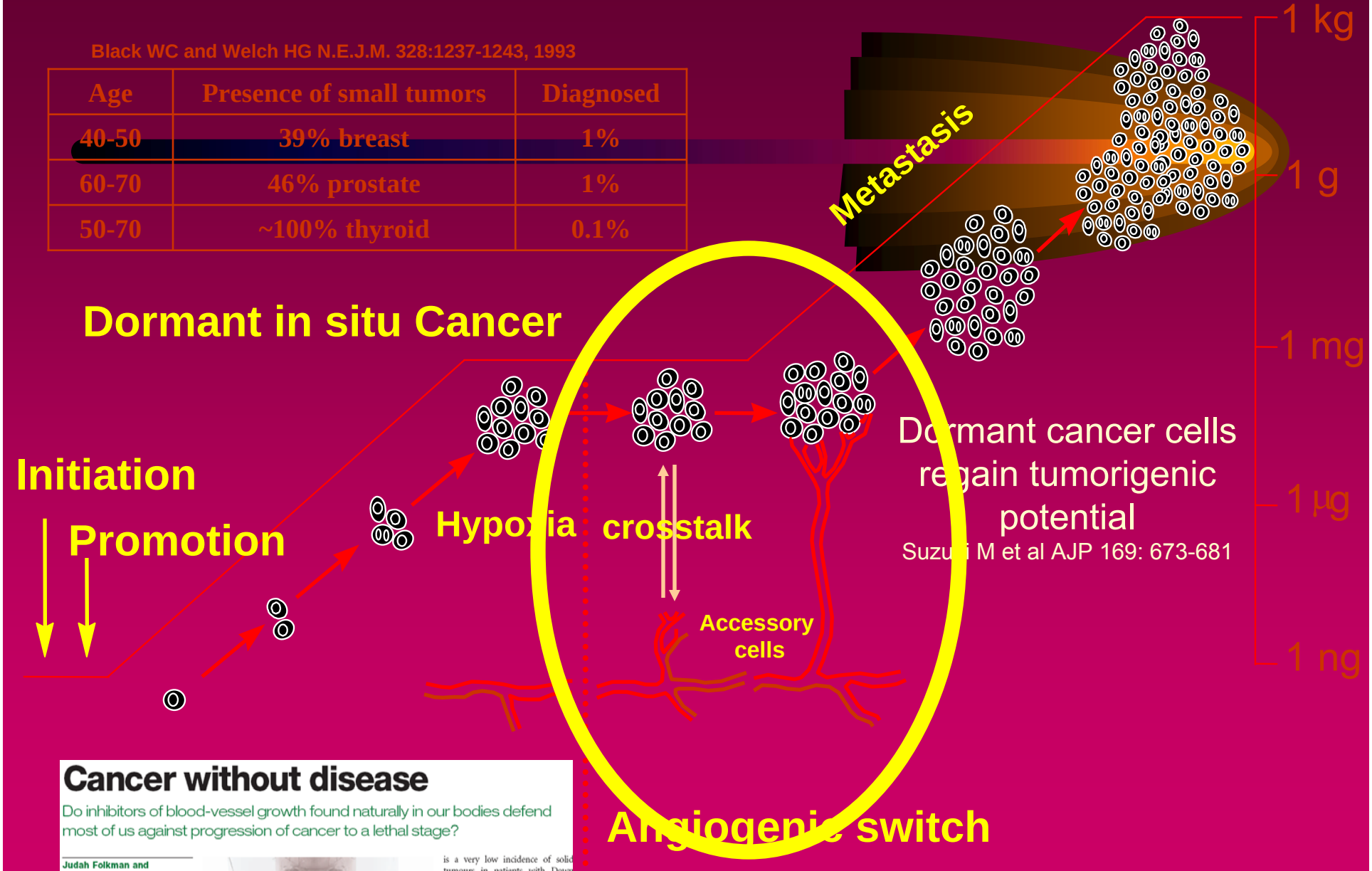
Physiological versus pathological angiogenesis

	Therapeutic goal	
	Inhibition of angiogenesis	**Stimulation of angiogenesis**
Embryogenesis Female reproductive system Development of follicles Corpus luteum formation Embryo implantation Successful wound healing	Hemangiomas Psoriasis Kaposi's sarcoma Ocular neovascularization Rheumatoid arthritis Endometriosis Atherosclerosis **Tumor growth and metastasis**	Myocardial ischemia Peripheral ischemia Cerebral ischemia Wound healing Reconstructive surgery Ulcer healing

Progression of Cancer

Black WC and Welch HG N.E.J.M. 328:1237-1243, 1993

Age	Presence of small tumors	Diagnosed
40-50	39% breast	1%
60-70	46% prostate	1%
50-70	~100% thyroid	0.1%



Cancer without disease

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

Judah Folkman and Raghu Kalluri

is a very low incidence of solid tumours in patients with Down Syndrome, who circulate elevated

The balance hypothesis for the angiogenic switch

VEGF family
FGF family
PDGF
TGF family
Angiogenin
Angiopoietin-1/Tie2
TNF-α
HGF/scatter factor
IGF family
IL-8
Nitric oxide
Prostaglandins
Tissue factor
MMPs
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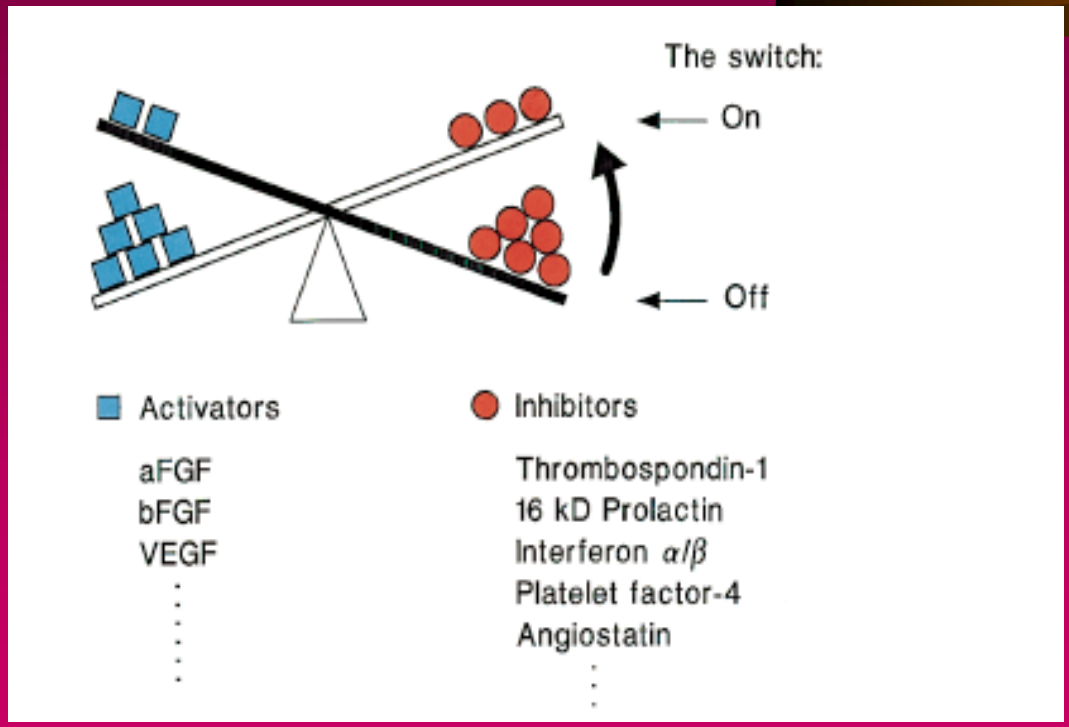

Hanahan D & Folkman J. Cell. 86:353, 1996

Angiostatin/other plasminogen kringles
Antithrombin (cleaved)
Endostatin
Fibronectin fragments
PEX
16-kDa Prolactin
Prothrombin kringle-2
Maspin
Restin
Vasostatin
IL-1, -4, -10, -12, -18
IFNs
TIMPs
1,25-$(OH)_2$-vitamin D
2-Methoxyestradiol
Angiopoietin-2
EMAP-II
gro-β
IP-10
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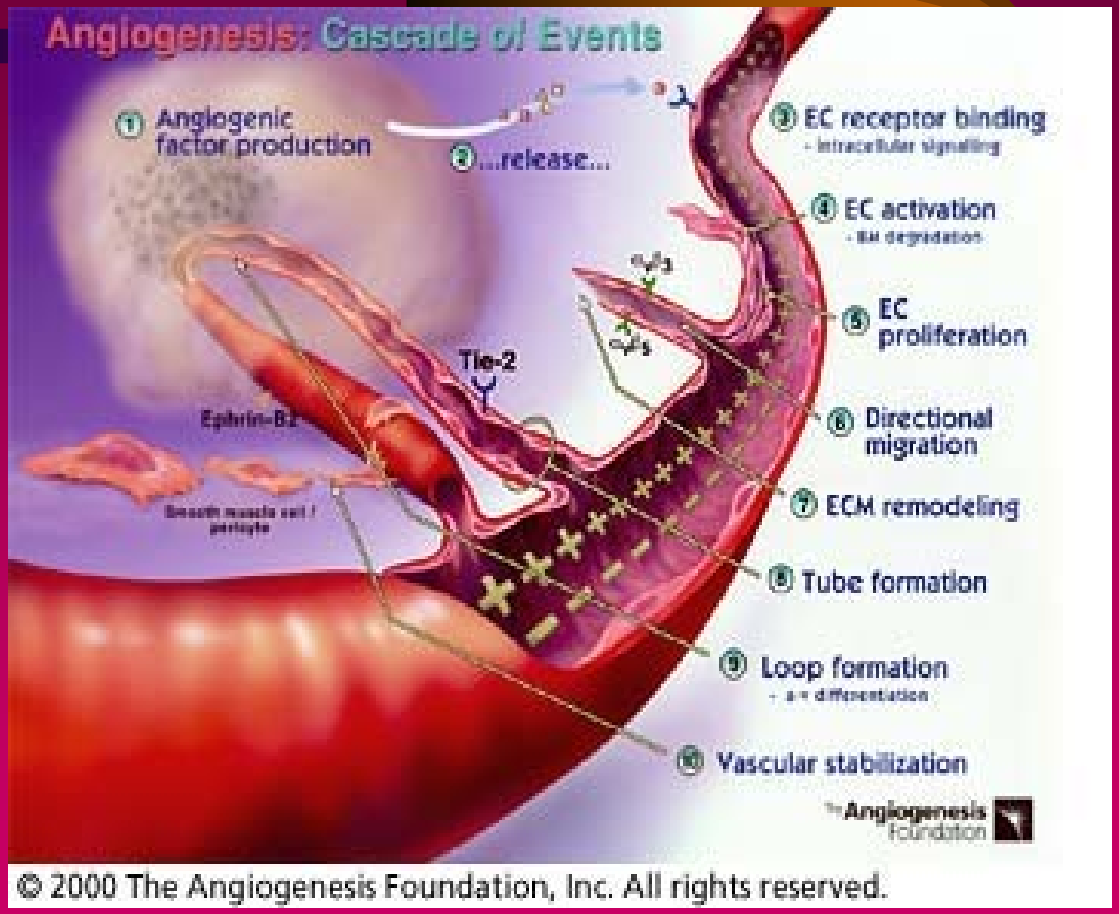
The angiogenesis process: How do new blood vessels grow?

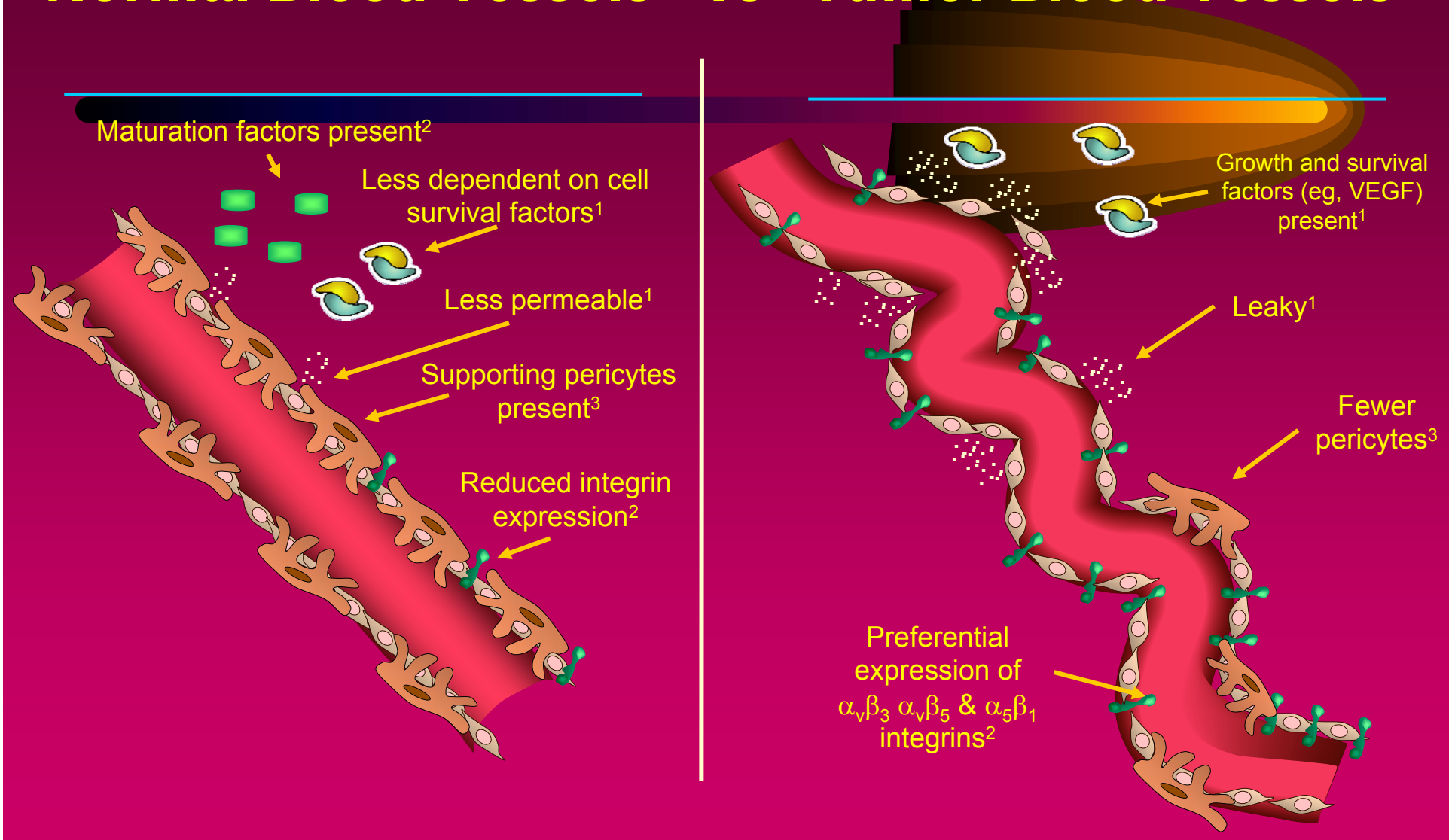
The process of angiogenesis occurs as an orderly series of events

1. Diseased or injured tissues produce and release angiogenic growth factors that diffuse into the nearby tissues
2. The angiogenic growth factors bind to specific receptors located on the endothelial cells (EC) of nearby preexisting blood vessels
3. Once growth factors bind to their receptors, the endothelial cells become activated. Signals are sent from the cell's surface to the nucleus. The endothelial cell's machinery begins to produce new molecules including enzymes
4. Enzymes dissolve tiny holes in the sheath-like covering (basement membrane) surrounding all existing blood vessels
5. The endothelial cells begin to proliferate, and they migrate out through the dissolved holes of the existing vessel towards the diseased tissue (tumor)
6. Specialized molecules called adhesion molecules, or integrins (avß3, avß5, avß1) serve as "grappling hooks" to pull the sprouting new blood vessels forward
7. Additional enzymes (matrix metalloproteinases, or MMPs) are produced to dissolve the tissue at the sprouting vessel tip in order to accommodate forward growth. As the vessel extends, the tissue is remolded around the vessel



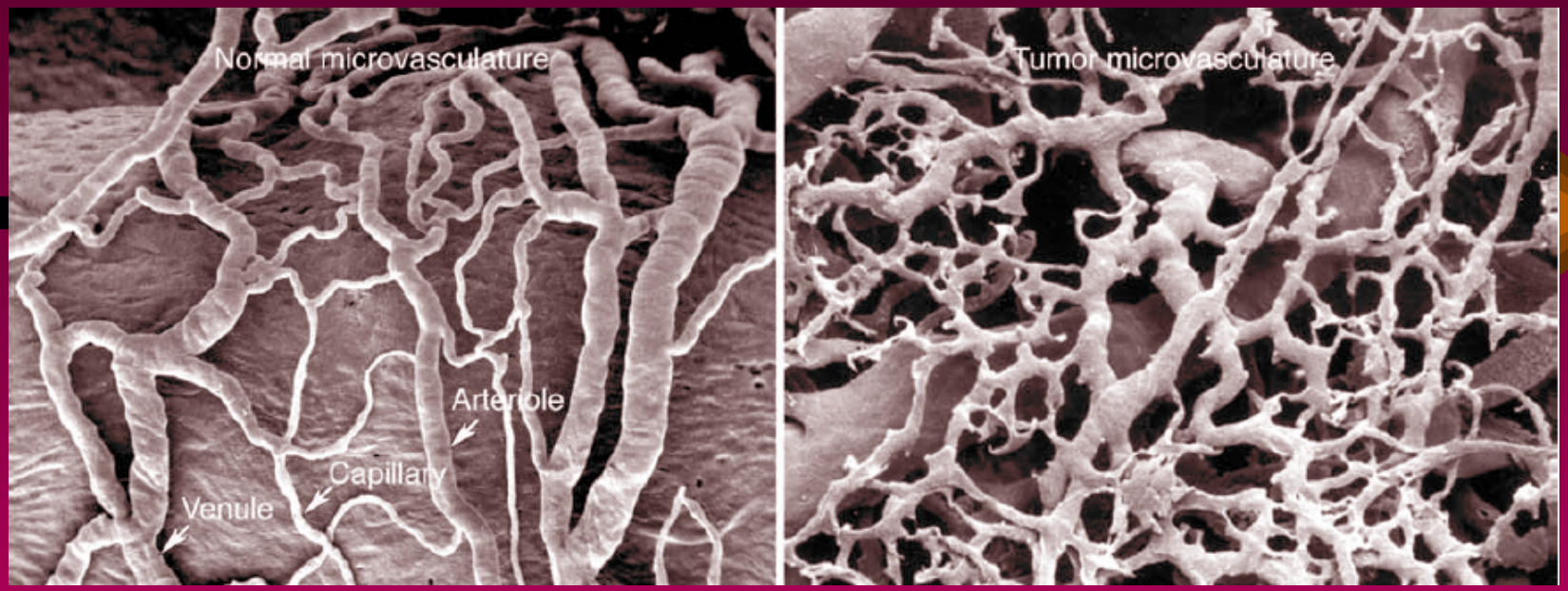
8. Sprouting endothelial cells roll up to form a blood vessel tube (tubular morphogenesis)
9. Individual blood vessel tubes connect to form vascular loops
10. Finally, newly formed blood vessel tubes are stabilized by specialized muscle cells (smooth muscle cells and pericytes) that provide structural support. Blood flow then begins

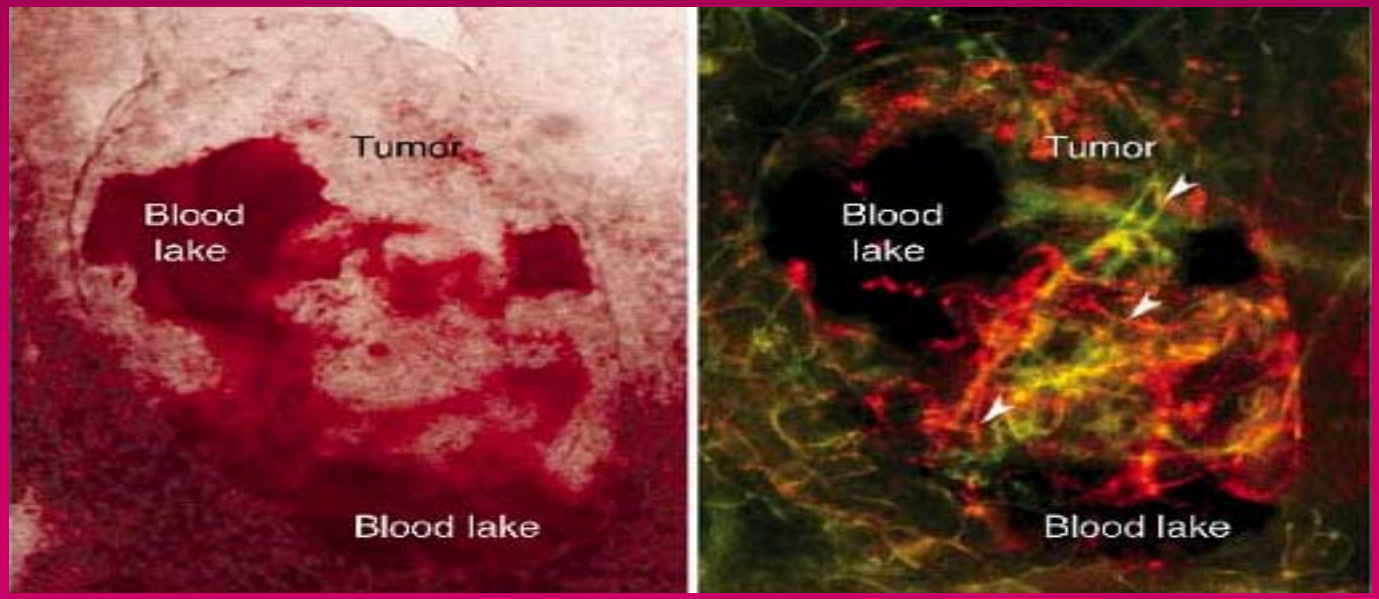
Normal Blood Vessels vs Tumor Blood Vessels



1. Jain. *Nat Med.* 2001;7:987. 2. Griffioen and Molema. *Pharmacol Rev*. 2000;52:237.3. Blau and Banfi. *Nat Med*. 2001;7:532.

Normal Blood Vessels vs Tumor Blood Vessels





McDonald & Choyke Nat Med 2003

Contrast Enhancement of malignant tumor

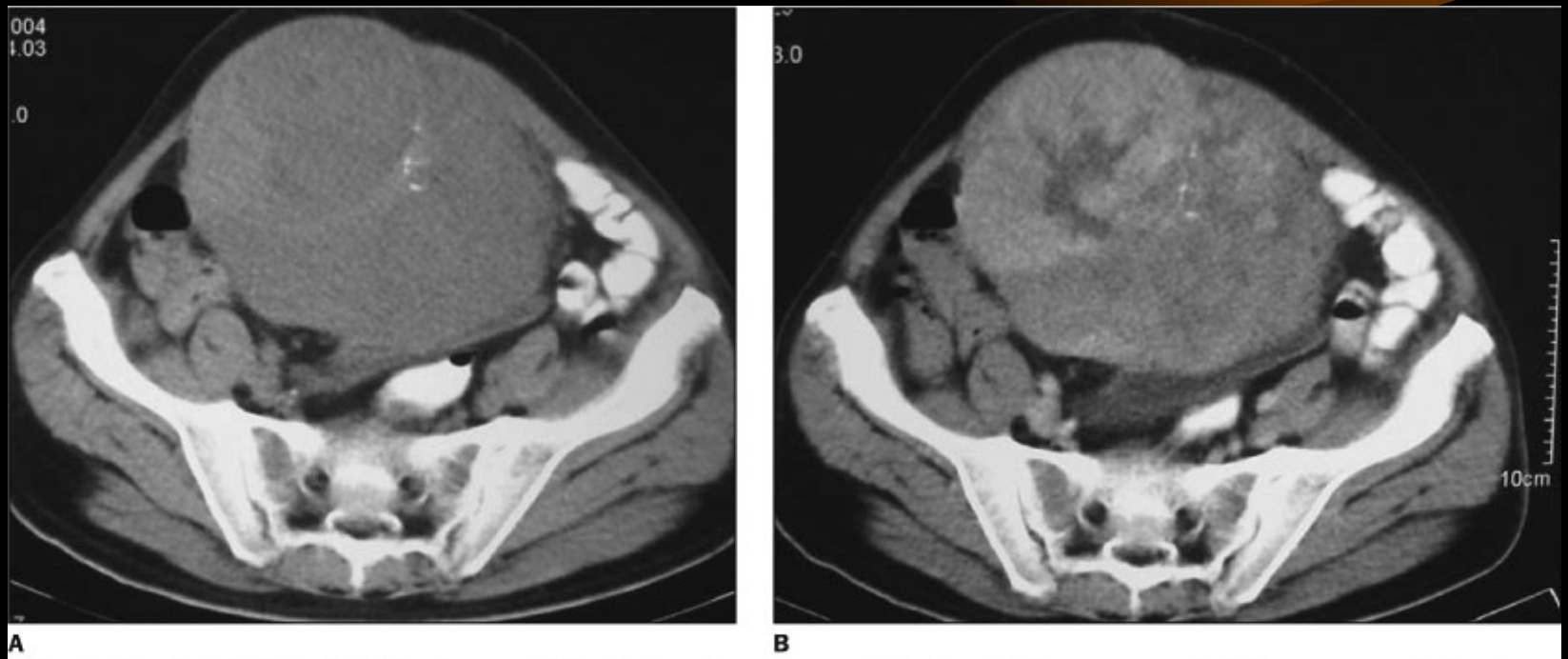
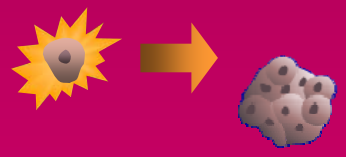


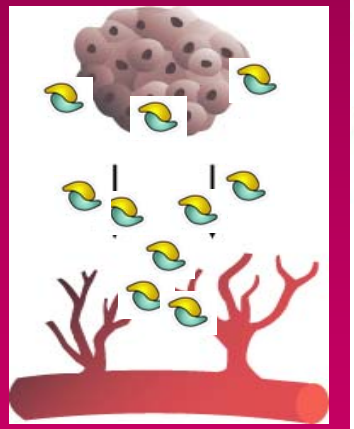
Figure 1. Mesenteric GIST. **A:** Well-defined mass with lobulated margins and some calcifications. **B:** The mass presents heterogeneous contrast-enhancement.

The Angiogenic Switch and Antiangiogenic Therapy

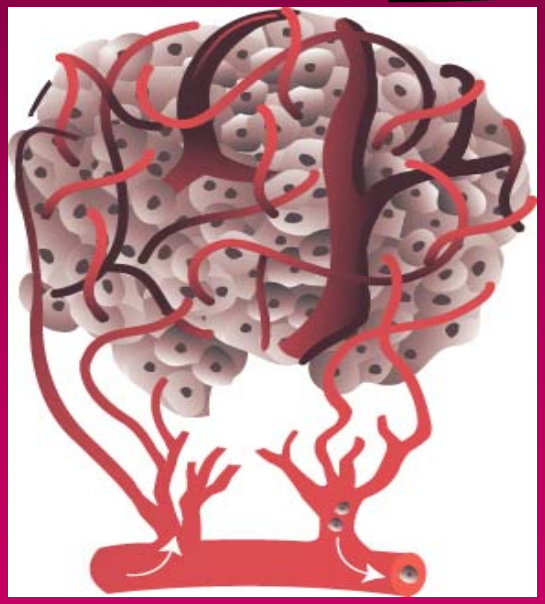


Somatic Mutation

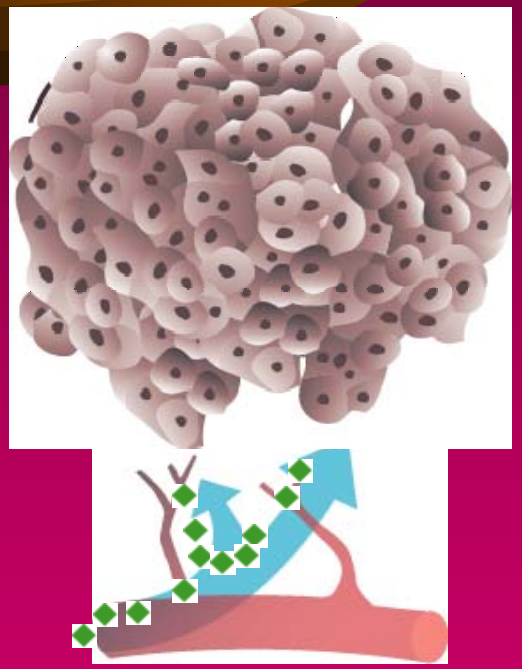
Small Avascular Tumor



Tumor secretion of angiogenic factors stimulates angiogenesis



Rapid tumor growth and metastasis



Angiogenic inhibitors may reverse this vascularization

Carmeliet and Jain. *Nature*. 2000;407:249.

Bevacizumab mAB against VEGF

- inhibits tumor growth by blocking the formation of new blood vessels
- approved by the U.S. FDA in 2004 for use in combination with standard chemotherapy in the treatment of metastatic colon cancer and most forms of metastatic non-small cell lung cancer
- 2008, it was approved by the FDA for use in breast cancer

Table 1 Summary of the main case

Table 1 Summary of the main case.

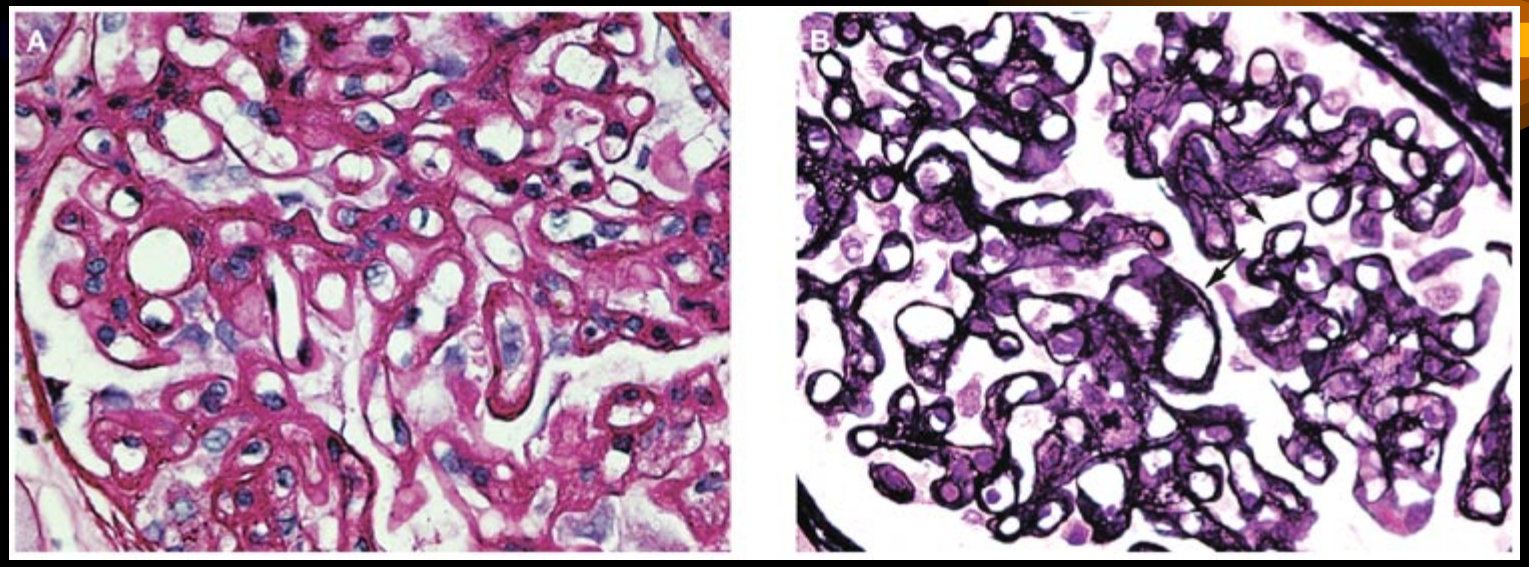
Time from start of treatment with interferon α2b and bevacizumab	Serum creatinine level (μmol/l)	Proteinuria dipstick test	Urine specific gravity	Proteinuria (mg/day)	Blood pressure (mmHg)	Medications and procedures
–2 months	106	ND	ND	ND	140/72	Quinapril 20 mg/day Hydrochlorothiazide 12.5 mg/day Glimepiride 2 mg/day
0 months (treatment with interferon α2b and bevacizumab started)	115	Negative	1.029	ND	148/80	Interferon α2b started at 9 million units subcutaneously 3 times/week Bevacizumab started at 10 mg/kg intravenously over 1.5 hours every 2 weeks
2 months	ND	ND	ND	ND	ND	Interferon α2b dose decreased to 6 million units 3 times/week
4 months	141	Negative	1.030	ND	ND	Quinapril discontinued
9 months	ND	1+	1.030	1,836	ND	No medication changes or procedures
11 months	ND	2+	ND	ND	150/74	Amlodipine started at 5 mg/day
11.5 months	ND	1+	ND	660	120/84	No medication changes or procedures
12 months	141	ND	ND	ND	140/80	No medication changes or procedures
13 months	124	1+	1.030	ND	148/70	Telmisartan added at 40 mg/day Amlodipine increased to 10 mg/day
13.5 months	124	ND	ND	1,045	148/84	No medication changes or procedures
14 months	133	ND	ND	1,593	148/84	No medication changes or procedures
15 months	150	3+	ND	ND	144/94	Interferon α2b discontinued because of anxiety and depression
15.5 months	ND	ND	ND	ND	157/100	Bevacizumab discontinued because of persistent proteinuria and elevated creatinine
16 months	141	4+	ND	6,958	ND	No medication changes or procedures
17 months	150	ND	ND	5,580	142/80	Lisinopril added at 10 mg/day
17.25 months	150	ND	ND	ND	ND	Renal biopsy performed
18 months	ND	ND	ND	4,640	ND	No medication changes or procedures
21 months	167	ND	ND	2,744	148/86	No medication changes or procedures
25 months	111	ND	ND	1,899	132/92	No medication changes or procedures

Abbreviation: ND, not done.

Roncone D *et al.* (2007) *Nat Clin Pract Nephrol* **3:** 277–293 doi:10.1038/ncpneph0476

nature CLINICAL PRACTICE NEPHROLOGY

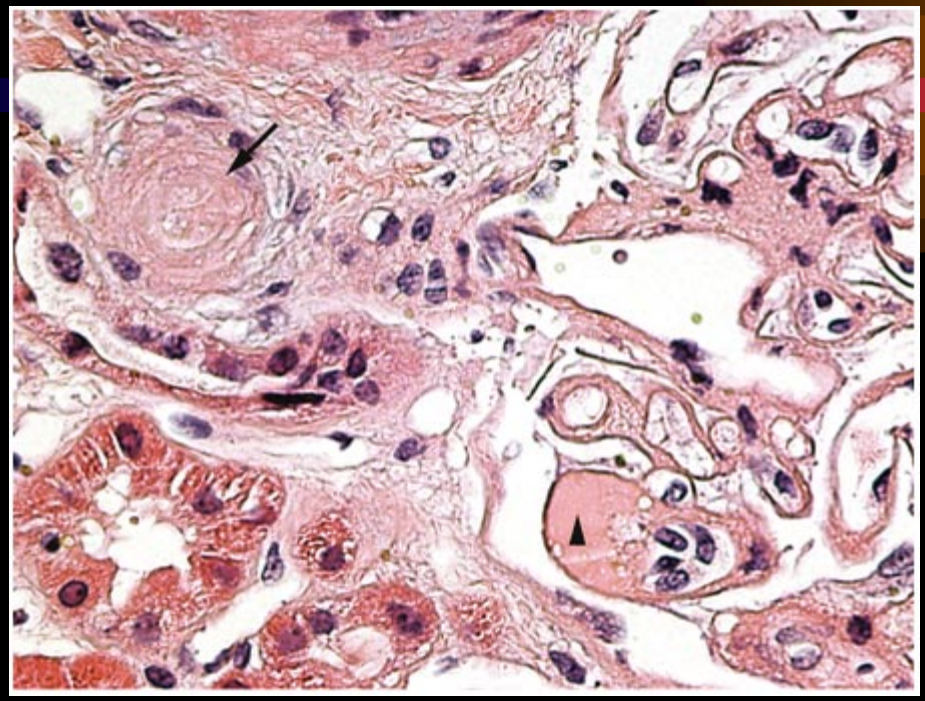
Figure 1 Light microscopy of the kidney biopsy



Roncone D *et al.* (2007) Proteinuria in a patient receiving anti-VEGF therapy for metastatic renal cell carcinoma *Nat Clin Pract Nephrol* **3:** 277–293 doi:10.1038/ncpneph0476

nature CLINICAL PRACTICE NEPHROLOGY

Figure 2 Light microscopy of the kidney biopsy



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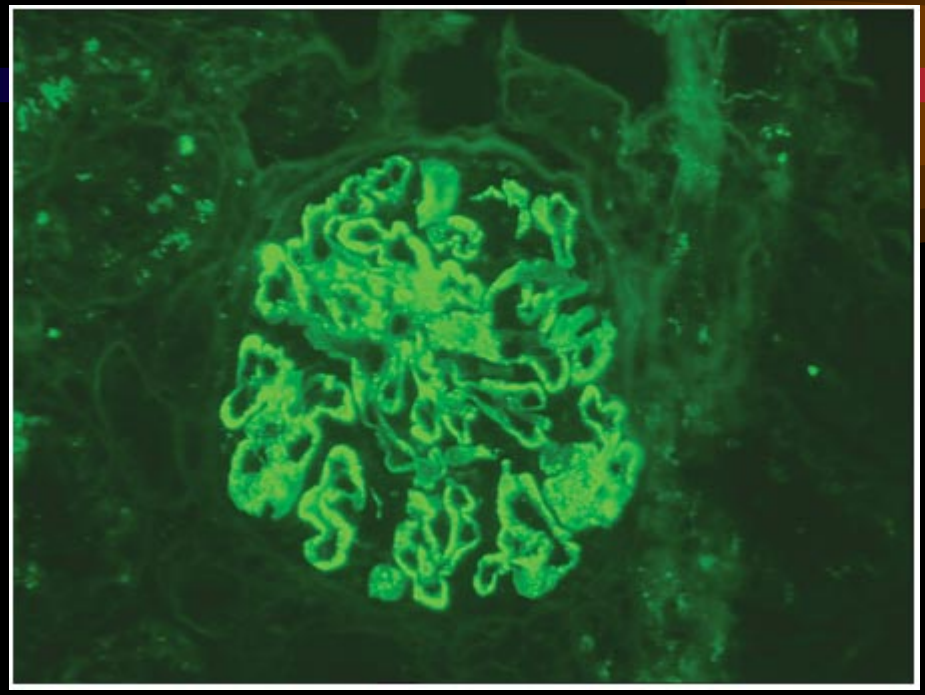
nature CLINICAL PRACTICE NEPHROLOGY

Figure 3 Immunofluorescence microscopy of the kidney biopsy



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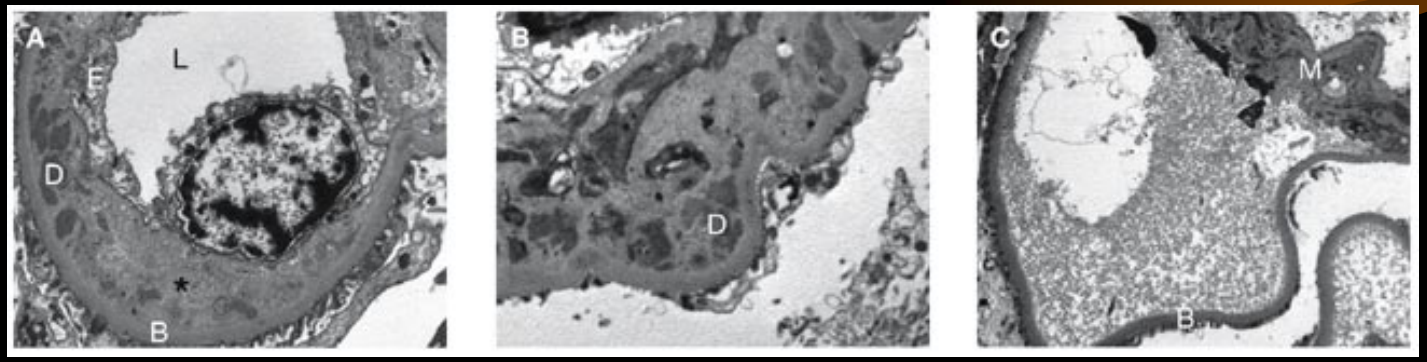
nature CLINICAL PRACTICE NEPHROLOGY

Figure 4 Electron microscopy of the kidney biopsy



Roncone D *et al.* (2007) Proteinuria in a patient receiving anti-VEGF therapy for metastatic renal cell carcinoma *Nat Clin Pract Nephrol* **3:** 277–293 doi:10.1038/ncpneph0476

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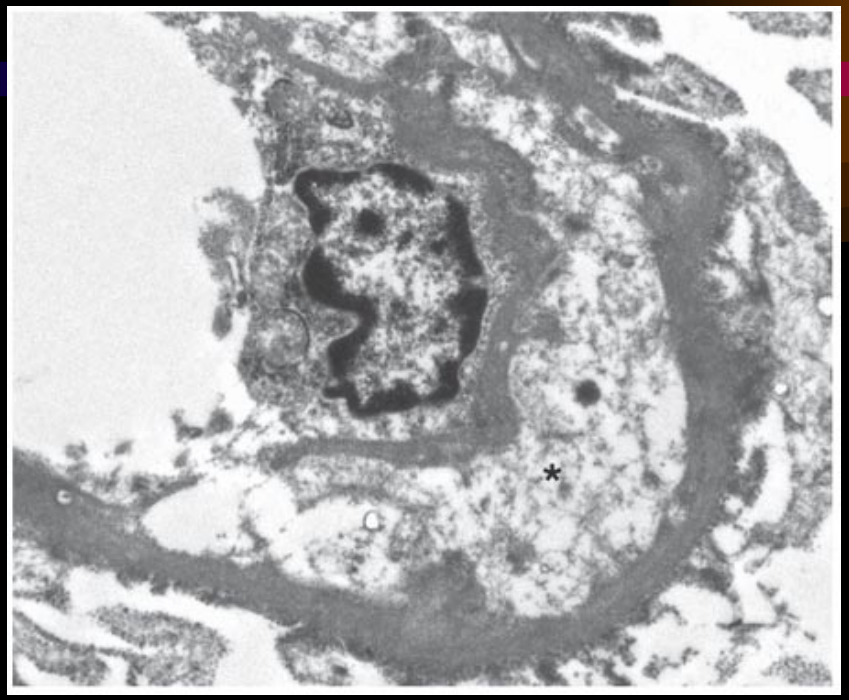
Figure 6 Electron micrograph of a glomerular capillary loop from a partial nephrectomy specimen from the patient described in Box 1



Roncone D *et al.* (2007) Proteinuria in a patient receiving anti-VEGF therapy for metastatic renal cell carcinoma *Nat Clin Pract Nephrol* **3:** 277–293 doi:10.1038/ncpneph0476

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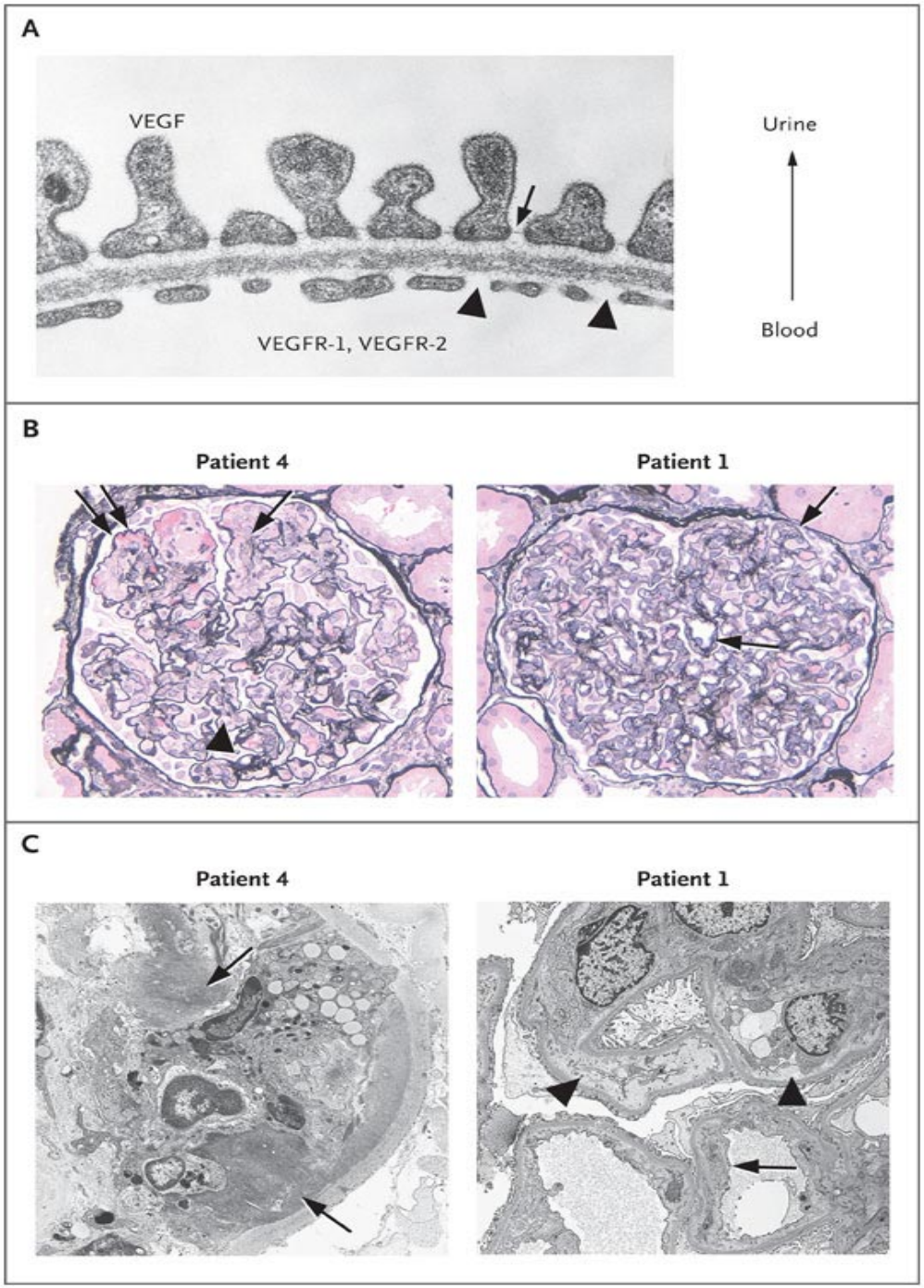
Microangiopathy in Patients Who Were Treated with Bevacizumab



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Eremina V et al. N Engl J Med 2008;358:1129-1136

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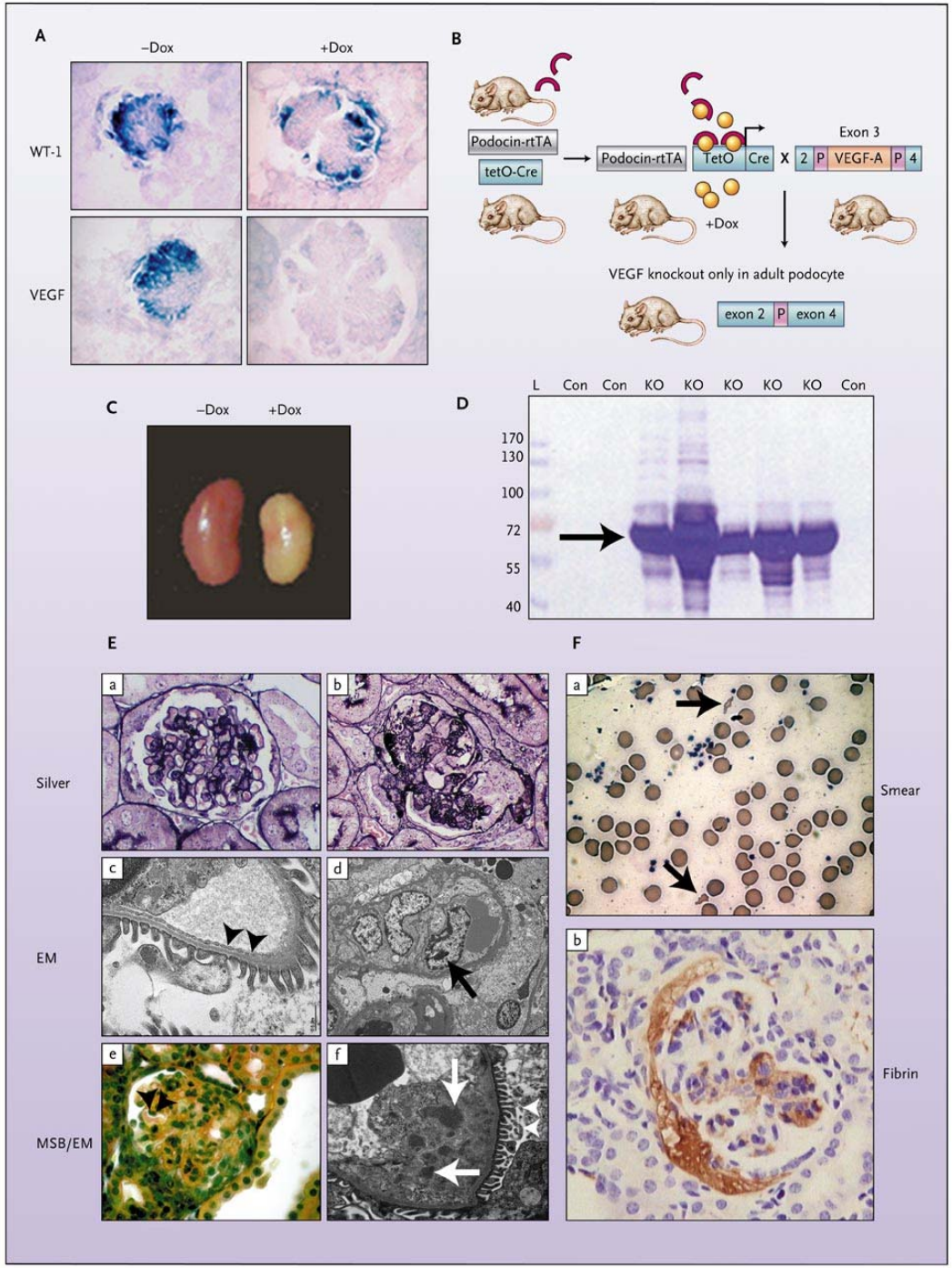
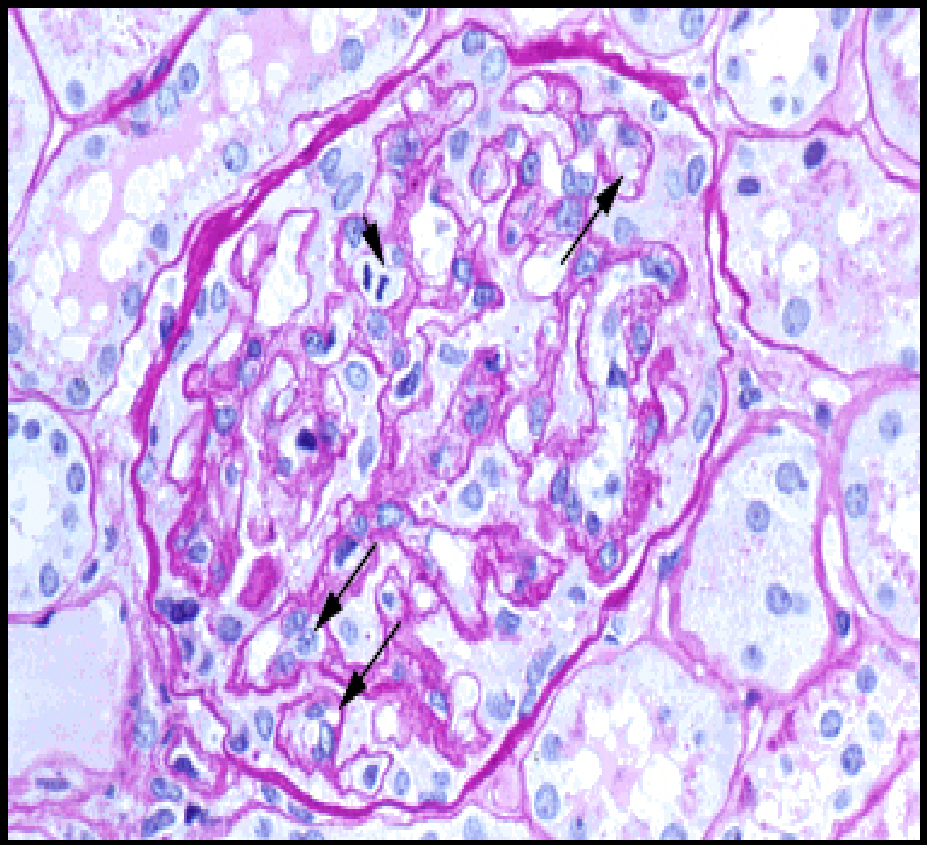
Thrombotic Microangiopathy Caused by Genetic Deletion of VEGF from Glomeruli in a Murine Model



Eremina V et al. N Engl J Med 2008;358:1129-1136

LIGHT MICROSCOPY

PREECLAMPSIA



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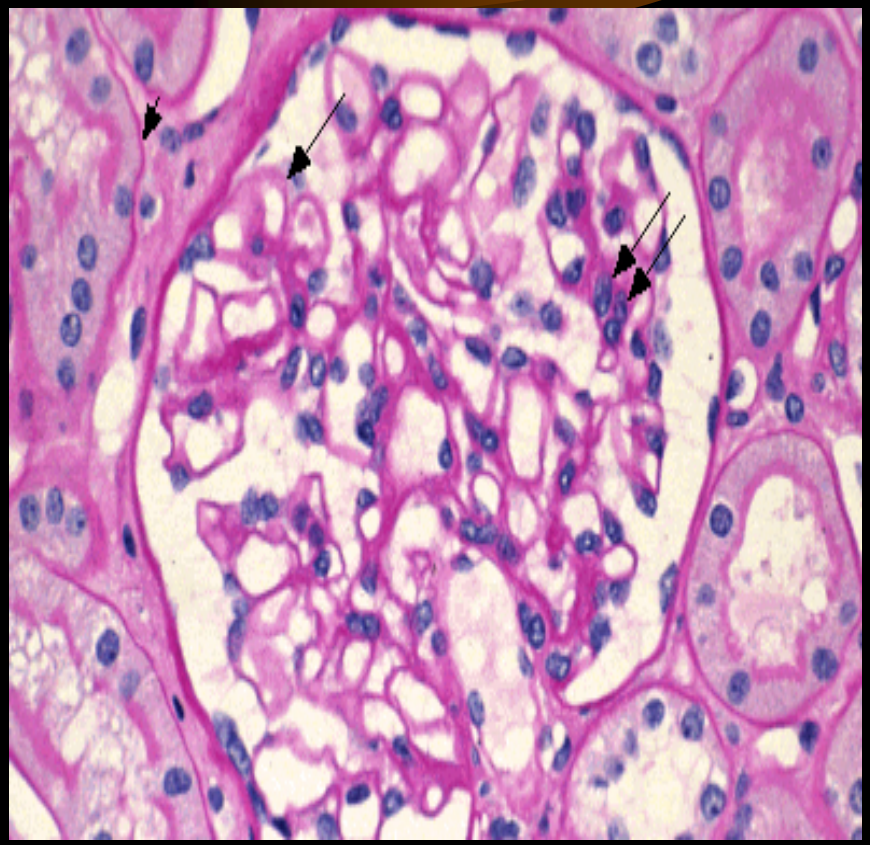

ELECTRON MICROSCOPY

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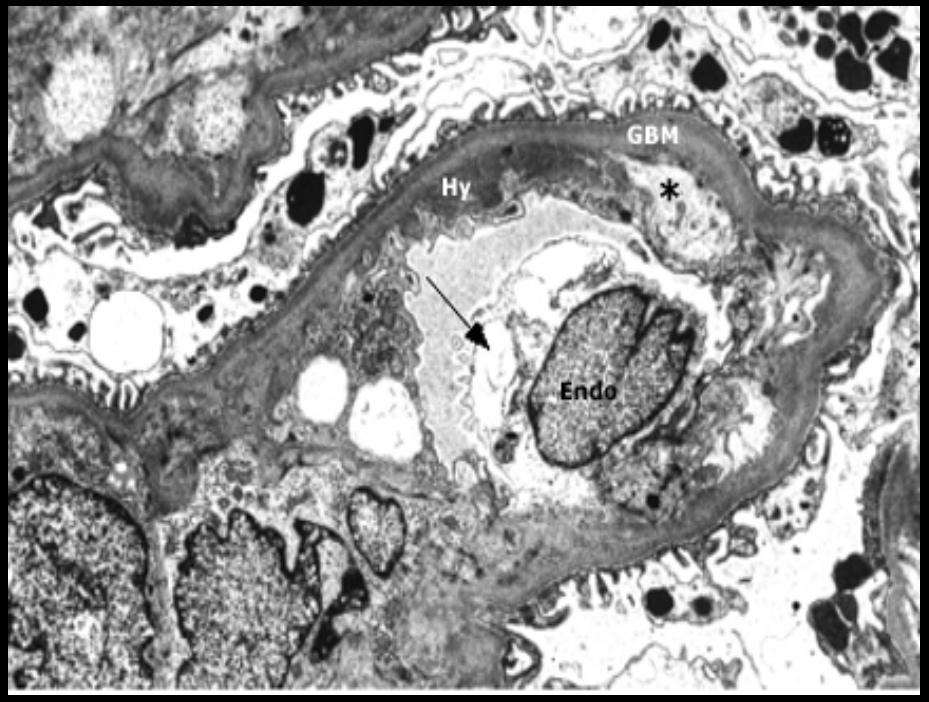

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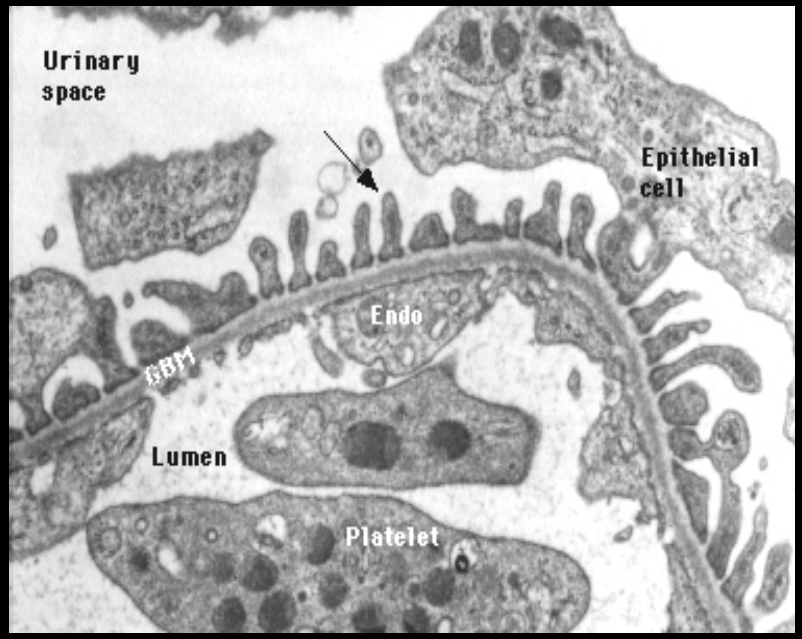
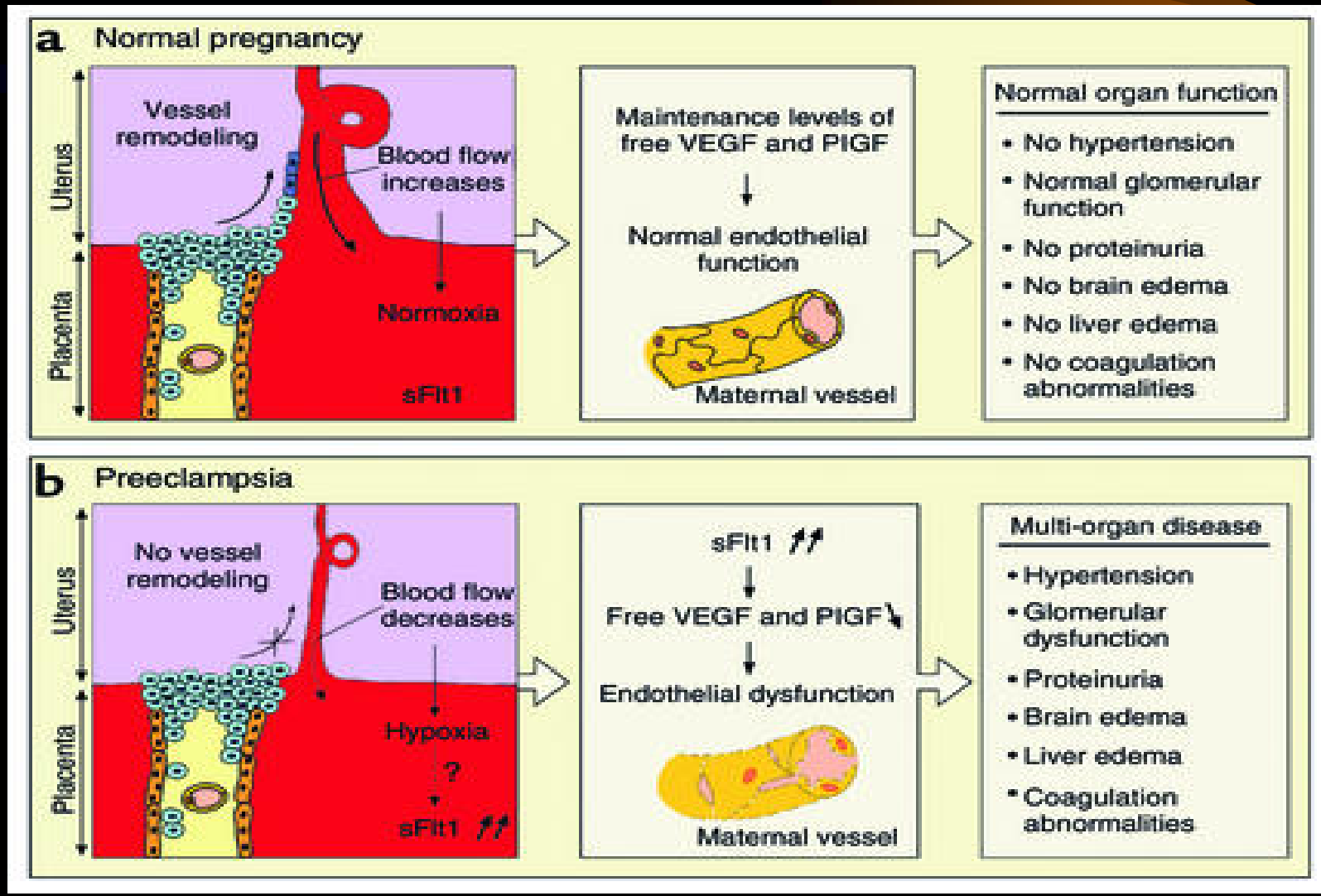
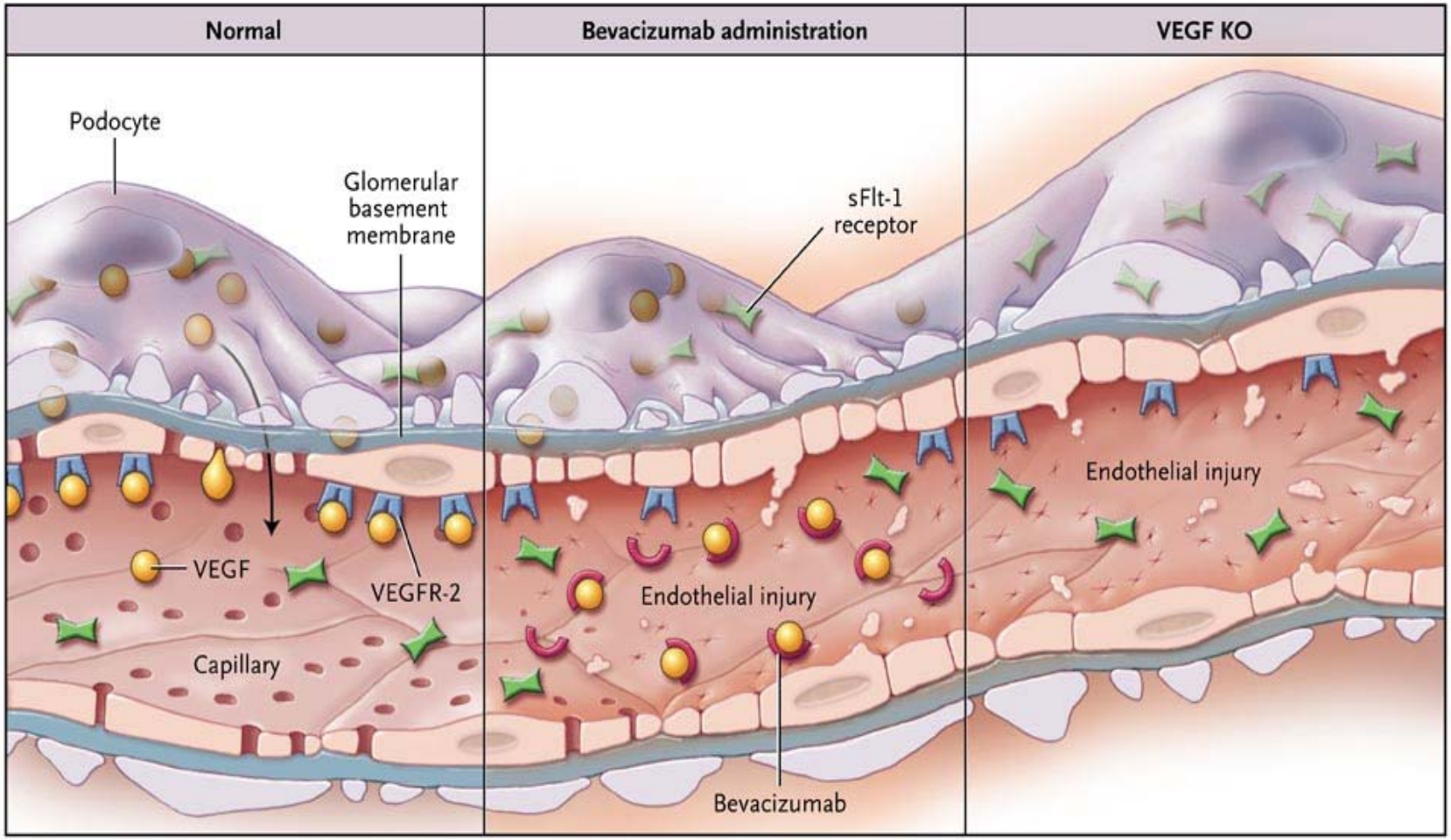


Soluble VEGF receptor Flt1



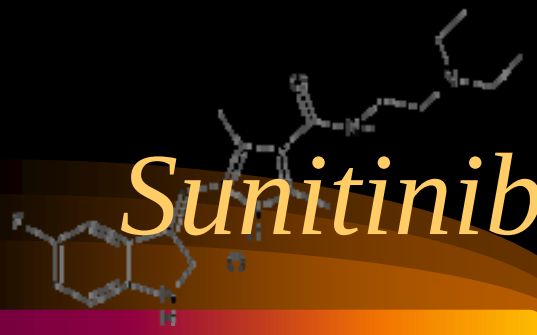
J. Clin. Invest. Aernout Luttun, et al. 111:600 doi:10.1172/JCI18015

Hypothetical Model of Disruption of VEGF Signaling in Renal Thrombotic Microangiopathy



Eremina V et al. N Engl J Med 2008;358:1129-1136

Sunitinib



- Sunitinib inhibits targets multiple RTKs. These include all platelet-derived growth factor receptors (PDGF-R) and vascular endothelial growth factor receptors (VEGF-R), which play a role in both tumor angiogenesis and tumor cell proliferation.
- first-line treatment of metastatic RCC.
- 11 months for sunitinib compared with 5 months for IFNa (P<.000001). (NEJM, 2007)
- Side effects: CHF, Hypertension

Who is going to pay?