2007 International Society for Biological Therapy of Cancer

Primer on Tumor Immunology and Biological Therapy.

Thursday, November 1,

6:55 to 7:30 pm

Angiogenesis as an "Organizing Principle" in Biology.

Judah Folkman, M.D.
Children's Hospital Boston
& Harvard Medical School

Nature Reviews Drug Discovery *6:273-286, April, 2007*

PERSPECTIVES

OPINION

Angiogenesis: an organizing principle for drug discovery?

Judah Folkman

Abstract | Angiogenesis — the process of new blood-vessel growth — has an essential role in development, reproduction and repair. However, pathological angiogenesis occurs not only in tumour formation, but also in a range of non-neoplastic diseases that could be classed together as 'angiogenesis-dependent diseases'. By viewing the process of angiogenesis as an 'organizing principle' in biology, intriguing insights into the molecular mechanisms of seemingly unrelated phenomena might be gained. This has important consequences for the clinical use of angiogenesis inhibitors and for drug discovery, not only for optimizing the treatment of cancer, but possibly also for developing therapeutic approaches for various diseases that are otherwise unrelated to each other.

New England Journal of Medicine,
285:1182-1186, ***1971***

TUMOR ANGIOGENESIS: THERAPEUTIC IMPLICATIONS

JUDAH FOLKMAN, M.D.

1. "solid tumors are **dependent** upon **new** capillary sprouts. . ."

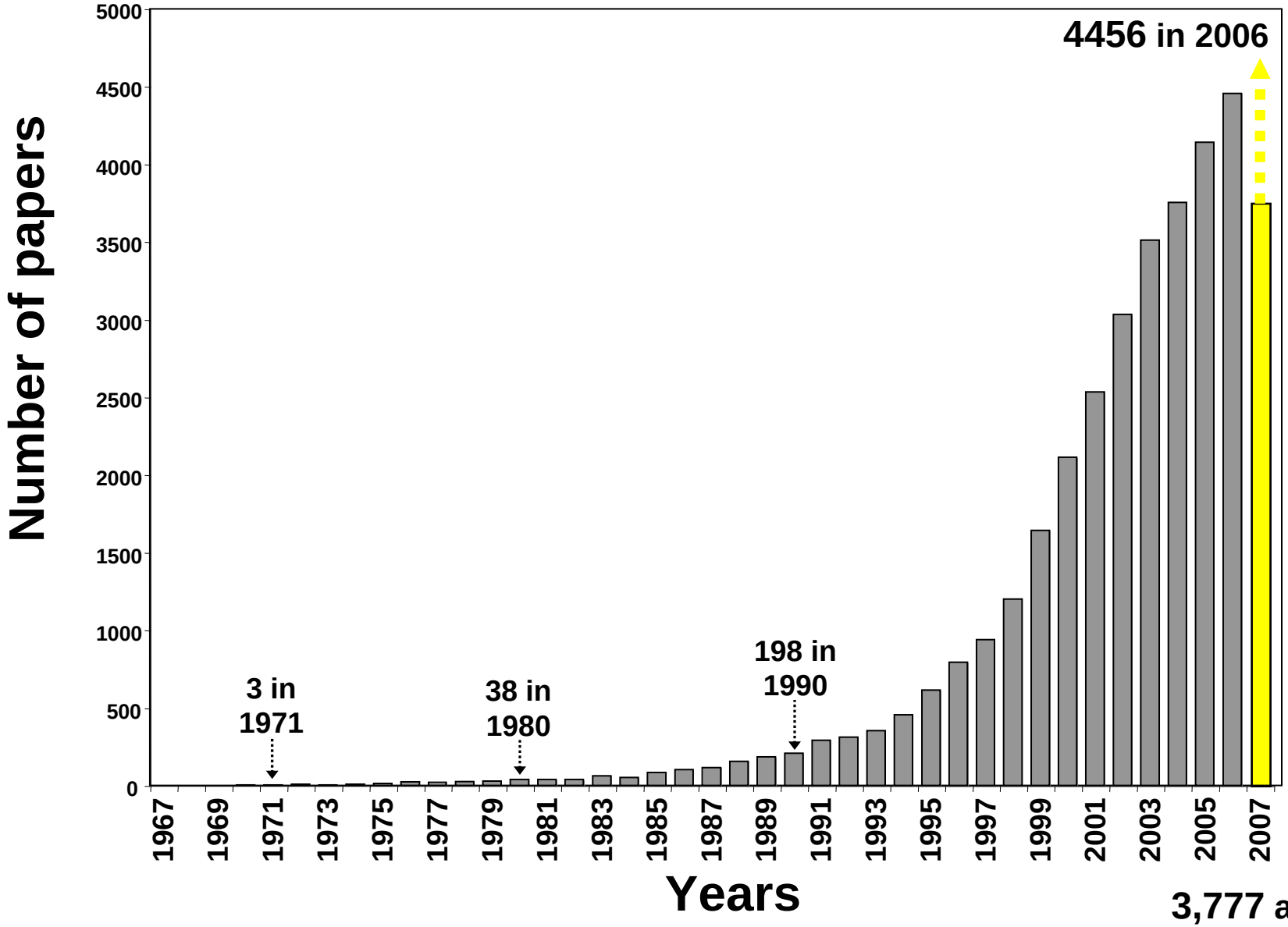
2. "without neovascularization solid tumors might become completely **dormant**…"

3. "the term "**anti-angiogenesis** is proposed to mean the prevention of new vessel **sprouts** from penetrating into an early tumor implant."

4. "this hypothesis predicts the possible future discovery of **angiogenesis inhibitors**,.."

5. "an **antibody** to a tumor angiogenic factor (TAF) could be therapeutic."

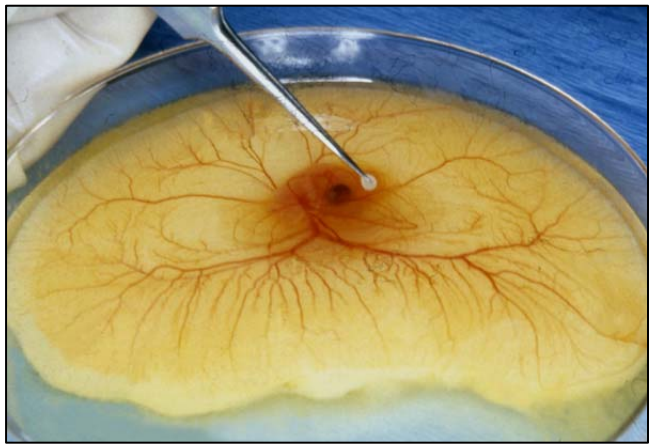
32,598 papers published on angiogenesis from 1971 - September 2007



3,777 as of September 1, 2007

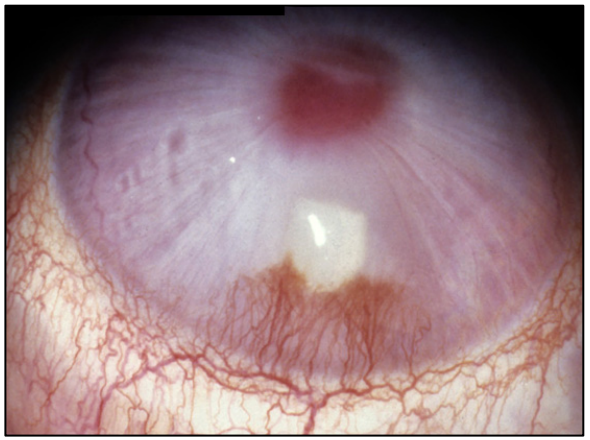
Development of bioassays for angiogenesis

Shell-less **chick embryo**:
Choriallantoic membrane



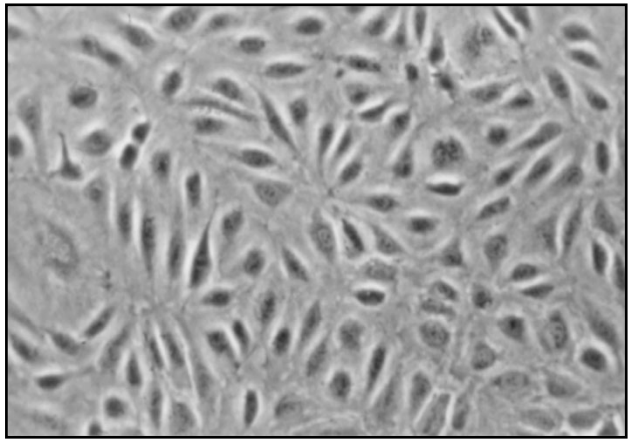
1974 *Devel Biology, 41: 391*

1) **Cornea micropocket** and
2) Sustained release **polymers**



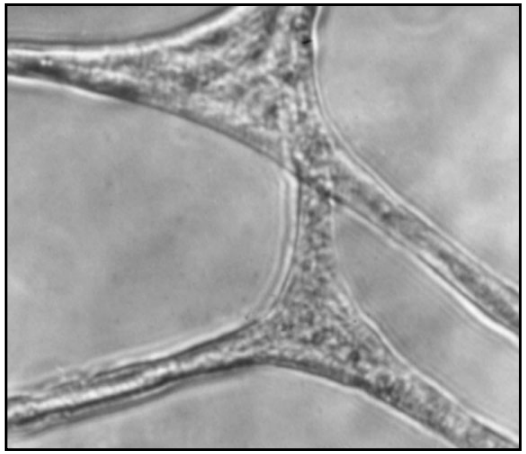
1974 *JNCI, 52: 514,* **1976** *Nature, 236: 797*

Cloned capillary **endothelial cells**



1973 *Series Haematol 6:453*
1979 *Proc Natl Acad Sci 76: 5217*

Angiogenesis *in vitro*



1980 *Nature, 288: 551*

Molecules with antiangiogenic activity

published by the Folkman lab **(1980 - 2005).**

1980	Interferon α/β, new activity	(Brouty-Boye, D. and Zetter, B.R. ***Science*** *208: 516-518, 1980*)
1982	Platelet factor 4, Protamine	(Taylor, S. and Folkman, J. ***Nature*** *297: 307-312, 1982*)
1985	Angiostatic steroids	(Crum, R. et al. (Folkman) ***Science*** *230: 1375-1378, 1985*)
1990	TNP-470 a fumagillin analogue	(Ingber, D. et al. (Folkman) ***Nature*** *348: 555-557, 1990*)
1994	Angiostatin	(O'Reilly, M. et al. (Folkman) ***Cell*** *79: 315-328, 1994*)
1994	Thalidomide	(D'Amato R.J. et al., (Folkman) ***PNAS*** *91: 4082-4085, 1994*)
1994	2-methoxyestradiol*	(D'Amato, R.J. et al. (Folkman) ***PNAS*** *91:3964-3968, 1994*)
1997	Endostatin	(O'Reilly, M. et al. (Folkman) ***Cell*** *88: 277-285, 1997*)
1999	Cleaved antithrombin III	(O'Reilly, M. et al. (Folkman) ***Science*** *285:1926-1928, 1999*)
2002	3-amino thalidomide	(Lentzsch, S. et al. (D'Amato) ***Cancer Res*** *62: 2300-2305, 2002*)
2003	DBP-maf	(Kisker, O. et al. (Folkman) ***Neoplasia*** *5: 32-40, 2003)*
2005	Caplostatin	(Satchi-Fainaro, R. et al. (Folkman) ***Cancer Cell*** *7: 251-261, 2005)*

A proteosome inhibitor with **antiangiogenic** activity.

NCI

Clinical Cancer Research, 7: 1419, 2001

Novel Proteasome Inhibitor PS-341 Inhibits Activation of Nuclear Factor-κB, Cell Survival, Tumor Growth, and Angiogenesis in Squamous Cell Carcinoma[1]

John B. Sunwoo, Zhong Chen, Gang Dong, Ning Yeh, Caren Crowl Bancroft, Edward Sausville, Julian Adams, Peter Elliott, and Carter Van Waes[2]

MD Anderson

Molecular Cancer Therapeutics, 1:1243, 2002

Effects of the Proteasome Inhibitor PS-341 on Apoptosis and Angiogenesis in Orthotopic Human Pancreatic Tumor Xenografts

Steffan T. Nawrocki,[1] Christiane J. Bruns,[1] Matthew T. Harbison, Richard J. Bold, Bridget Sweeney Gotsch, James L. Abbruzzese, Peter Elliott, Julian Adams, and David J. McConkey[2]

Dana-Farber

Cancer Research, 62: 4996, 2002

Proteasome Inhibitor PS-341 Inhibits Human Myeloma Cell Growth *in Vivo* and Prolongs Survival in a Murine Model[1]

Richard LeBlanc,[2] Laurence P. Catley,[2] Teru Hideshima, Suzanne Lentzsch, Constantine S. Mitsiades, Nicholas Mitsiades, Donna Neuberg, Olga Goloubeva, Christine S. Pien, Julian Adams, Deepak Gupta, Paul G. Richardson, Nikhil C. Munshi, and Kenneth C. Anderson[3]

May 14, 2003

THE BOSTON GLOBE

Millennium cancer fighter Velcade gets FDA approval

By Naomi Aoki
GLOBE STAFF

Millennium Pharmaceuticals Inc. won regulatory approval for its cancer-fighting drug Velcade, putting the Cambridge biotechnology company a year ahead of its initial schedule in bringing its second drug to market.

Angiogenesis inhibitors approved for clinical use.

Date approved	Drug	Place	Disease
May 2003	**Velcade** (Bortezomib)	U.S. (FDA)	**Multiple myeloma**
December 2003	**Thalidomide**	Australia	**Multiple myeloma**
February 2004	**Avastin** (Bevacizumab)	U.S. (FDA)	**Colorectal cancer**
February 2004	**Erbitux**	U.S. (FDA)	**Colorectal cancer**
November 2004	**Tarceva** (Erlotinib)	U.S. (FDA)	**Lung cancer**
December 2004	**Avastin**	Switzerland	**Colorectal cancer**
December 2004	**Macugen** (Pegaptanib)	U.S. (FDA)	**Macular degeneration**
January 2005	**Avastin**	E. U. (27 countries)	**Colorectal cancer**
September 2005	**Endostatin** (Endostar)	China (SFDA)	**Lung cancer**
December 2005	**Nexavar** (Sorafenib)	U.S. (FDA)	**Kidney cancer**
December 2005	**Revlimid** (Lenalidomide)	U.S. (FDA)	**Myelodysplastic syn**.
January 2006	**Sutent** (Sunitinib)	U.S. (FDA)	**GIST**
June 2006	**Lucentis** (Ranibizumab)	U.S. (FDA)	**Macular degeneration**
June 2006	**Revlimid**	U.S. (FDA)	**Multiple myeloma**
August 2006	**Lucentis**	Switzerland	**Macular degeneration**
September 2006	**Lucentis**	India	**Macular degeneration**
October 2006	**Avastin**	U.S. (FDA)	**Lung cancer**
January 2007	**Lucentis**	E. U. (27 countries)	**Macular degeneration**
March 2007	**Avastin**	E. U., Iceland, Norway	**Metastatic breast**
April 2007	**Avastin**	Japan	**Colorectal cancer**
May 2007	**Torisel** (CCI-779)	U.S. (FDA)	**Kidney cancer**

Clinical application of antiangiogenic therapy.

Patients who have received **prescriptions** for pure angiogenesis inhibitors, or drugs with potent anti-angiogenic activity, during 2006.
(U.S. and other countries).

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1. 2006: **1,257,600** patients have received FDA-approved angiogenesis inhibitors, for cancer and age-related macular degeneration.

2. 2009: Low estimate: **7,336,000**
   High estimate: **14,000,000**

From a professional search of world-wide databases by “info2go”, using Thomson Pharma, Chemical Market Reporter, & Business Communications Co.

Phase III - Drugs with varying degrees of antiangiogenic activity. May, 2007. (All drugs except those in blue are not yet FDA approved).

Agent	Target
AG3340 (Prinomastat) (Agouron Phamaceuticals)	MMP inhibitor
Avastin *(Genentech)*	**VEGF**
AZD2171 (AstraZeneca)	**VEGFR-1, 2, 3,** PDGFR
BMS-275291 *(Bristol Myers Squibb)*	MMP inhibitor
CCI-779 *(Wyeth)*	**VEGFR,** MTOR inhibitor
Ceflatonin (Homoharringtonine) (ChemGenex)	Downregulates BEG in leukemic cells
Celebrex (Celecoxib) *(Pfizer)*	Increases endostatin
GW786034 (Pazopanib) *(GlaxoSmithKline)*	**VEGFR**
LY317615 (Enzastaurin) *(Eli Lilly & Company)*	**VEGF**
Neovastat (Benefin/AE941) *(Aeterna Zentaris)*	**VEGFR-2** and MMP inhibitor
Nexavar (Sorafenib/BAY439006) *(Bayer/Onyx)*	**VEGFR-2** and PDGFR-beta
PTK787 (Vatalanib) *(Novartis)*	**VEGFR-1, 2,** PDGFR
RAD001 (Everolimus) *(Novartis)*	**VEGFR,** MTOR
Revlimid (Lenalidomide/CC5013) *(Celgene)*	**VEGF, precursor endothelial cells**
Suramin *(NCI)*	IGF-1, EGFR, PDGFR, TGF-b, inhibits **VEGF** & bFGF
Sutent (SU11248) *(Pfizer)*	**VEGFR-1, 2, 3,** PDGFR
Tarceva (OSI774/Erlotinib) *(Genentech/OSI)*	HER1, EGFR
Tetrathiomolybdate (TM) *(Univ. of Michigan)*	**VEGF,** Copper chelator
Thalidomide *(Celgene Corporation)*	**VEGF, precursor endothelial cells**
VEGF Trap *(Regeneron Pharm.)*	**VEGF**
Velcade (PS341/Bortezomib) *(Millennium Pharm.)*	**VEGF**
ZD6474 (Zactima/Vandetanib) *(AstraZeneca)*	**VEGFR-2**, EGFR

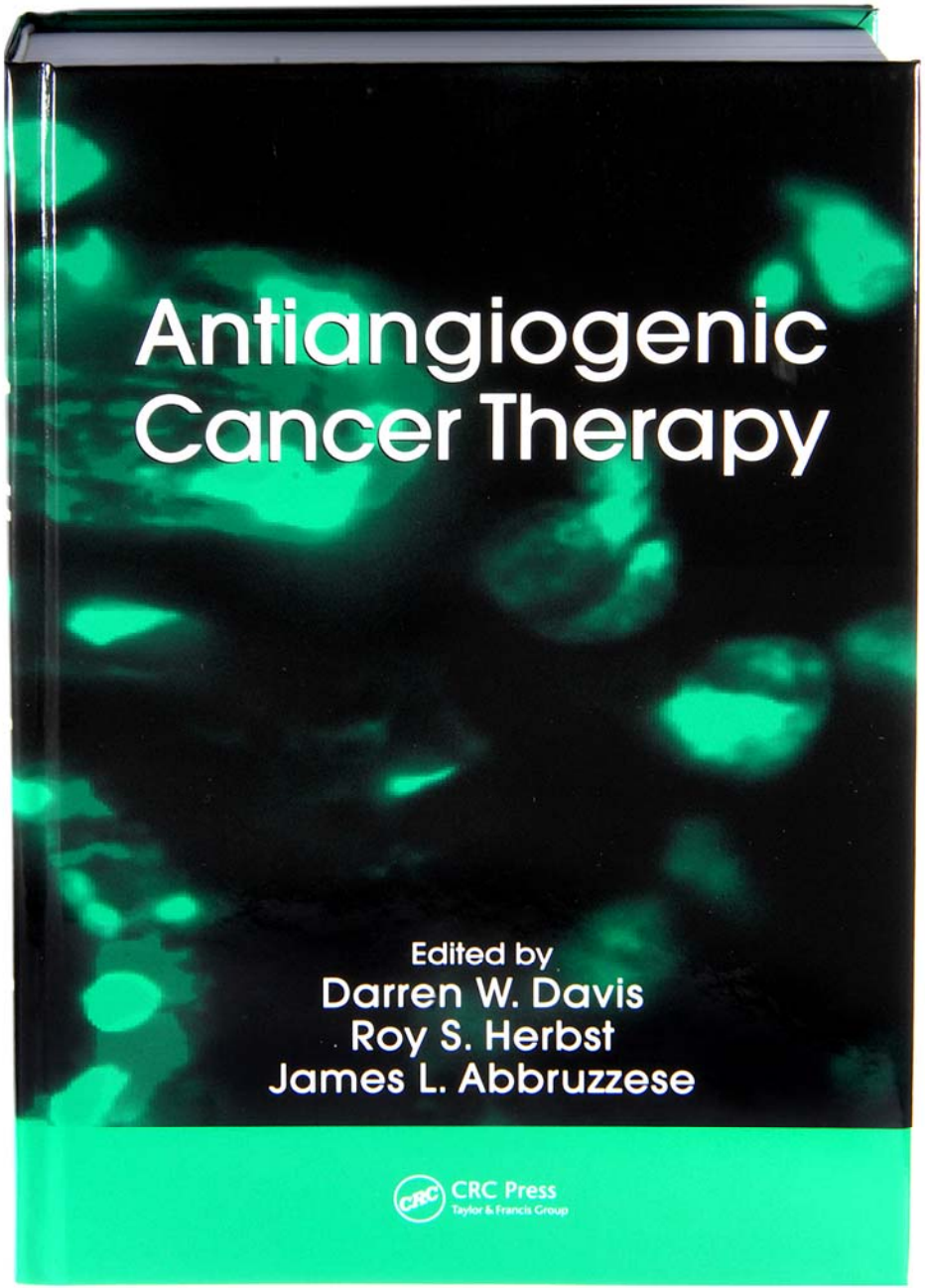
Drugs in blue are FDA approved for one tumor and are in Phase III clinical trials for other tumors.

Phase II - Drugs with varying degrees of antiangiogenic activity.
May 2007. (Not yet FDA approved)

Agent	Target
A6 *(Angstrom Pharmaceuticals)*	Binds to uPA cell surface receptor
ABT-510 *(Abbott Laboratories)*	Thrombospondin-1 receptor CD36
Actimid (CC4047) *(Celgene Corp.)*	Downregulate TNF-α
AHD-1 (Exherin) *(Adherex Technologies, Inc.)*	VE-Cadherin
AEE788 *(Novastis)*	ErbB, **VEGFR**
AG-013736 *(Pfizer)*	**VEGF**, PDGFR
AMG706 *(Amgen)*	**VEGF,** PDGFR, KITR, RetR
AP23573 *(Ariad Pharmaceuticals)*	**VEGF,** MTOR Inhibitor
AS1404 *(Antisoma)*	Vascular disrupting; releases TNF-α & vWF
Atiprimod *(Callisto Pharm.)*	**VEGF**, bFEGF, IL6
ATN-161 *(Attenuon)*	Alpha 5 beta 1 antagonist
BIBF1120 *(Boehringer Ingelheim LTD)*	**VEGF,** PDGF, FGF receptor kinases
BMS-582664 *(Bristol-Meyers-Squibb)*	**VEGFR-2**
CDP-791 *(ImClone)*	**VEGFR-2,** KDR
Combretastatin *(Oxigene)*	VE-Cadherin
E7820 *(Elsai)*	**Inhibits integrin alpha 2 subunit on endothelium**
EMD 121974 (Cilengitide) *(EMD)*	Alpha v beta 3 and 5 antagonist
Genistein *(McKesson Health Solutions)*	**Suppresses VEGF, neuropilin, & MMP-9**
INGN 241 *(Introgen Therapeutics)*	**VEGF**, MDA-7
Interleukin-12 *(NCI)*	Upregulates IP10
MEDI 522 (Abergrine) *(MedImmune)*	Antibody Alpha V beta 3
MLN518 (Tandutinib) *(Millennium)*	**FLT3, PDGFR, c-Kit, CSF-1R**
Panzem (2ME2) *(EntreMed)*	Inhibits tubulin polymerization
PI-88 *(Progen Industries/Medigen)*	bFGF, stimulates release of TSP1
PKC412 *(Novartis)*	**VEGFR-2**
PXD101 *(CuraGen Corporation)*	HDAC inhibitor
SU-O14813 *(Pfizer)*	**VEGFR-3, PDGFR-a, PDGFR-b, RET, FLT3**
Tempostatin *(Collard Biopharm.)*	Extracellular matrix proteins
XL647 *(Exelisis)*	VEGFR-2, EGFR, ErbB2, EphB4
XL784 *(Exelisis)*	ADAM-10, MMPs
XL880 *(Exelisis)*	**VEGFR-2, C-met, RTK**
XL999 *(Exelisis)*	**VEGFR, PDGFR, FGFR, Flt-3, Src**

Leukemia: Drugs with **anti-angiogenic** activity and varying degrees of other activities. Phase II & III (April, 2007).

Agent	Target	Leukemia
ABT-869 *(Abbott Laboratories)*	**VEGFR, RTK inhibitor, PDGFR**	**AML, MDS (I)**
AG-013736 *(Pfizer)*	**VEGFR, PDGFR**	**AML, MDS**
AP23573 *(Ariad Pharmaceuticals)*	**VEGF, mTOR**	**Heme malignancies**
Avastin *(Genentech)*	**VEGF**	**AML, CLL, CML**
AZD2171 *(AstraZeneca)*	**VEGFR 1, 2, 3, PDGFR**	**CLL**
CCI-779 *(Wyeth)*	**VEGFR, mTOR**	**CML(I), CLL**
Ceflatonin/Homoharringtonine/HHT *(ChemGenex Therapeutics)*	**?VEGF**	**CML, APML (CML III)**
MLN518/Tandutinib *(Millenium)*	**FLT3, PDGFR, c-Kit, CSF-1R**	**AML**
Nexavar/Sorafenib/BAY439006 *(Bayer/Onyx)*	**VEGFR 2, PDGFR**	**ALL, CML, MDS (I) CLL, AML, CML**
PKC412 *(Novartis)*	**VEGFR 2**	**AML(I), mast cell leukemia AML, MDS**
PXD101 *CuraGen Corporation)*	**HDAC inhibitor**	**Heme malignancies(I), AML**
Revlimid/CC5013/Lenalidomide *(Celgene)*	**VEGF, precursor endothelial cells**	**AML, CLL, B-cell CLL (III)**
Sutent/SU11248 *(Pfizer)*	**VEGFR 1, 2, 3, PDGFR**	**CLL**
Thalidomide *(Celgene)*	**VEGF, precursor endothelial cells**	**AML, CLL**
TKI258/CHIR-258 *(Novartis / Chiron Corporation)*	**VEGFR, FLT-3 FGFR**	**AML**
Velcade/PS341/Bortezomib *(Millenium Pharmaceuticals)*	**VEGF**	**AML, CLL, CML (I), leukemia/lymphoma**



2007. 841 pages. CRC Press London, New York. http//www.crcpress.com

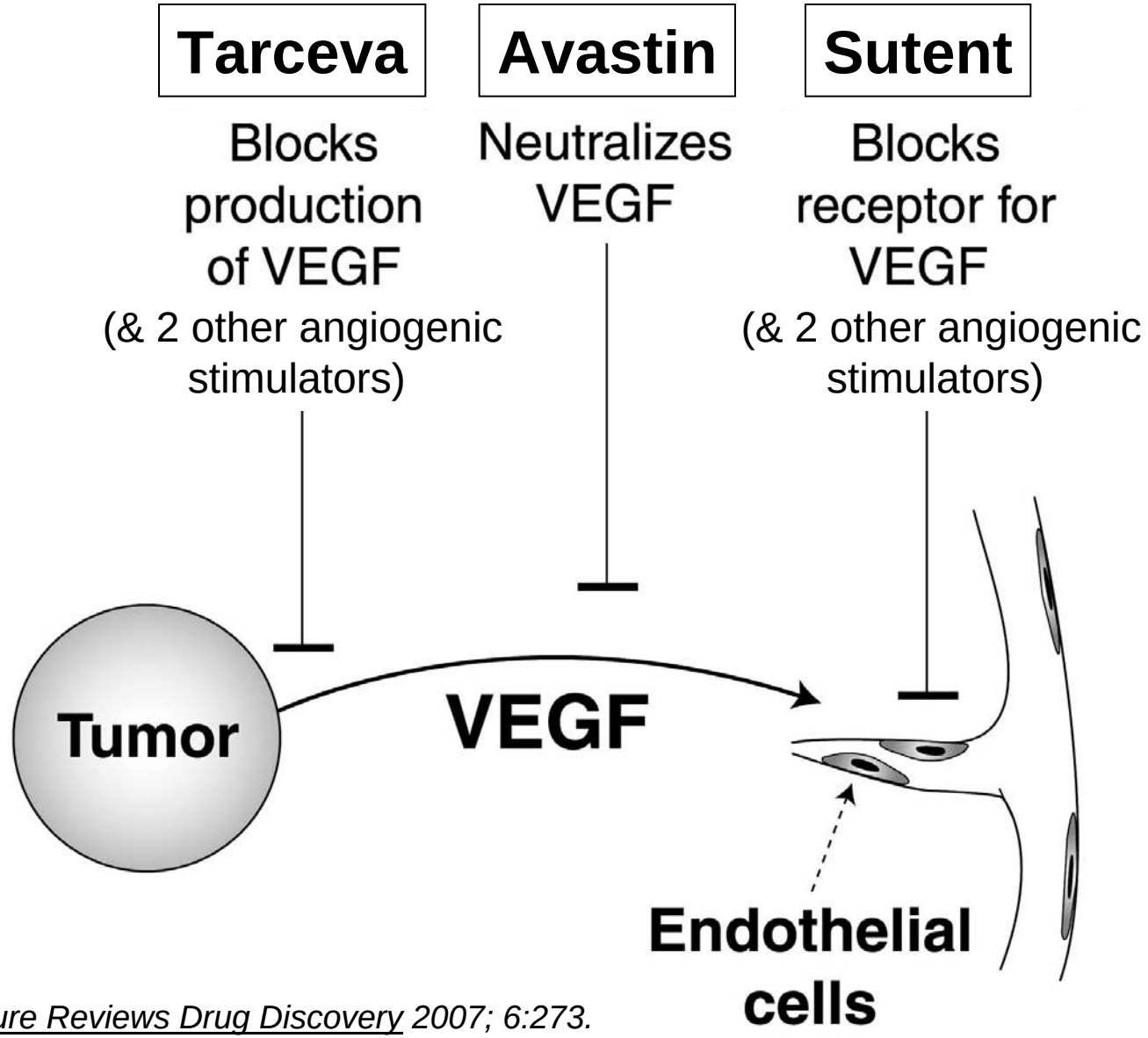
Conclusion 1.

- **10** drugs with **anti-angiogenic** activity have been approved by the FDA in the U.S., and by regulatory agencies in more than **30** other countries, **. . . . . .**

- to treat cancer and age-related macular degeneration, **. . . . .**

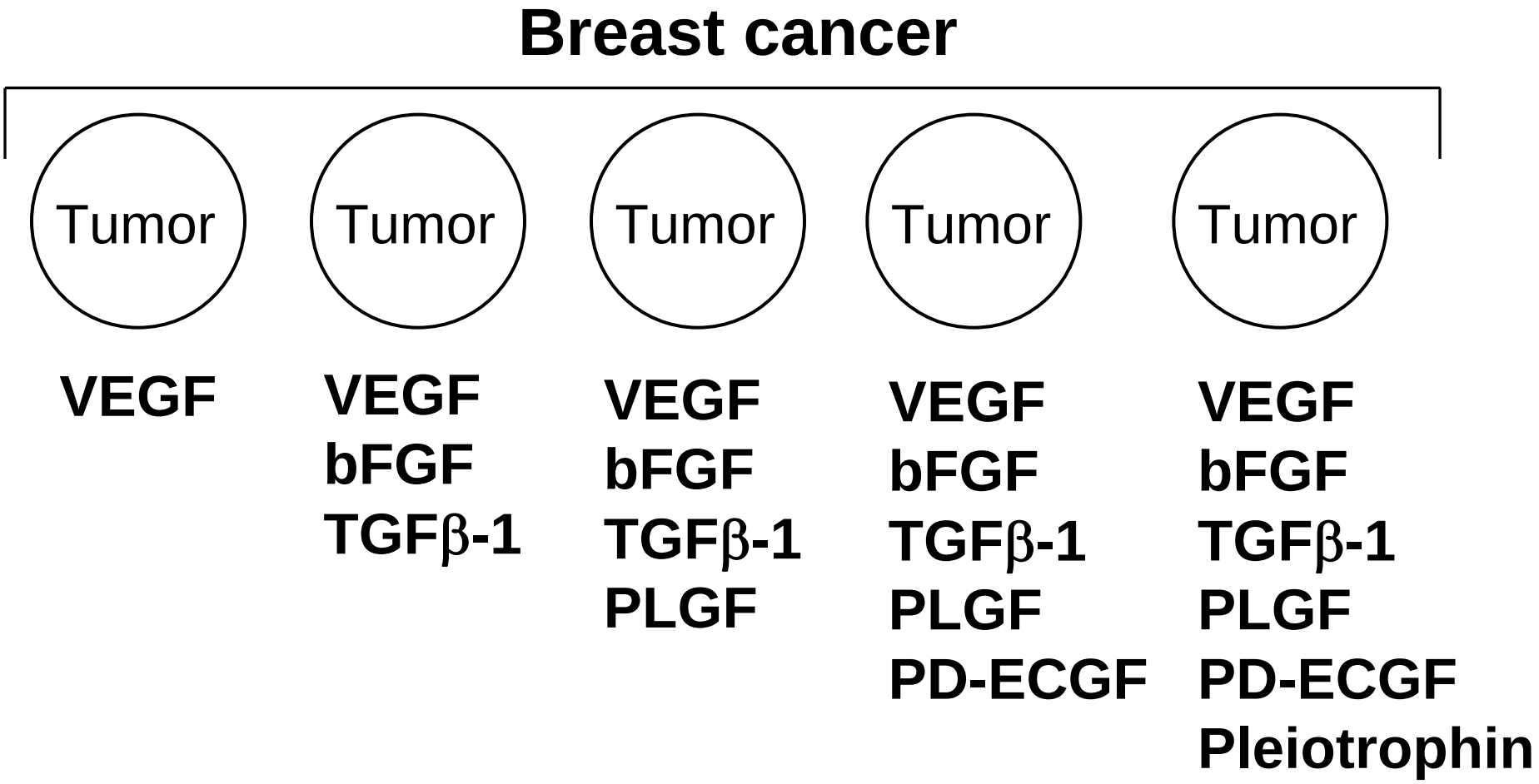
- But, the **majority** of these drugs provide only **mono-anti-angiogenic therapy**, i.e., . they block only a **single** pro-angiogenic protein produced by tumors.

"Indirect" angiogenesis inhibitors target tumor cell products and usually inhibit only 1 or 2 pro-angiogenic proteins.



Folkman, Nature Reviews Drug Discovery 2007; 6:273.

Most human tumors can produce up to **6** or more pro-angiogenic proteins.



Adapted from Relf et al., Cancer Research 1997, 57:963

- Certain **compensatory** mechanisms in the angiogenesis network permit tumors to eventually **"evade"** **mono-**anti-angiogenic therapy.
- **"Evasion"** of antiangiogenic therapy differs from resistance to chemotherapy.

★ Inhibition of only **VEGF,** eventually results in **upregulation** of **bFGF** and **PLGF.**

(Willett 2005) (Casanovas [Hanahan], 2005)

★ Inhibition of the **EGF receptor** eventally results in **upregulation** of **VEGF.**

(Viloria-Petit 2004) (Bianco et al., 2005)

★ Genetic silencing of **integrin β3** or **HIF-1** eventually results **in upregulation of VEGF receptor-2** and **IL-8,** respectively.

(Carmeliet 2002) (Reynolds 2002) (Mizukami 2005)

Proc. Nat'l Acad. Sci. USA, 104: 967, ***2007***

Combination angiostatic therapy completely inhibits ocular and tumor angiogenesis

Michael I. Dorrell, Edith Aguilar, Lea Scheppke, Faith H. Barnett, and Martin Friedlander*

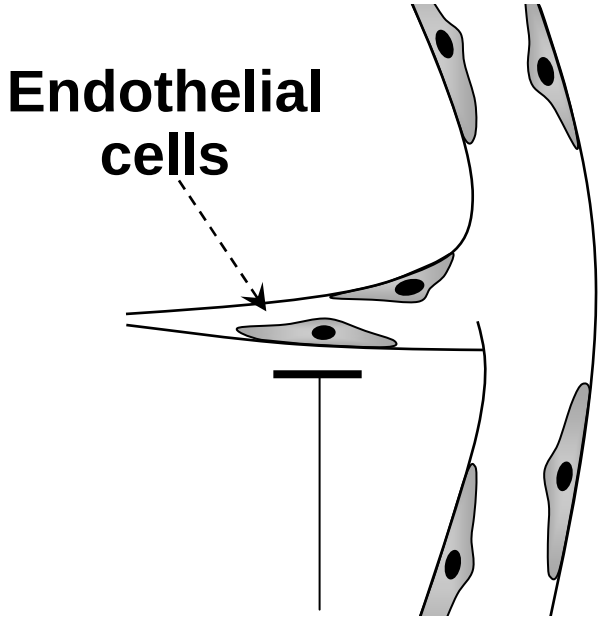
1. Individual angiostatic **mono-therapies** resulted in **compensatory upregulation** of other pro-angiogenic proteins.

2. When **VEGF** was blocked in the neonatal mouse retina, there was **compensatory upregulation** in the **vitreous** of:

   aFGF **TNF-alpha** **IL-1a** **Leptin**
   bFGF **TGF-alpha** **IL-6**

3. **Triple-combination angiostatic** therapy resulted in **complete inhibition** of ocular neovascularization, & potent reduction in **brain tumor** angiogenesis in mice.

"Direct" angiogenesis inhibitors target proliferating endothelium directly, and inhibit multiple angiogenic proteins.



Angiostatin

Endostatin

Platelet factor 4

Thrombospondin

Tumstatin

Downregulates

VEGF

bFGF

bFGF receptor

HIF1α

EGF receptor

Upregulates

Thrombospondin-1

Maspin

HIF1α inhibitor

TIMP-2

Neuropilin

Folkman, Nature Reviews Drug Discovery 2007; 6:273-286.

Endogenous angiogenesis inhibitors in blood or tissues.

1980	**Interferon α/β, new activity**	(Brouty-Boye, D. and Zetter, B.R. ***Science*** *208: 516-518, 1980*)
1982	**Platelet factor 4,** Protamine	(Taylor, S. and Folkman, J. ***Nature*** *297: 307-312, 1982*)
1985	**Angiostatic steroids**	(Crum, R. et al. (Folkman) ***Science*** *230: 1375-1378, 1985*)
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1994	**2-methoxyestradiol***	(D'Amato, R.J. et al. (Folkman) ***PNAS*** *91:3964-3968, 1994*)
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2005	Caplostatin	(Satchi-Fainaro, R. et al. (Folkman) ***Cancer Cell*** *7: 251-261, 2005)*

Folkman, Nature Reviews Drug Discovery 2007; 6:273.

Additional **endogenous** angiogenesis inhibitors.

J. Folkman, *Acta Pathologica Microbiol. et Immunol. Scandinavica* , *112:496,* ***2004***

Also see: Nyberg et al (Kalluri) Cancer Research 65:3967, ***2005****.*

1.	Alphastatin		
2.	Angiostatin		
3.	Arresten		
4.	Anti-thrombin III (truncated)		
5.	Canstatin		
6.	**Endostatin**	(under p53 control)	Approved, China
7.	Fibulin-5		
8.	Interferon-beta		*Phase III*
9.	Maspin		
10.	2-methoxyestradiol		*Phase II*
11.	Pigment epithelial derived factor (PEDF)		
12.	Platelet factor 4 (PF4)		
13.	Tetrahydrocortisol		*Phase II*
14.	**Thrombospondin-1**	(under p53 control)	*Phase II*
15.	TIMP-2		
16.	**Tumstatin**	(under p53 control)	
17.	Fragment of histidine-rich glycoprotein		
18.	Semaphorin 3F		

Experiment #1:

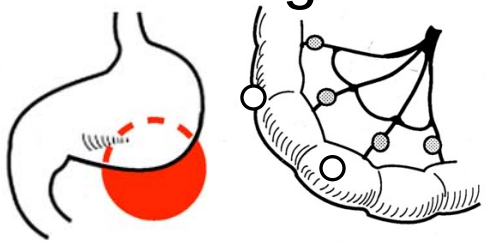
“Can endogenous, broad-spectrum angiogenesis inhibitors, induce durable blockade of tumor angiogenesis in a specific **anatomical region** of the body**?”**

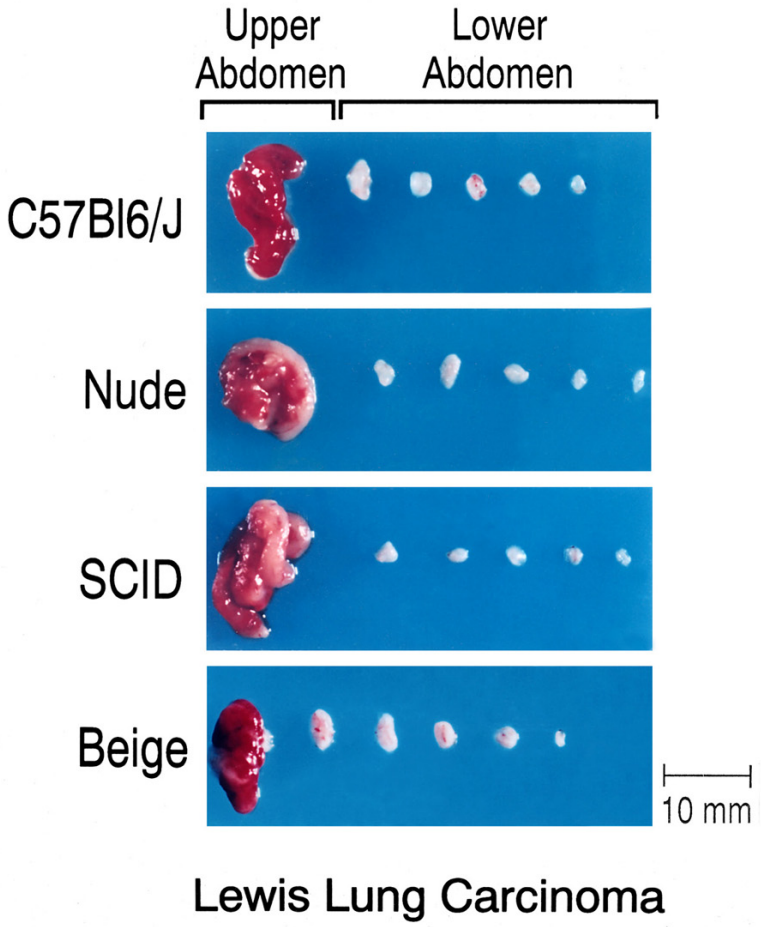
Example: ***Lower abdominal cavity.***

(Constituitive host response)

Mouse model of human ovarian cancer:

Tumors in the **lower** peritoneal cavity remain **non-angiogenic** and do not grow beyond **~ 1 mm^3**



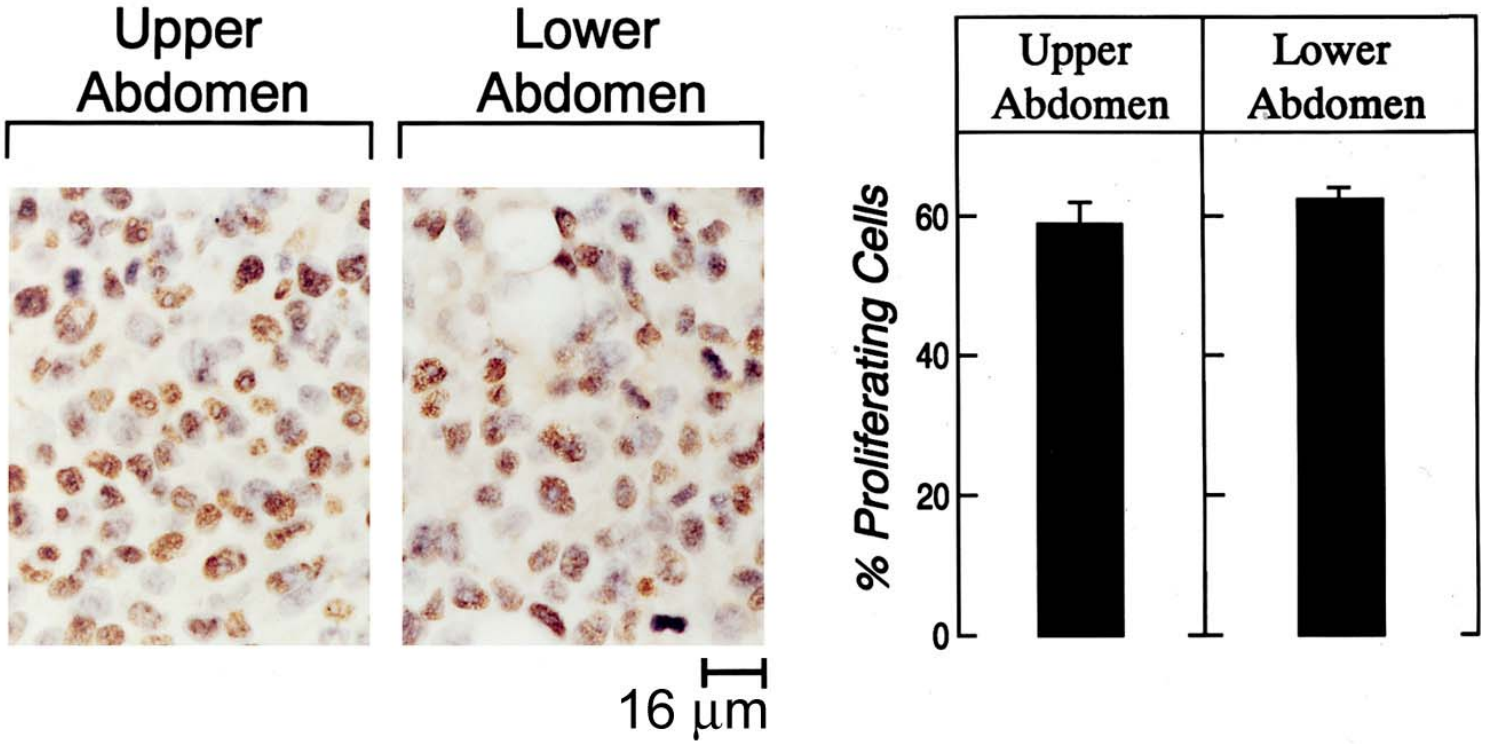


Lewis Lung Carcinoma

Catherine Chen, & Folkman

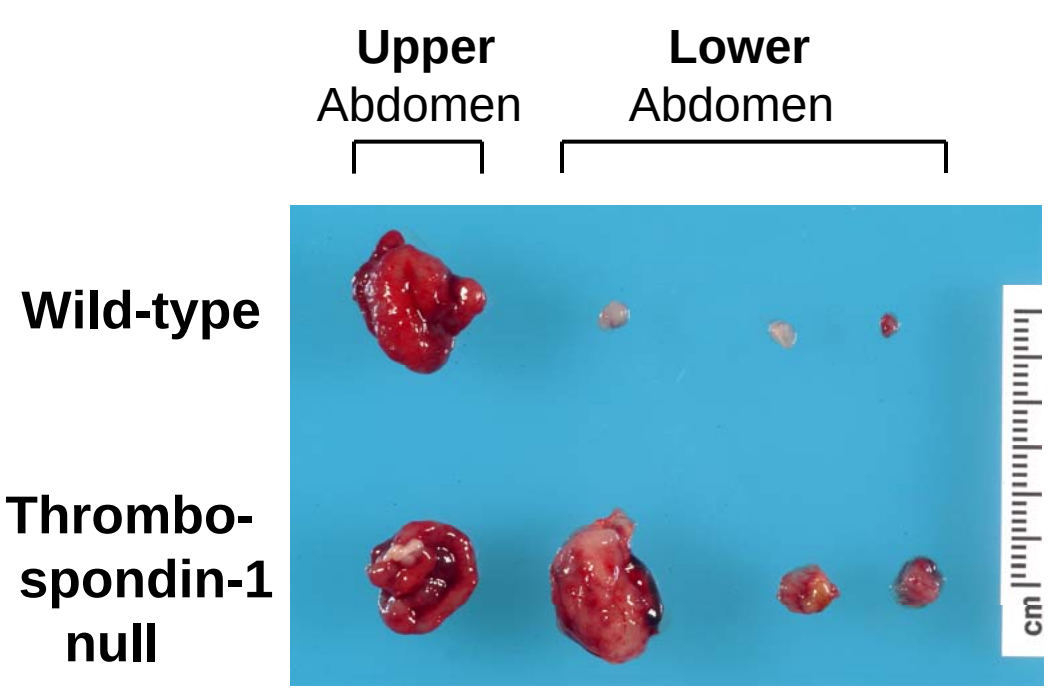
Intraperitoneal tumor cells all **proliferate** at the same rate for a given tumor type, whether it is in the upper or lower abdomen.

PCNA = ~ 60% in tumors in upper and lower abdomen.

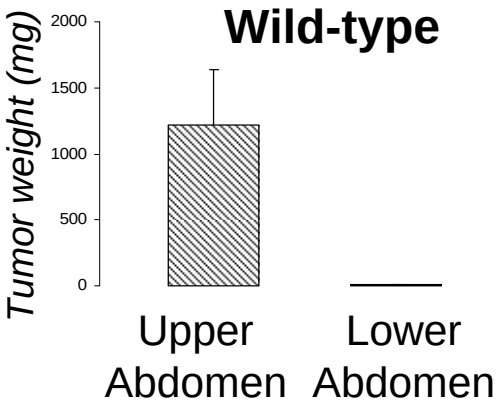


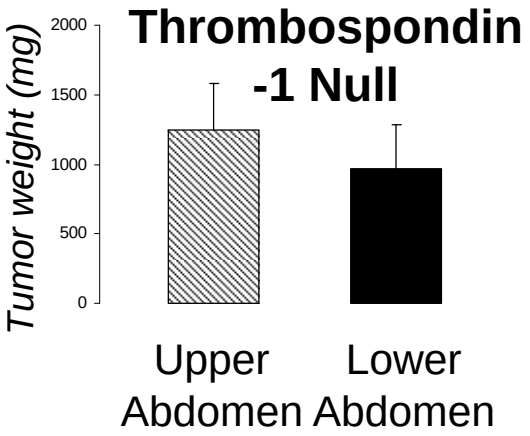
Lewis lung carcinoma

In mice lacking **thrombospondin-1,** **angiogenic** tumors grow in the **lower** and **upper** abdomen.



Lewis lung carcinoma





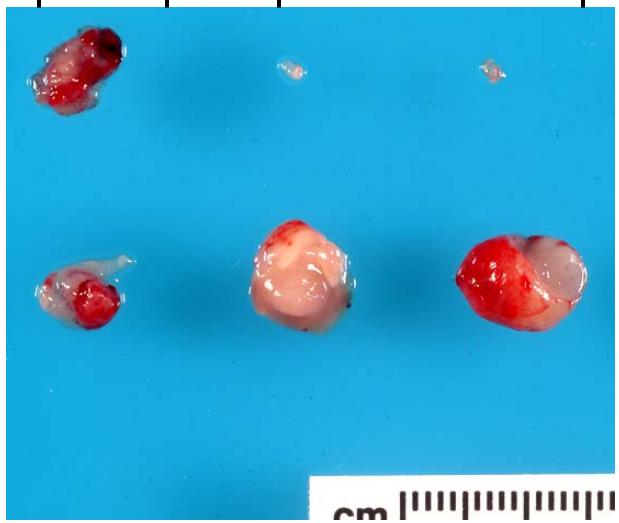
Ryeom, Zaslavsky, Chen, Folkman, et al. **2007**

In mice lacking **thrombospondin-1** & **endostatin,** **angiogenic** tumors in the lower abdomen are **3-fold** larger than **angiogenic** tumors in the upper abdomen.

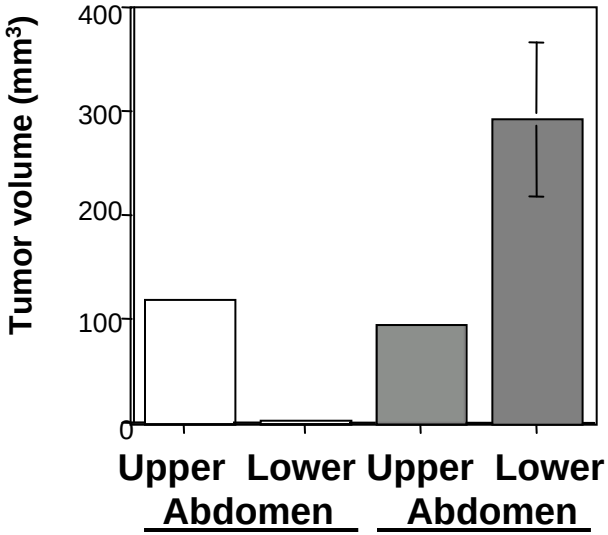
Upper Abdomen **Lower** Abdomen

Wild-type

Thrombospondin and **endostatin null**



Lewis lung carcinoma



Sandra Ryeom, Alex Zaslavsky, Chen et al (Folkman) unpublished, ***2007.***

- *Endostatin null mice from Bjorn Olsen.*
- *Thrombospondin-1 null mice from Jack Lawler.*
- *Double null mice by Sandra Ryeom, Folkman lab, (BCRF supported)*

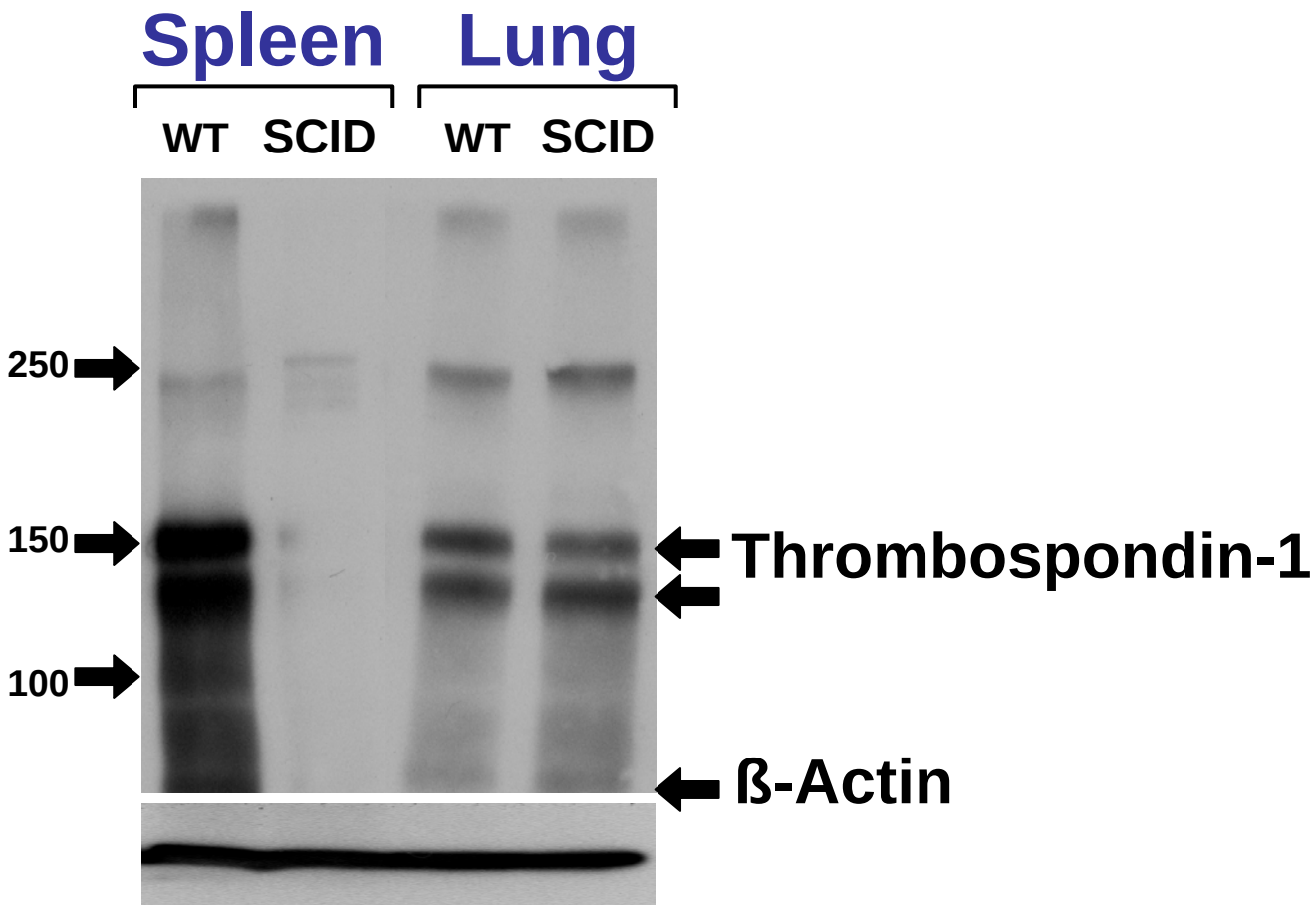
Experiment #2:

“Can endogenous, broad-spectrum angiogenesis inhibitors, induce durable blockade of tumor angiogenesis in a specific **anatomical region** of the body**?”**

Example: ***Spleen***

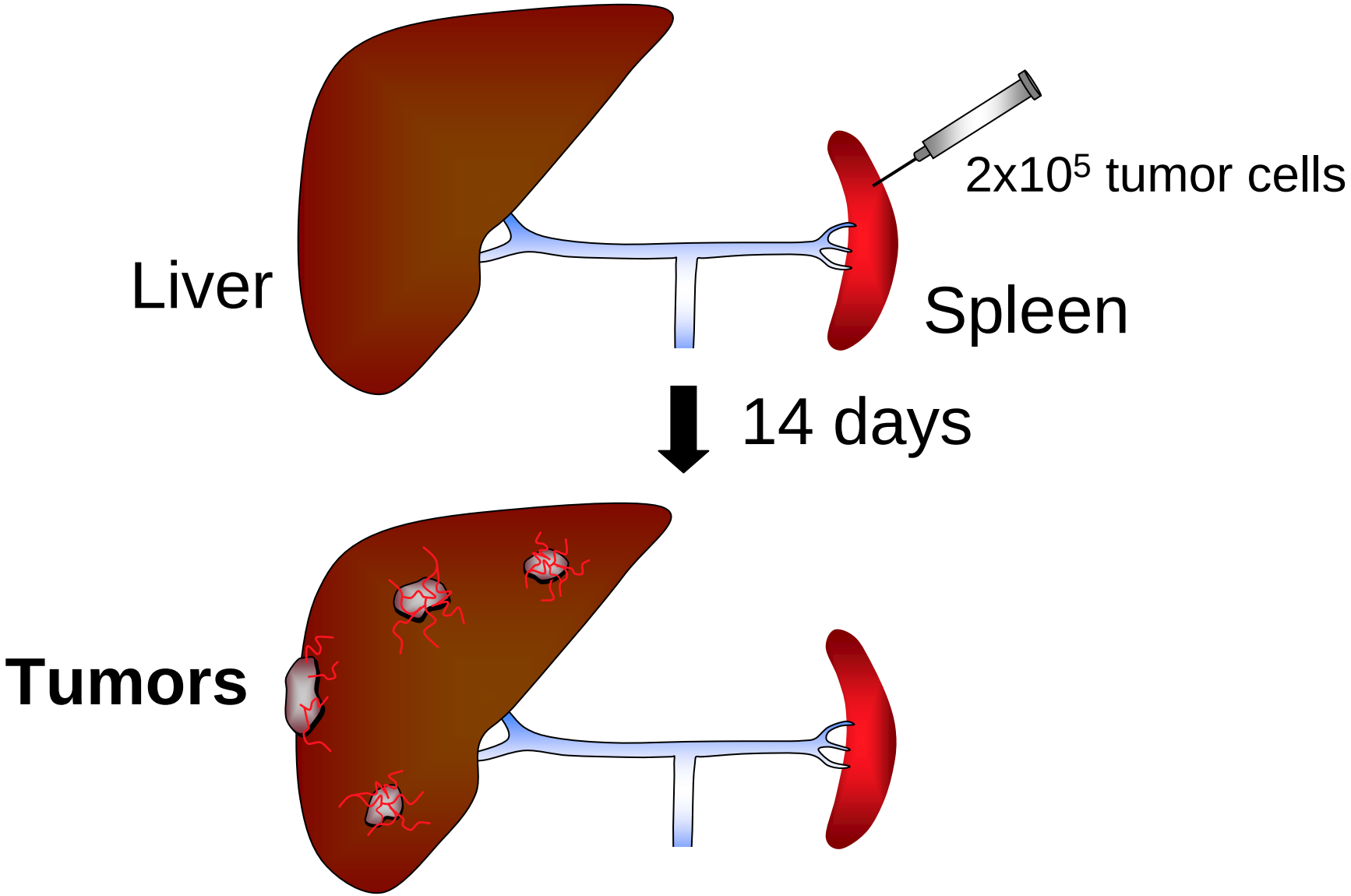
(Constituitive host response)

Thrombospondin-1 is **over-expressed** in spleen of wild-type mice, but **disappears** in **SCID** **spleens.**



Sandra Ryeom, Kwan-Hyuck Baek, Folkman **2007**

Experimental model of liver metastasis in mice.

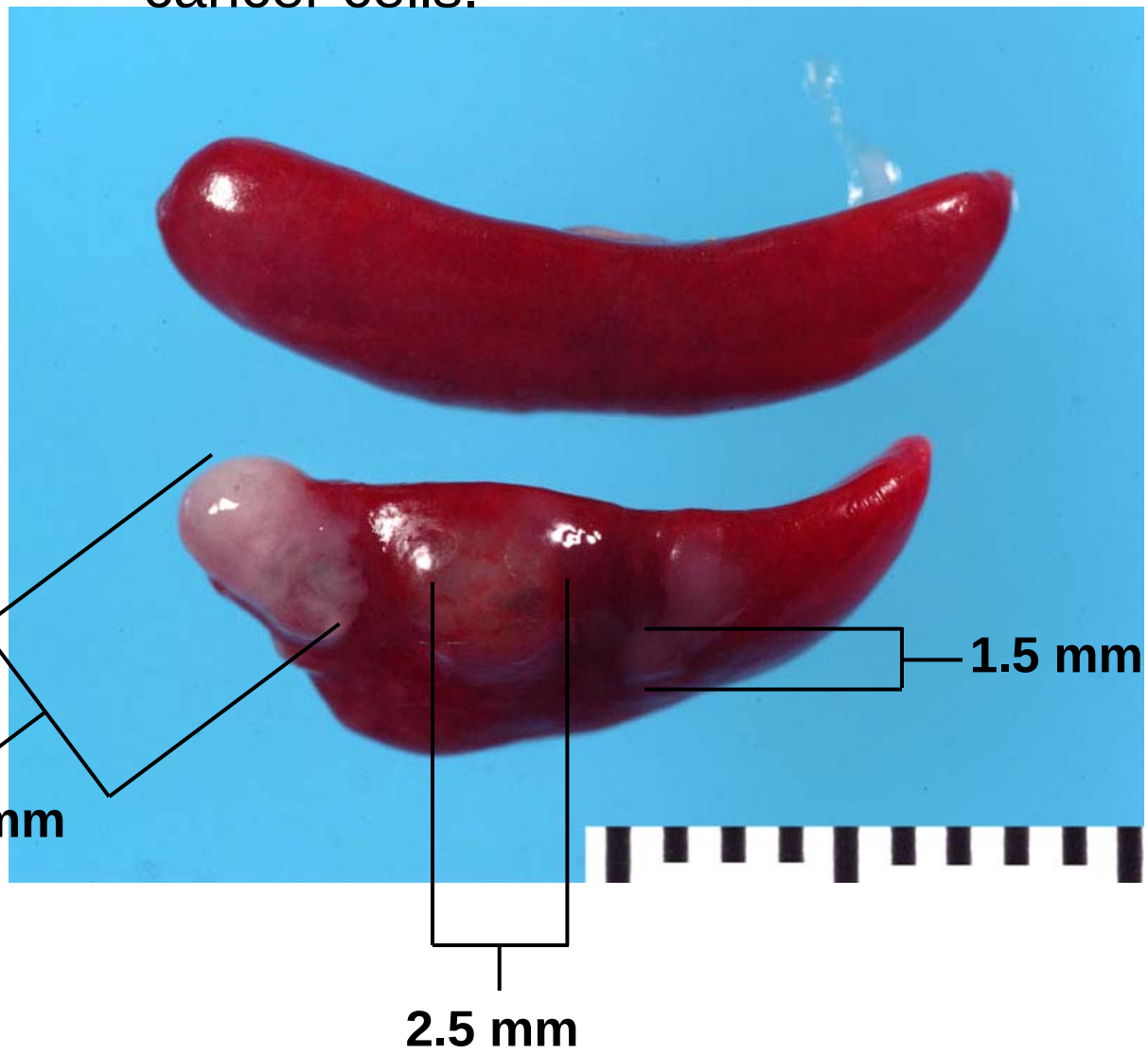


Mouse spleens

C57Bl/6 mice. **10 days** after intrasplenic injection of Lewis lung cancer cells.

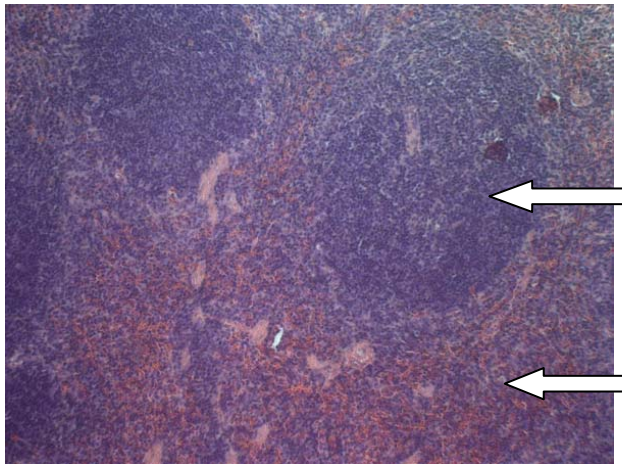
Wild type

Thrombospondin-1 null
&
endostatin null mouse.

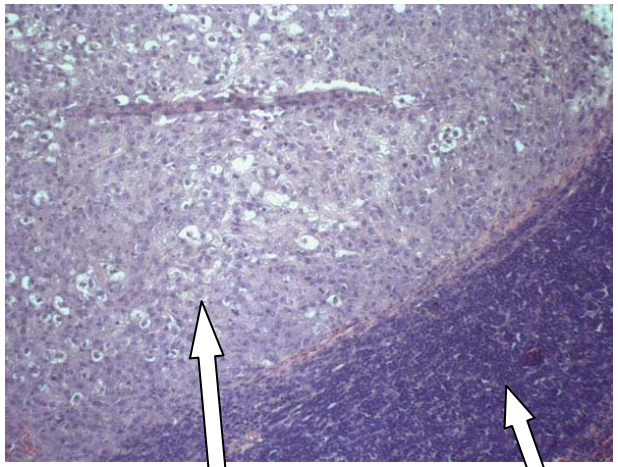


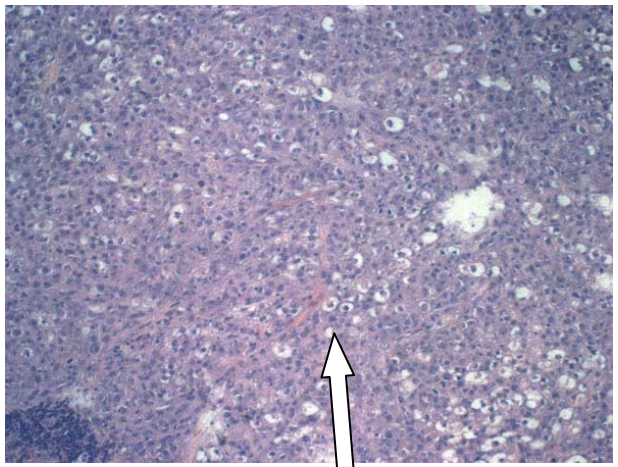
Intrasplenic injection of Lewis lung **carcinoma** cells in mice.

Wild type



Thrombospondin-1 null & endostatin null





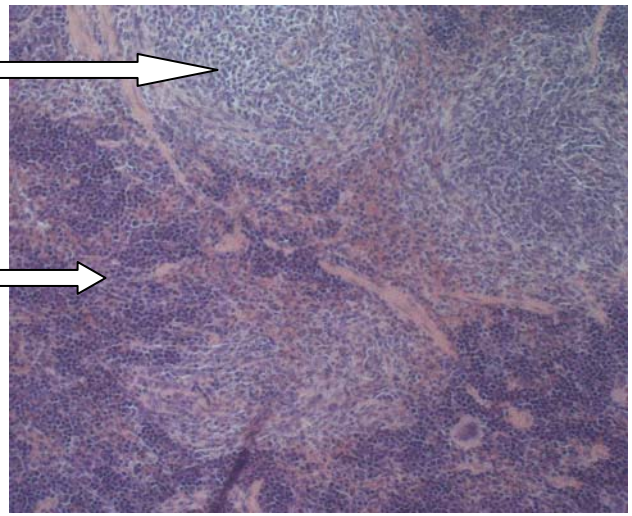
SCID mouse spleens after intrasplenic injection of Lewis lung carcinoma cells

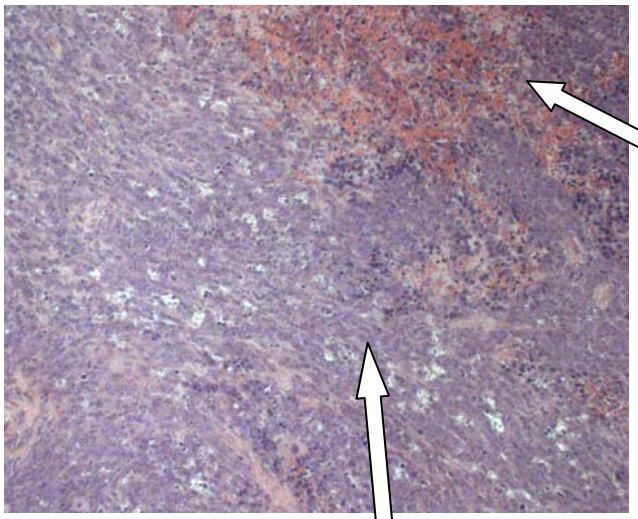
Thrombospondin-1 *is* ***absent*** *in spleens of* ***SCID*** *mice.*

No tumor **Tumor cells injected**

Splenic nodules

Red pulp (Disorganized)

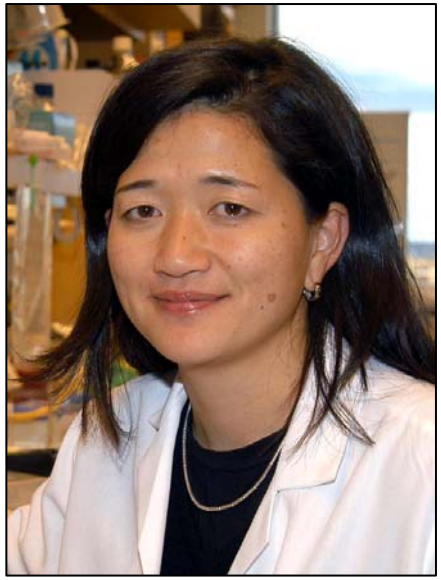




Red pulp

Tumor

Thanks to:



Sandra Ryeom, Ph.D.

Alexander Zaslavsky, Ph.D.

Kwan Hyuck Baek, Ph.D.

Catherine Chen, M.D., Ph.D.

Experiment #3:

"Can endogenous, broad-spectrum angiogenesis inhibitors induce durable blockade of angiogenesis in a **tumor?**

Example: ***Neutrophils*** *that deliver* ***thrombospondin-1*** *&* ***endostatin*** *to the tumor.*

(Deletion of a single gene).

*PLoS One, 2: e260, February, **2007***

PPARα Deficiency in Inflammatory Cells Suppresses Tumor Growth

Arja Kaipainen[1], Mark W. Kieran[1,2], Sui Huang[1], Catherine Butterfield[1], Diane Bielenberg[1], Gustavo Mostoslavsky[3], Richard Mulligan[3], Judah Folkman[1], Dipak Panigrahy[1]*

1 Vascular Biology Program, Department of Surgery, Children's Hospital, Harvard Medical School, Boston, Massachusetts, United States of America, **2** Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, United States of America, **3** Department of Genetics, Harvard Medical School, Boston, Massachusetts, United States of America

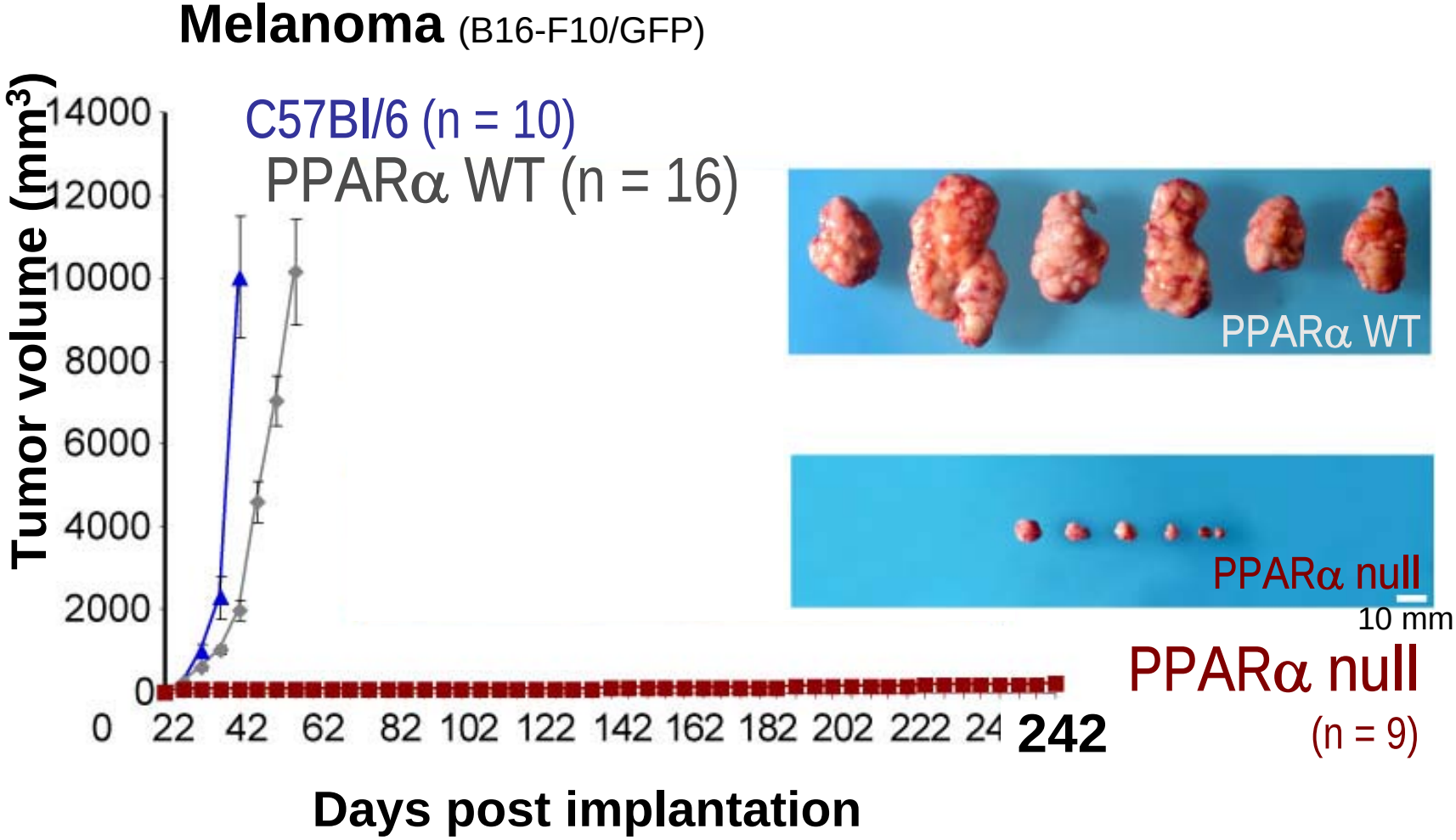
Inflammation in the tumor bed can either promote or inhibit tumor growth. Peroxisome proliferator-activated receptor (PPAR)α is a central transcriptional suppressor of inflammation, and may therefore modulate tumor growth. Here we show that PPARα deficiency in the host leads to overt inflammation that suppresses angiogenesis via excess production of the endogenous angiogenesis inhibitor thrombospondin-1 and prevents tumor growth. Bone marrow transplantation and granulocyte depletion show that PPARα expressing granulocytes are necessary for tumor growth. Neutralization of thrombospondin-1 restores tumor growth in PPARα-deficient mice. These findings suggest that the absence of PPARα activity renders inflammatory infiltrates tumor suppressive and, thus, may provide a target for inhibiting tumor growth by modulating stromal processes, such as angiogenesis.

- In **PPAR-*alpha*** deficient mice, **neutrophils** (granulocytes) overexpress **thrombospondin-1** and **endostatin**.

- The neutrophils **accumulate** in tumors, and **suppress angiogenesis** and **tumor growth**.

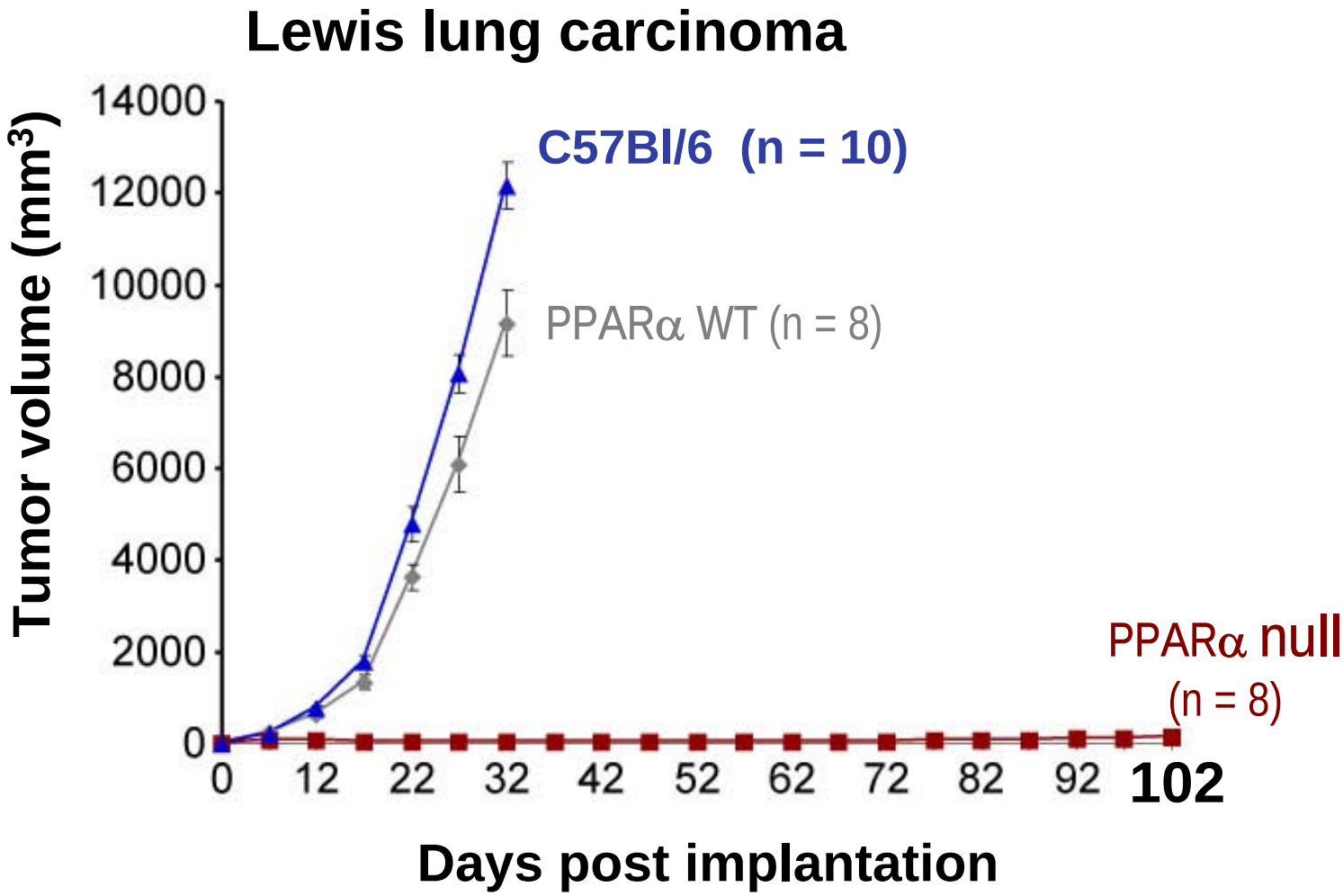
(PPAR-alpha = peroxisome proliferator-activated receptor *alpha).*

Angiogenesis, tumor growth, and metastasis are inhibited by deletion of a single gene: **PPARα**



A. Kaipainen et al., (J. Folkman and D. Panigrahy) Plos One, 2: e260, 2007

Angiogenesis, tumor growth, and metastasis are inhibited in PPARα null mice.

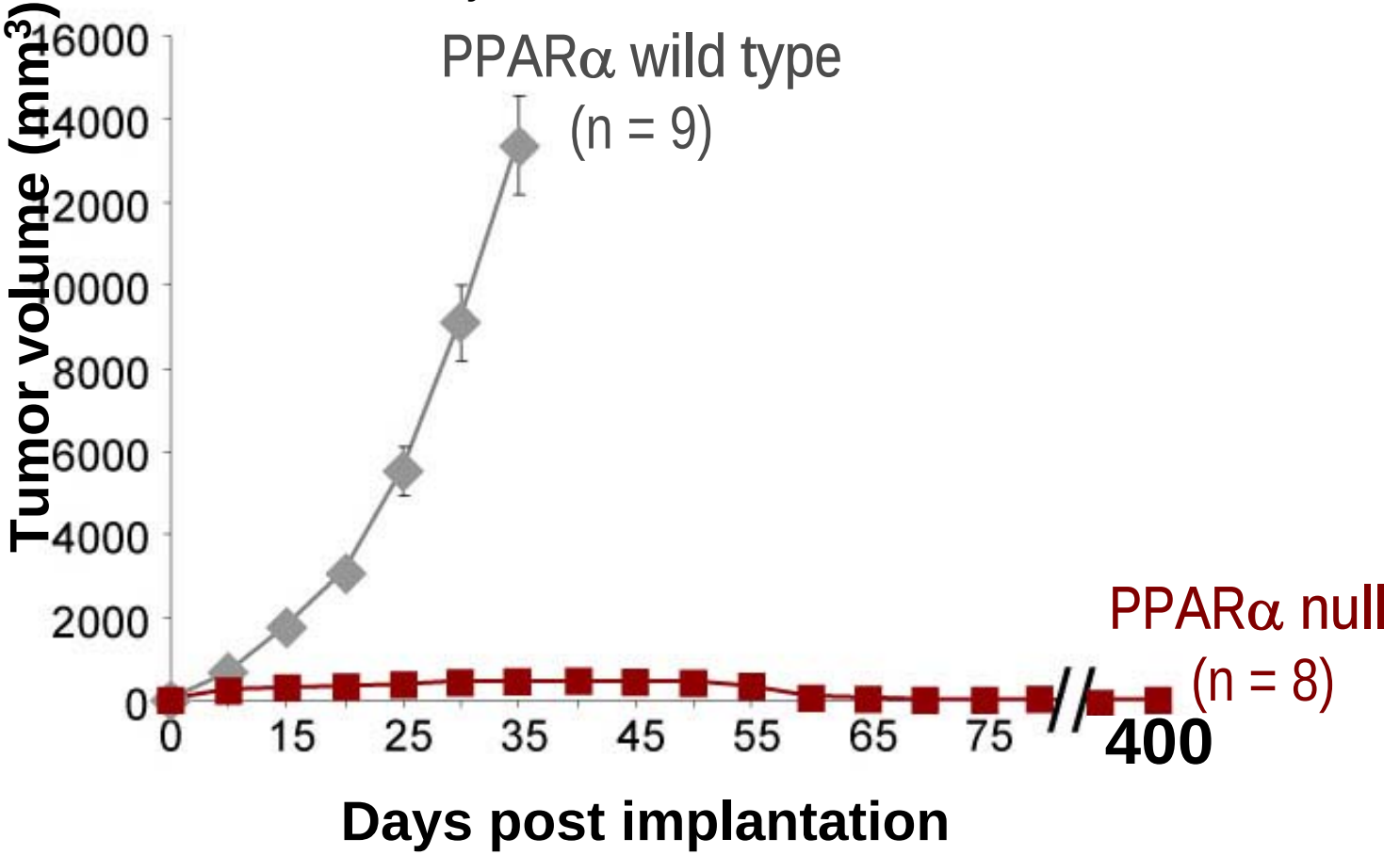


A. Kaipainen et al., (J. Folkman and D. Panigrahy) Plos One, 2: e260, 2007

Angiogenesis, tumor growth, and metastasis are inhibited in PPARα null mice.

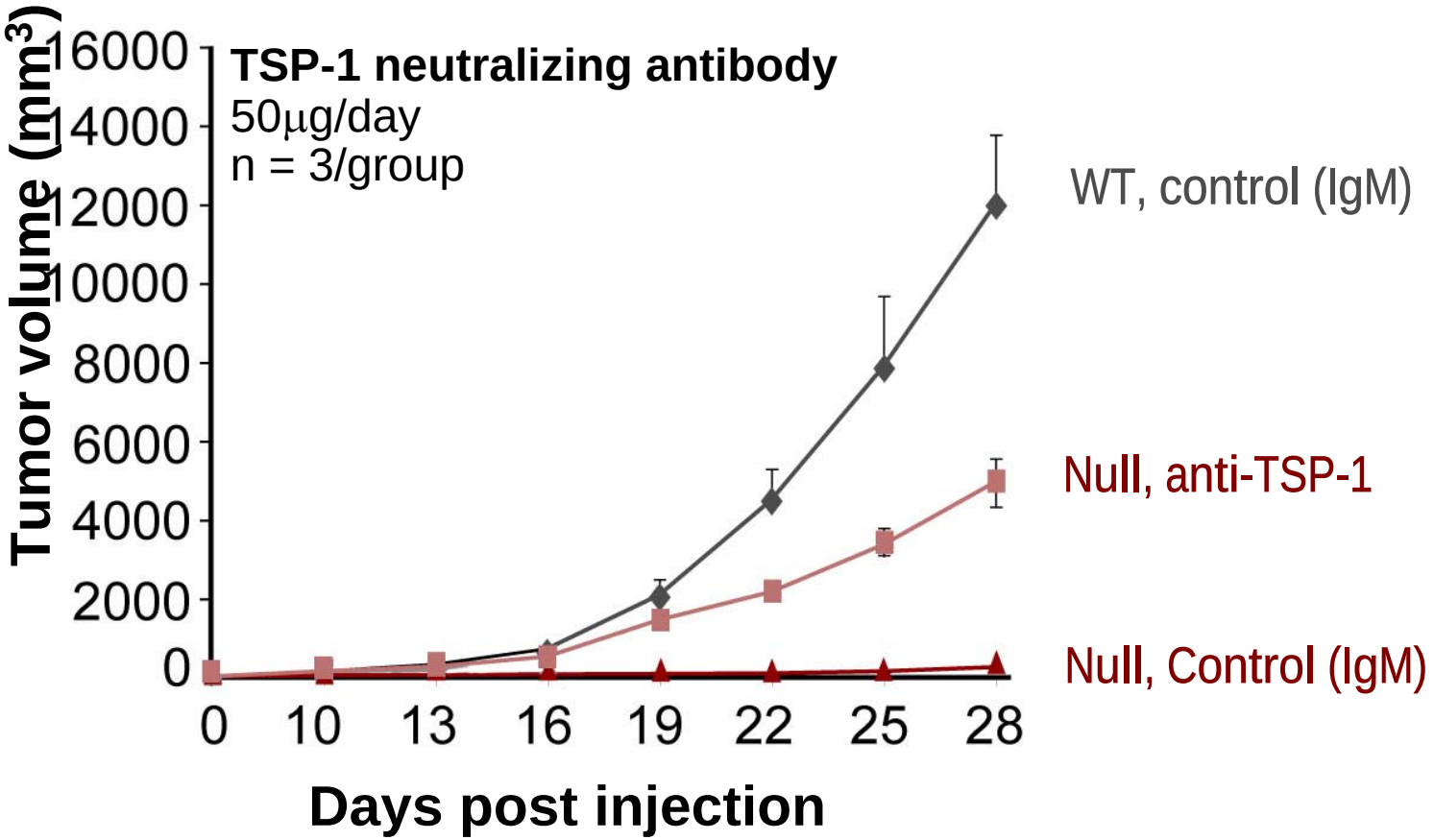
Sarcoma

(PPARα–/–) from mouse embryo fibroblasts transformed by SV40 & H-ras



A. Kaipainen et al., (J. Folkman and D. Panigrahy) Plos One, 2: e260, 2007

Melanoma in PPARα null mice, treated with **thrombospondin-1 neutralizing antibody**, (**anti-TSP-1**) or, control antibody (IgM).



A. Kaipainen et al., (J. Folkman and D. Panigrahy) Plos One, 2: e260, 2007

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PPARα Deficiency in Inflammatory Cells Suppresses Tumor Growth

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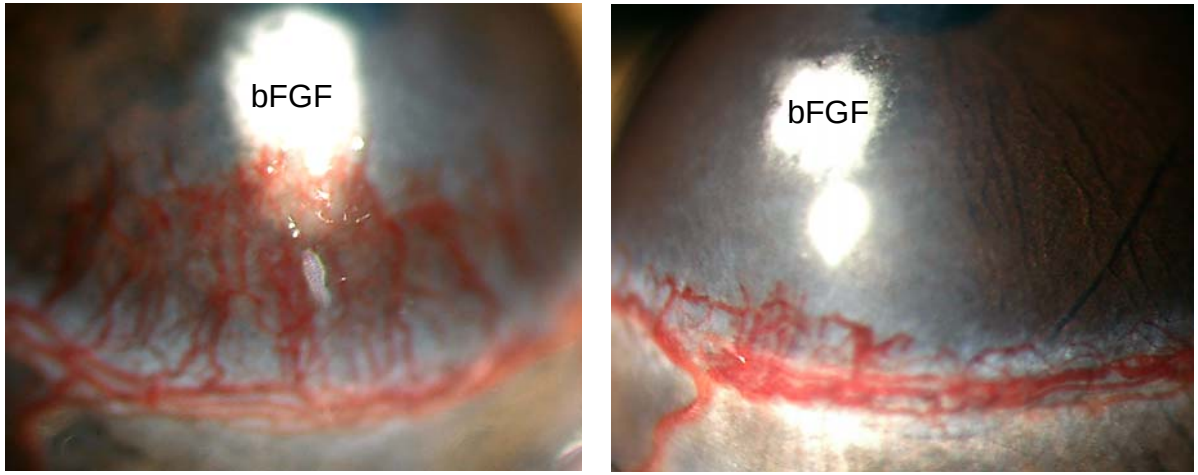
1 Vascular Biology Program, Department of Surgery, Children's Hospital, Harvard Medical School, Boston, Massachusetts, United States of America, 2 Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, United States of America, 3 Department of Genetics, Harvard Medical School, Boston, Massachusetts, United States of America

PPARα wild type **PPARα null**



1. PPAR-*alpha* deficiency in the host leads to excess production of the endogenous angiogenesis inhibitor **thrombospondin-1** in granulocytes.

2. These granulocytes inflitrate the vascular bed induced by **bFGF,** and suppress angiogenesis.

Thanks to:

Arja Kaipainen, Ph.D.

Mark Kieran, M.D, Ph.D.

Dipak Panigrahy, M.D.

Conclusion 2.

- In PPAR-alpha null mice:
  - (i) Weight gain is normal;
  - (ii) Wound healing is delayed by only a day;
  - (iii) Pregnancies are normal.

- These results demonstrate that:
  - (i) Complete blockade of tumor angiogenesis can lead to permanent tumor dormancy that is harmless to the host.

  - (ii) PPAR-alpha could become a novel drug screening target to discover broad-spectrum angiogenesis inhibitors.

Other angiogenesis regulatory proteins that also regulate the immune response.

---

1. **Hepatocyte growth factor (HGF),** stimulates angiogenesis, but **supresses dendritic** cell function.

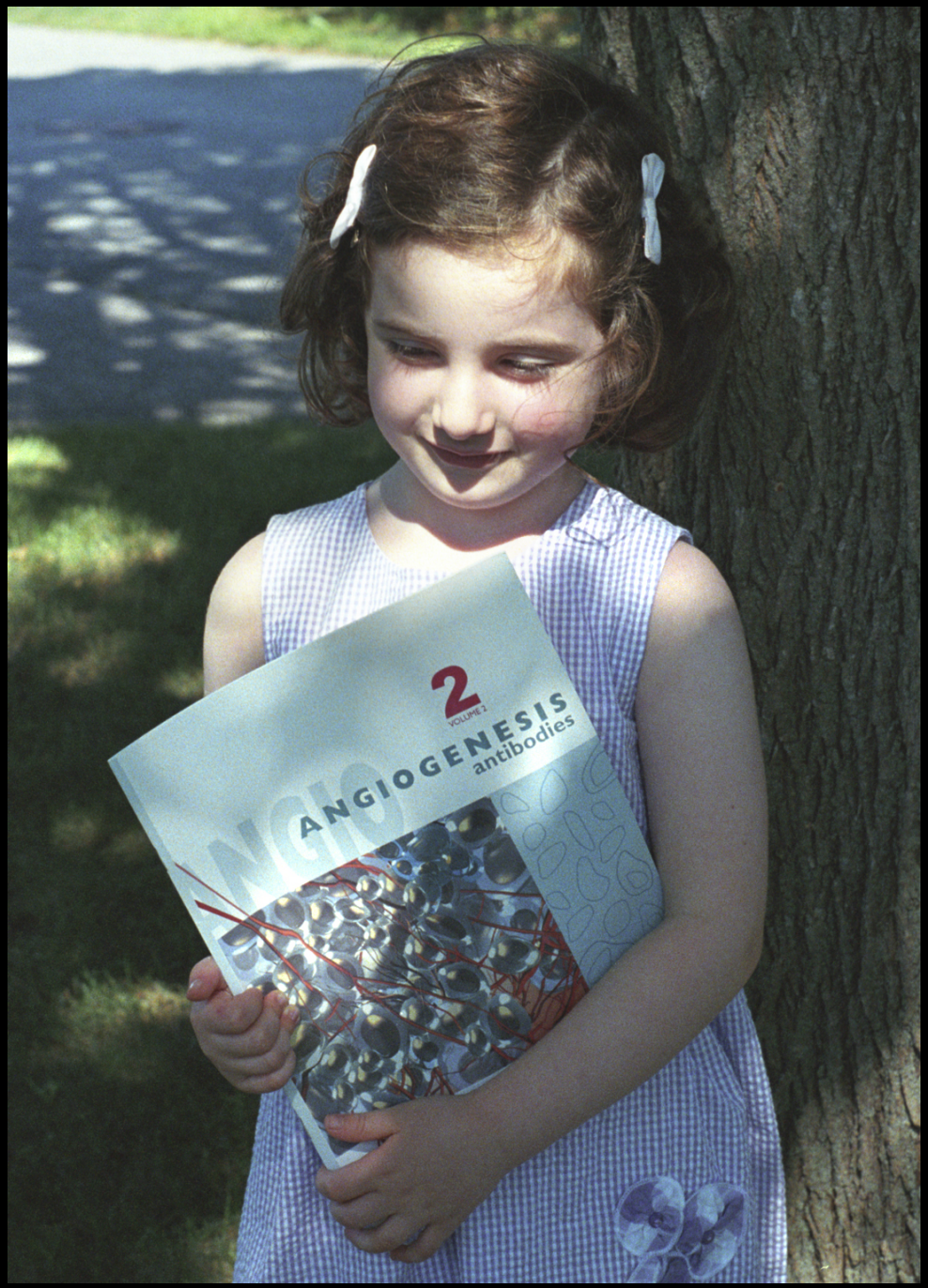
2. **VEGF** stimulates angiogenesis, but **inactivates T-cells**.

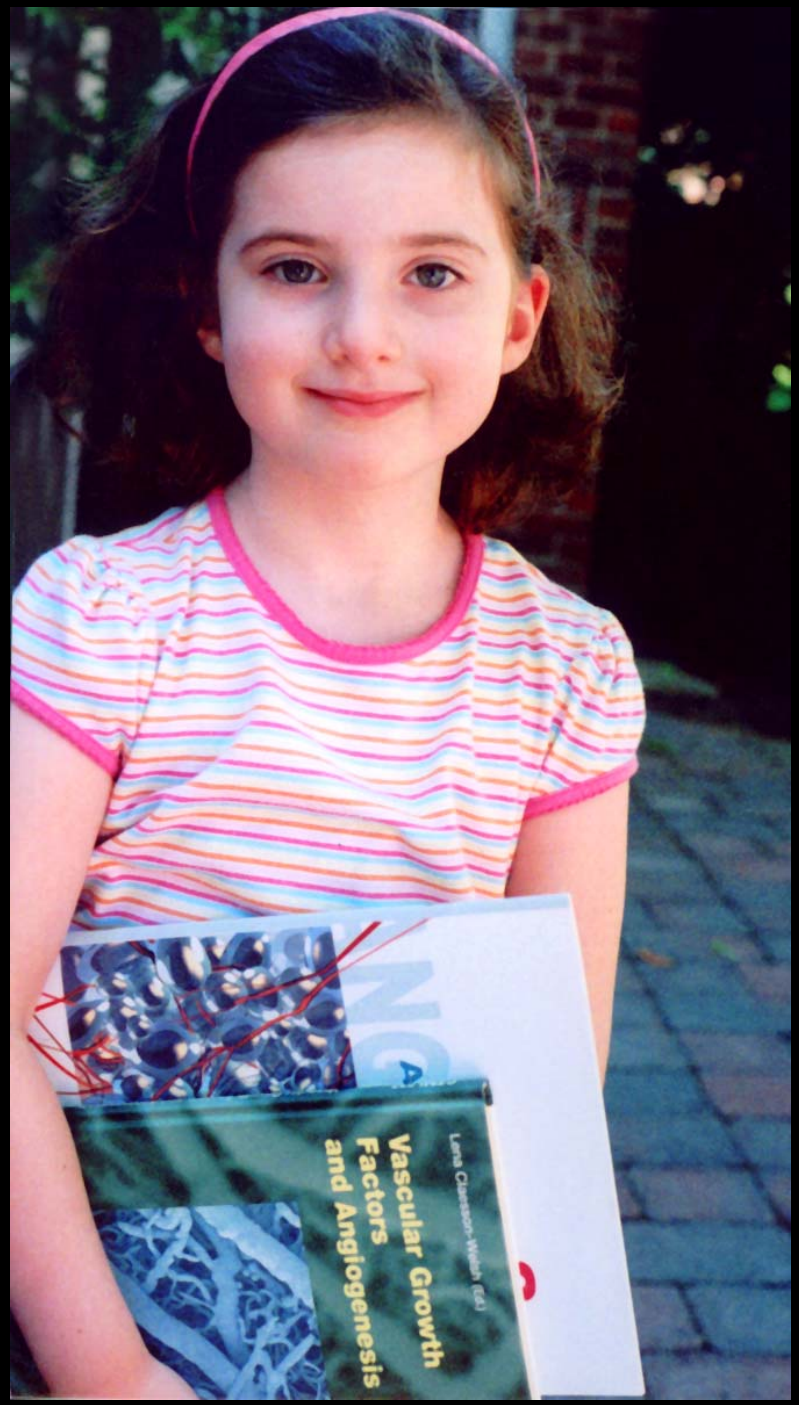
3. **Interleukin 12** inhibits angiogenesis, but **activates T-cells**, & **NK** cells, and **increases interferon-gamma** expression by spleen cells.

4. **Platelet factor 4** inhibits angiogenesis, and **activates T cells**.

5. **Interleukin-27** inhibits angiogenesis and **activates T-cells**.


2
ANGIOGENESIS
antibodies
www.labvision.com
NeoMarkers


2
VOLUME 2
ANGIOGENESIS
antibodies


Vascular Growth
Factors
and Angiogenesis


MC100

p53 upregulates 3 endogenous **angiogenesis inhibitors:**

Thrombospondin-1

K.M. Dameron, et al., Science, 265: 1582, 1994.

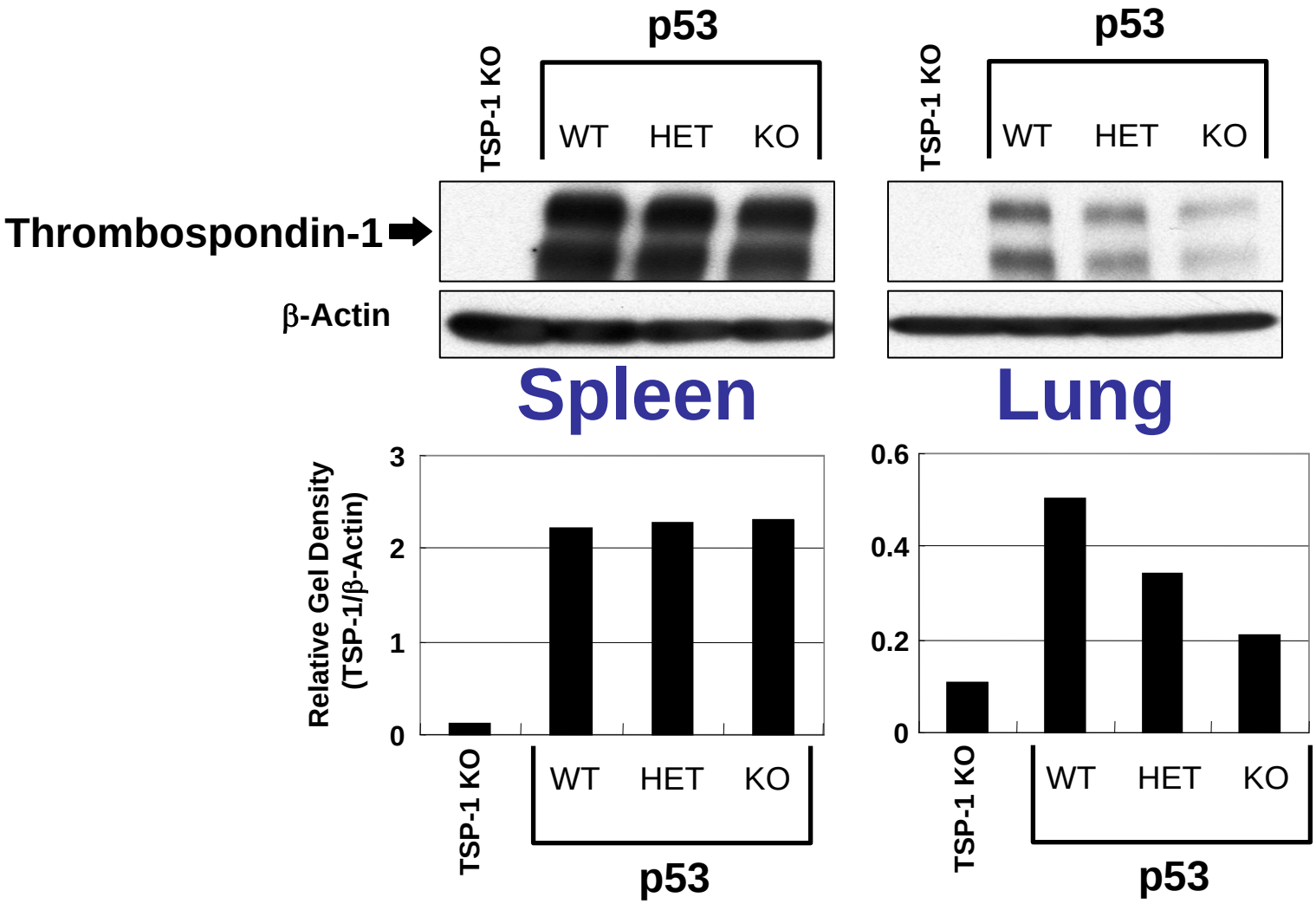
Endostatin

J.G. Teodoro, et al., Science, 313: 968, 2006.

Tumstatin

J.G. Teodoro, et al., Science, 313: 968, 2006.

Organ specific regulation of thrombospondin-1 by p53.



Kwan-Hyuck Baek, Christiana Dellorusso, Sandra Ryeom, Folkman **2007** unpublished data.

*Science, 313: 968, (August 18, **2006**)*

p53-Mediated Inhibition of Angiogenesis Through Up-Regulation of a Collagen Prolyl Hydroxylase

Jose G. Teodoro, Albert E. Parker, Xiaochun Zhu, Michael R. Green*

The tumor suppressor gene, **p53** normally up-regulates a specific enzyme, **collagen prolyl hydroxylase**, that releases two potent angiogenesis inhibitors into the circulation**:**

- **Endostatin** from collagen 18;
- **Tumstatin** from collagen 4.

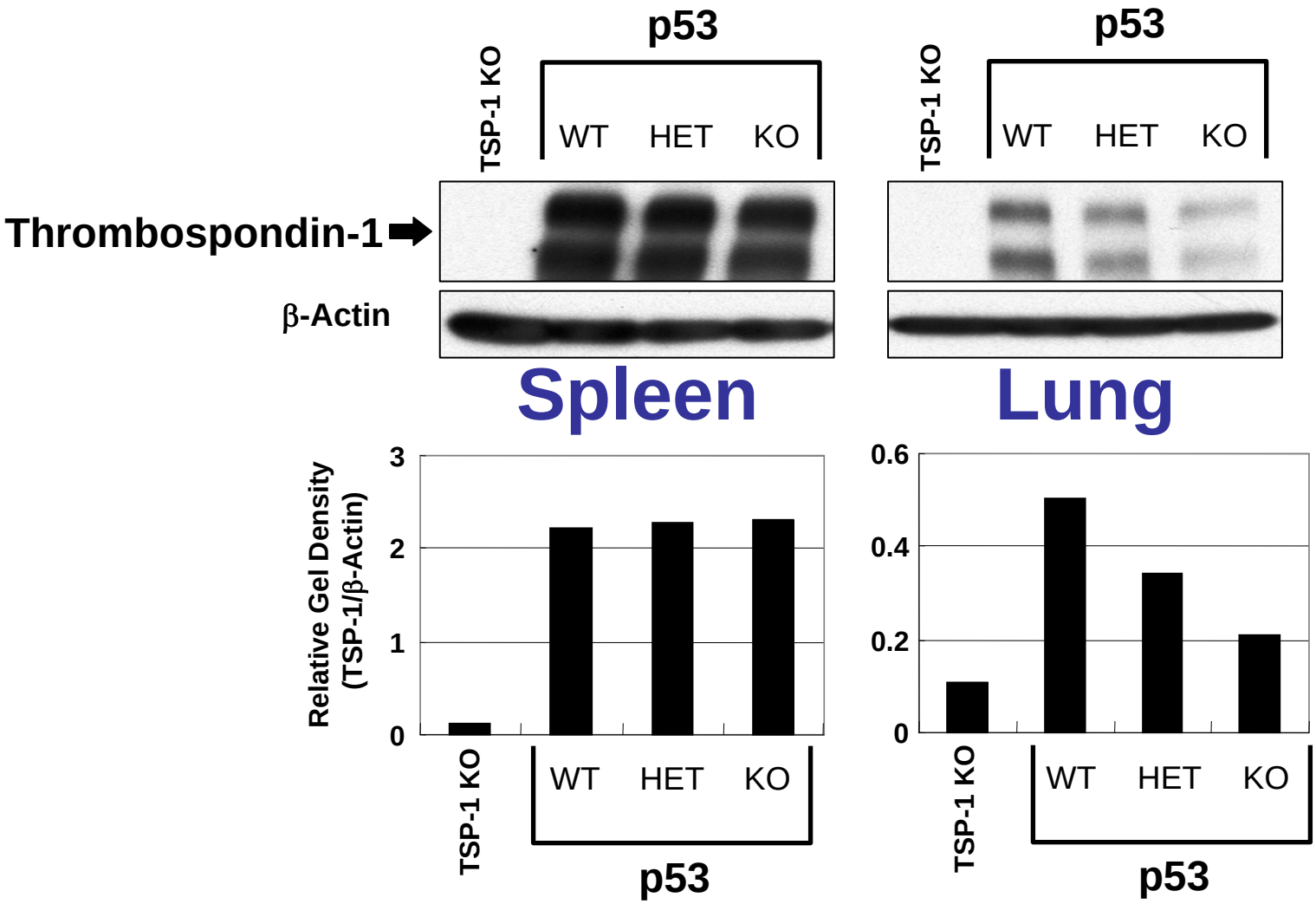
p53 downregulates 3 **pro-angiogenic proteins.**

1. p53 degrades **hypoxia inducible factor-1.**
   Ravi R, Genes and Development, 2000.

2. p53 suppresses expression of **VEGF**.
   Zhang L, Cancer Research, 2000.

3. p53 suppresses expression of **bFGF-binding protein**.
   Sherif, ZA, Cancer Gene Therapy, 2001.

Organ specific regulation of thrombospondin-1 by p53.



Kwan-Hyuck Baek, Christiana Dellorusso, Sandra Ryeom, Folkman **2007** unpublished data.