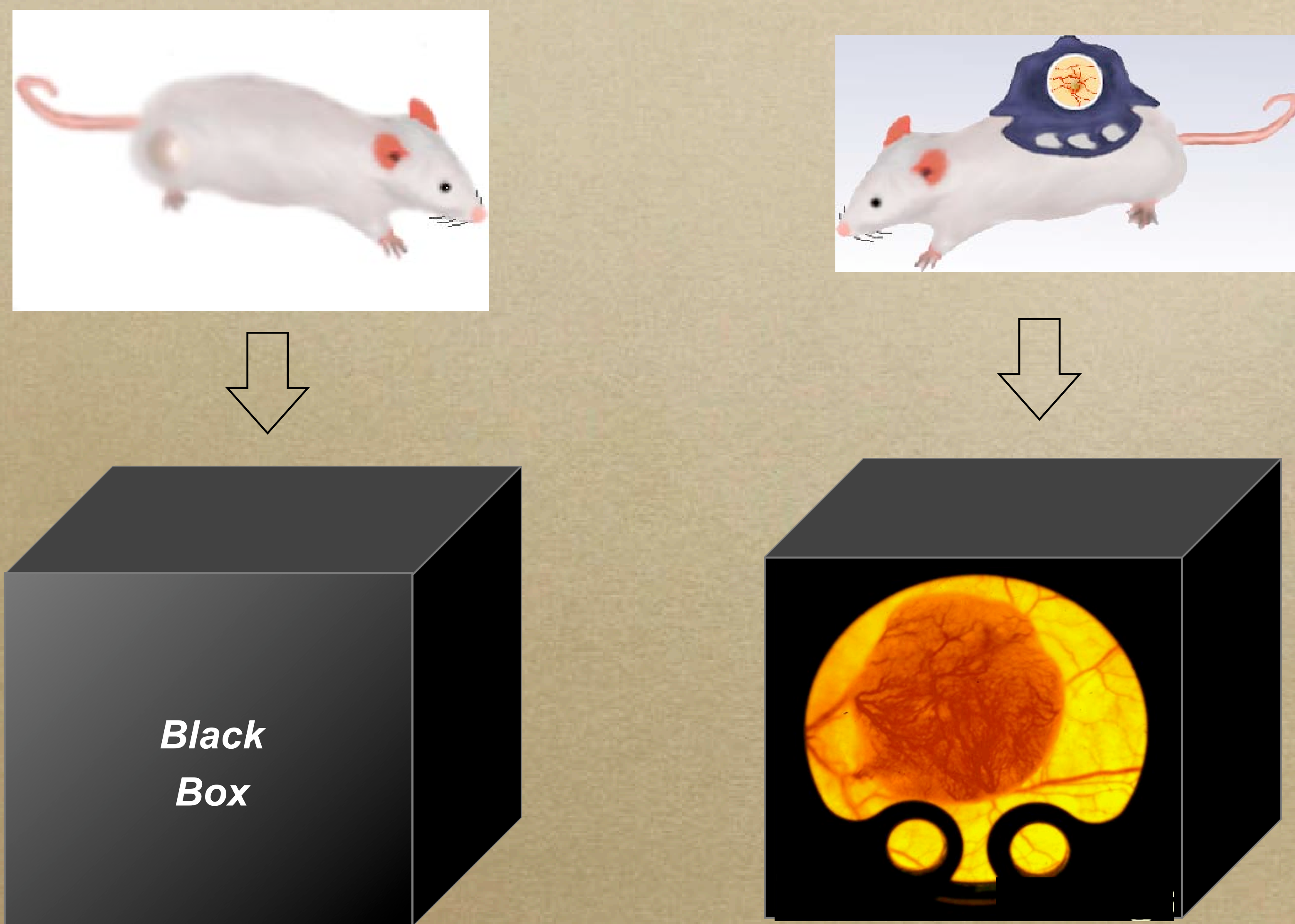
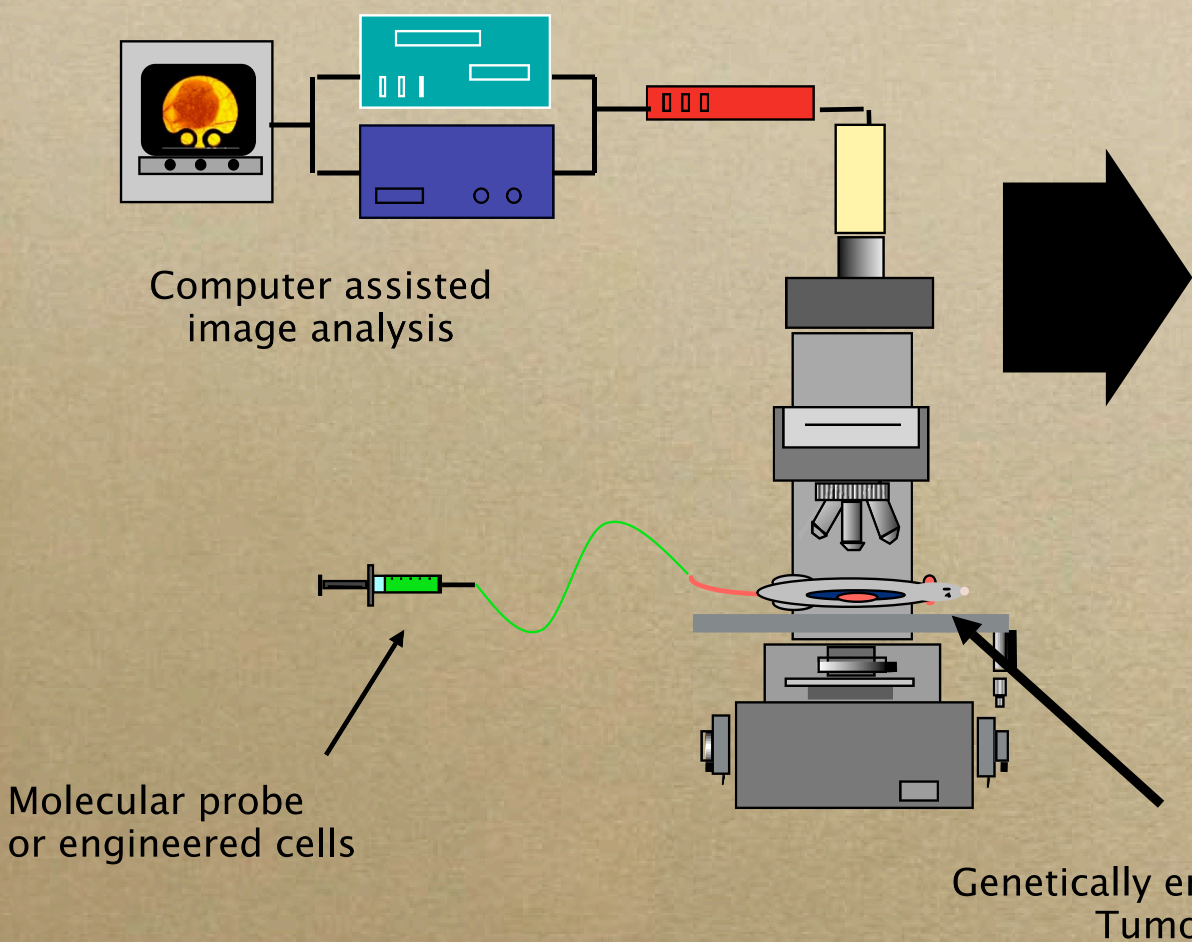
Opening the Black Box



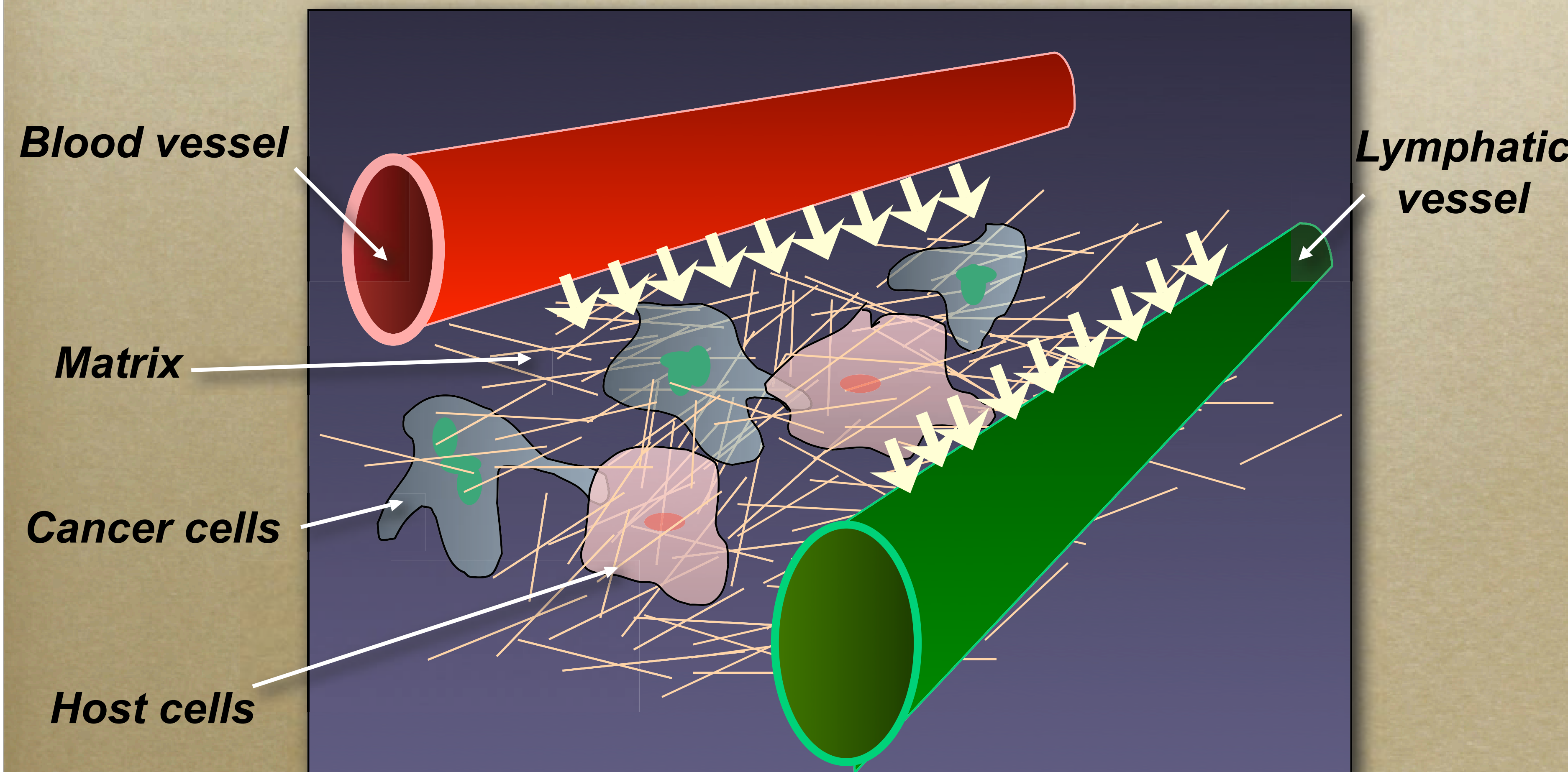
Dissecting Tumors using Intravital Microscopy



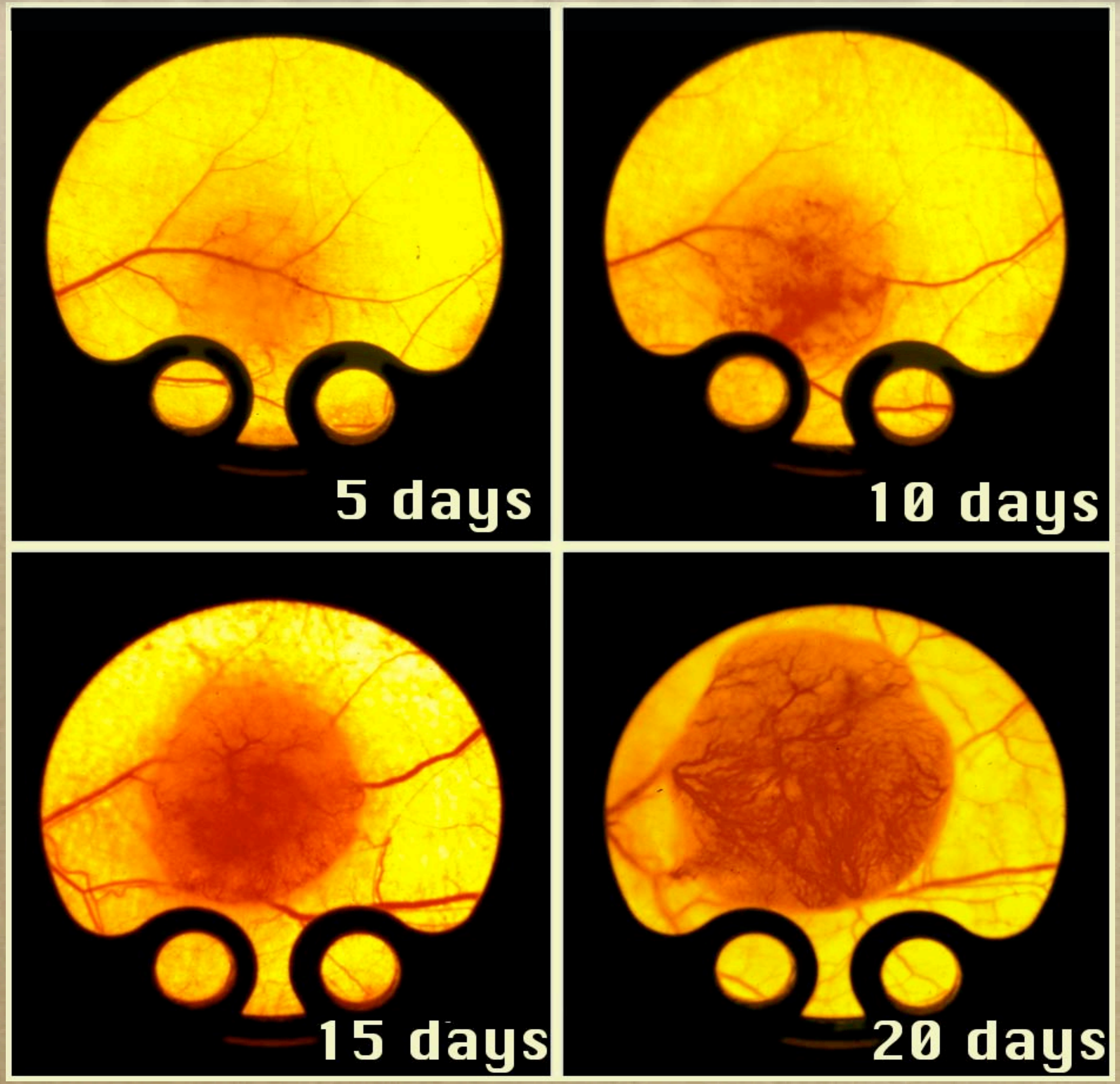
- Molecular imaging
- Cellular imaging
- Anatomical imaging
- Functional imaging
- Therapeutic response

Jain, Munn, Fukumura, Nature Reviews Cancer (2002)

Deconstructing Solid Tumors

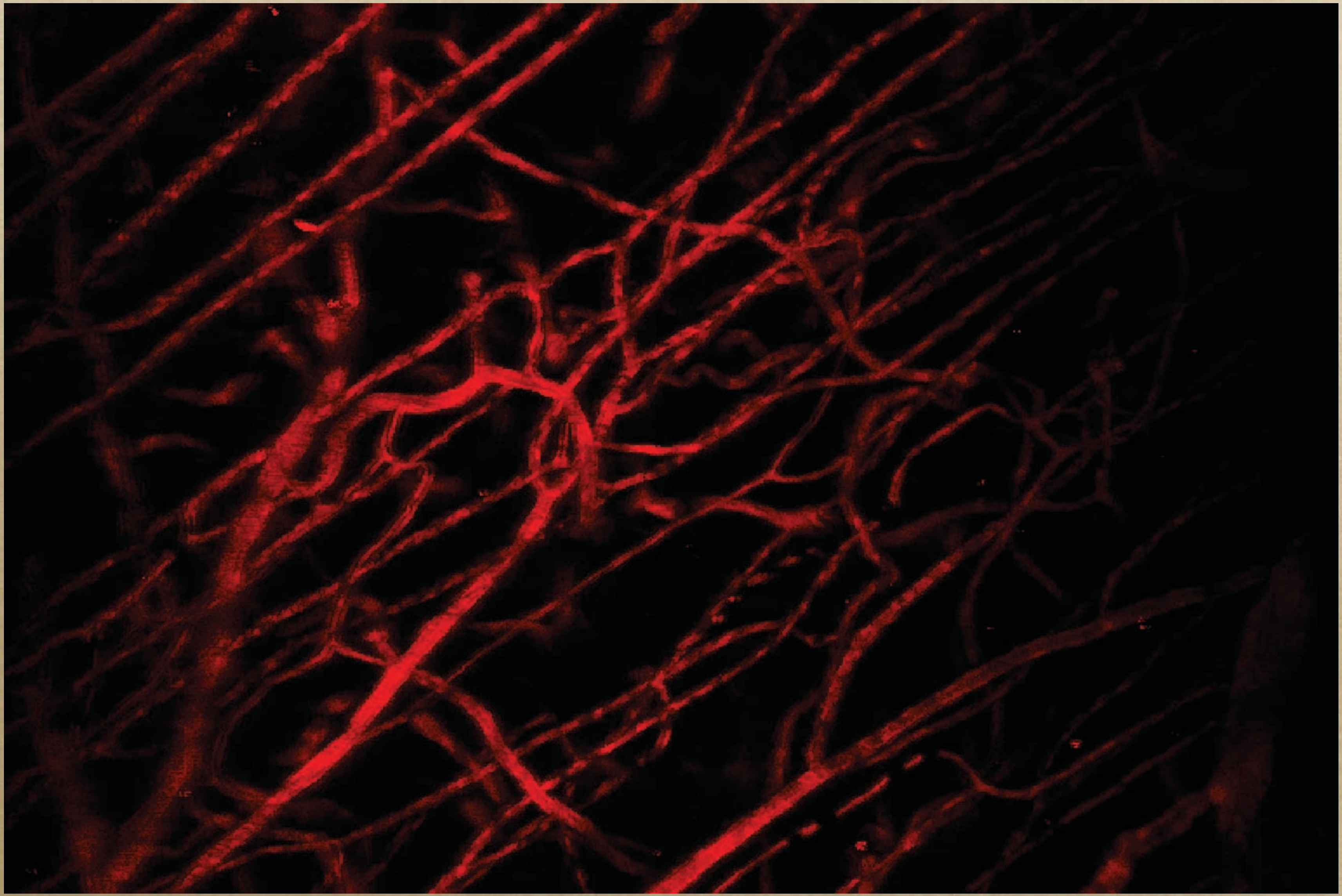


Growth of LS174T in Dorsal Window



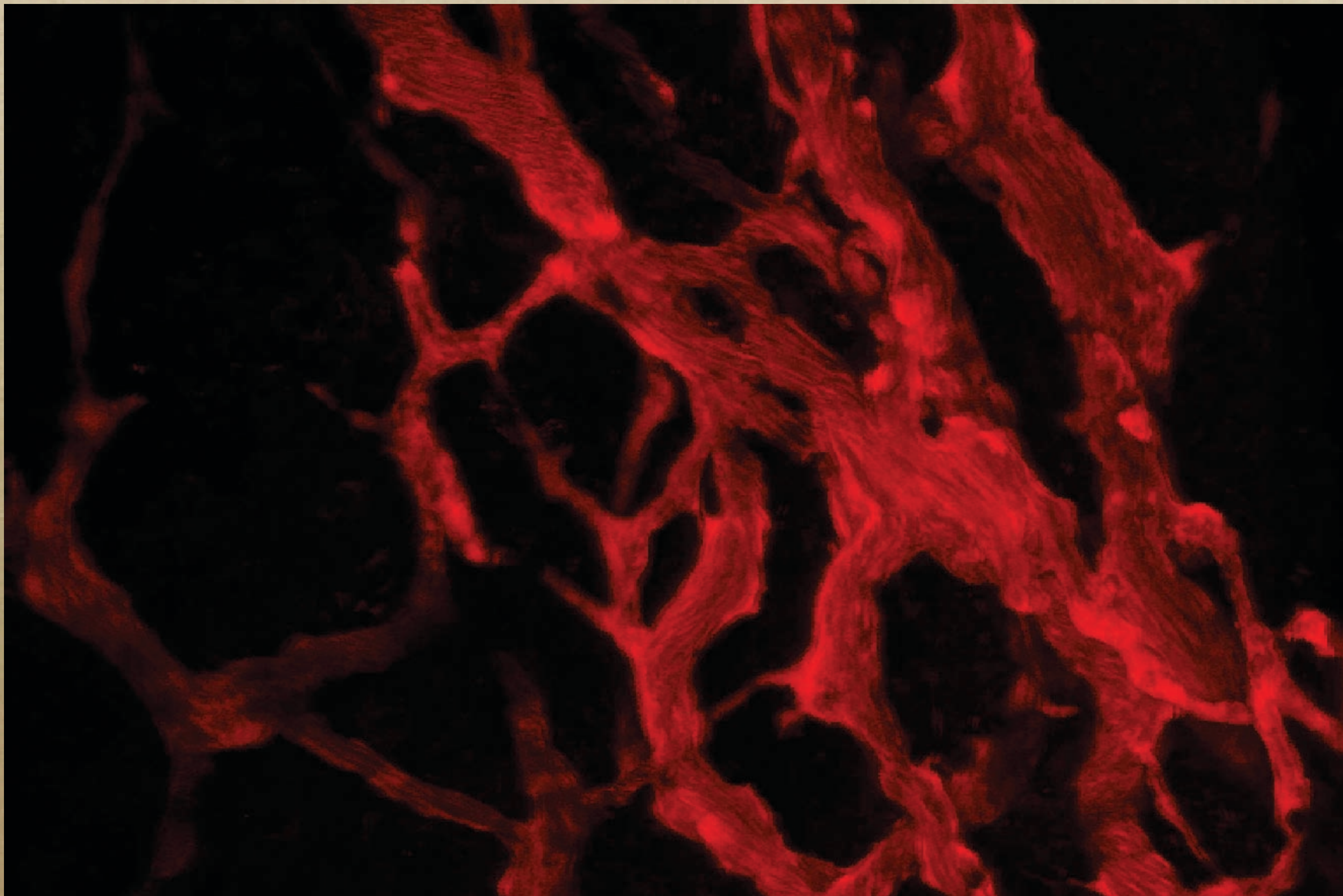
Leunig et al. Cancer Research (1992)

3D imaging of normal vasculature



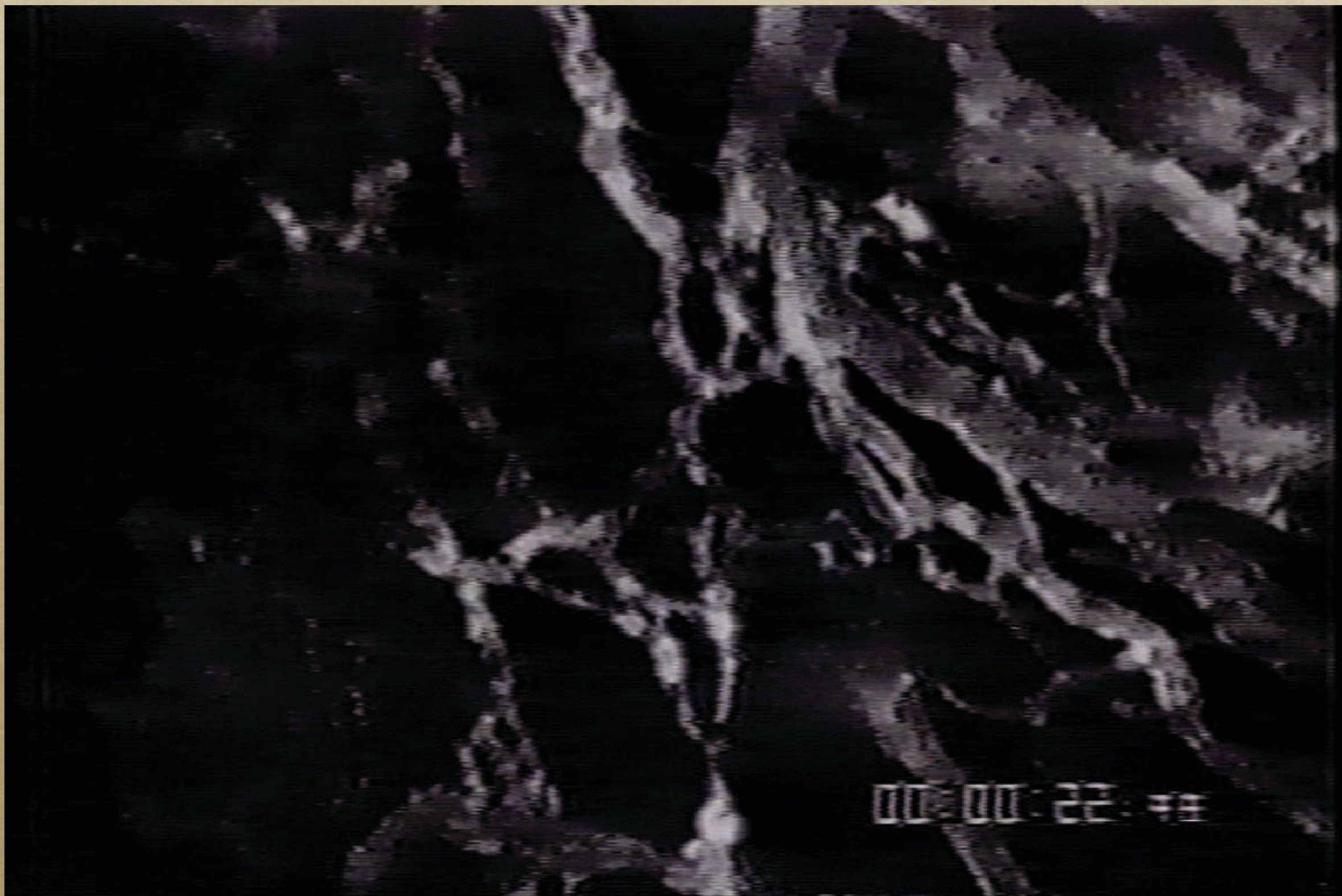
Brown et al. Nature Medicine (2001)

3D imaging of tumor vasculature

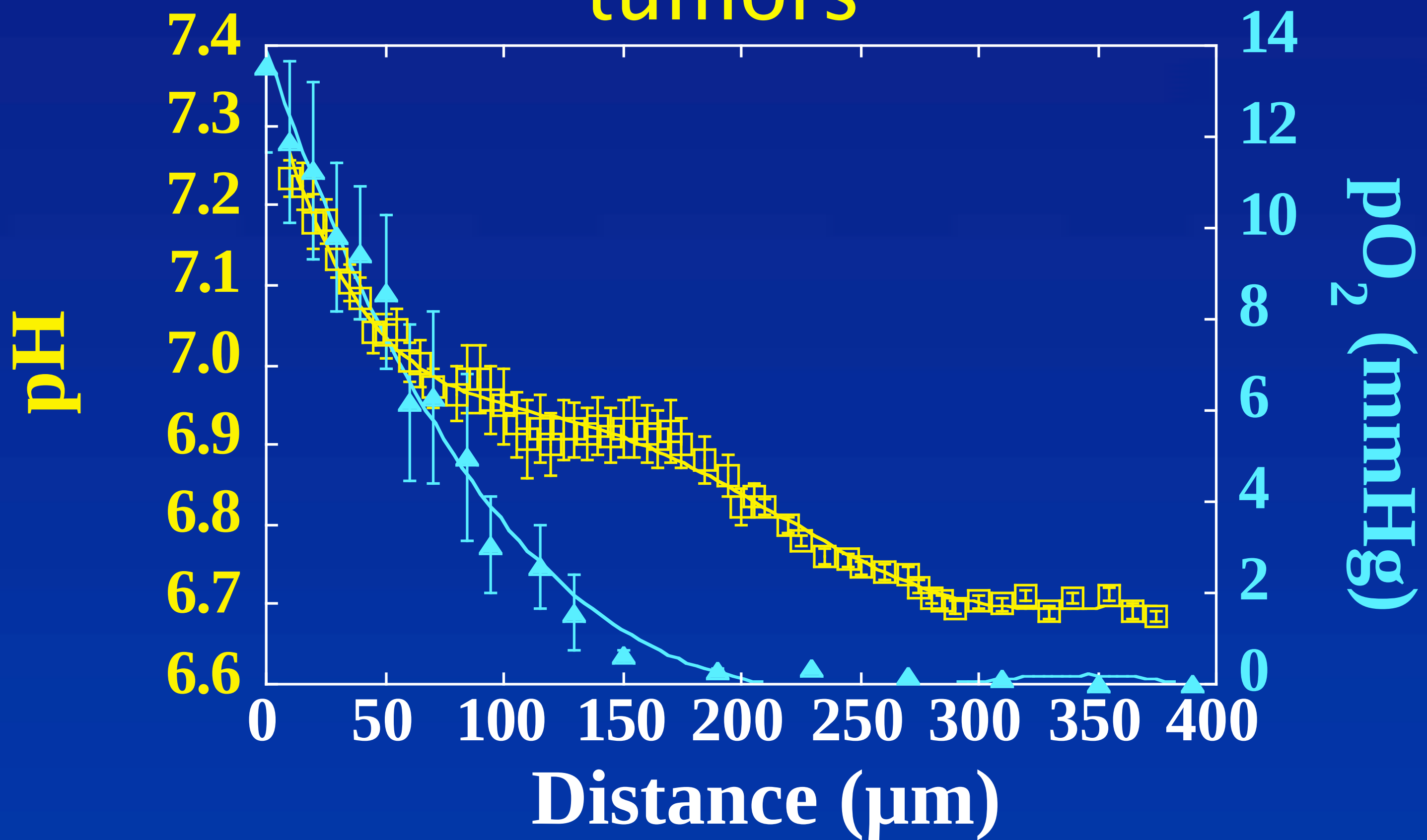


Brown et al. Nature Medicine (2001)

Tumor blood vessels:
abnormal structure and function

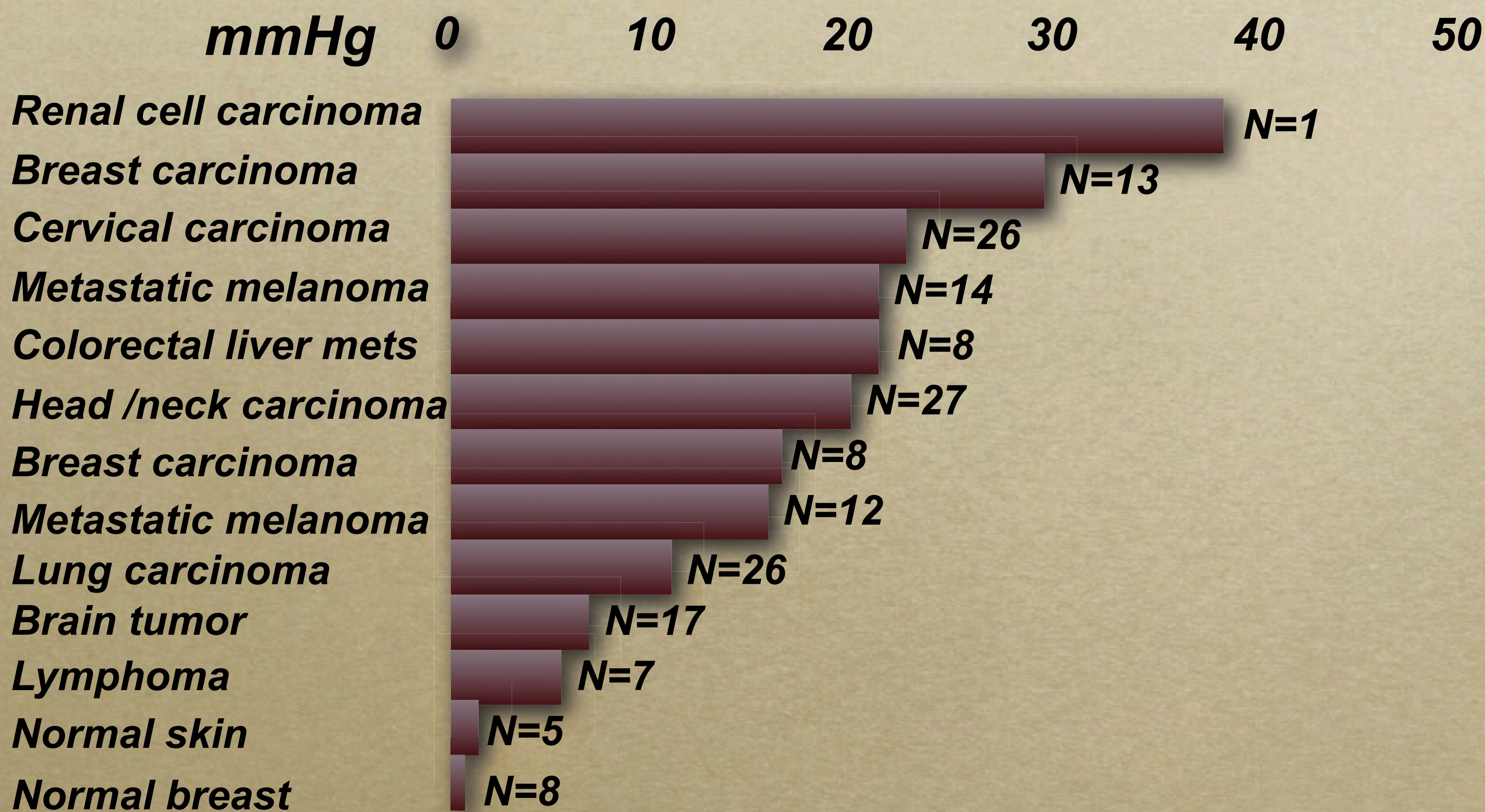


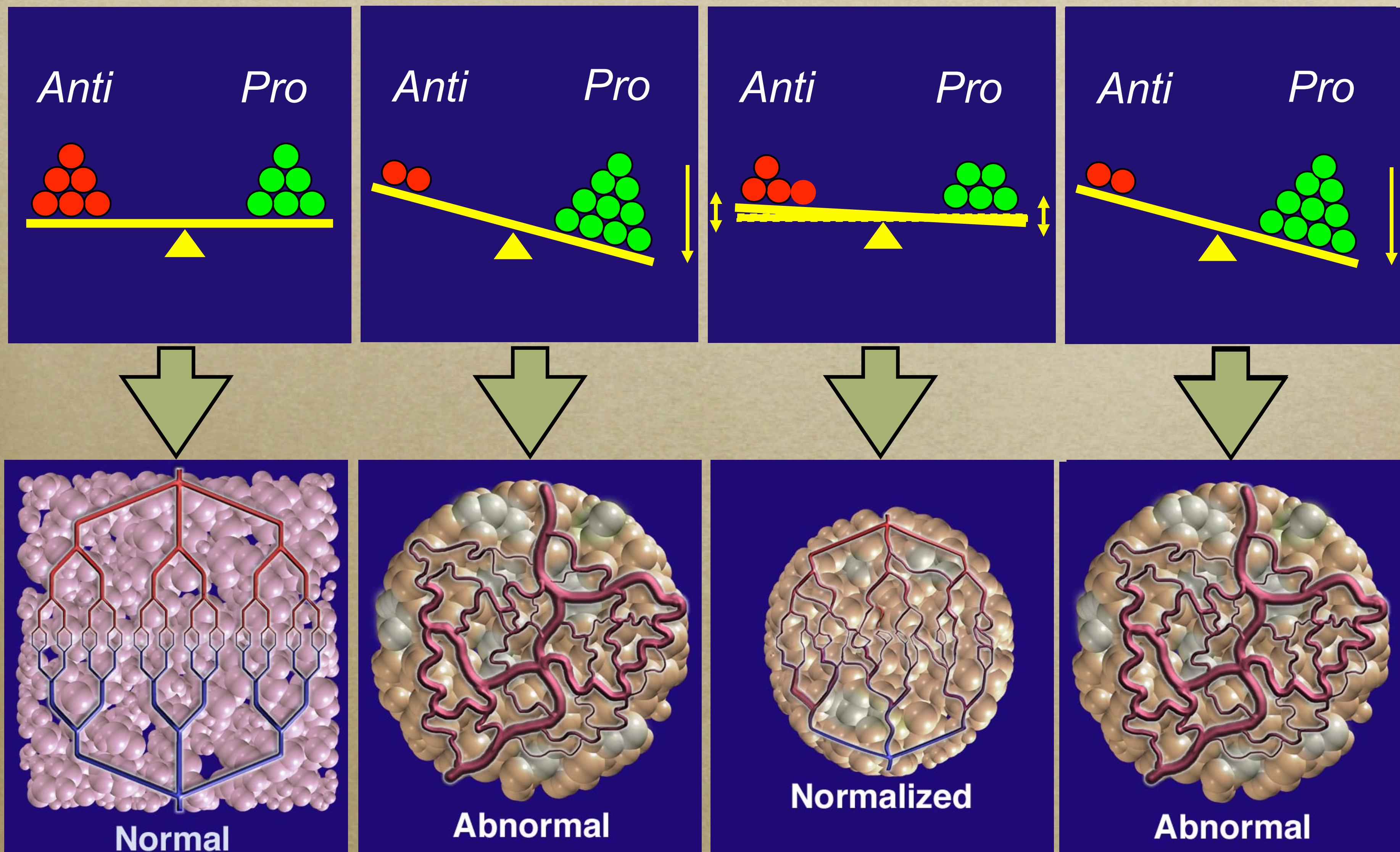
Mean interstitial profile of pO_2 and pH in tumors



Helmlinger et al. Nature Med (1997)

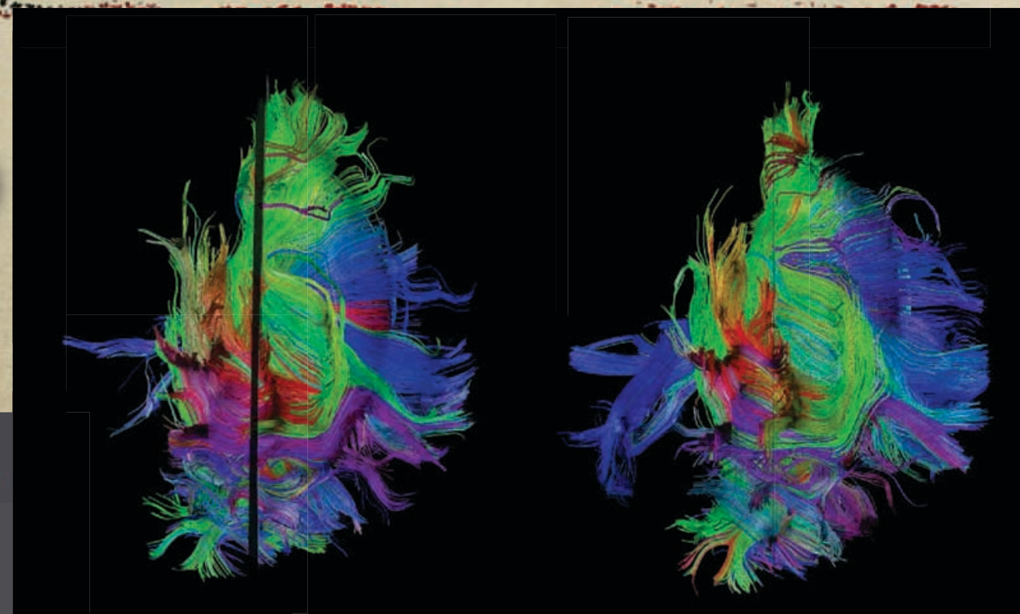
Interstitial Fluid Pressure in Human Tumors





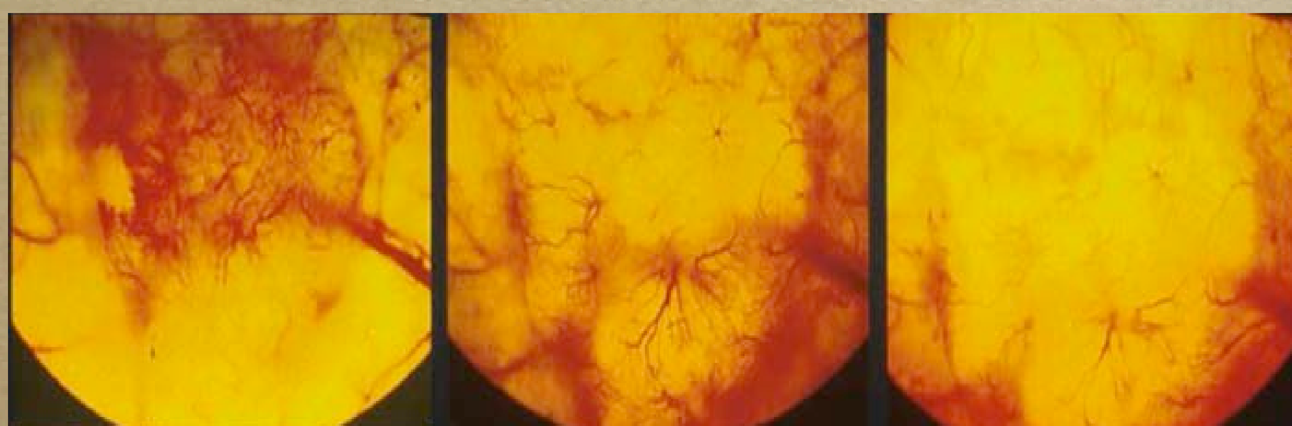
Jain, Nat Med (2001); Science (2005)

Bench to Bedside and Back



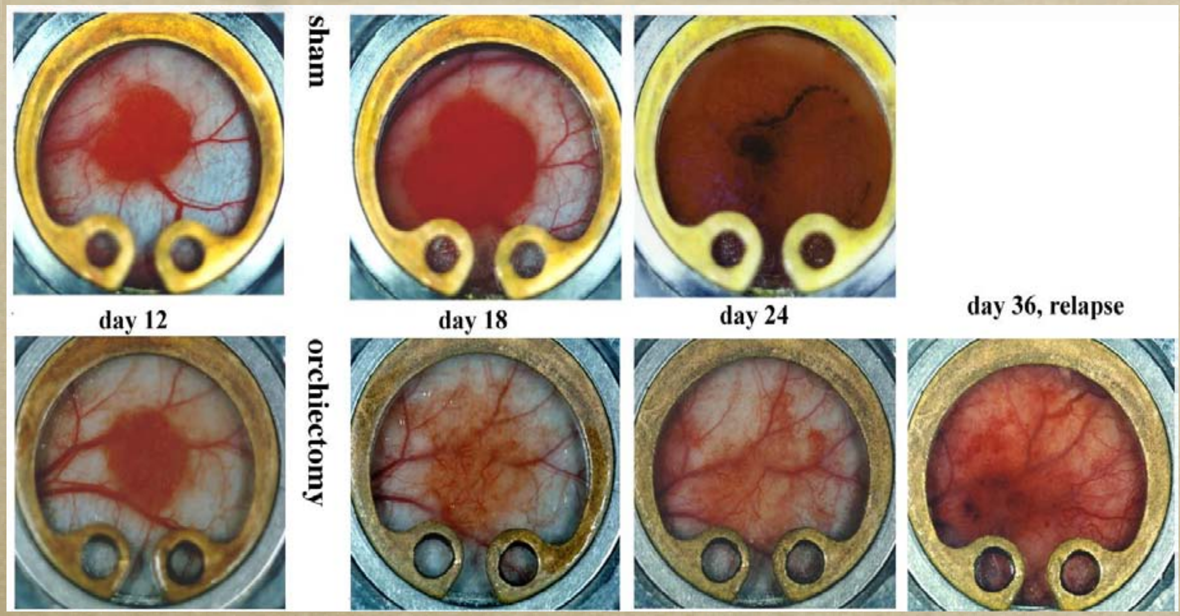
Regression of LS174T by anti-VEGF antibody

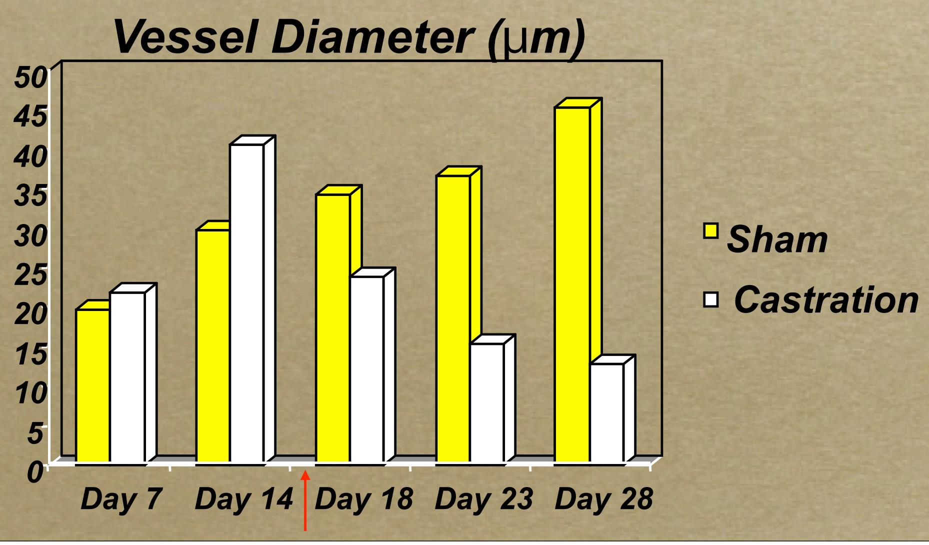
Before treatment | **3 days** | **7 days**

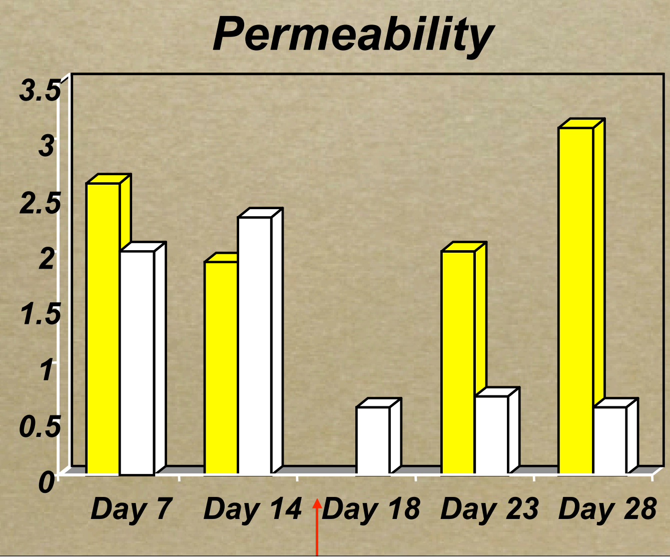


Yuan et al. PNAS (1996)

Normalization of tumor vasculature by hormone withdrawal

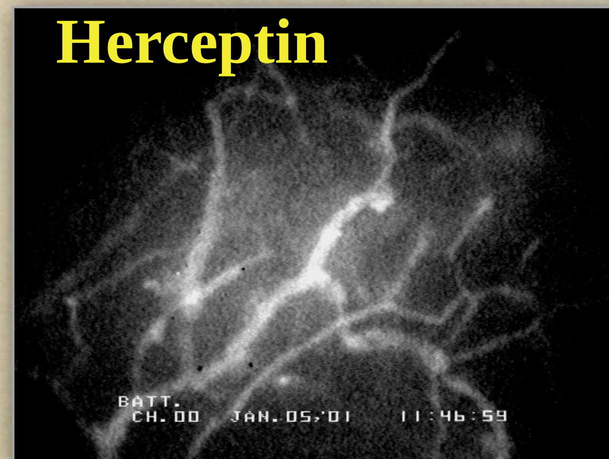


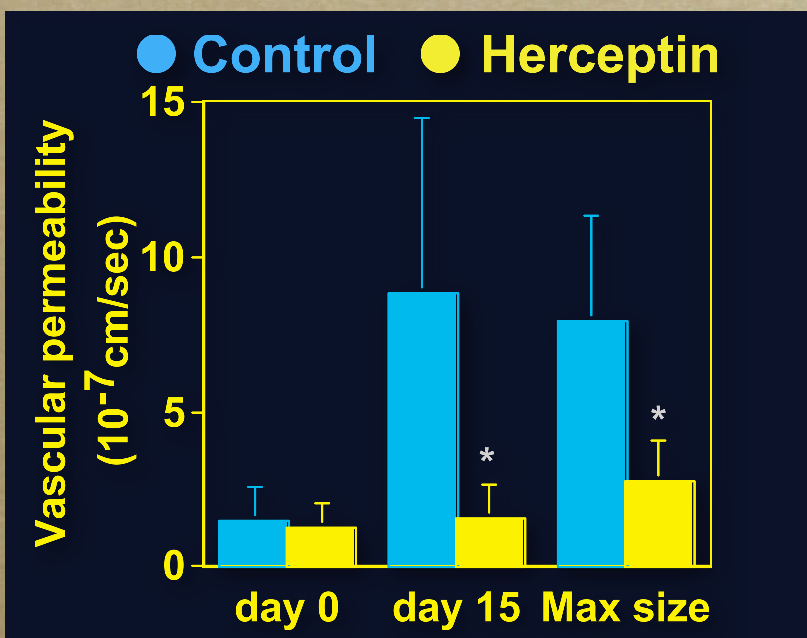


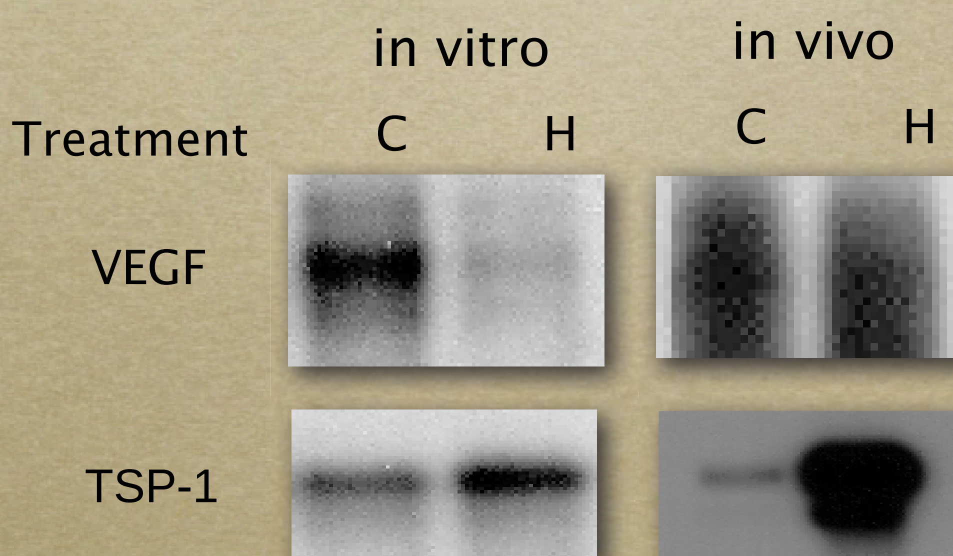


Jain et al. PNAS (1998)

Can Herceptin normalize tumor vessels?

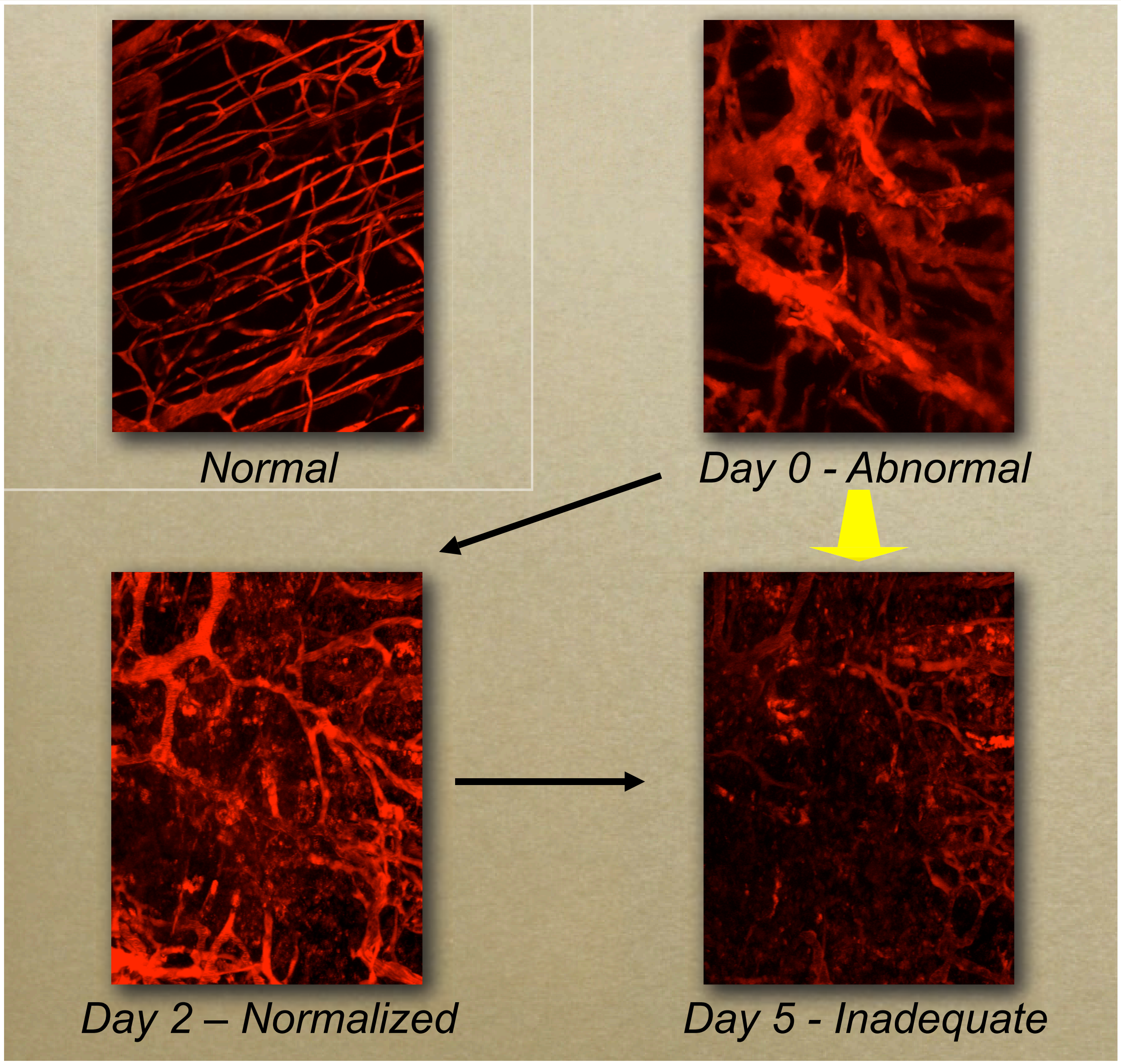




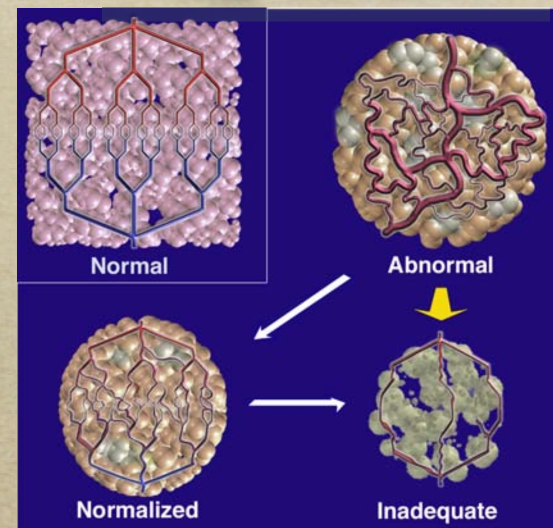


Izumi et al. Nature (2002)

VEGF Blockade Normalizes Tumor Vasculature

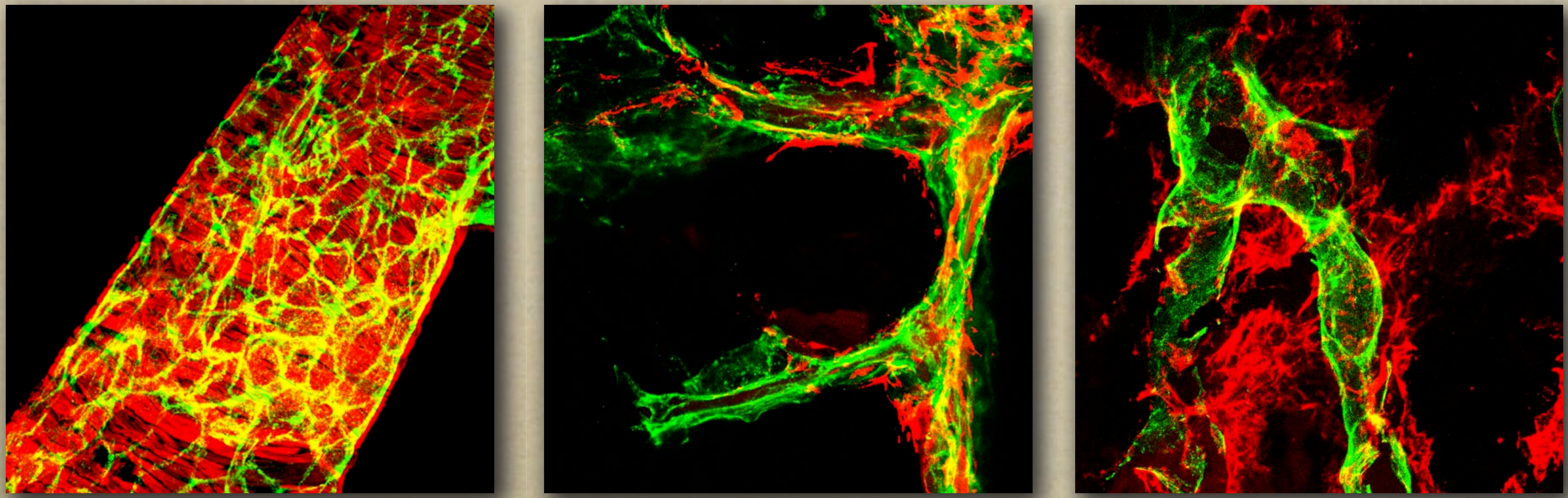


Normalization Hypothesis

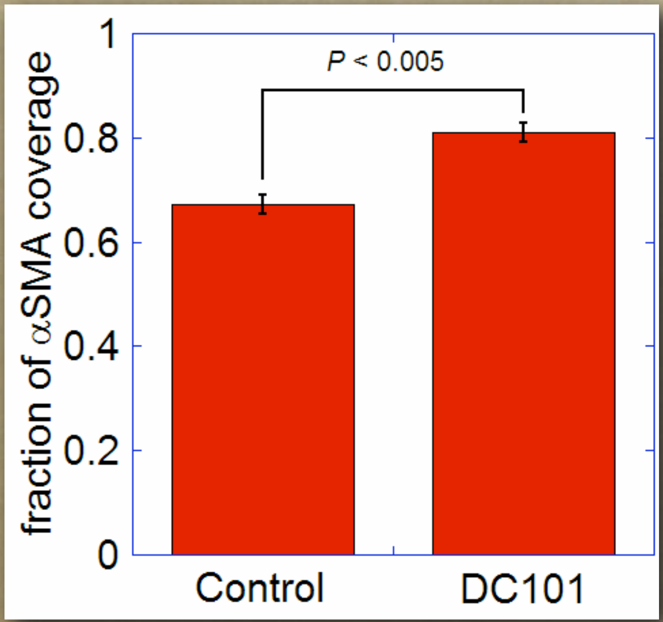


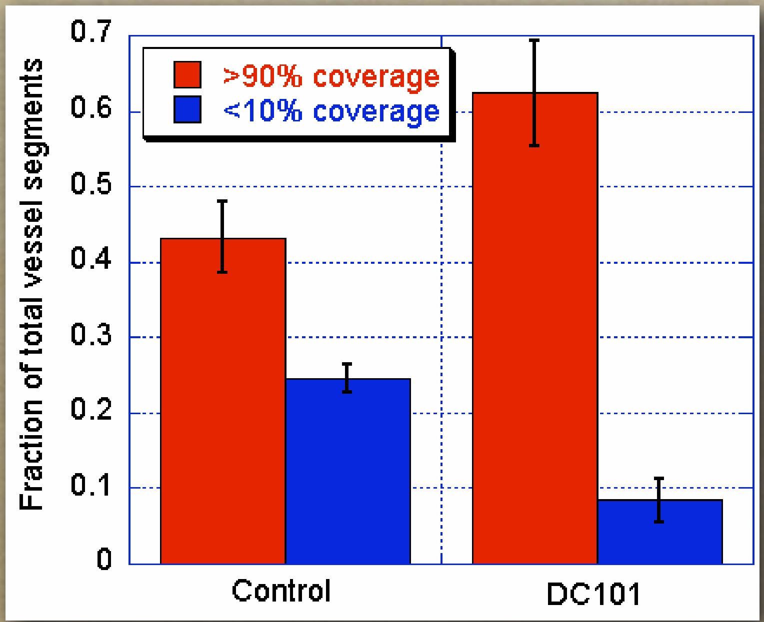
Tong et al. (Cancer Research 2004)
Jain, Nature Medicine (2001)

DC101 fortifies tumor vessels



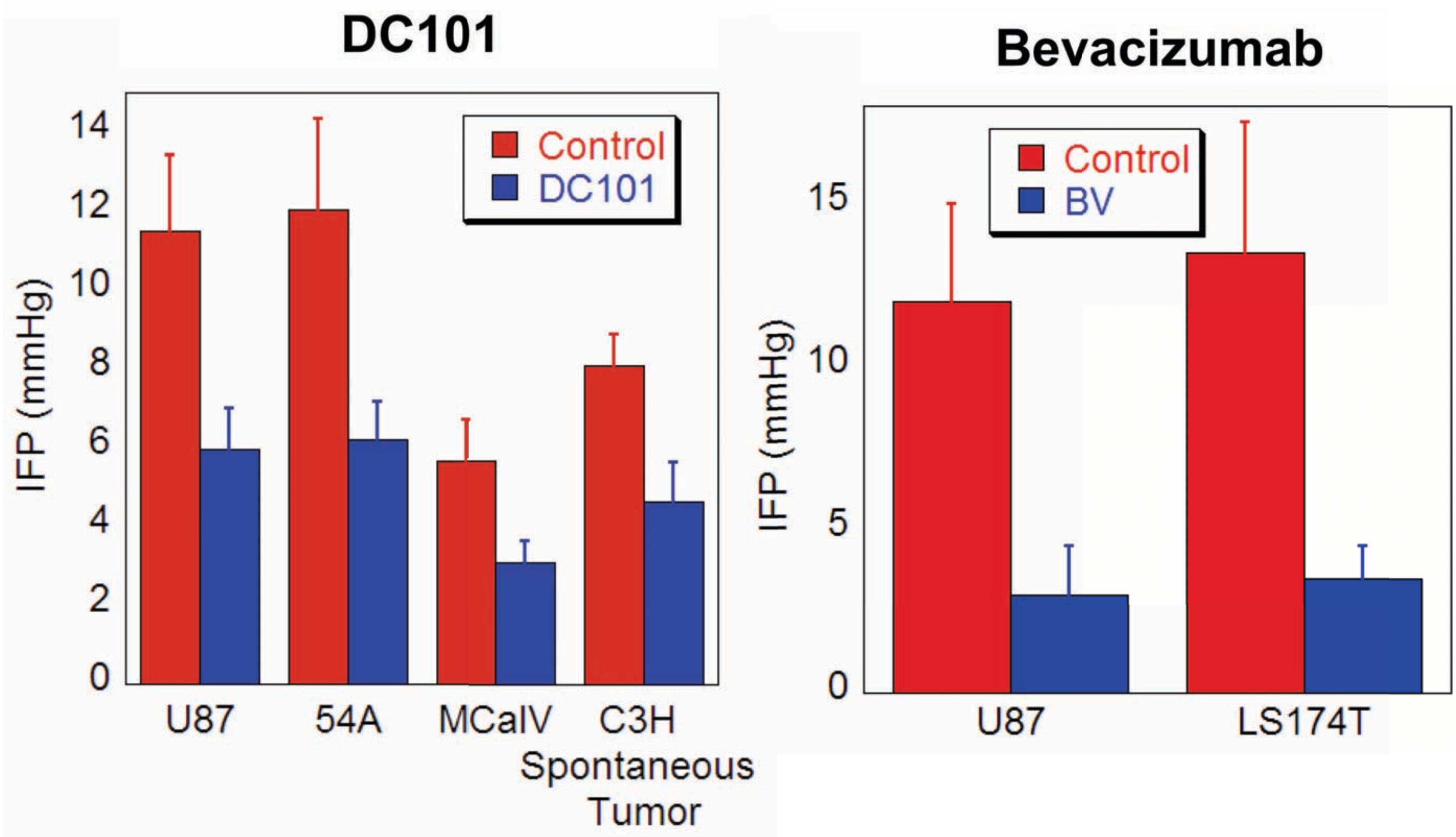
Normal arteriole *control* *DC101*





Tong et al. Cancer Research (2004)

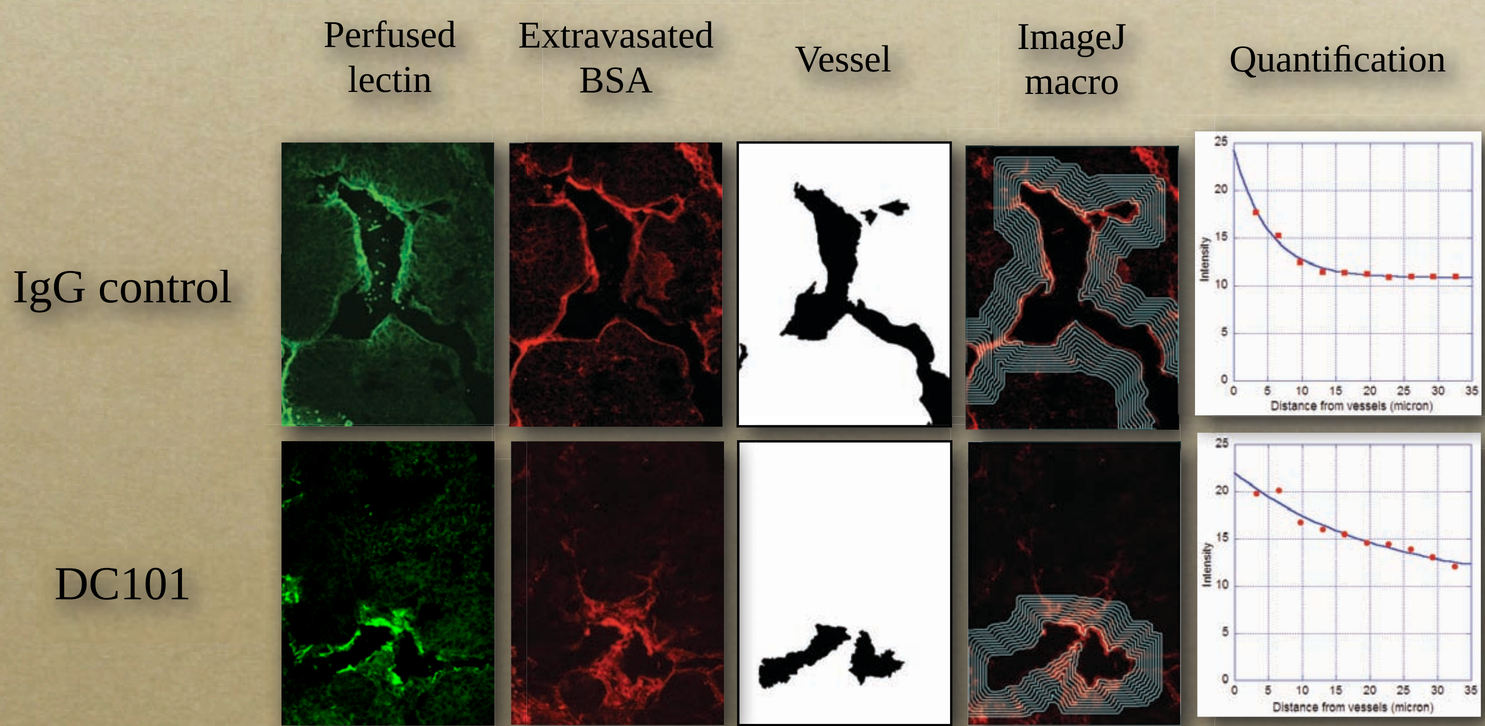
Decrease in interstitial fluid



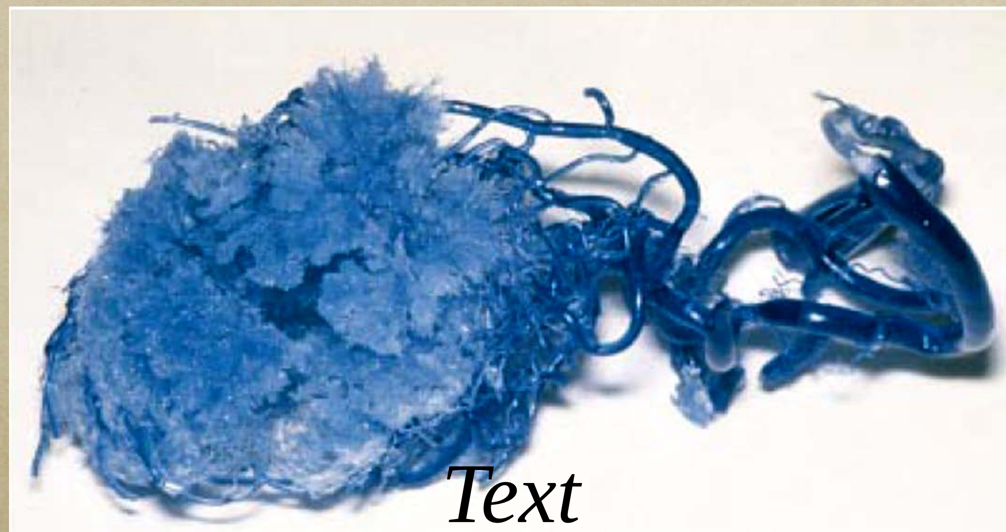
Tong et al. Cancer Research (2004)

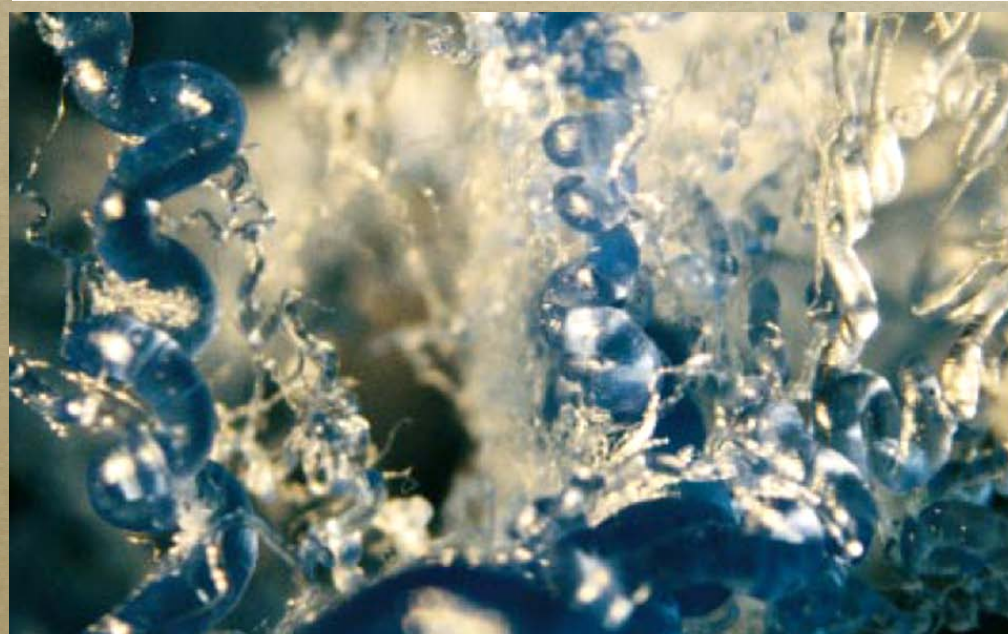
Lee et al. Cancer Research (2000)

Fluorecent BSA penetration in tumor tissue after VEGFR2 blockade



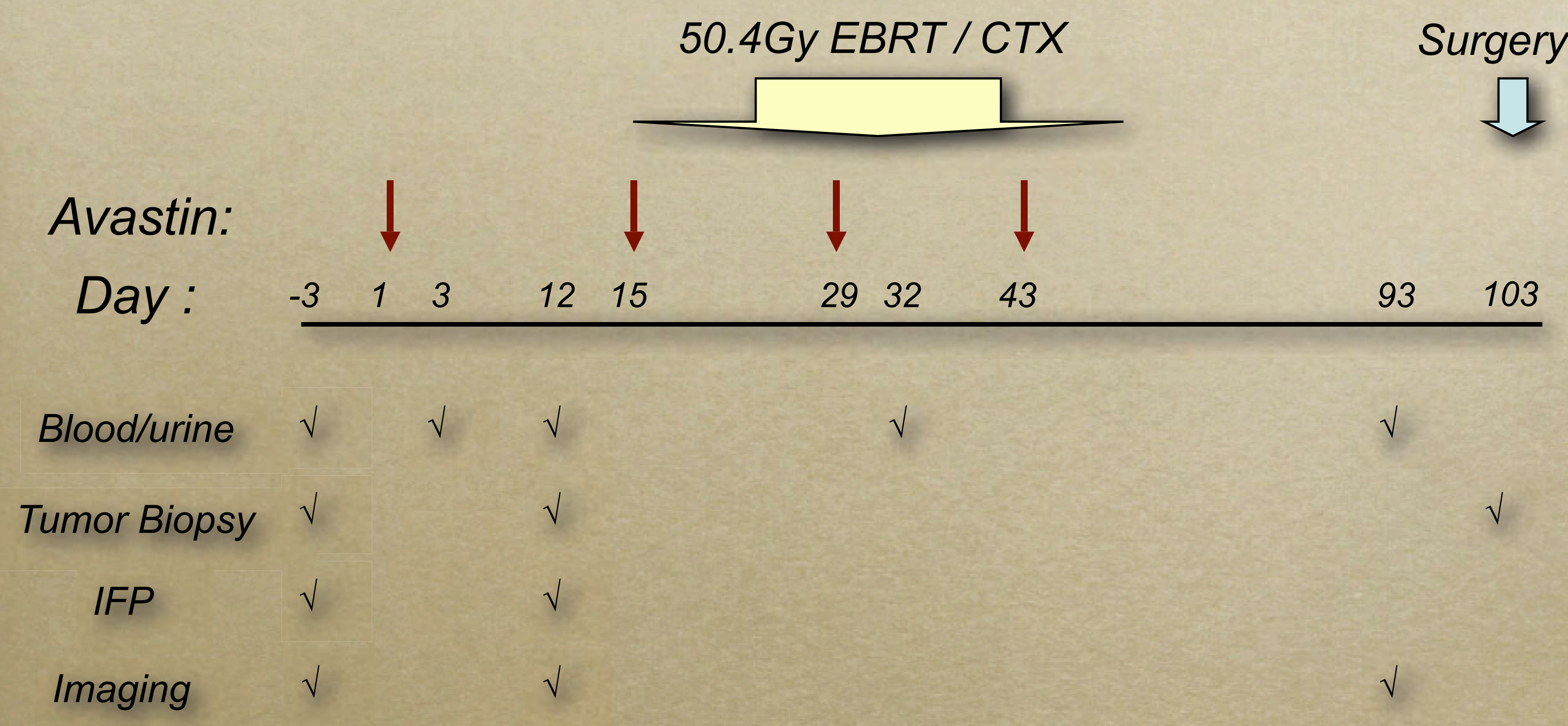
Human colon cancer casts





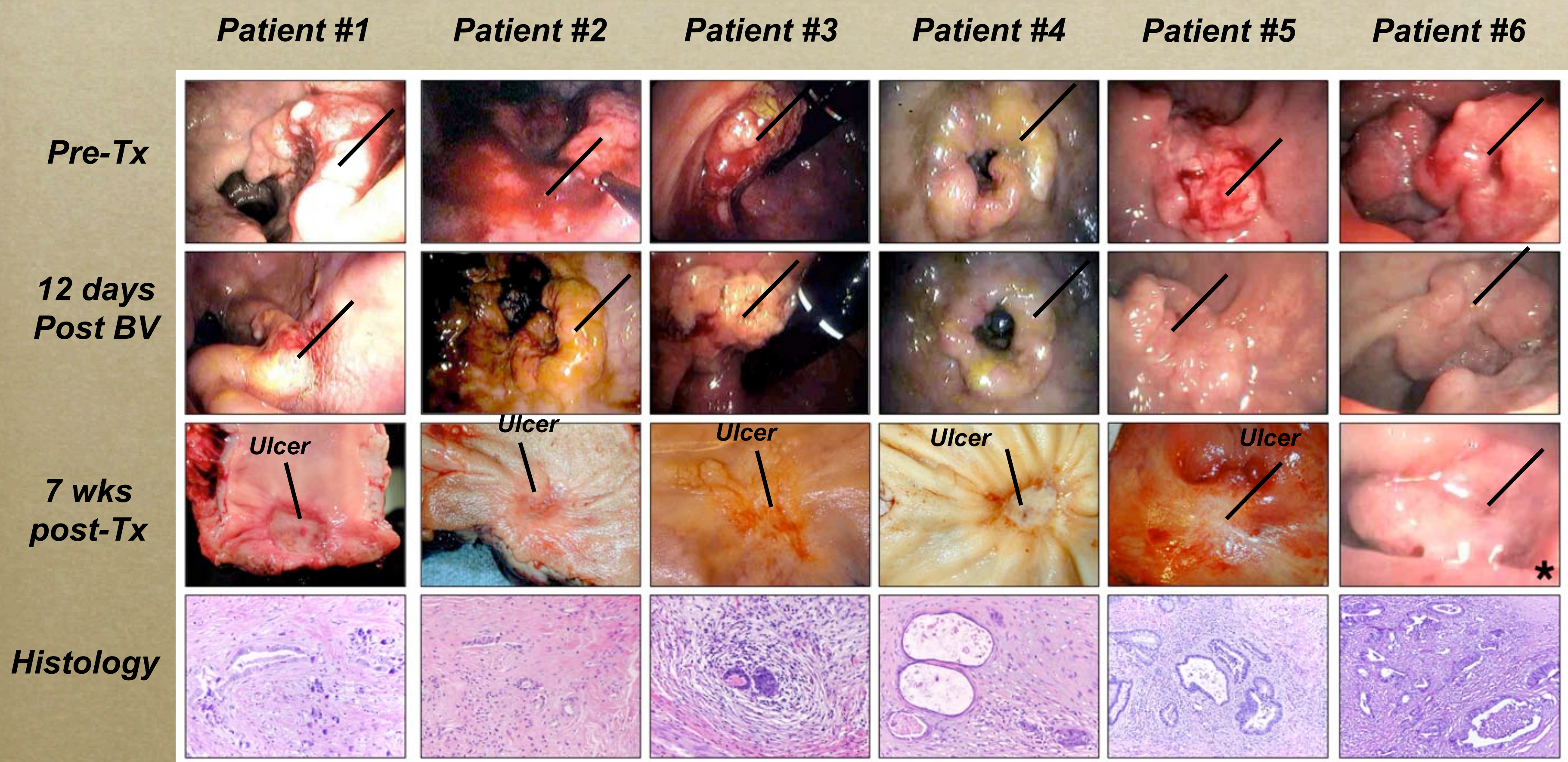
Jain,
Sci Am 1994

Protocol of Clinical Trial



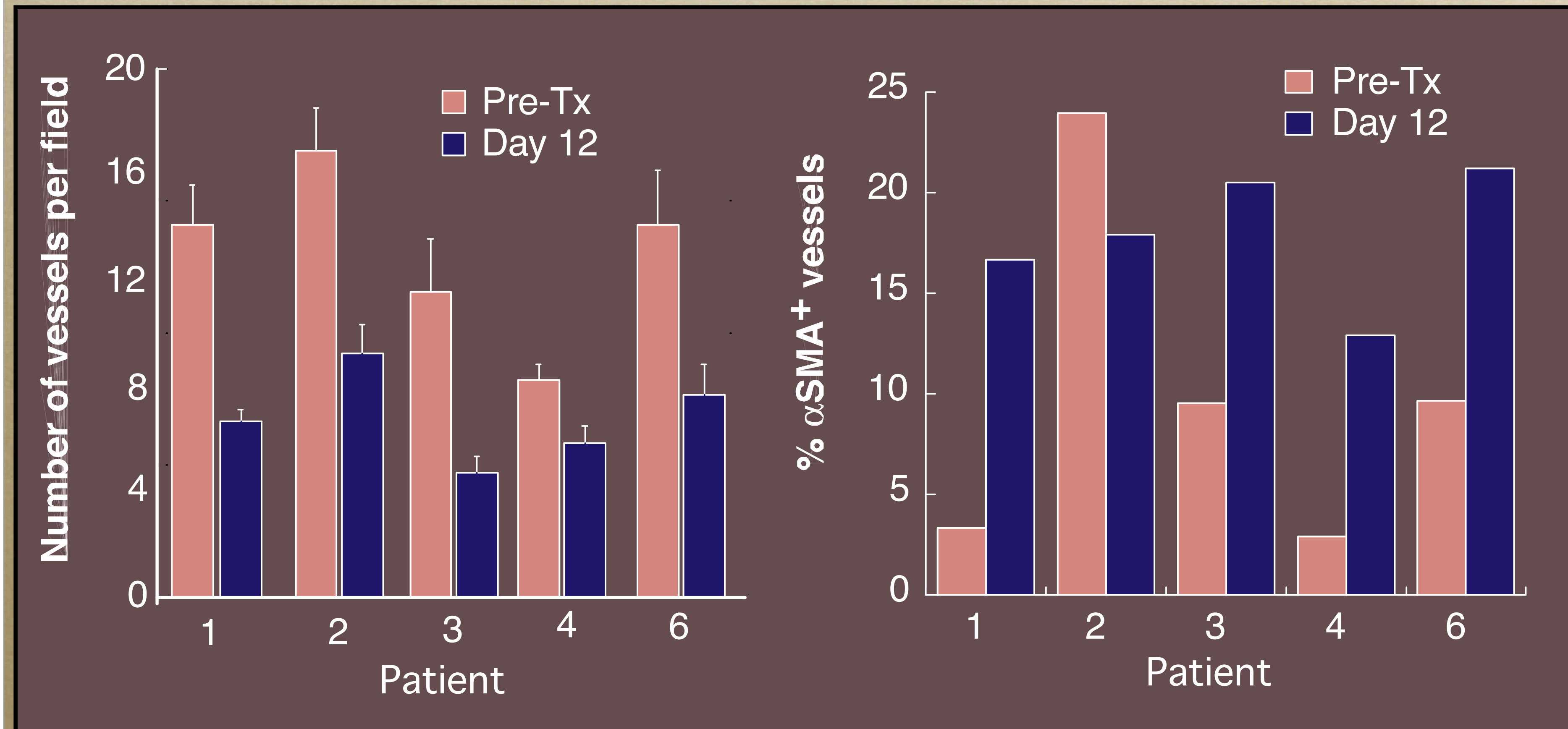
Willett et al. Nature Medicine (2004)

Effect of BV plus chemoradiation in rectal cancer patients (BV: 5mg/kg)



Willett et al., Nature Medicine 2004

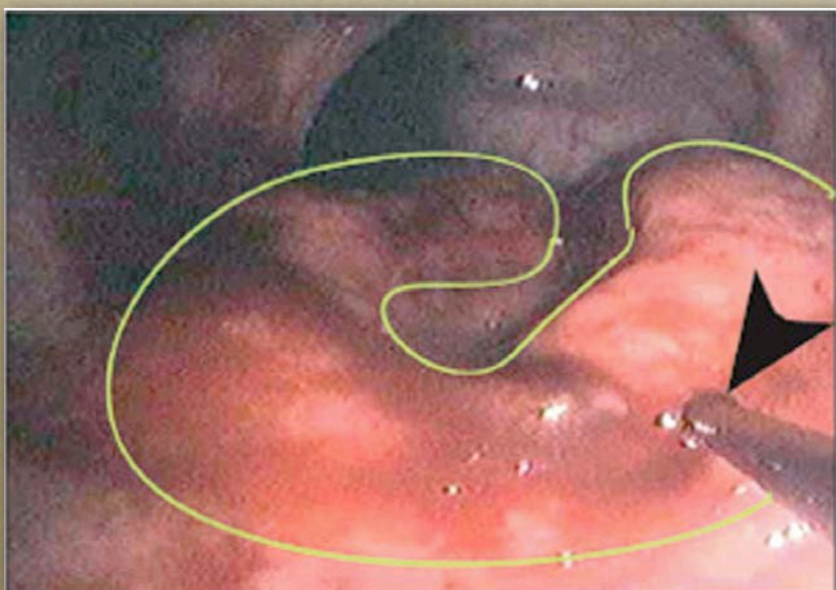
Tumor vascular parameters from histology

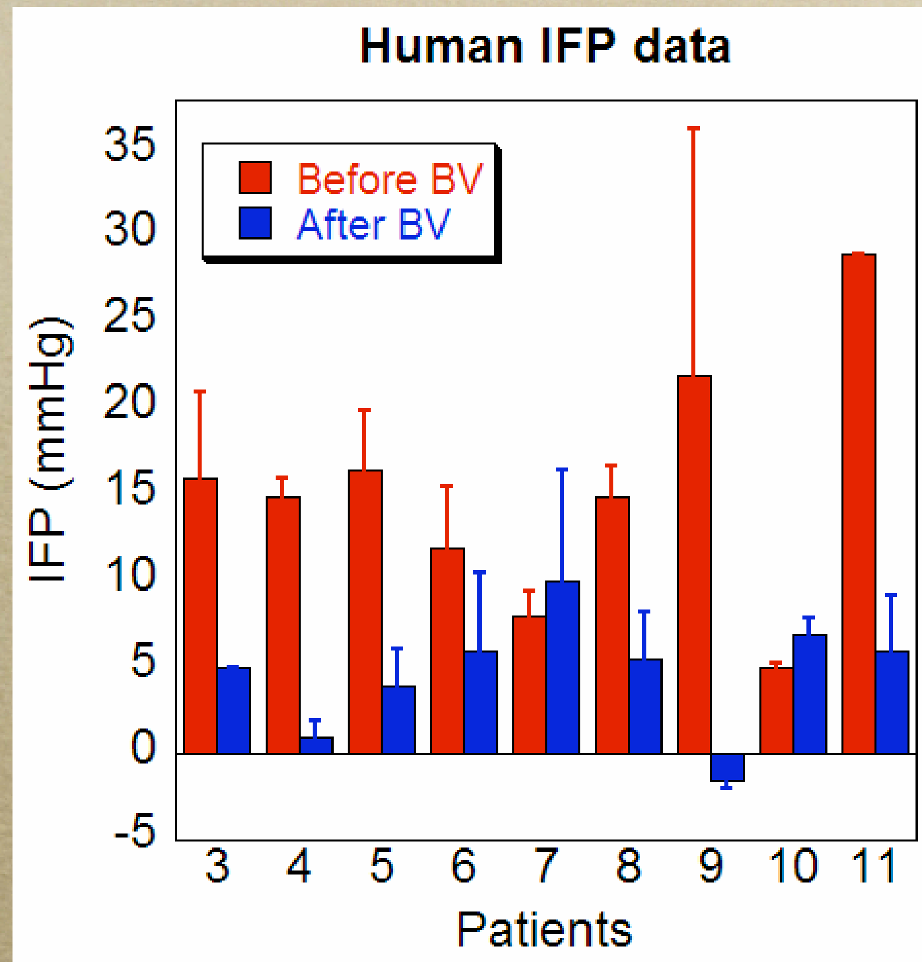


Willett et al. Nature Medicine (2004)

Willett et al. J Clin Oncol (2005)

Endoscopic IFP Measurements





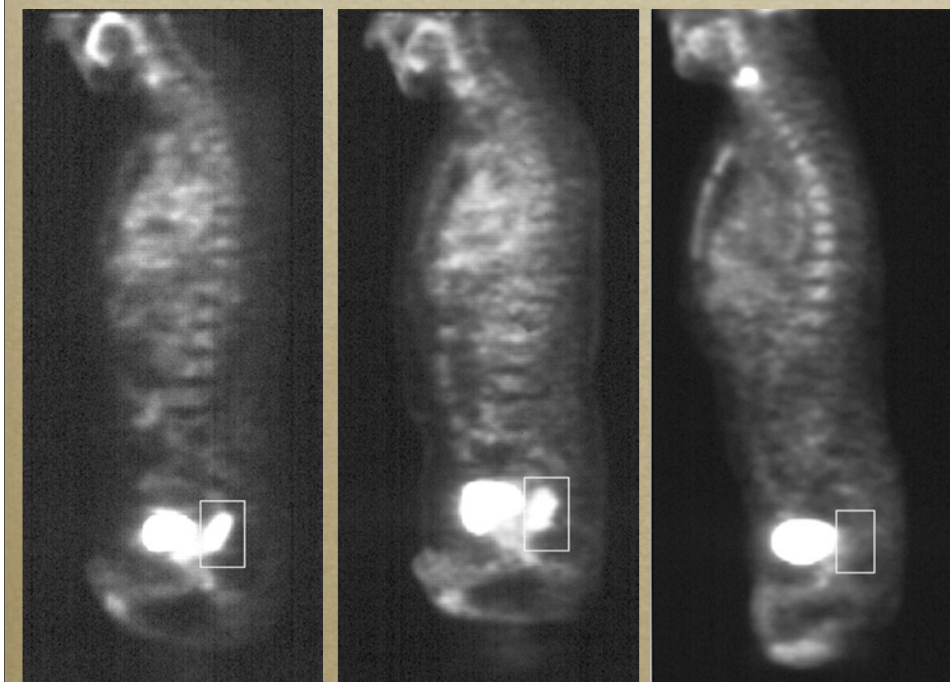
Willett et al. Nature Medicine (2004)

Willett et al. J Clin Oncol (2005)

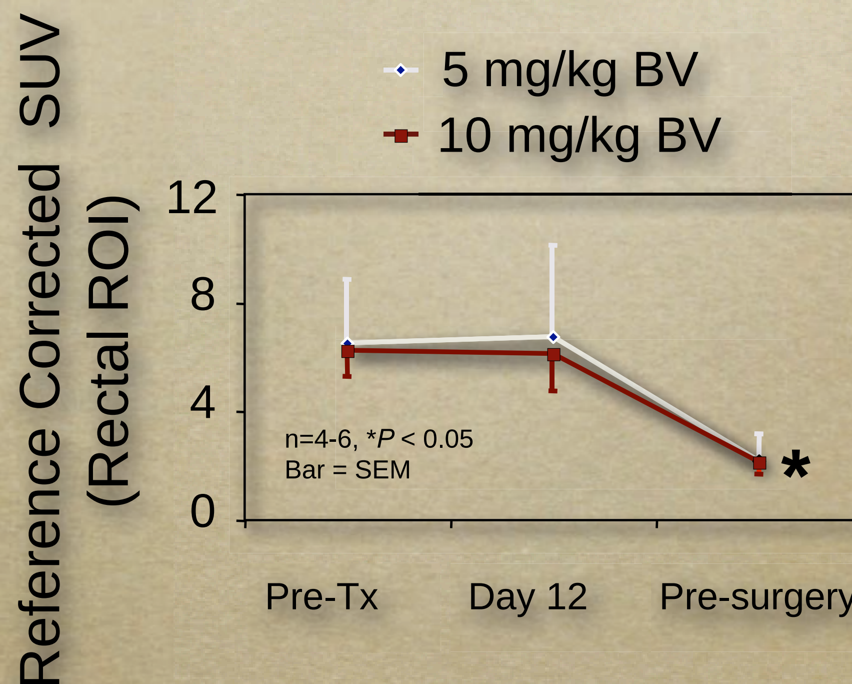
low dose | *high dose*

PET Scan: Tumor FDG Uptake

Sagittal PET scans: Patient #1



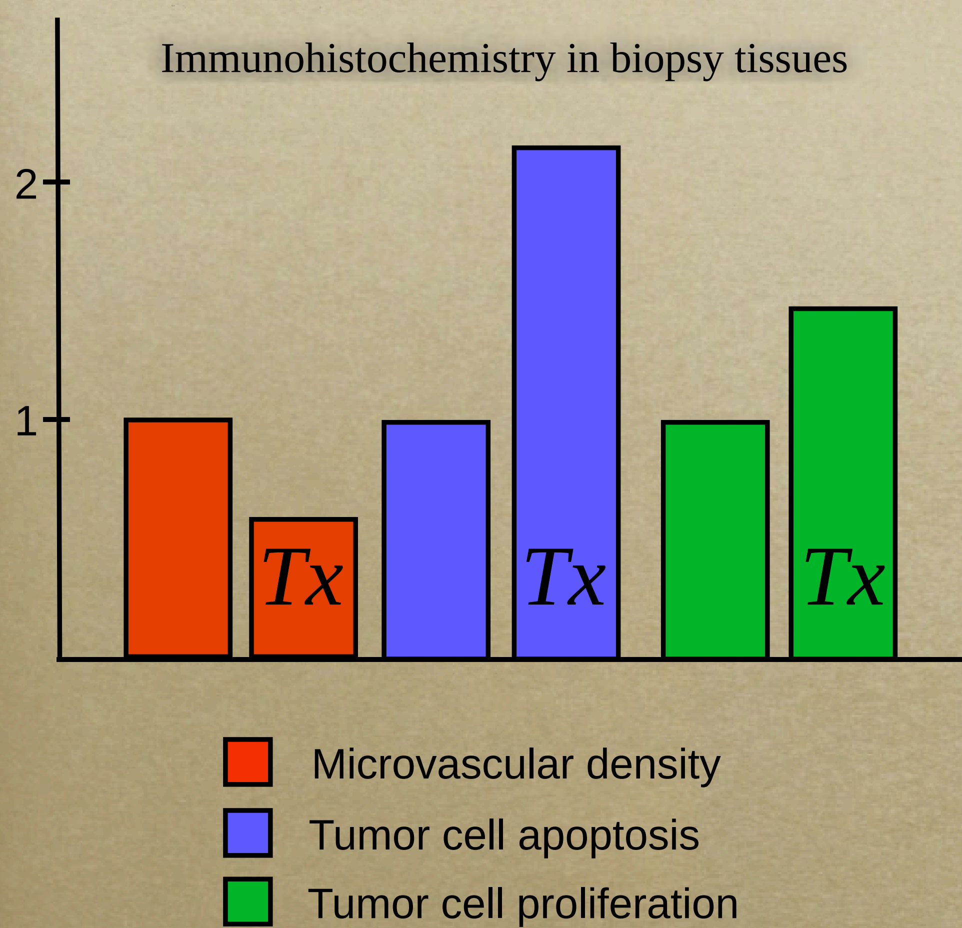
Pre-Tx ***Day 12*** ***Pre-Surgery***

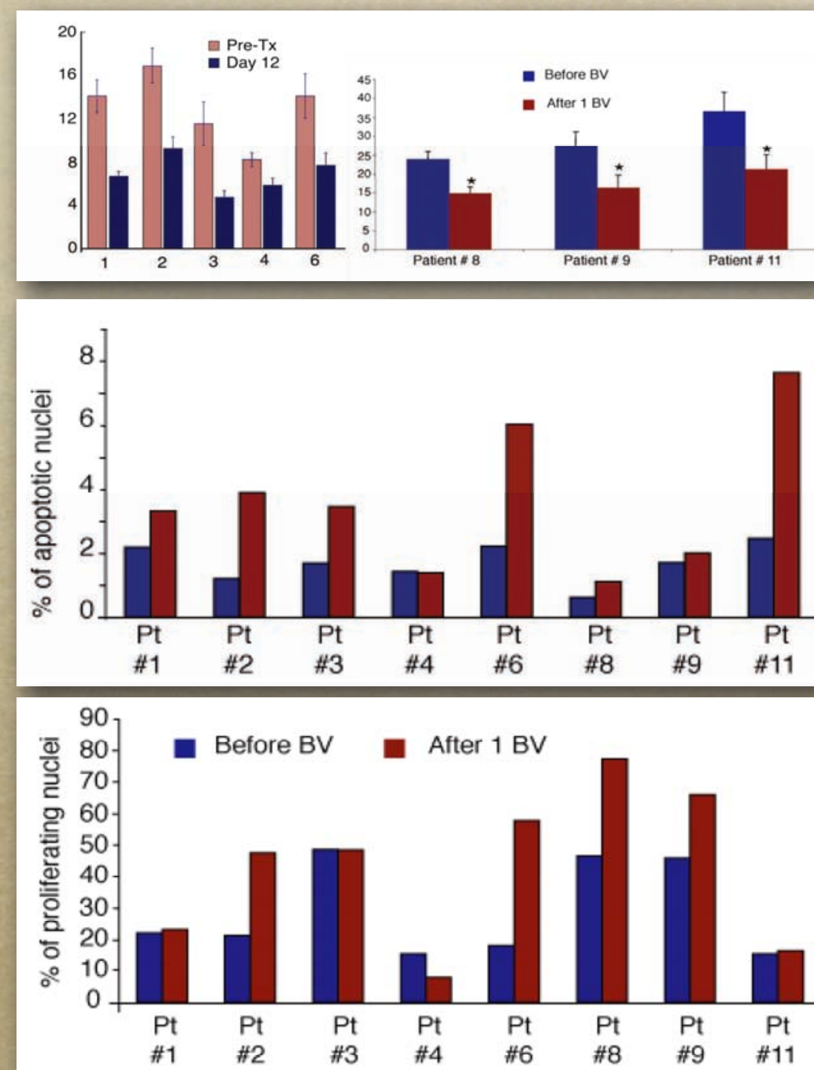


Willett et al. Nature Medicine (2004)

Willett et al. J Clin Oncol (2005)

Tumor response after first BV infusion





Willett et al., Nature Medicine (2004),
Willett et al., Journal of Clinical Oncology (2005)

Pre-clinical and clinical data

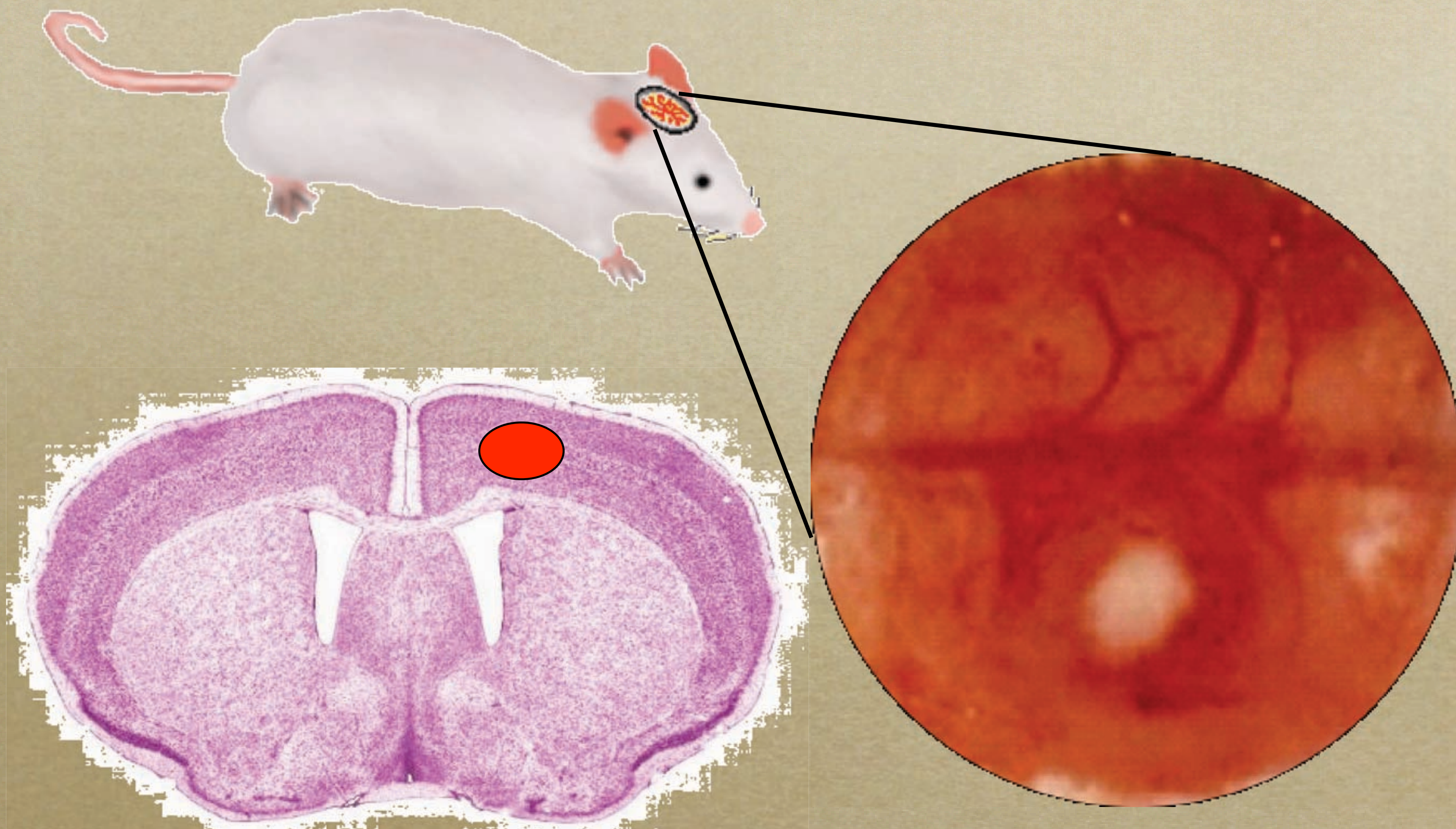
Effects of anti-angiogenic therapy

	Pre-clinical data	***Clinical data***
Blood volume	↓	↓
Vascular density	↓	↓
Permeability (high MW)	↓	
PS product (low MW)		*- (no changes)*
Interstitial fluid pressure	↓	↓
Perivascular cell coverage	↑	↑
Apoptosis/ Proliferation	↑ */?*	↑ ↑
Plasma VEGF /PIGF	↑ ↑	↑ ↑
EPC/CEC	↓ ↓	↓ ↓

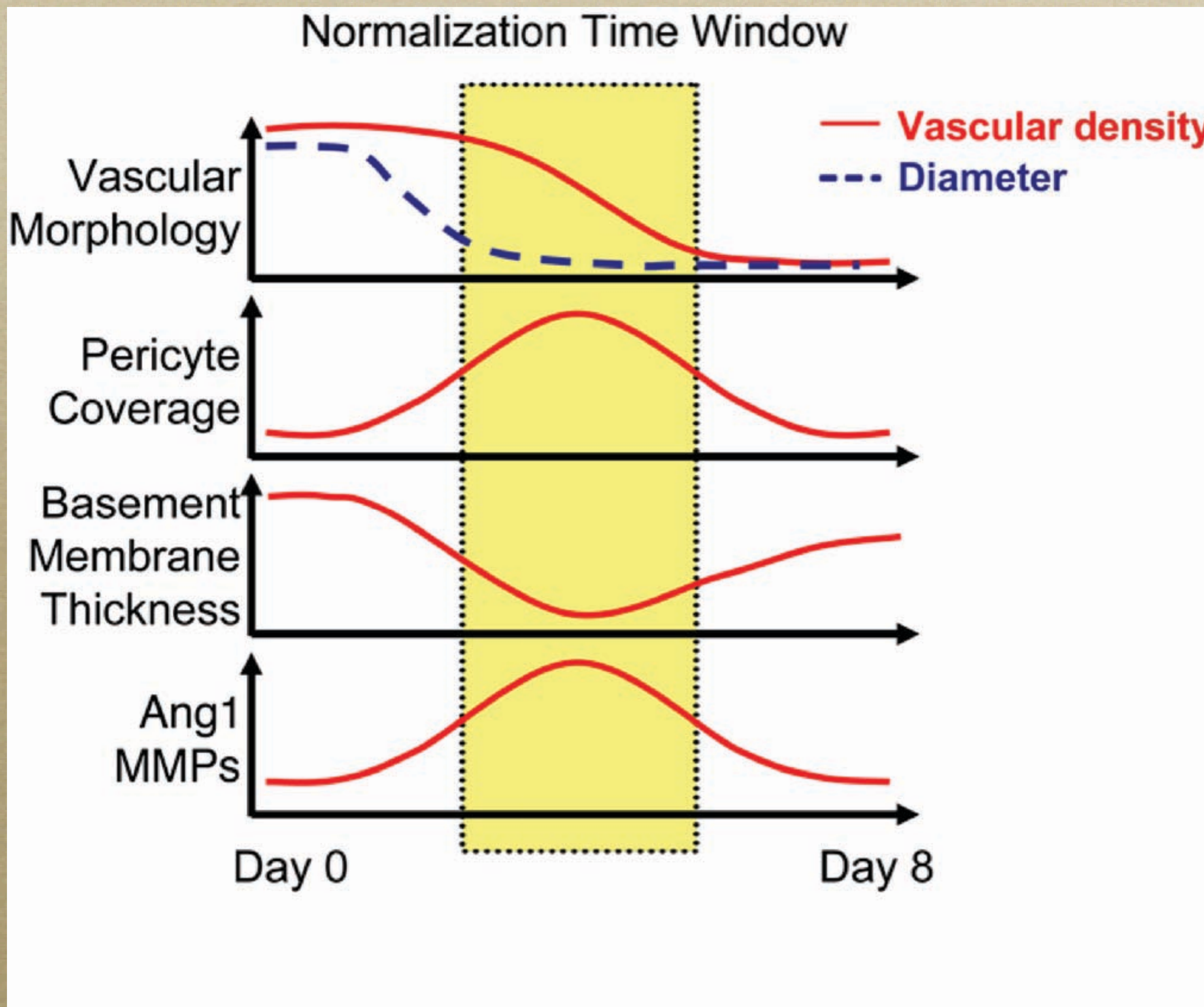
Winkler, Kozin et al. (Cancer Cell 2004)
Willett et al. (Nat. Med. 2004)
Tong et al. (Cancer Res. 2004)

Willett et al. (J. Clin. Oncol. 2005)
Duda et al. (J. Clin. Oncol. 2006)

Cranial Model – Orthotopic Tumor Model

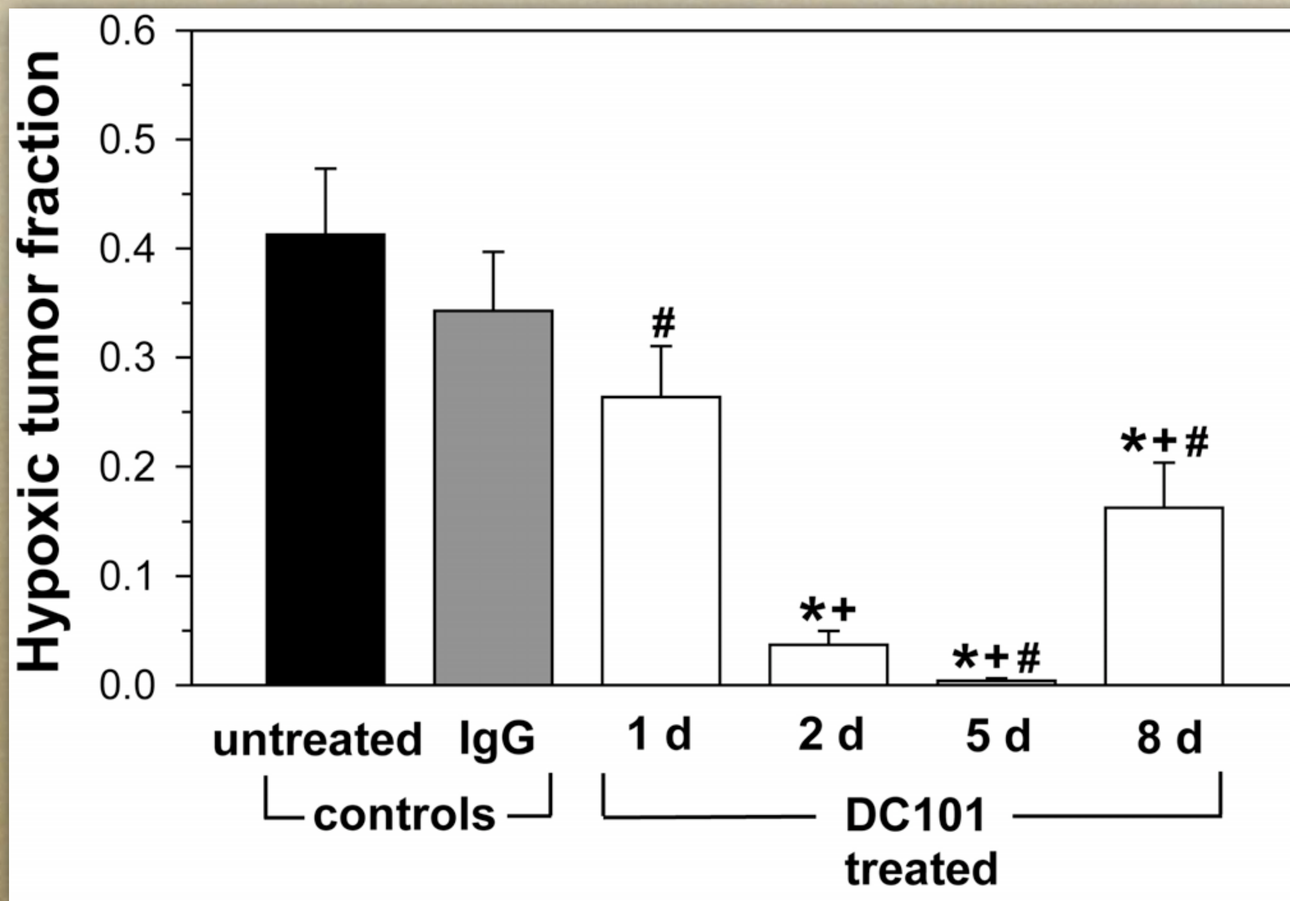


The Vascular Normalization Time Window



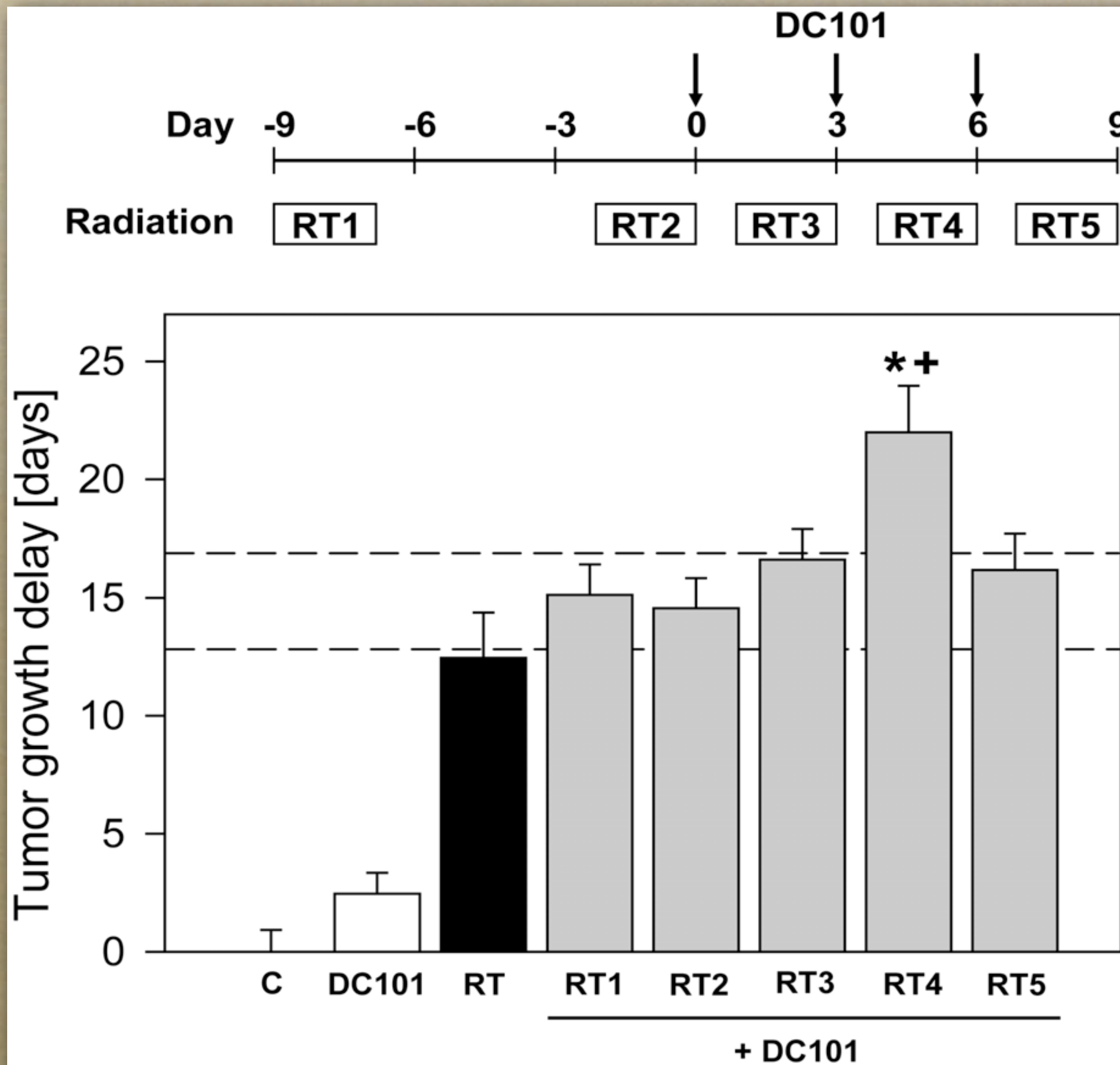
Winkler, Kozin et al., Cancer Cell 2004

DC101 Decreases Tumor Hypoxia During the



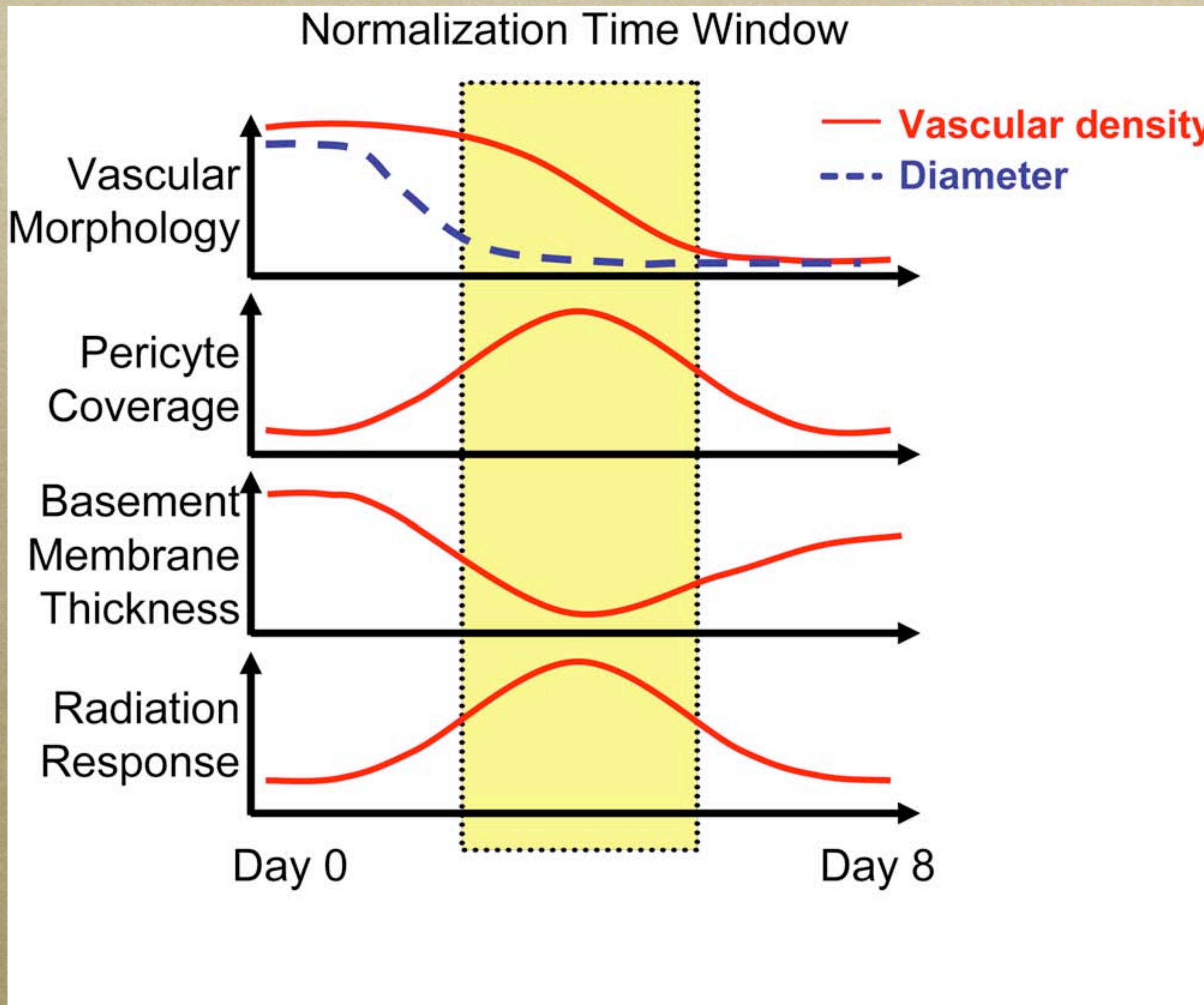
Winkler, Kozin et al., Cancer Cell 2004

Radiation Therapy Acts Synergistically with VEGFR2 Blockade During the Normalization Time Window



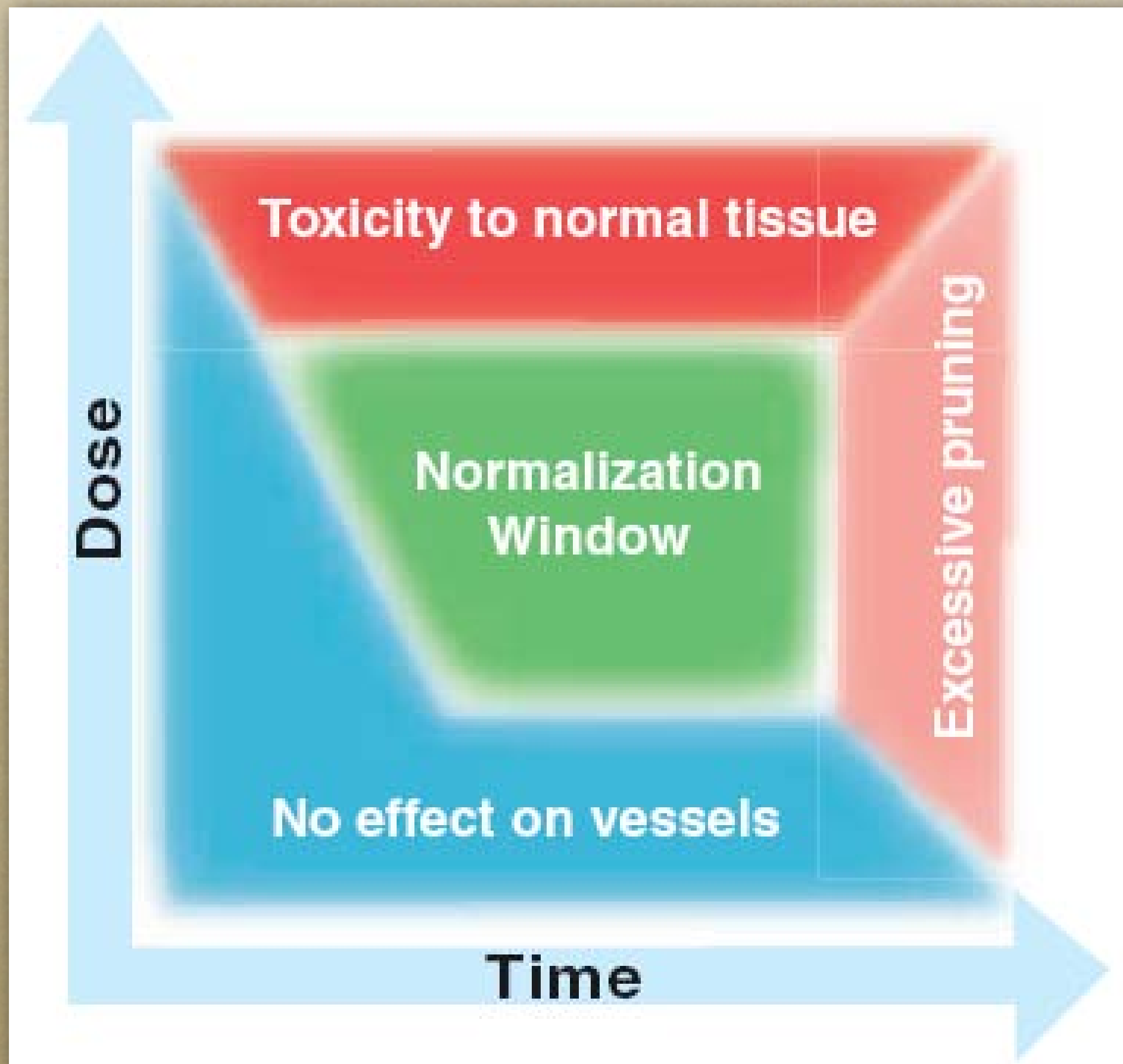
Winkler, Kozin et al., Cancer Cell 2004

The Vascular Normalization Time Window



Winkler, Kozin et al., Cancer Cell 2004

Proposed Normalization Window



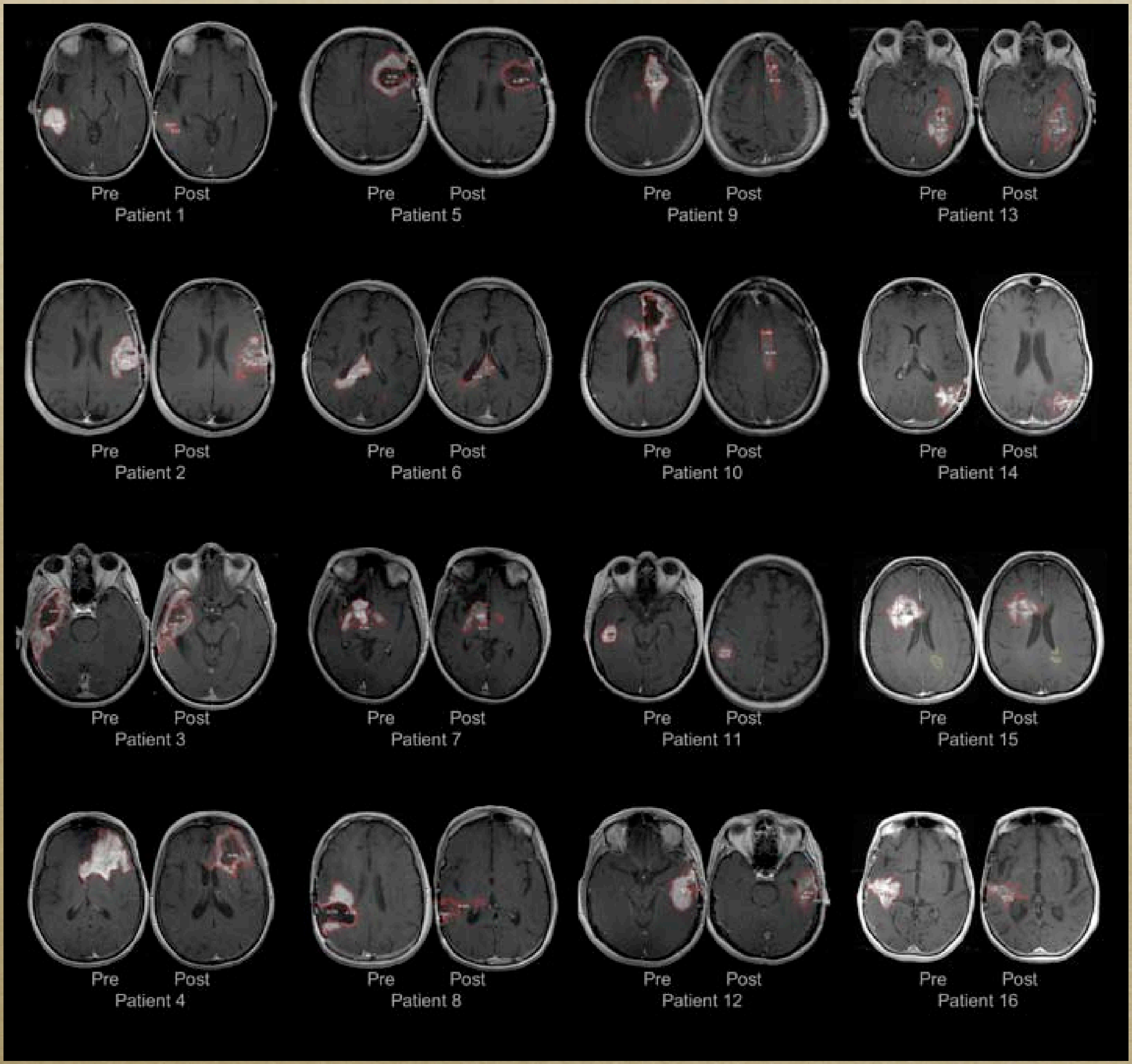
Jain, Science 2005

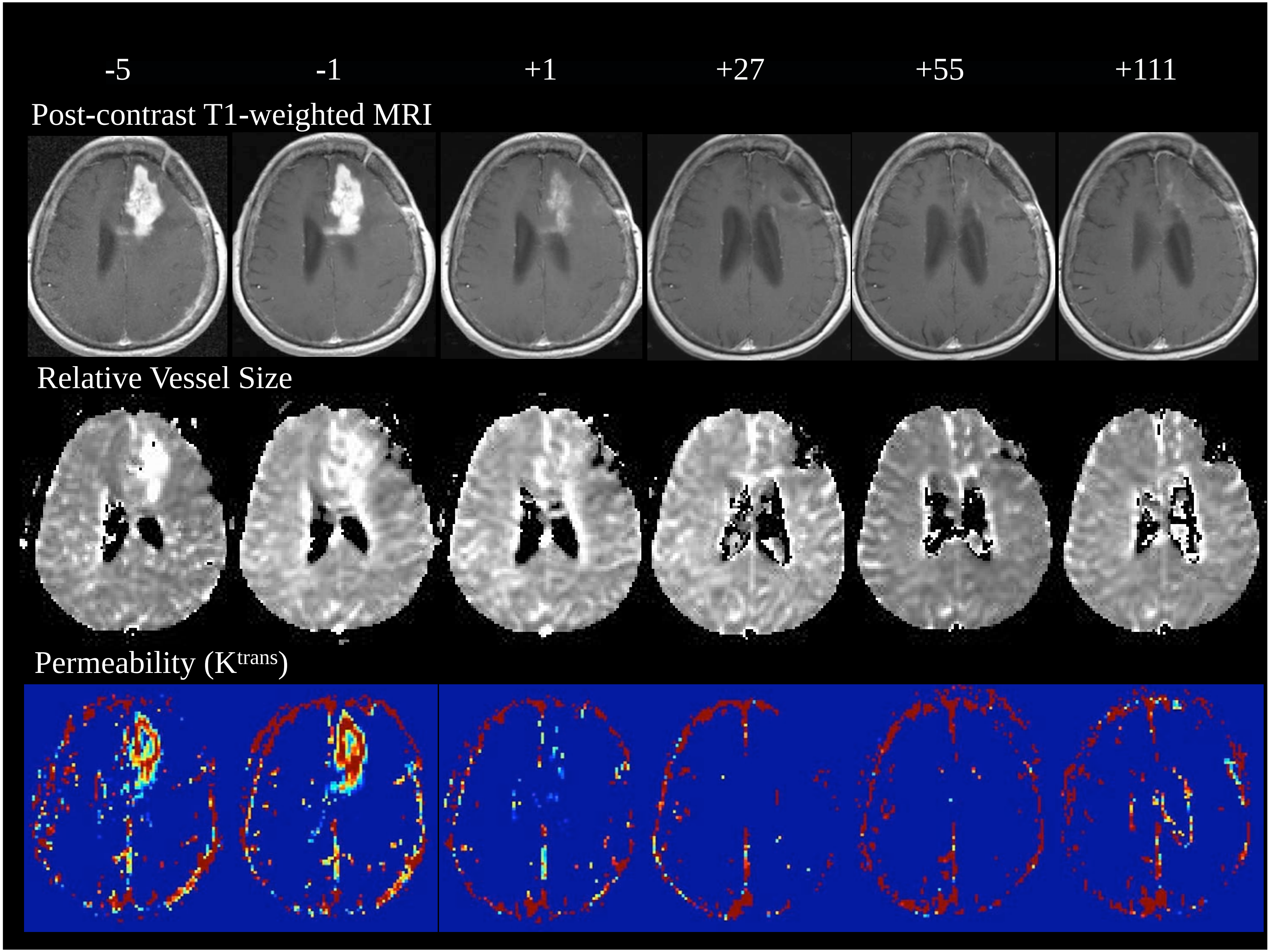
Study Design

			AZD treatment, 45 mg QD						
Day	-5 to -3	-1	8 hours	1-2	9-10	28	56	84	112
MRI	●	●		●		●	●		●
Blood		●	●	●	●	●	●	●	●
Urine*		●	●	●	●	●	●	●	●

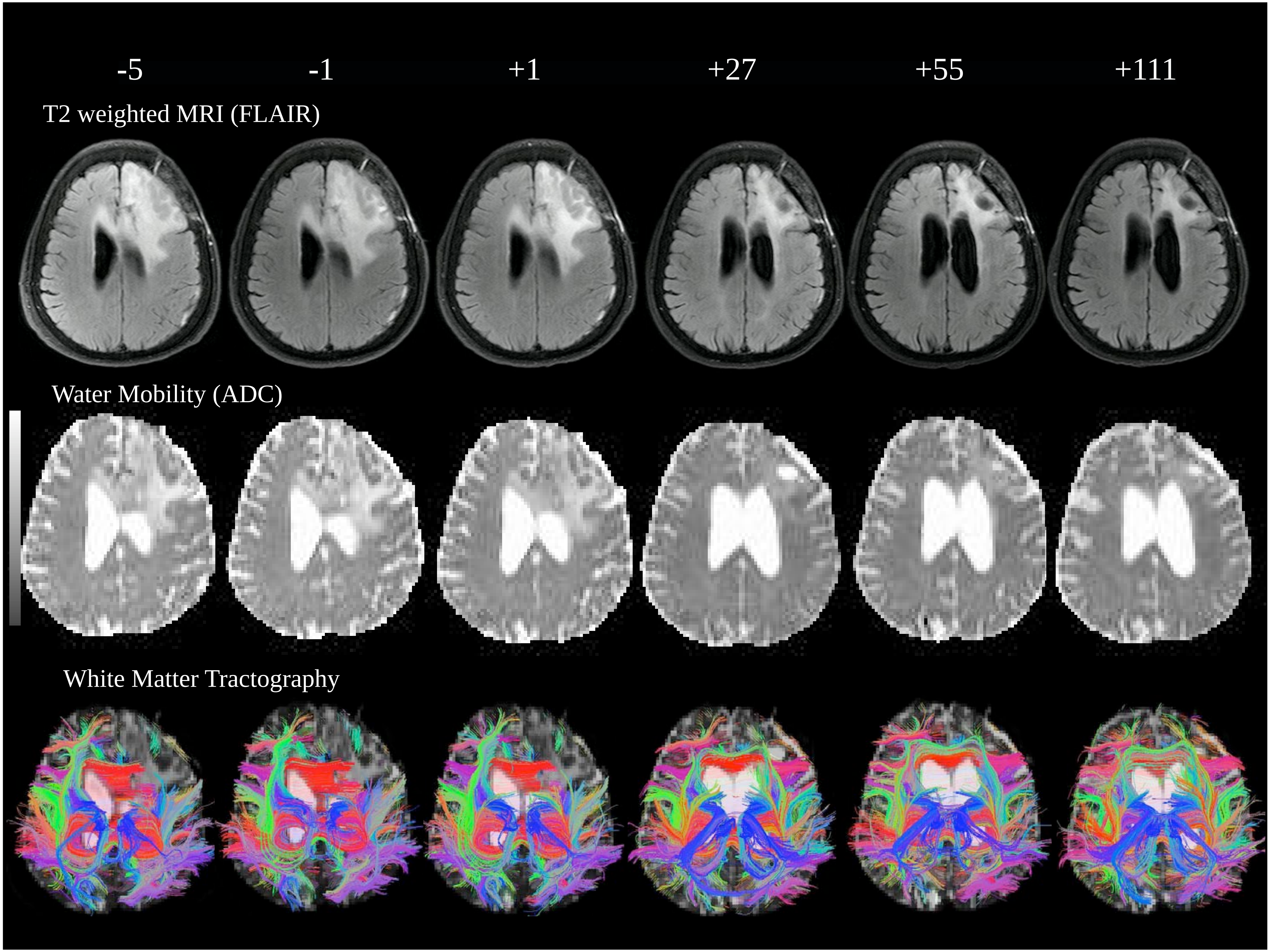
Batchelor et al,
Cancer Cell, 2007

Radiographic responses in the first 16 patients

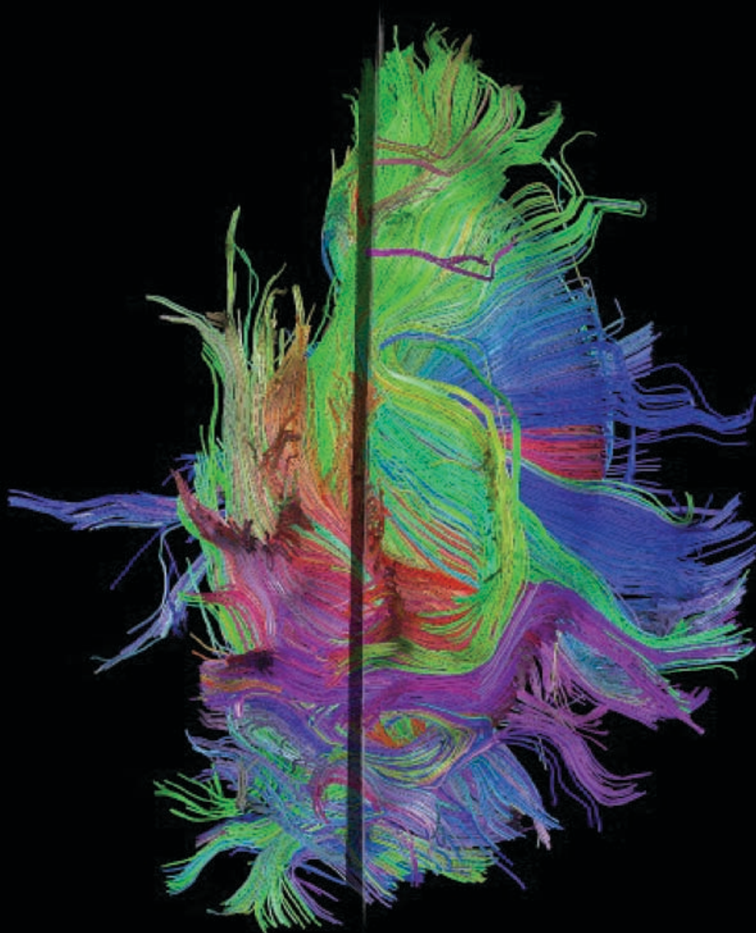




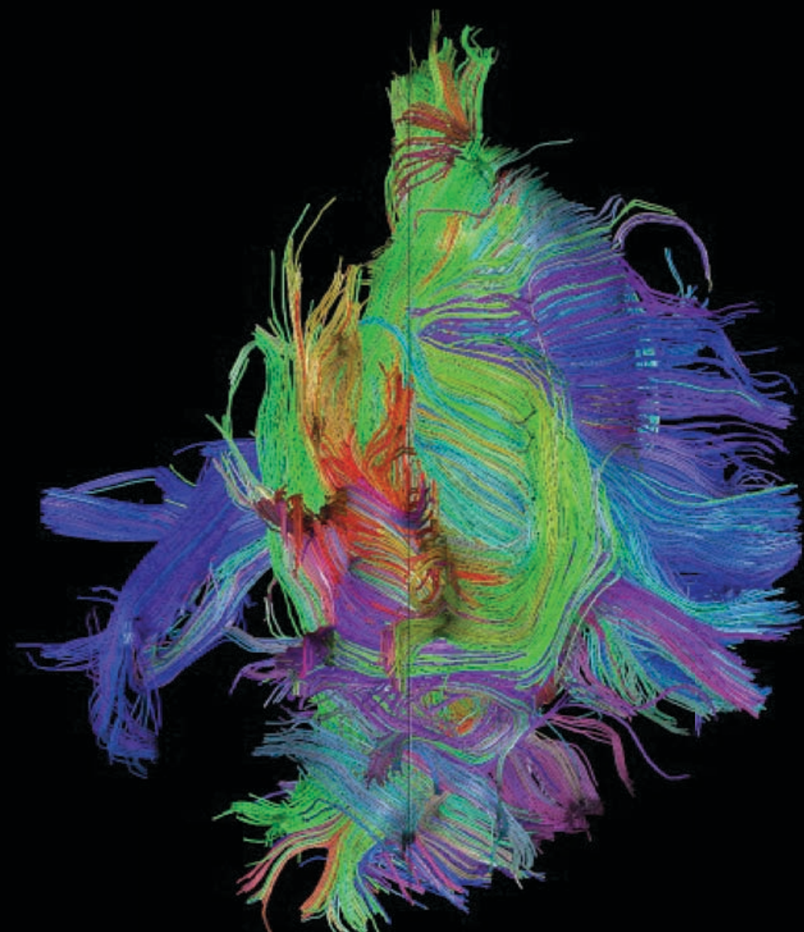
-5
-1
+1
+27
+55
+111
Post-contrast T1-weighted MRI
Relative Vessel Size
Permeability (K^{trans})


-5
-1
+1
+27
+55
+111
T2 weighted MRI (FLAIR)
Water Mobility (ADC)
White Matter Tractography

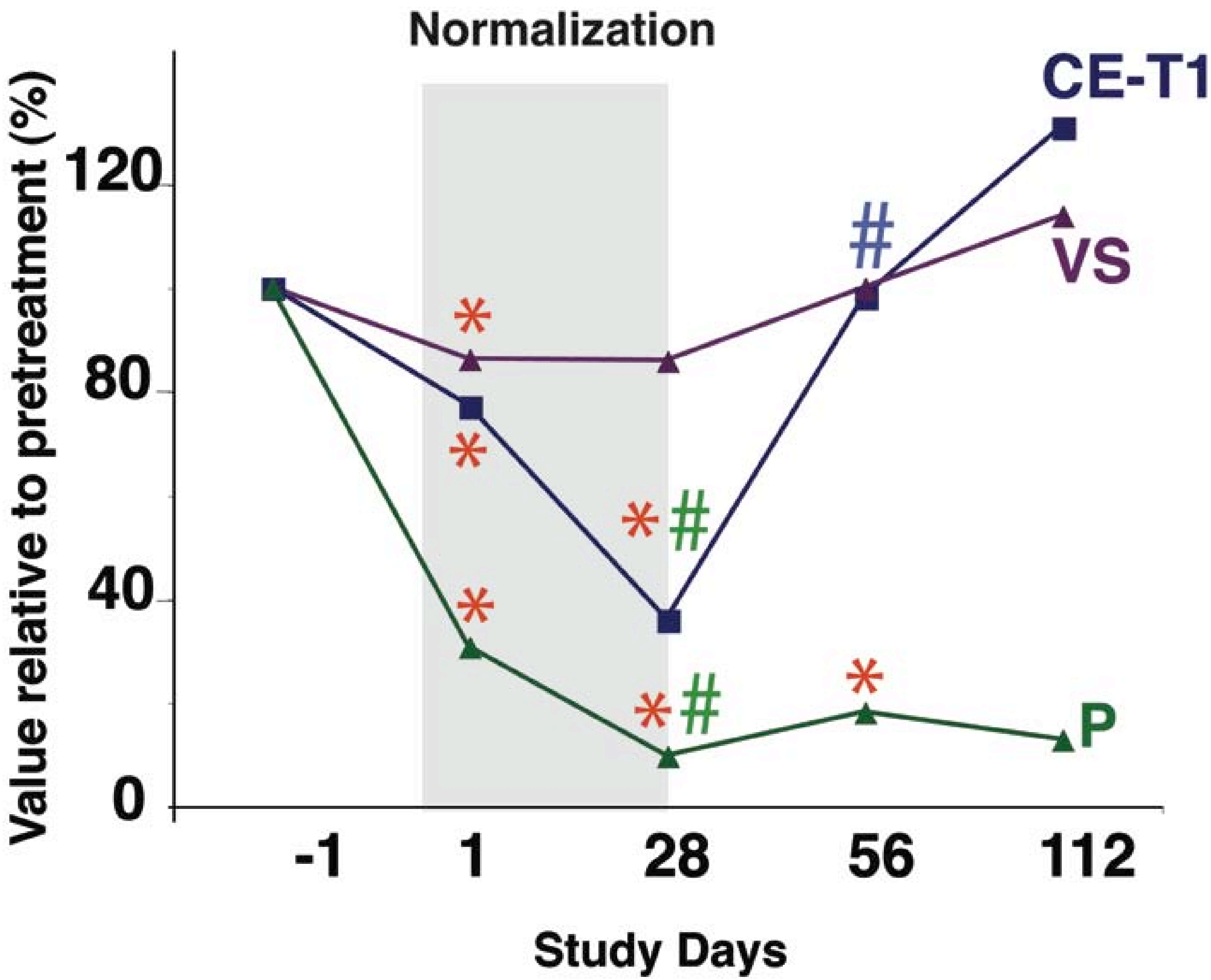
Re-emergence of White Matter Tracts



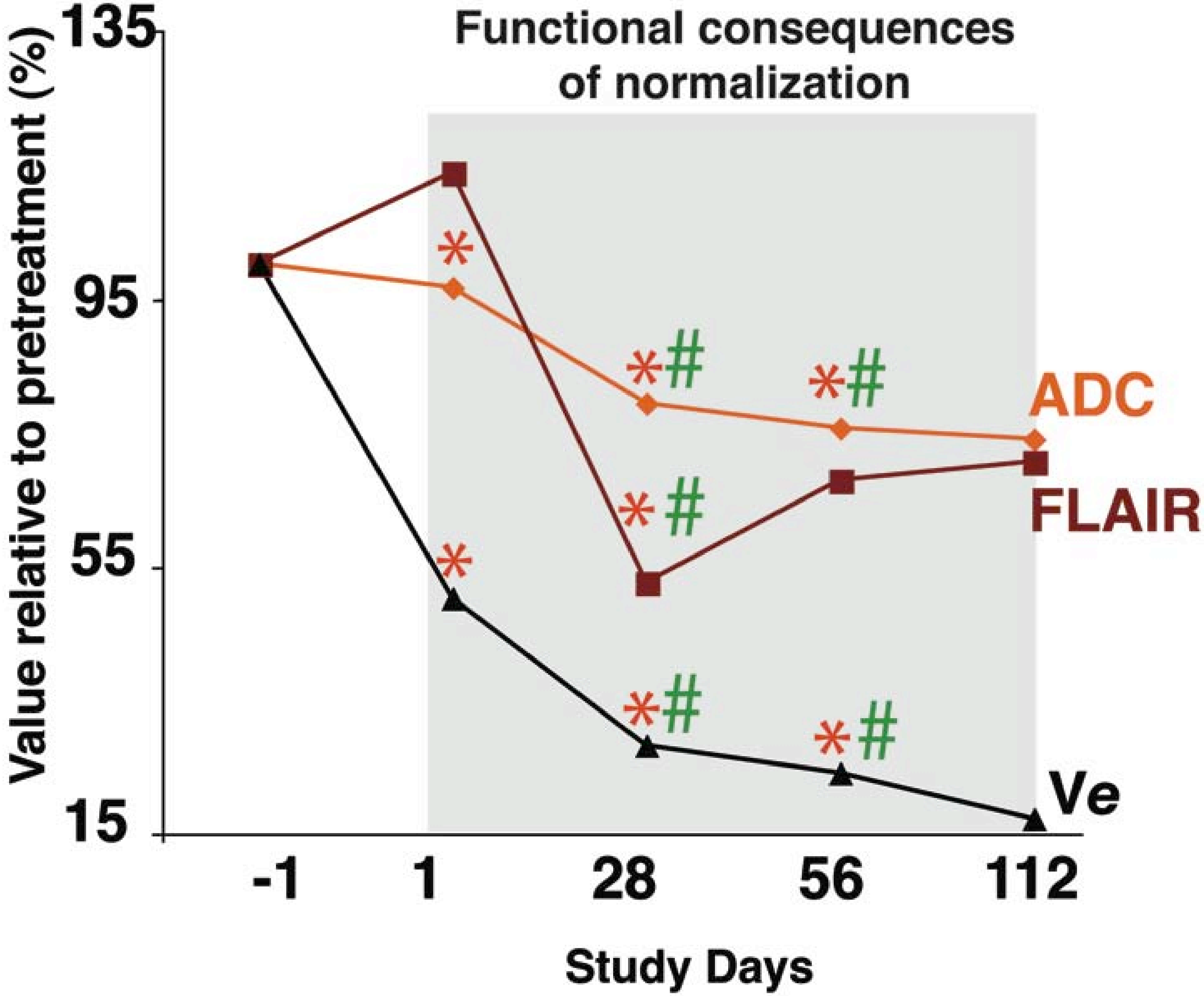
Day -1



Day +27

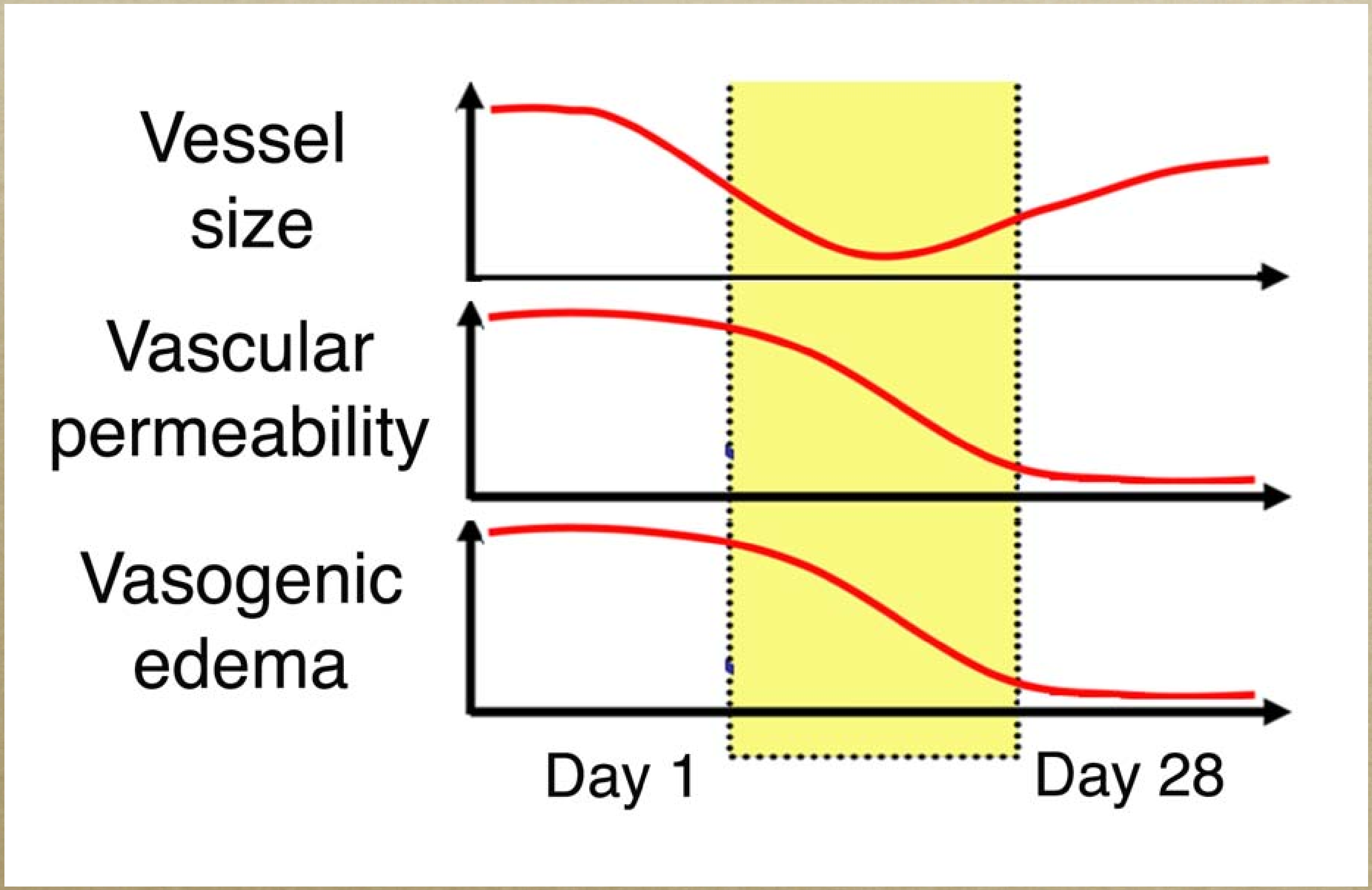


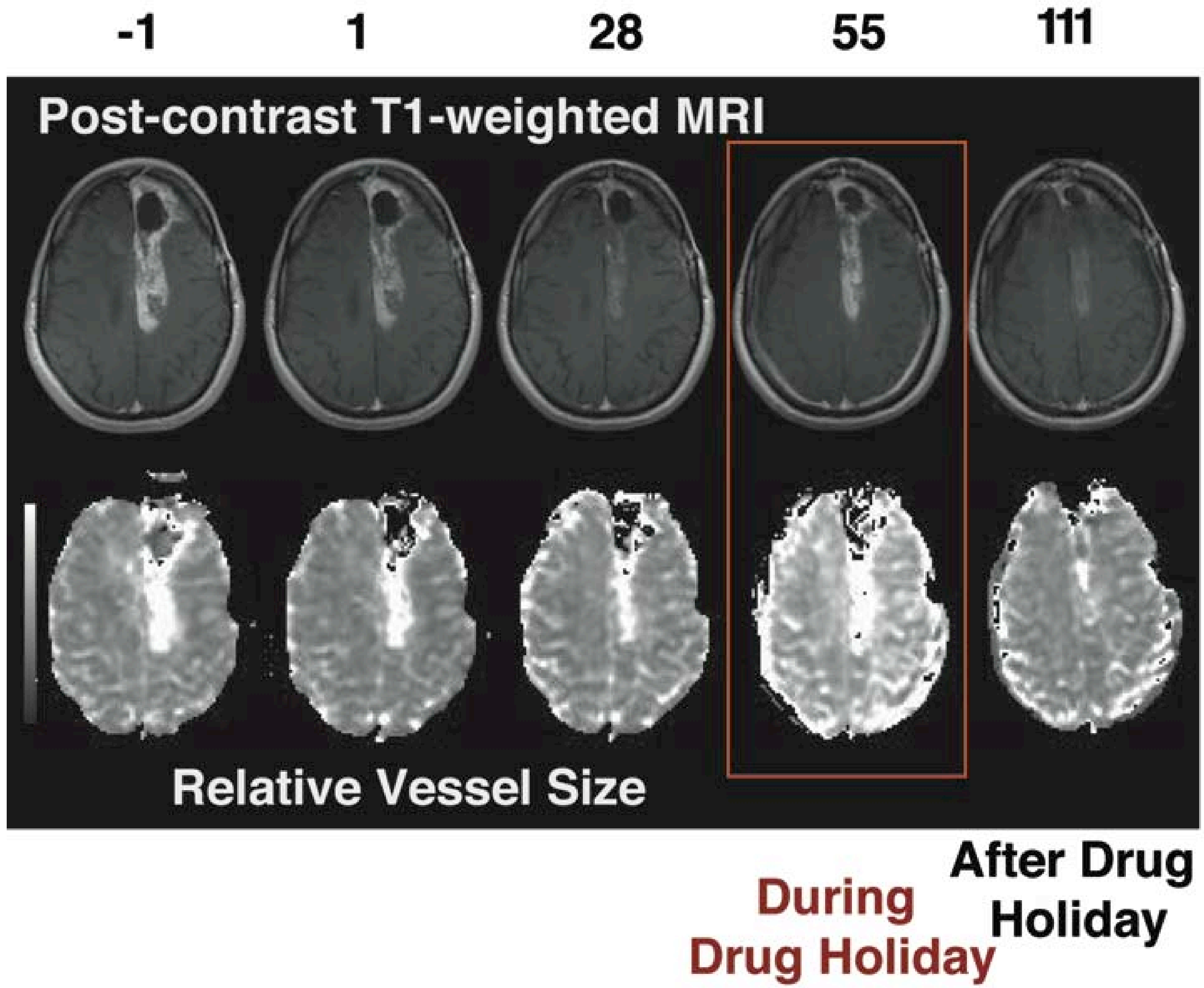
Normalization
CE-T1
VS
P
Value relative to pretreatment (%)
120
80
40
0
-1
1
28
56
112
Study Days



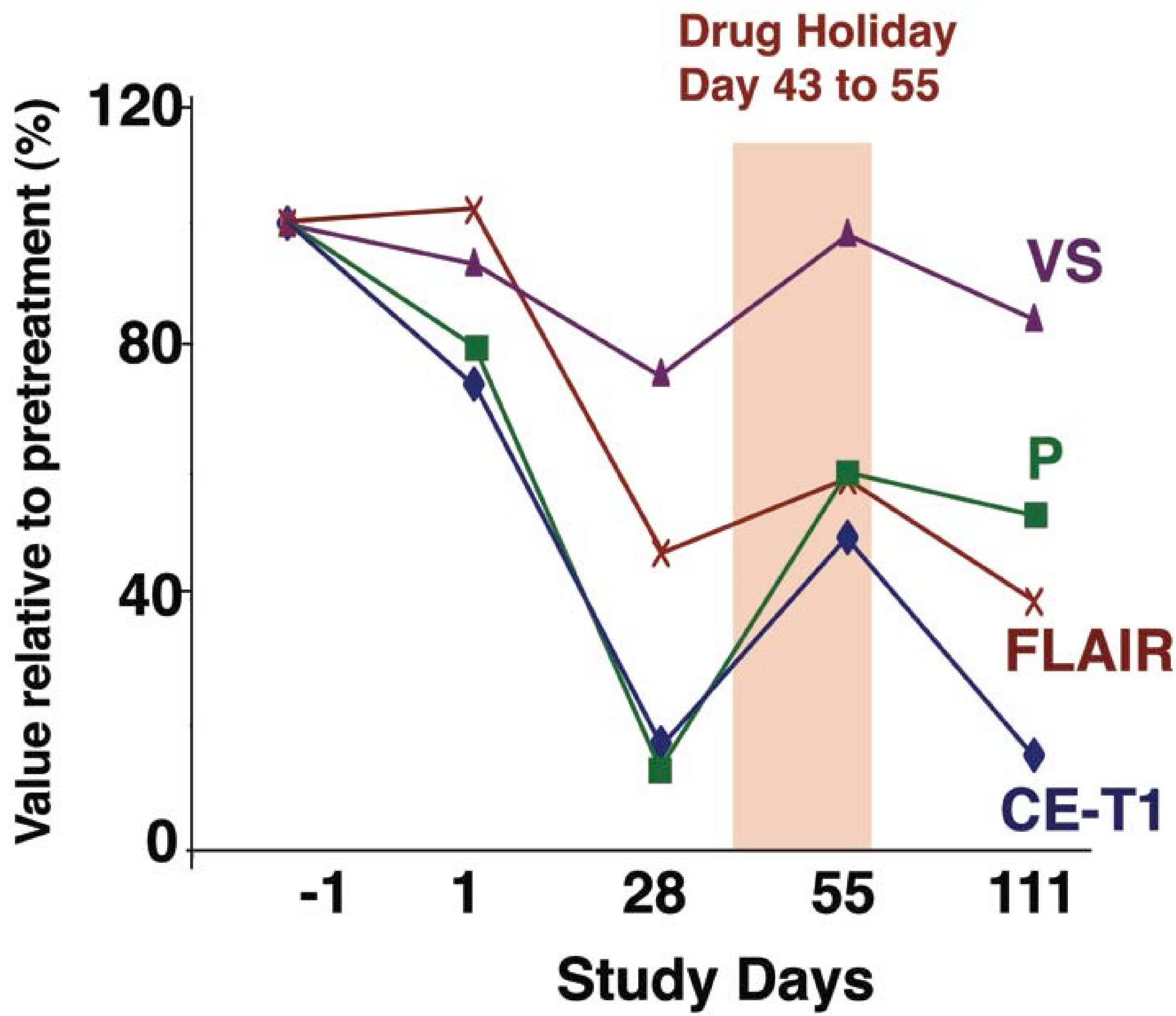
Functional consequences
of normalization
Value relative to pretreatment (%)
135
95
55
15
*
*#
*#
*#
*
*#
*#
ADC
FLAIR
Ve
-1
1
28
56
112
Study Days

Normalization time window in patients





-1
1
28
55
111
Post-contrast T1-weighted MRI
Relative Vessel Size
During Drug Holiday
After Drug Holiday



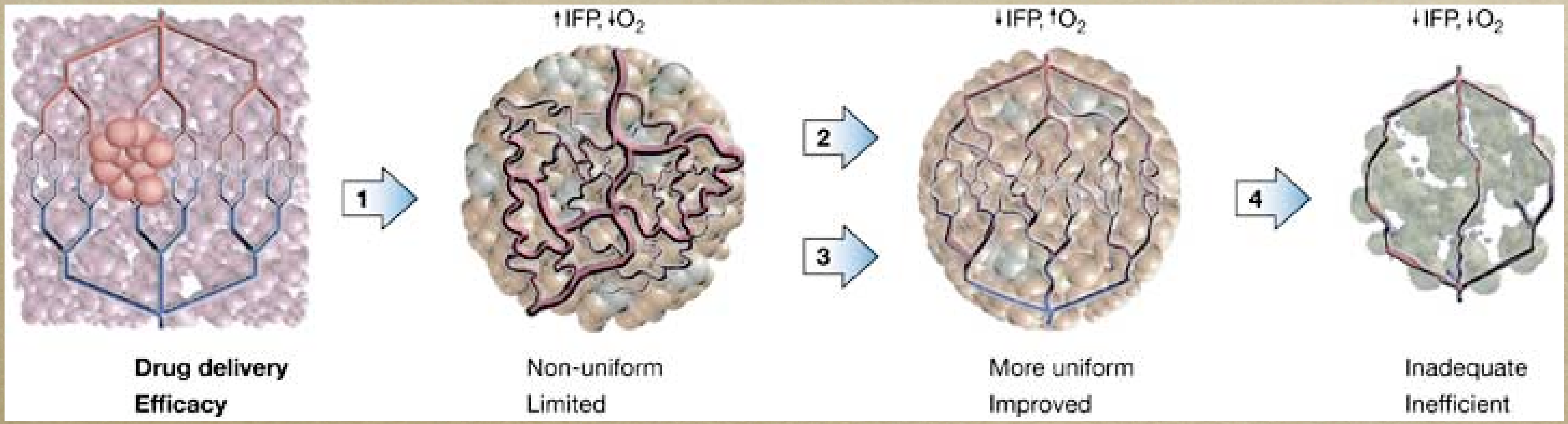
Drug Holiday
Day 43 to 55
120
80
40
0
Value relative to pretreatment (%)
VS
P
FLAIR
CE-T1
-1
1
28
55
111
Study Days

Blood biomarkers

	Effect of tumor progression	Effect of drug holidays	Effect on vessel size
VEGF	NS	-43% [-58% – -22%] $p = 0.0005$	NS
PlGF	-32% [-49% – -9%] $p = 0.0094$	-58% [-72% – -38%] $p < 0.0001$	NS
sVEGFR2	+13% [0% – +26%] $p = 0.0420$	NS	NS
bFGF	+59% [+2% – +148%] $p = 0.0417$	NS	0.138 [0.008 – 0.268] $p = 0.0372$
SDF1α	+12% [+2% – +22%] $p = 0.0158$	NS	0.667 [0.075 – 1.258] $p = 0.0227$
Viable CECs	+53% [+3% – +128%] $p = 0.0347$	NS	NS
CPCs	NS	+75% [+15% – +167%] $p = 0.0097$	NS

Batchelor *et al*, *Cancer Cell* 2007

Potential mechanisms of action of anti-VEGF agents on tumor vasculature



Jain RK et al. (2006) Lessons from phase III clinical trials on anti-VEGF therapy for cancer
Nat Clin Pract Oncol ***3:*** *24–40 doi:10.1038/ncponc0403*

nature CLINICAL PRACTICE ONCOLOGY

Collaborators

Chris Willett
Duke Radiation Oncology

Daniel Chung
Gastrointestinal Unit

Dushyant Sahani and Sanjeeva Kalva
Department of Radiology

Kenneth Cohen and David Scadden
Center for Regenerative Medicine & Harvard Stem Cell Institute

Alan Fischman
Department of Nuclear Medicine

Jeffrey Clark, David Ryan, Andrew Zhu & Lawrence Blaszkowsky
Division of Hematology/Oncology

Paul Shellito
Department of Surgery

Mari Mino-Kenudson and Gregory Lauwers
Department of Pathology

Collaborators

Tracy Batchelor, PI
Pappas Center for Neuro-Oncology/Radiation Oncology, MGH

Dan Duda, Emmanuelle di Tomaso, Kevin Kozak, Marek Ancukiewicz, Jay Loeffler
Steele Laboratory for Tumor Biology/Radiation Oncology, MGH

Gregory Sorensen, Wei-Ting Zhang, Poe-Jou Chen, Mingwang Zhu, Thomas Benner
Department of Radiology, MGH

Kenneth Cohen & David Scadden
Center for Regenerative Medicine, MGH & Harvard Stem Cell Institute

Maciej Mrugala, Scott Plotkin, Jan Drappatz & Patrick Wen
Department of Neuro-Oncology, MGH/DFCC

Daniel Cahill & David Louis
Department of Pathology, MGH

Percy Ivy
National Cancer Institute

Steele Lab

Emmanuelle di Tomaso

Yves Boucher

Dan Duda

Dai Fukumura

Sergey Kozin

Lance Munn

Ricky Tong