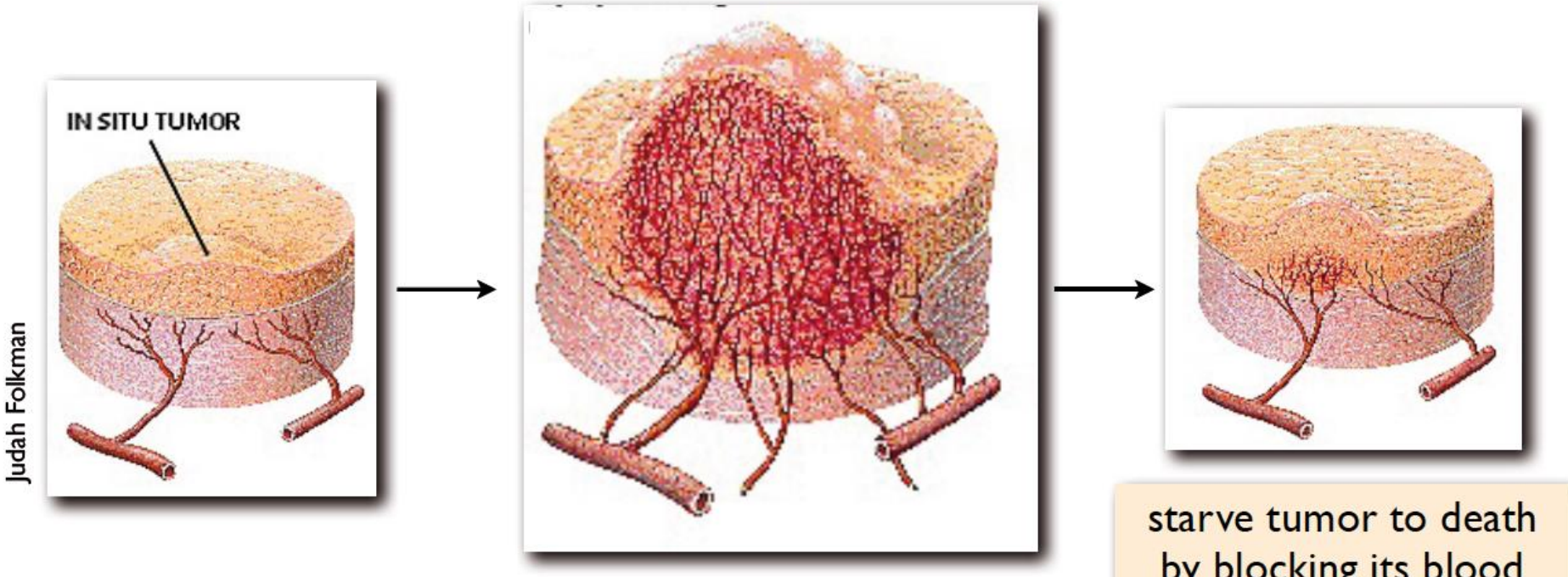
ANTI-ANGIOGENIC THERAPY

Miki De Palma, PhD
ISREC, EPFL

The phylosophy of anti-angiogenic therapy



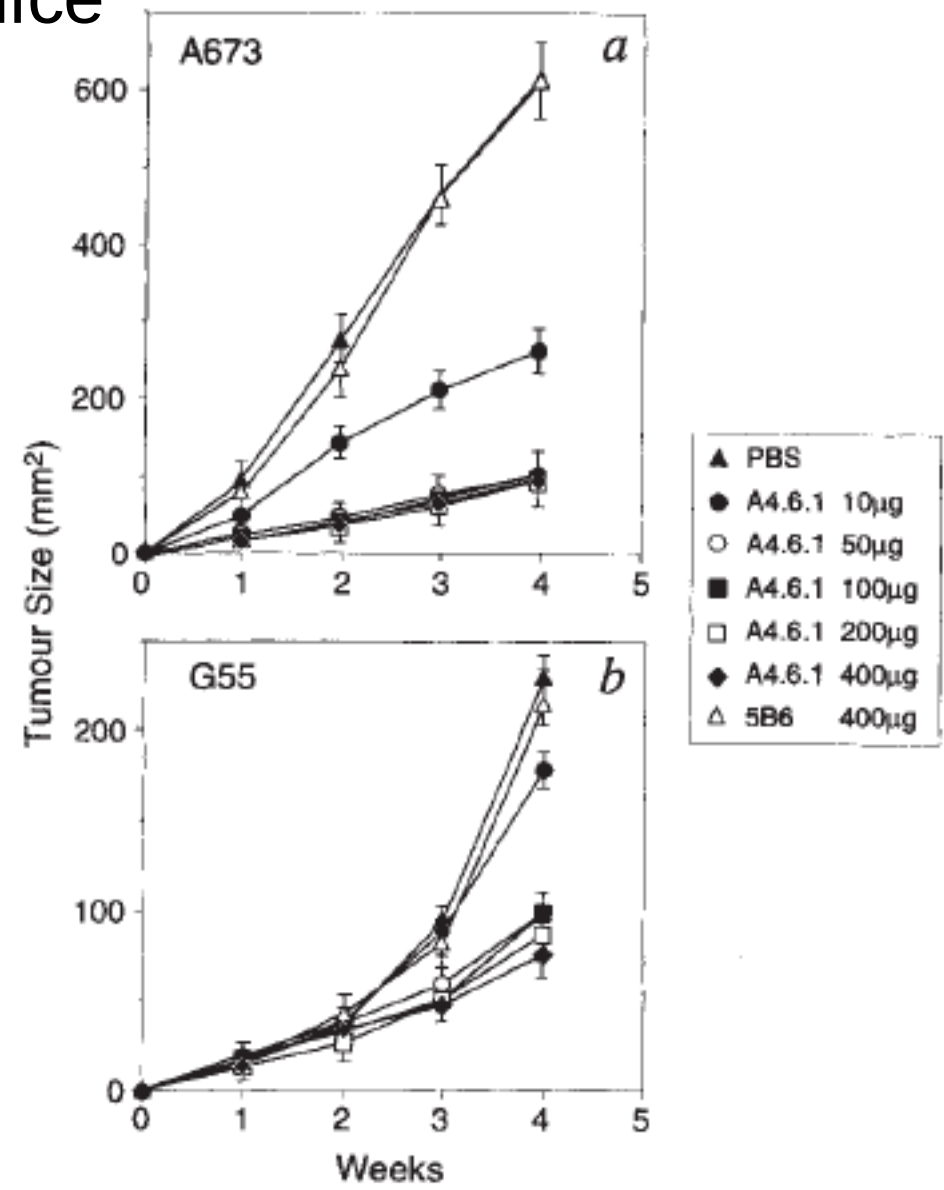
An anti-VEGFA monoclonal antibody delays tumor growth in mice

Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*

K. Jin Kim, Bing Li, Jane Winer, Mark Armanini, Nancy Gillett, Heidi S. Phillips & Napoleone Ferrara*

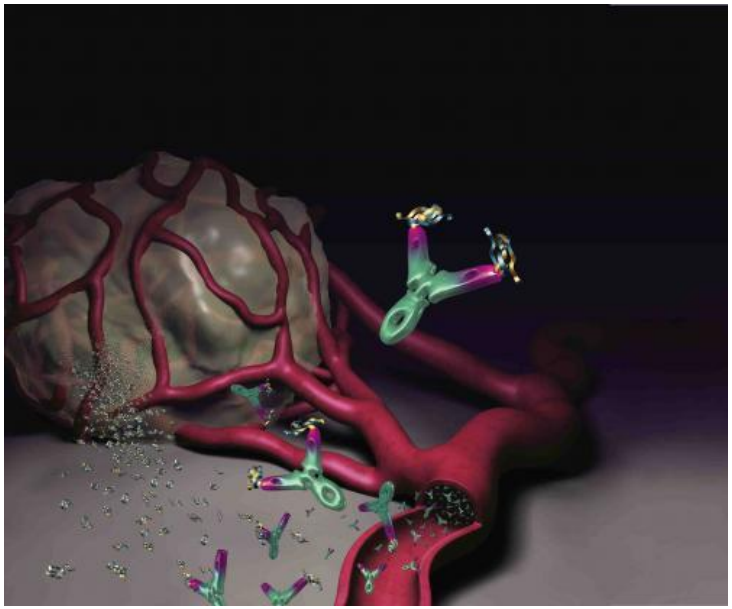
Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA

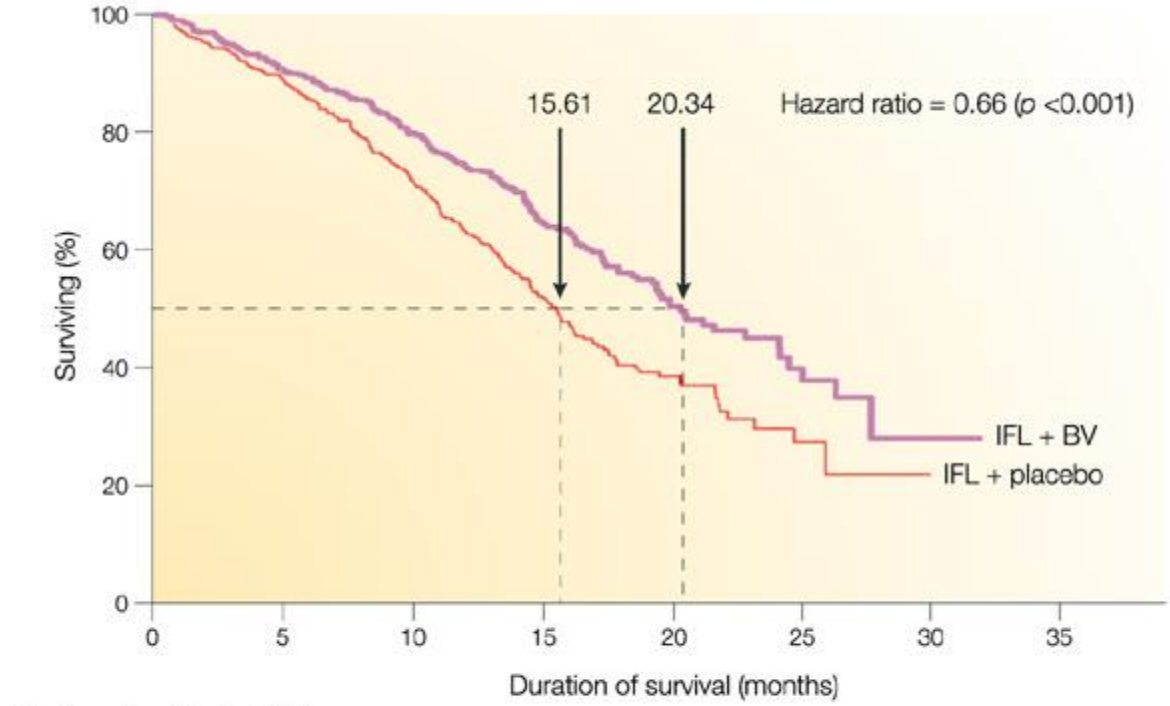
NATURE · VOL 362 · 29 APRIL 1993



Targeting tumor angiogenesis: efficacy of an anti-VEGF monoclonal antibody (bevacizumab)

Bevacizumab plus Irinotecan, Fluorouracil and Leucovorin for Metastatic Colorectal Cancer (FDA approval)





Number of subjects at risk

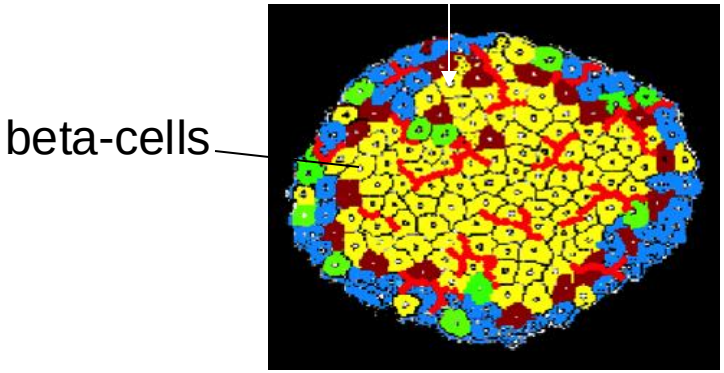
IFL + BV	402	362	320	178	73	20	1	0
IFL + placebo	411	363	292	139	51	12	0	0

Hurwitz et al., N Engl J Med. 2004

RIP1-Tag2 transgenic mouse model of pancreatic neuroendocrine cancer

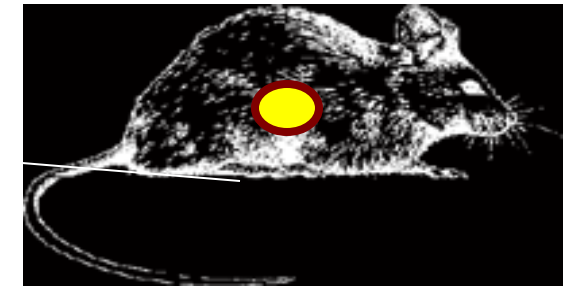
(Rat insulin gene promoter region)

(Potent oncoprotein, inactivates p53 and pRb)



Islet of Langerhans

RIP1-Tag transgenic mice



Hanahan, Nature 1984

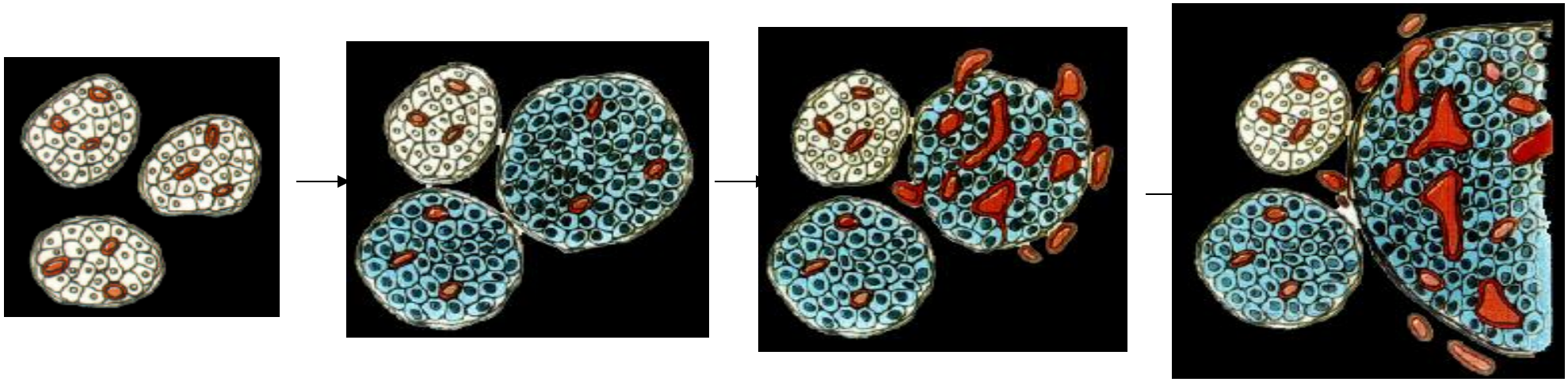
The model system: Multistage tumorigenesis of pancreatic islets in RIP-Tag transgenic mice

Normal stage (onc+)

Hyperplastic/ dysplastic stage

Angiogenic stage (transition to malignancy)

Tumor stage



<5 wks

100%

5-7 wks

~50%

7-12 wks

~10%

12-14 wks

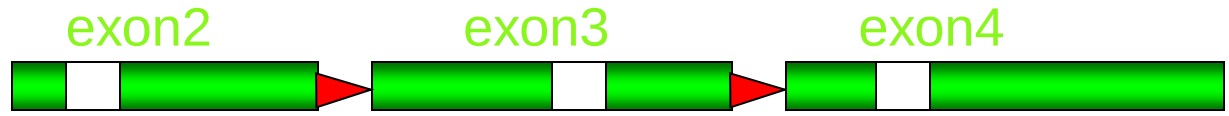
2-4%

Genetic knockout of VEGFA in cancer cells of RIP1-Tag2 mice

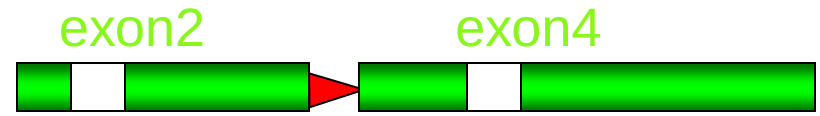
Rip-Cre
Transgene
(ß cell selective)

VEGF gene
(functional)

= loxP sites
flanking exon 3

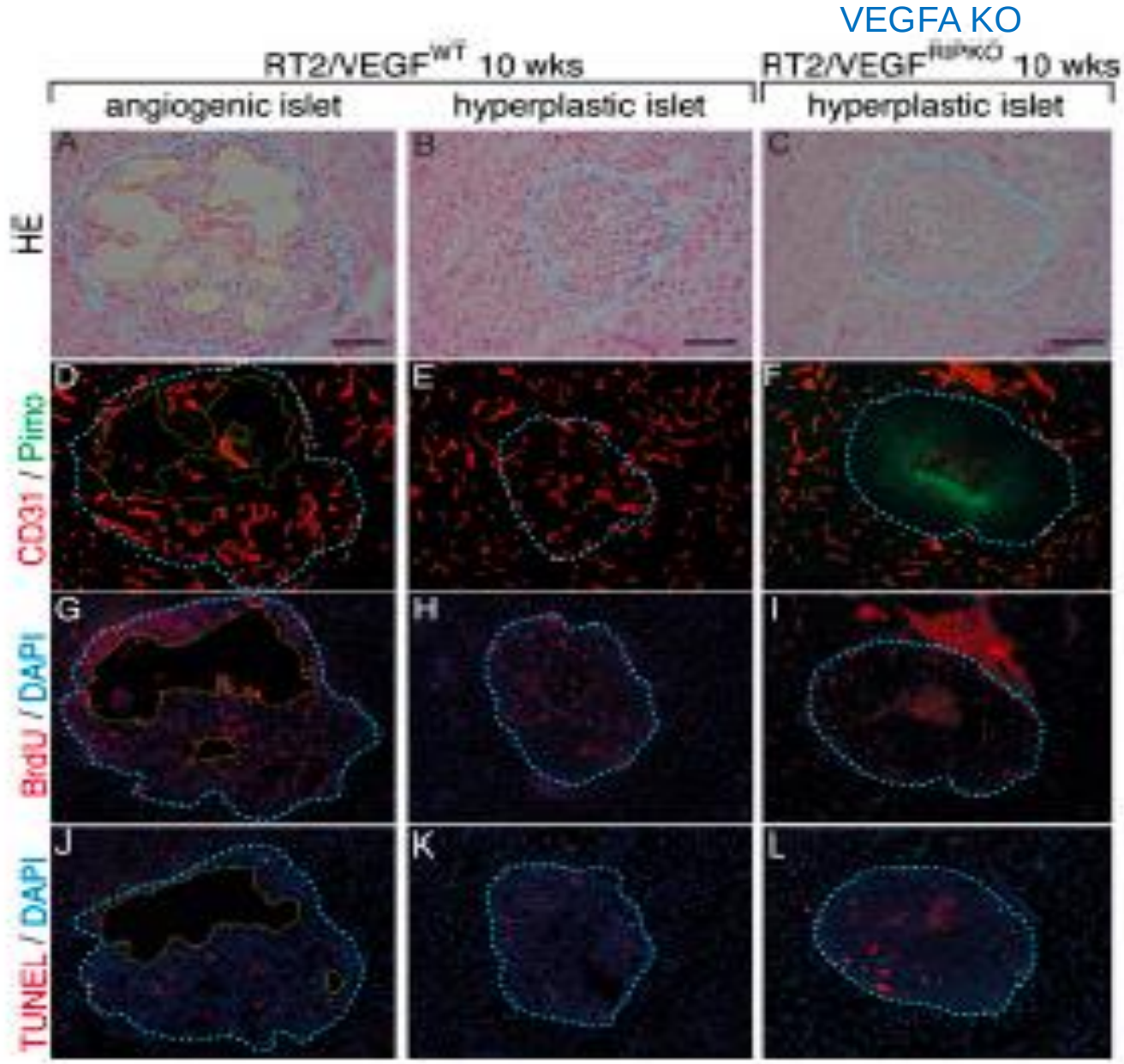


ß cell-specific gene
KO of VEGFA



Genetic deletion of VEGFA shows its importance for the angiogenic switch

VEGFA KO

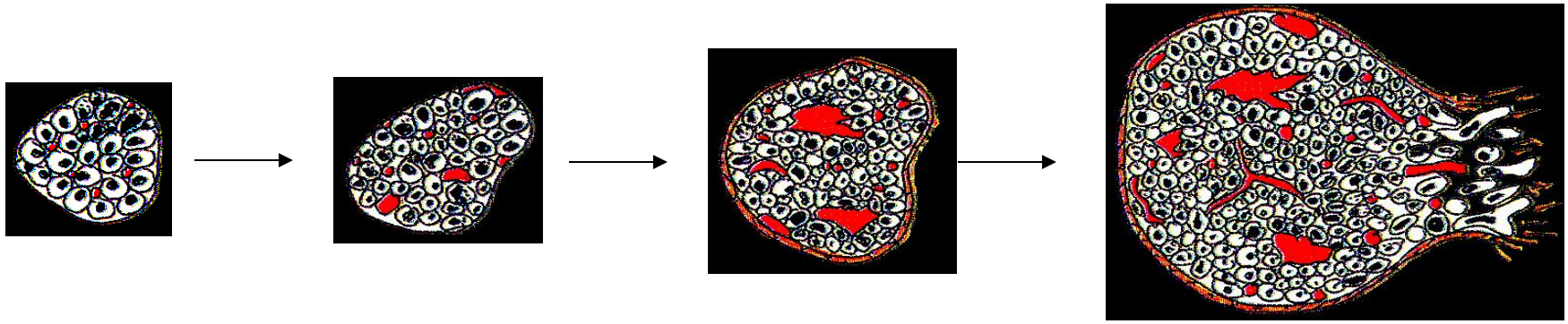


Hypoxia
Endothelial cells

Nuclei
Proliferation

Nuclei
Apoptosis

Stage-specific therapeutic trials in RIP-Tag mice



Prevention

5-10.5 wk treatment

Intervention

11- 14.5 wk treatment

Regression

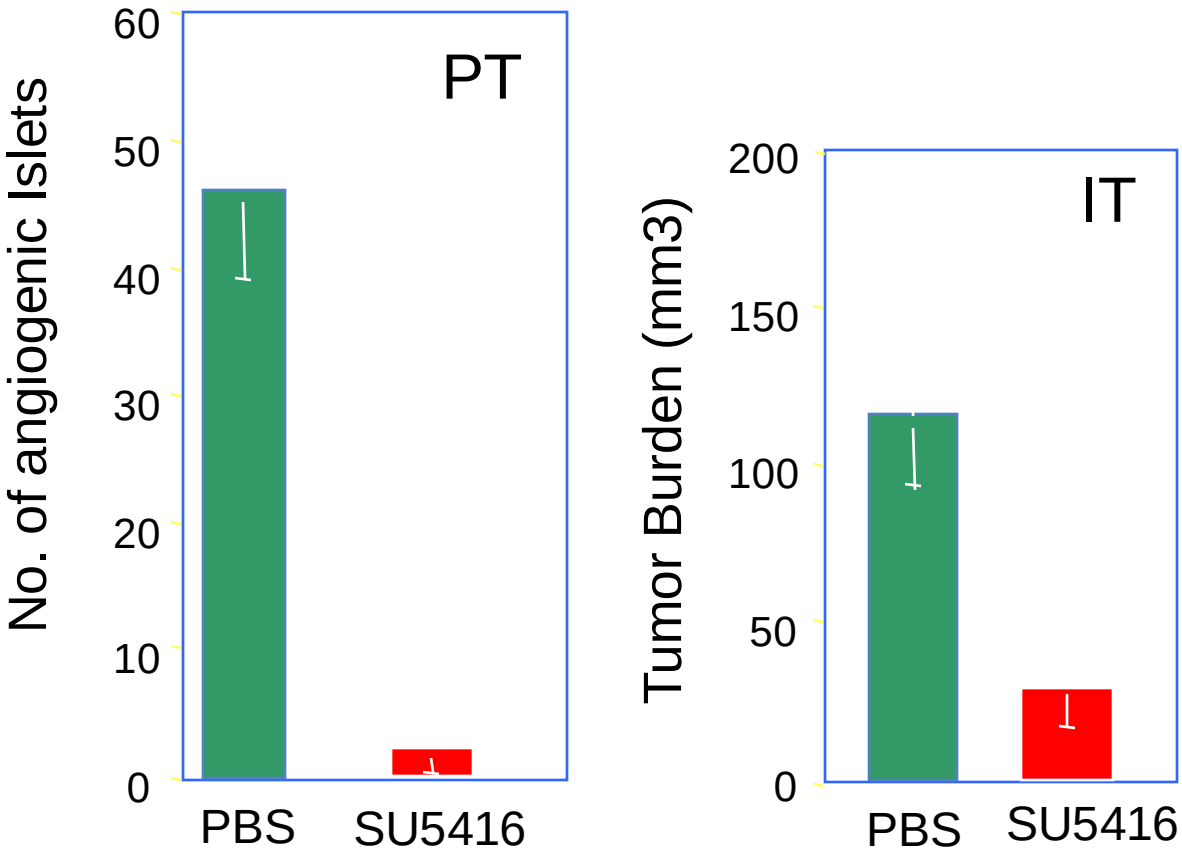
12- 16 wk treatment

Prevention trial (PT): can angiogenic switching be prevented?

Intervention trial (IT): can tumor progression be slowed or stopped?

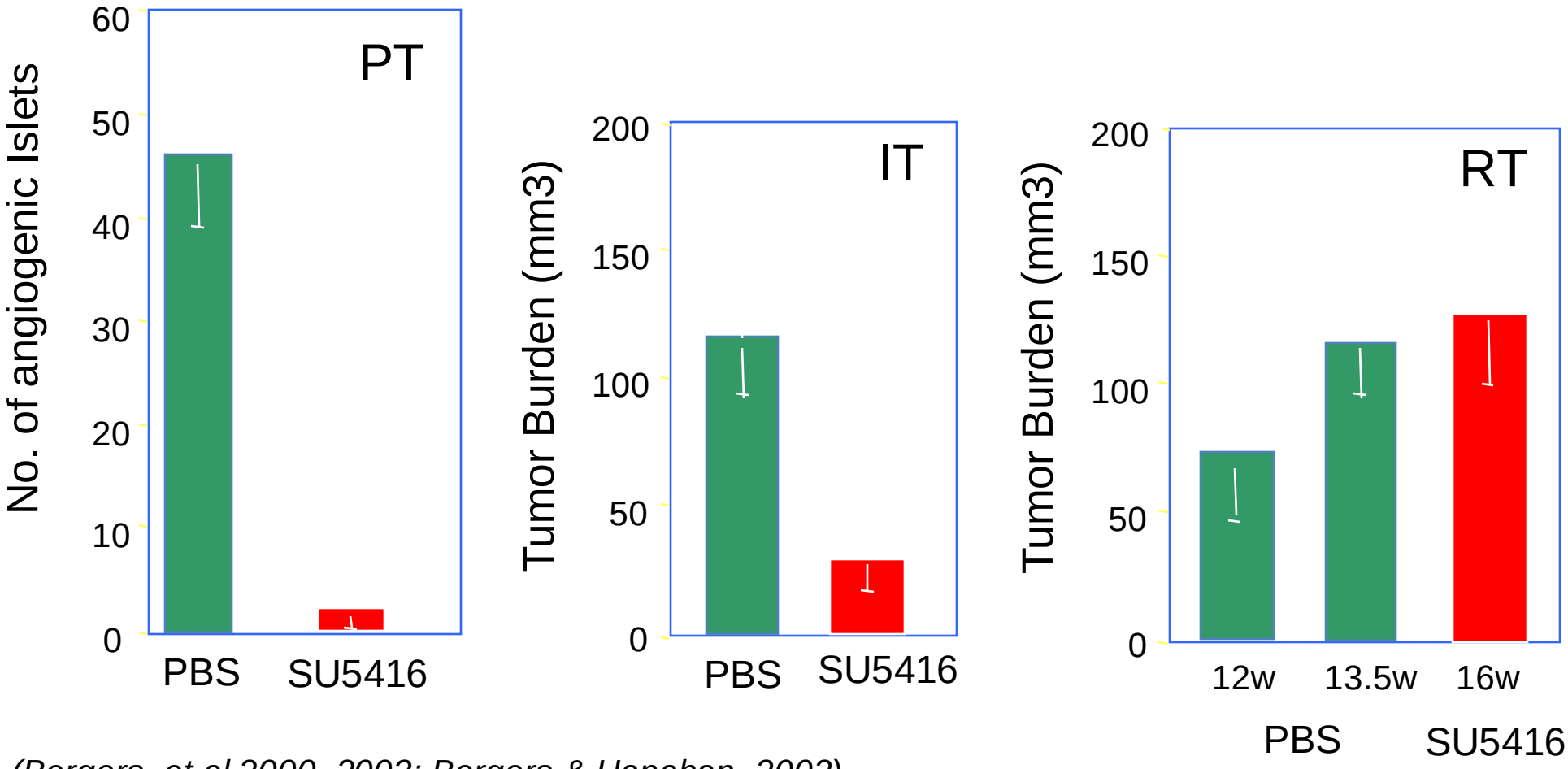
Regression trial (RT): can tumor growth be stabilized or regressed and can lifespan be extended?

The VEGFR inhibitor SU5416 blocks the angiogenic switch and impairs growth of small tumors



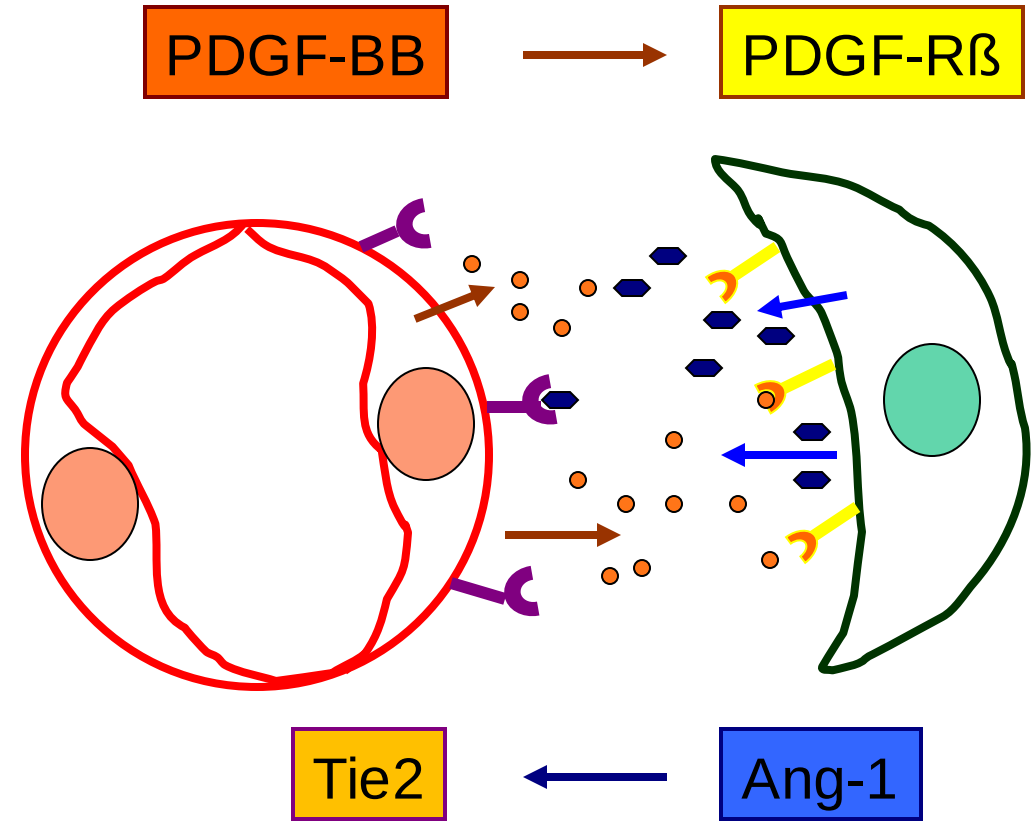
(Bergers, et al 2000, 2003; Bergers & Hanahan, 2002).

The VEGFR inhibitor SU5416 blocks the angiogenic switch and impairs growth of small tumors – but does not inhibit established tumors

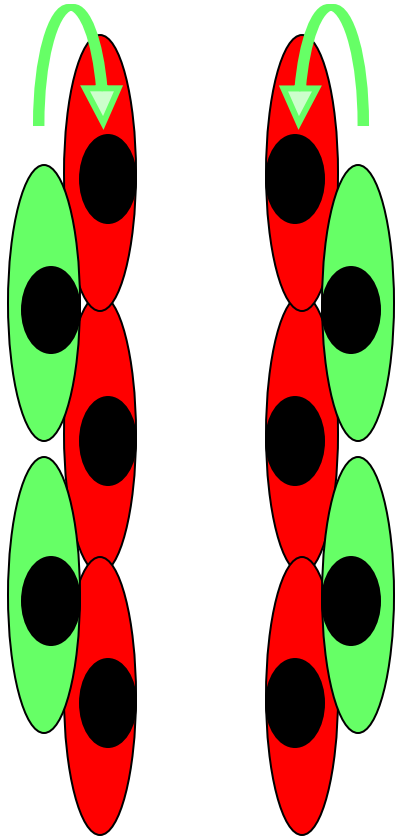


(Bergers, et al 2000, 2003; Bergers & Hanahan, 2002).

Endothelial cells and pericytes associate via reciprocal paracrine interactions of regulatory ligands binding signaling receptors



Pericytes support and protect the endothelial cells of the tumor (and normal tissue) vasculature

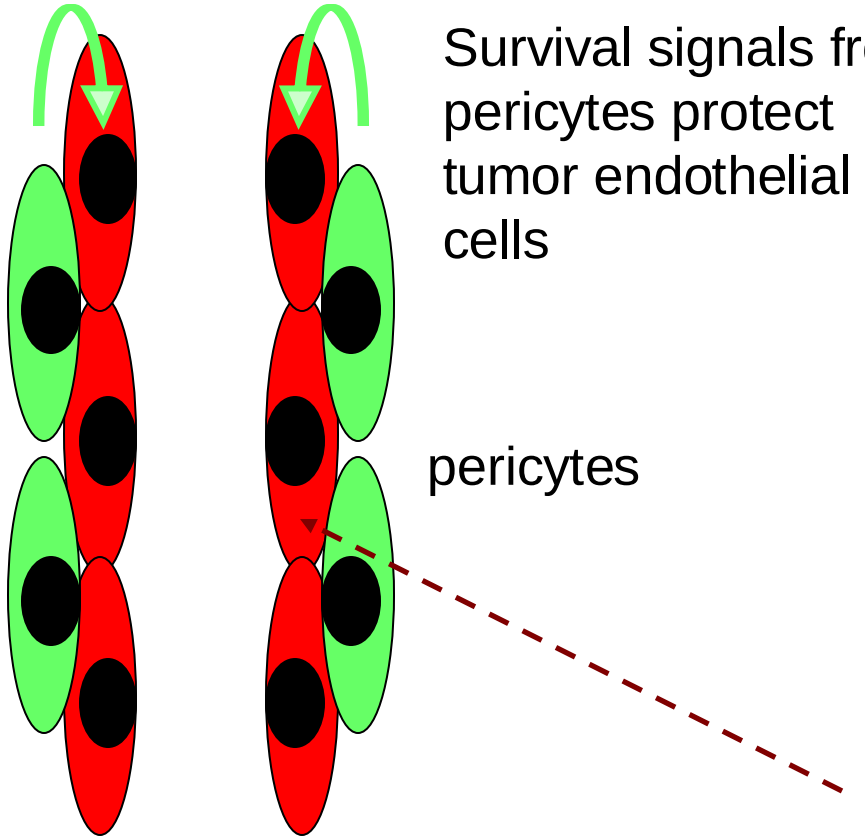


Survival signals from pericytes protect tumor endothelial cells

pericyte

endothelial cells

Pericytes support and protect the endothelial cells of the tumor (and normal tissue) vasculature



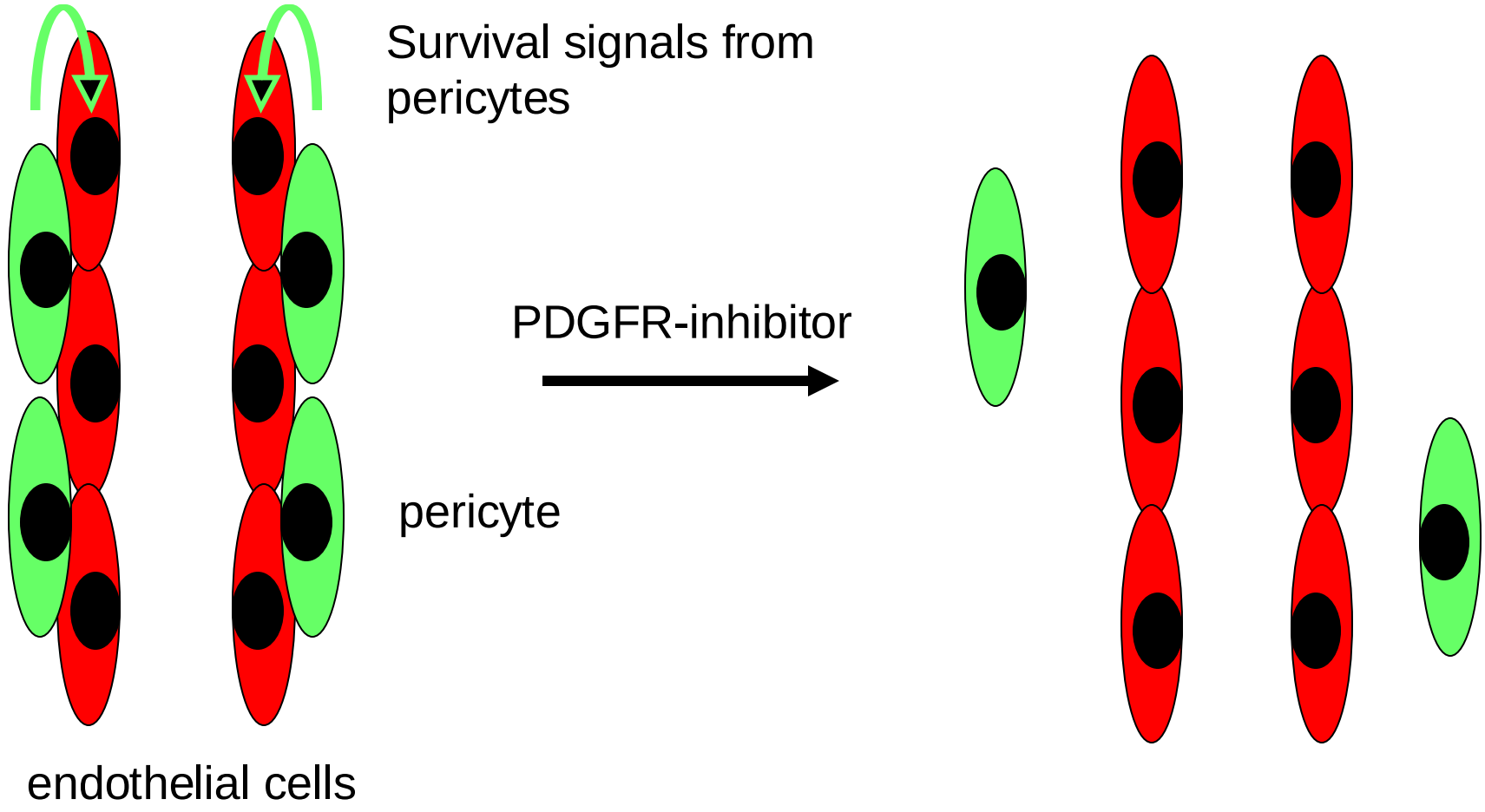
Survival signals from pericytes protect tumor endothelial cells

pericytes

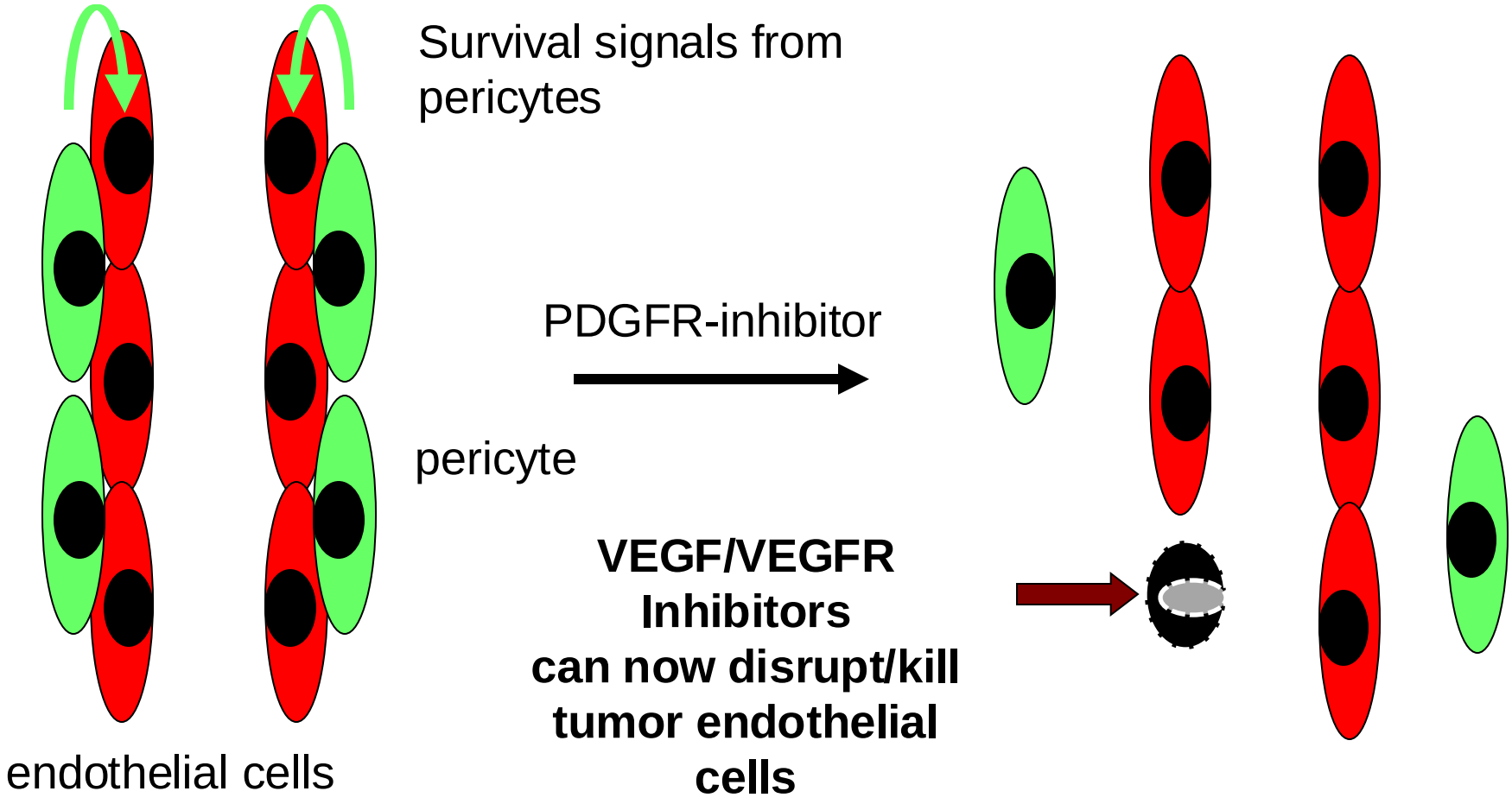
endothelial cells

VEGF/VEGFR inhibitors have limited effect on pericyte-covered vessels

PDGF receptor inhibitors dissociate pericytes from tumor endothelial cells, abolishing their supportive functions



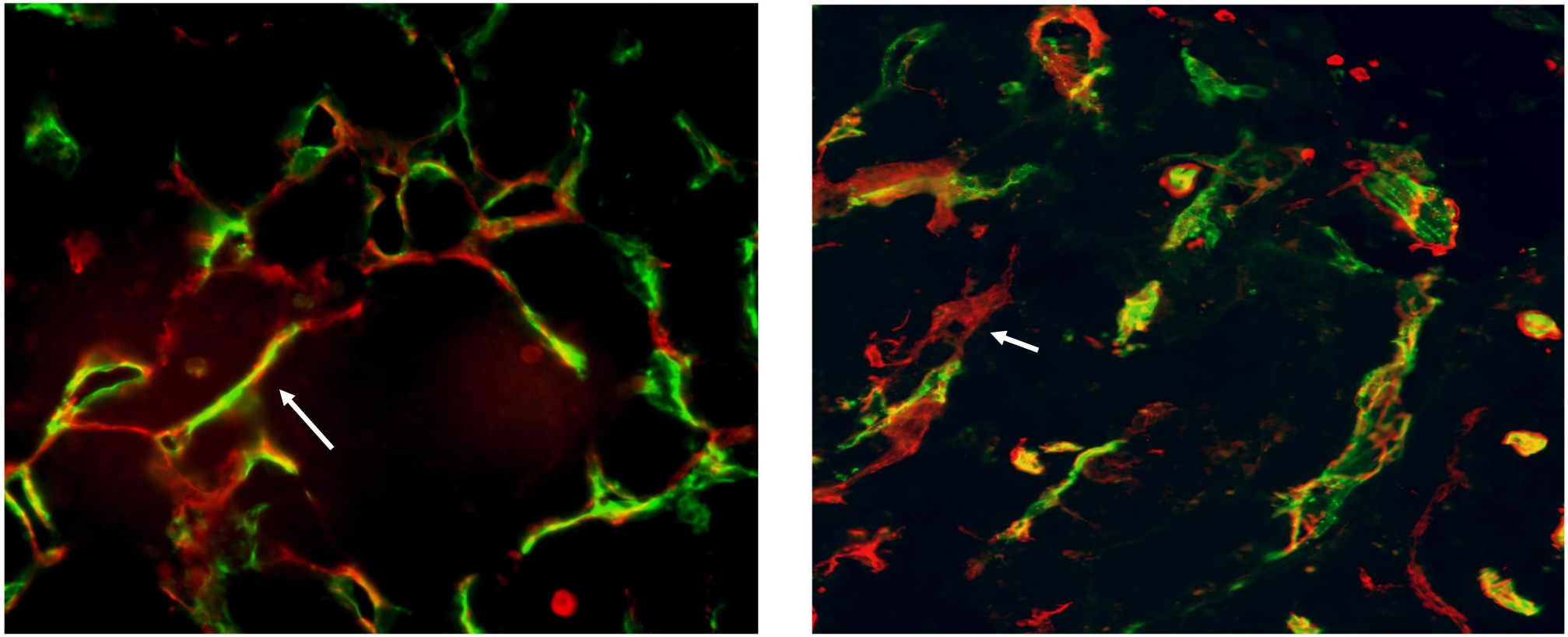
PDGF Receptor inhibitors dissociate pericytes, increasing tumor endothelial cell killing by VEGF signaling inhibitors



PDGFR inhibitors disrupt pericyte association with tumor vasculature

Untreated

SU6668 (or Gleevec, or MAb)

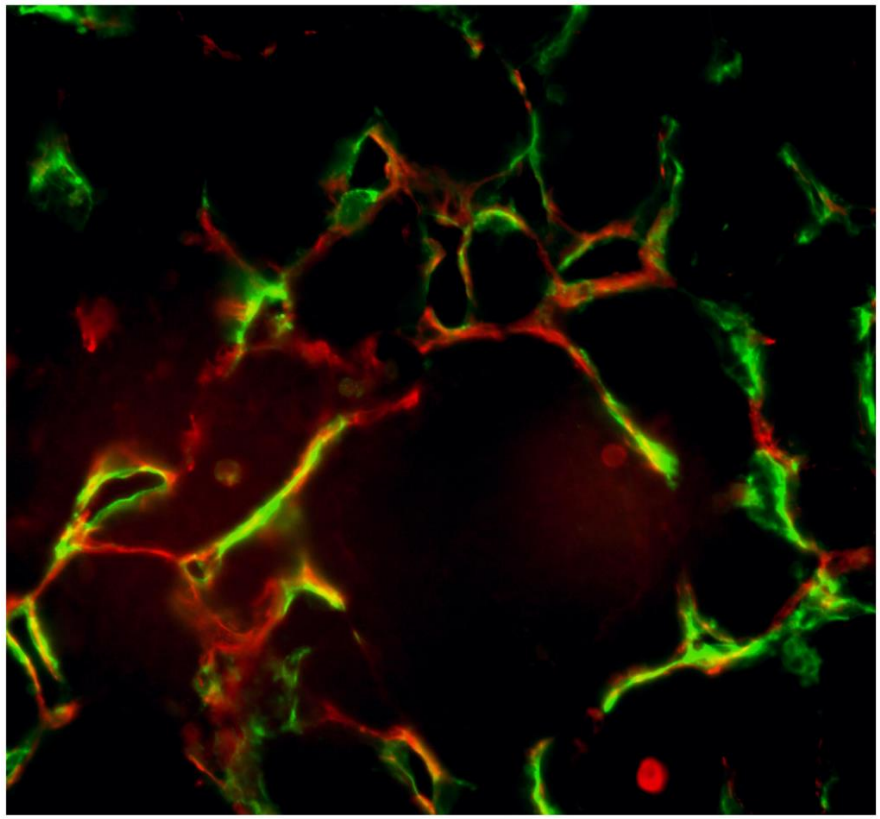


FITC-Lectin (vessels, green) / Cy3-Desmin (pericytes, red)

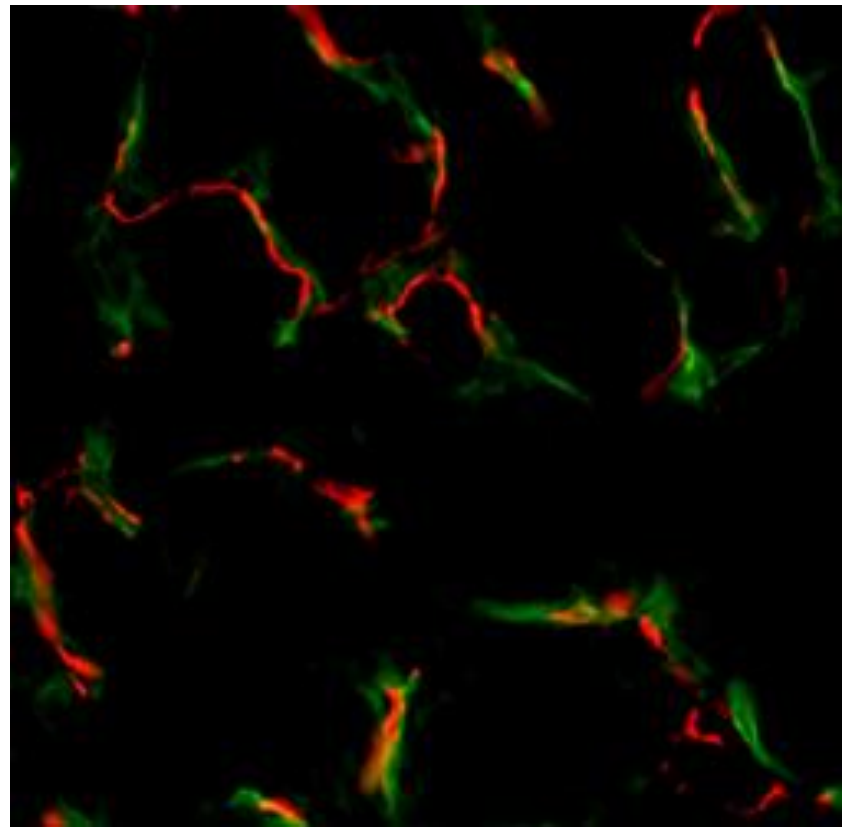
Bergers, et al (2003). Benefits of targeting both pericytes and endothelial cells in tumor vasculature with kinase inhibitors. J.C.I., 111: 1287-95.

Pure VEGFR inhibitors prune the angiogenic vasculature, leaving vessels with more intimate and extensive pericyte coverage, that are evidently resistant

Untreated tumor



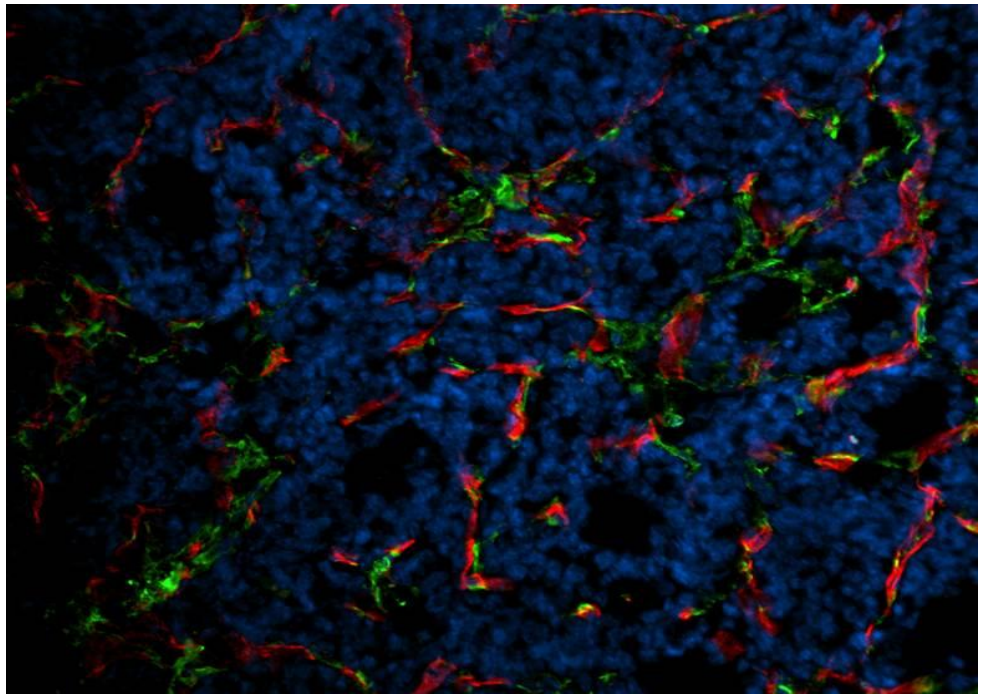
Tumor from mouse treated with SU5416



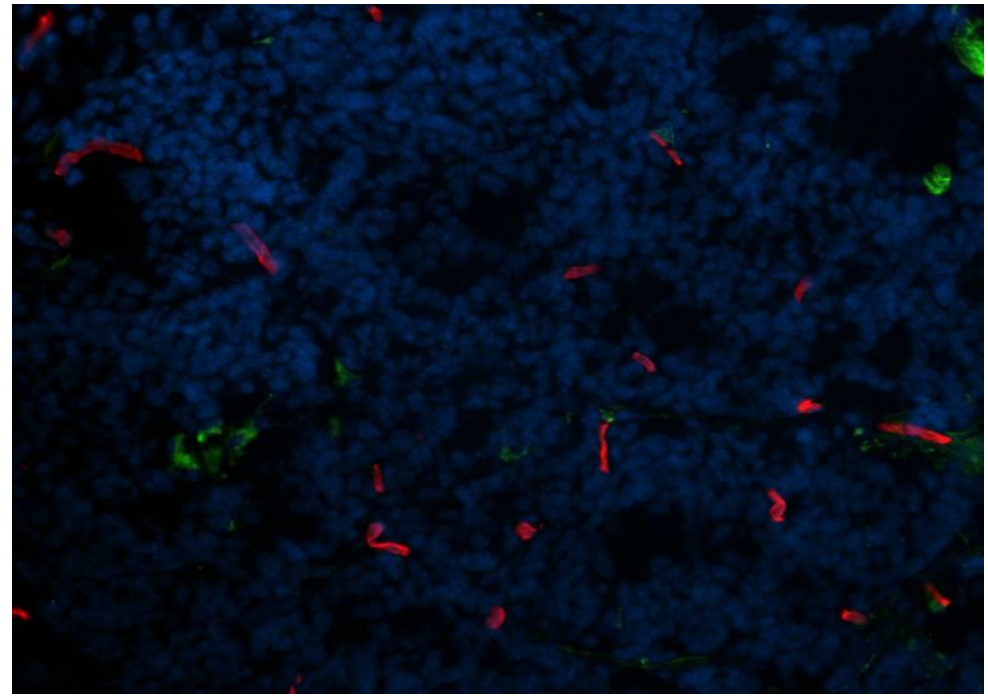
FITC-Lectin (vessels, green) / Cy3-Desmin (pericytes, red)

The multi-kinase inhibitor sunitinib (which hits both VEGFR and PDGFR) impairs angiogenesis, reduces vascularity and disrupts pericyte coverage

Control



Treated

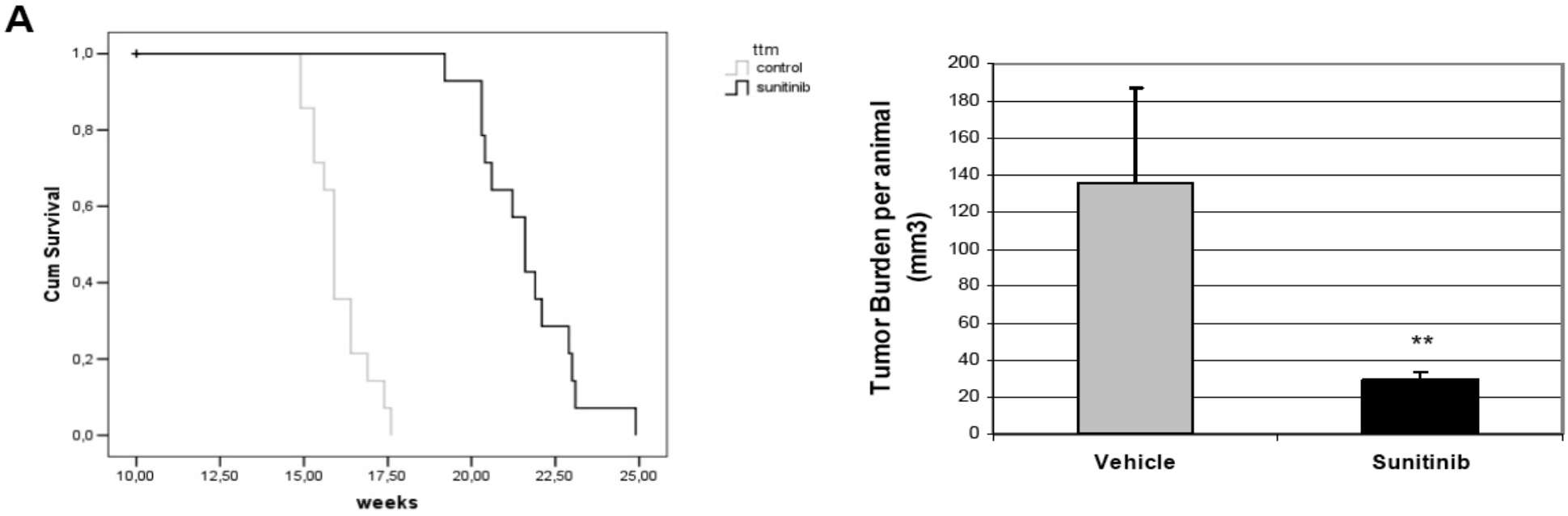


Meca32 = endothelial cells (red)
NG2 = pericytes (green)

Sunitinib has demonstrable efficacy in the RIP-Tag model of PNET

Survival

Tumor burden



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 FEBRUARY 10, 2011 VOL. 364 NO. 6

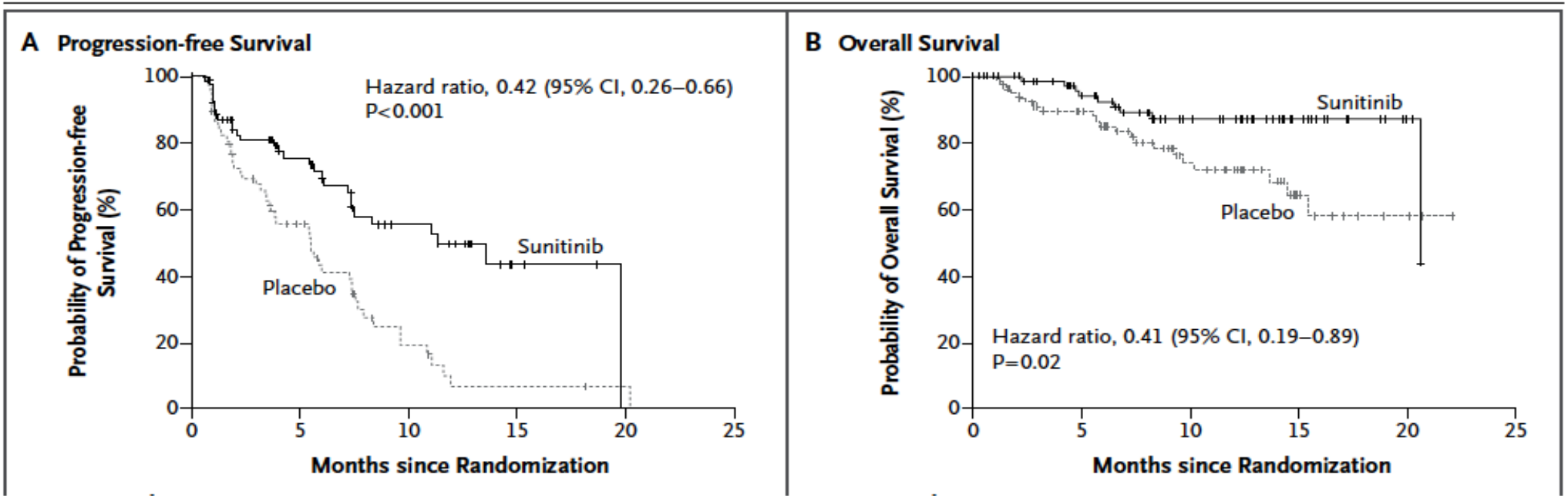
Sunitinib Malate for the Treatment of Pancreatic Neuroendocrine Tumors

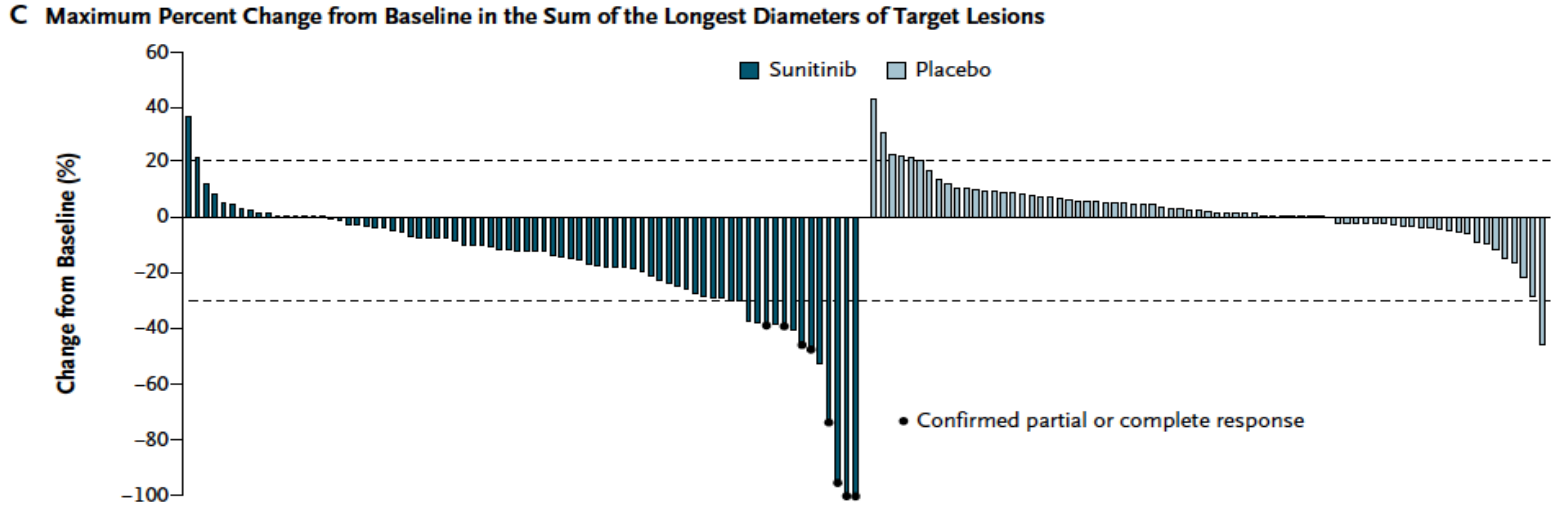
Eric Raymond, M.D., Ph.D., Laetitia Dahan, M.D., Ph.D., Jean-Luc Raoul, M.D., Ph.D., Yung-Jue Bang, M.D., Ivan Borbath, M.D., Ph.D., Catherine Lombard-Bohas, M.D., Juan Valle, M.D., Peter Metrakos, M.D., C.M., Denis Smith, M.D., Aaron Vinik, M.D., Ph.D., Jen-Shi Chen, M.D., Dieter Hörsch, M.D., Pascal Hammel, M.D., Ph.D., Bertram Wiedenmann, M.D., Ph.D., Eric Van Cutsem, M.D., Ph.D., Shem Patyna, Ph.D., Dongrui Ray Lu, M.Sc., Carolyn Blanckmeister, Ph.D., Richard Chao, M.D., and Philippe Ruszniewski, M.D.

CONCLUSIONS

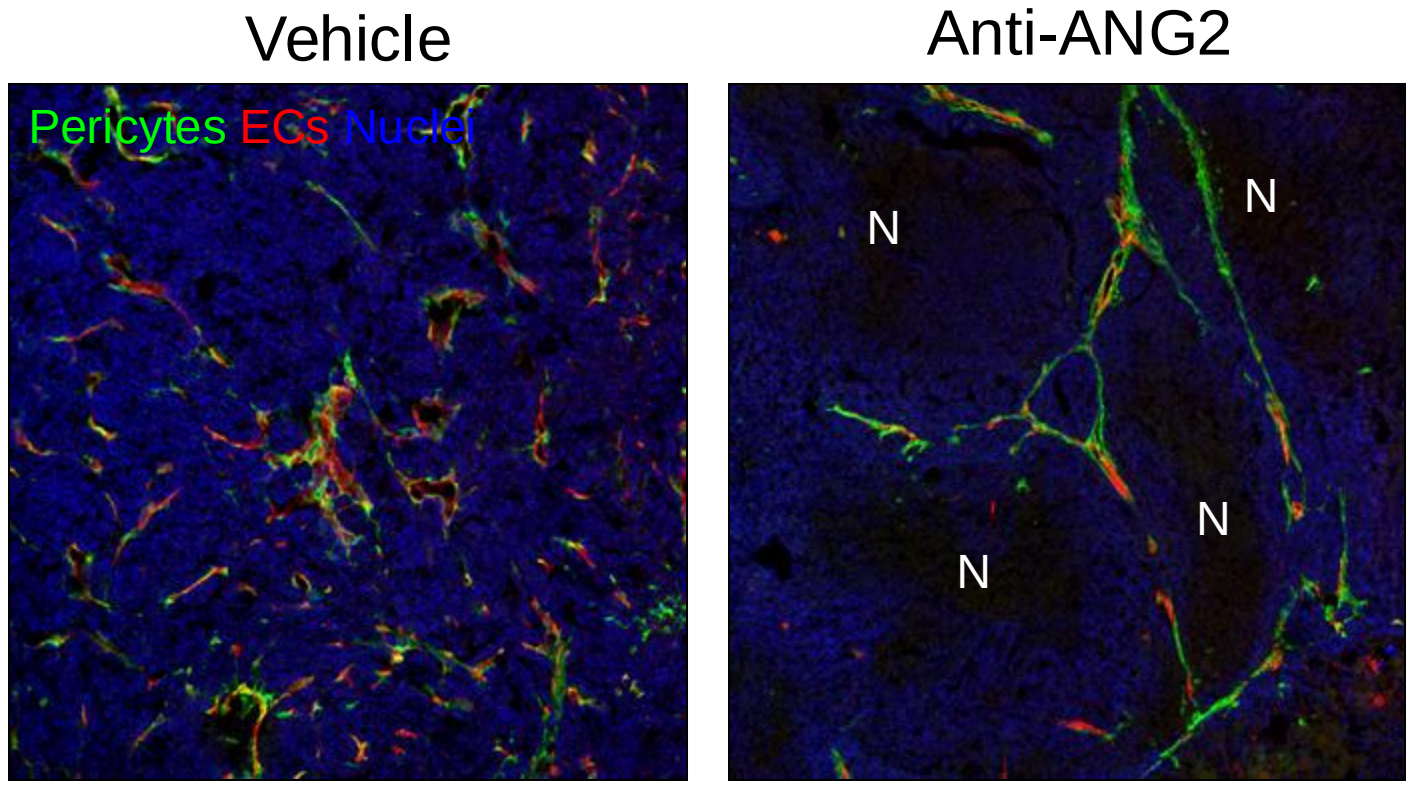
Continuous daily administration of sunitinib at a dose of 37.5 mg improved progression-free survival, overall survival, and the objective response rate as compared with placebo among patients with advanced pancreatic neuroendocrine tumors.

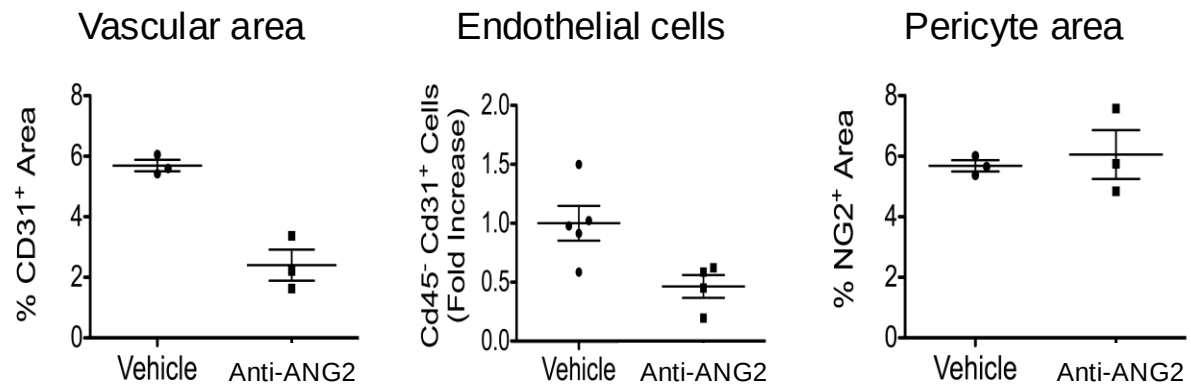
Efficacy of sunitinib in human PNET





Blocking ANG2 inhibits angiogenesis in mouse tumor models





Mazzieri et al., Cancer Cell 2011

Still, there is a conundrum

- Six angiogenesis inhibitors have been approved for clinical use as therapeutic agents in particular tumor types.
- The drugs, both in preclinical models and in human trials, have demonstrable but transitory benefits, after which tumors start growing again (progression)
- We were expecting greater and more enduring effect, so what's going on?

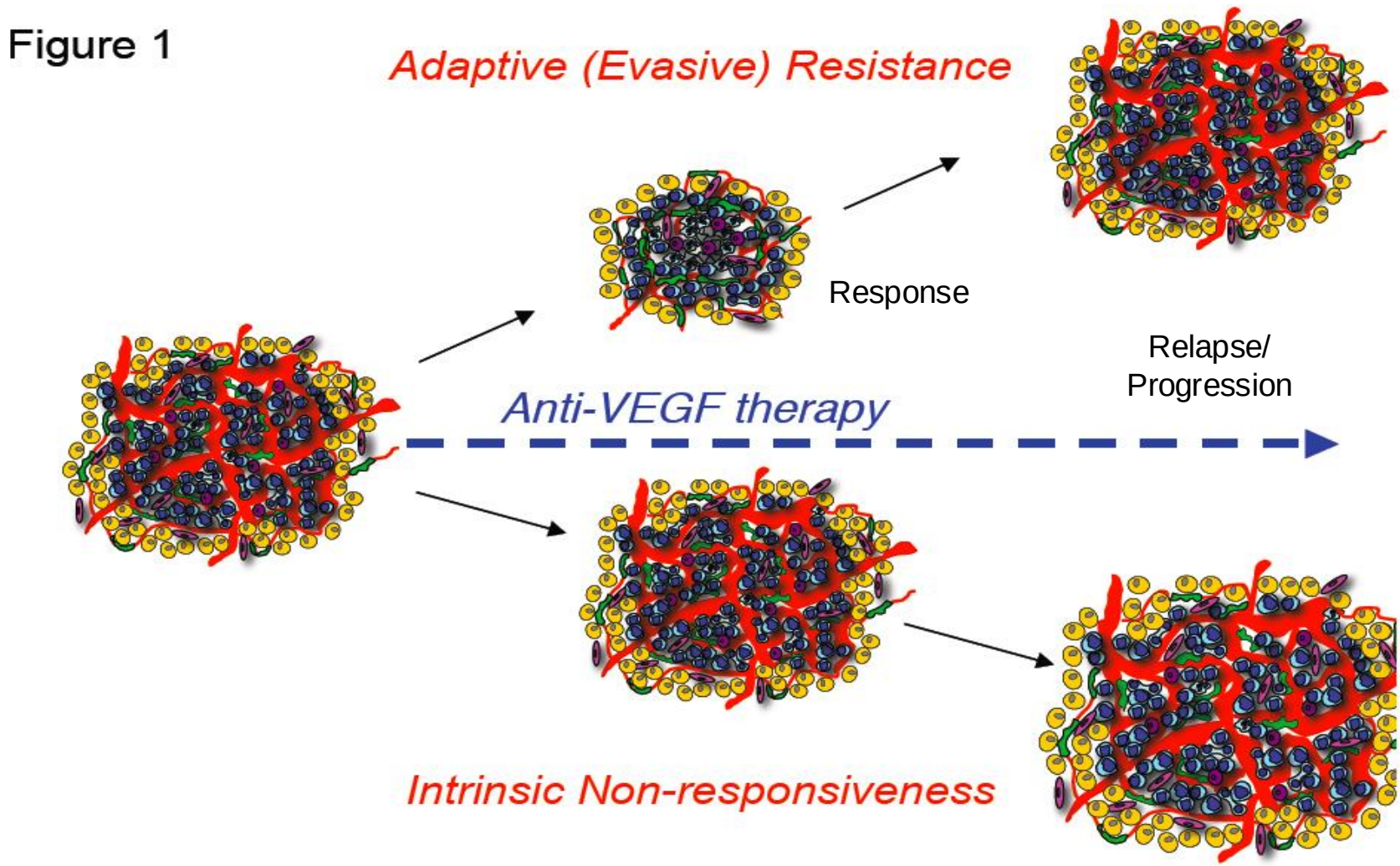
A rationale for resistance

- Bevacuzimab, sunitinib, and sorafinib variously inhibit VEGFR2 signaling so as to inhibit tumor angiogenesis

- Each has been approved for certain late-stage cancers, representing a proof of principle for therapeutic targeting of tumor angiogenesis;

- Each only produces a transitory survival benefit against such late stage tumors, a “delayed time to progression” to renewed tumor growth after a period of response or stable disease

Modes of resistance to angiogenesis inhibitors

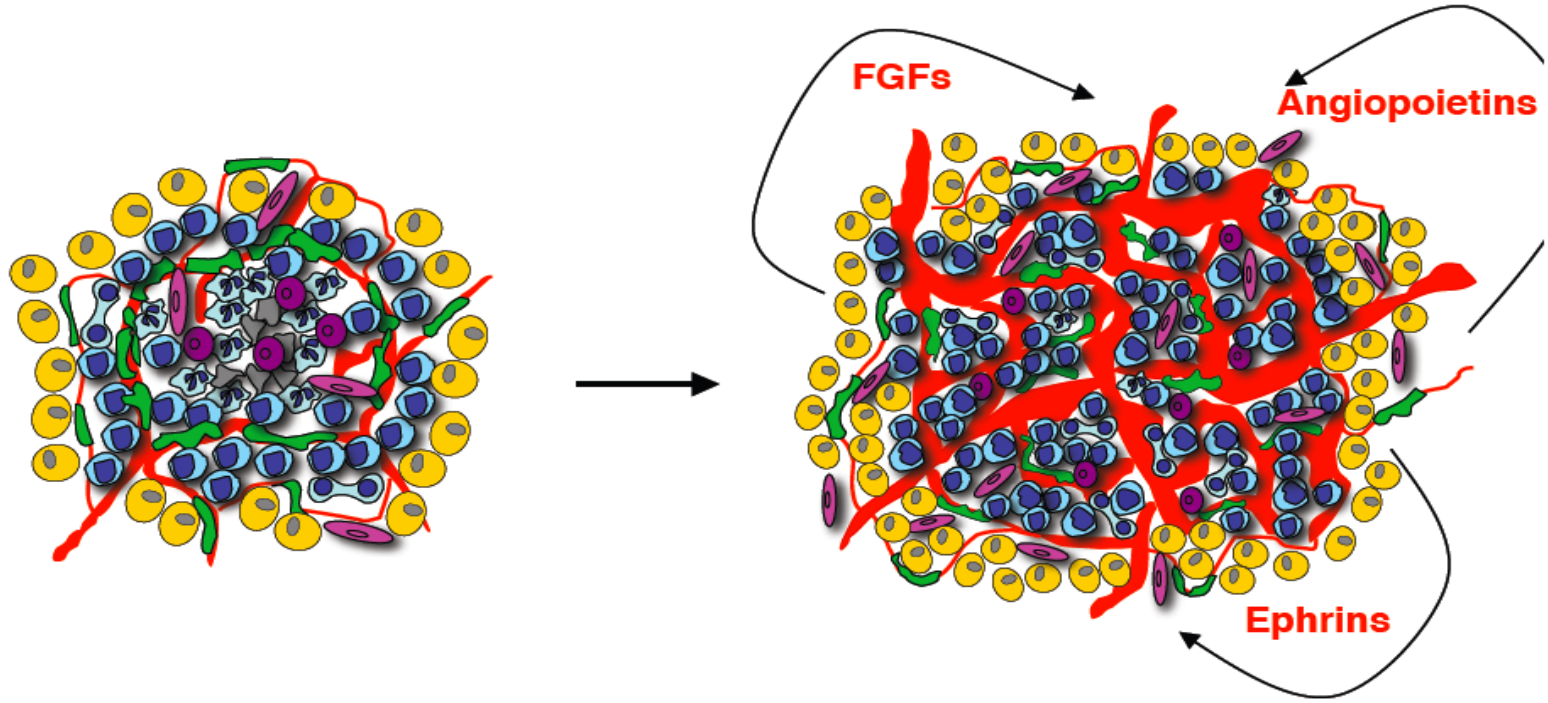


Bergers & Hanahan. Nature Reviews Cancer, 2008

Evasive resistance to angiogenesis inhibitors

-- by upregulating alternative pro-angiogenic signaling circuits to promote revascularization

Figure 2



Evasive resistance to VEGFA signaling blockade in RIP-Tag2 mice

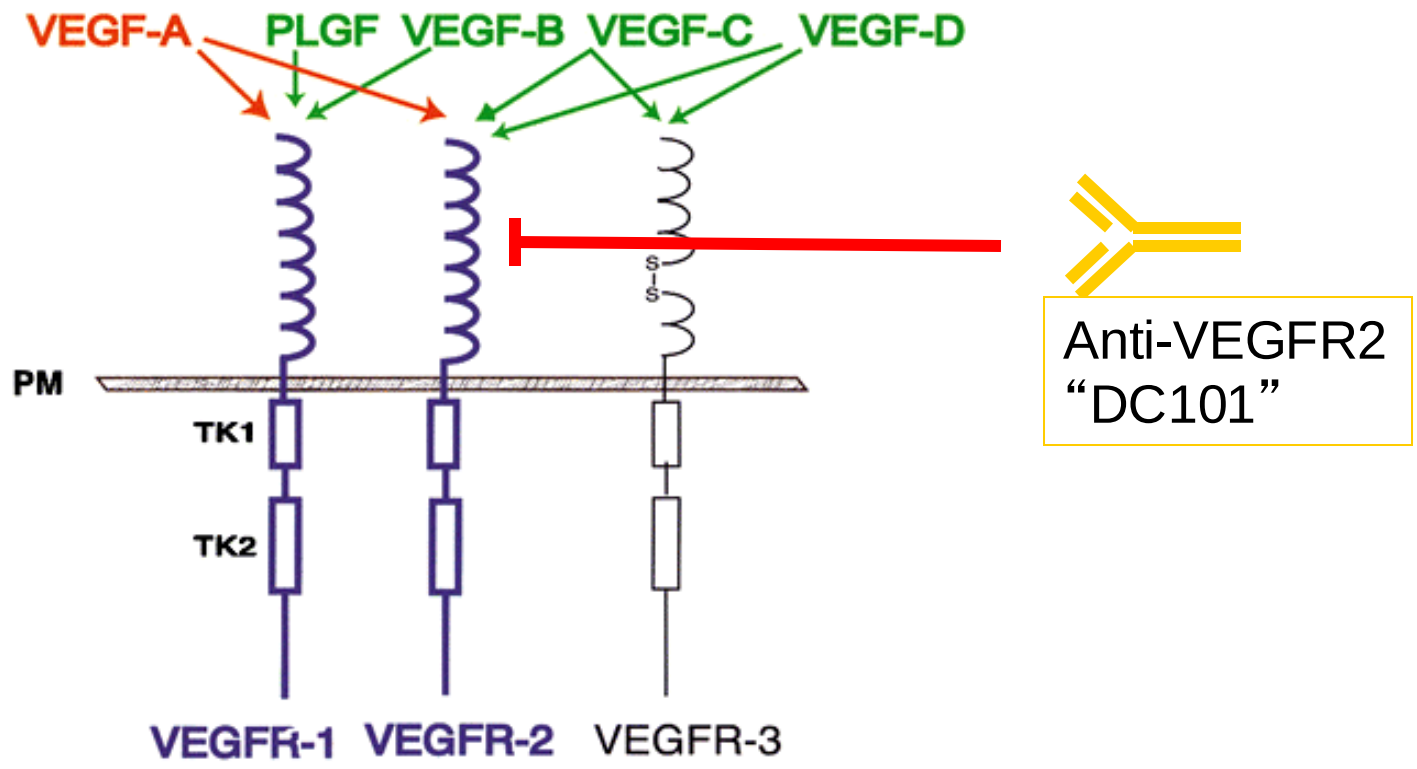
ARTICLE

Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors

Oriol Casanovas,[1] Daniel J. Hicklin,[2] Gabriele Bergers,[3] and Douglas Hanahan[1,*]

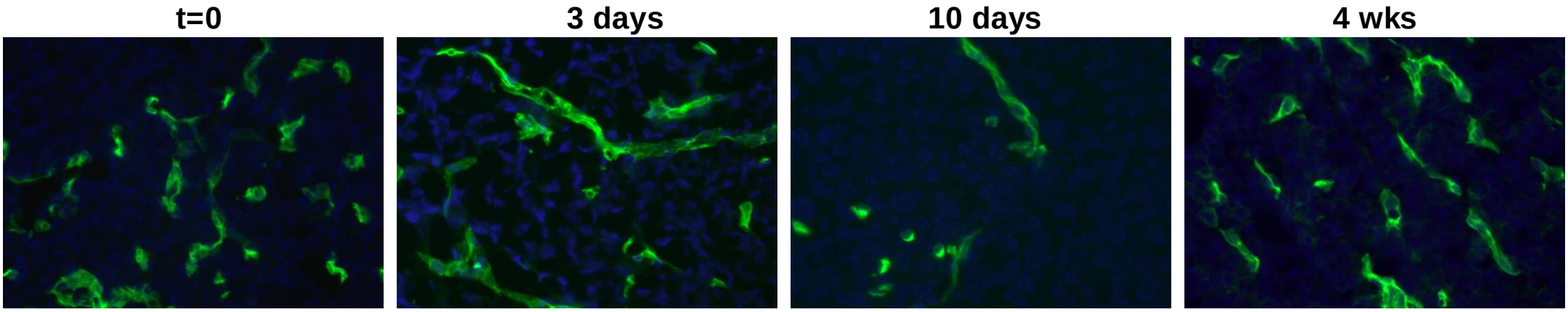
[1]Department of Biochemistry and Biophysics, Comprehensive Cancer Center, and Diabetes Center, University of California, San Francisco, San Francisco, California 94143
[2]ImClone Systems Inc., New York, New York 10014
[3]Brain Tumor Research Center and Department of Neurosurgery, University of California, San Francisco, San Francisco, California 94143
*Correspondence: dh@biochem.ucsf.edu

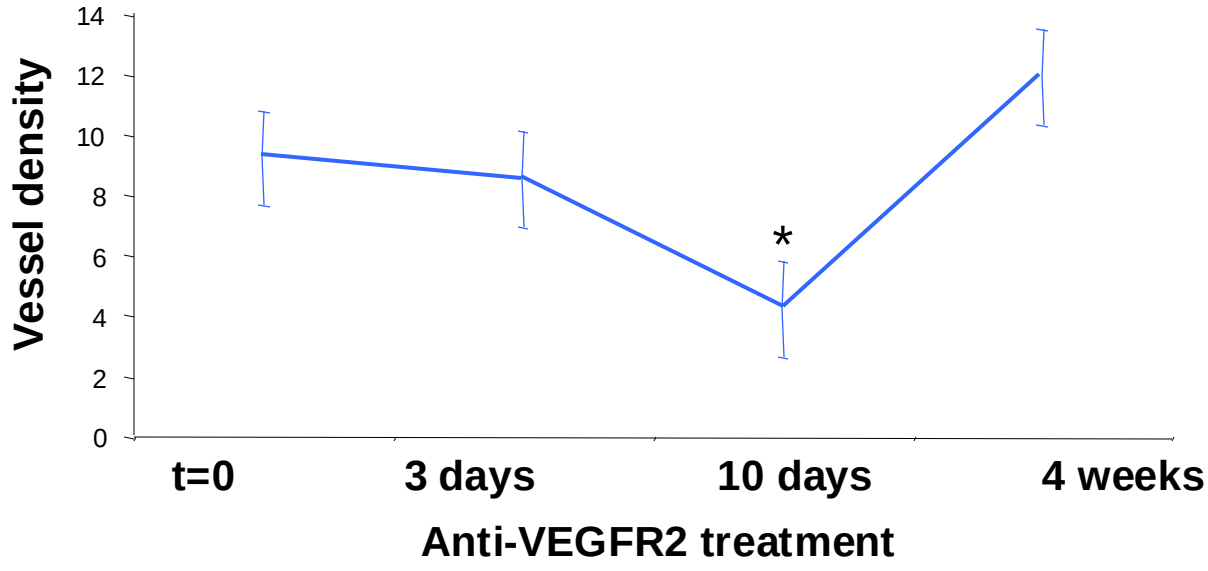
Trials with blocking MAb to VEGFR2



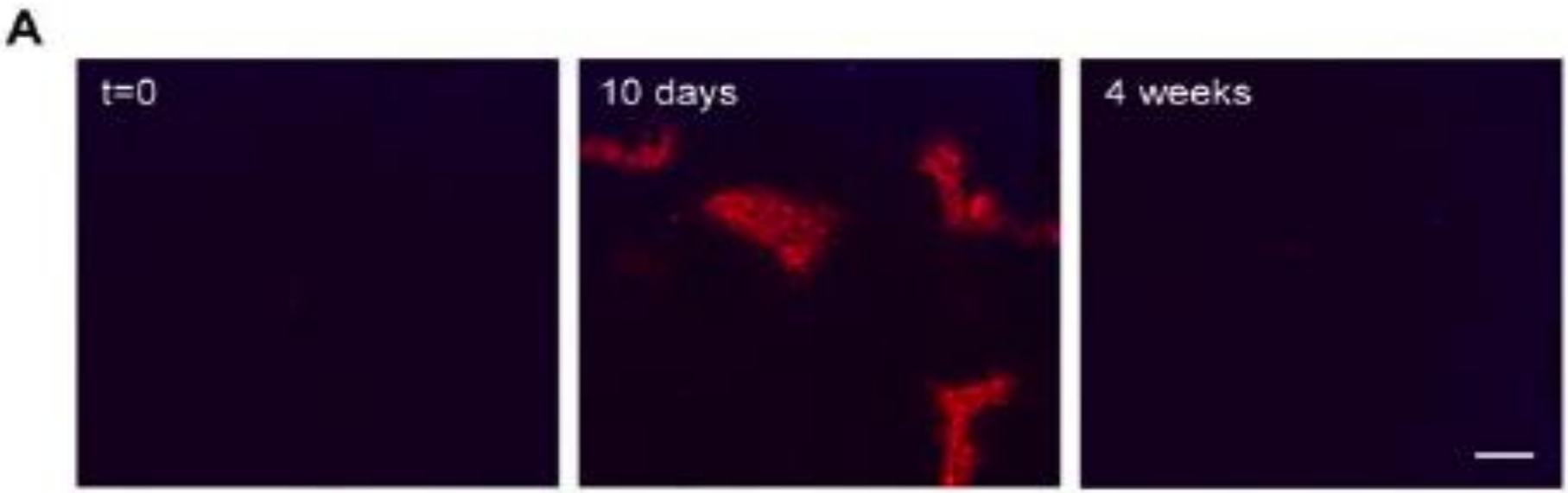
DC101: Blocking antibody, rat-anti-mouse VEGFR2 (Imclone Inc.)

Re-vascularization occurs concomitant with tumor re-growth

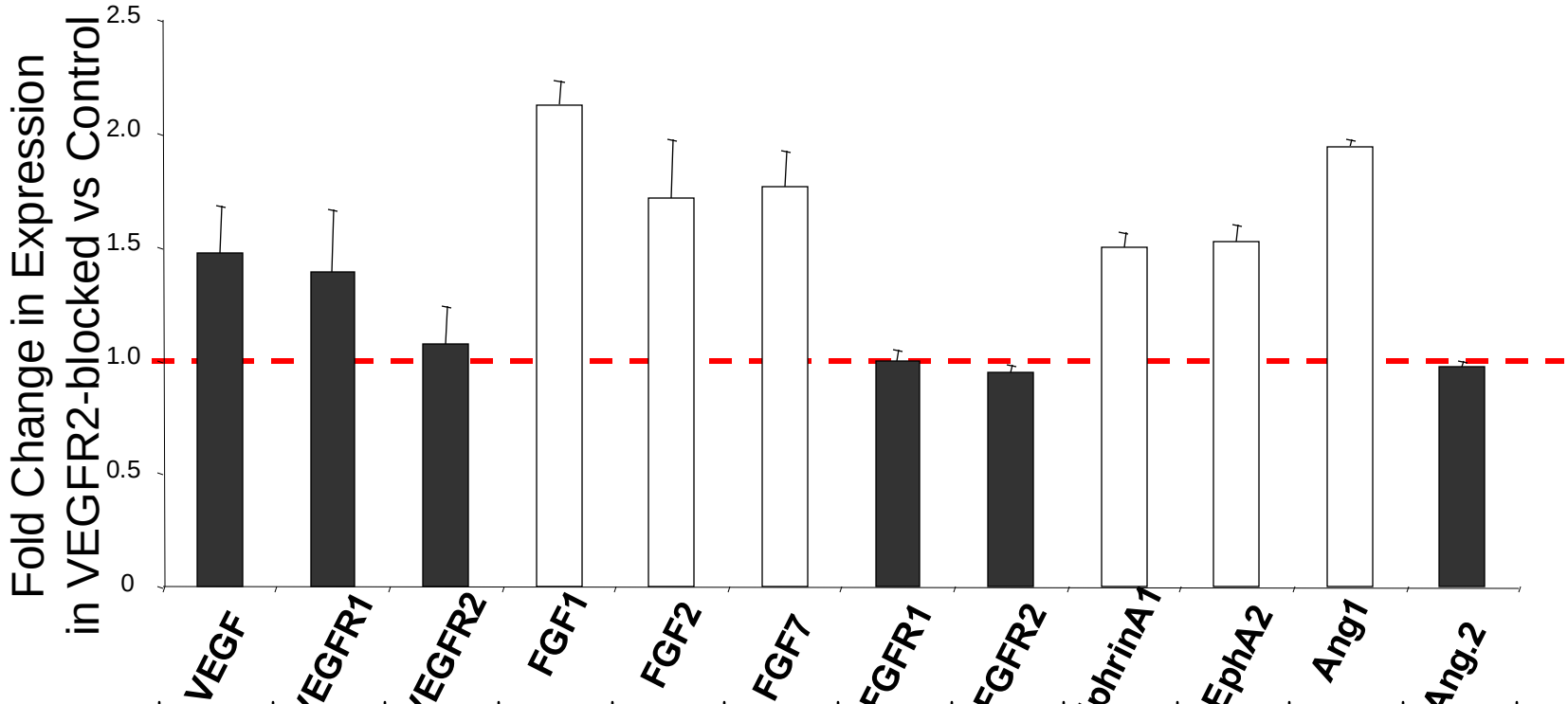




Hypoxia is induced during the response phase, concomitant with vascular dropout



Other pro-angiogenic factors are upregulated, possibly in a hypoxia-dependent manner



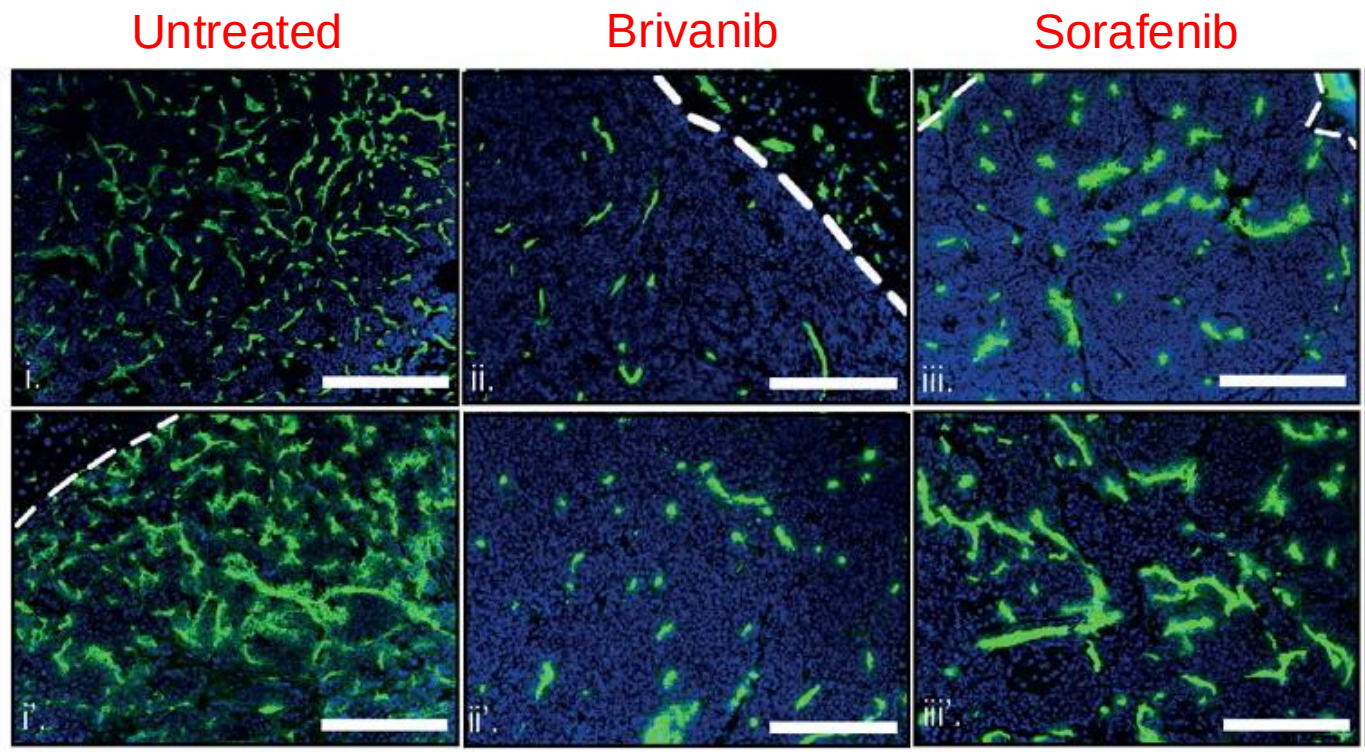
	VEGF	VEGFR1	VEGFR2	FGF1	FGF2	FGF7	FGFR1	FGFR2	EphrinA1	EphA2	Ang1	Ang.2
CC	+++	-	-	+++	++	+	++	++	+	-	+	-
EC	-	+++	+++	+	++	-	++	++	++	+++	-	+

FACS purified cancer cells and tumor endothelial cells

Double VEGFR/PDGFR and FGFR blockade inhibits angiogenesis more effectively and stably than single VEGFR/PDGFR blockade

Brivanib: VEGFR and FGFR inhibitor

Sorafenib: VEGFR and PDGFR inhibitor



Allen et al, Clin Can Res 2011

ANG2 sustains tumor angiogenesis in VEGFA-depleted tumors

OPEN ACCESS
CellPress

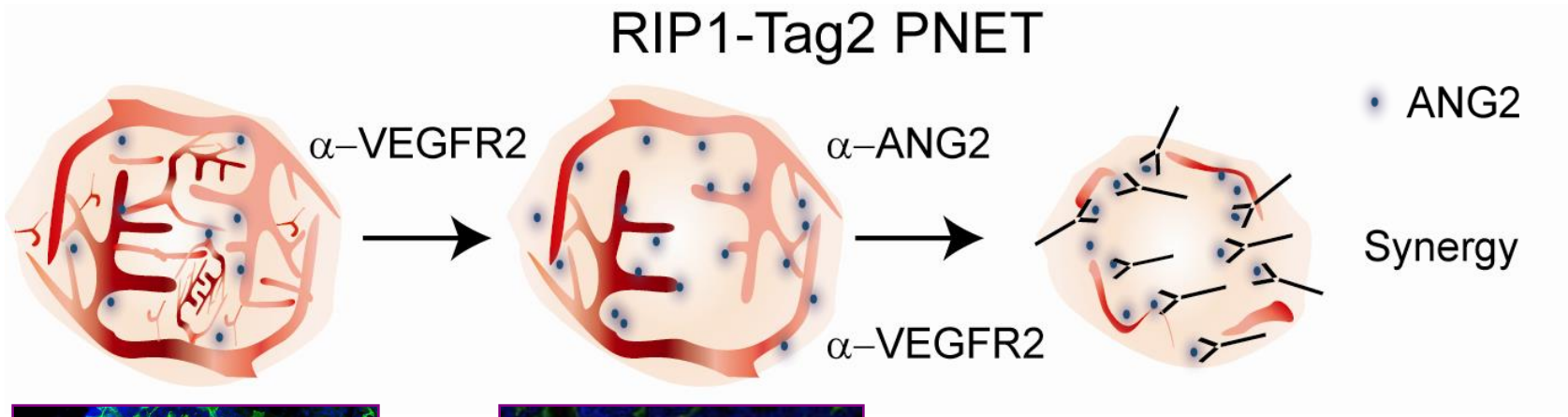
Cell Reports
Report

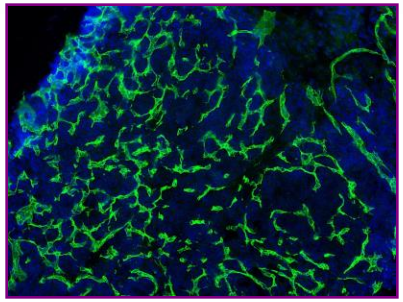
Role of Angiopoietin-2 in Adaptive Tumor Resistance to VEGF Signaling Blockade

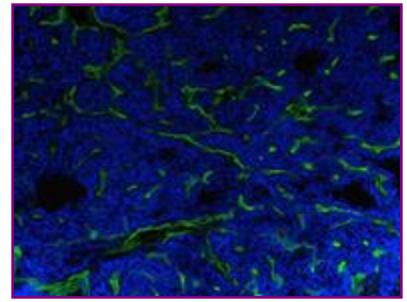
Nicolò Rigamonti,[1,3] Ece Kadioglu,[1,3] Ioanna Keklikoglou,[1] Céline Wyser Rmili,[1] Ching Ching Leow,[2] and Michele De Palma[1,*]
[1]Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland
[2]Translational Medicine Oncology, MedImmune, Gaithersburg, MD 20878, USA
[3]Co-first author
*Correspondence: michele.depalma@epfl.ch
http://dx.doi.org/10.1016/j.celrep.2014.06.059

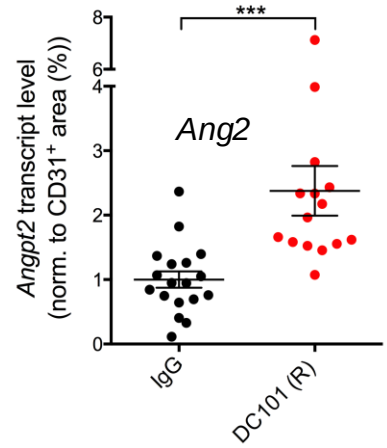
Cell Reports 8, 696–706, August 7, 2014

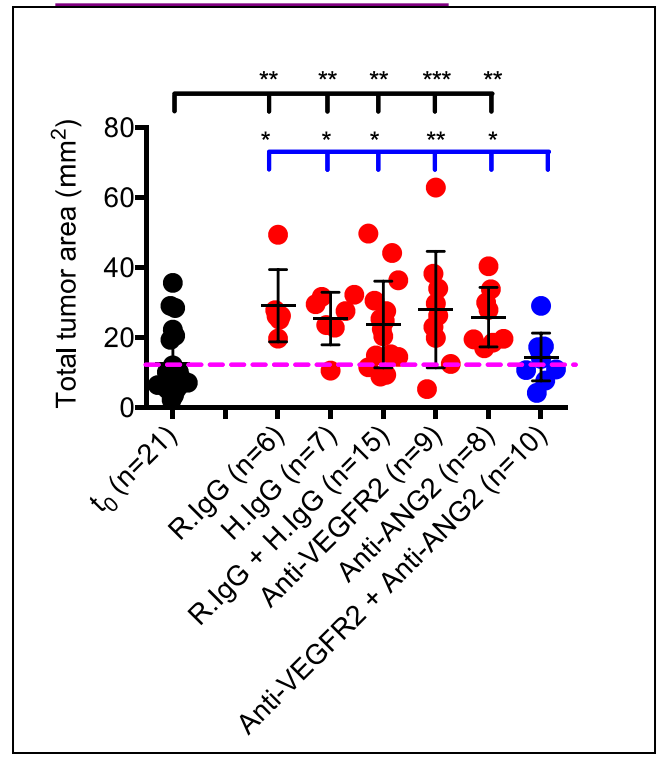
Double VEGFR/ANG2 blockade limits resistance to anti-VEGF therapy







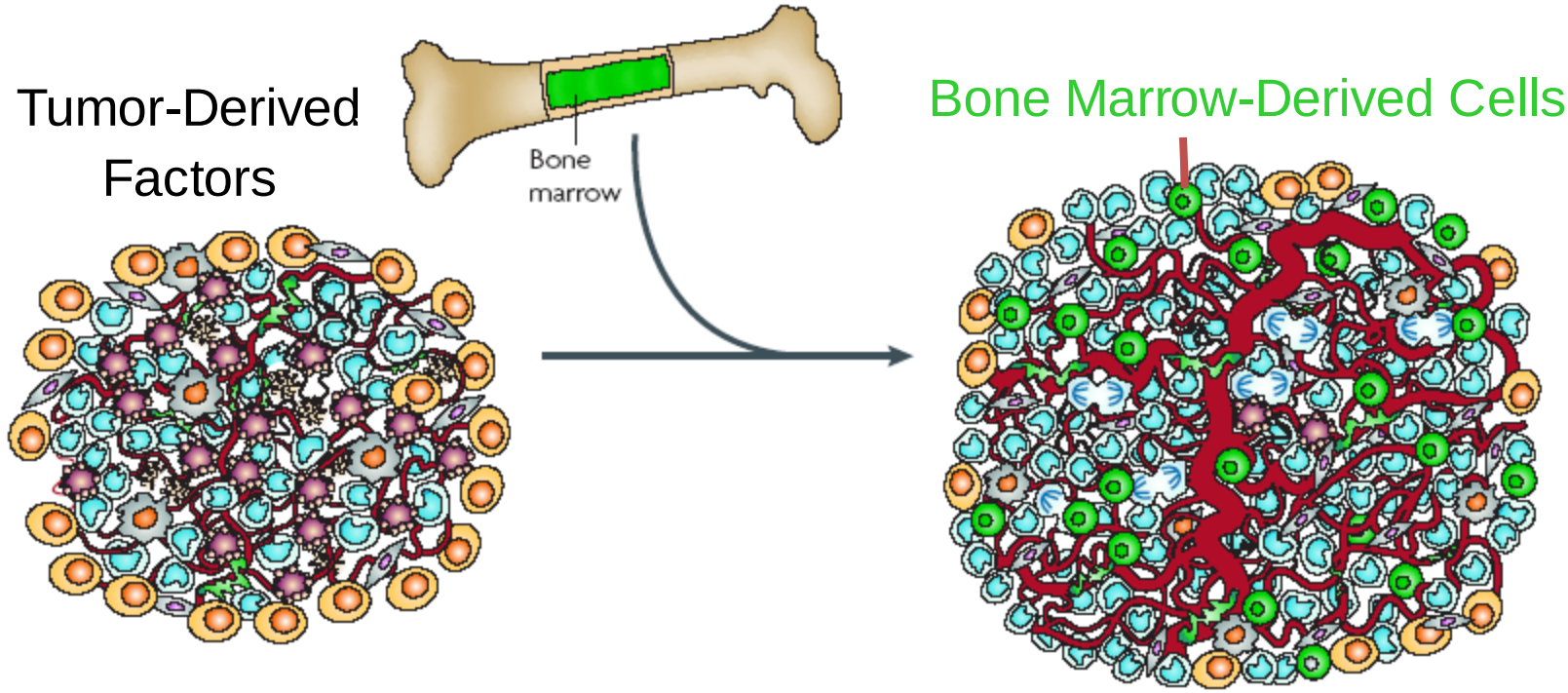




Rigamonti et al, Cell Rep 2014

Evasive resistance to angiogenesis inhibitors

-- by recruitment of pro-angiogenic myeloid cells



Adapted from Bergers and Hanahan, Nat Rev Cancer 2008

Evasive resistance to angiogenesis inhibitors

- VEGFA signaling blockade enhances recruitment of myeloid cells in some tumors

nature biotechnology

Tumor refractoriness to anti-VEGF treatment is mediated by CD11b^{+}Gr1^{+} myeloid cells

Farbod Shojaei[1], Xiumin Wu[1], Ajay K Malik[1], Cuiling Zhong[1], Megan E Baldwin[1], Stefanie Schanz[1], Germaine Fuh[1], Hans-Peter Gerber[1,2] & Napoleone Ferrara[1]

/naturebiotechnology

Adaptive-evasive resistance in human cancer: Role of myeloid cells

-- Notably, macrophages and myeloid cells *are associated (and potentially implicated)* in resistance to VEGF inhibitors in glioblastoma patients failing therapy

Neuro-Oncology 15(8):1079–1087, 2013.
doi:10.1093/neuonc/not082

NEURO-ONCOLOGY

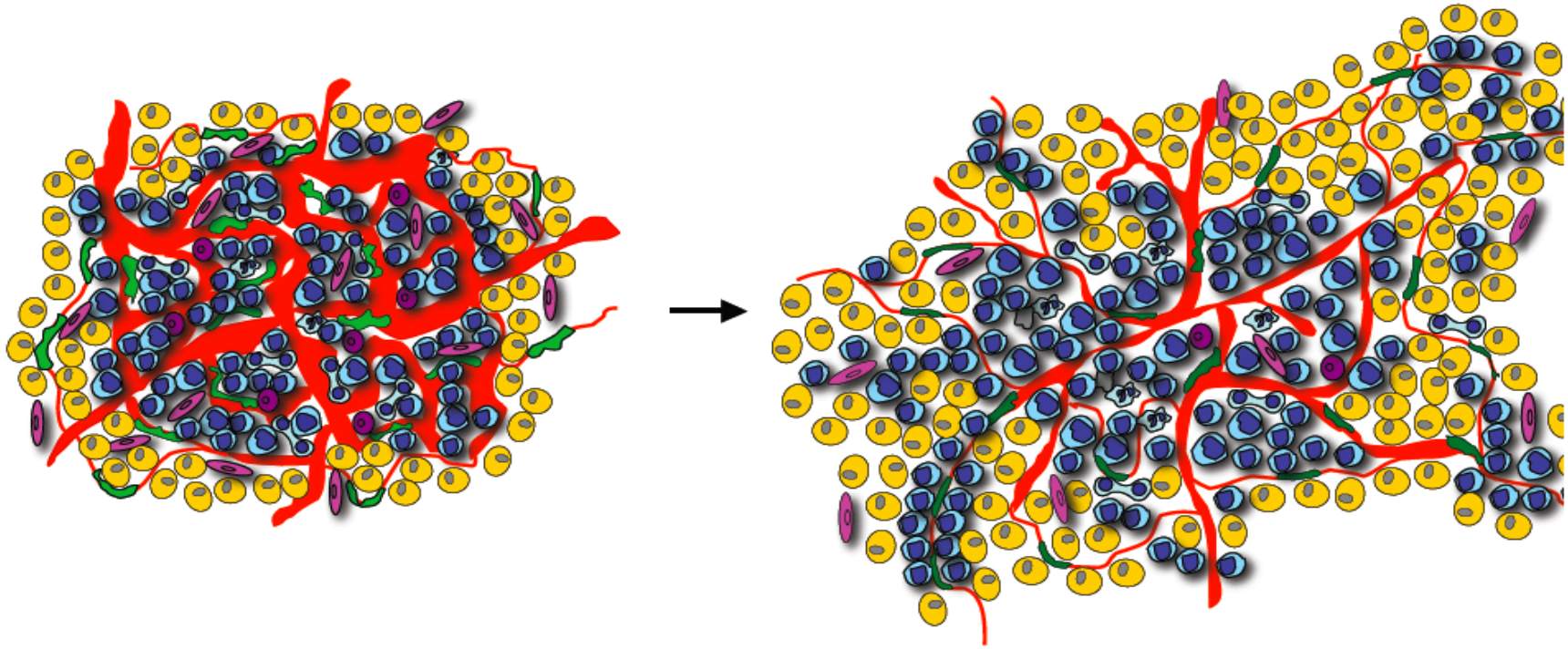
Increase in tumor-associated macrophages after antiangiogenic therapy is associated with poor survival among patients with recurrent glioblastoma

Christine Lu-Emerson, Matija Snuderl[†], Nathaniel D. Kirkpatrick[†], Jermaine Goveia, Christian Davidson, Yuhui Huang, Lars Riedemann, Jennie Taylor, Percy Ivy, Dan G. Duda, Marek Ancukiewicz, Scott R. Plotkin, Andrew S. Chi, Elizabeth R. Gerstner, April F. Eichler, Jorg Dietrich, Anat O. Stemmer-Rachamimov, Tracy T. Batchelor[‡], and Rakesh K. Jain[‡]

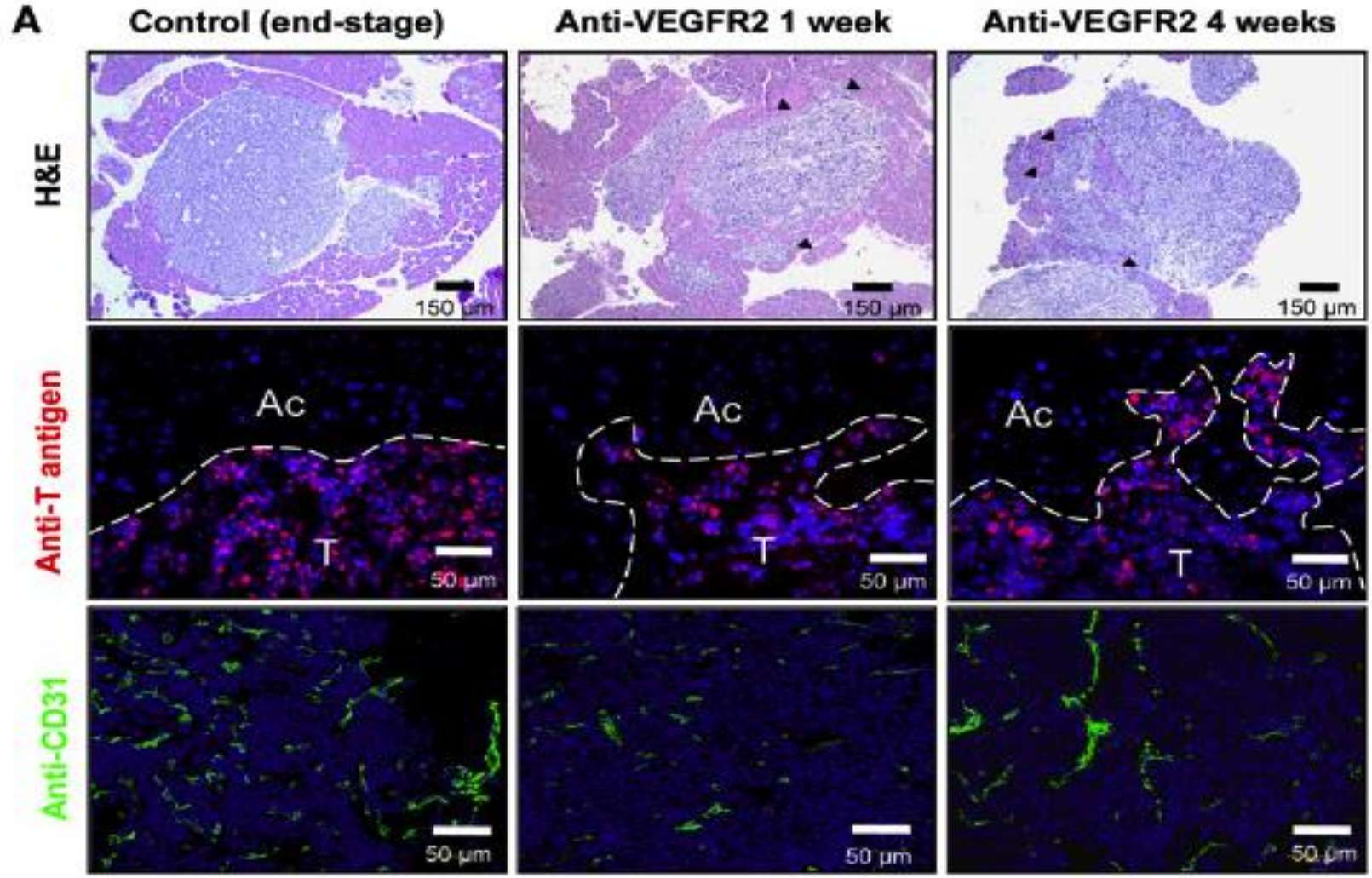
Evasive resistance to angiogenesis inhibitors

-- by increased local invasion and metastasis

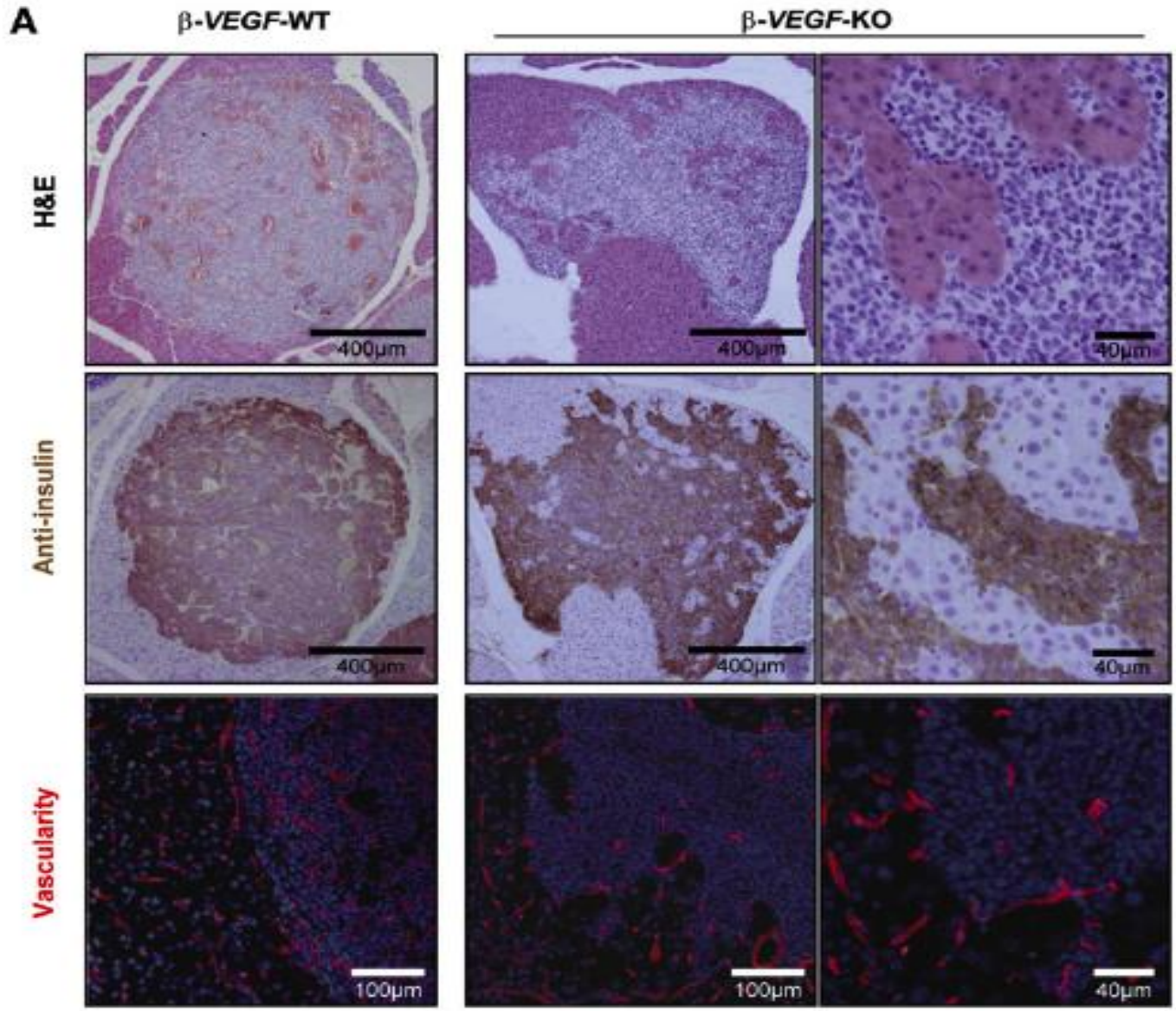
Figure 5



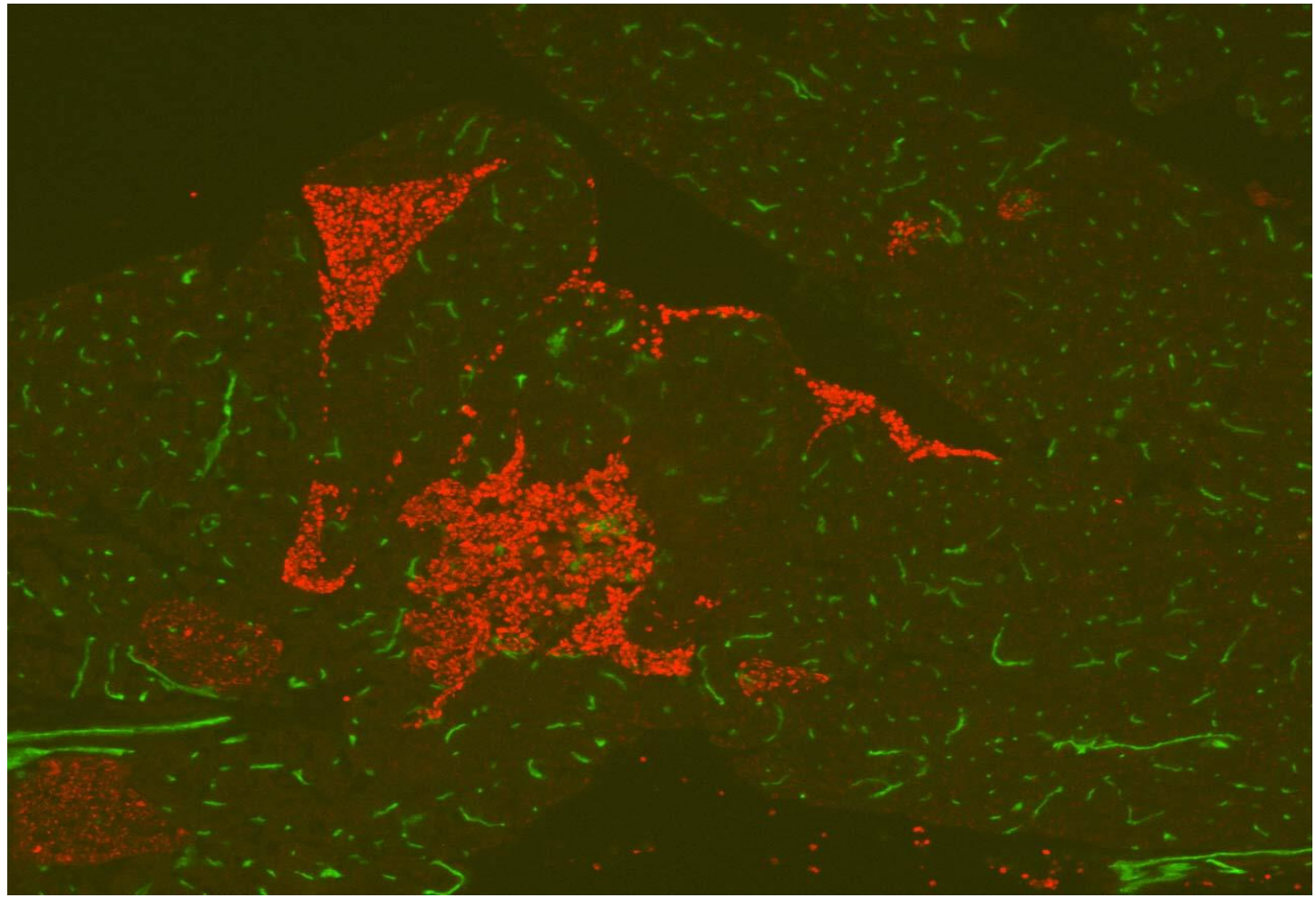
DC101, the monoclonal Ab that blocks VEGFR2 signaling, may elicit increased tumor invasiveness in Rip-Tag mice



Small, invasive lesions arise in response to genetic deletion of VEGFA

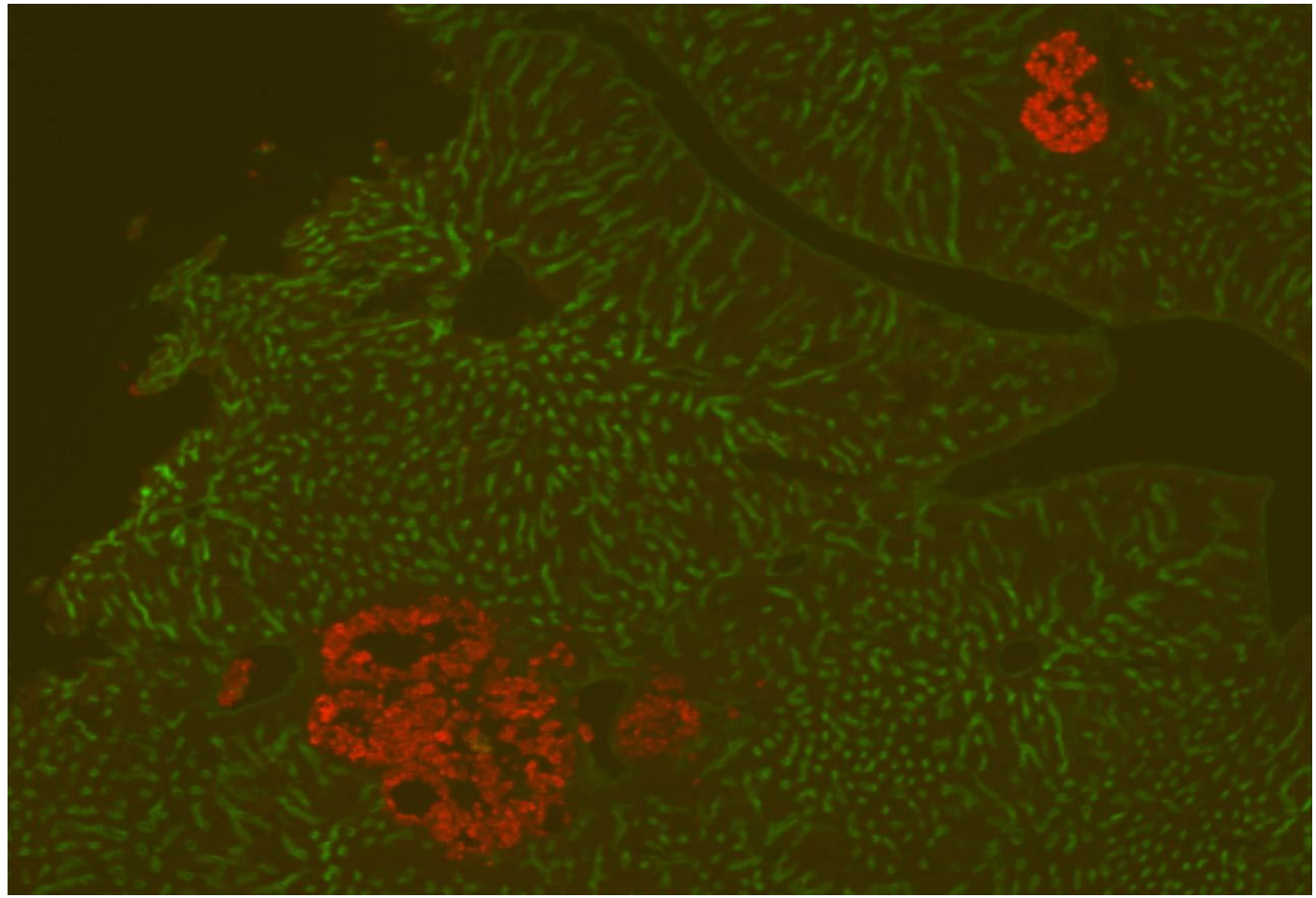


Invasive lesions in a RIP-Tag2 mouse treated for 5 weeks with sunitinib



Red – Tag oncoprotein (cancer cells)
Green – FITC-Lectin (vessels)

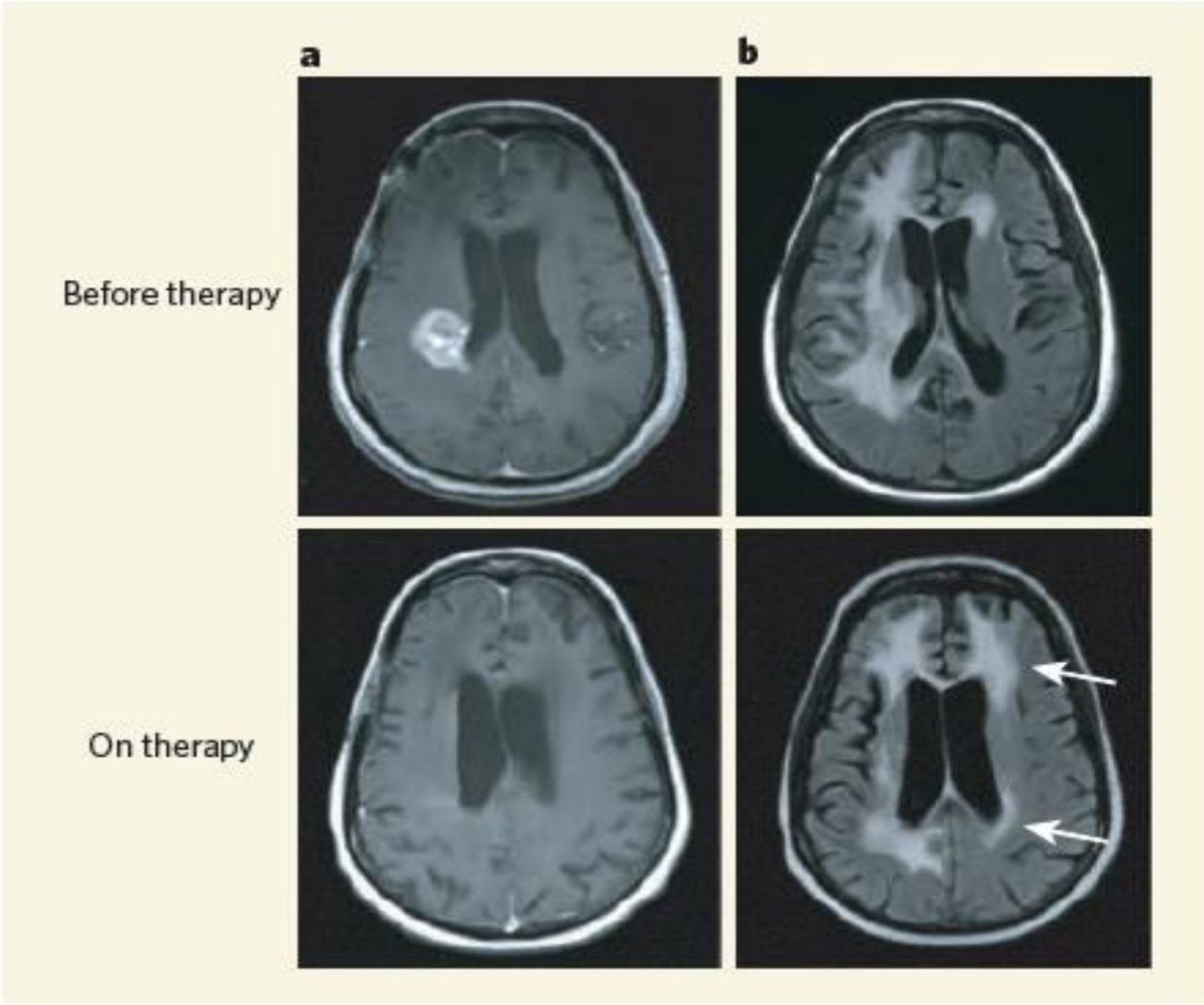
Liver metastases are more frequent in RIP-Tag2 mice treated for 5 weeks with sunitinib



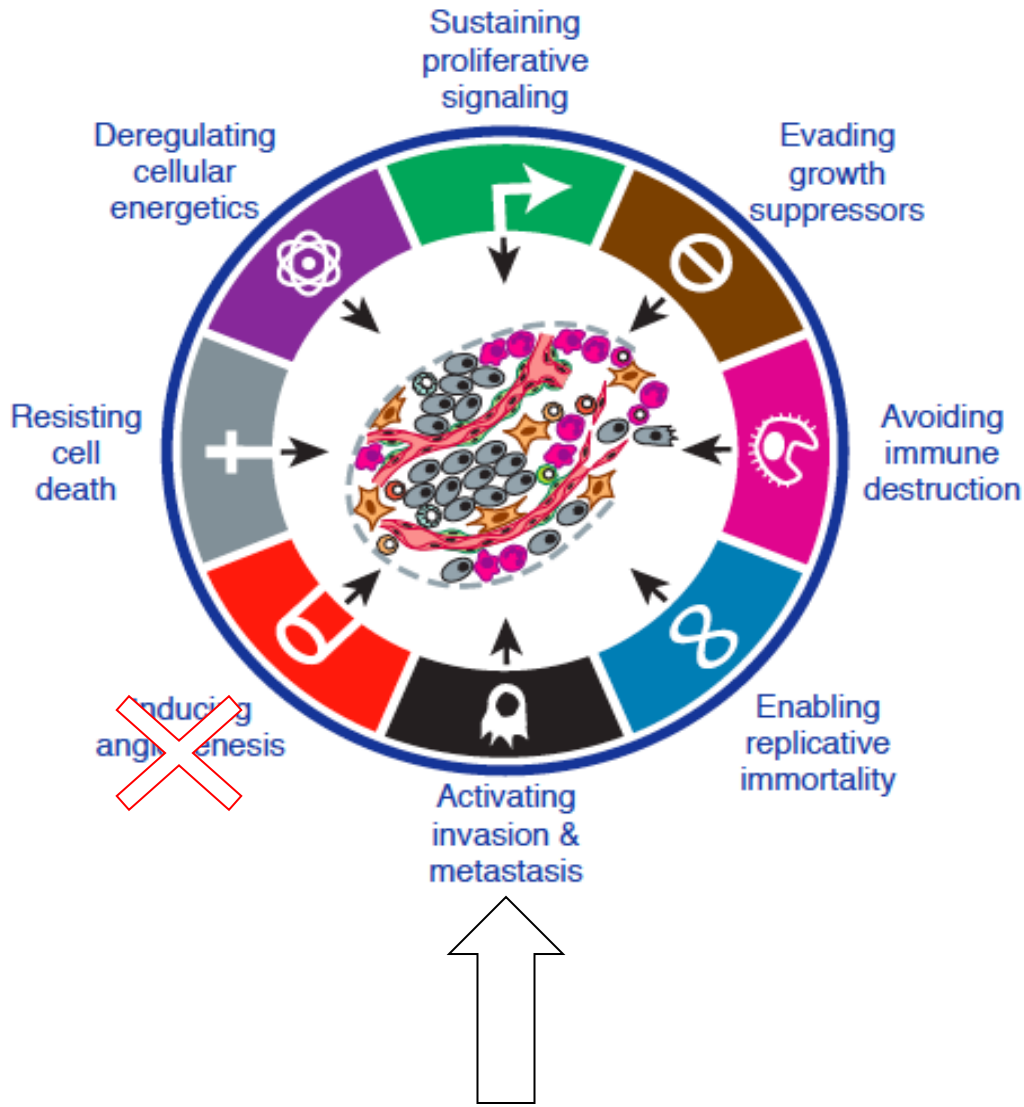
Red – Tag oncoprotein (cancer cells)
Green – FITC-Lectin (vessels)

VEGFR inhibition is apparently eliciting increased invasion in some GBM patients

Figure 1 | The MRI evidence. **a**, In agreement with the latest preclinical data[1,2], MRI scans from a patient with recurrent glioblastoma show that, after treatment with the VEGF-neutralizing antibody bevacizumab and the chemotherapeutic agent irinotecan, the macroscopic 'enhancing' tumour disappears, consistent with a complete response. **b**, However, microscopic tumour infiltration to other brain regions (arrows) is detectable in the patient after this therapy, using a different type of MRI that highlights brain inflammation and swelling[11].



Drug resistance by hallmark switching: Shifting dependence from angiogenesis to increased invasion & metastasis

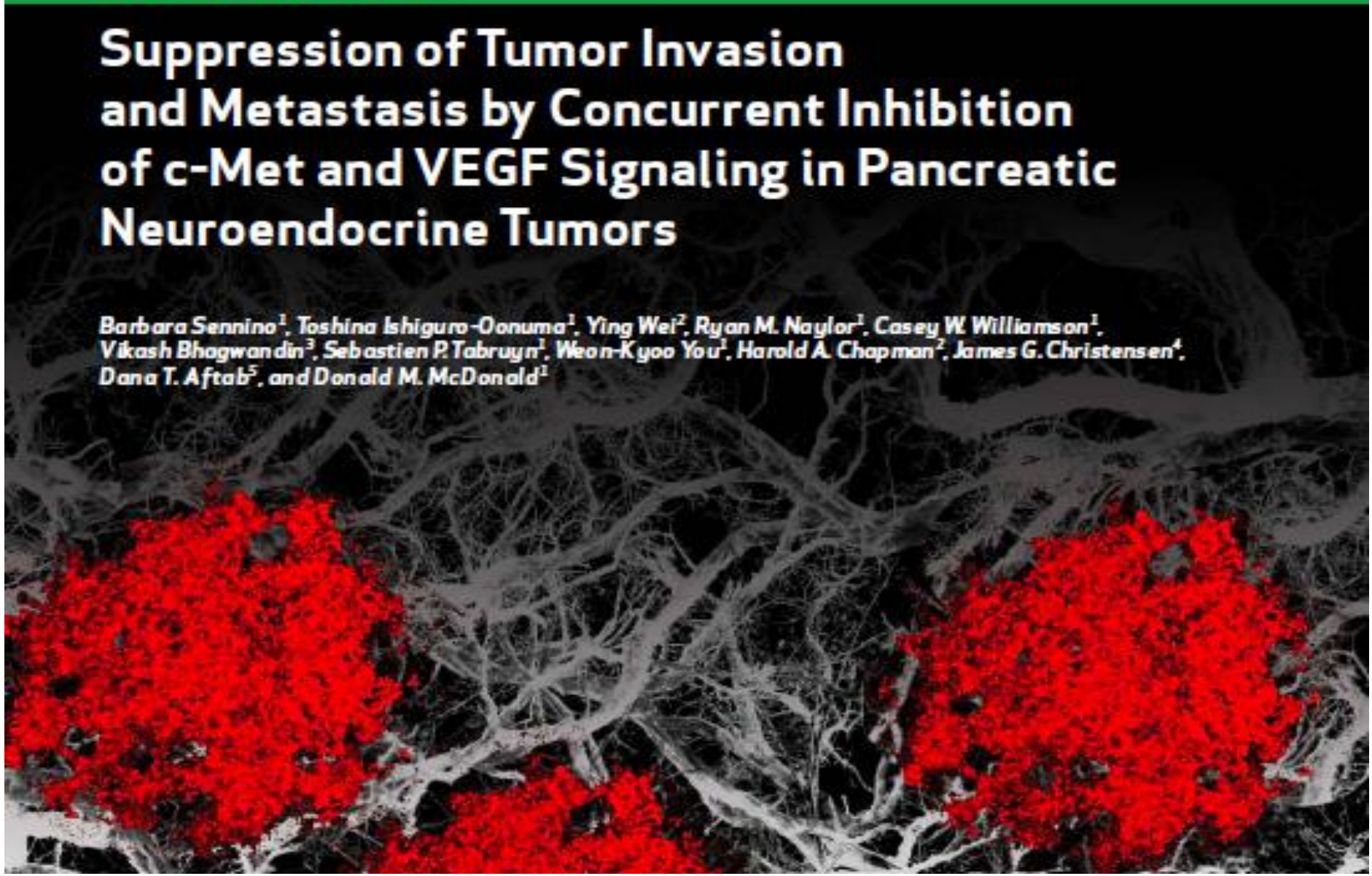


Published OnlineFirst on February 24, 2012; DOI:10.1158/2159-8290.CD-11-0240

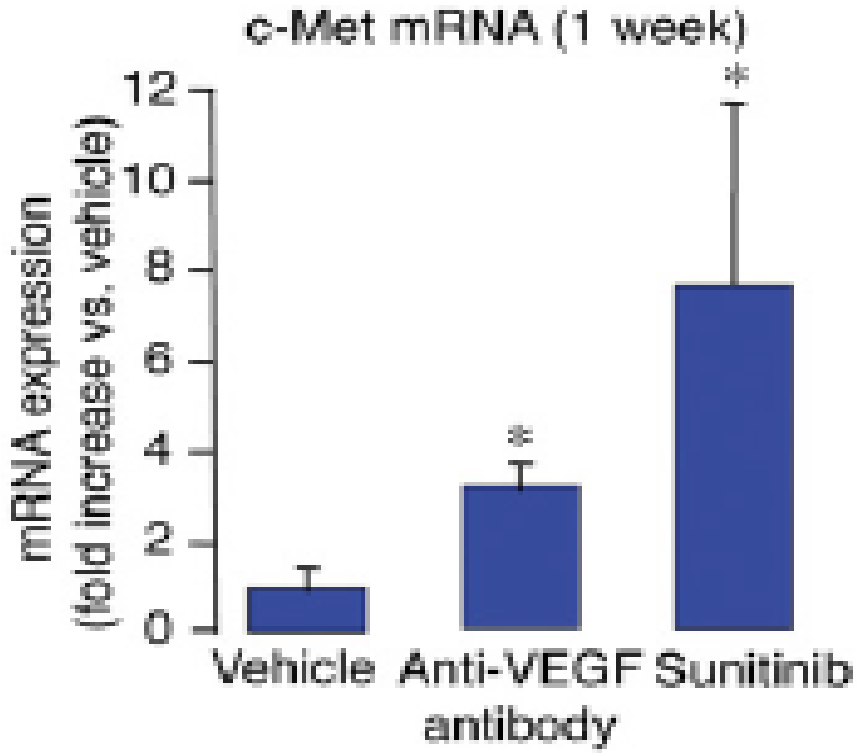
RESEARCH ARTICLE

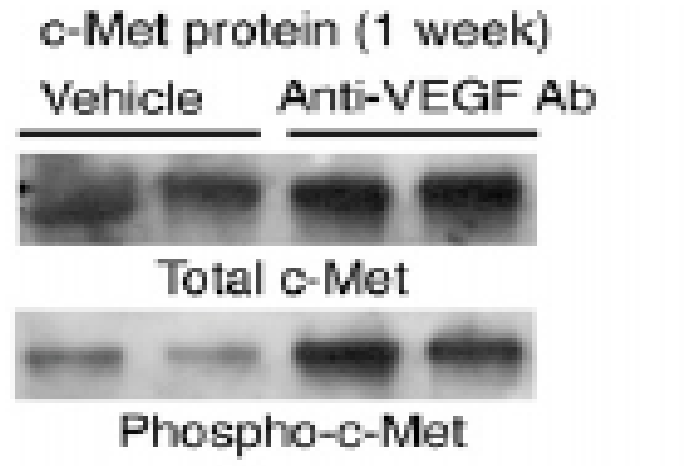
Suppression of Tumor Invasion and Metastasis by Concurrent Inhibition of c-Met and VEGF Signaling in Pancreatic Neuroendocrine Tumors

Barbara Sennino[1], Toshina Ishiguro-Oonuma[1], Ying Wei[2], Ryan M. Naylor[1], Casey W. Williamson[1], Vikash Bhagwandin[3], Sebastien P. Tabruyn[1], Weon-Kyoo You[1], Harold A. Chapman[2], James G. Christensen[4], Dana T. Aftab[5], and Donald M. McDonald[1]



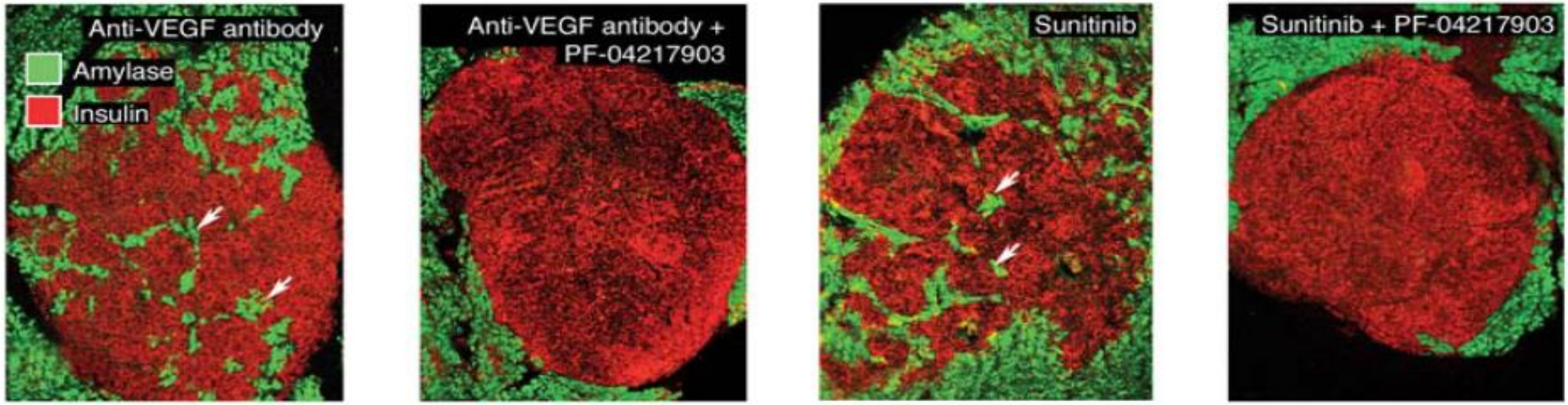
c-Met is induced in tumors treated with angiogenesis inhibitors (hypoxia-dependent)





Sennino et al Cancer Discovery, March 2012

Dual inhibition of VEGF/VEGFR and c-Met reduces invasiveness and prolongs survival

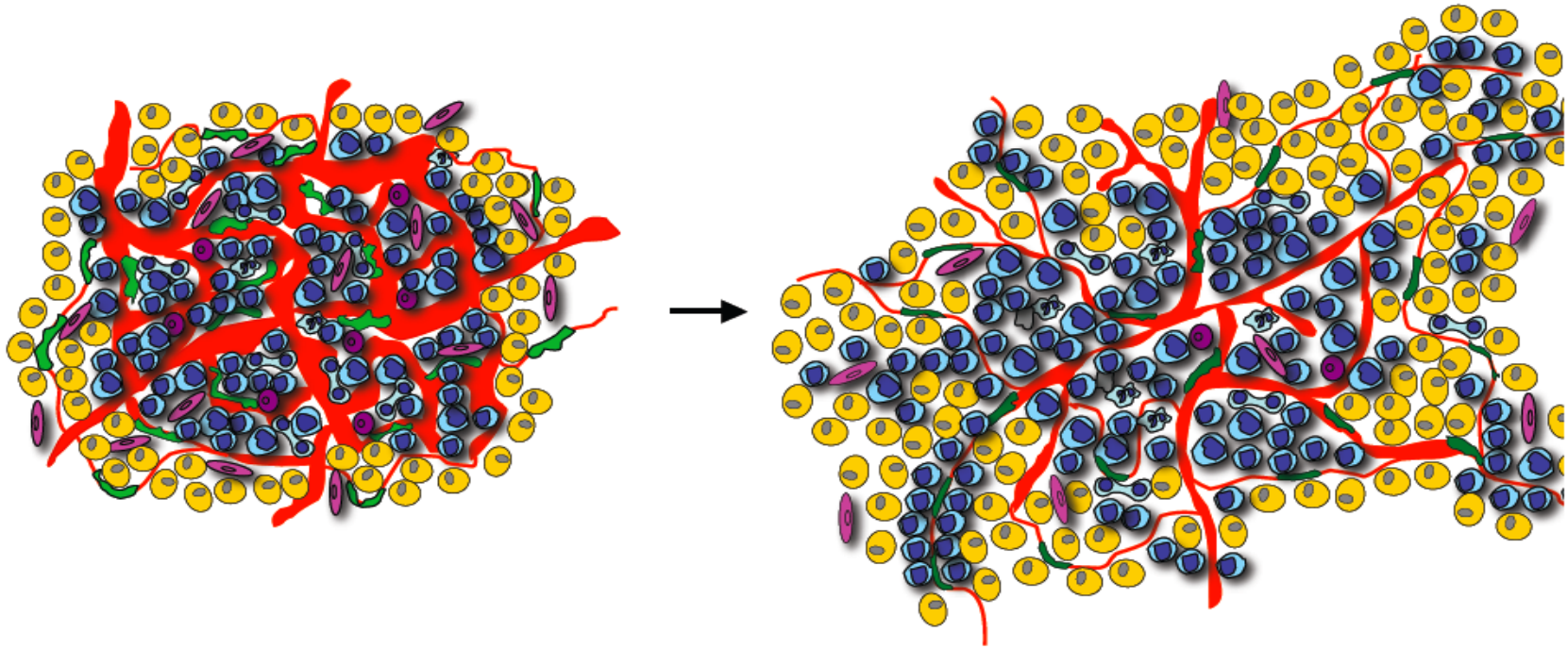


Sennino et al Cancer Discovery, March 2012

How do invasion and metastasis evade the necessity for angiogenesis to produce tumor neovessels?

- by co-opting normal tissue vessels to fuel disseminated tumor growth

Figure 5



There is, in addition, an even broader question, beyond adaptive resistance to anti-angiogenic therapy

-- Is the angiogenic switch and chronic tumor angiogenesis necessary for the development and progression of all types and subtypes of cancer?

-- an increasing body of histopathological evidence implicates co-option of normal tissue vessels as a means for cancer cells to access oxygen and nutrients to fuel tumor growth

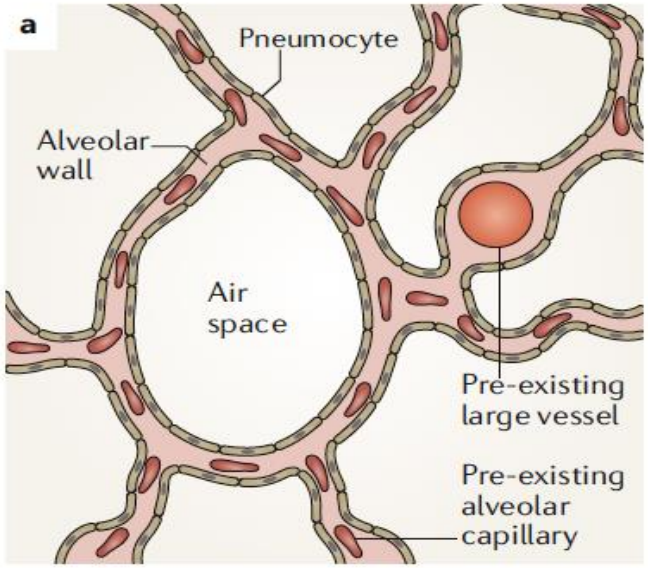
Vessel co-option in cancer

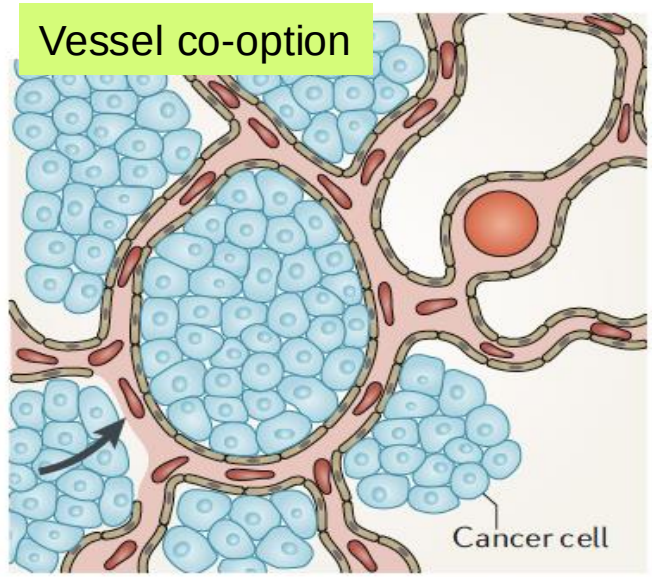
Elizabeth A. Kuczynski[1,2], Peter B. Vermeulen[3,4,5], Francesco Pezzella[6], Robert S. Kerbel[2,7] and Andrew R. Reynolds[5,8]**

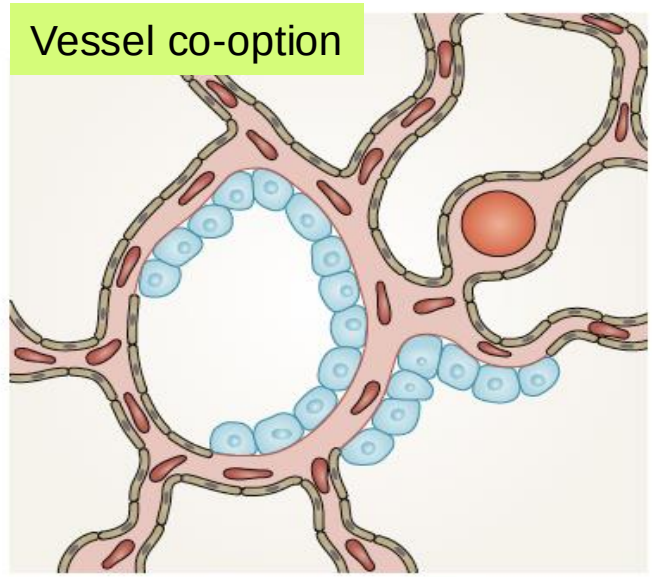
Abstract | All solid tumours require a vascular supply in order to progress. Although the ability to induce angiogenesis (new blood vessel growth) has long been regarded as essential to this purpose, thus far, anti-angiogenic therapies have shown only modest efficacy in patients. Importantly, overshadowed by the literature on tumour angiogenesis is a long-standing, but continually emerging, body of research indicating that tumours can grow instead by hijacking pre-existing blood vessels of the surrounding nonmalignant tissue. This process, termed vessel co-option, is a frequently overlooked mechanism of tumour vascularization that can influence disease progression, metastasis and response to treatment. In this Review, we describe the evidence that tumours located at numerous anatomical sites can exploit vessel co-option. We also discuss the proposed molecular mechanisms involved and the multifaceted implications of vessel co-option for patient outcomes.

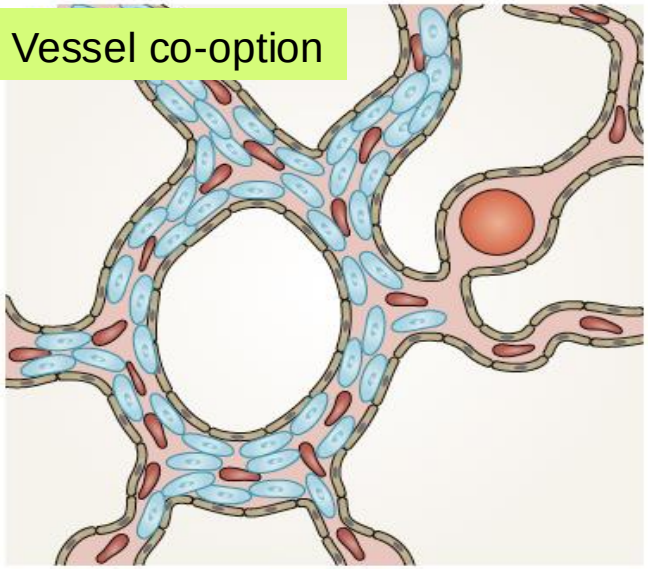
Nature Reviews Clinical Oncology, 2019

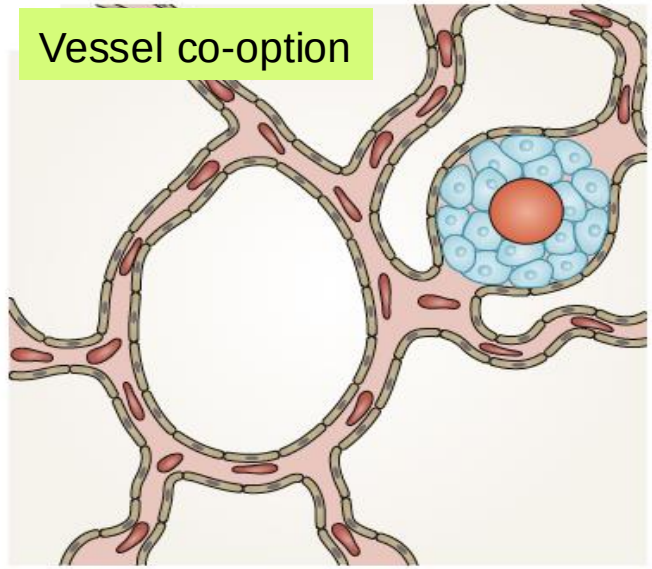
Neoplastic patterns of vessel co-option vs angiogenesis

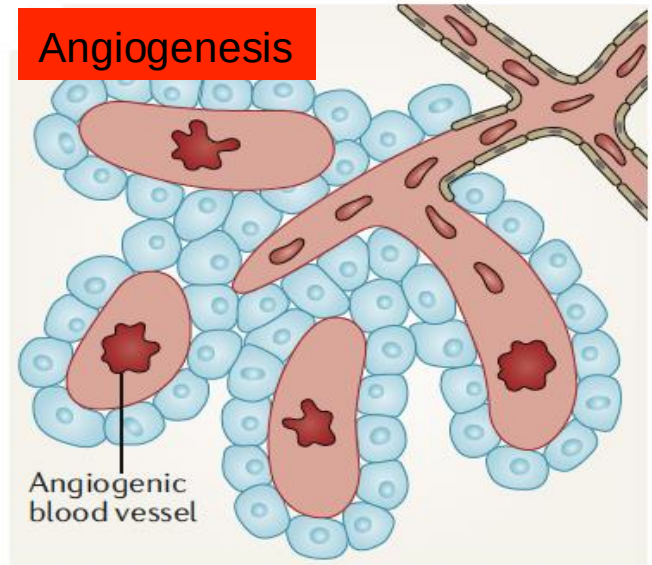












Distinguishing parameters of non-angiogenic vessel co-option

- Infrequent endothelial cell proliferation
- No sprouting
- Dense coverage by pericytes
- Intact basement membrane between endothelial cells and pericytes

A number of unanswered questions

- What are the regulatory mechanisms?
- Is more than an invasive capability required?
- What keeps angiogenesis switched off?
- How might vascular co-option be targeted therapeutically?

A different strategy for targeting the tumor vasculature: “vascular normalization”

- Rather than blocking new blood vessel growth (angiogenesis)

- Rather than disrupting and ablating the existing tumor vasculature, causing acute hypoxia leading to adaptive resistance

=> Instead “normalize” the tumor vasculature, producing vessels with better blood flow, more complete pericyte coverage

Normalizing the tumor vasculature

- Better delivery of chemo-therapies through the circulation

- Better extravasation of T cells into tumors via normalized blood vessels

This may happen after short-term treatment with angiogenesis inhibitors, or by using suboptimal doses.

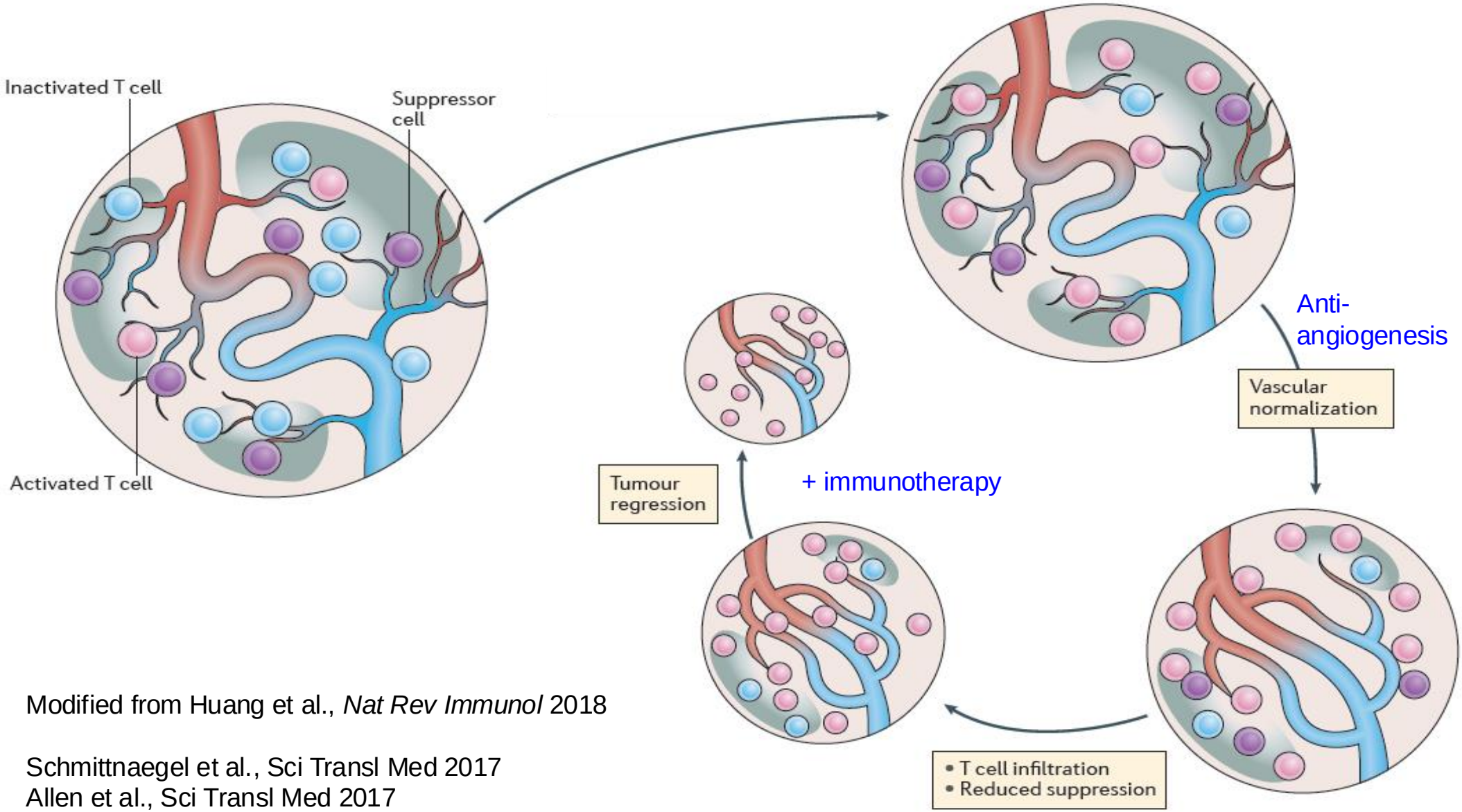
CANCER

Dual angiopoietin-2 and VEGFA inhibition elicits antitumor immunity that is enhanced by PD-1 checkpoint blockade

Martina Schmittnaegel,[1]* Nicolò Rigamonti,[1]*† Ece Kadioglu,[1] Antonino Cassará,[1] Céline Wyser Rmili,[1] Anna Kiialainen,[2] Yvonne Kienast,[3] Hans-Joachim Mueller,[3] Chia-Huey Ooi,[2,3] Damya Laoui,[1] Michele De Palma[1]‡

Pathological angiogenesis is a hallmark of cancer and a therapeutic target. Vascular endothelial growth factor A (VEGFA) and angiopoietin-2 (ANGPT2; also known as ANG2) are proangiogenic cytokines that sustain tumor angiogenesis and limit antitumor immunity. We show that combined ANGPT2 and VEGFA blockade by a bispecific antibody (A2V) provided superior therapeutic benefits, as compared to the single agents, in both genetically engineered and transplant tumor models, including metastatic breast cancer (MMTV-PyMT), pancreatic neuroendocrine tumor (RIP1-Tag2), and melanoma. Mechanistically, A2V promoted vascular regression, tumor necrosis, and antigen presentation by intratumoral phagocytes. A2V also normalized the remaining blood vessels and facilitated the extravasation and perivascular accumulation of activated, interferon-γ (IFNγ)–expressing $CD8^+$ cytotoxic T lymphocytes (CTLs). Whereas the antitumoral activity of A2V was, at least partly, CTL-dependent, perivascular T cells concurrently up-regulated the expression of the immune checkpoint ligand programmed cell death ligand 1 (PD-L1) in tumor endothelial cells. IFNγ neutralization blunted this adaptive response, and PD-1 blockade improved tumor control by A2V in different cancer models. These findings position immune cells as key effectors of antiangiogenic therapy and support the rationale for cotargeting angiogenesis and immune checkpoints in cancer therapy.

Anti-angiogenic immunotherapy



Modified from Huang et al., *Nat Rev Immunol* 2018

Schmittnaegel et al., Sci Transl Med 2017
Allen et al., Sci Transl Med 2017
Kashyap et al., PNAS, in press
Ragusa et al., JCI, in press

Clinical benefits of anti-angiogenic immunotherapy

The NEW ENGLAND JOURNAL of MEDICINE

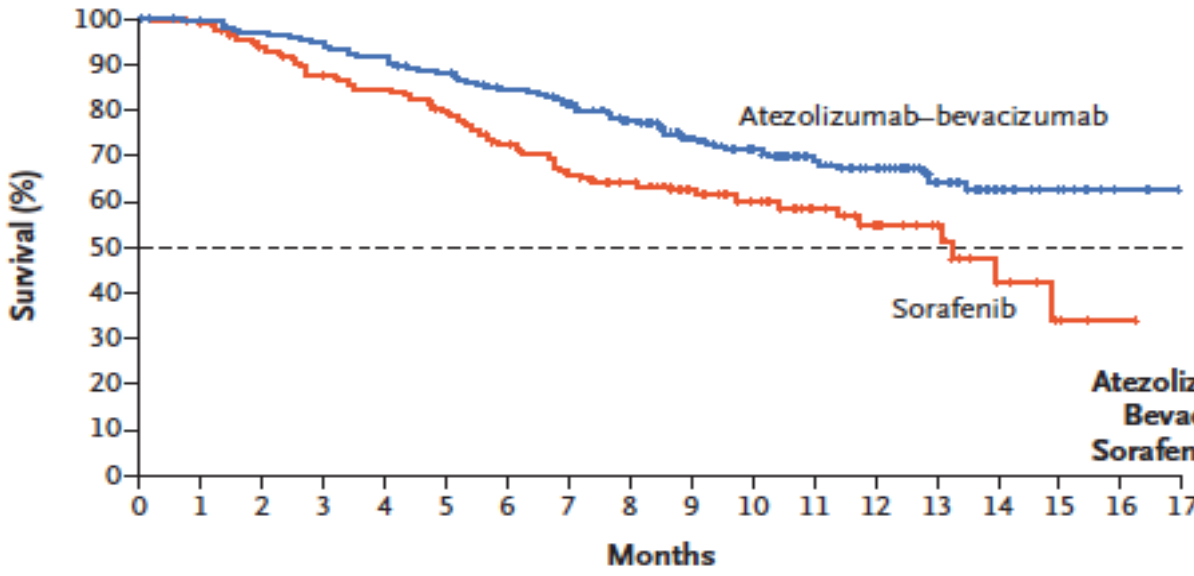
ORIGINAL ARTICLE

Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma

Richard S. Finn, M.D., Shukui Qin, M.D., Masafumi Ikeda, M.D., Peter R. Galle, M.D., Michel Ducreux, M.D., Tae-You Kim, M.D., Masatoshi Kudo, M.D., Valeriy Breder, M.D., Philippe Merle, M.D., Ahmed O. Kaseb, M.D., Daneng Li, M.D., Wendy Verret, Ph.D., Derek-Zhen Xu, M.D., Sairy Hernandez, Ph.D., Juan Liu, Ph.D., Chen Huang, M.D., Sohail Mulla, Ph.D., Yulei Wang, Ph.D., Ho Yeong Lim, M.D., Andrew X. Zhu, M.D., Ph.D., and Ann-Lii Cheng, M.D., for the IMbrave150 Investigators*

Clinical benefits of anti-angiogenic immunotherapy

A Overall Survival



	No. of Events/ No. of Patients (%)	Median Overall Survival (95% CI)	Overall Survival at 6 Mo
		mo	%
Atezolizumab–Bevacizumab	96/336 (28.6)	NE	84.8
Sorafenib	65/165 (39.4)	13.2 (10.4–NE)	72.2

Stratified hazard ratio for death, 0.58 (95% CI, 0.42–0.79)
P<0.001

No. at Risk

Atezolizumab–bevacizumab	336	329	320	312	302	288	275	255	222	165	118	87	64	40	20	11	3	NE
Sorafenib	165	157	143	132	127	118	105	94	86	60	45	33	24	16	7	3	1	NE