BMDCs and Cancer Therapy

1. BMDCs for cell-based therapies

Engineered embryonic EPCs for tumor targeting

Images removed for copyright reasons.

See: Fig. 1 and 8 in Wei, et al. "Embryonic endothelial progenitor cells armed with a suicide gene target hypoxic lung metastases after intravenous delivery." *Cancer Cell* 5 (2004): 477-488.

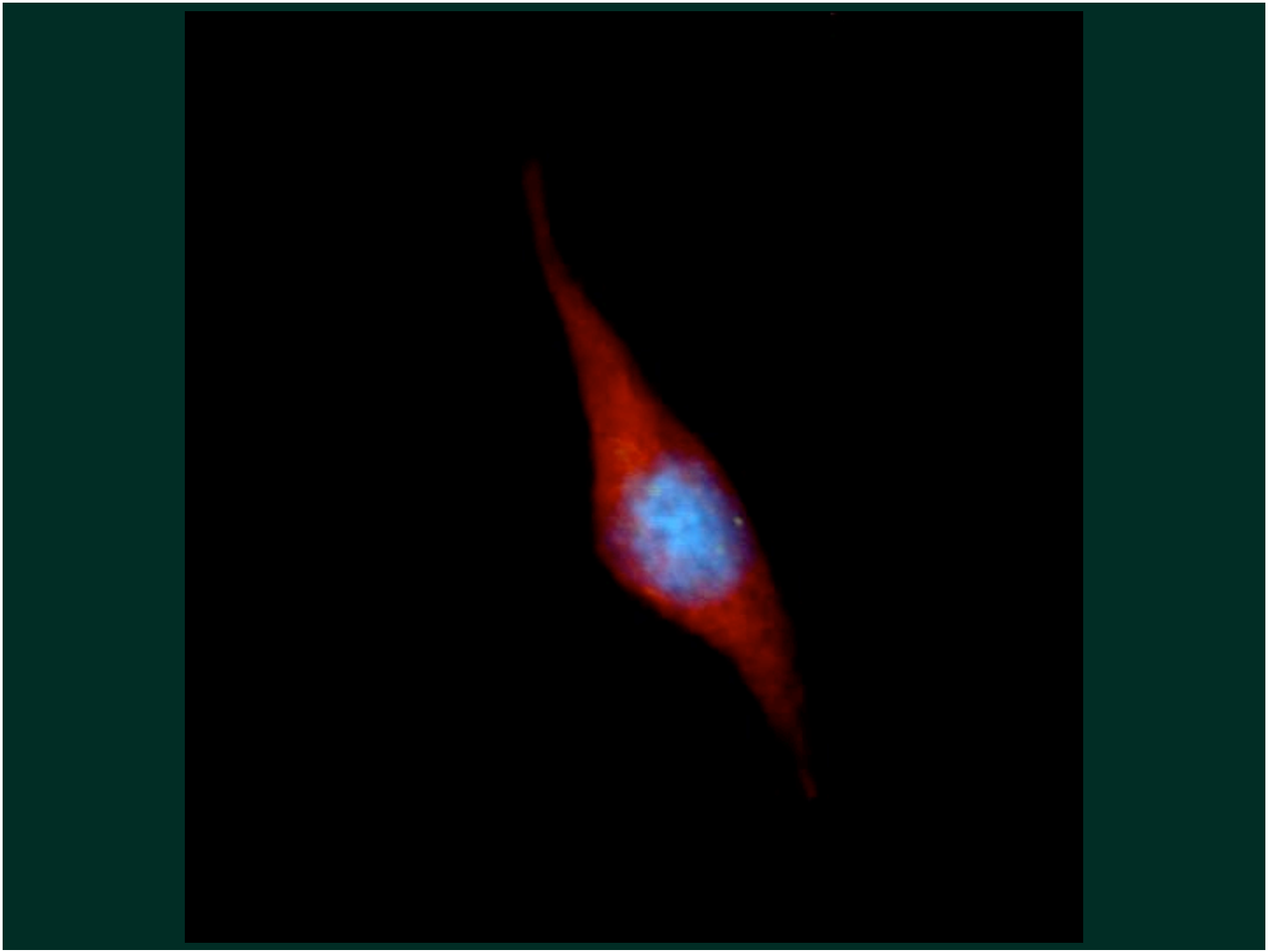
Homing kinetics after cell infusion

Cell homing after a lineage negative cell delivery, local and systemic, in tumor bearing mice (mammary carcinoma in CW)

Images removed for copyright reasons.

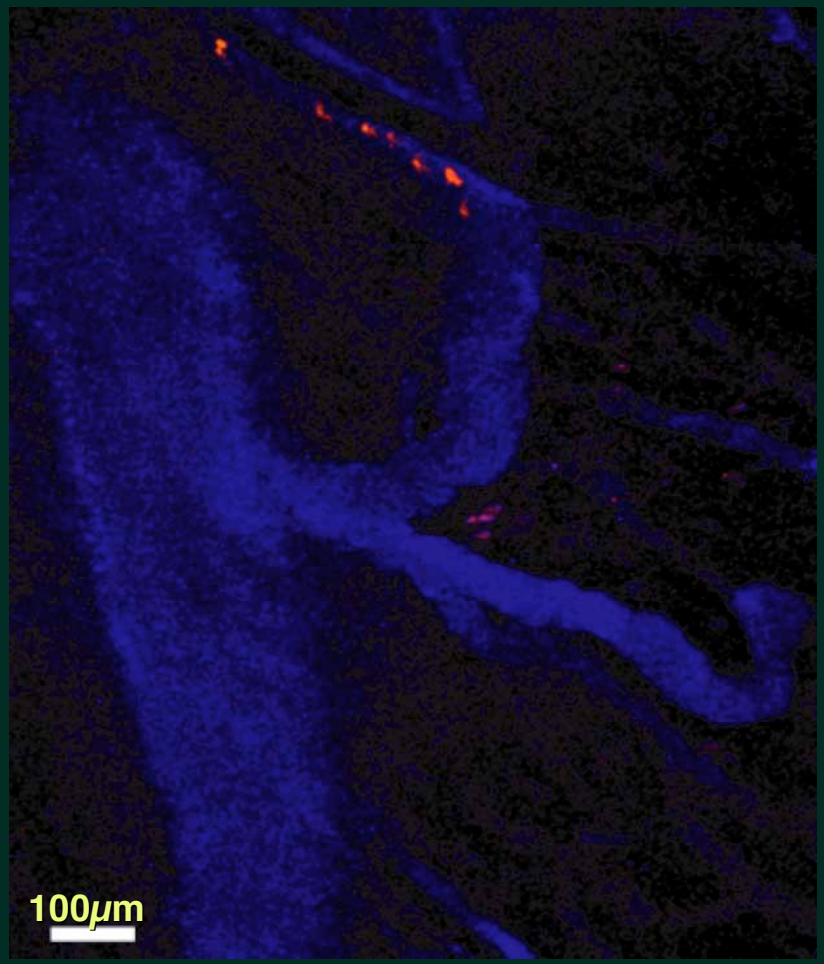
90 minutes, x6.25, IVM

10 minutes, x20, MPLSM



Source: Stroh, M., J. P. Zimmer, D. G. Duda, T. S. Levchenko, K. S. Cohen, E. B. Brown, D. T. Scadden, V. P. Torchilin, M. G. Bawendi, D. Fukumura, and R. K. Jain. "Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo." *Nature Medicine* 11 (2005): 678-682.

Cell cytoplasm labeling using Quantum dots micelle and tat bioconjugate



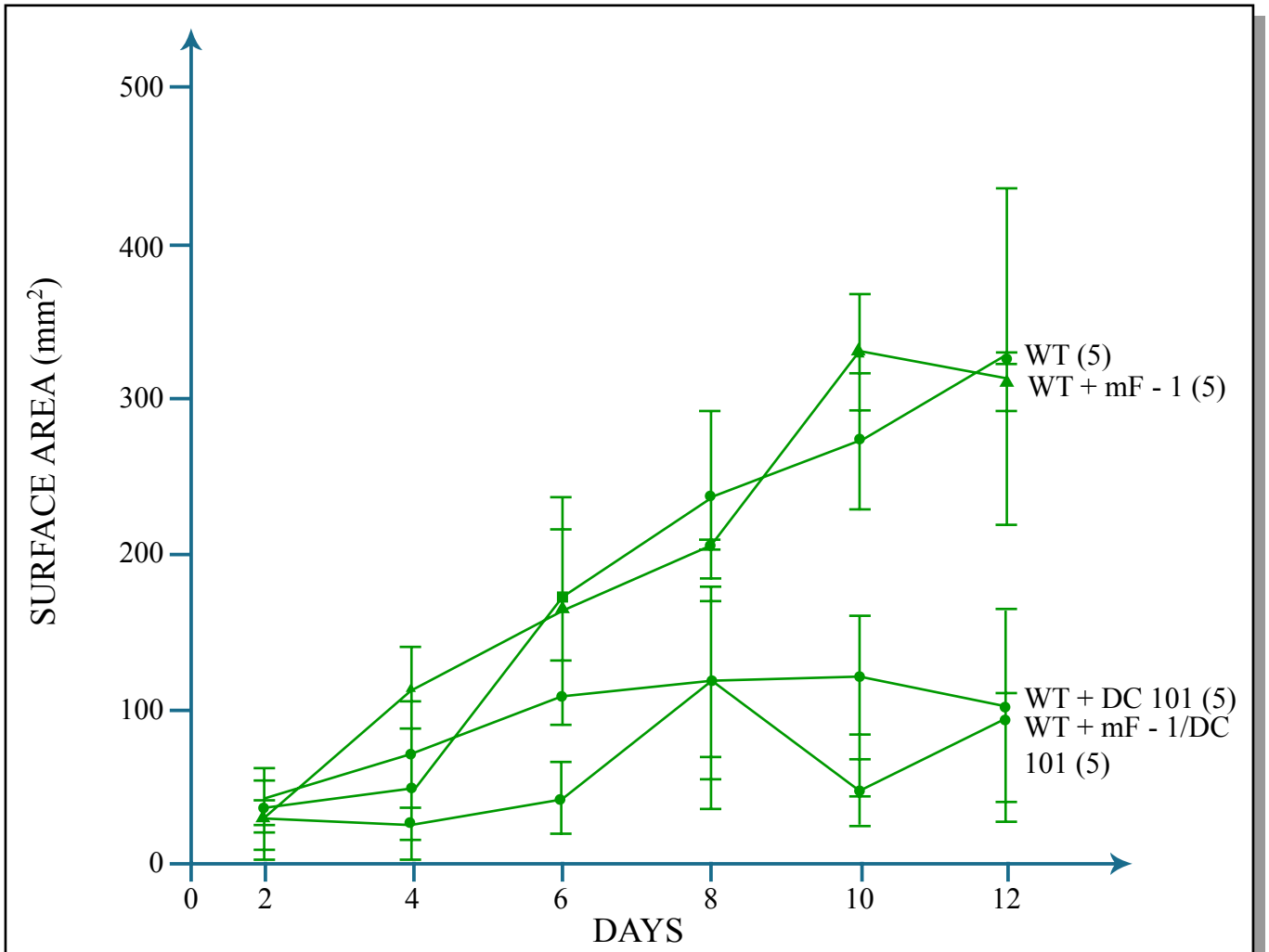
Mammary carcinoma in CW

2-photon image of Q-dots labeled progenitor cells (red)
Vessel enhancement by Q-dots (blue) infusion

Source: Stroh, M., J. P. Zimmer, D. G. Duda, T. S. Levchenko, K. S. Cohen, E. B. Brown, D. T. Scadden, V. P. Torchilin, M. G. Bawendi, D. Fukumura D, R. K. Jain "Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo." *Nature Medicine* 11 (2005): 678-682.

2. Role of BMDCs in tumor relapse after treatment

BMDC targeting delays tumor growth



Neutralizing the BMDCs with antibodies to both murine VEGFR1 (mF-1) and VEGFR2 (DC101) blocks tumor angiogenesis and thus blocks tumor growth.

Images removed for copyright reasons.
See: Fig. 6 in Lyden, Rafii et al. "Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth." *Nature Medicine* 7 (2001): 1194 - 1201.

Figure by MIT OCW.

Tumor response to radiation is BMDC-dependent

Images removed for copyright reasons.
See: Fig. 2 in Garcia-Barros, et al. "Tumor Response to Radiotherapy Regulated by Endothelial Cell Apoptosis." *Science* 300, (May 16, 2000): 1155-1159.

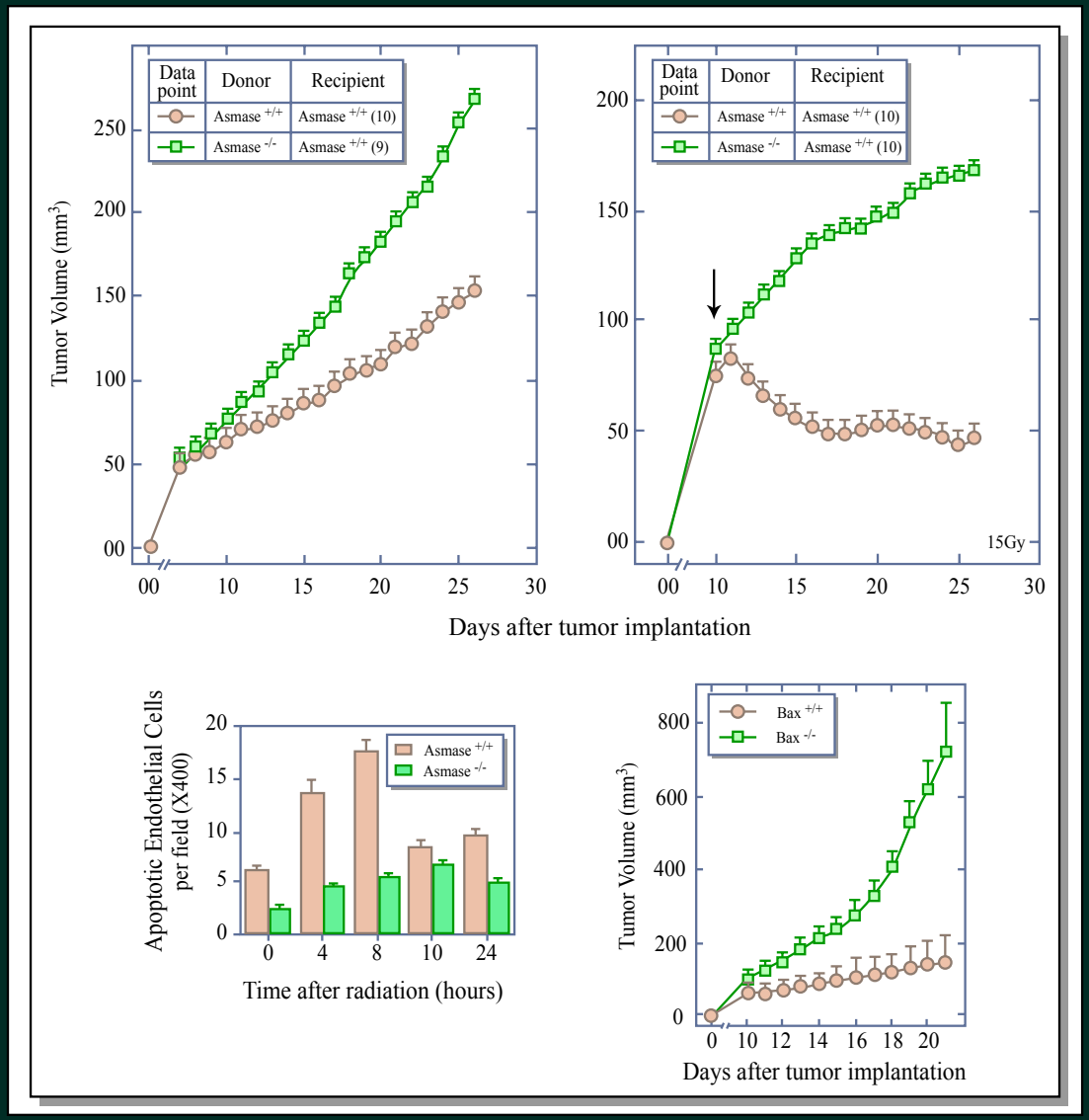


Figure by MIT OCW.

Mammary tumor relapse after 25 Gy irradiation

Tie2-GFP **Tie2-GFP-BMT** **Wild-type-BMT-Tie2-GFP**

Images removed for copyright reasons.

BMDC infiltrate during LLC recurrence after local irradiation using a single dose of 50Gy

Images removed for copyright reasons.

Antiangiogenic therapy by VEGFR2 blockade for LLC tumors implanted in Actb-GFP/BMT

%

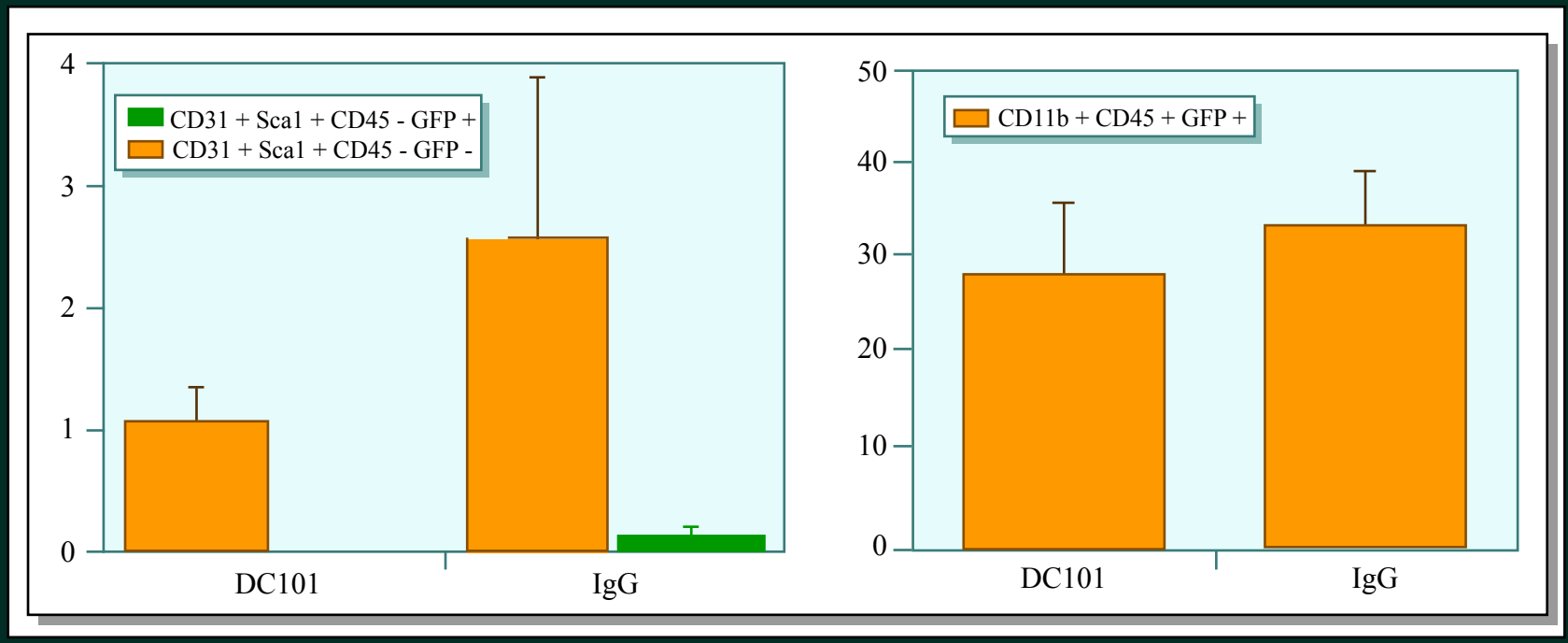
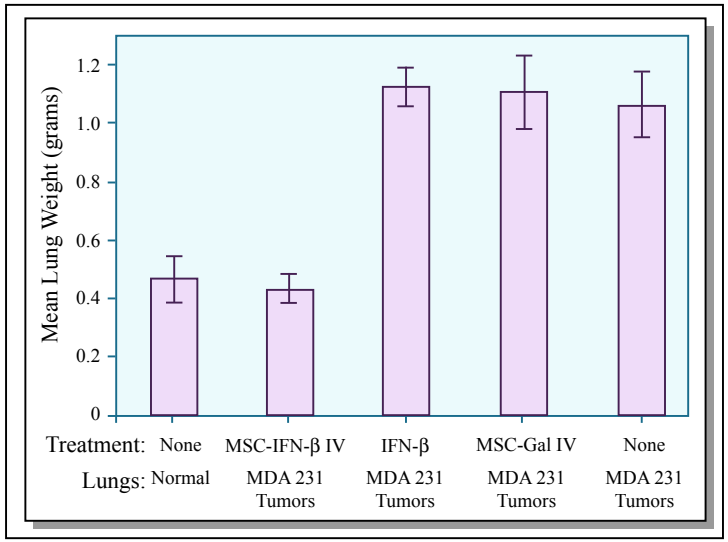


Figure by MIT OCW.

Tumor targeting using genetically engineered MSCs



Images removed for copyright reasons.
See: Figs. 2 and 4 in Studeny, et al. "Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents." *JNCI* 96 (2004): 1593-1603.

Figure by MIT OCW.

Reference: Andreeff and collab., JNCI 2004

3. BMDCs as surrogate markers for antiangiogenic therapies

Bevacizumab:
A blocking VEGF-specific antibody

- **Bevacizumab monotherapy** significantly prolonged the time to progression in a Phase II trial for metastatic **renal cell carcinoma** patients (Yang *et al.*, *New Engl J Med* 2003)
- **Bevacizumab (5mg/kg) + IFL** (Irinotecan-5-FU-Leucovorin)- increased the overall survival in a Phase III trial for metastatic **colorectal cancer** patients (Hurwitz *et al.*, *New Engl J Med* 2004)
- *Based on these results, bevacizumab was the first antiangiogenic drug approved by the Food and Drug Administration (February 2004)*

- **Bevacizumab (5mg/kg) + FOLFOX4** (oxaliplatin, 5-fluorouracil and leucovorin) increased overall survival in a Phase III trial for recurrent **colorectal cancer** patients*
- **Bevacizumab + paclitaxel and carboplatin** increased progression-free survival in a Phase III trial for **lung cancer** patients*
- **Bevacizumab + paclitaxel** increased progression-free survival in a Phase III trial for **breast cancer** patients*

**NCI Newcenter*

Background

Image removed for copyright reasons. Avastin (cancer therapeutic) advertisement.

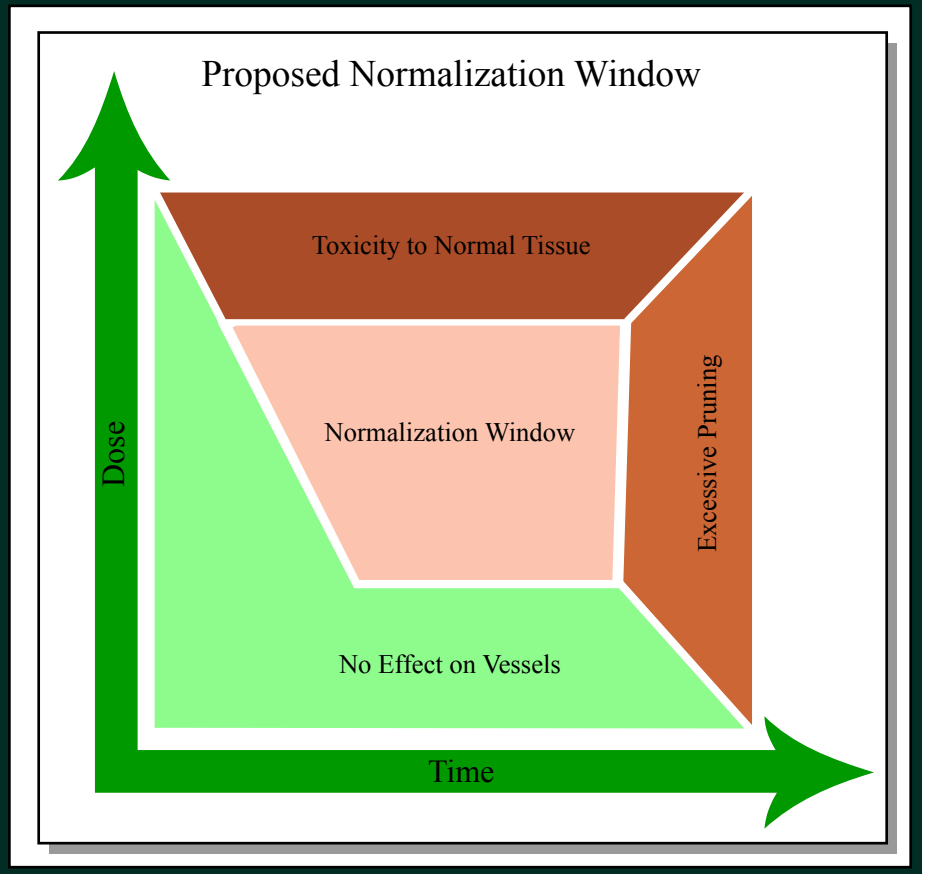
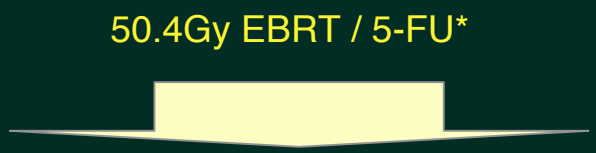


Figure by MIT OCW.

The unprecedented success of Avastin in the clinic was explained by its antivascular effects and by the ability to “normalize” tumor vasculature. The latter may improve the delivery of cytotoxic treatments.

To date, no established, valid surrogate marker exists in the clinic to guide the optimal dosing and scheduling of Avastin to achieve these effects

Protocol of Clinical Trial



Surgery

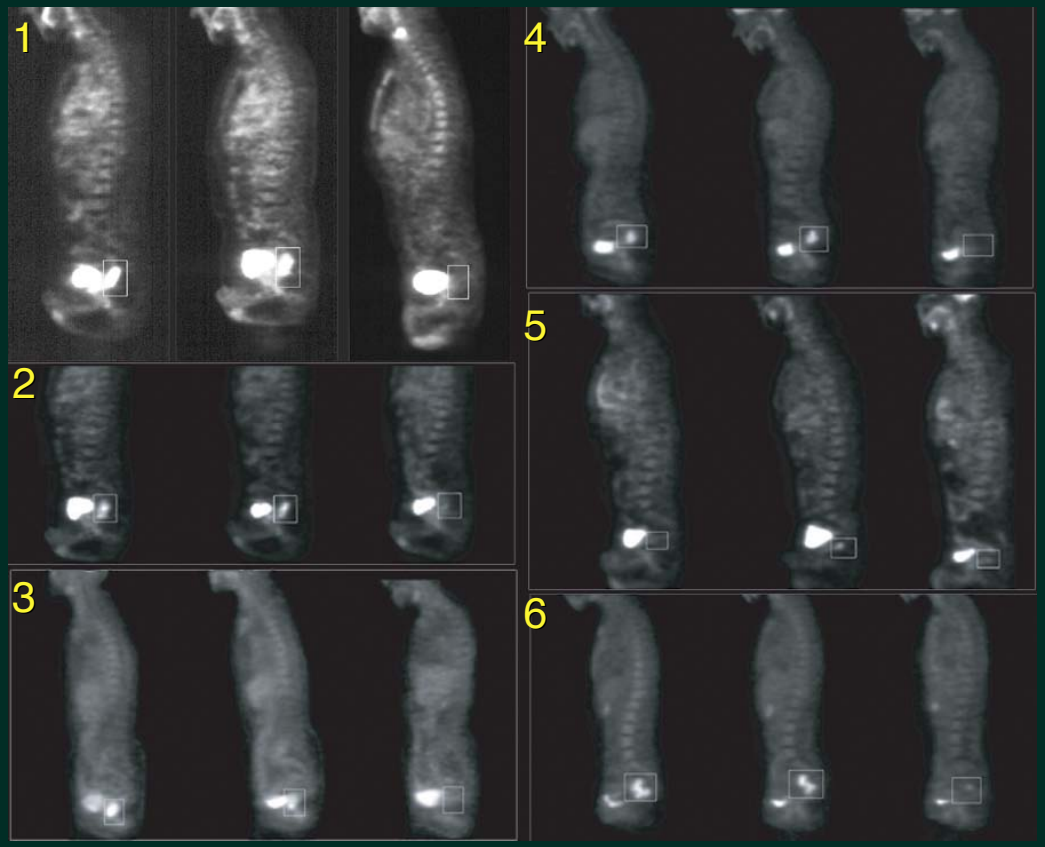
Avastin
5 or 10 mg/kg

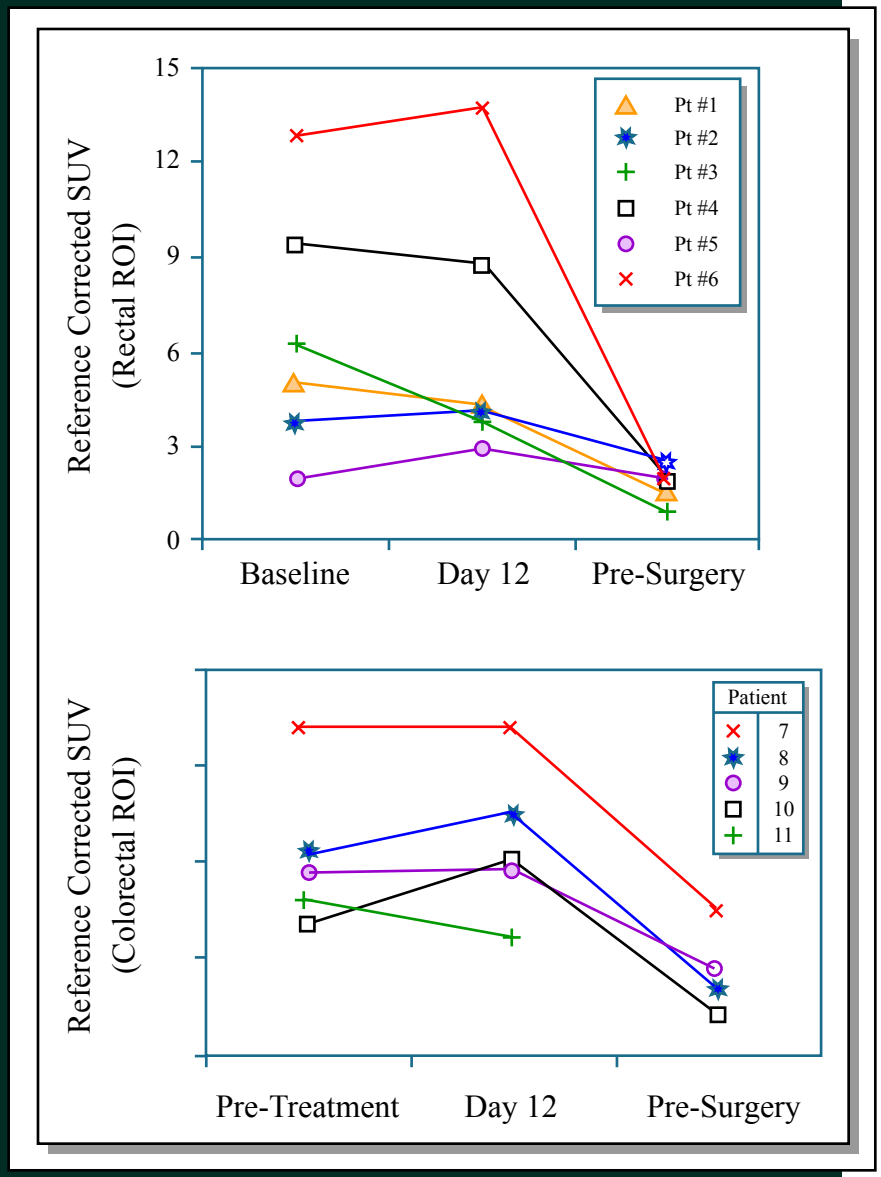
Day	-3	1	3	12	15	29	32	43	93	103
Blood/urine	√		√	√			√		√	
Tumor Biopsy	√			√						√
IFP	√			√						
Imaging	√			√					√	

*Daily Radiation Therapy (50.4 Gy/ 28 Fx) and continuous infusion of 5-Fluorouracil (225 mg/m^2/day)

Tumor Response: FDG Uptake (PET)

Sagittal PET scans: Patient #1-6





Reference:
Willett *et al*, *Nat Med* 2004 (1), Willett *et al*, *Nat Med* 2004 (2), Willet *et al*, *J Clin Oncol* 2005

Tumor Response: Biopsy Tissue Histological Analyses

Proliferation of epithelial cells

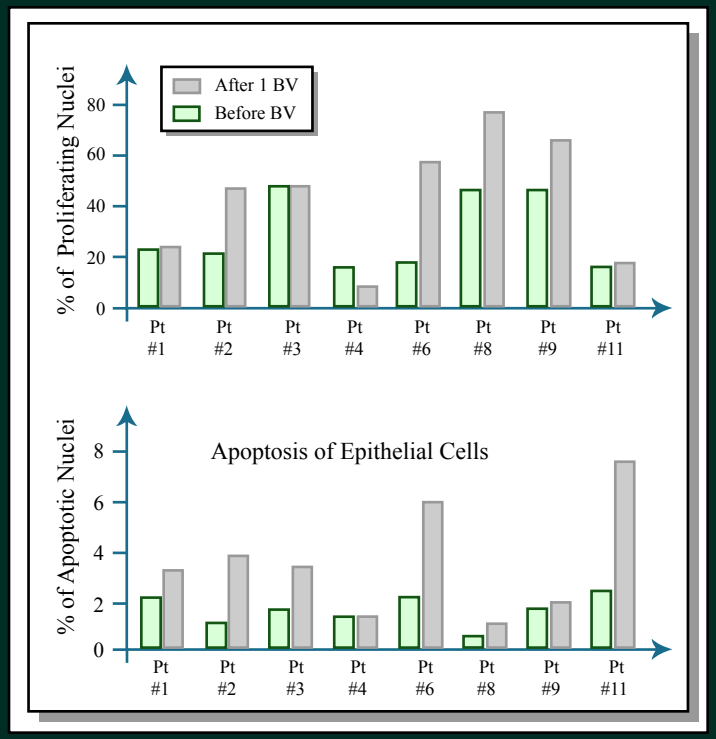


Figure by MIT OCW.

Microvascular Density

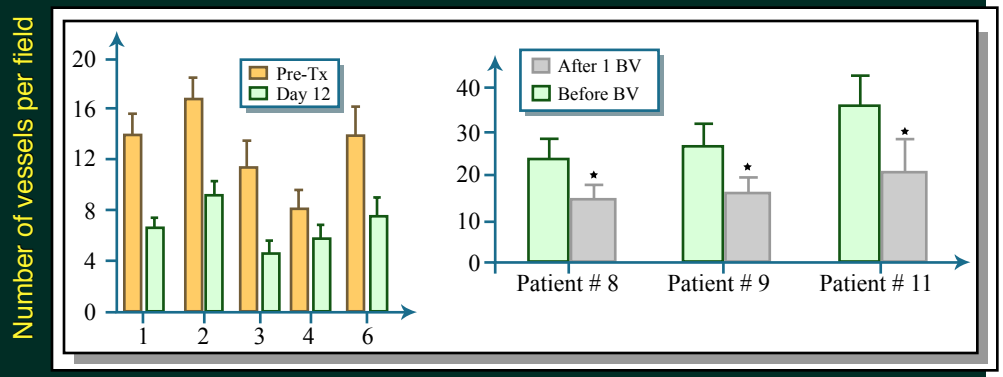


Figure by MIT OCW.

Pericyte Coverage

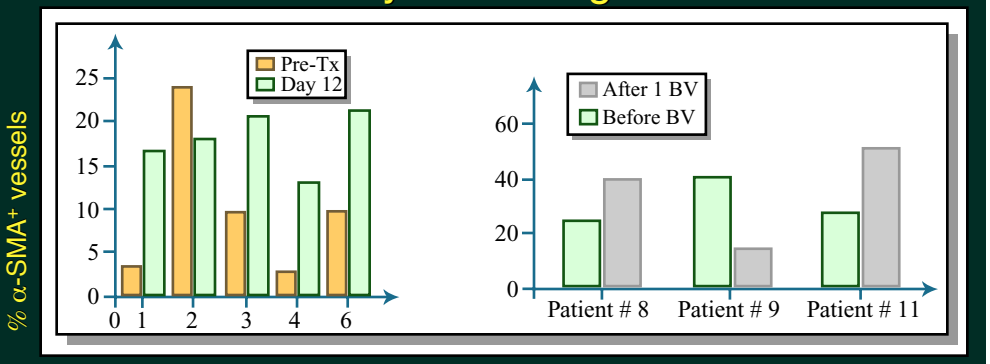


Figure by MIT OCW.

Functional Tumor Vascular Parameters after Avastin Treatment

CT Scan

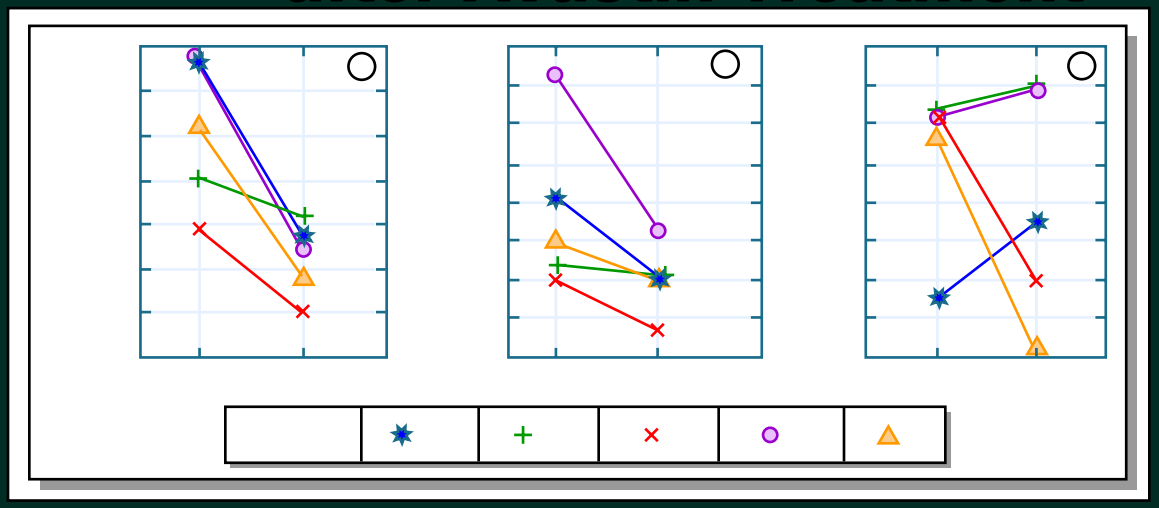


Figure by MIT OCW.

Solid line, $p<0.05$

Endoscopy: IFP measurements

Patient (dose)	IFP (mm Hg) Before B V	IFP During BV (Day12)
# 3 (5mg/kg)	16.0 ± 7.0, 5.0*	5.0
# 4 (5mg/kg)	15.0 ± 1.4, 1.0	1.0 ± 1.5, 0.9
# 5 (5mg/kg)	16.5 ± 5.0, 3.5	4.0 ± 3.7, 2.1
# 6 (5mg/kg)	12.0 ± 5.0, 3.5	6.0 ± 9.0, 4.5
# 7 (10mg/kg)	8.0 ± 2.1, 1.5	10 ± 9.2, 6.5
# 8 (10mg/kg)	15.0 ± 2.5, 1.8	5.5 ± 3.9, 2.8
# 9 (10mg/kg)	22.0 ± 20.0, 14.3	-1.5 ± 0.7, 0.5
# 10 (10mg/kg)	5.0 ± 0.6, 0.3	7.0 ± 1.5, 0.9
# 11 (10mg/kg)	29	6.0 ± 5.5, 3.2

*Mean IFP ± SD, SEM

Reference: Willett *et al*, *Nat Med* 2004, Willet *et al*, *J Clin Oncol* 2005

The Need for Surrogate Markers for Treatment

In summary,

- Addition of Avastin as neoadjuvant to chemotherapy was demonstrated in the clinical trials to increase survival in cancer patients (for three tumor types)

- We have shown that Avastin alone has rapid and potent effects on tumor vascular structure and function

- Increasing evidence supports the concept that the mechanism of action of Avastin is to prune abnormal vasculature and fortify and improve the function of the remaining vasculature in tumors

- *To date, no surrogate marker has been established in the clinic to evaluate the anti-vascular effects and no marker has been proposed for the normalizing effects of Avastin*

- Based on the increasing preclinical evidence that blood markers may be useful for the evaluation of antiangiogenic drugs' efficacy, we investigated the:

  i) kinetics of circulating endothelial cells (CECs) and progenitor cells (CPCs); and

  ii) changes plasma protein

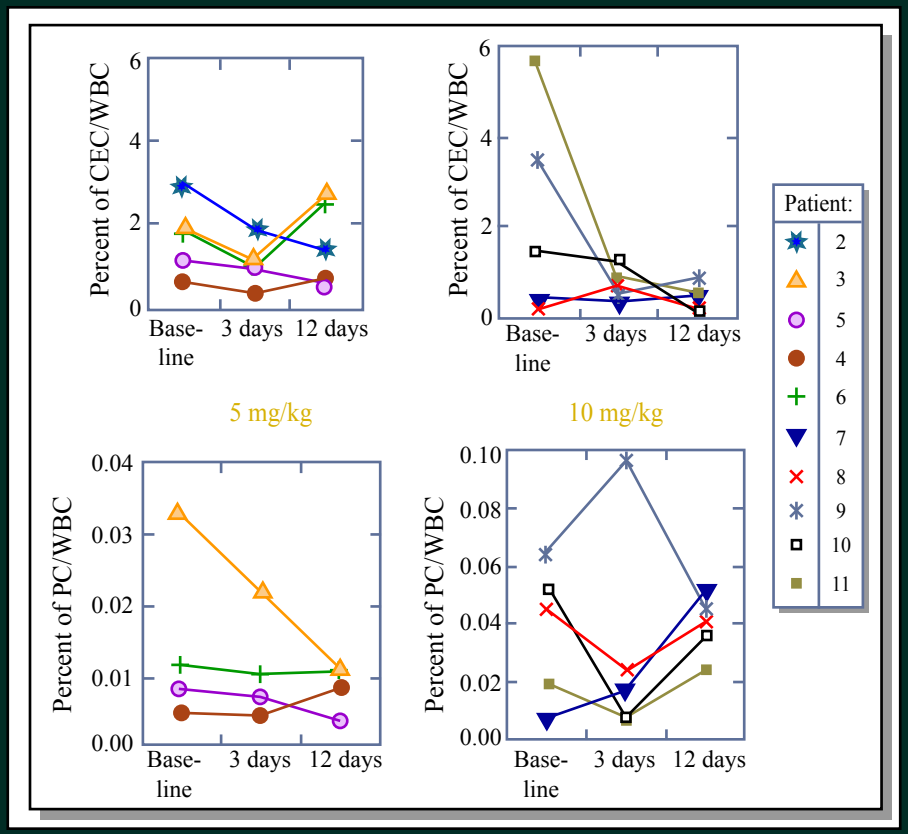
  in rectal cancer patients before and during Avastin treatment.

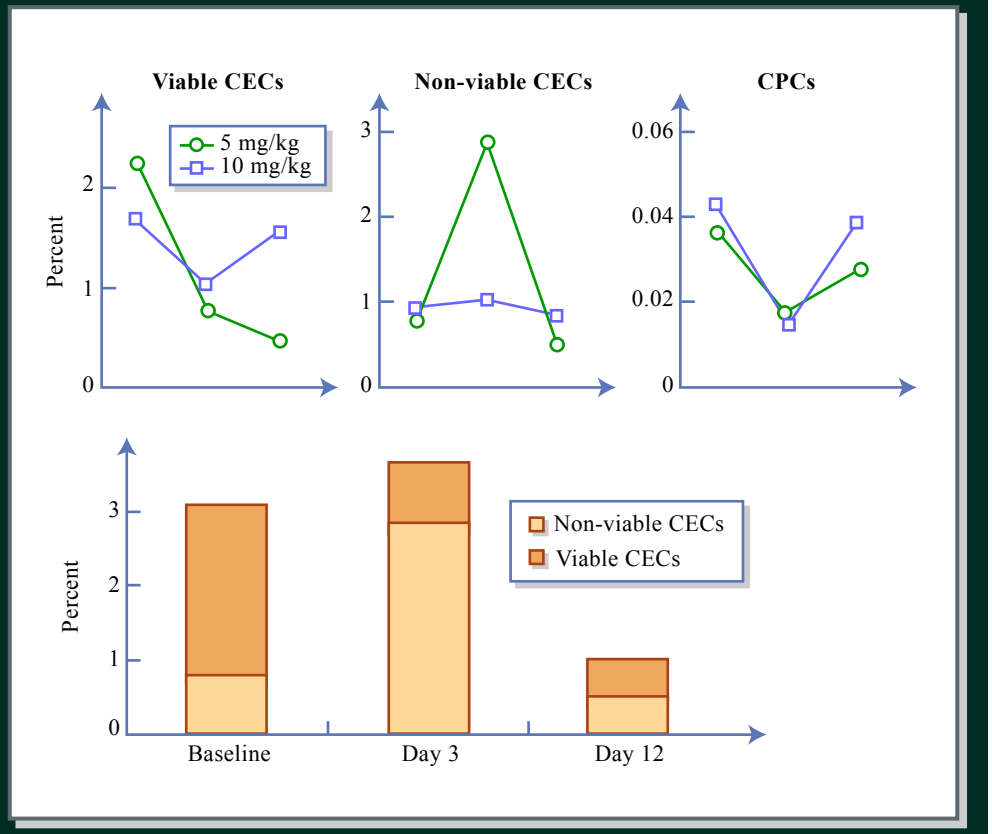
Characterization of Peripheral Blood Cells' Phenotype by Four-color Flow Cytometry

Images removed for copyright reasons.
Sources: Duda, D. G., K. S. Cohen, E. di Tomaso, P. Au, R. Klein, D. Scadden, C. G. Willett, and R. K. Jain. "Differential CD146 expression on circulating versus tissue endothelial cells in cancer patients: Implications for circulating endothelial cells as biomarker for antiangiogenic therapy." *J Clin Oncol* 24 (2006): 1449-53.

Kinetics of Circulating Cells in Response to Avastin Treatment Alone in Cancer Patients by Flow Cytometry

Viable CECs





CPCs

N = 5 patients

Figures by MIT OCW.

Kinetics of plasma proteins after Avastin treatment

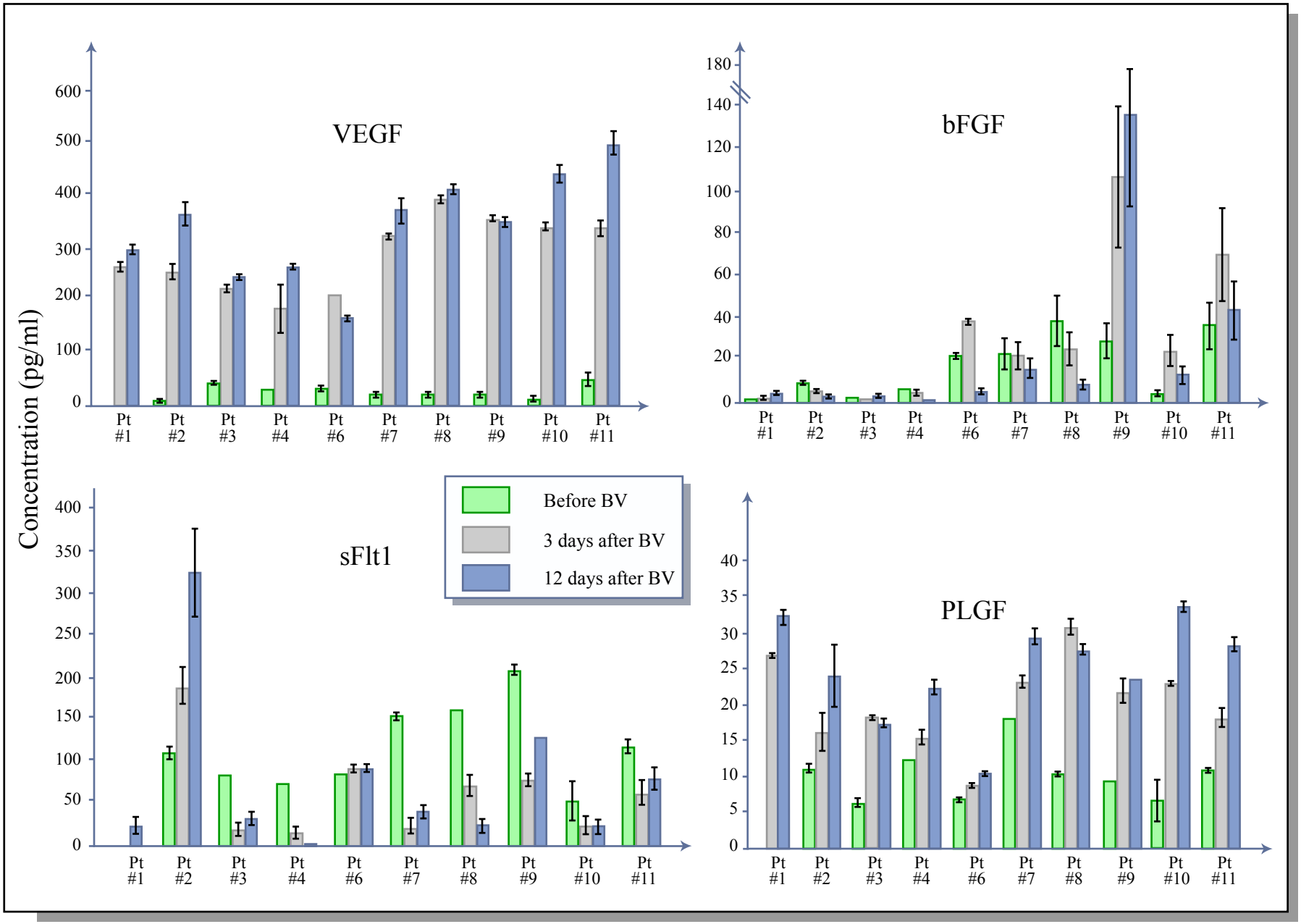


Figure by MIT OCW.

Effects of VEGF on Bone Marrow Precursor Cells

VEGF mobilizes precursors/stem cell for hematopoiesis and vasculogenesis

VEGF is a survival factor for hematopoietic stem cells and endothelial cells

VEGFRs are present on hematopoietic precursors/stem cells, endothelial cells and mesenchymal stem cells

Bevacizumab blockade of VEGF may affect bone marrow precursor cells

Surrogate marker candidates:

i) kinetics of circulating endothelial cells (CECs) and progenitor/stem cells (CPCs)

ii) changes plasma protein

Characterization of Endothelial Cell Phenotype in Peripheral Blood and in Tumor Tissue in Mice

Images removed for copyright reasons.

CEC kinetics in Actb-GFP/BMT mice bearing LLC

Image removed for copyright reasons.

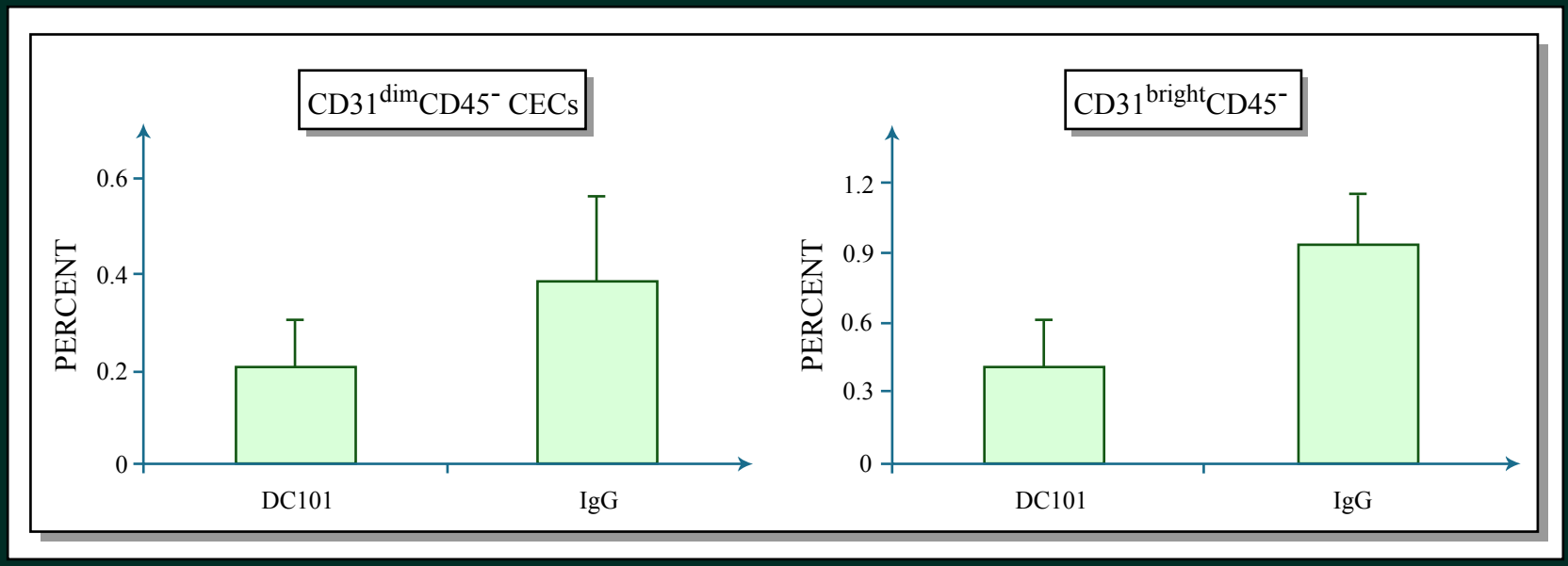


Figure by MIT OCW.

Analysis of CD133 (Prominin 1) Expression on Mouse Bone Marrow Cells

Images removed for copyright reasons.

Expression of CD146 (P1H12, pan-endothelial cell marker) and CD133 (AC133, progenitor/stem cell marker) on Peripheral Blood Mononuclear Cells in Cancer Patients

Rectal carcinoma patient peripheral blood

Images removed for copyright reasons.
Source: Duda, D. G., K. S. Cohen, E. di Tomaso, P. Au, R. Klein, D. Scadden, C. G. Willett, and R. K. Jain. "Differential CD146 expression on circulating versus tissue endothelial cells in cancer patients: Implications for circulating endothelial cells as biomarker for antiangiogenic therapy." *J Clin Oncol* 24 (2006): 1449-53.

What Type of Cells Is CD146 Identifying in Human Tissues?

Images removed for copyright reasons.

Source: Duda, D. G., K. S. Cohen, E. di Tomaso, P. Au, R. Klein, D. Scadden, C. G. Willett, and R. K. Jain. "Differential CD146 expression on circulating versus tissue endothelial cells in cancer patients: Implications for circulating endothelial cells as biomarker for antiangiogenic therapy." *J Clin Oncol* 24 (2006): 1449-53.

Lung carcinoma biopsy

Images removed for copyright reasons.

Source: Duda, D. G., K. S. Cohen, E. di Tomaso, P. Au, R. Klein, D. Scadden, C. G. Willett, and R. K. Jain."Differential CD146 expression on circulating versus tissue endothelial cells in cancer patients: Implications for circulating endothelial cells as biomarker for antiangiogenic therapy." *J Clin Oncol* 24 (2006): 1449-53.

Glioblastoma biopsy

Images removed for copyright reasons.

Conclusions and Future Directions

- The study of VEGF blockade in cancer patients suggested that blood analyses may provide useful surrogate markers for response to bevacizumab therapy
- VEGF withdrawal reduces early after treatment the viable (bone marrow-derived) CECs and progenitor cells, and increases plasma levels of VEGF and PlGF
- The increase and then decline in non-viable CECs after bevacizumab treatment may correlate with the *anti-vascular effect* (at day 3) and *normalizing effect* (at day 12) on tumor vessels

Conclusions and Future Directions

- Unlike tissue ECs, viable CECs do not express CD146; CD133 progenitor cells are $CD45^{+}$; further understanding of biology of CECs and CPCs (markers, viability, differentiation, etc.) is warranted

- The changes in plasma levels of other factors affecting angiogenesis (e.g., TSP1) should also be evaluated and correlated with circulating cell kinetics

- We are evaluating these markers after VEGF-specific blockade (bevacizumab) or growth factor receptor TKI treatment in six ongoing trials for five different cancer types

- Translation on a patient-by-patient basis of these markers will require more sensitive technologies as well as unitary guidelines for CEC and CPC evaluation

Acknowledgements

Dr. Jain
Dr. Jones
Dr. Scadden
Dr. Seed
Dr. Suit
Dr. Willett

All Steele lab

National Cancer Institute, Bethesda
(PO1 to RK Jain and R21 to CG Willett)

National Cancer Foundation
(RK Jain)

Cancer Research Institute, New York
(DG Duda)

American Association for Cancer Research & Genentech-BioOncology
(DG Duda)