Contribution of bone marrow-derived cells during tumor progression and treatment

Reference:
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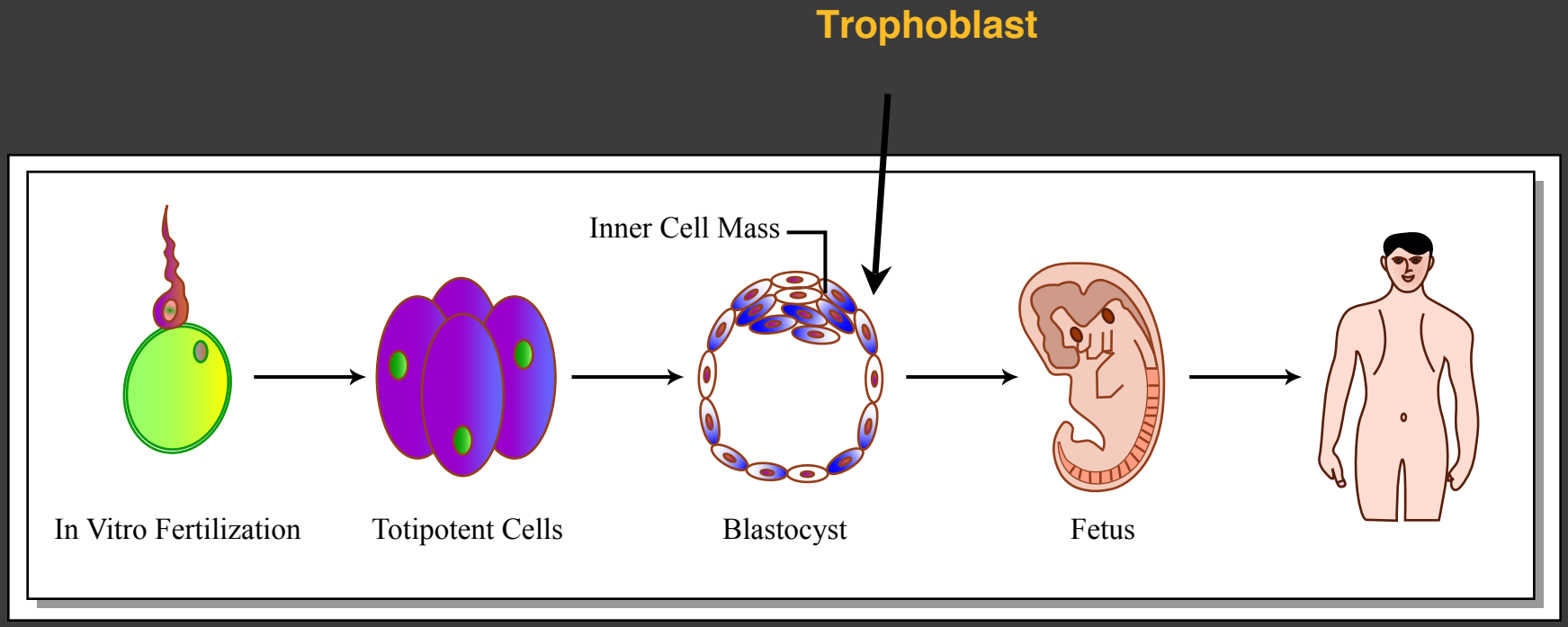
Steele Laboratory
Massachusetts General Hospital
Harvard Medical School

1. Introduction: Stem cells and neovascularization
2. BMDCs involvement in neovascularization during tumor growth
3. BMDCs and the therapy of cancer

Embryonic stem cells
Vasculogenesis



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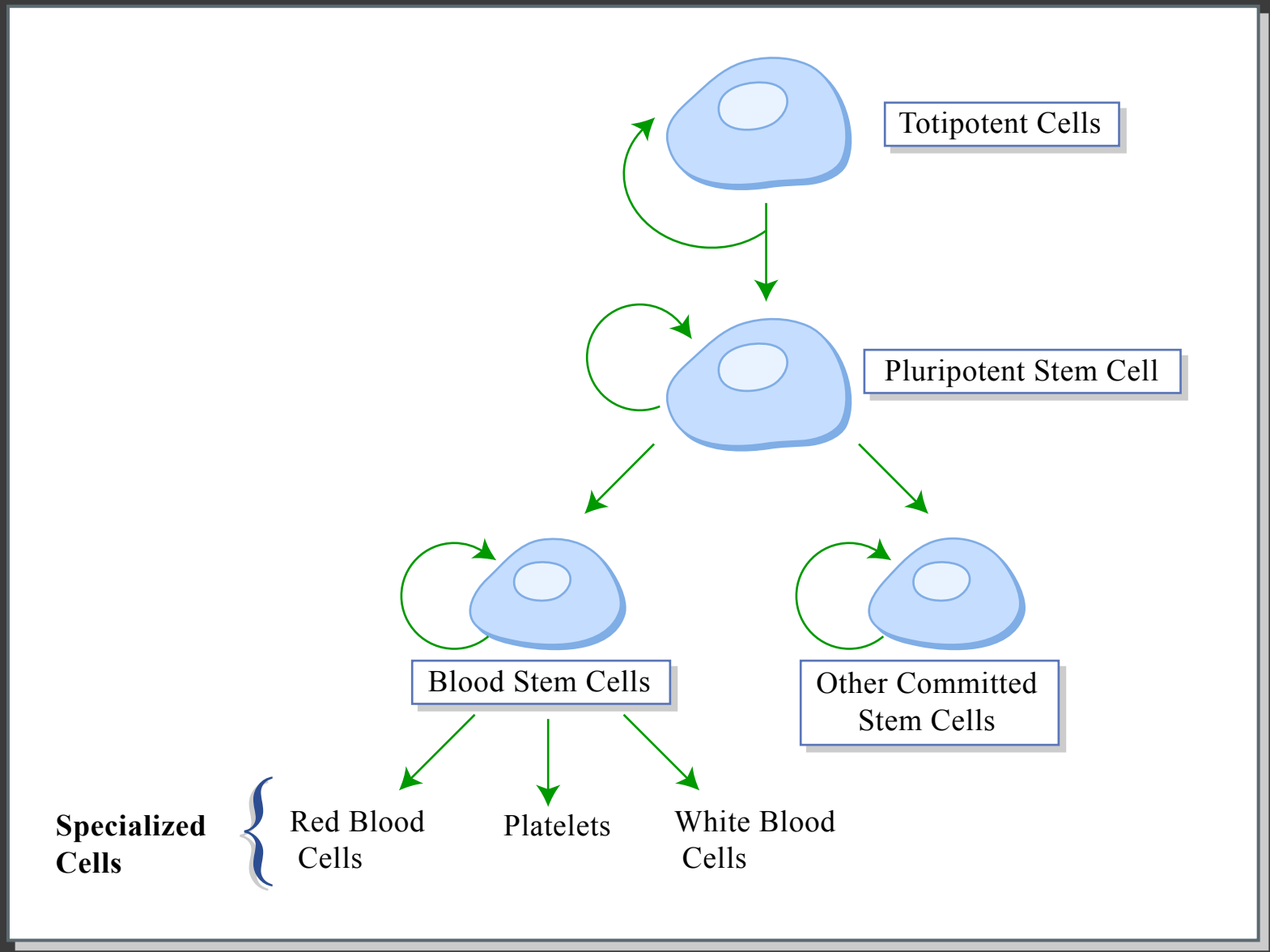
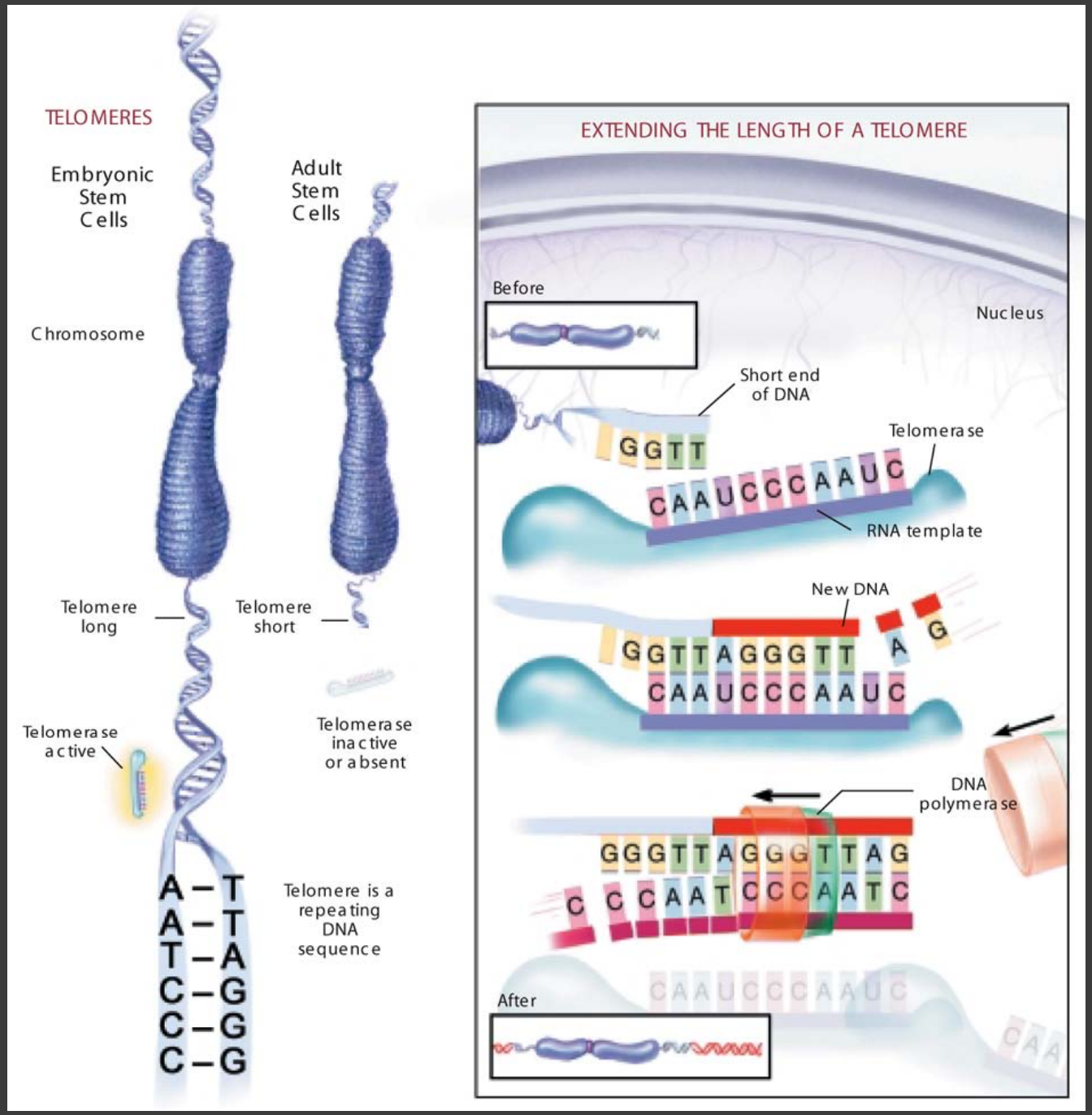


Figure by MIT OCW.



Courtesy of the National Institutes of Health.

Vasculogenesis in Embryo

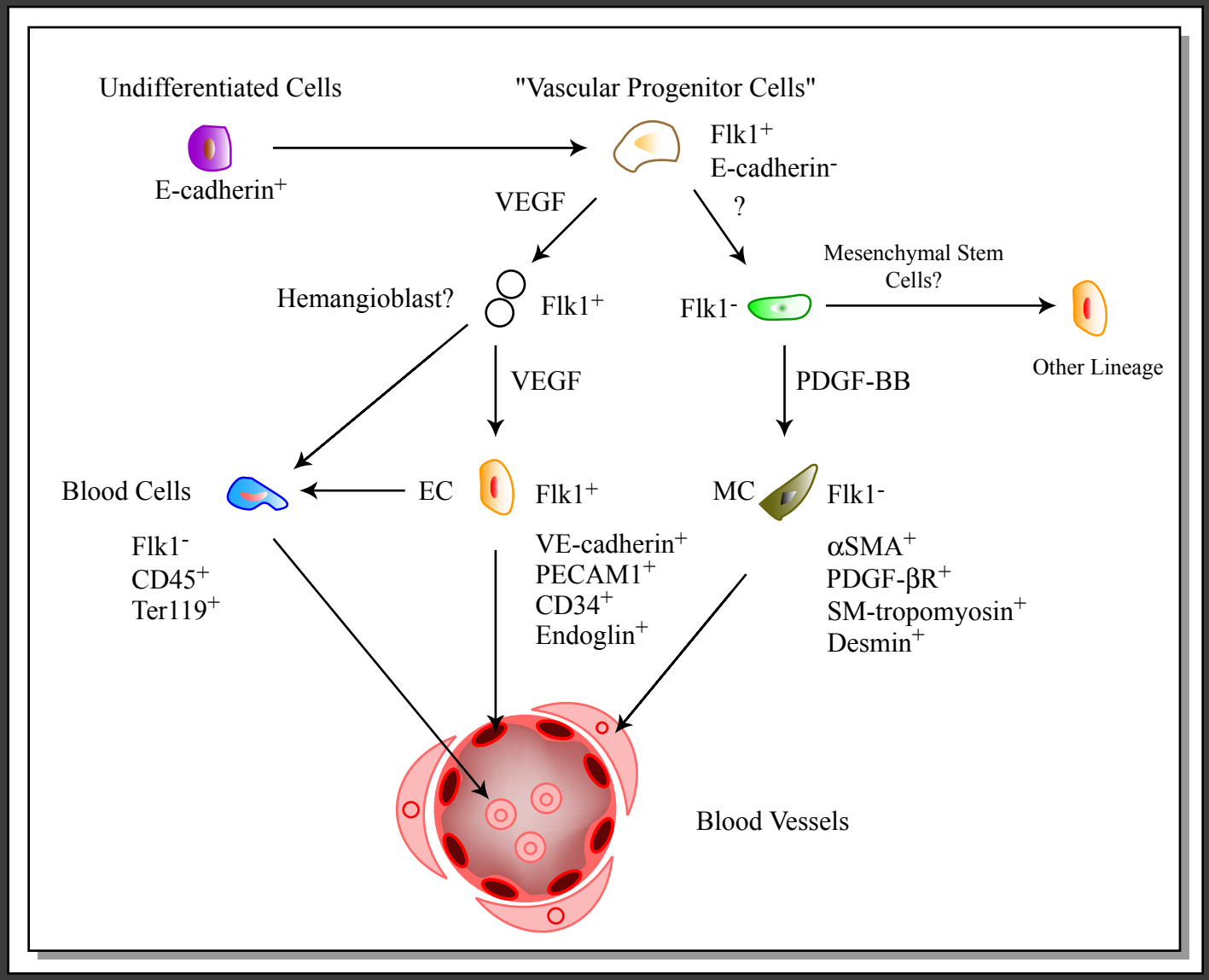


Figure by MIT OCW. After Yamashita et al., 2000.

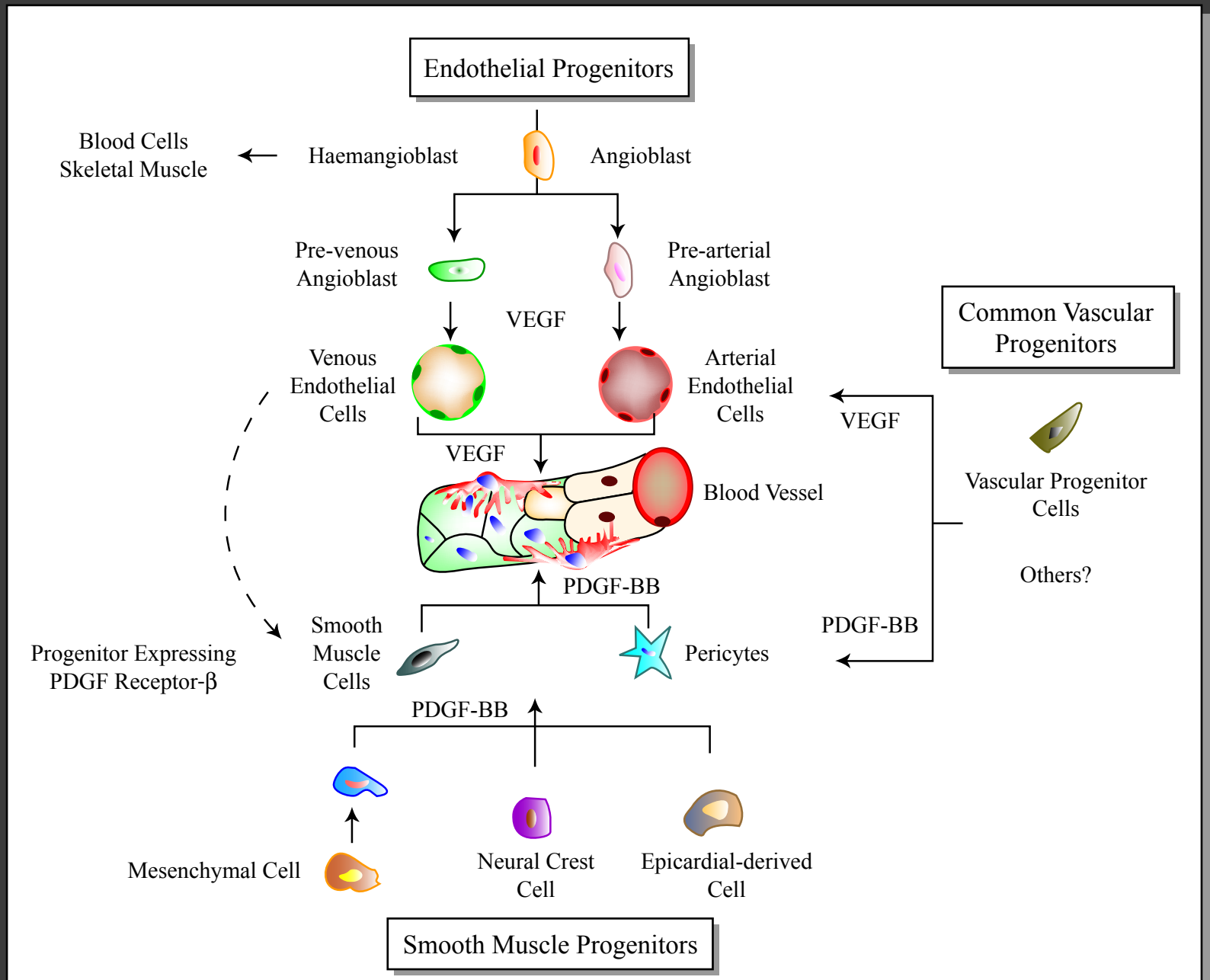


Figure by MIT OCW. After Carmeliet, 2001.

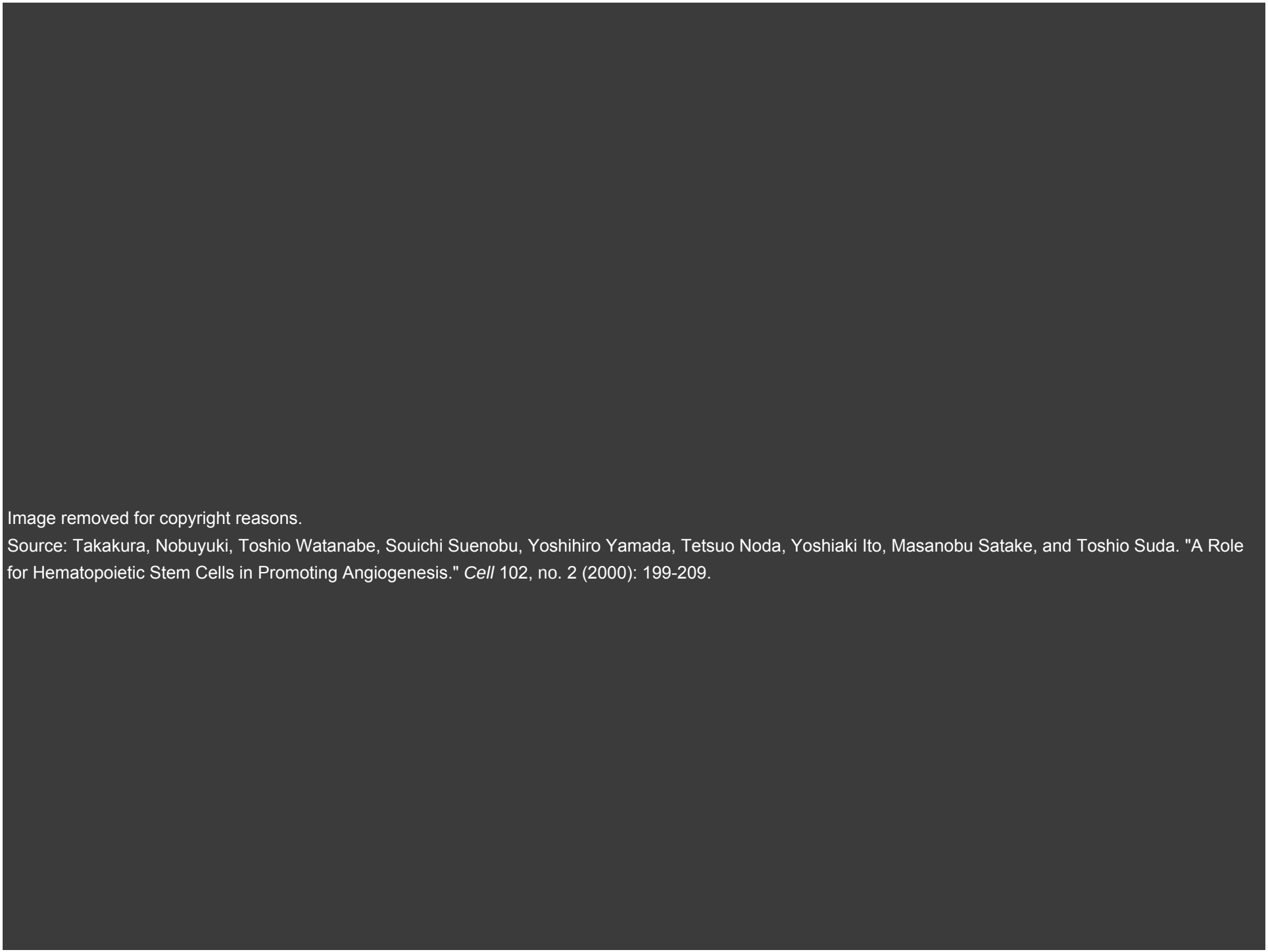


Image removed for copyright reasons.
Source: Takakura, Nobuyuki, Toshio Watanabe, Souichi Suenobu, Yoshihiro Yamada, Tetsuo Noda, Yoshiaki Ito, Masanobu Satake, and Toshio Suda. "A Role for Hematopoietic Stem Cells in Promoting Angiogenesis." *Cell* 102, no. 2 (2000): 199-209.

- Adult stem cells
- Vasculogenesis

Adult Hematopoiesis

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Stem Cell Sorting

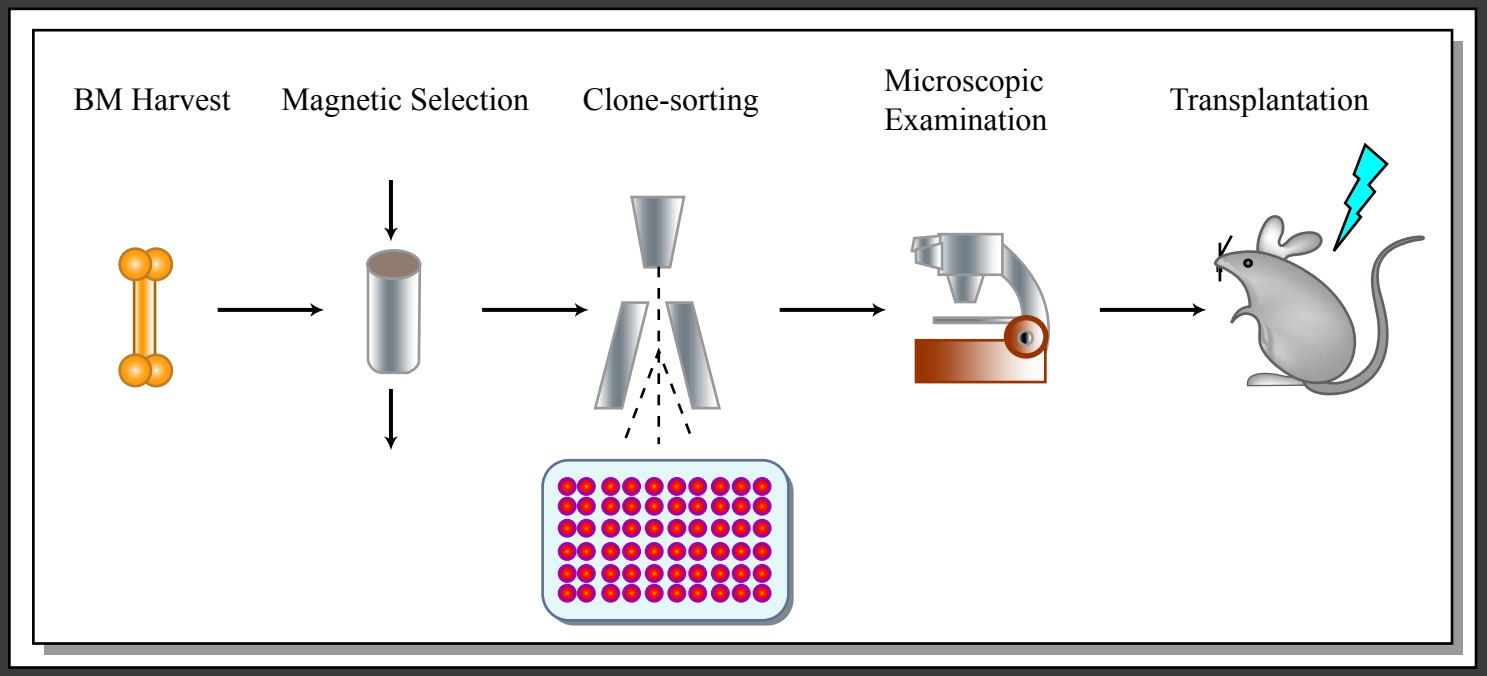


Figure by MIT OCW

(CD3, CD4, CD8, CD45, Gr-1, Mac-1, B220, and TER-119)

Hematopietic stem cells assays

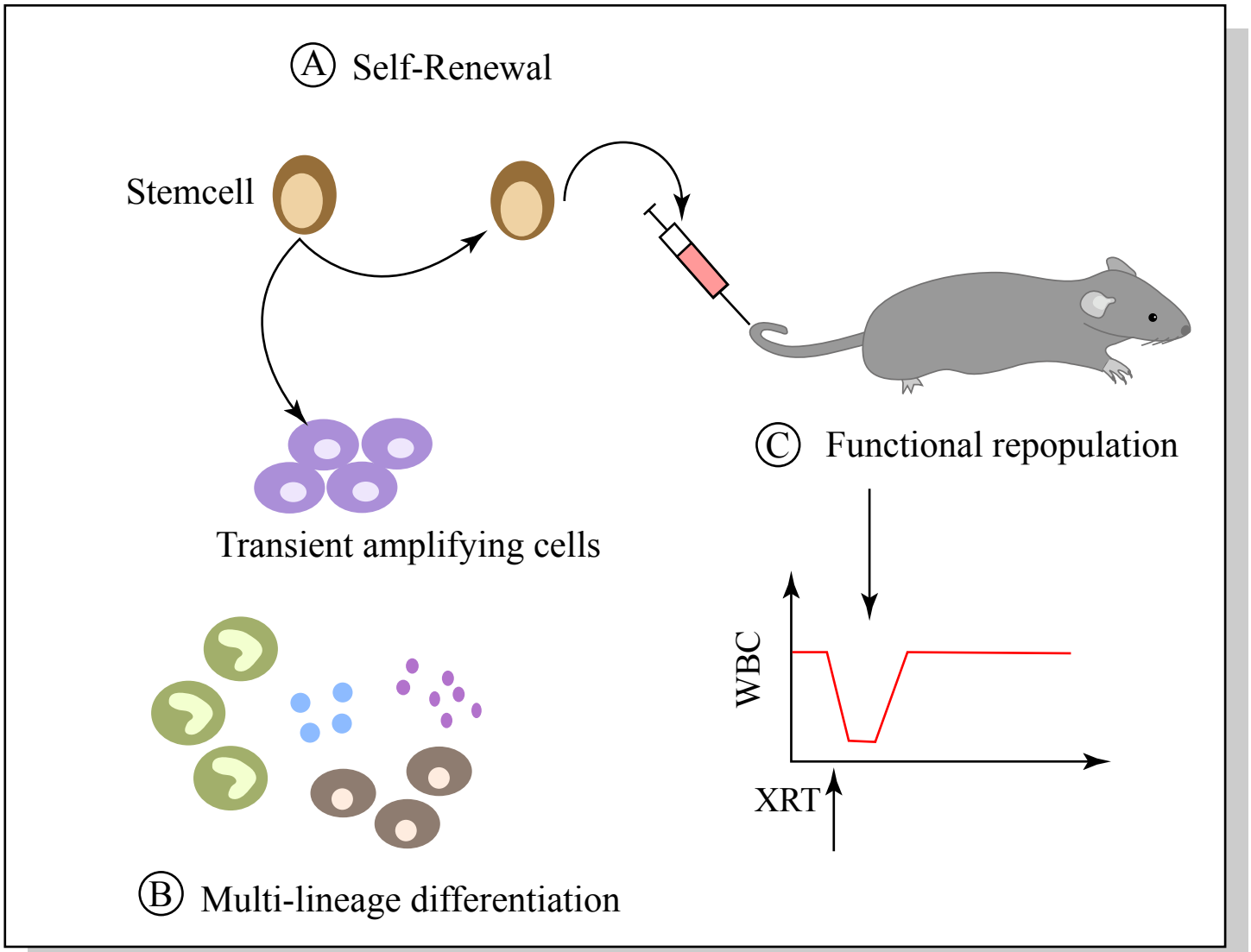


Figure by MIT OCW.

Tracking bone marrow progeny

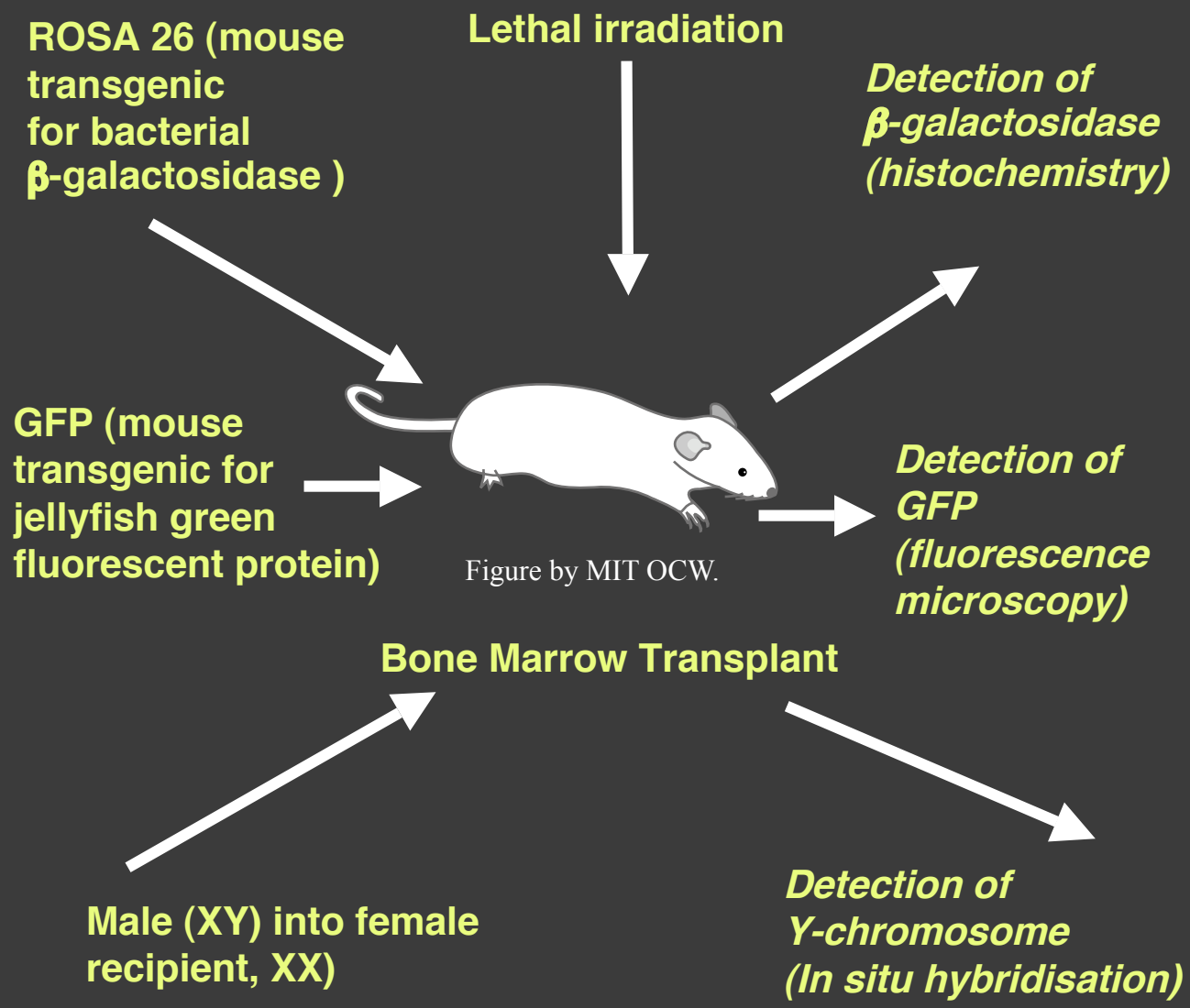


Figure by MIT OCW.

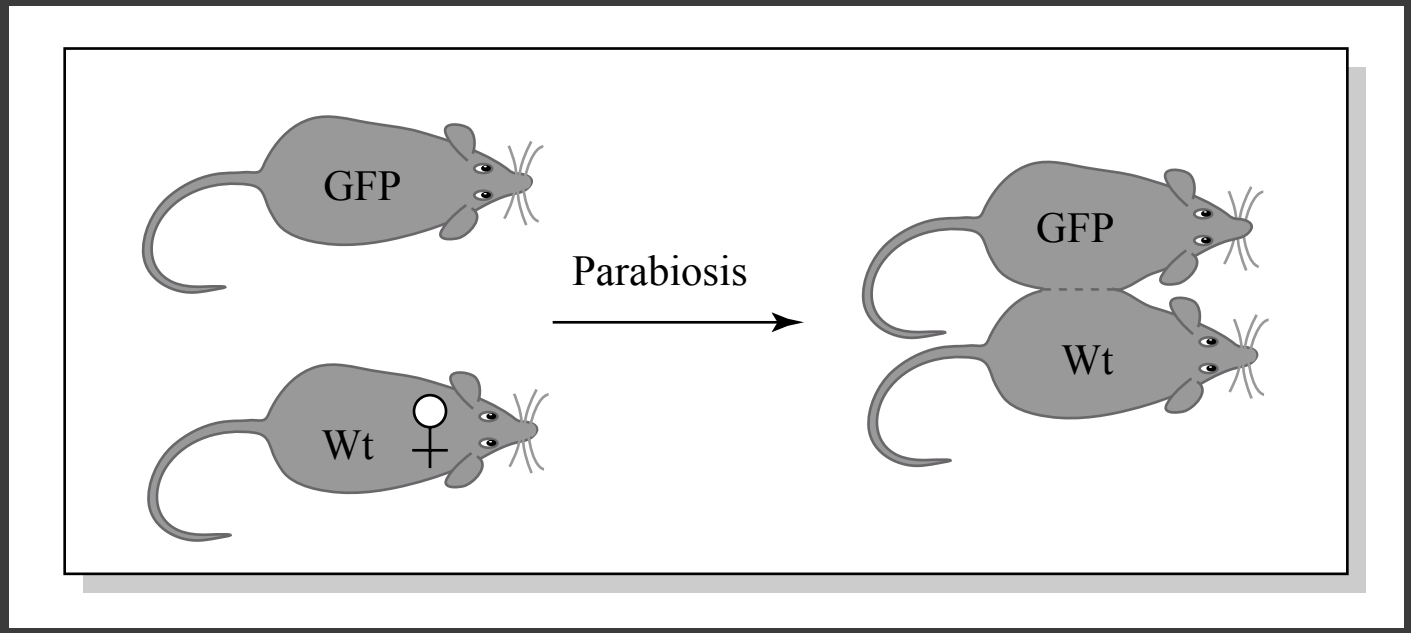


Figure by MIT OCW

Bone Marrow hosts multipotent stem cells

Haematopoietic Stem Cells

Mesenchymal Stem Cells

Endothelial Precursor Cells

Stem Cell Plasticity?

Images removed for copyright reasons.

Source: Krause, D., and N. Theise, et al. "Multi-Organ, Multi-Lineage Engraftment by a Single Bone Marrow-Derived Stem Cell." *Cell* 105, no. 3 (2001): 369-377.

Percent donor engraftment of nonhematopoietic tissues 11 months post transplant

	Bronchi	Alveoli	Esoph	Stomach	Sm. Bowel	Large Bowel	Skin	Bile Duct
M1	3.6	14.8	0	0.5	0.3	0.2	2.6	0.4
M2	2.3	10.3	0.4	0.5	0.4	0.1	2.4	0
M3	3.5	18.7	2.2	0	0	0	1.2	0
M4	2.2	10.1	2.5	0.2	0.4	0.3	1.6	2.2
M5	0	9	0.5	0.4	1.6	0	2.7	0
Mean +/- SD	2.32 +/- 1.45	12.58 +/- 4.07	1.12 +/- 1.14	0.32 +/- 0.21	0.54 +/- 0.61	0.12 +/- 0.13	2.1 +/- 0.66	0.52 +/- 0.95
Corr.*	3.74	20.30	1.81	0.52	0.87	0.19	3.39	0.84

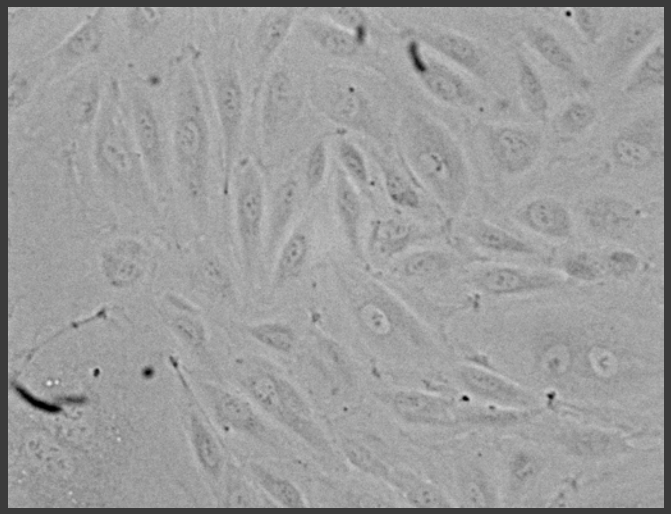
Figure by MIT OCW.

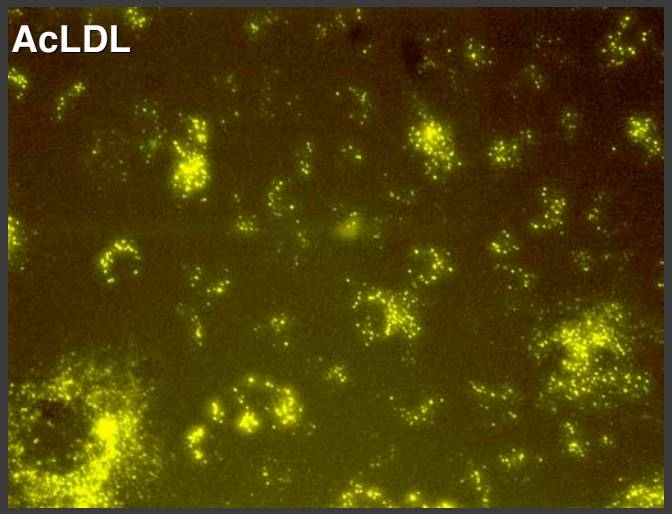
What is the evidence for BMDC vasculogenesis in tumors?

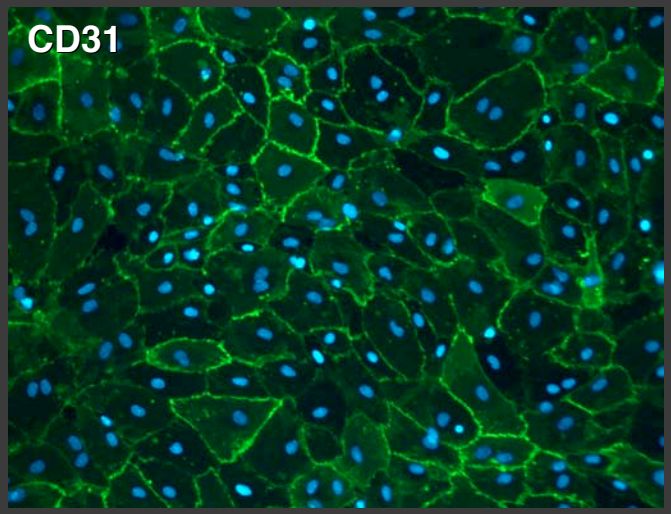
Putative endothelial progenitor cells

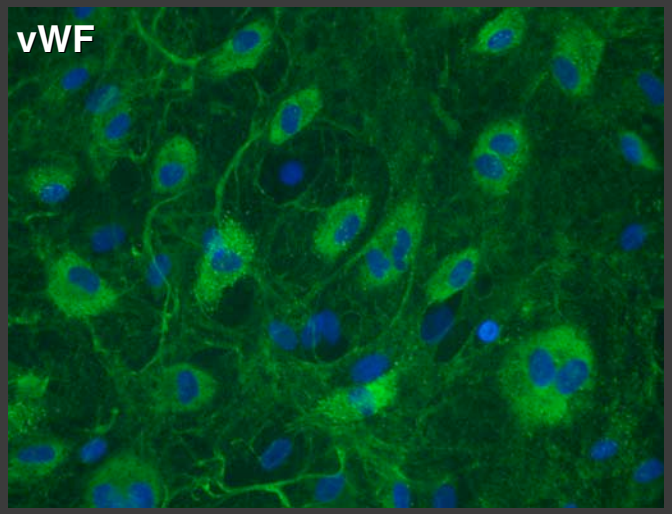
Images removed for copyright reasons.
See: Fig. 1 and 2 in Asahara, Takayuki, Toyoaki Murohara, Alison Sullivan, Marcy Silver, Rien van der Zee, Li Tong, Bernhard Witzenbichler, Gina Schatteman, and M. Isner Jeffrey. "Isolation of Putative Progenitor Endothelial Cells for Angiogenesis." *Science* 275 (1997): 964-966.

Human peripheral blood mononuclear cells



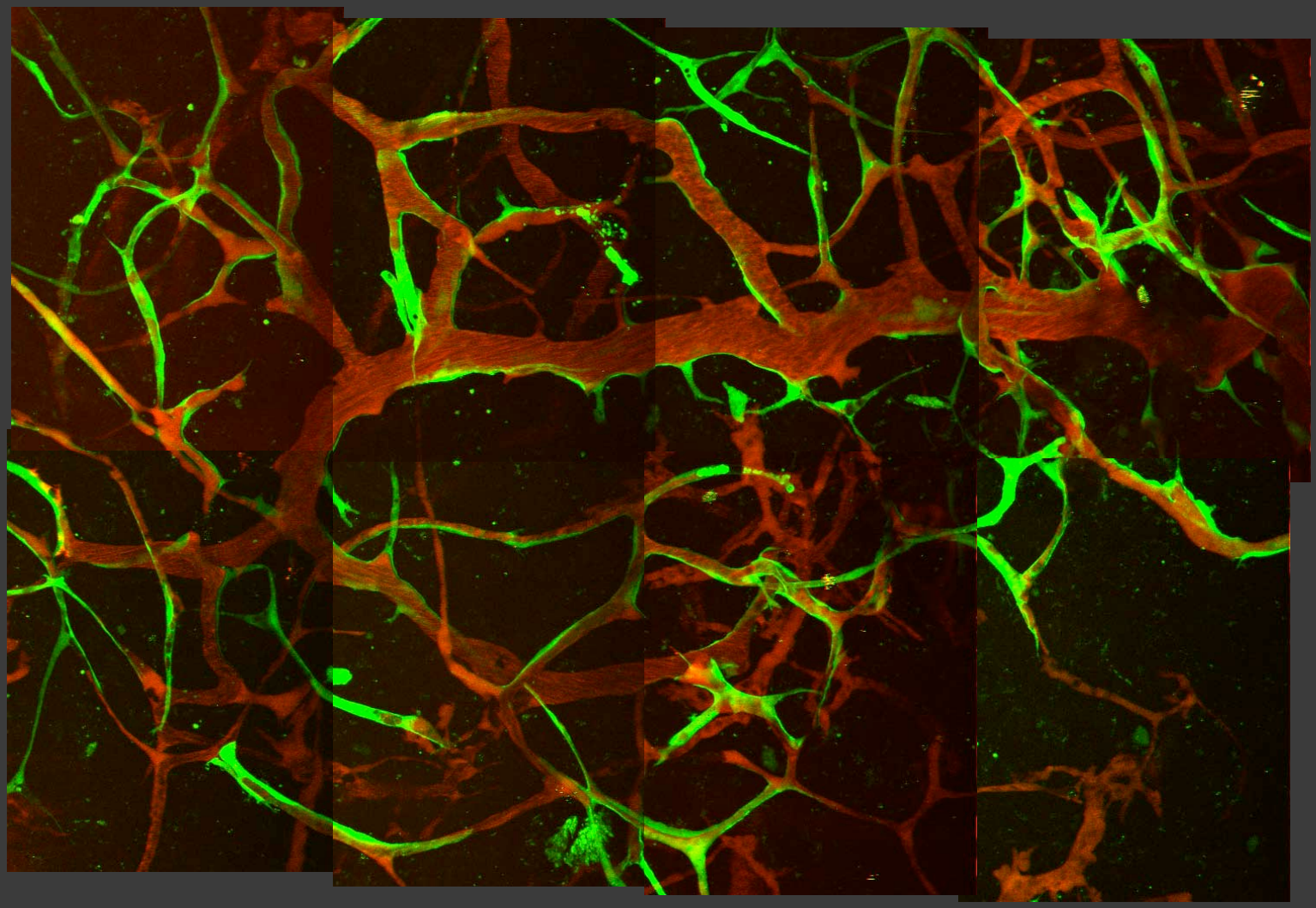






Reference: Au, *unpublished* 2003

GFP-HPBMC in a collagen gel w/10T1/2 pericyte precursors- 3 weeks



Reference: Koike *et al.*, *Nature 2004*; Au, *unpublished*

BMDC rescue angiogenesis deficiency in $Id1^{-/-}Id3^{+/-}$ mice

Images removed for copyright reasons.

Source: Fig. 3 and 4 from Lyden, D., et al. "Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth." *Nature Medicine* 7 (2001): 1194 - 1201.

Gr+CD11b+ BMDCs directly promote tumor angiogenesis

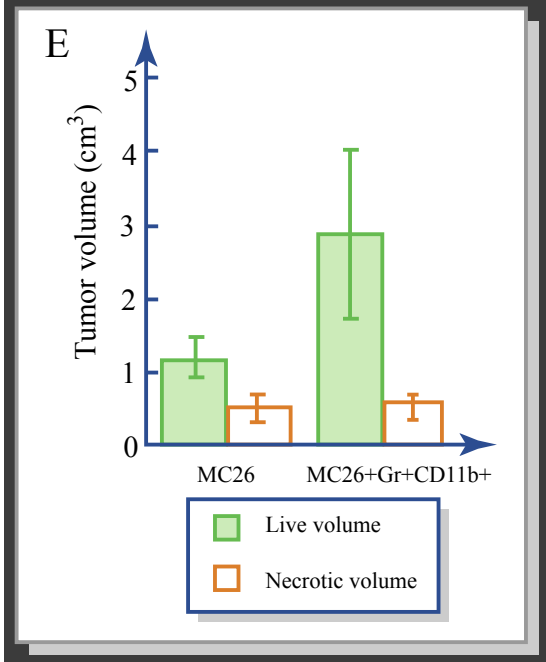


Figure by MIT OCW.
After Yang et al., 2004.

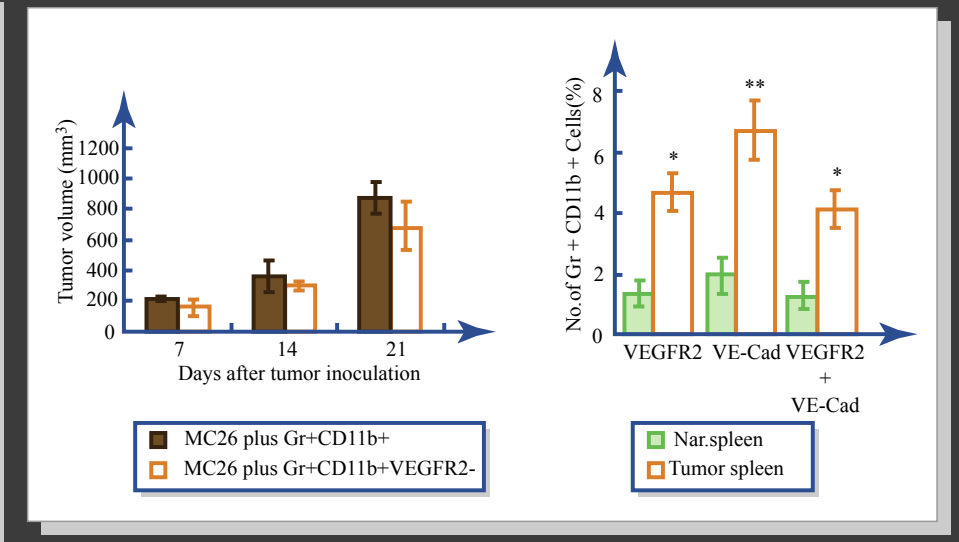


Figure by MIT OCW.
After Yang et al., 2004.

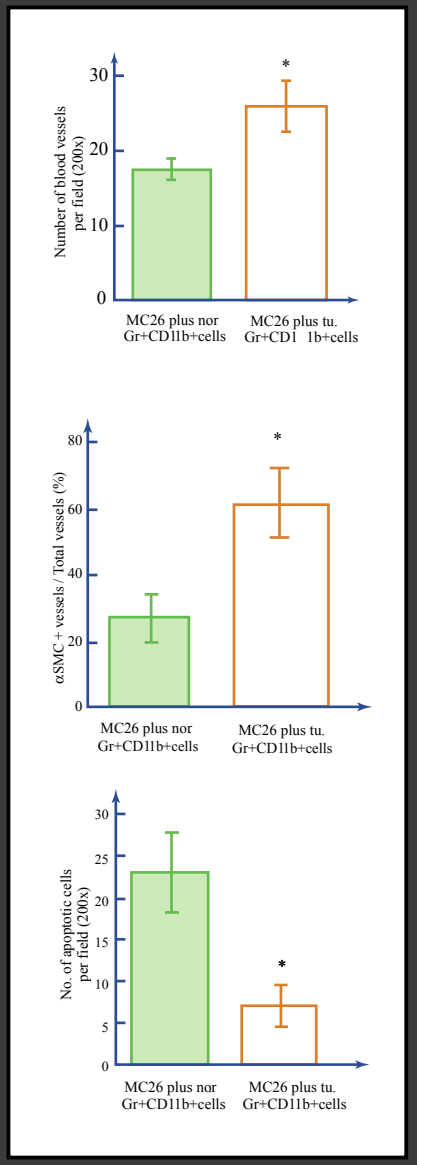


Figure by MIT OCW.
After Yang et al., 2004.

Endothelial Cell Precursors

Vasculogenesis in adults - Asahara T., Isner M.J., et al.

- putative endothelial progenitor cell (EPC, angioblast), $CD34^+$

Postnatal vasculogenesis - Rafii S., et al.

- circulating endothelial progenitors (CEP), $Flk\text{-}1^+/AC133^+$

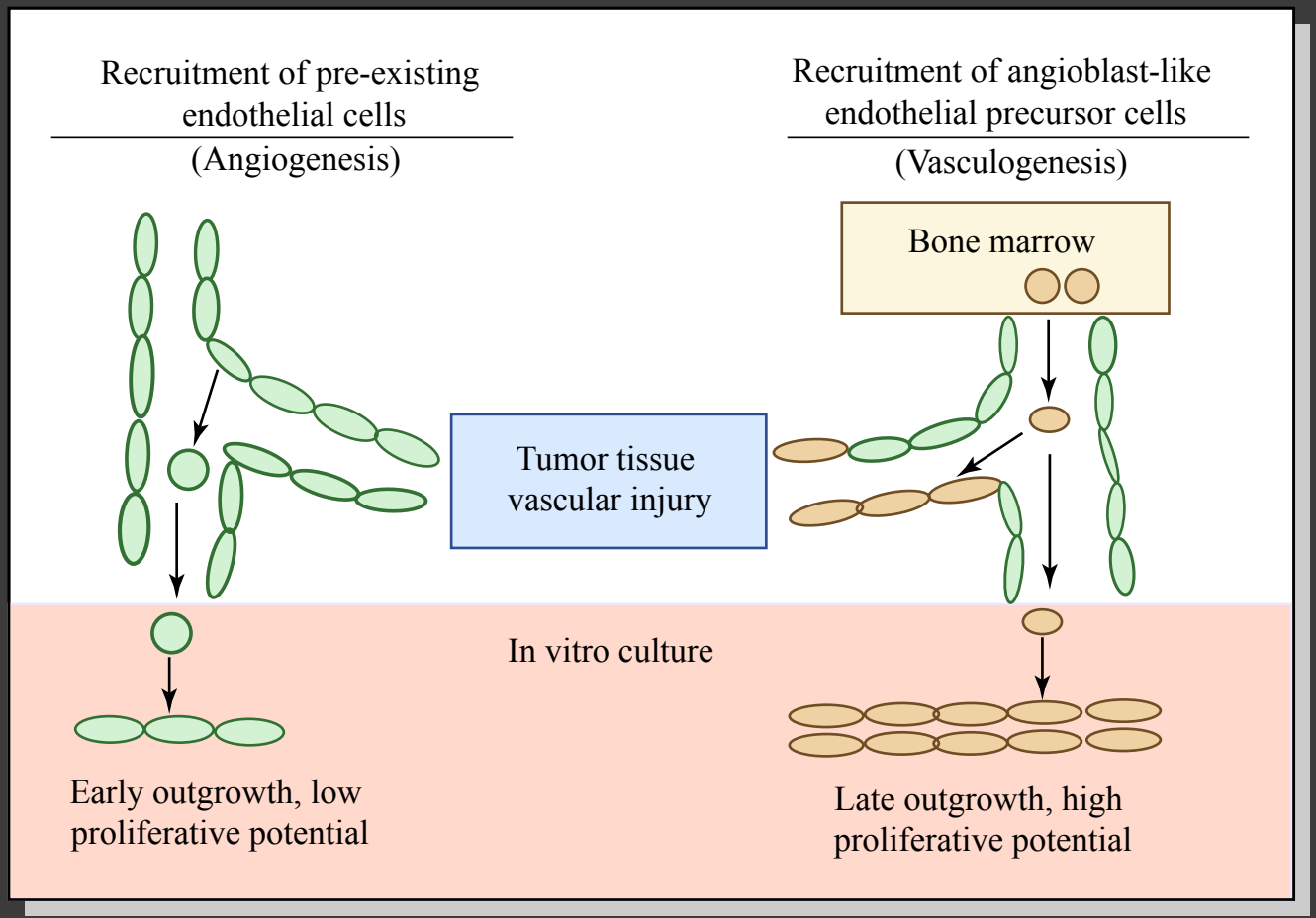


Figure by MIT OCW. After Rafii, 2002.

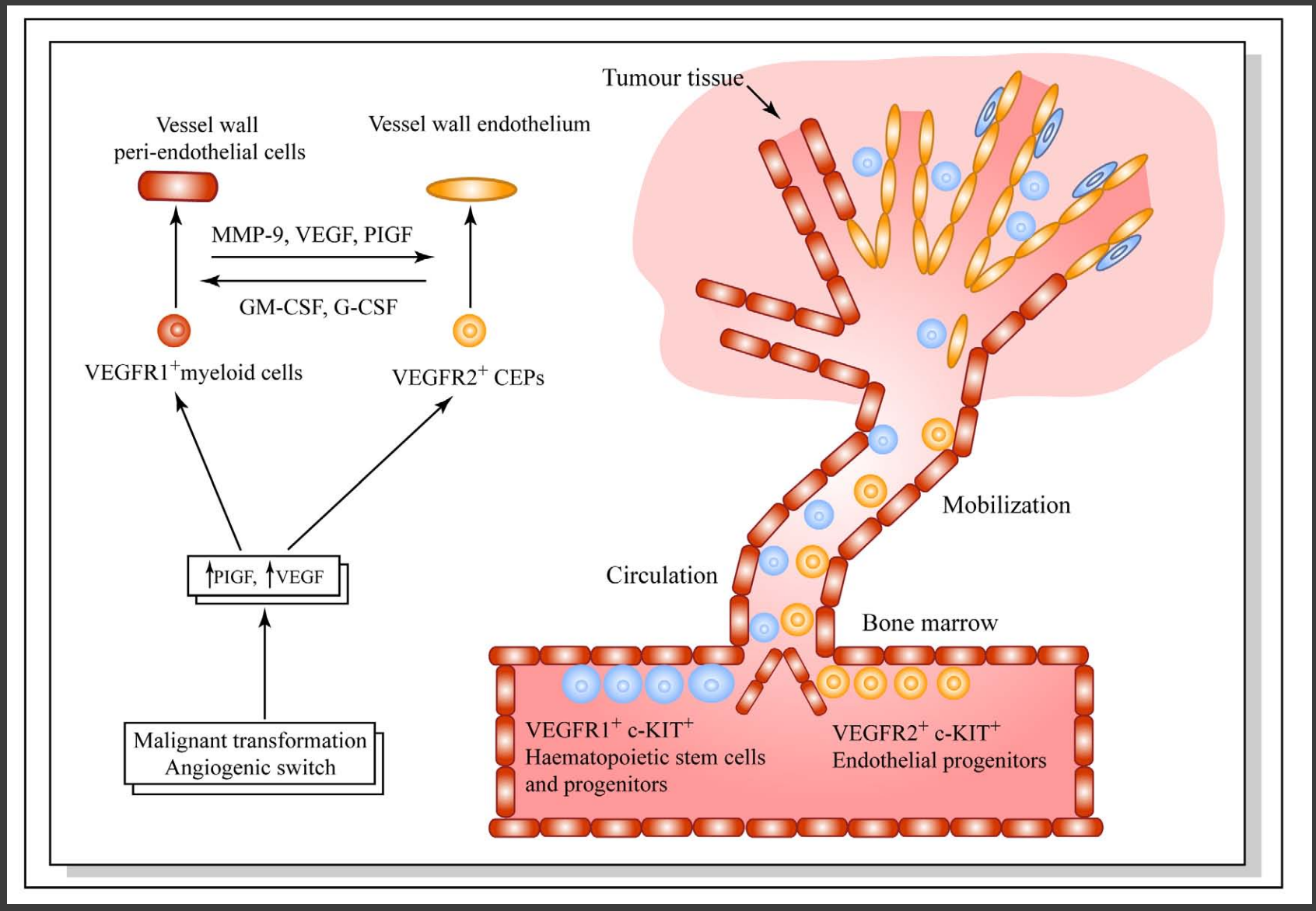


Figure by MIT OCW.

Source: Wade, Nicholas. "Scientists Link Tumors to Bone Marrow Cells." *The New York Times,* November 1, 2001: A22.

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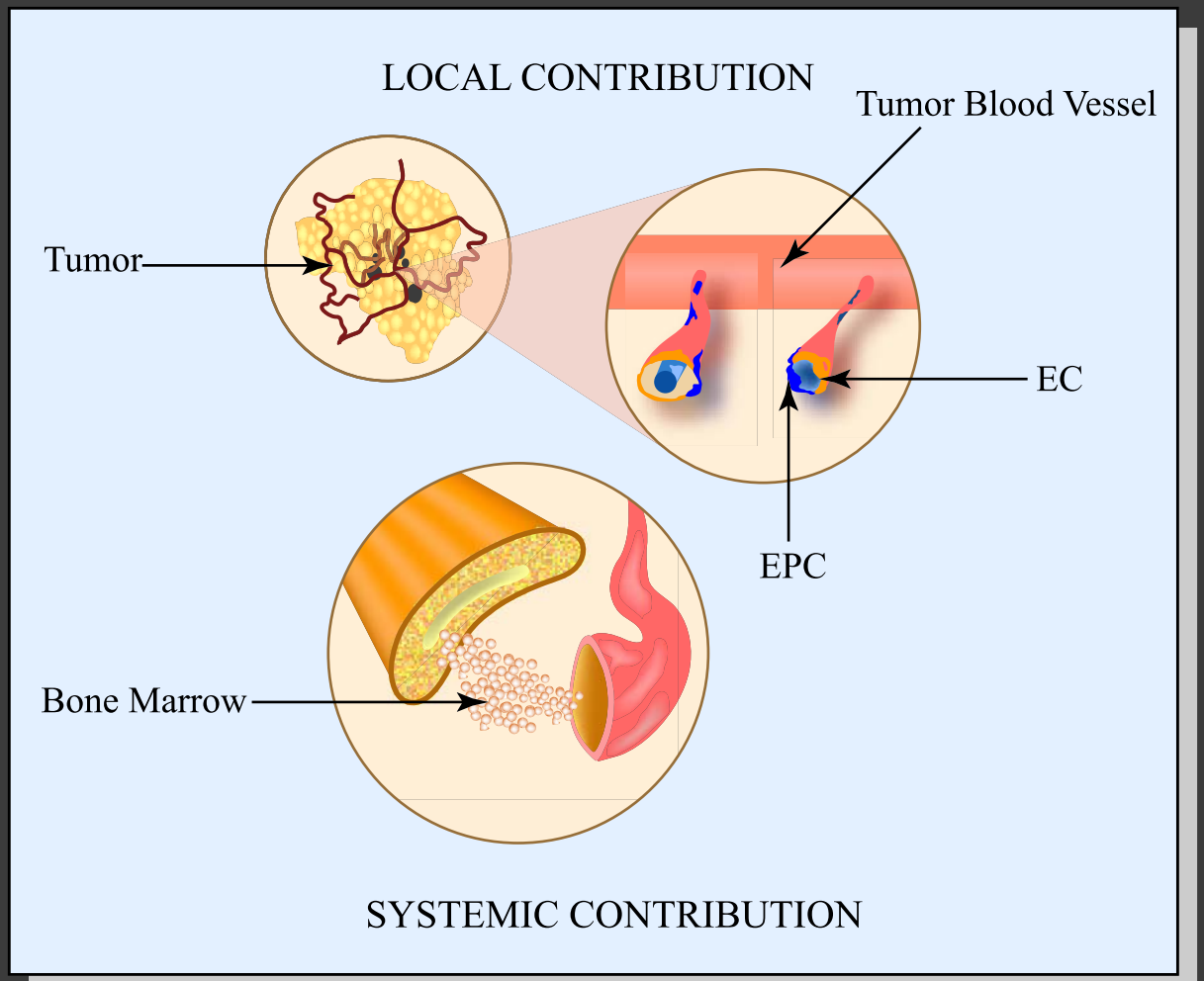


Figure by MIT OCW. After Stoll et al., 2003.

Bone marrow cell mobilization for neovascularization

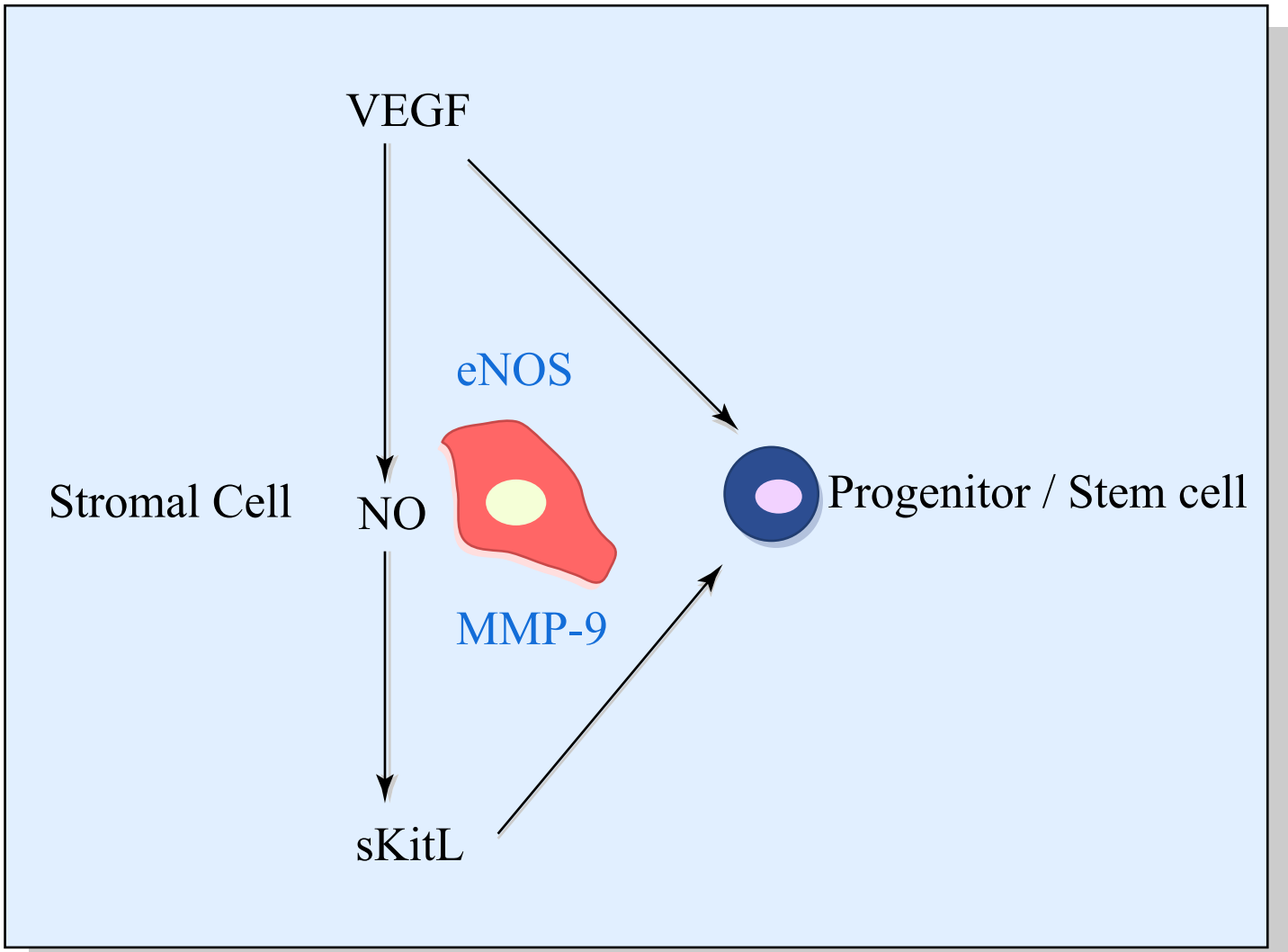


Figure by MIT OCW. After Duda et al., 2004.

The Weinberg hypothesis

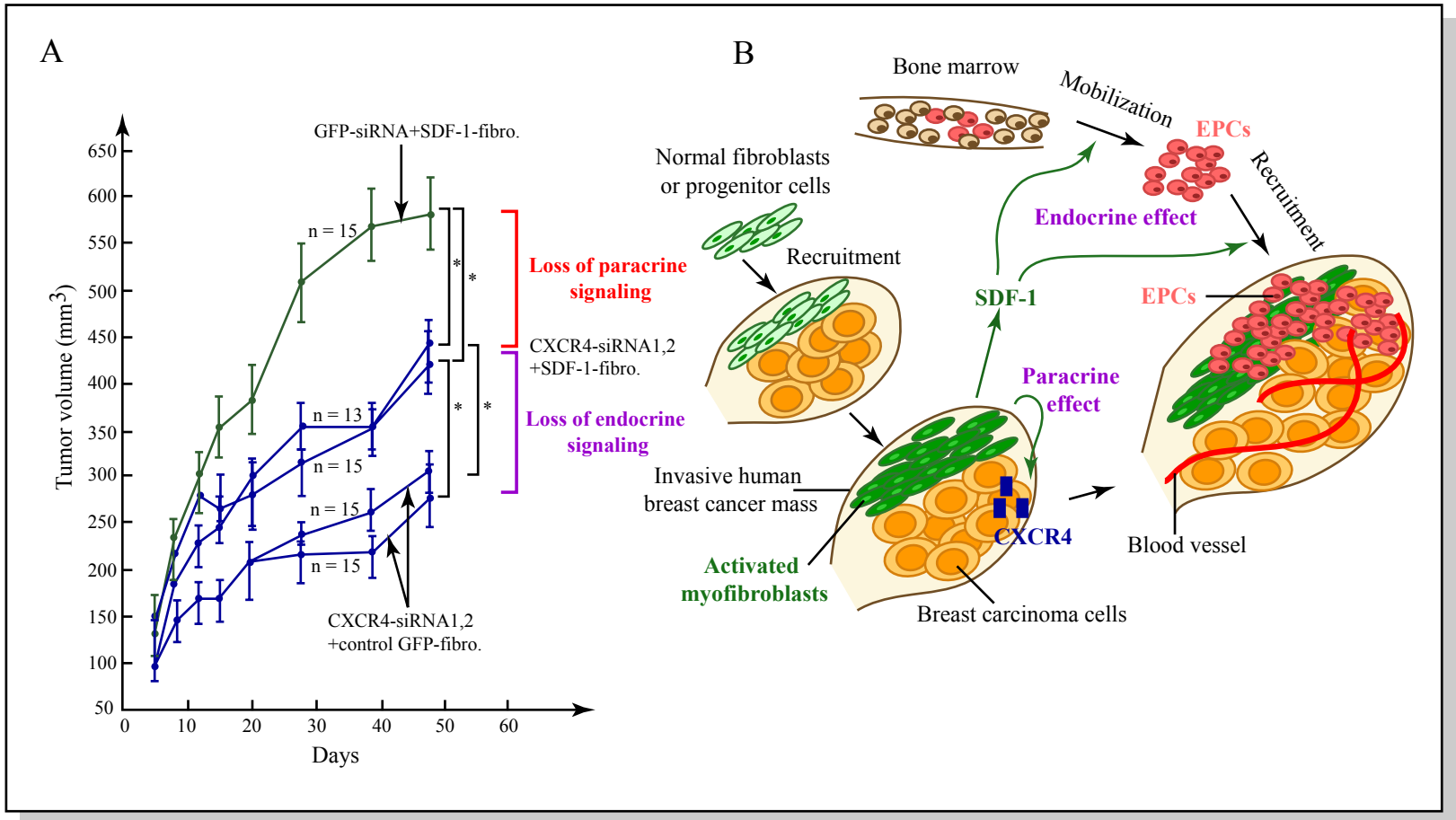


Figure by MIT OCW.

Sample	Patient sex	Donor sex	Number of vessels snalyzed	vWF^+ $CD45^-$ cells	BMDC	BMDC (percent)	BMDC (percent) normalized
Spindle cell sarcoma, head and neck	F	M	58	215	1	0.5	1.0
Hodgkin lymphoma	F	M	37	219	11	5.0	12.1
Mucoepidermoid carcinoma, submandibular	F	M	38	192	7	3.6	7.0
Thyroid carcinoma	F	M	95	752	4	0.5	1.1
Osteogenic carcinoma	M	F	36	293	3	1.0	4.1
Mucoepidermoid carcinoma, glossal	M	F	11	94	1	1.1	4.0

BMDC: bone marrow-derived cells.

Combined Immunofluorescence and FISH Data

Figure by MIT OCW. After Peters et al., 2005.

Image removed for copyright reason.

Please see Fig. 1 in Peters, B. A., L. A. Diaz, K. Polyak, L. Meszler, K. Romans, E. C. Guinan, E. C. Antinx, D. Myerson, S. R. Hamilton, B. Vogelstein, K. W. Kinzler, and C. Lengauer. "Contribution of bone marrow-derived endothelial cells to human tumor vasculature." *Nature Medicine* 11 (2005): 261-262.

Tie2-expressing myeloid cells

Images removed for copyright reasons.

Source: Palma, Michele De, Mary Anna Venneri, Rossella Galli, Lucia Sergi Sergi, S. Politi Letterio, Sampaolesi Maurilio, and Luigi Naldini. "Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors." *Cancer Cell* 8 (2005): 211-226.

No detectable BMDC in tumor endothelium

Images removed for copyright reasons. See: Fig. 6 in Gothert, J. R. et al. "Genetically tagging endothelial cells in vivo: bone marrow-derived cells do not contribute to tumor endothelium." *Blood* 104 (2004): 1769-1777.

Garcia-Barros et al.
Sciencer, May 18

De Palma et al.
Nature Medicine, June

Acid sphingomyelinase$^{-/-}$

Tie 2p/e-GFP or PGKp
lentiviruses

BM Transplant

Tumor Transplant

MCA fibrosarcoma
B16 melanoma

TS/A mammary carcinoma
LLC
B16 melanoma

EPCs- critical for
tumor angiogenesis and growth

No EPCs
TEMs- critical for
tumor angiogenesis and growth

Bone marrow derived cell contribution to neovascularization

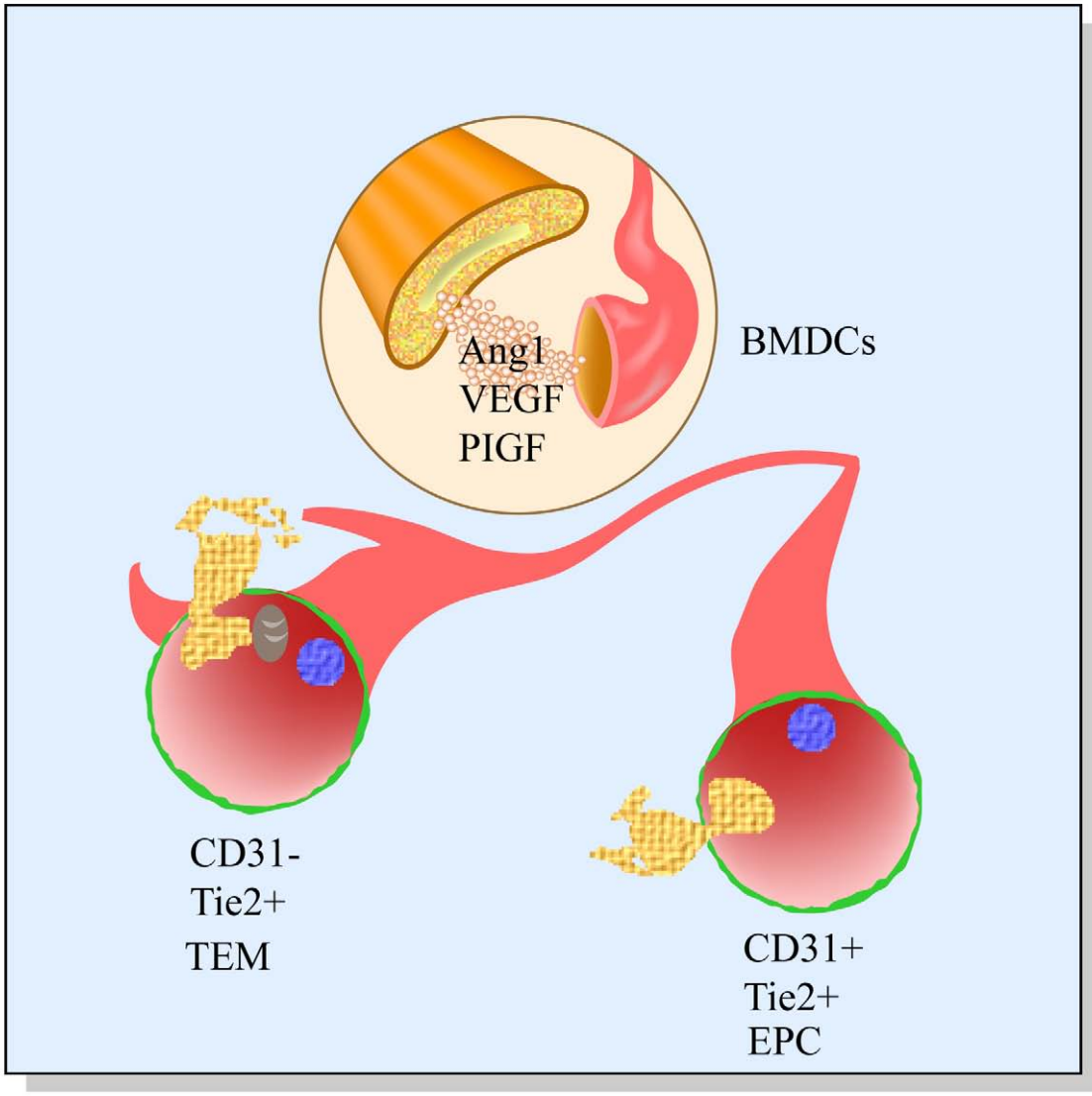


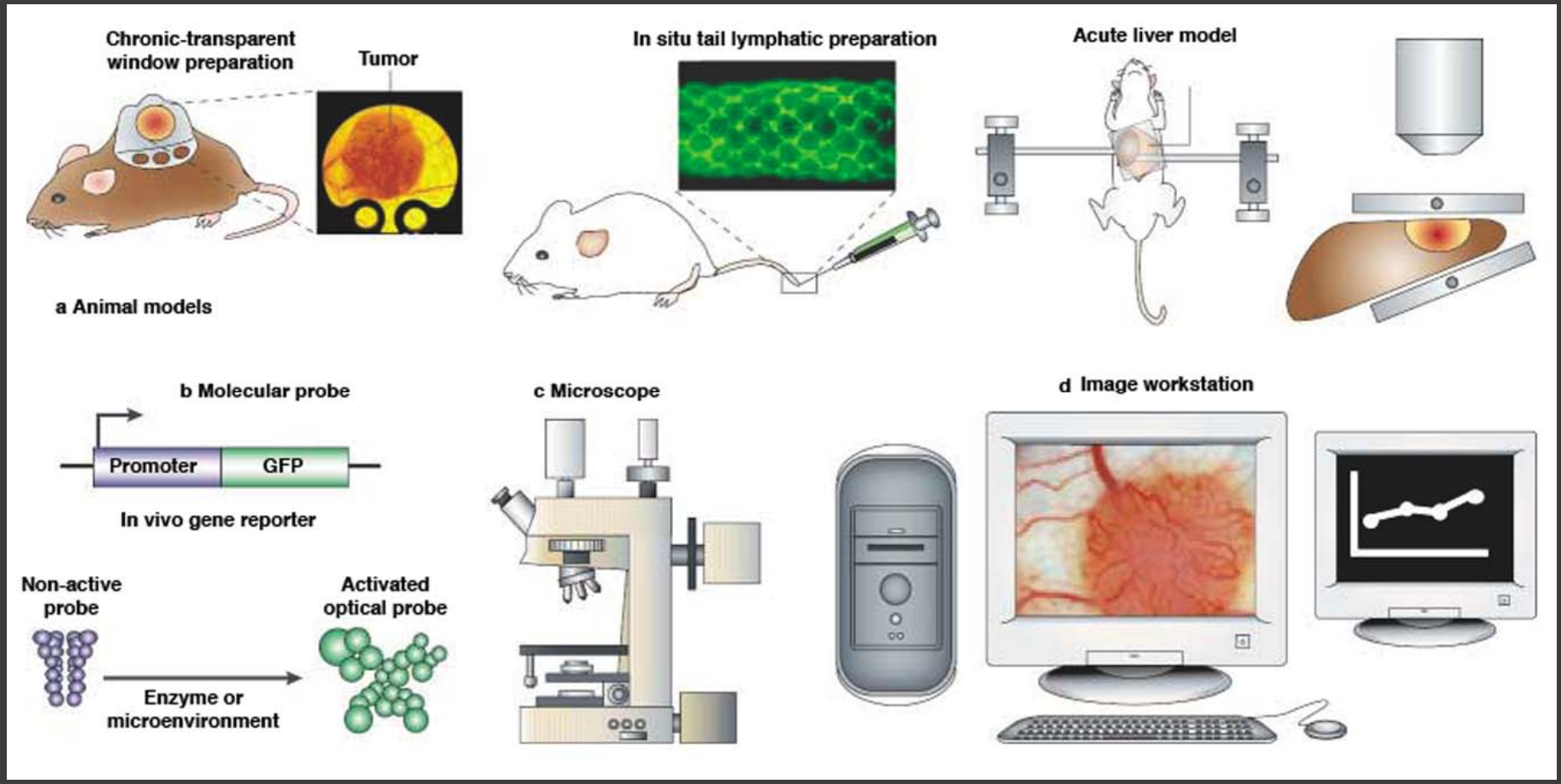
Figure by MIT OCW. After Jain and Duda, 2003.

Research problems

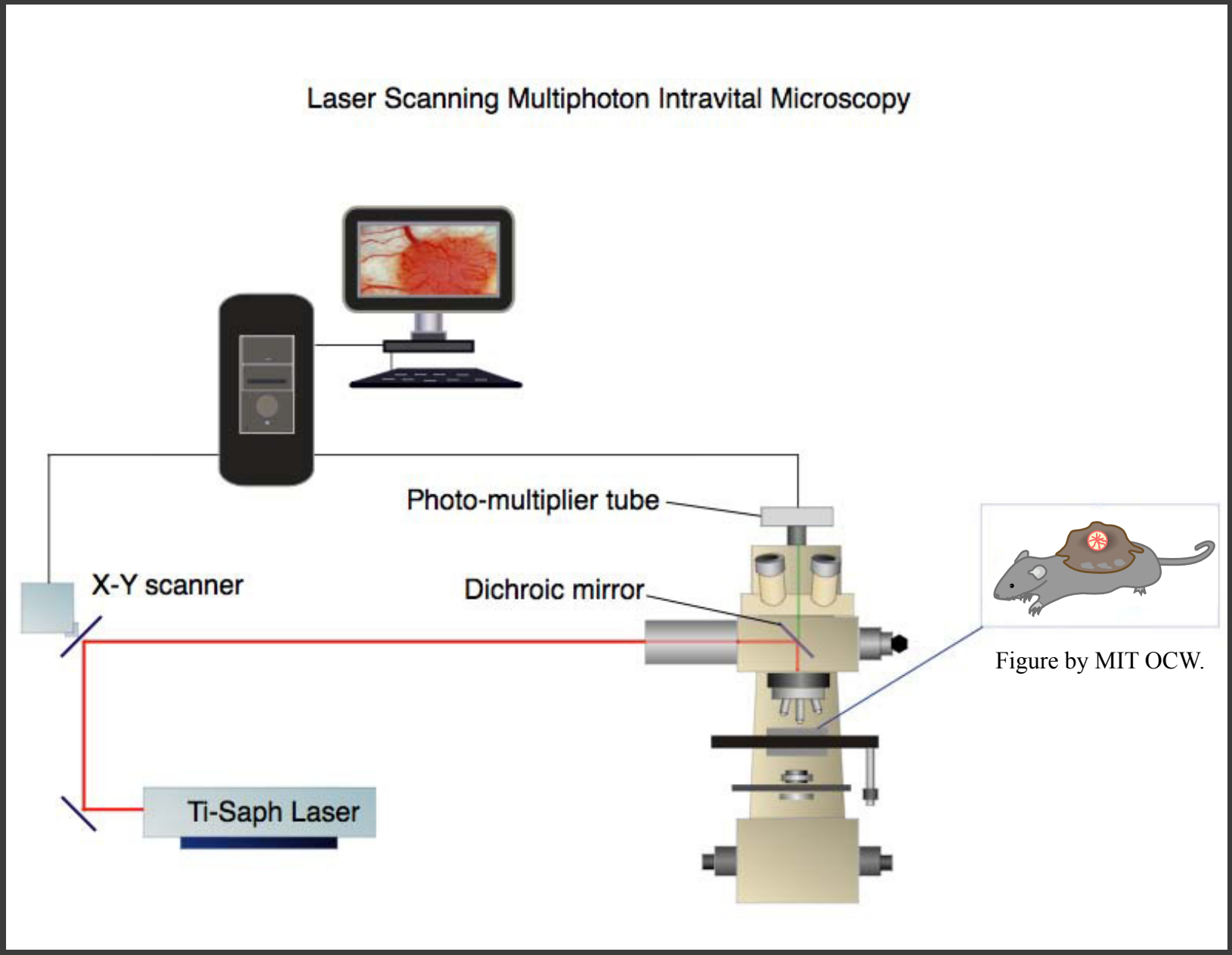
Bone marrow-derived cells infiltrate tumors and modulate neovascularization:
-endothelial precursors (eg, Rafii, Lyden & Benezra, Nat Med 2001, Science 2003)
- mezenchymal stromal precursors (eg, Direkze, Cancer Res 2004, Bergers, Nat Cell Biol 2005)
-hematopoietic precursors (eg, De Palma, *Nat Med* 03, Cancer Cell 2005, Rafii *Nat Med* 01)

BM cells are mobilized, home to tumors and contribute to neovascularization

Ionizing radiation kills cancer and normal cells, and BMDCs can rescue tumor growth (Fuks and colab., *Science* 01 & 03);
Is this a mechanism of relapse?



Courtesy of RK Jain, et al. Used with permission.



Courtesy of RK Jain, et al. Used with permission.

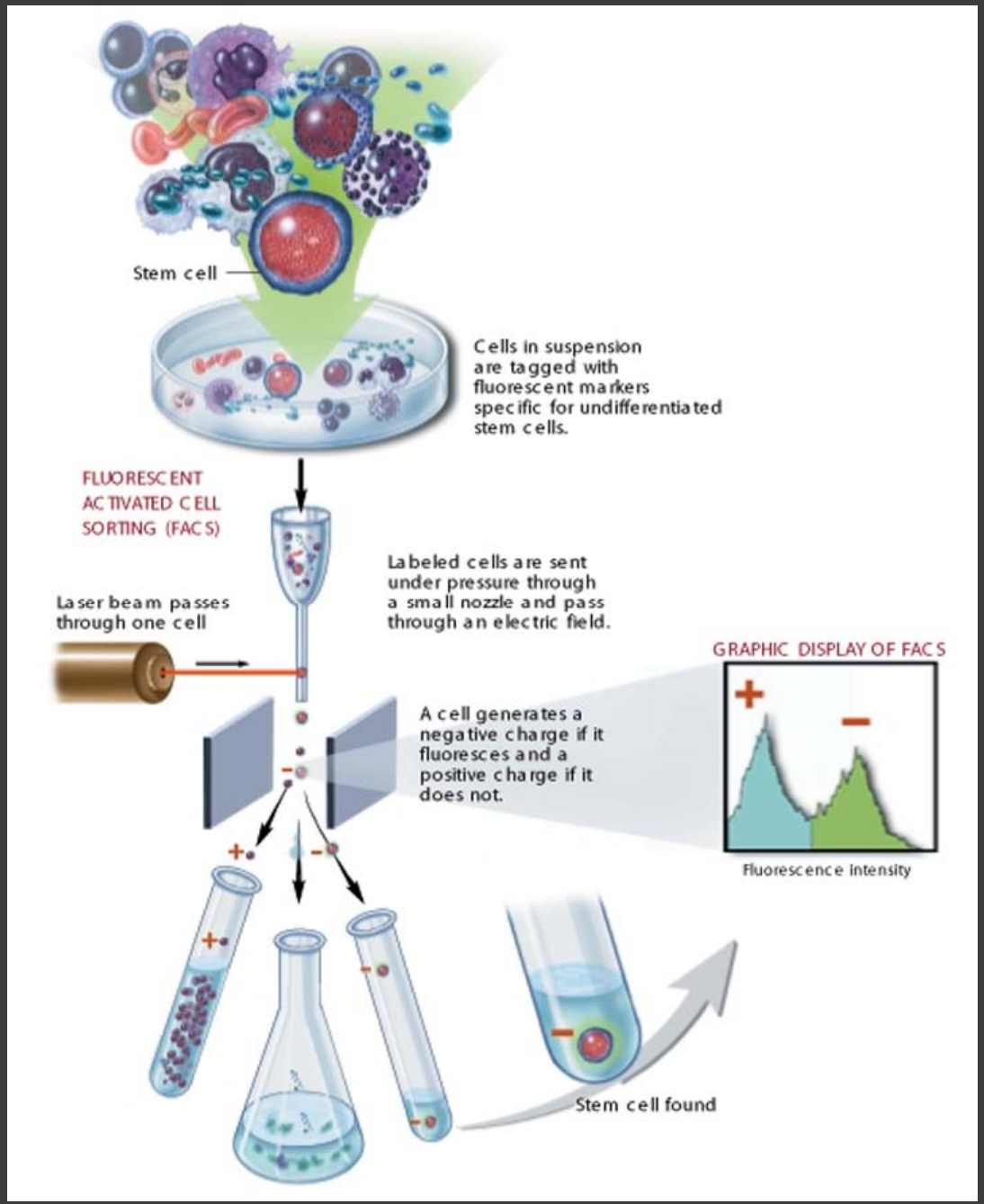


Image courtesty of NIH.

What is the evidence for BMDC incorporation as Perivascular cells in tumors?

Smooth muscle precursor cells in blood

Images removed for copyright reasons.

Source: Fig. 4 and 6 in Simper, D., P. G. Stalboerger, C. J. Panetta, S. Wang, and N. M. Caplice. "Smooth muscle progenitor cells in human blood." *Circulation* 106 (2002): 1199-1204.

Quantification of number and diameter of EPC-derived vessels

Tumor type	Implantation site	Percent GFP+ functional vessels	
		Actb-GFP/BMT (C57BL6)[1]	Tie2-GFP/BMT (FVB)[2]
Lung adenocarcinoma	Subcutaneous	< 1%[4]	4.5 ± 1.6[4]
Lung adenocarcinoma metastasis	Lung[3]	< 1%[5]	10.6 ± 3.6[5]
Melanoma	Subcutaneous	< 1%[4]	N/A[6]
Mammary carcinoma	Subcutaneous/ mammary fat pad	N/A	1.3 ± 0.1[7]
Mammary carcinoma metastasis	Brain	N/A	58.4 ± 8.4[7]
Spontaneous mammary carcinoma	–	N/A	20.7[8]
Spontaneous fibrosarcoma	–	N/A	3.2[8]
Spontaneous hemangiosarcoma	–	N/A	< 1%[8]
Spontaneous squamous cell carcinoma	–	N/A	5.3[8]

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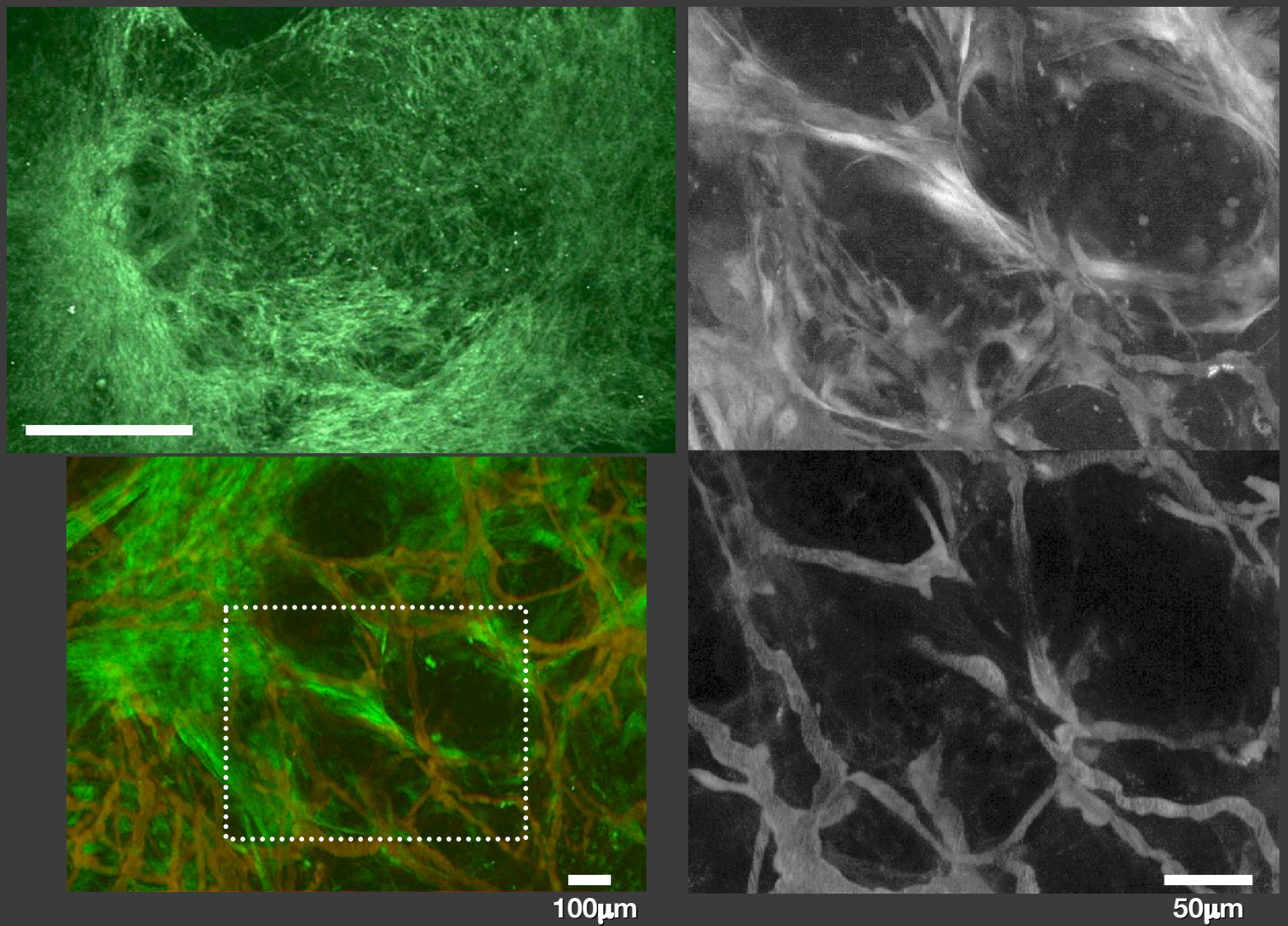
TG1-1 in CW (VEGF-GFP/FVB mouse, 2 weeks, rho dextran perfusion)



Reference: Fukumura et al., Cell 1998; Duda et al. Cancer Res 2004

αSMA staining in mammary carcinoma tissue in VEGF-GFP mouse

Image removed due to copyright reasons.

Reference: Tong et al., PhD Thesis 2005

Image removed for copyright reasons.

Bone marrow-derived stromal cells in mouse insulinoma after BMT

Perivascular cells originate from Sca1^{+} hematopoietic cells

Image removed for copyright reasons.

See Fig. 4 in Song, Steven, J. Ewald Andrew, William Stallcup, Zena Werb, and Gabriele Bergers. "PDGFRß+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival." *Nature Cell Biology* 7 (2005): 870-879.

Circulating VEGF-GFP+ cells in orthotopic mammary carcinoma-bearing immunodeficient mice

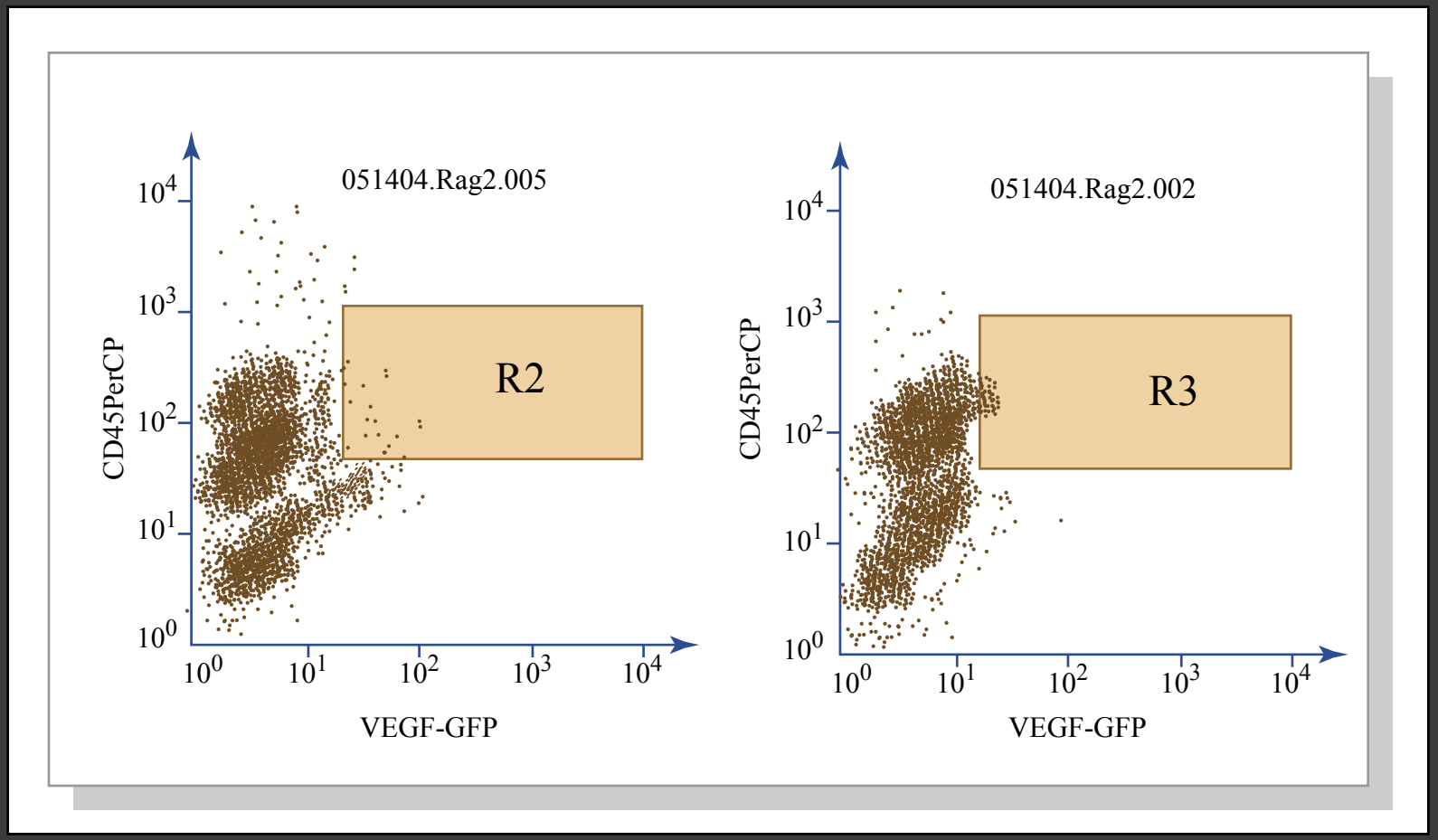


Figure by MIT OCW.

Tumor **Control**

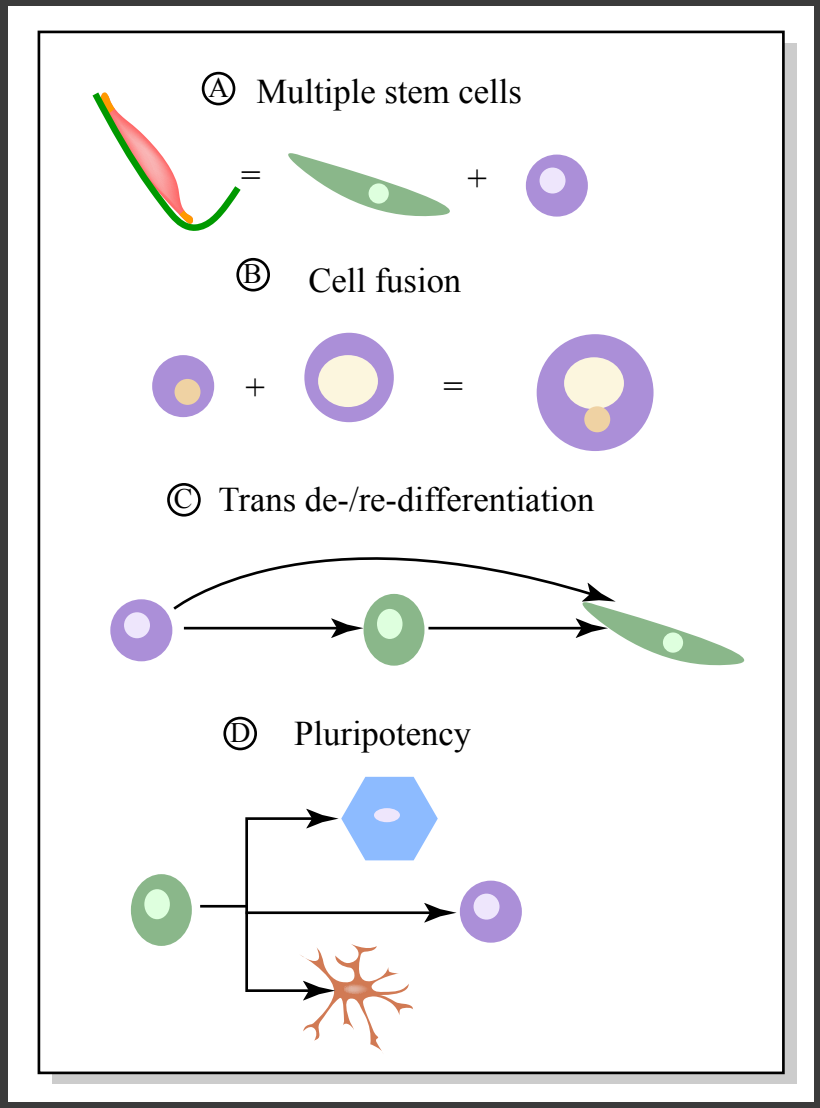


Figure by MIT OCW.

Images removed for copyright reasons.

See: Figs. 1, 4, 6 and 7 in Blau, H. M., T. R. Brazelton, and J. M. Weimann. "The Evolving Concept of a Stem Cell: Entity or Function?" *Cell* 105 (2001): 829-841.

Stem Cell- entity or function

MAPC, Verfaillie, Nature 2001

Images removed for copyright reasons.

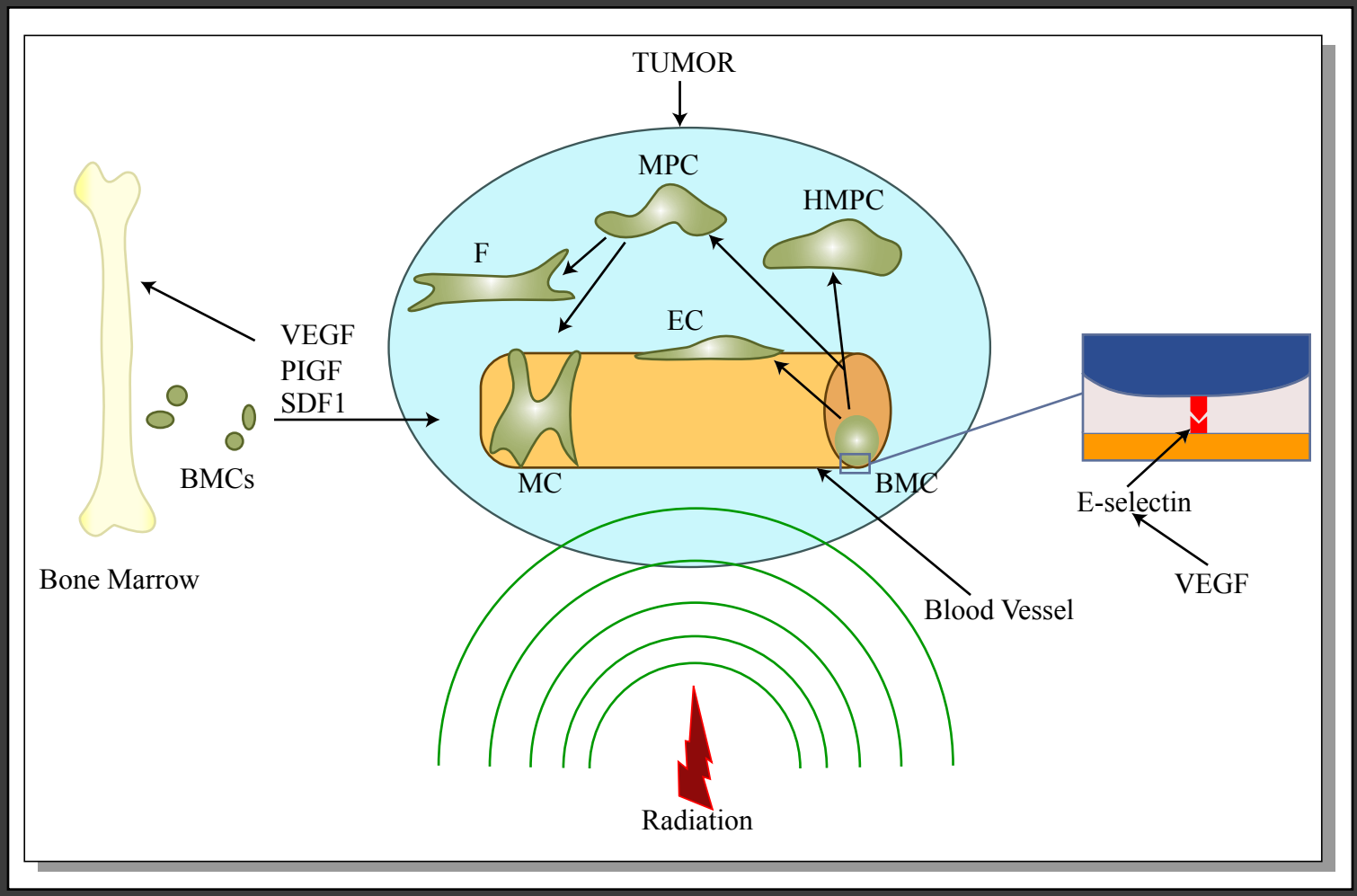


Figure by MIT OCW.