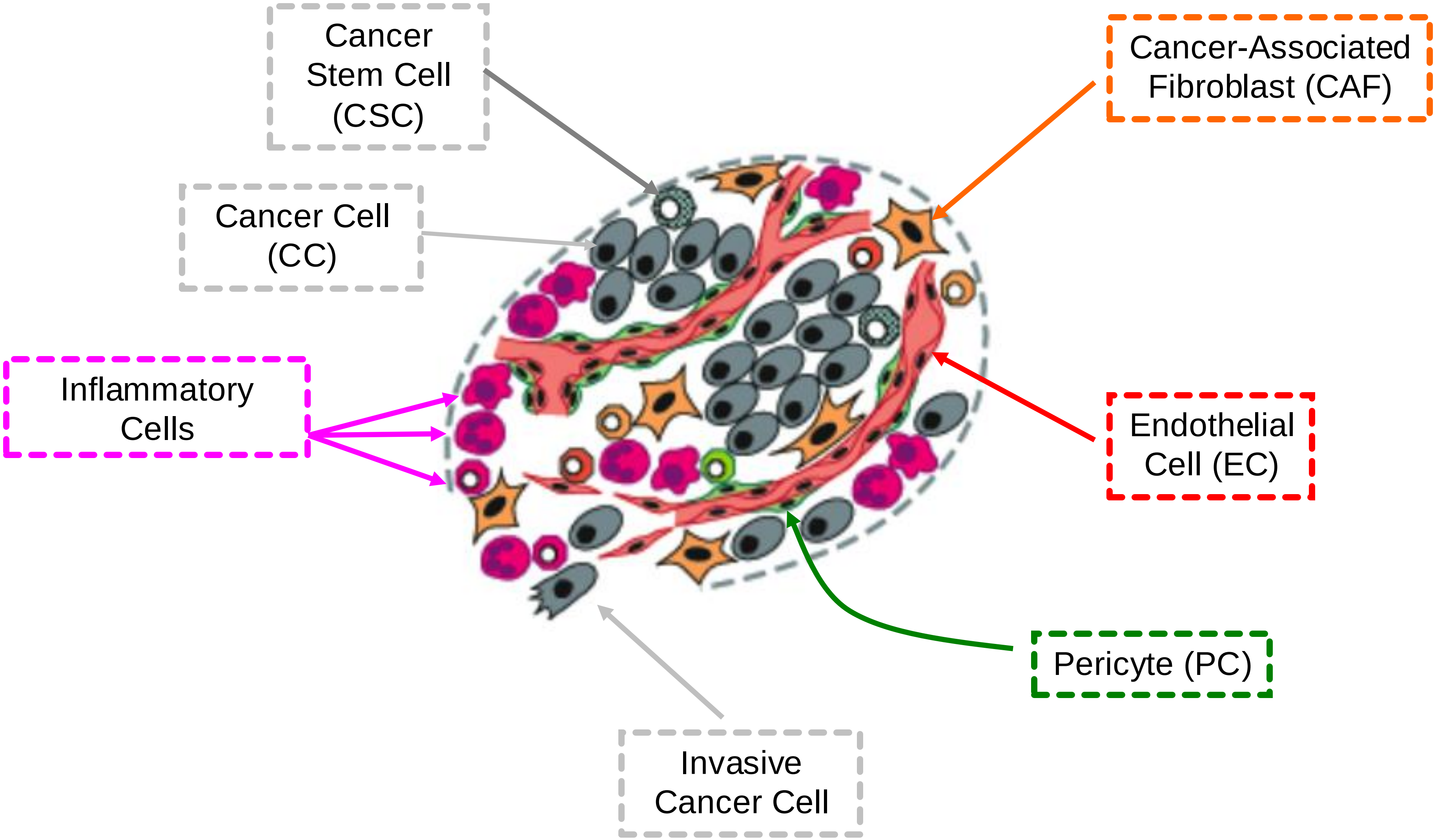
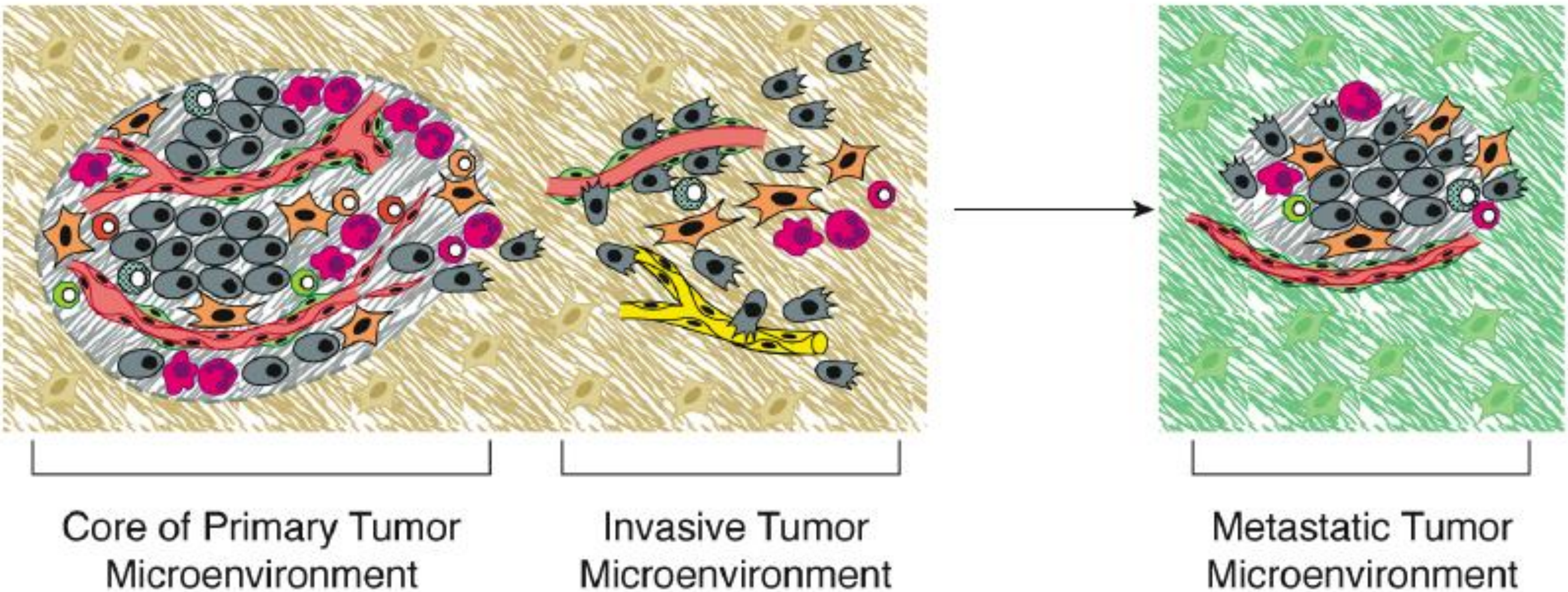
Pro-tumoral inflammation

Multiple “normal” cell types are present in tumors

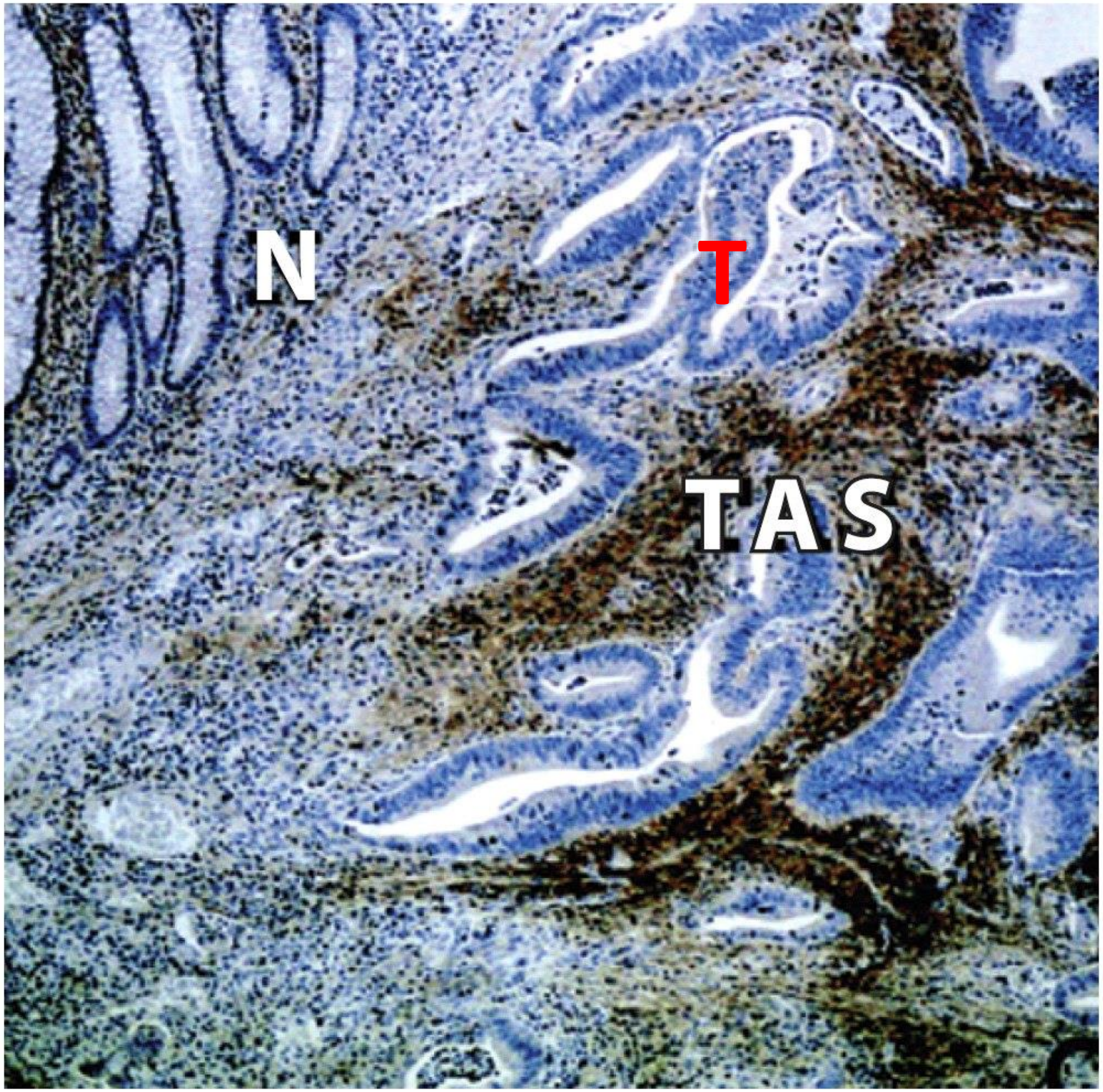


The tumor microenvironment evolves during malignant progression…



Hanahan & Weinberg (Cell, 2011)

Stromal cell components of carcinomas



Invasive colon carcinoma

N: normal colonic mucosa

T: tumor

TAS: tumor-associated stroma, mostly comprising fibroblasts (brown).

In this invasive tumor, the stromal cell component is conspicuous (note the abundance of TAS compared to normal colon).

Figure 13.1b *The Biology of Cancer* (© Garland Science 2007)

Hypotheses: Stromal cell components of carcinomas

- Possible origins and functions of tumor-associated stromal cells:
  1. ? They are just the remnants of cells that pre-existed at the site where the tumor has developed. They do not play fundamental roles (either positive or negative) during tumor progression (bystander role).
  2. ? They are recruited actively, and possibly activated/induced, by the growing cancer cells. They largely support tumor progression.
  3. ? They represent an attempt by the host to eradicate the tumor.

Hypotheses: Stromal cell components of carcinomas

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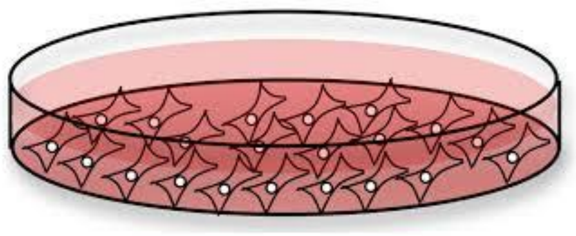
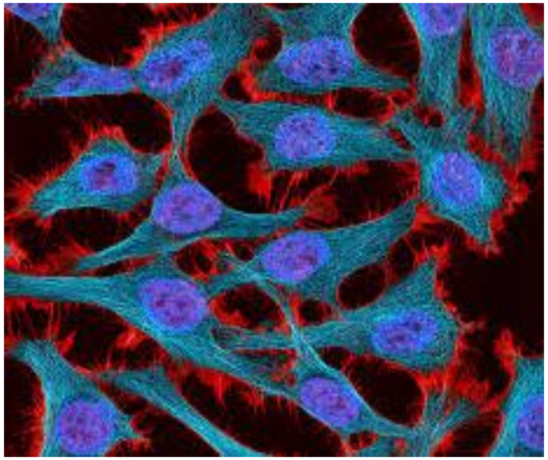
  2. V They are recruited actively, and possibly activated/induced, by the growing cancer cells. They largely support tumor progression.
  3. ~ They represent an attempt by the host to eradicate the tumor.

Question

If cancer cell-stromal cell interactions are essential for tumor progression (as are epithelial cell-stromal cell interactions in normal organs), how can cancer cells be routinely propagated *in vitro* in Petri dishes in the absence of stromal cells??

Henrietta Lacks
cervical cancer (1952)





HeLa cells

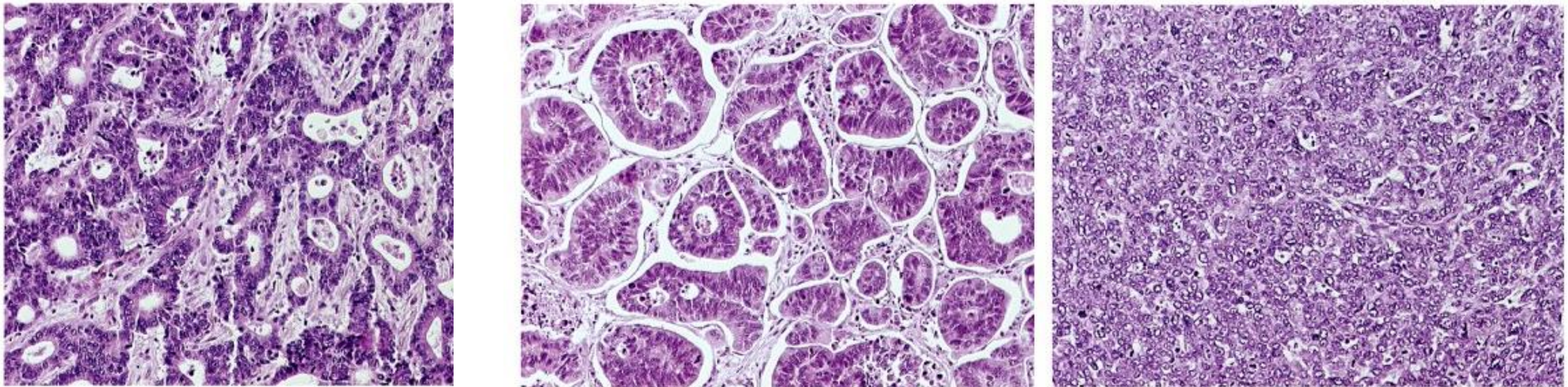
Response

Cancer cell lines are the result of Darwinian selection:

Some cancer cells may be selected that can propagate *in vitro* following extended culture of dissociated tumor fragments. **So, cells emerge and expand that can grow independent of stromal cells**. Serum is provided as a source of growth factors.

These cancer cell lines generally grow as aggressive tumors when inoculated in mice (**tumor xenografts**), and may represent a stage of tumor progression that goes beyond the one reached by the cancer cells in the primary tumor. They can be propagated for decades *in vitro* and *in vivo*…

Tumor xenografts derived from cancer cell lines lack the histological features of the primary tumor



surgical specimen | **engrafted patient specimen** | **engrafted cell line Colo205**

Grown in mouse

Why cancer cell lines do not make tumors with the same histology (including architecture and stromal cell components) as the parental tumor?

- Site of injection (ectopic, generally subcutaneous)
- Independence from heterotypic cell interactions (evolved *in vitro*)
- It is a clone: Lack of stem / progenitor cell hierarchy or heterogeneity w/in the cancer cell population

Consequences: Tumor xenograft models (in mice) that ignore heterotypic cell interactions amongst cancer and stromal cells may fail to reproduce the biology of the parental tumor and predict tumor responses to anti-cancer therapies.

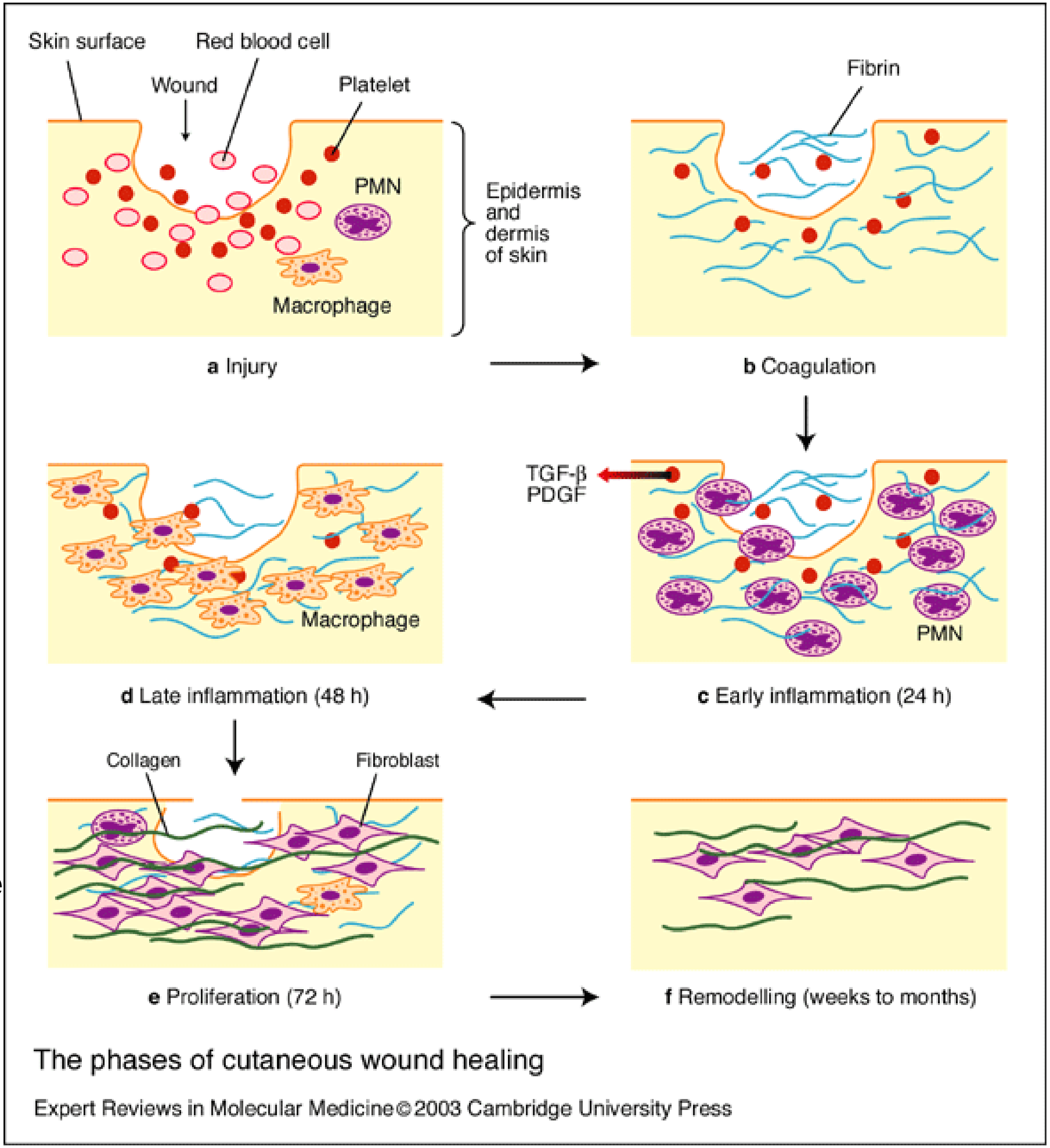
How do tumors orchestare a stromal tissue?

Tumors are wounds that never heal (recap)

How do cancer cells establish heterotypic interactions with (recruited) stromal cells?

They do so by activating a complex, physiological genetic program that is encoded in the genome of normal (epithelial) cells: the **wound healing response**.

Wound healing (stromal response)



Hemostasis phase

Inflammatory phase

Proliferative phase

Remodeling phase

Wound healing (stromal response)

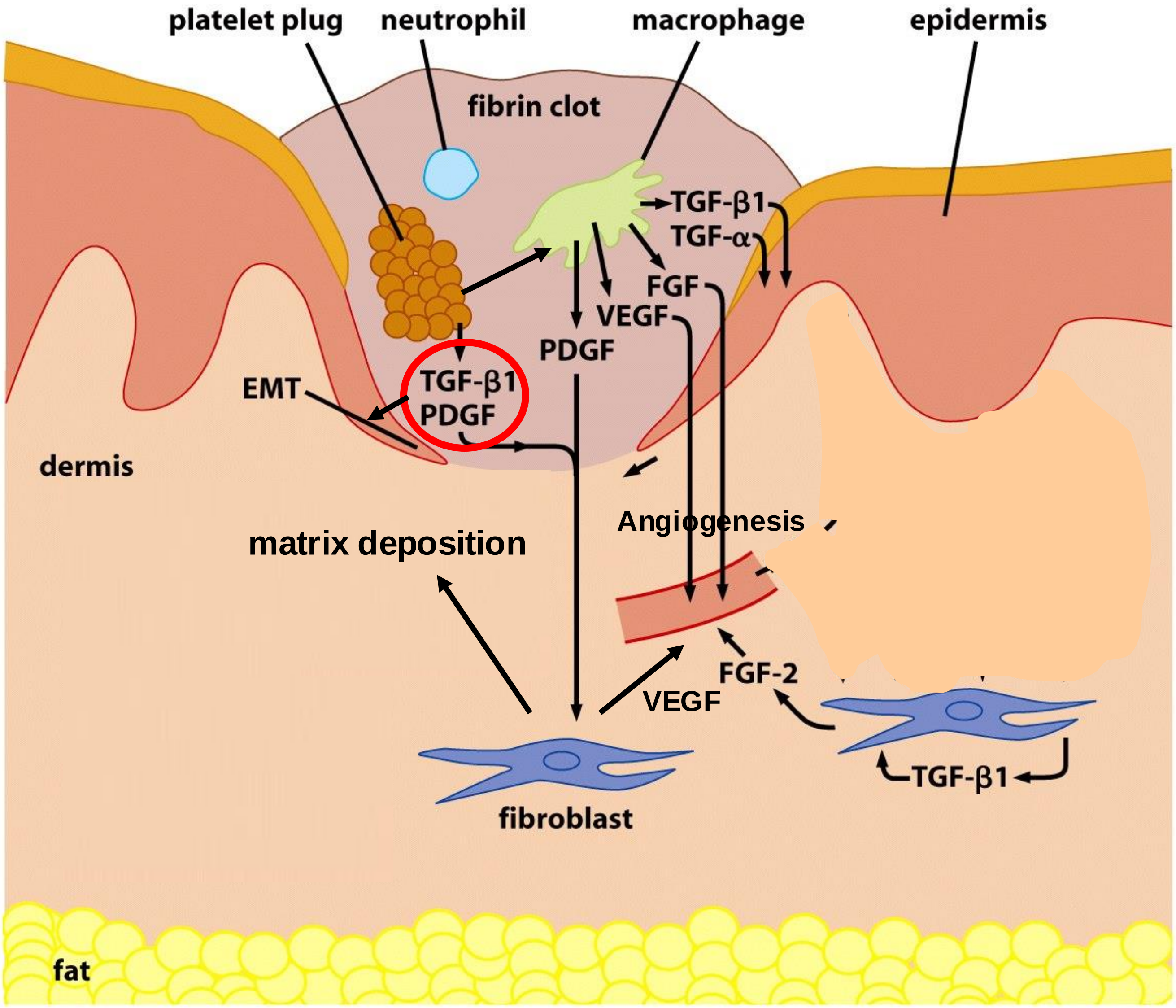
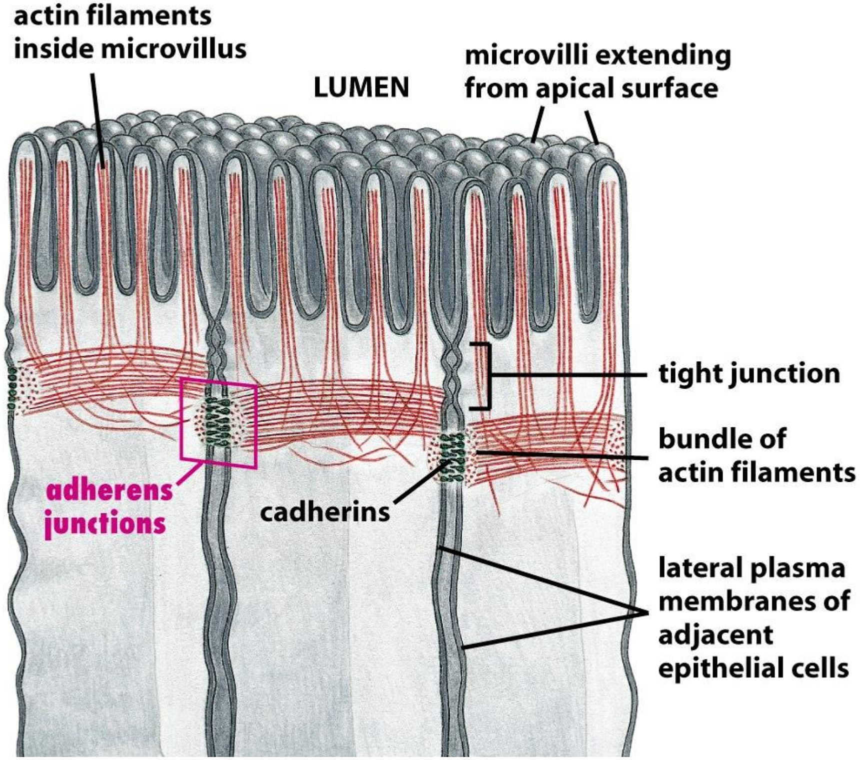
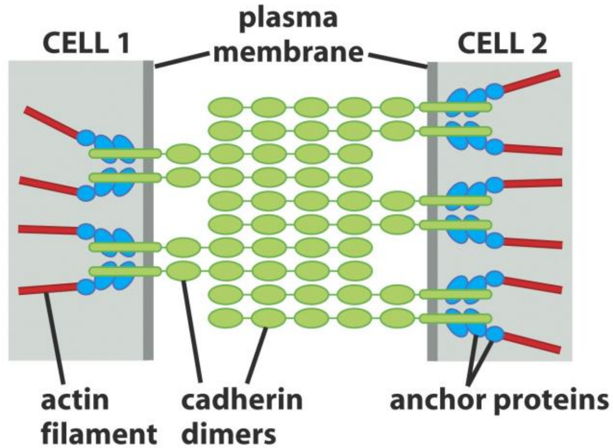
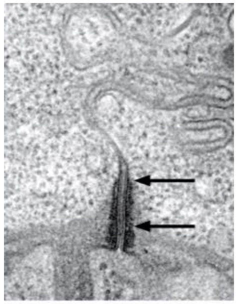


Figure 13.14 *The Biology of Cancer* (© Garland Science 2007)

Adherens junctions in epithelia

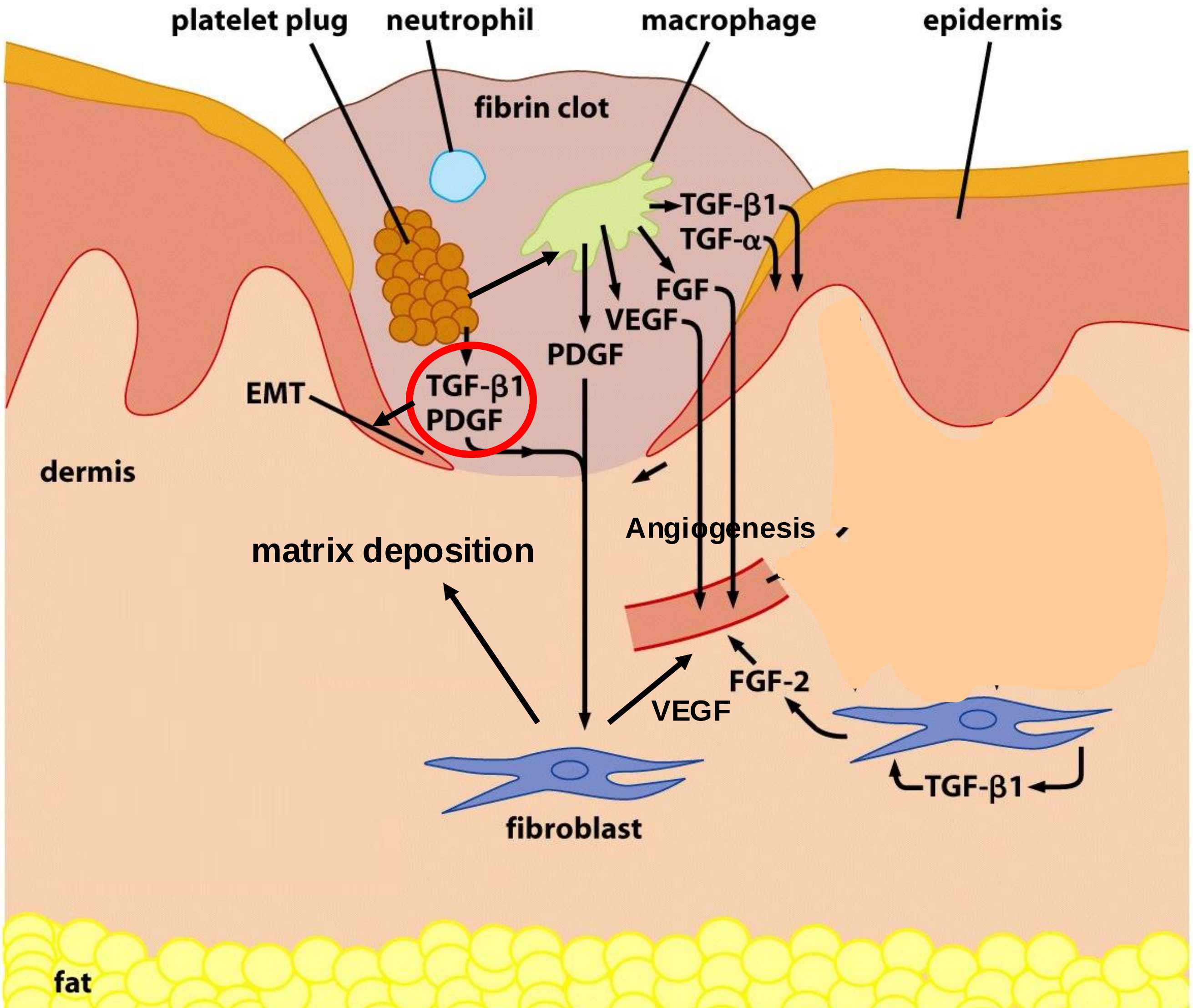






E-Cadherin

Wound healing (epithelial response)



EPITHELIUM

epithelial cells

↓ TGF-β1, MMPs from stroma

morph into mesenchymal cells (EMT)

↓

move into wound site and cover wound

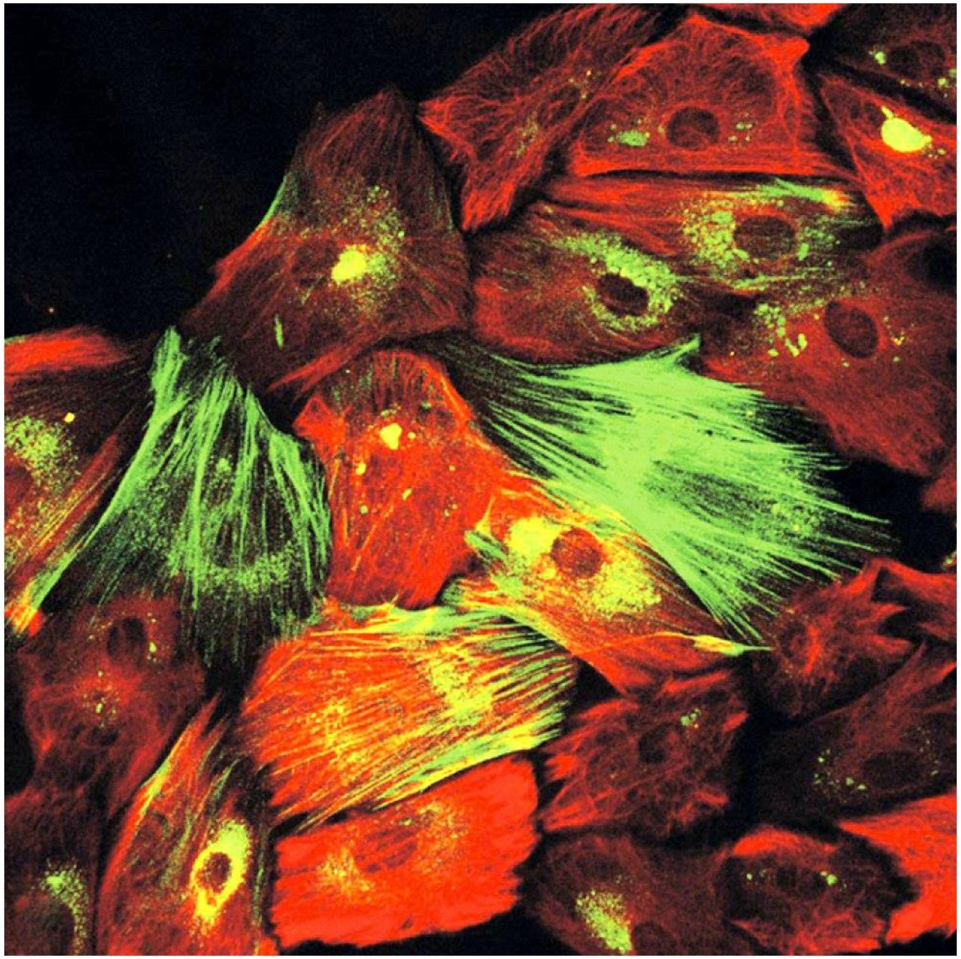
↓

morph into epithelial cells (MET)

↓

reconstruct epithelium

Epithelial-to-mesenchymal transition (EMT)



Cytokeratin (epithelial marker)

Alpha-smooth muscle actin (mesenchymal marker)

EMT

Process whereby epithelial cells acquire some of the features of mesenchymal cells (e.g., fibroblast-like phenotype). This is associated with the downregulation of E-cadherin and the upregulation of N-cadherin, which cannot form adherens junctions.

Such phenotypic transition is used by epithelial cells to acquire increased motility and invasive properties.

EMT occurs at the edge of wounds, enabling the epithelial cells to migrate and "regenerate" the damaged epithelium. It is a reversible genetic program.

Platelets and macrophages release TGFb. Fibroblasts and macrophages also produce MMPs that liberate matrix-bound TGFb. It is a major inducer of the EMT program.

Figure 13.13b *The Biology of Cancer* (© Garland Science 2007)

Tumors are wounds that never heal

Why?
(Recap from angiogenesis class)

Growing tumors rapidly induce new blood vessels (angiogenesis) around and within the tumor

skin or brain windows

Cancer cells proliferate rapidly and trigger a robust pro-angiogenic response mediated by hypoxia (previous lectures)

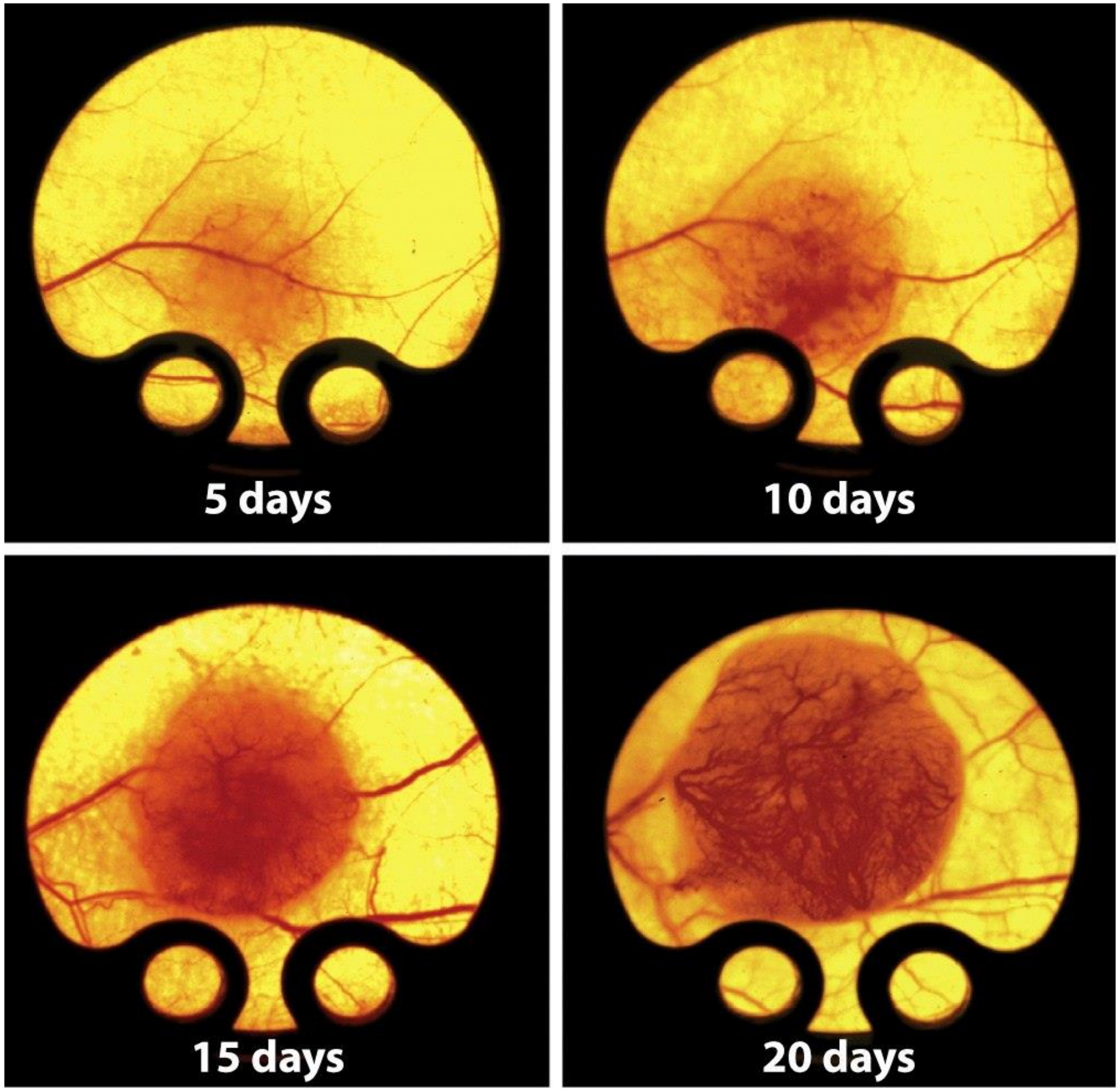
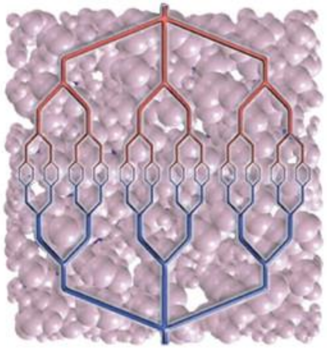


Figure 13.32a *The Biology of Cancer* (© Garland Science 2007)

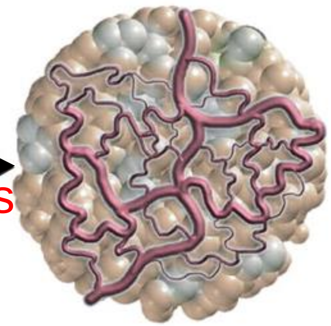
Features of tumor blood vessels

Normal | **Tumor**

Organization in arteries, veins and capillaries

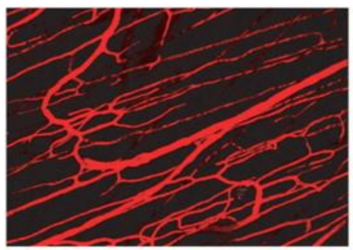


Tumor angiogenesis



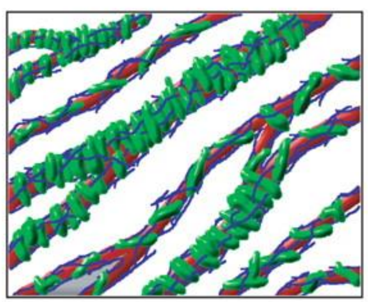
No distinct arteries and veines

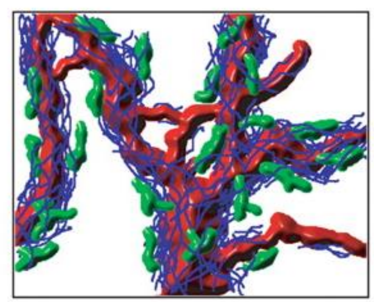
Regular morphology



Irregular morphology

High and even pericyte coverage (maturation; stability)



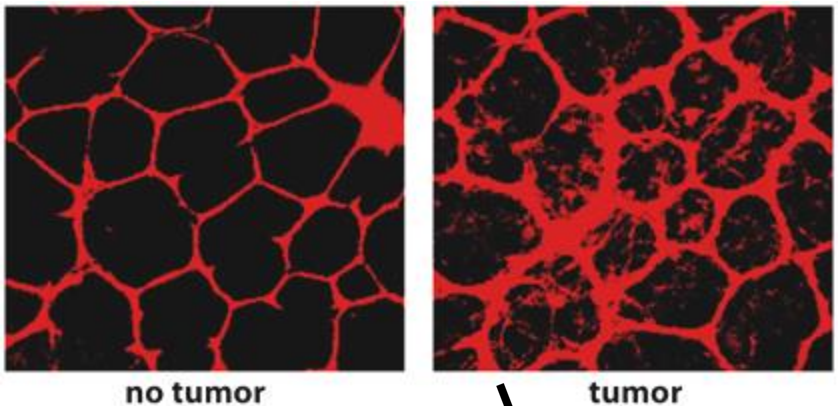


Uneven pericyte coverage (immature, unstable, leaky)

Because of these features, tumor blood vessels are poorly functional, leaky and provide **inadequate** oxygen and nutrient levels to the tumor mass

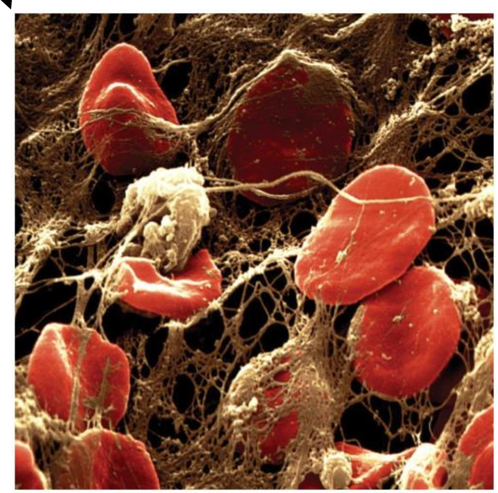
Vascular leakiness, fibrin deposition, platelet degranulation, PDGF/TGF, fibroblast activation, desmoplastic stroma….

Chronic angiogenesis



Platelet degranulation (PDGF, TGFb) and fibrin bundles stimulate **fibroblast proliferation and activation**. These cells secrete abundant ECM and form a **stiff** stromal tissue (**desmoplastic stroma**).

Like in chronic wounds, persistent angiogenesis and vascular leakage at the tumor-host interface generates a peritumoral, “clot-like” tissue.



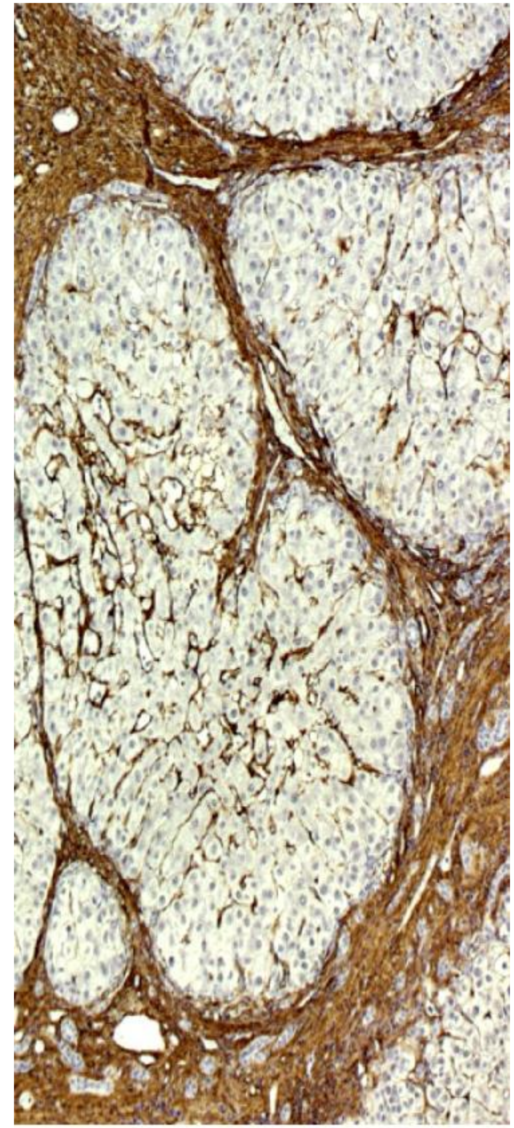
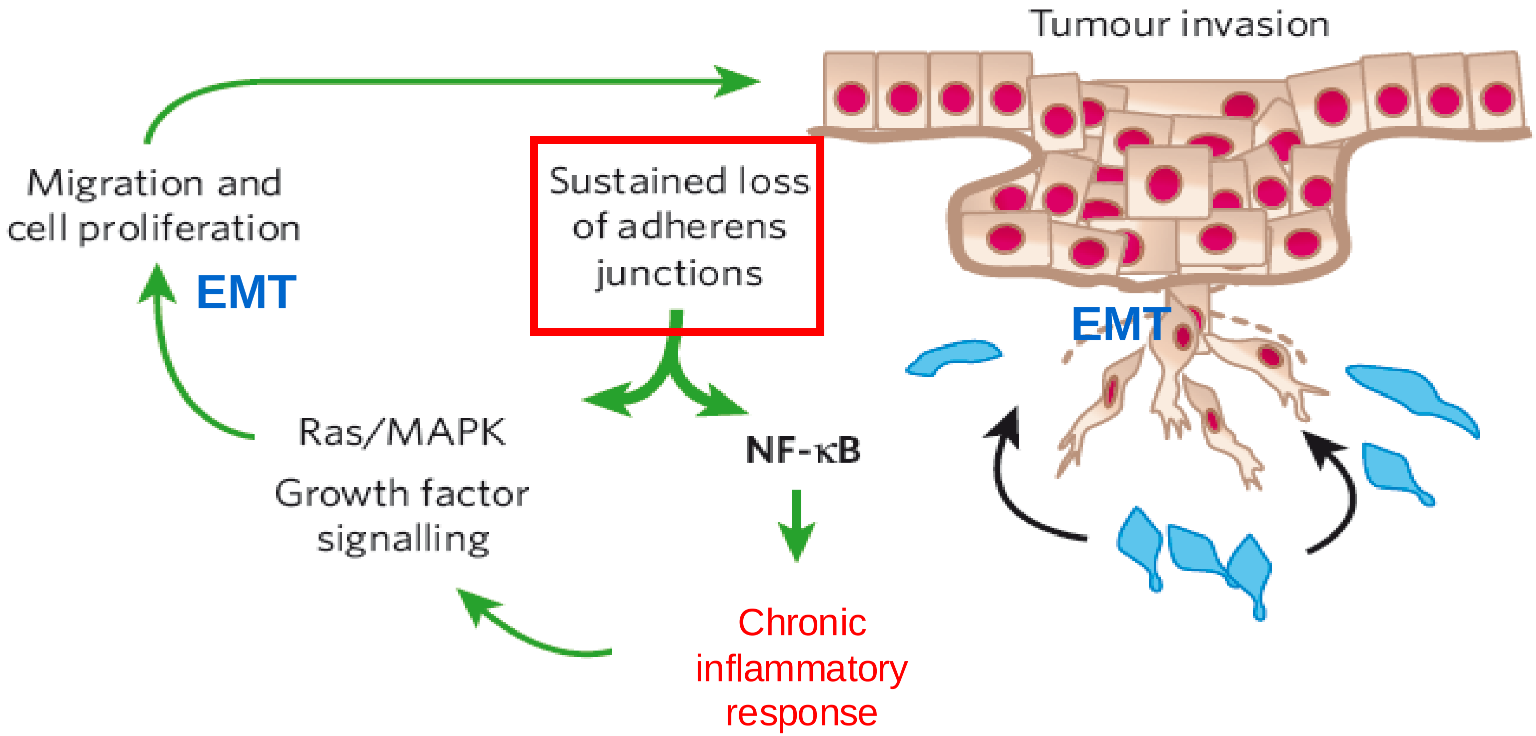


Figure 13.16b *The Biology of Cancer* (© Garland Science 2007)

Cancer: Wounds that never heal



Inflammation and cancer

A reminder: Tumor initiators and promoters

Tumor initiator: Generally a mutagen, causes genetic or epigenetic changes (mutations) in normal cells that are necessary (but often not sufficient) for tumor development.

Tumor promoter: Fosters the growth (proliferation) of "initiated" cancer cells, enabling their acquisition of additional features, also genetic, leading to cancer. A tumor promoter may not be a mutagen.

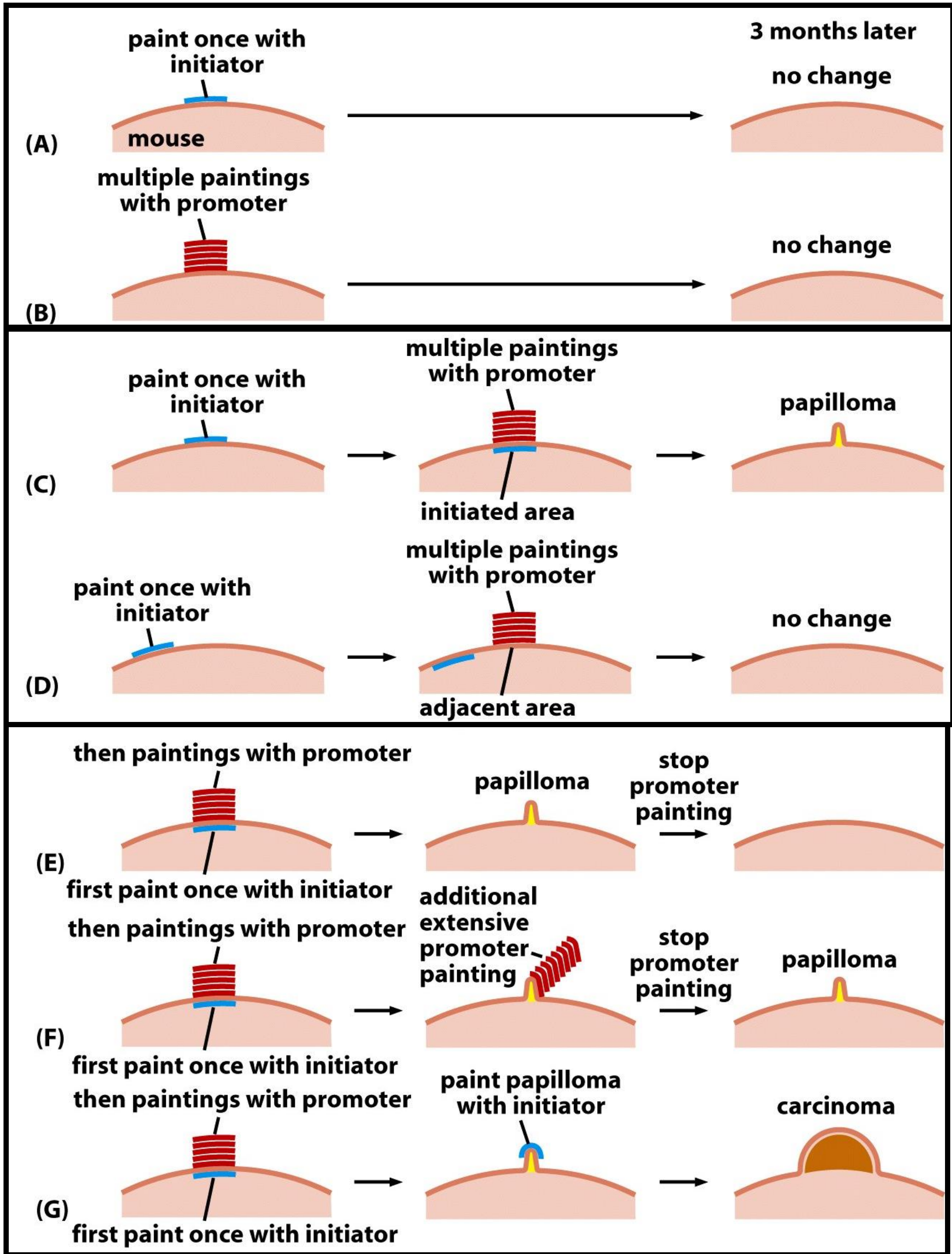
Tumor initiators and promoters: the skin carcinogenesis model

Tumor initiator: DMBA, 7,12-dymethylbenzanthracene (a highly carcinogenic tar constituent)

Tumor promoter: TPA, 12-O-tetradecanoylphorbol-13-acetate

Papilloma: benign cell proliferation (adenoma-like)

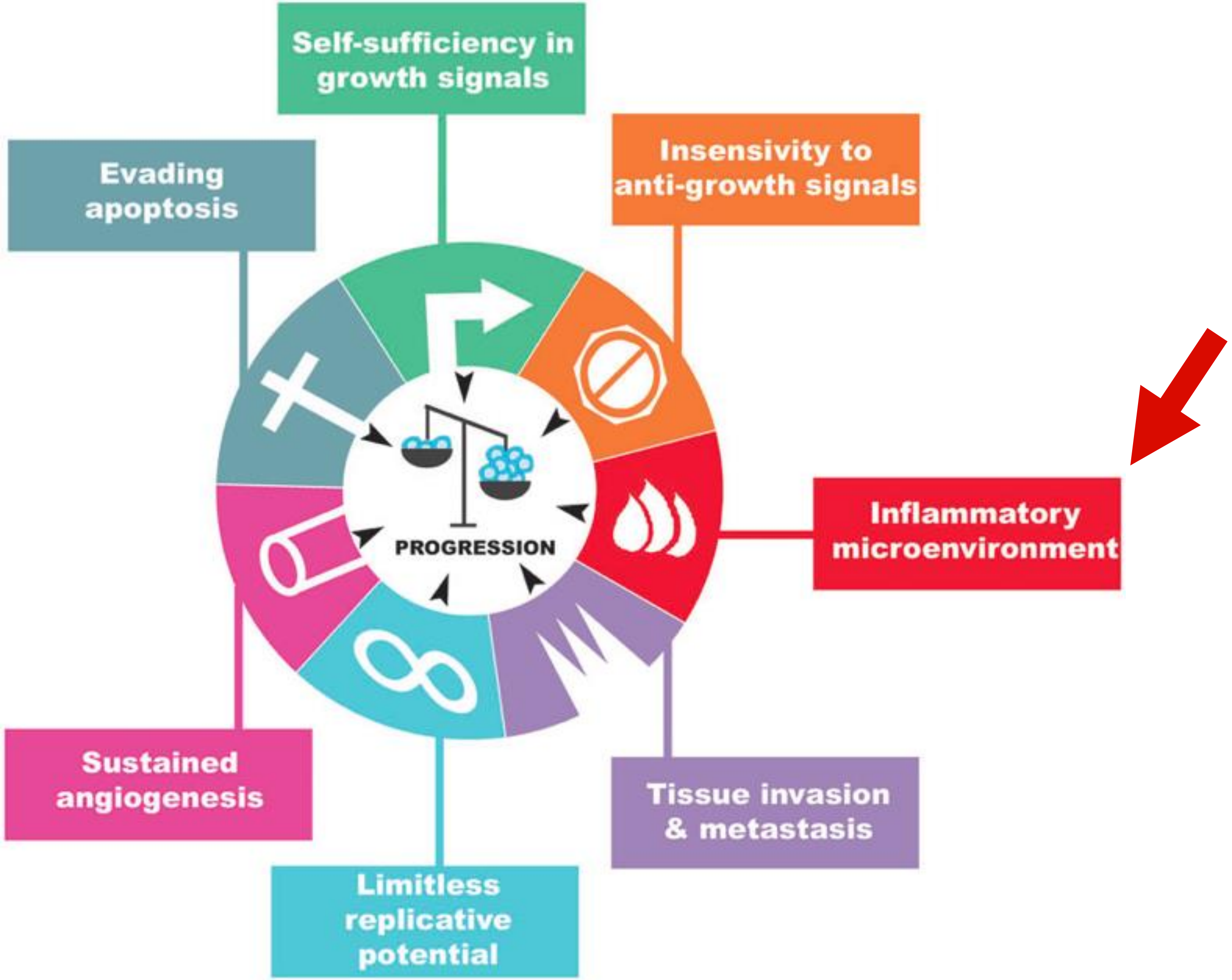
Carcinoma: malignant



Two mutagenic hits

Figure 11.28 part 2 of 2 *The Biology of Cancer*

Tumor promotion by inflammation, the seventh hallmark of cancer

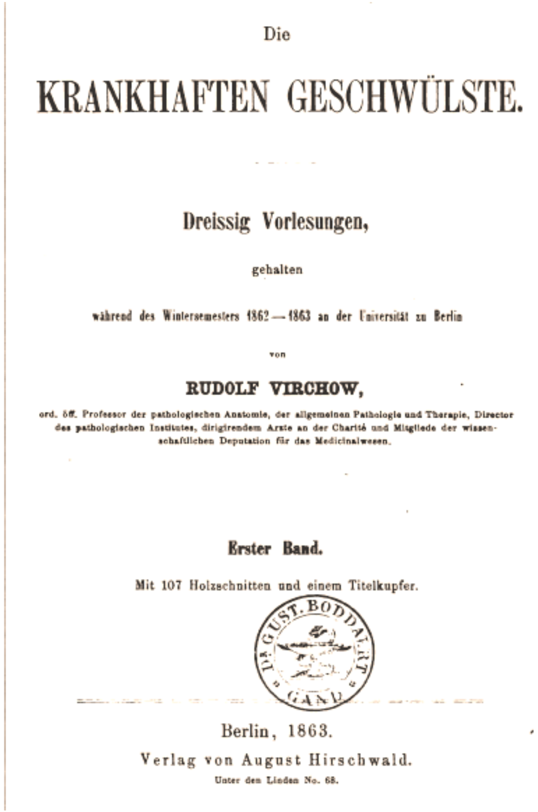


Colotta et al., Carcinogenesis, 2009

Inflammation is part of the biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.

Rudolf Virchow, 1863

He first noted the association of **tumors** with **chronically inflamed tissues** (tissues characterized by unusually high numbers of infiltrating inflammatory cells, or leukocytes)



Die

KRANKHAFTEN GESCHWÜLSTE.

Dreissig Vorlesungen,

gehalten

während des Wintersemesters 1862—1863 an der Universität zu Berlin

von

RUDOLF VIRCHOW,

ord. öff. Professor der pathologischen Anatomie, der allgemeinen Pathologie und Therapie, Director des pathologischen Institutes, dirigirendem Arzte an der Charité und Mitgliede der wissenschaftlichen Deputation für das Medicinalwesen.

Erster Band.

Mit 107 Holzschnitten und einem Titelkupfer.

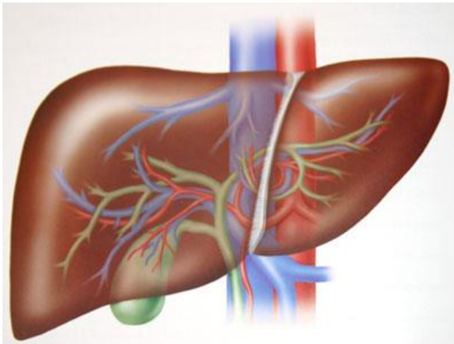
Dr. AUGUST. BODDAERT GAND

Berlin, 1863.

Verlag von August Hirschwald.

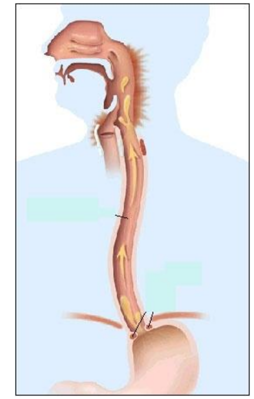
Unter den Linden No. 68.

In many (most?) cases, **chronic** inflammation functions as a **tumor promoter**, which supports the expansion/evolution of initiated (mutated) cancer cells



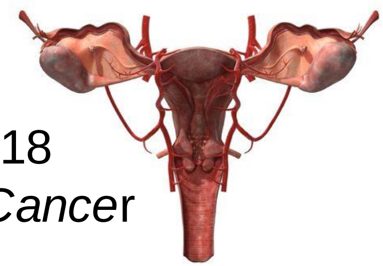
Viral hepatitis / Liver cirrhosis
→ *Hepatocellular Carcinoma*

H. pylori → Gastritis
Gastric Cancer



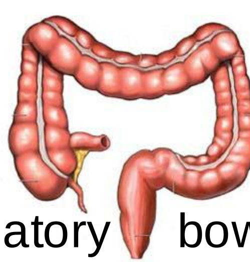
Reflux → Esophagitis
Esophageal Cancer

Inflammation
&
Cancer

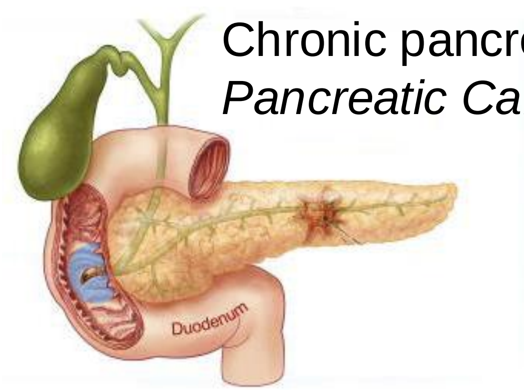


HPV-16 / 18
Cervical Cancer

Recurrent cholangitis
Cholangiocellular Carcinoma
Recurrent gallstones
Gallbladder Carcinoma



Inflammatory bowel disease
Colorectal Cancer



Chronic pancreatitis
Pancreatic Cancer

Human papilloma virus (HPV) infection and cervical / oro-pharyngeal cancers

HPV: small DNA viruses that infect keratinocytes and integrate into their genomes.
HPV 16 and 18 are the two most oncogenic strains.
They encode two oncogenic proteins, which provide the initiating event:

- E6: inactivates p53 (ubiquitinase activity; via ubiquitination and degradation)
- E7: inactivates pRB (via inhibition of hypophosphorylated pRB and deployment of E2F, which promotes cell cycle progression by transactivating E2F-responsive genes)

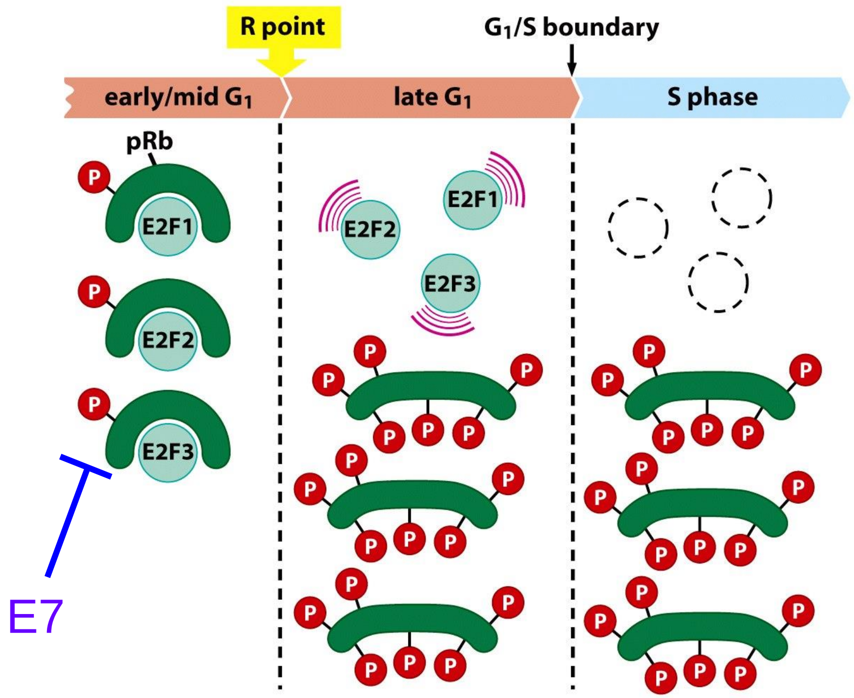
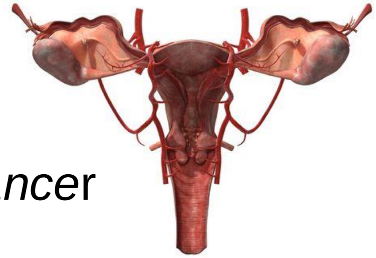


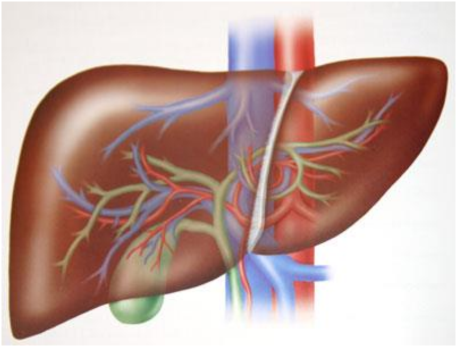
Figure 8.23a *The Biology of Cancer* (© Garland Science 2007)



HPV-16/18
Cervical cancer

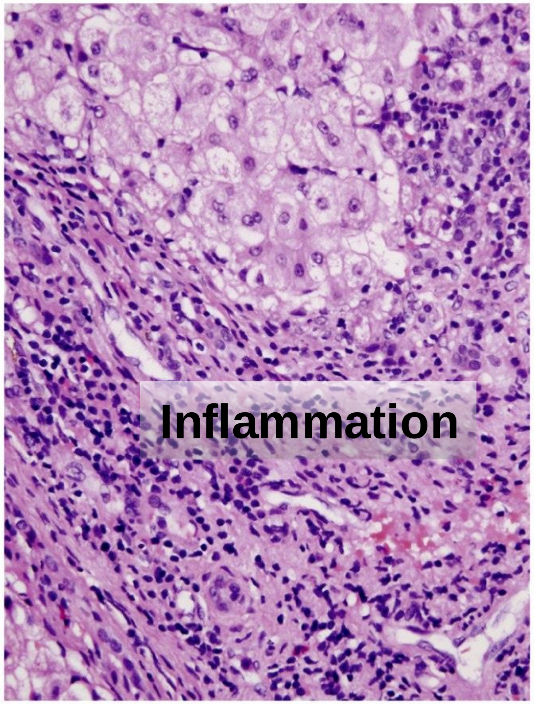
Tumor promotion by inflammation: The virus is immunogenic, so infected cells are generally cleared. In those cases when the immune system does not eliminate the infection, chronic inflammation promotes increased epithelial turnover and augments cancer risk significantly. HPV may cause cancer within 10-15 years from infection.

Chronic hepatitis B and C virus (HBV, HCV) infection and hepatocellular carcinoma (HCC)

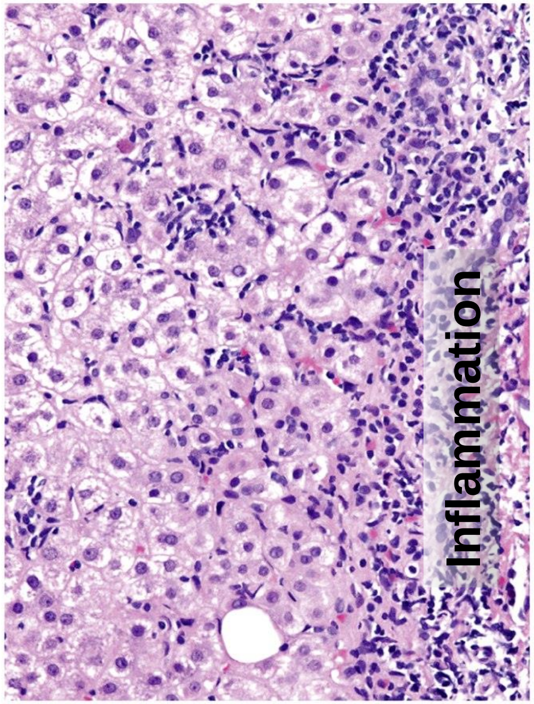


Viral Hepatitis / Liver Cirrhosis
→ *Hepatocellular Carcinoma*

High incidence in Asia (and Africa), where HBV is endemic; low incidence in USA and Europe.



HBV



HCV

Initiator: HBV-associated HCC is most frequent in subjects exposed to *Aspergillus*-derived aflatoxin-B1, a potent mutagen and carcinogen, which may be the main initiator in this tumorigenesis process. (Viral genes are not oncogenic *per se*; HBV does not cause insertional mutagenesis.)

Tumor promotion by inflammation: Continuous cycles of hepatocyte apoptosis and proliferation (due to viral replication and consequent T-cell mediated killing of infected cells) lead to the selection/expansion of mutated cancer cells.

Non-steroidal anti-inflammatory drugs (NSAIDs) as cancer preventive agents

Initiation → Promotion → Progression → CANCER

Insulting agents:
- Carcinogens
- Infections

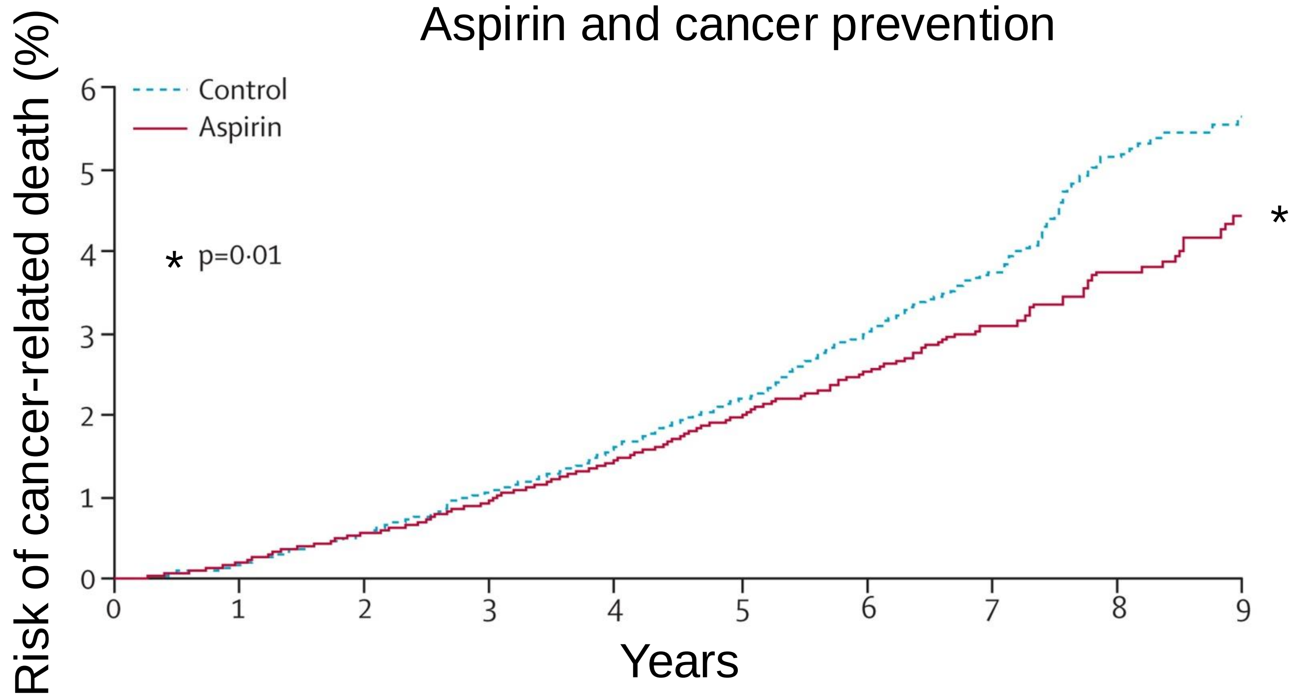
→ Mutations

Increased leukocyte infiltration:
- Growth factors
- ROS and NROS

→ Increased genetic instability

Risk of cancer-related death in 7 clinical trials (23570 patients)

Aspirin and cancer prevention



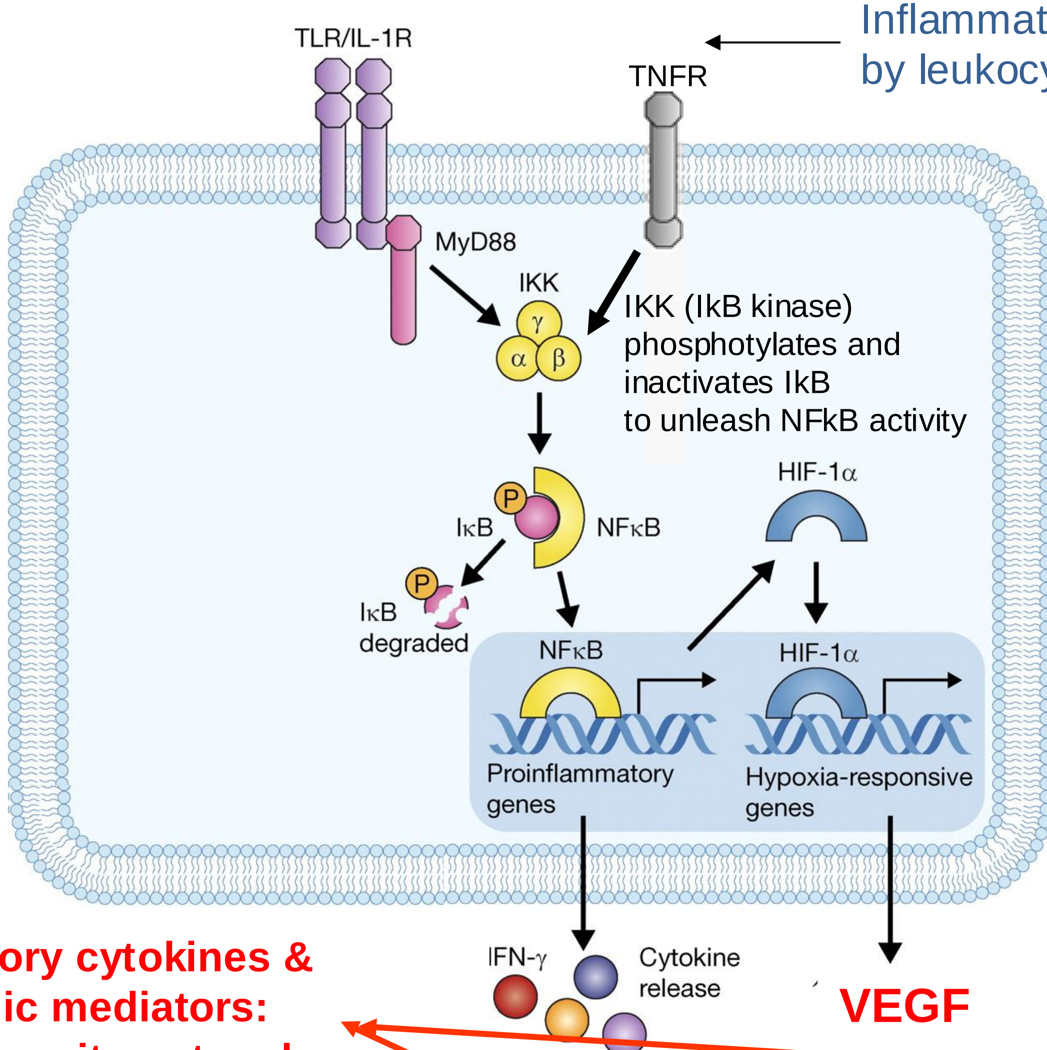
Adapted from Rothwell *et al.*, *Lancet* 2010

When a tumor develops, it instigates an inflammatory microenvironment
(wound healing response)

Cancer cell NFκB amplifies tumor inflammation

Inflammatory mediators produced by leukocytes (TNFα, IL1)

Cancer cell



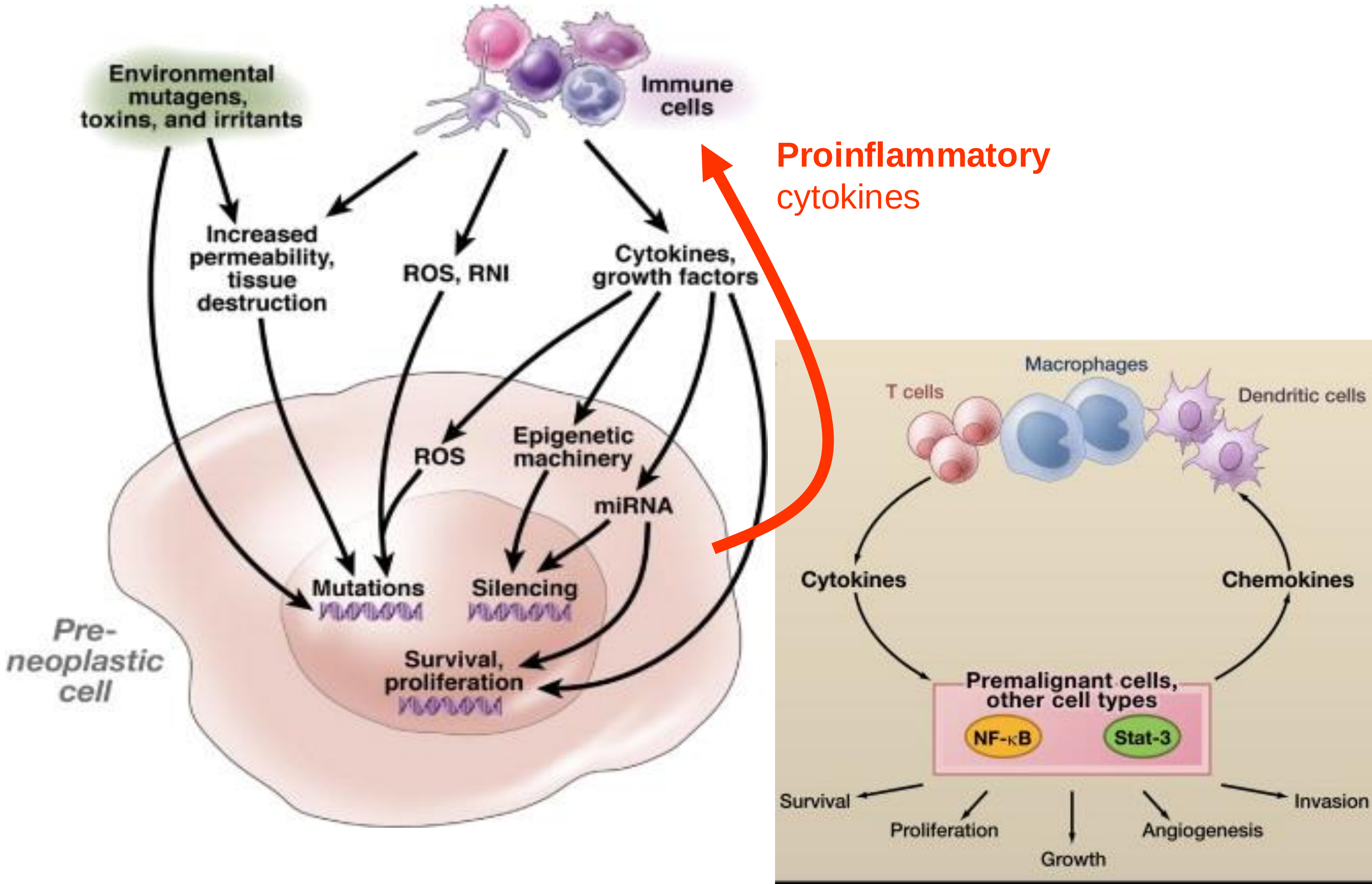
Proinflammatory cytokines & prongiogenic mediators: Leukocyte recruitment and angiogenesis

VEGF

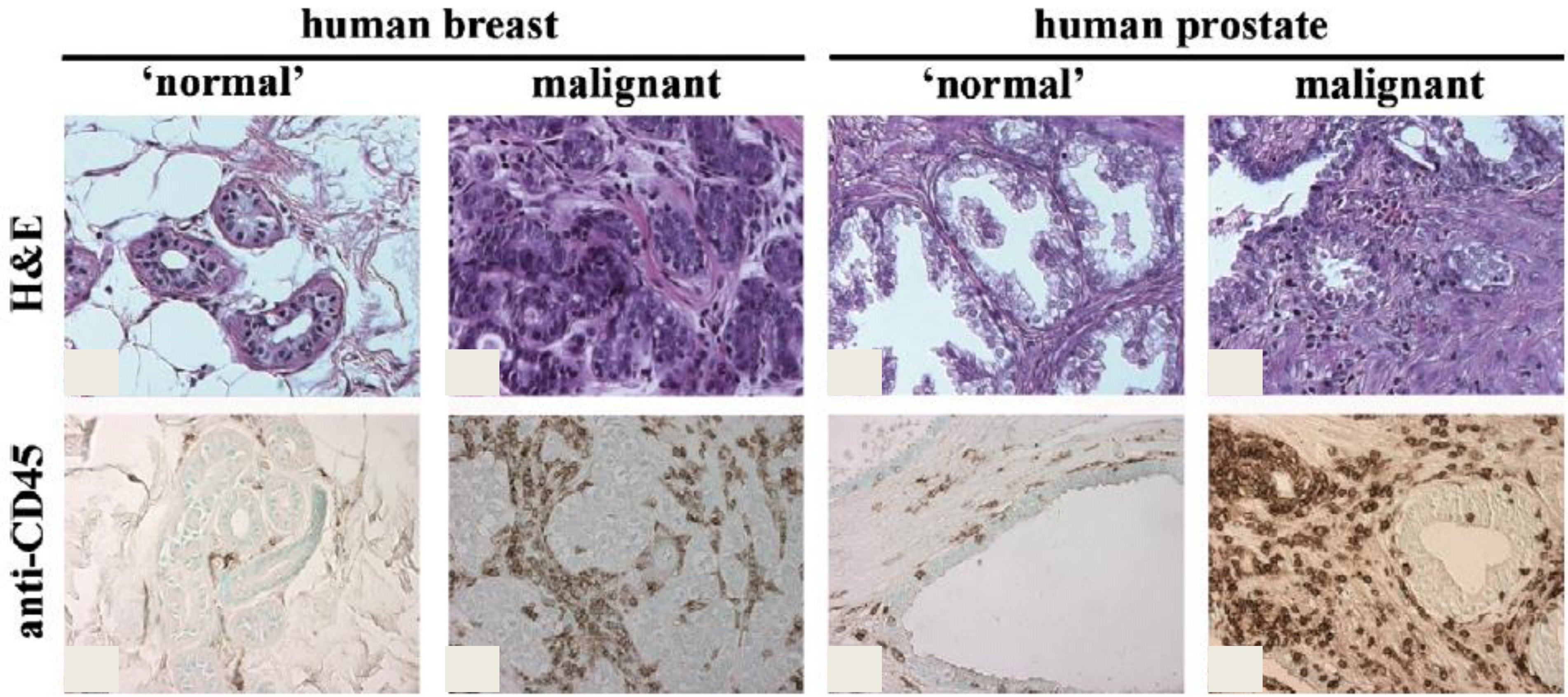
Cytokines

Adapted from Timmer & Nizet, *J Exp Med* 2008

Cross-talk between cancer and inflammatory cells fosters malignant progression



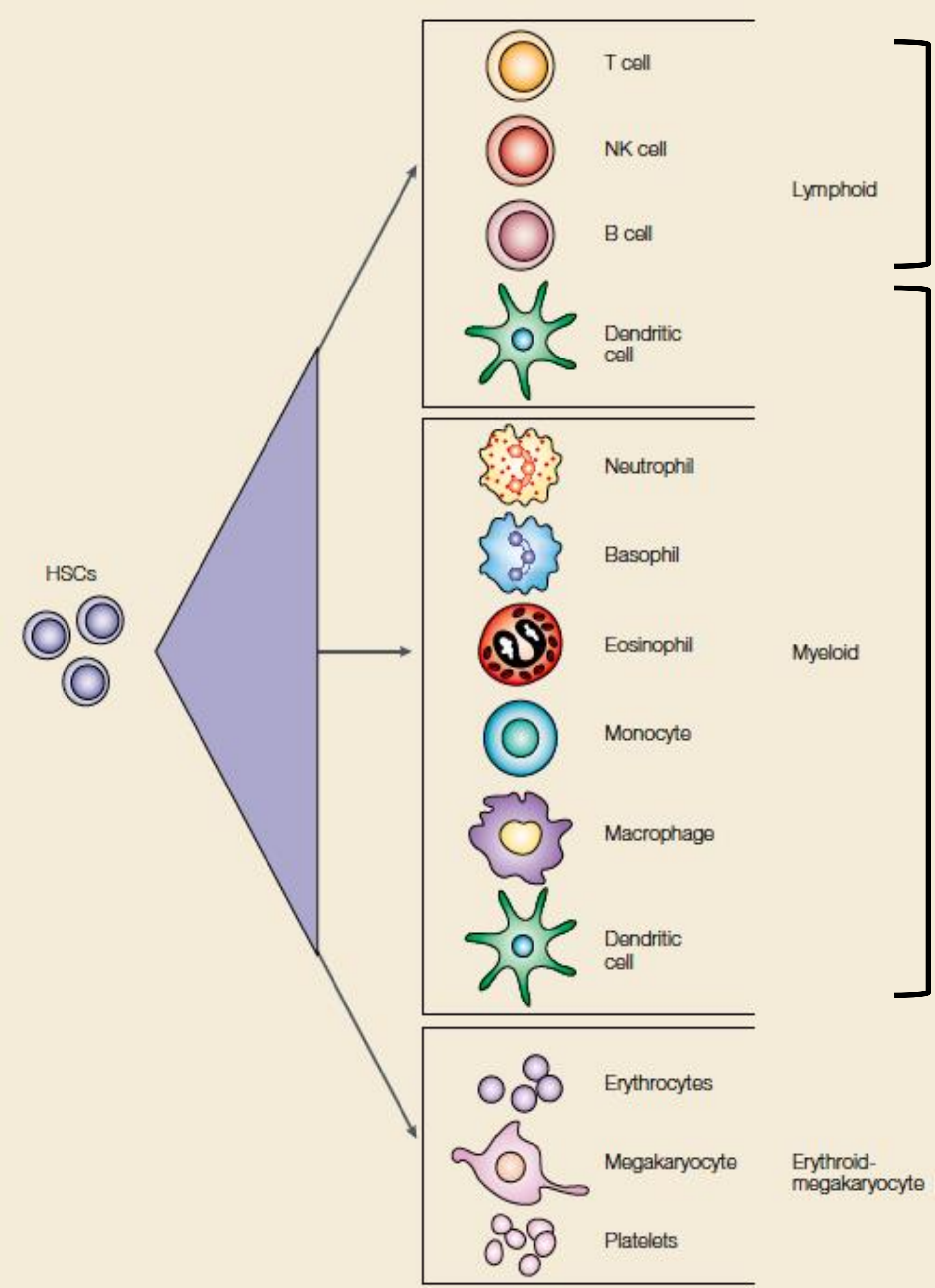
Human tumors are heavily infiltrated by leukocytes (inflammatory/immune cells)



CD45: total leukocytes (inflammatory cells)

Adapted from van Kempen *et al.*, *Eur J Cancer* 2006

Hematopoietic cells



Adaptive immunity
(antigen specific, induced)

Innate immunity
(aspecific, pre-existing)

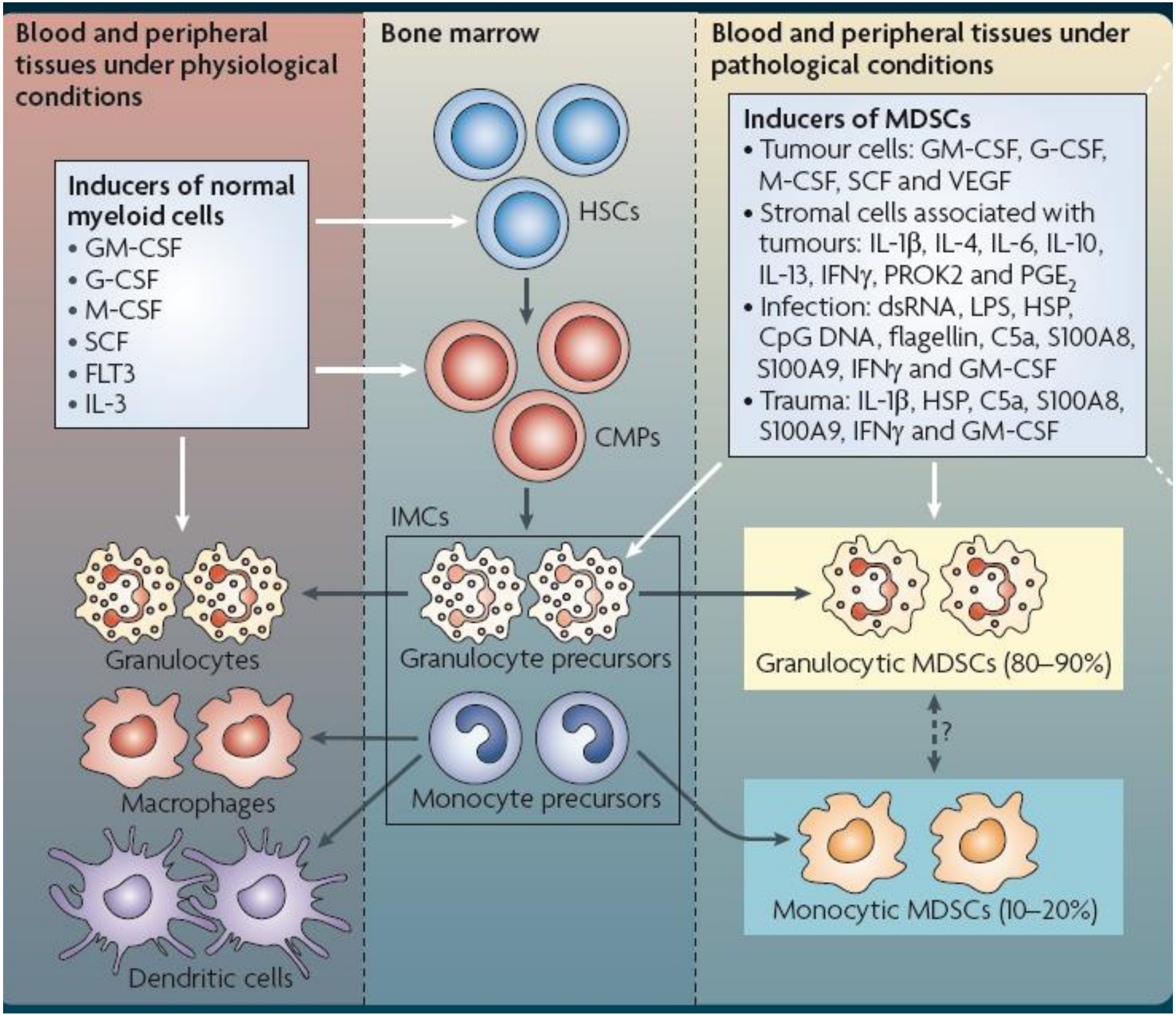
Katsura et al., *Nat. Rev. Immunol.*, 2002

Immunosuppression by myeloid cells (“MDSCs”) in cancer

Tumor-infiltrating myeloid cells often express an immature phenotype and are termed “meyloid-derived suppressor cells (MDSCs)”

These immature myeloid cells expand in subjects with cancer and are known to support tumor progression, in part, by suppressing tumor-antagonizing immune cells (T cells, etc.)

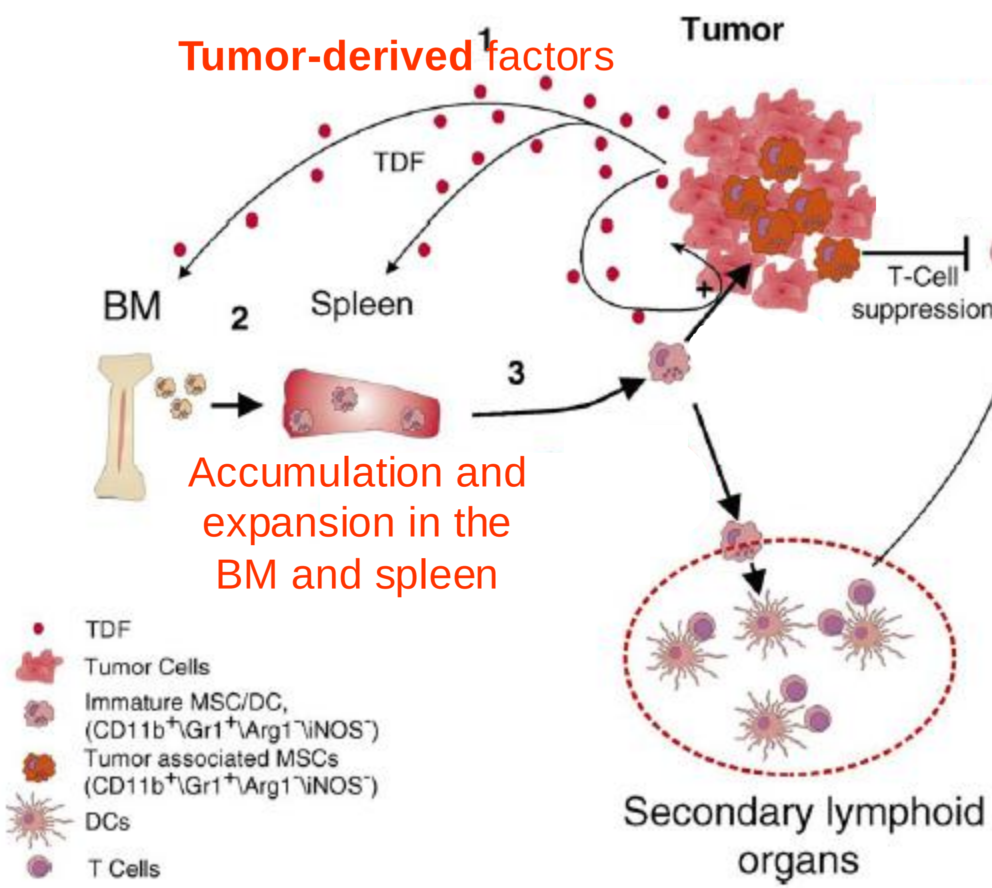
Tumor-derived factors promote the expansion of MDSCs



Expansion of immature myeloid cells (MDSCs) during tumor growth

MDSCs: myeloid-derived suppressor cells

Identified as CD11b+Gr1+ cells

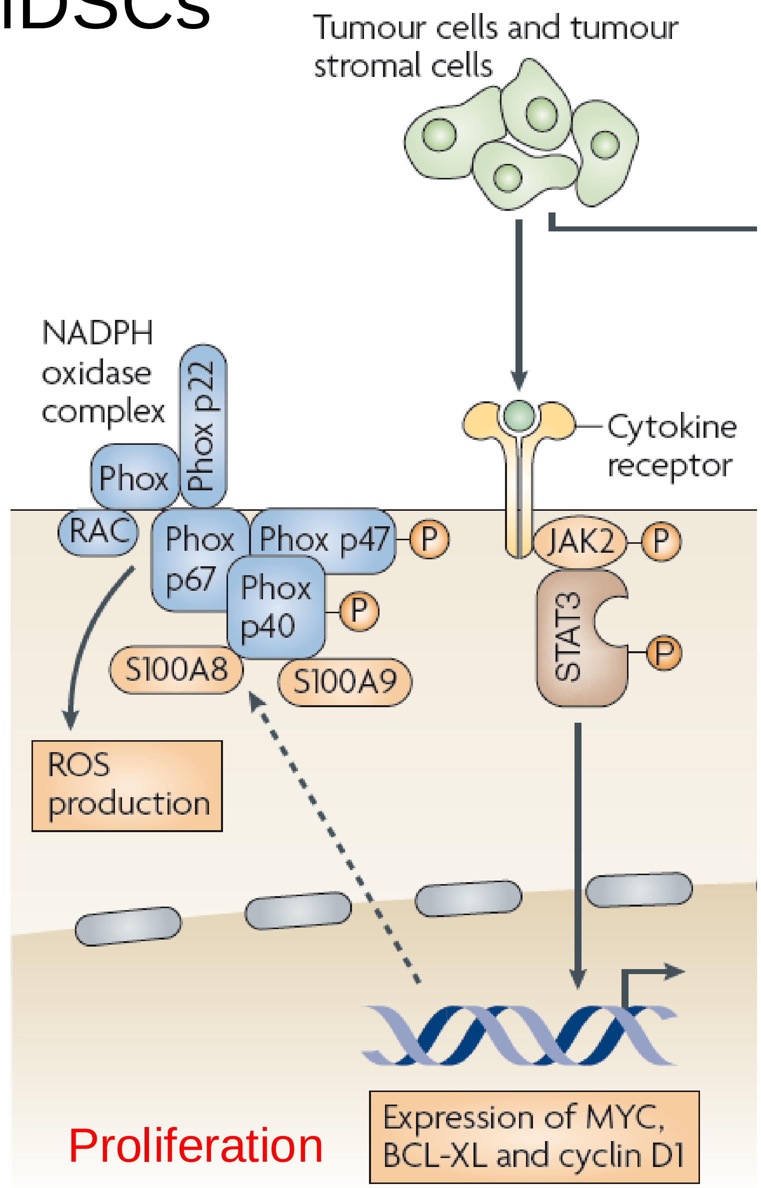


Accumulation in the tumor and suppression of tumor-antagonizing T cells in the tumor microenvironment

Impaired differentiantion into antigen-presenting cells (APCs)/dendritic cells (DCs) in the lymph nodes

Sica and Bronte, *J Clin Invest,* 2007

STAT3 promotes the expansion (& defective maturation) of MDSCs



Gabrilovich & Nagaraj, *Nat. Rev. Immunol.*, 2009

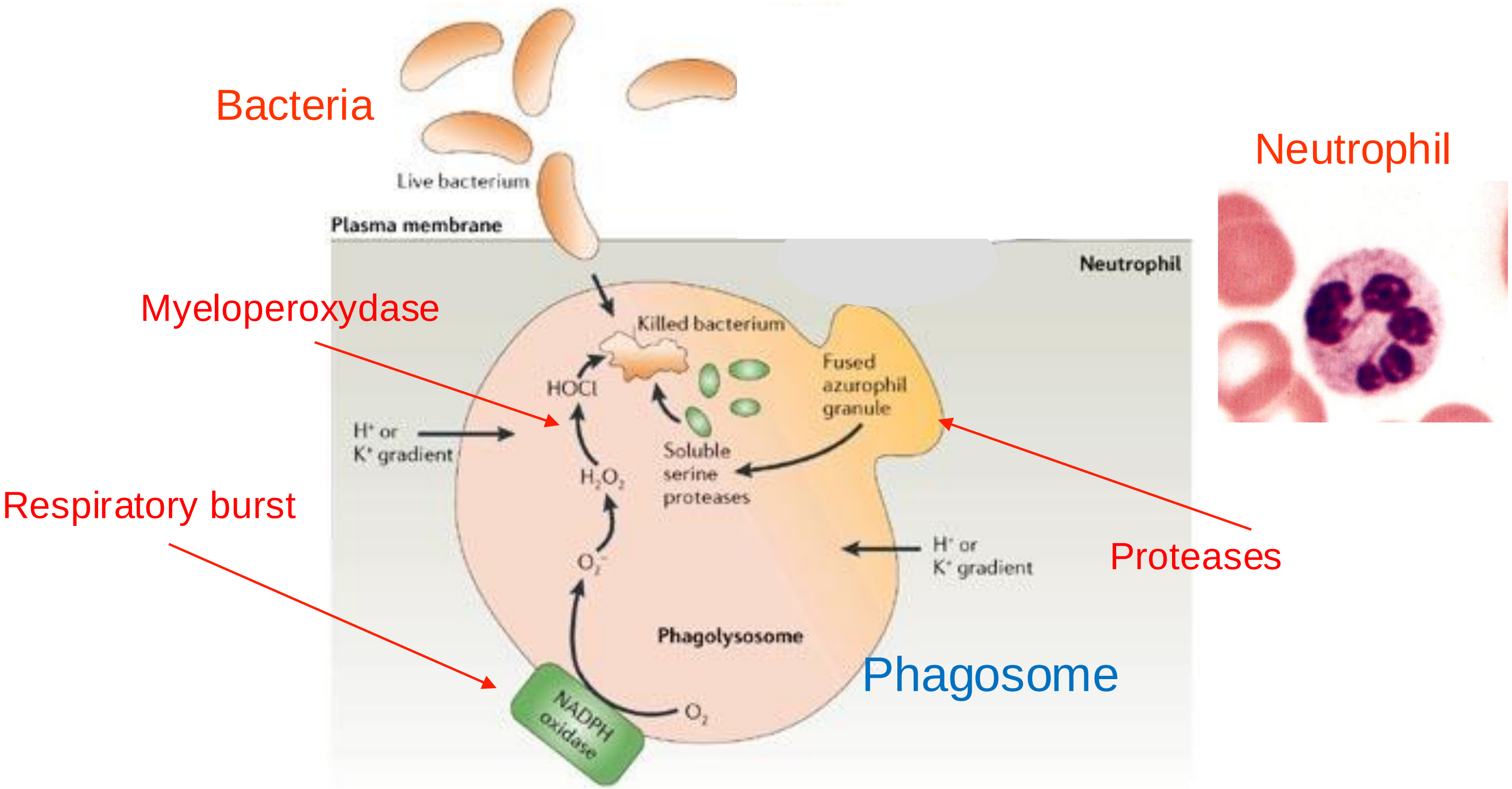
STAT3 is arguably the main transcription factor that regulates the expansion of MDSCs. MDSCs from tumour-bearing mice have markedly increased levels of phosphorylated STAT3 compared with IMCs from naive mice[45]. Exposure of haematopoietic progenitor cells to the supernatant from tumour-cell cultures resulted in the activation of JAK2 and STAT3, and was associated with an expansion of MDSCs *in vitro*. However, this expansion was abrogated when STAT3 expression in haematopoietic progenitor cells was inhibited[46]. Moreover, ablation of STAT3 expression through the use of conditional knockout mice or selective STAT3 inhibitors markedly reduced the expansion of MDSCs and increased T-cell responses in tumour-bearing mice[45,47]. STAT3 activation is associated with increased survival and proliferation of myeloid progenitor cells, probably through the upregulation of the expression of B-cell lymphoma XL, cyclin D1, MYC and survivin. So, abnormal and persistent activation of STAT3 in myeloid progenitor cells prevents their differentiation into mature myeloid cells and thereby promotes MDSC expansion.

Recent findings suggest that STAT3 also regulates MDSC expansion by inducing the expression of S100 calcium-binding protein A8 (S100A8) and S100A9, the receptors for which are also expressed on the cell surface of MDSCs. S100A8 and S100A9 belong to the family of S100 calcium-binding proteins that have been reported to have an important role in inflammation[48]. STAT3-dependent upregulation of S100A8 and S100A9 expression by myeloid progenitor cells prevented their differentiation and resulted in the expansion of MDSCs in the spleens of tumour-bearing and naive transgenic mice that overexpress S100A9. By contrast, MDSCs did not expand in the peripheral blood and spleens of mice that were deficient for S100A9 following challenge with tumour cells or

Tumor-infiltrating myeloid cells often express an immature phenotype and are termed “meyloid-derived suppressor cells (MDSCs)”

These inflammatory myeloid cells produce reactive free radicals that abate anti-tumor immunity, e.g. by blocking T cells, a process termed immunosuppression...

Role of ROS in bacterial killing by inflammatory cells



Pham *Nature Reviews Immunology* **6**, 541–550 (July 2006) | doi:10.1038/nri1841

Neutrophils (and macrophages) are inflammatory cells that, once activated at sites of inflammation, produce **reactive oxygen species (ROS)** to kill invading pathogens. The enzyme NADPH oxydase – activated by the respiratory burst – produces **superoxyde (O_2^-)**. This is converted to **hydrogen peroxyde (H_2O_2)**. The enzyme myeloperoxydase produces the cytotoxic molecule **hypoclorous acid (HOCl)** from H_2O_2 and Cl^-.

Mechanisms of immunosuppression by MDSCs

Production of ROS and RNOS

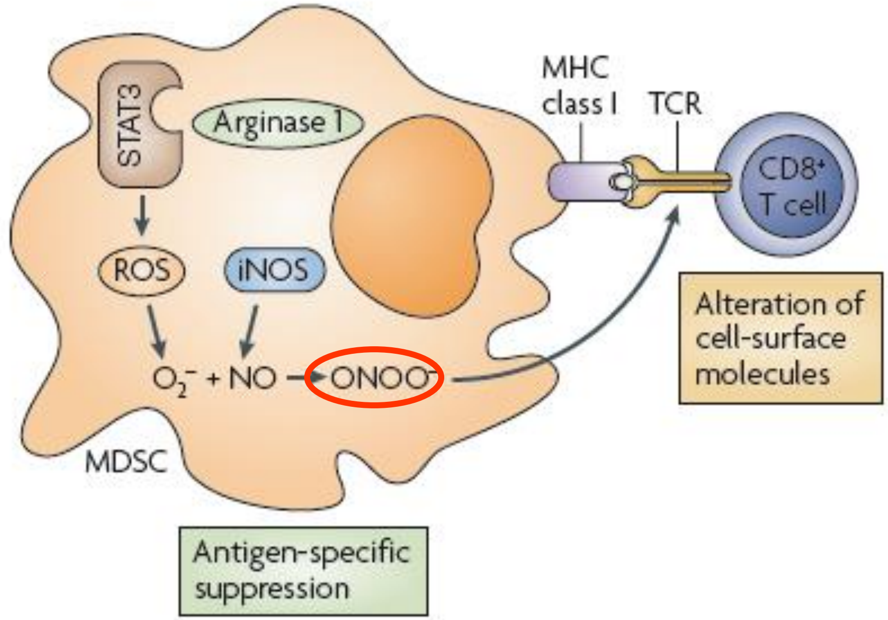
ROS. Another important factor that contributes to the suppressive activity of MDSCs is ROS. Increased production of ROS has emerged as one of the main characteristics of MDSCs from both tumour-bearing mice and patients with cancer[6,10,13,53,67–70]. Inhibition of ROS production by MDSCs isolated from tumour-bearing mice and patients with cancer completely abrogated the suppressive effect of these cells *in vitro*[10,13,67].

Gabrilovich et al., *Nat Rev Immunol* 2012

Mechanisms of immunosuppression by MDSCs

Peroxynitrite. More recently, it has emerged that peroxynitrite is a crucial mediator of MDSC-mediated suppression of T-cell function. Peroxynitrite is a product of a chemical reaction between NO and superoxide anion, and is one of the most powerful oxidants that are produced in the body. It induces the nitration and nitrosylation of the amino acids cysteine, methionine, tryptophan and tyrosine[72]. Increased levels of peroxynitrite are present at sites in which MDSCs and inflammatory cells accumulate, including sites of ongoing immune reactions. In addition, high levels of peroxynitrite are associated with tumour progression in many types of cancer[72,73,74–78], an effect that has been linked with T-cell unresponsiveness. One study[79] reported that human prostate adenocarcinomas were infiltrated by terminally differentiated CD8⁺ T cells that were in an unresponsive state. High levels of nitrotyrosine were present in the T cells, which suggested that peroxynitrite was produced in the tumour microenvironment. Inhibiting the activity of arginase 1 and iNOS, which are expressed in malignant but not in normal prostate tissue, led to decreased tyrosine nitration and restoration of T-cell responsiveness to tumour antigens. In addition, we have shown that peroxynitrite production by MDSCs during direct contact with T cells resulted in nitration of the T-cell receptor and CD8 molecules, which altered the specific peptide binding of the T cells and rendered them unresponsive to antigen-specific stimulation[80]. However, the T cells maintained their responsiveness to non-specific stimuli. This phenomenon of MDSC-mediated antigen-specific T-cell unresponsiveness was also observed *in vivo* in tumour-bearing mice[53].

A very reactive RNOS, peroxynitrite, can directly inactivate the T-cell receptor via nitration



Gabrilovich et al., *Nat Rev Immunol* 2012

Other mechanisms of immunosuppression by MDSCs

Depletion of arginine, which impairs T-cell function

Arginase 1 and iNOS. Historically, the suppressive activity of MDSCs has been associated with the metabolism of L-arginine. L-arginine serves as a substrate for two enzymes, iNOS (which generates NO) and arginase 1 (which converts L-arginine to urea and L-ornithine). MDSCs express high levels of both arginase 1 and iNOS, and a direct role for both of these enzymes in the inhibition of T-cell function is well established; this has been reviewed recently[59,60]. Recent data suggest that there is a close correlation between the availability of L-arginine and the regulation of T-cell proliferation[11,61]. The increased activity of arginase 1 in MDSCs leads to enhanced L-arginine catabolism, which depletes this non-essential amino acid from the microenvironment. The shortage of L-arginine inhibits T-cell proliferation through several different mechanisms, including decreasing their expression of CD3 ζ-chain[62] and preventing their upregulation of the expression of the cell cycle regulators cyclin D3 and cyclin-dependent kinase 4 (REF. 63). NO suppresses T-cell function through various different mechanisms that involve the inhibition of JAK3 and STAT5 function in T cells[64], the inhibition of MHC class II expression[65] and the induction of T-cell apoptosis[66].

Gabrilovich et al., *Nat Rev Immunol* 2012

Mechanisms of immunosuppression by MDSCs

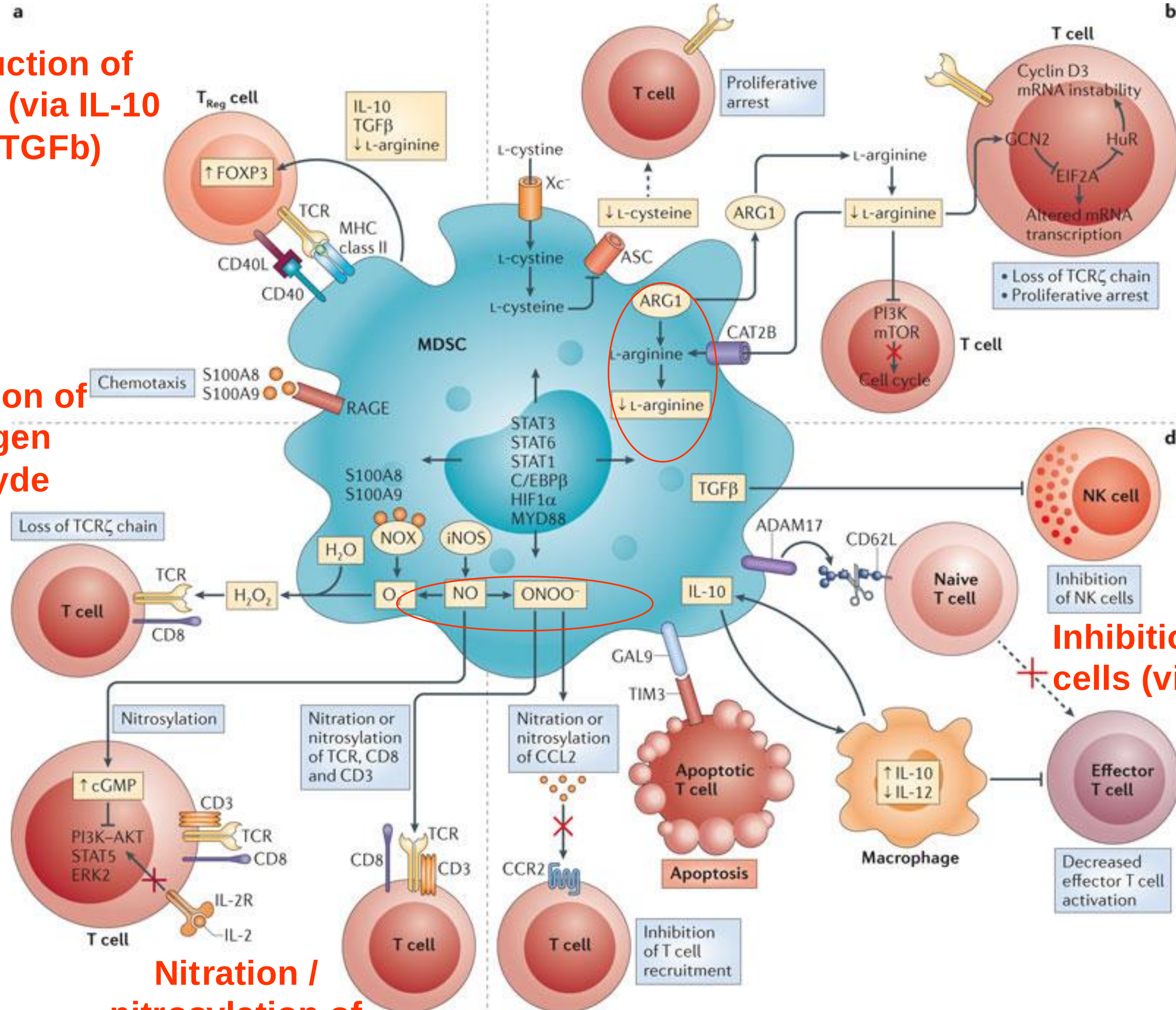
Induction of Tregs (via IL-10 & TGFb)

Depletion of L-arginine in microenv and inhibition of T cell proliferation

Production of hydrogen peroxyde

Inhibition of NK cells (via TGFb)

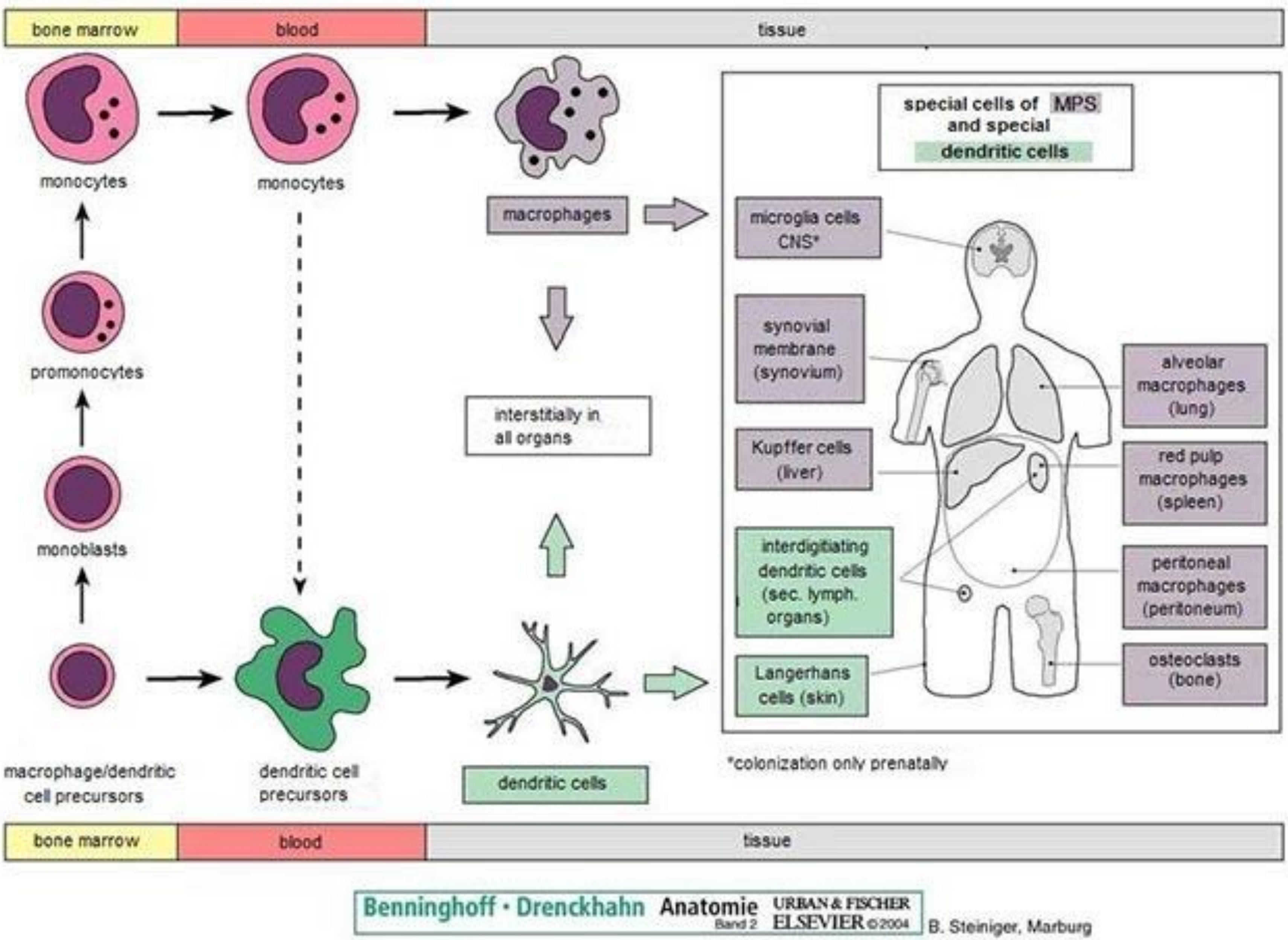
Nitration / nitrosylation of T cell receptors



Gabrilovich et al., *Nat Rev Immunol*, 2012

Tumor-associated macrophages

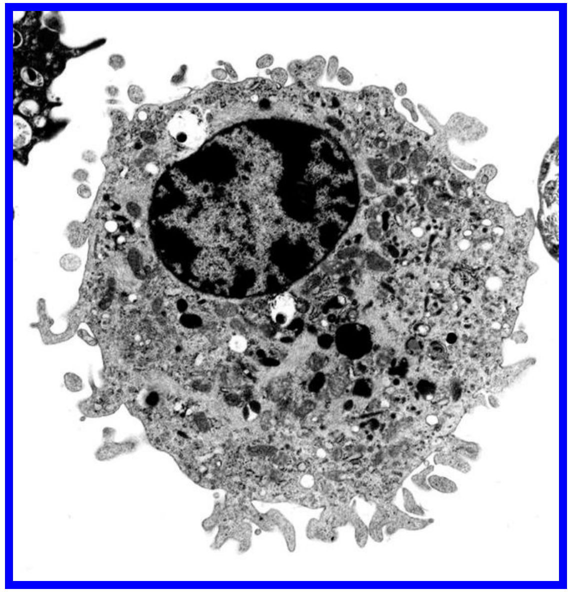
Tissue macrophages



Macrophages

Inflammation

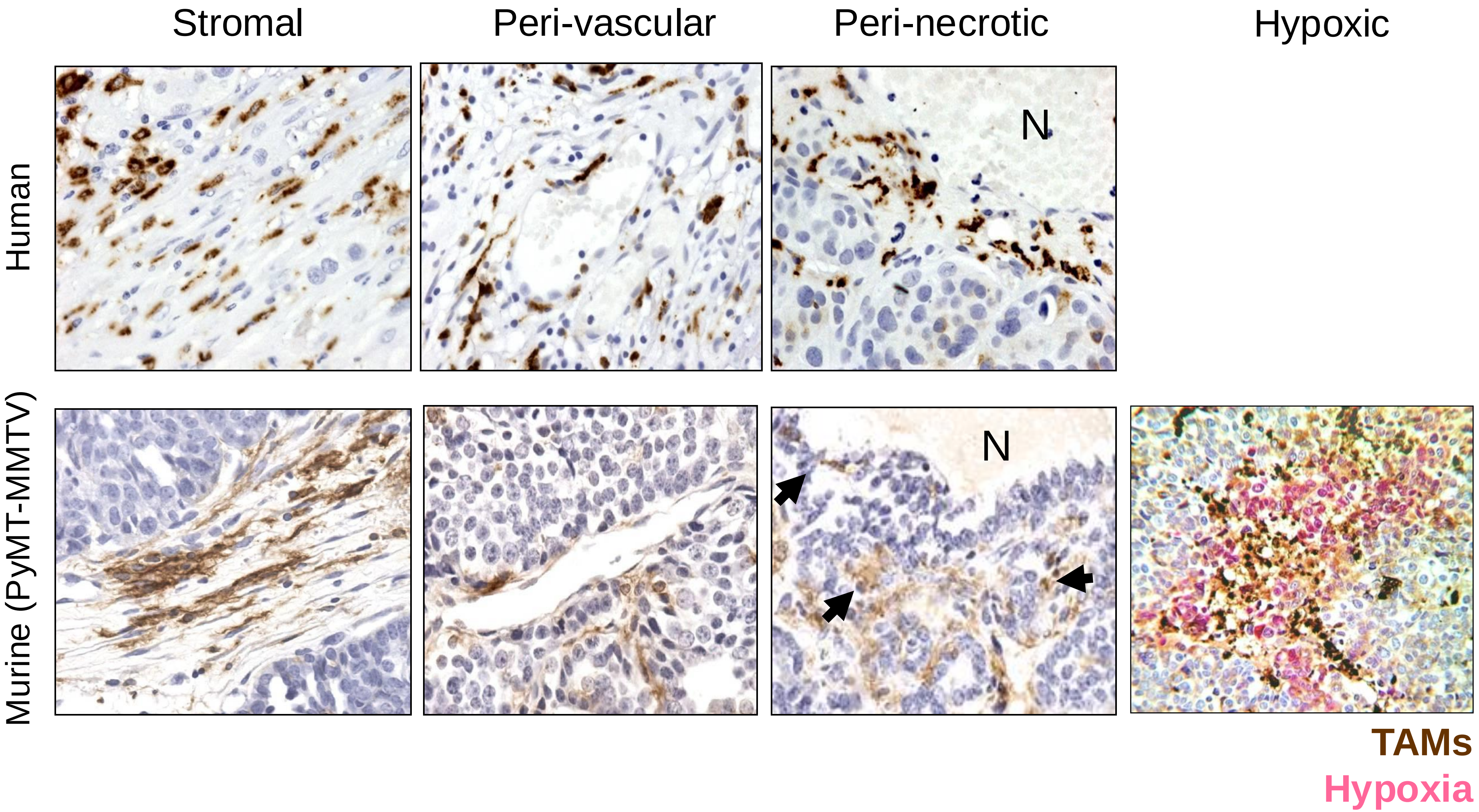
Tissue
homeostasis



Innate immunity
(phagocytosis)

Angiogenesis
Tissue remodeling

Tumor-associated macrophages (TAMs) are present in different tumor microenvironments



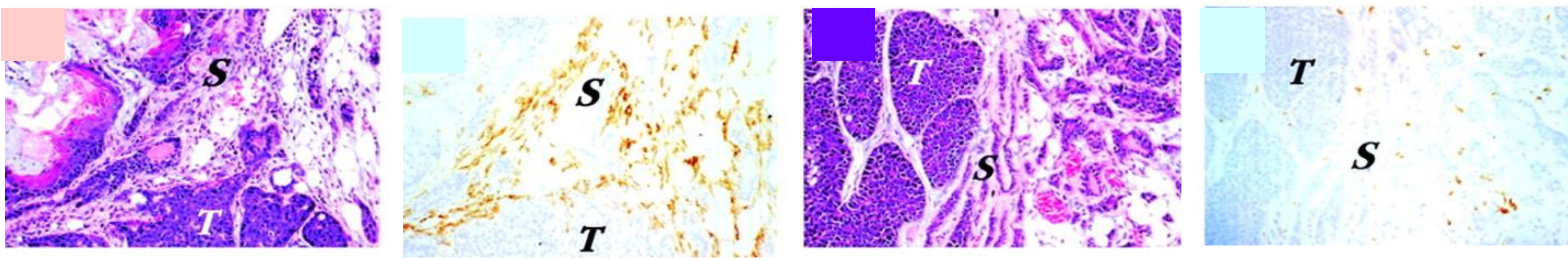
Adapted from Lewis & Pollard, *Cancer Res.* 2006
Adapted from Murdoch *et al*. *Blood*. 2004

TAMs accelerate tumor progression in mouse models

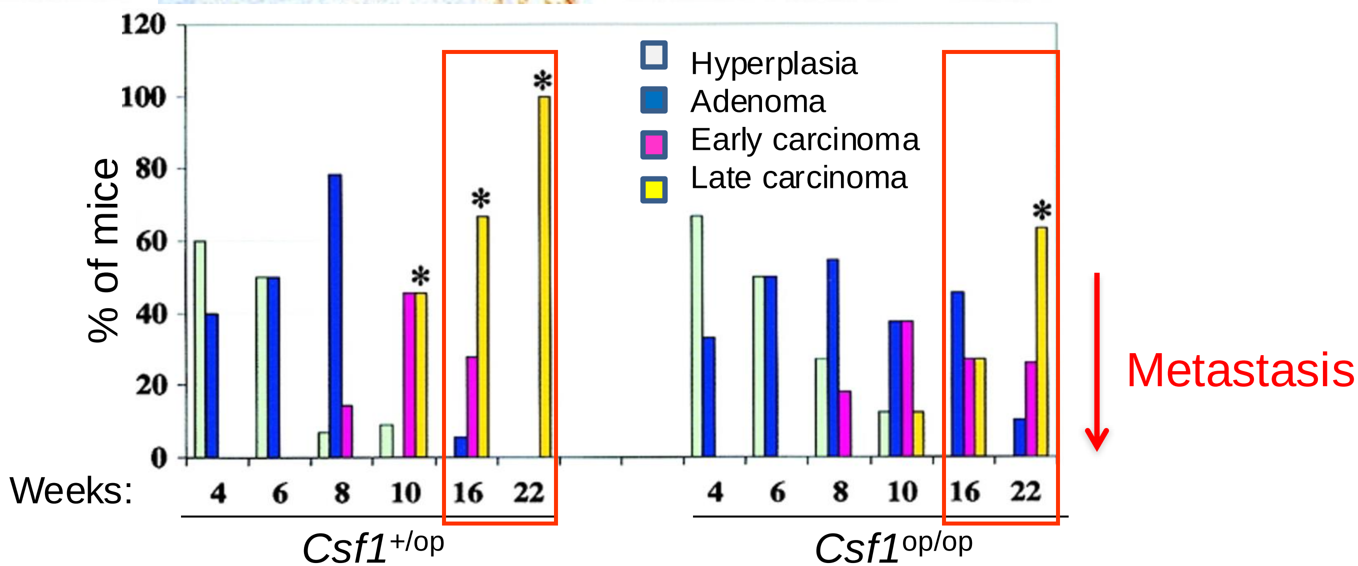
Colony stimulating factor-1 (CSF1) is a monocyte/macrophage growth and pro-survival factor. Mice lacking CSF1 (*Csf1*$^{op/op}$) have reduced macrophage numbers in several tissues.

MMTV-PyMT (mammary tumor model)

Csf1$^{+/op}$ | *Csf1*$^{op/op}$ (macrophage deficient)



T = tumor
S = stroma

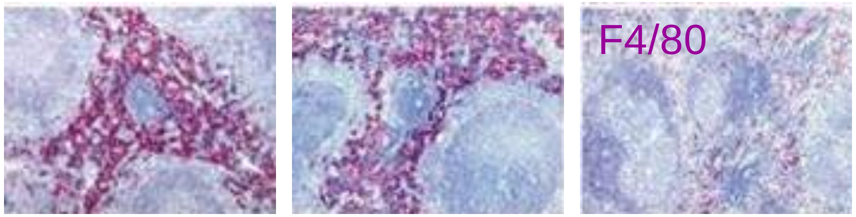


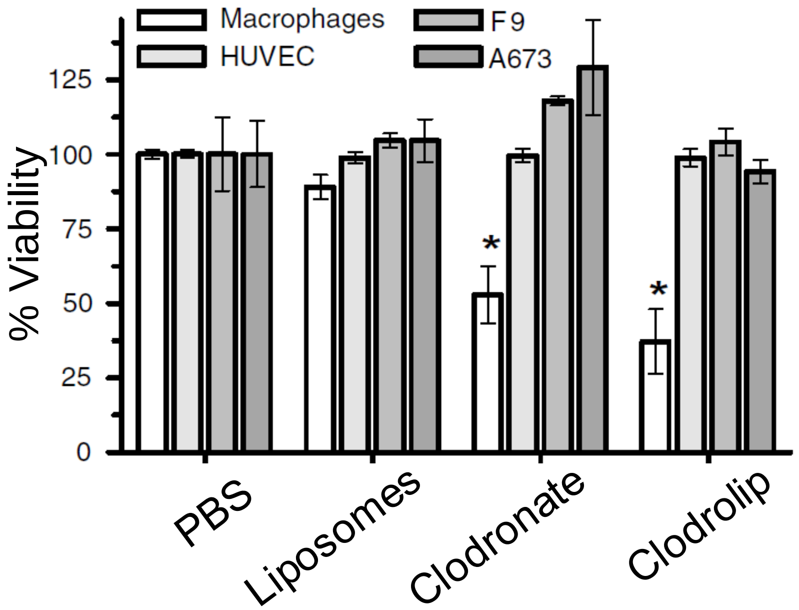
Adapted from Lin *et al*., *J Exp Med* 2001

TAM ablation decreases tumor growth in mice

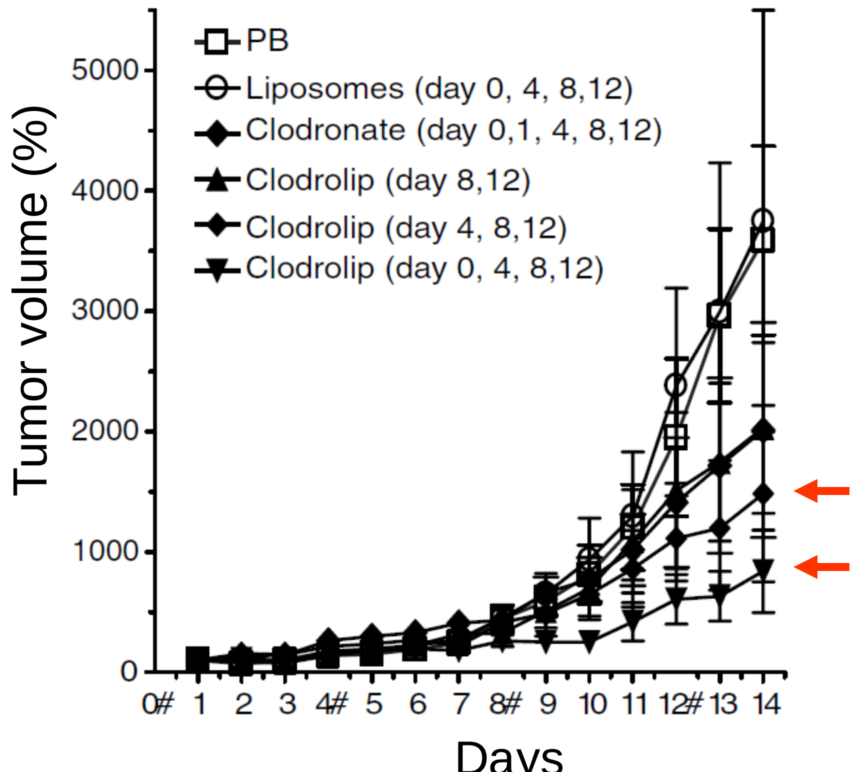
Clodronate is a biphosphonate that depletes macrophages from tissues. Delivery of clodronate is improved by combination with lipid carriers (clodrolip)

PBS Clodronate Clodrolip



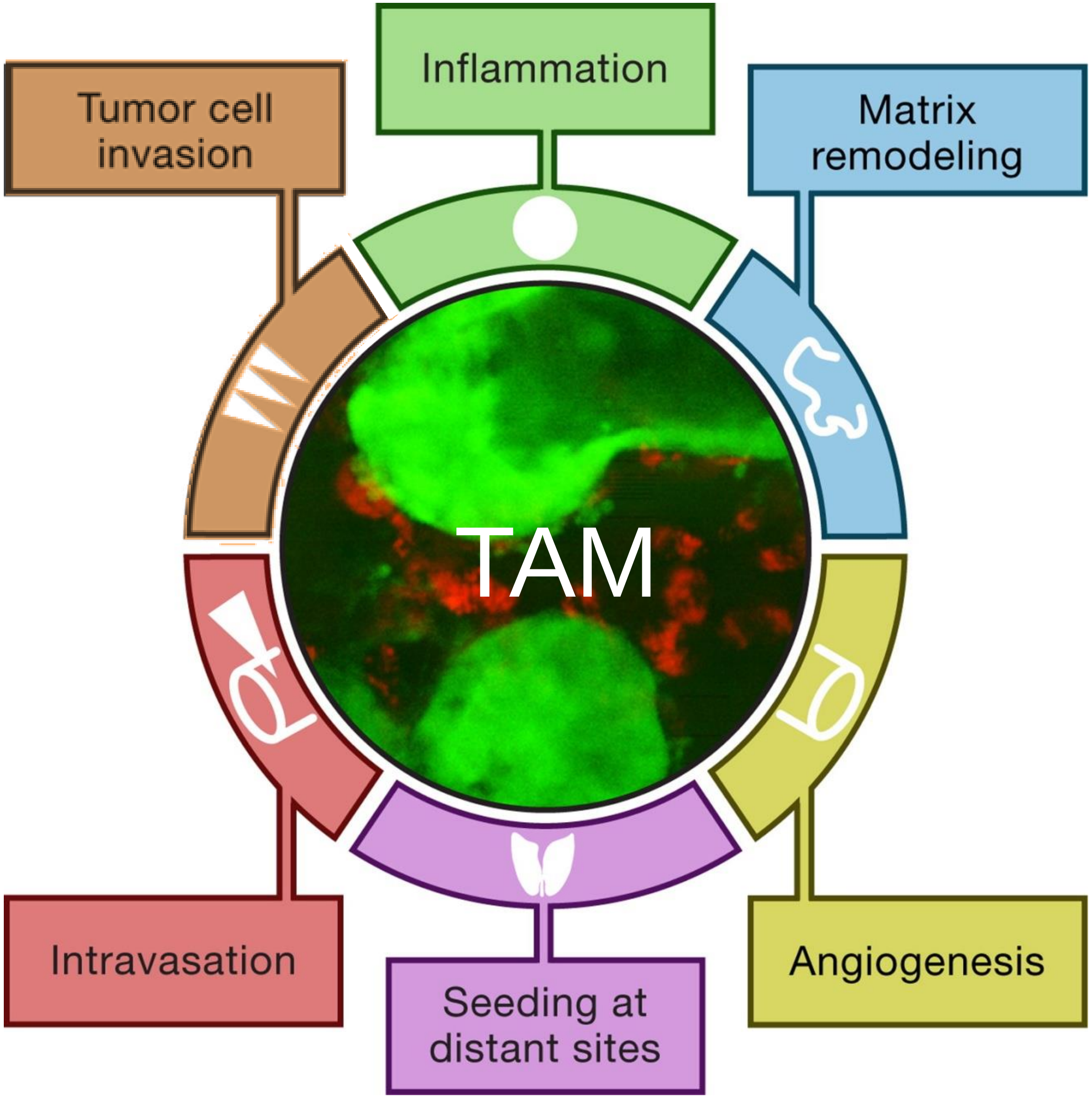


F9 teratocarcinoma



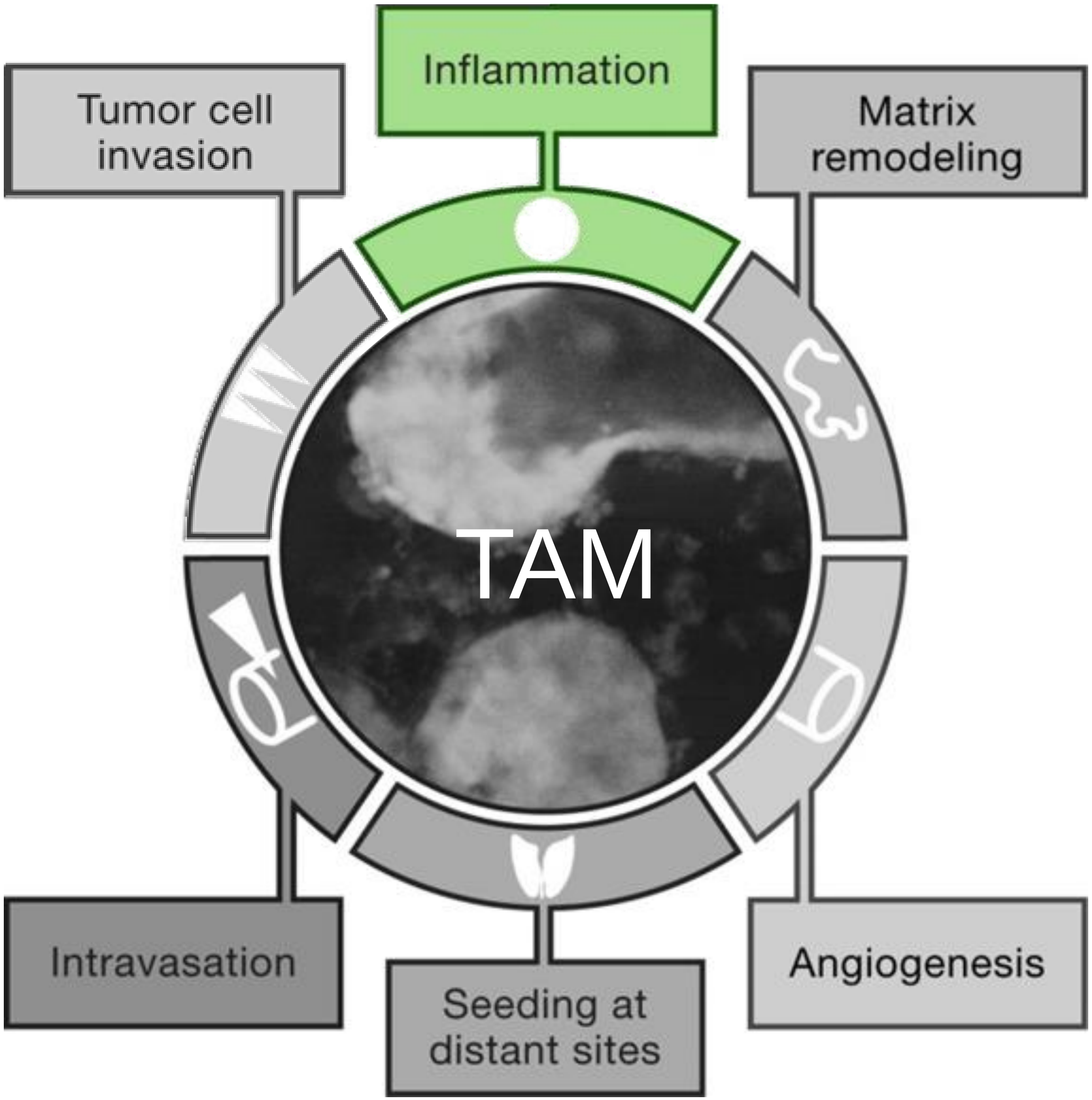
Adapted from Zeisberger *et al*., *Br J Cancer* 2006

TAMs provide extrinsic support to tumor growth



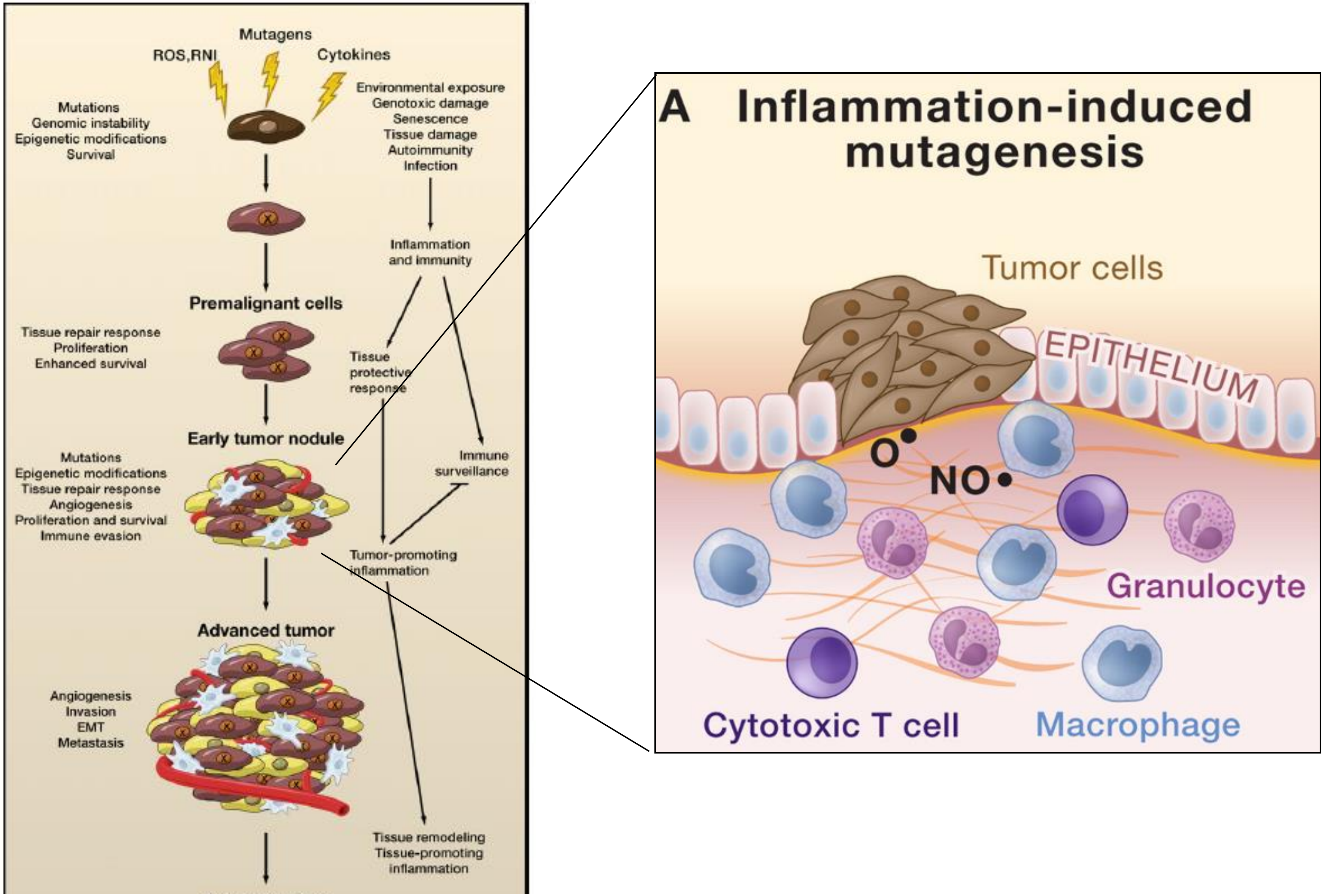
Adapted from Condeelis & Pollard, *Cell* 2006

Inflammation and cancer: Role of TAMs



Adapted from Condeelis & Pollard, *Cell* 2006

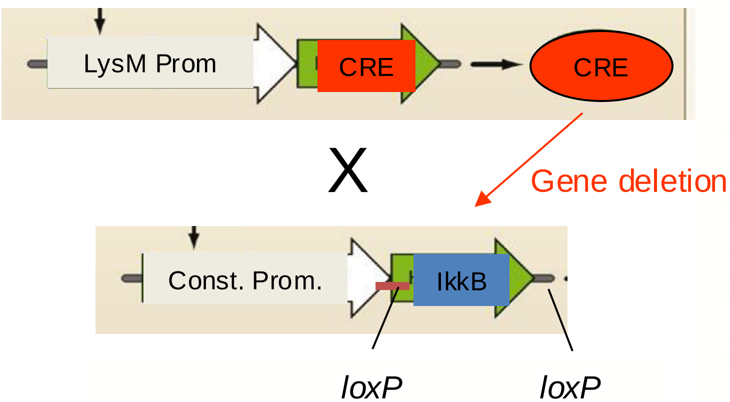
TAMs sustain tumor-promoting inflammation

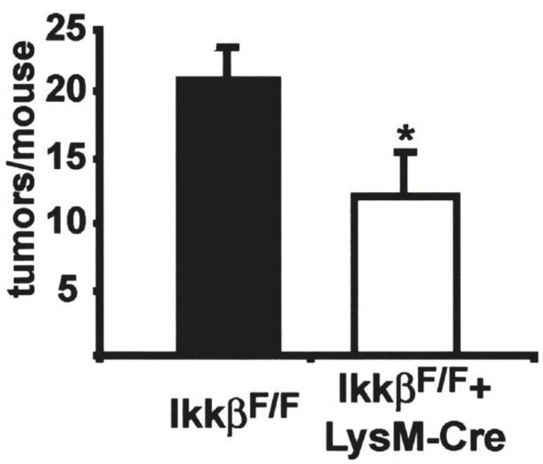


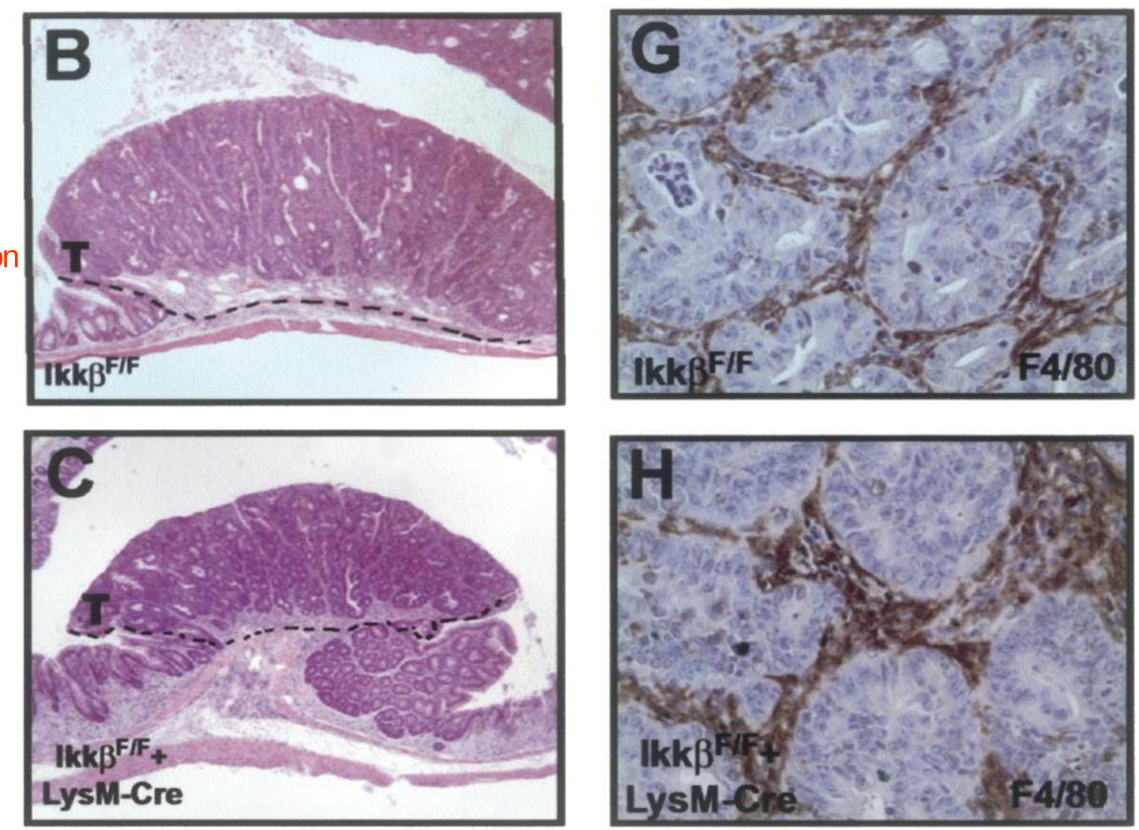
Adapted from Qian & Pollard, *Cell* 2010

Blocking NF-κB activation in macrophages delays colorectal carcinogenesis in mice

macrophage-cell specific deletion of Ikkβ

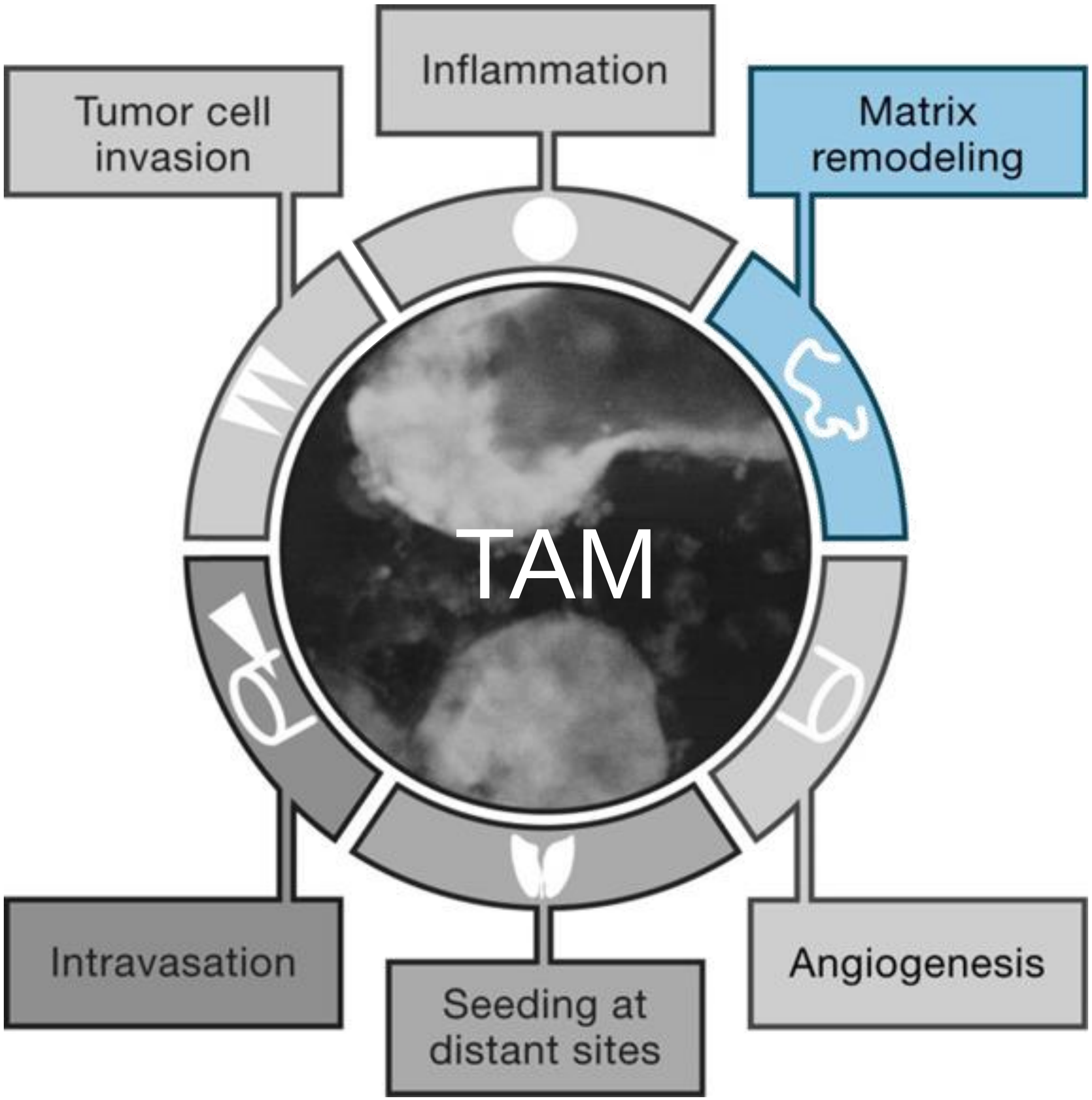






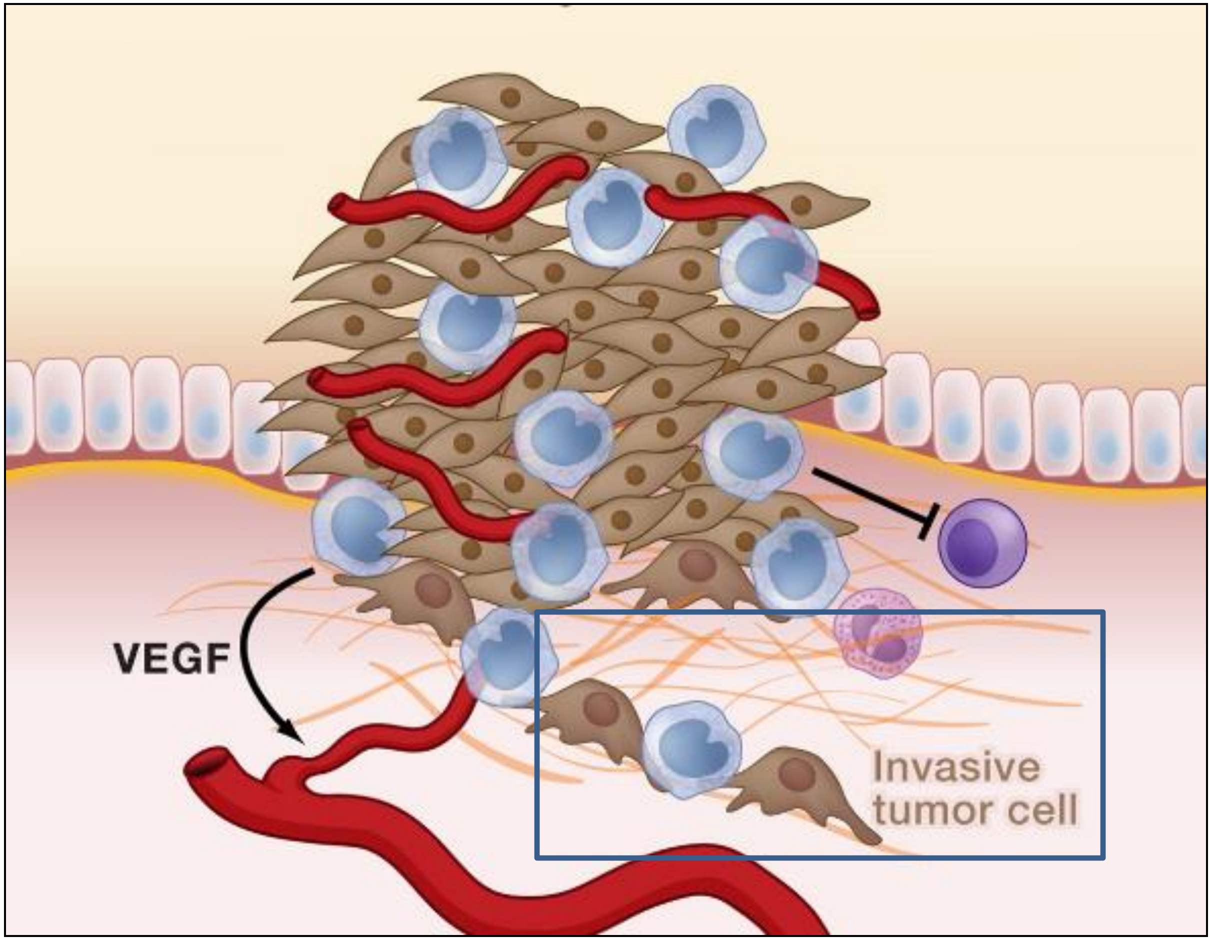
Adapted from Greten *et al.*, *Cell* 2004

TAMs promote extracellular matrix remodeling



Adapted from Condeelis & Pollard, *Cell* 2006

TAMs release ECM-remodeling factors that support angiogenesis and cancer cell motility



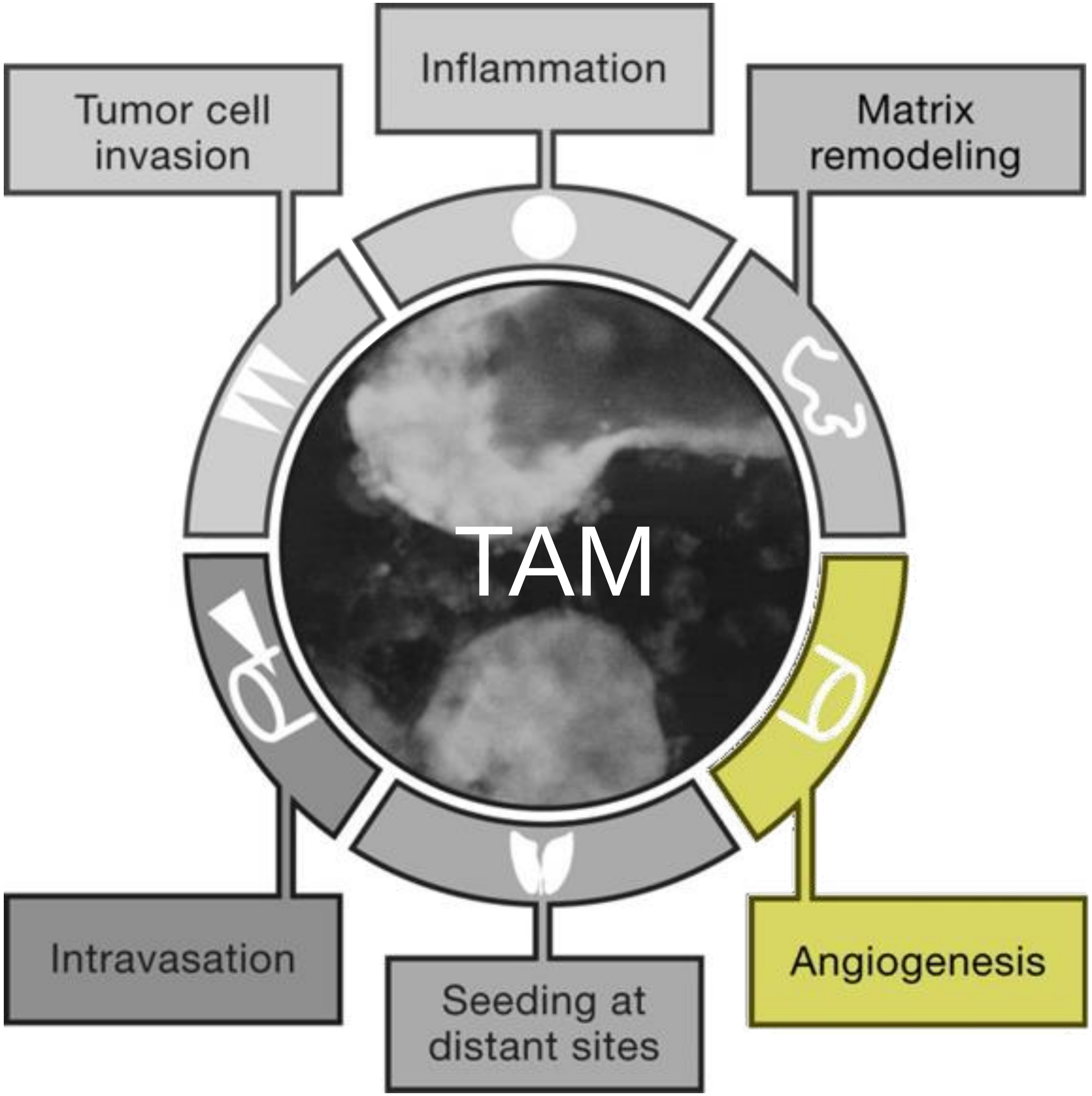
Matrix metalloproteinases (MMPs)*

Cathepsins

Other proteases

Adapted from Qian & Pollard, *Cell* 2010

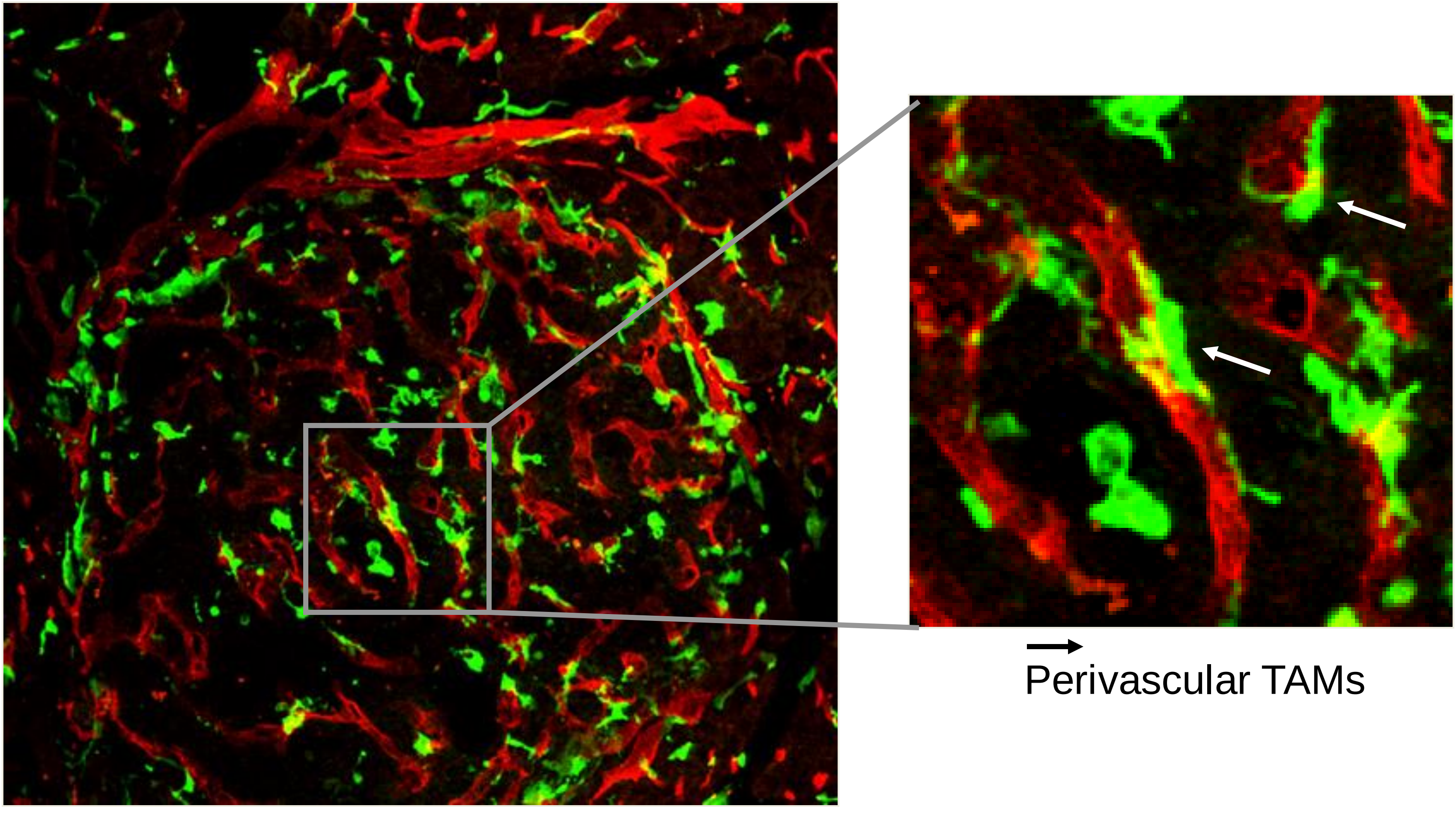
TAMs promote angiogenesis



Adapted from Condeelis & Pollard, *Cell* 2006

A fraction of TAMs localize around tumor blood vessels

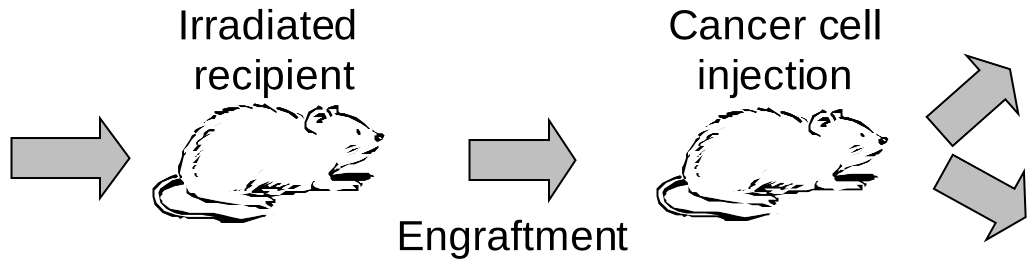
RIP1-Tag2 pancreatic islet tumor

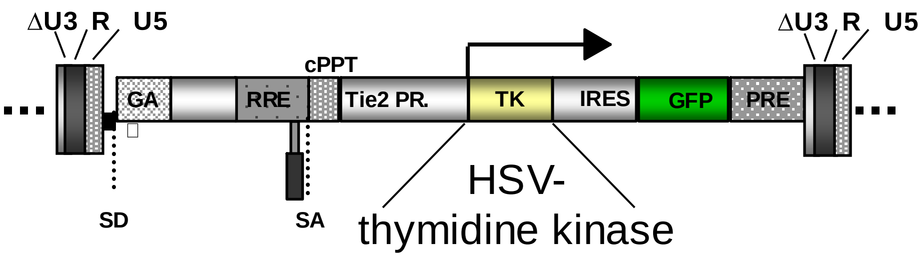


Adapted from De Palma *et al*., *Cancer Cell* 2005

TAMs sustain tumor angiogenesis

Bone marrow hematopoietic stem cells transduced with *conditional suicide gene*

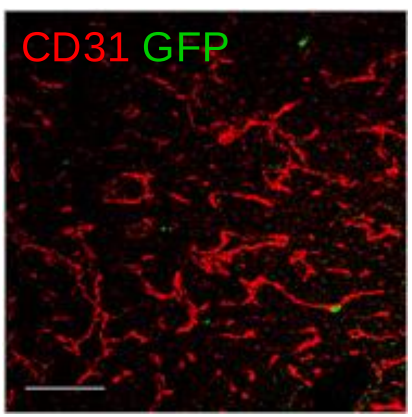




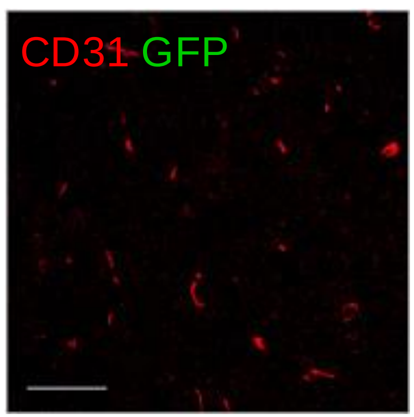
The gene is expressed only in the TAMs that derive from the transplant

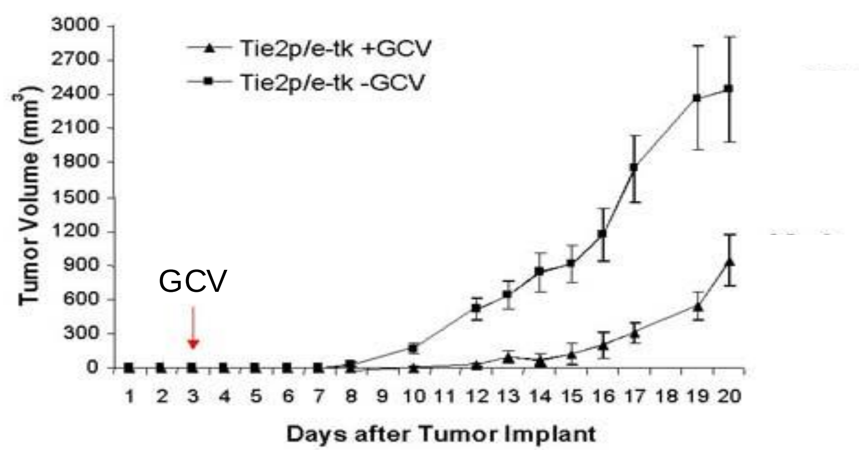
Lewis lung carcinoma

– GCV



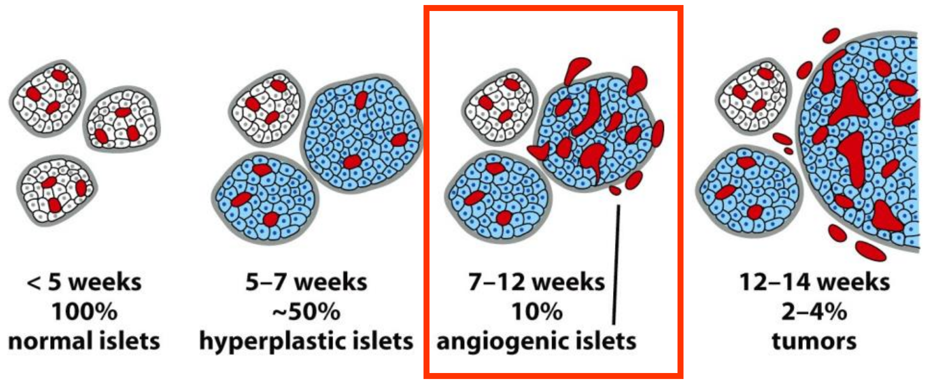
+ GCV



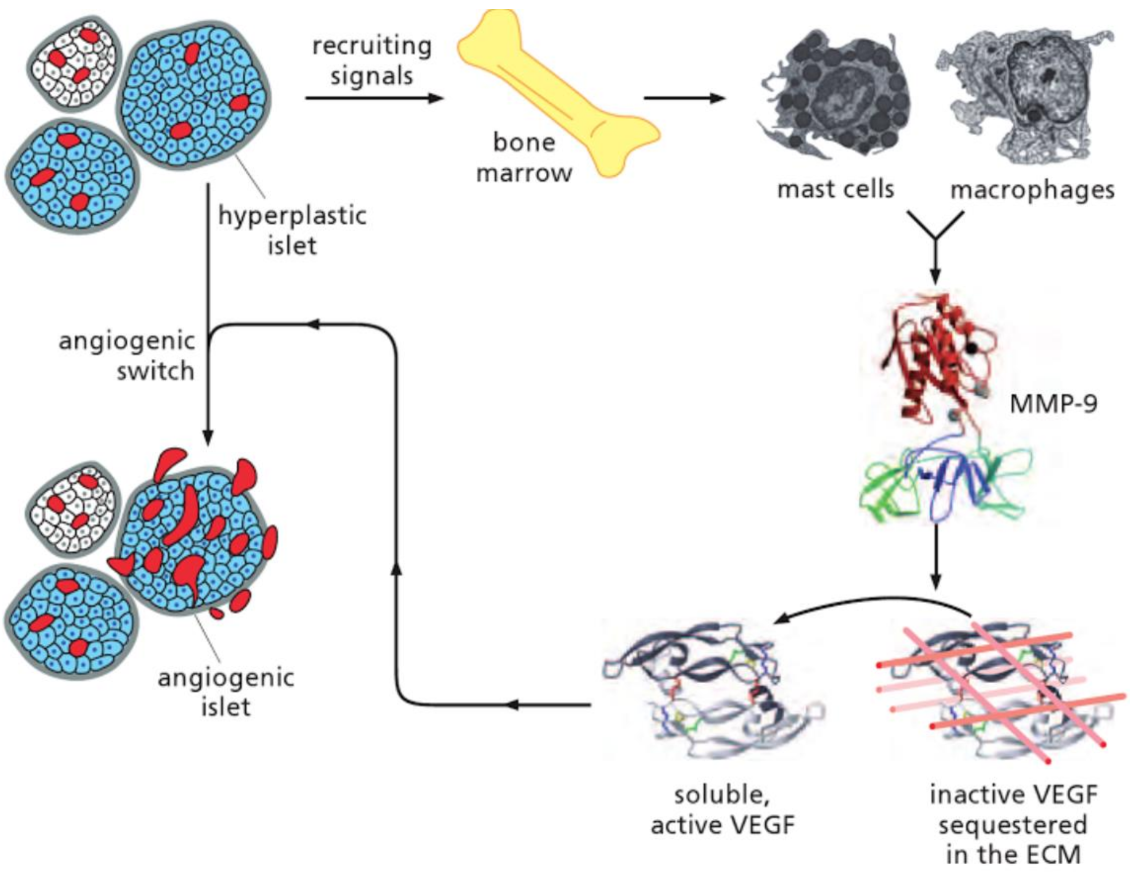


Adapted from De Palma *et al.*, *Nat Med* 2003

MMP9 expression by macrophages triggers the angiogenic switch

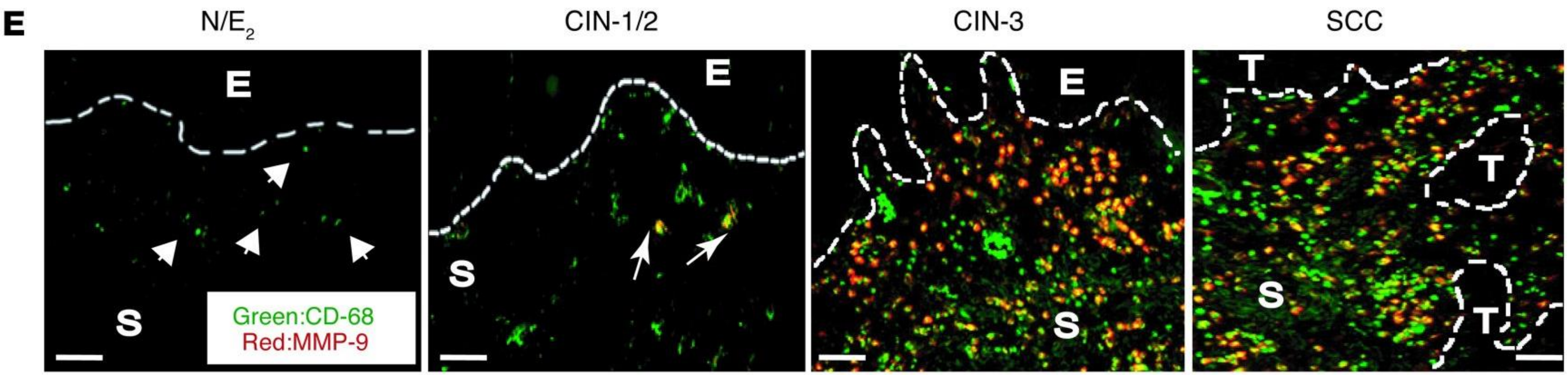


GEMM of pancreatic islet carcinogenesis: RIP1-Tag2 model



TAM-derived MMP9 supports tumor angiogenesis

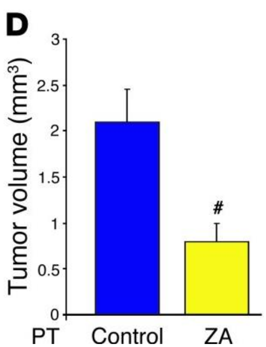
K14-HPV cervical cancer model

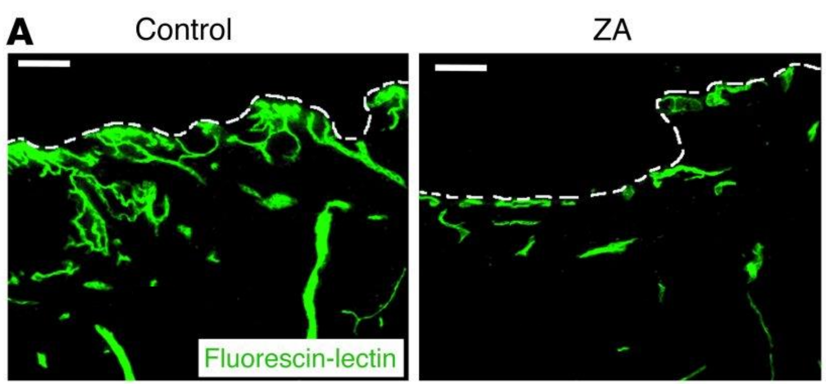


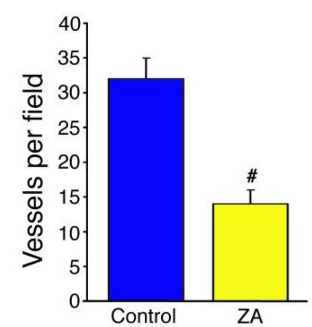
Tumor progression

S: stroma
E: epithelium
CD68: TAMs

Zoledronic acid (ZA) is a biphosphonate and MMP inhibitor

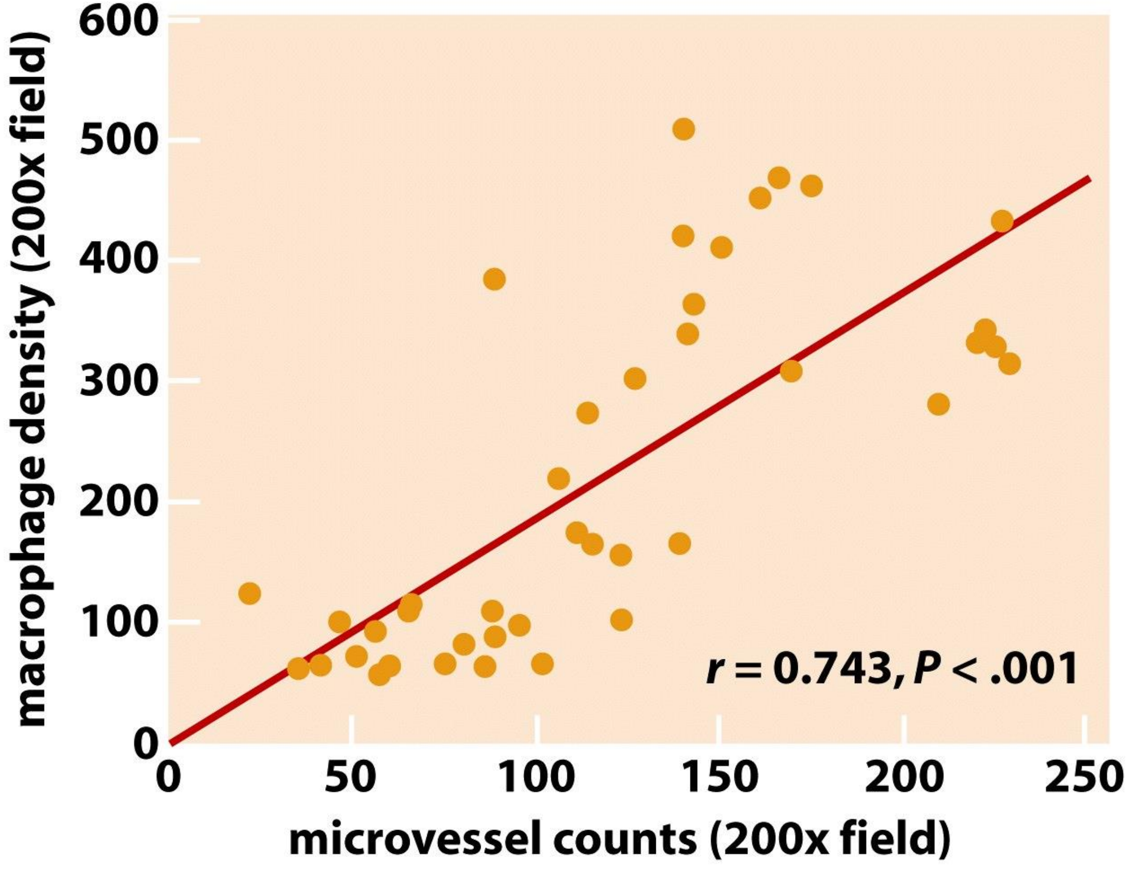






Adapted from Giraudo *et al.*, *J Clin Invest* 2004

High TAM numbers correlate with increased tumor angiogenesis

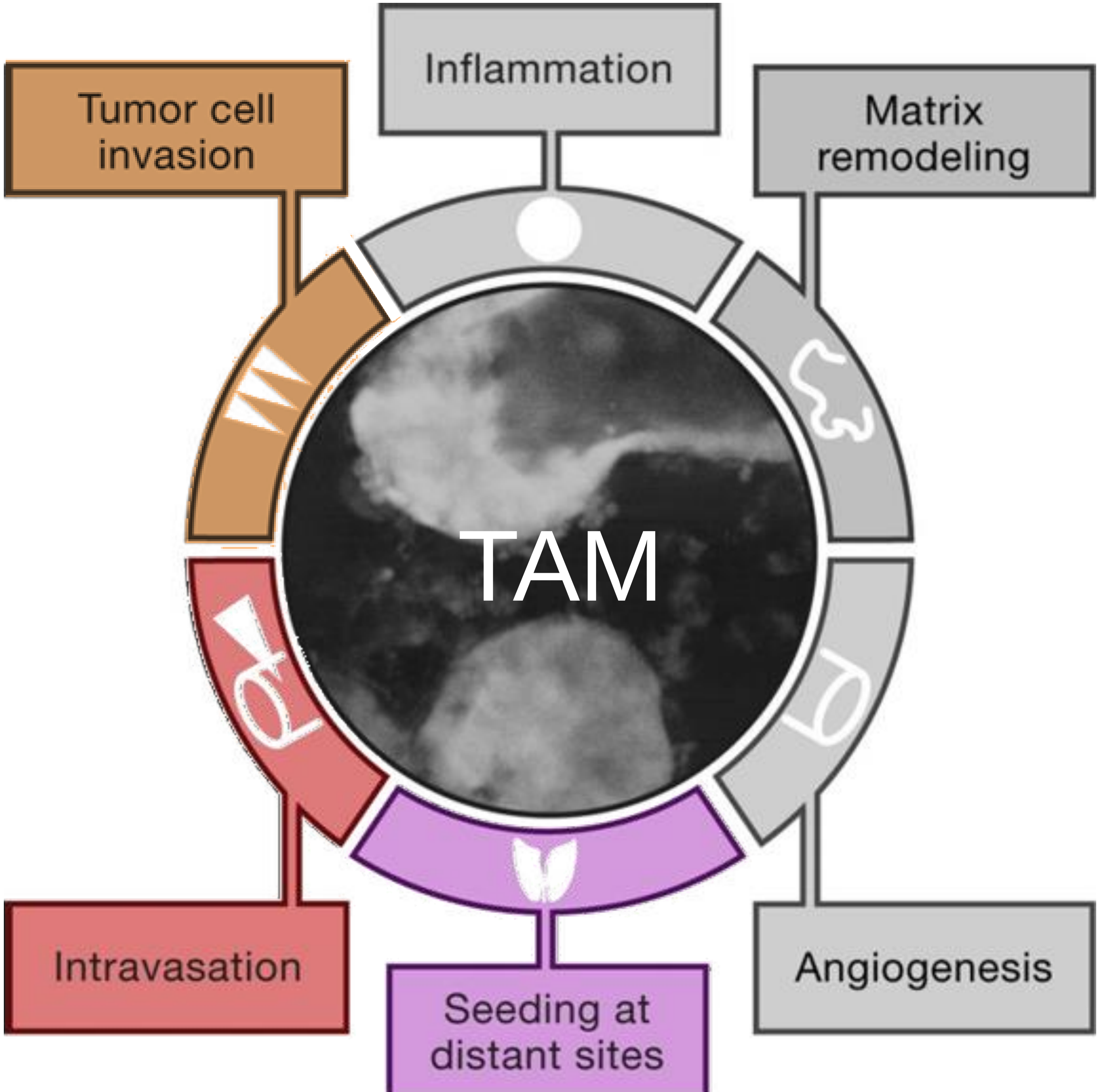


VEGF
FGF
Proteases

(more in next lectures on tumor angiogenesis)

Figure 13.25c *The Biology of Cancer* (© Garland Science 2007)

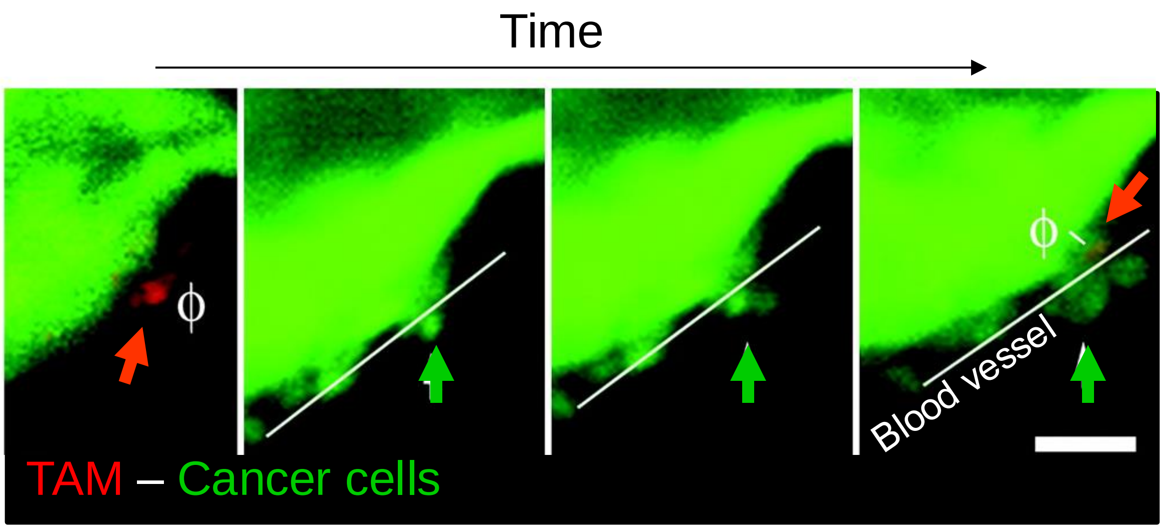
TAMs promote cancer cell invasion, intravasation and metastasis

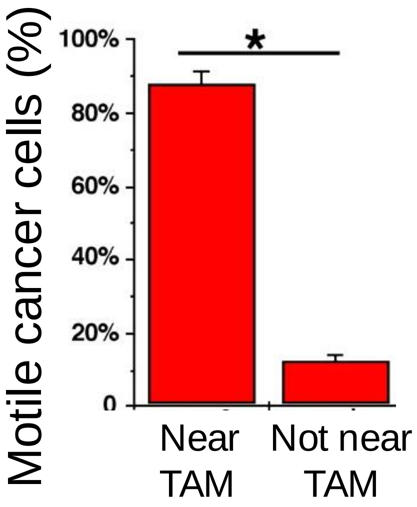


Adapted from Condeelis & Pollard, *Cell* 2006

Perivascular TAMs facilitate cancer cell intravasation by enhancing their motility

MMTV-PyMT mammary carcinoma

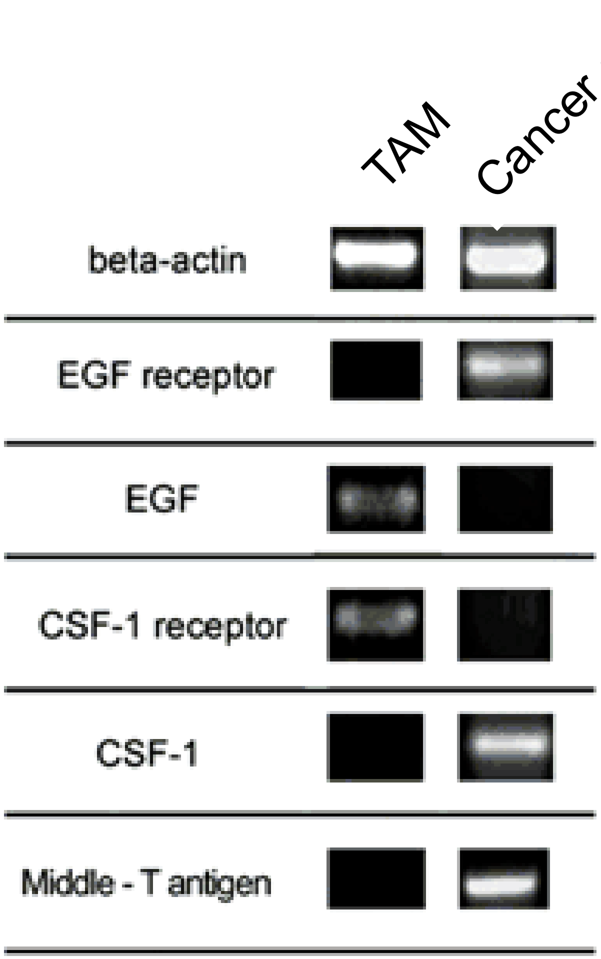


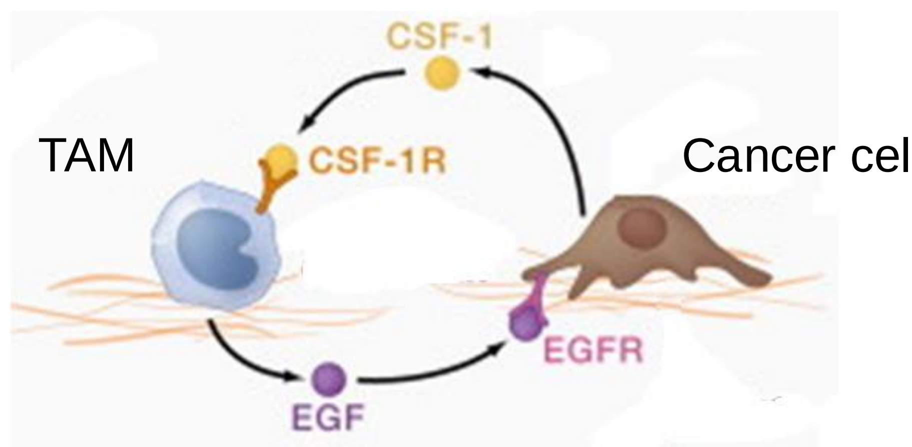


Adapted from Wyckoff *et al.*, *Cancer Res* 2007

A CSF1 / EGF paracrine loop between TAMs and tumor cells promotes cancer cell invasion

MMTV-PyMT mammary carcinoma



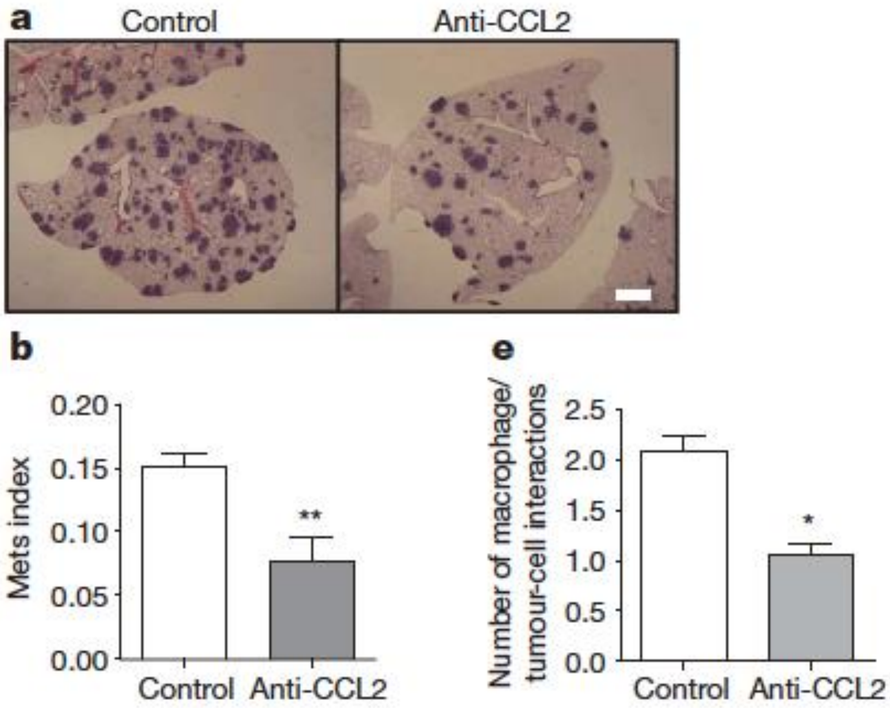


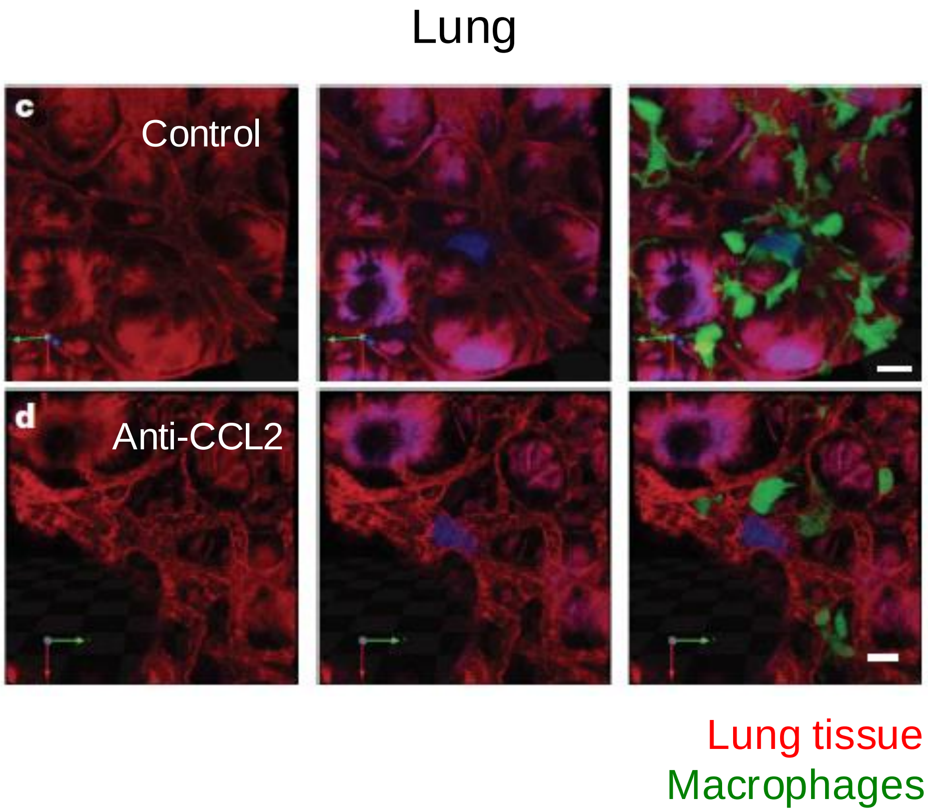
Adapted from Wyckoff *et al*., *Cancer Res* 2004

Adapted from Qian & Pollard, *Cell* 2010

Blocking CCL2 inhibits metastasis

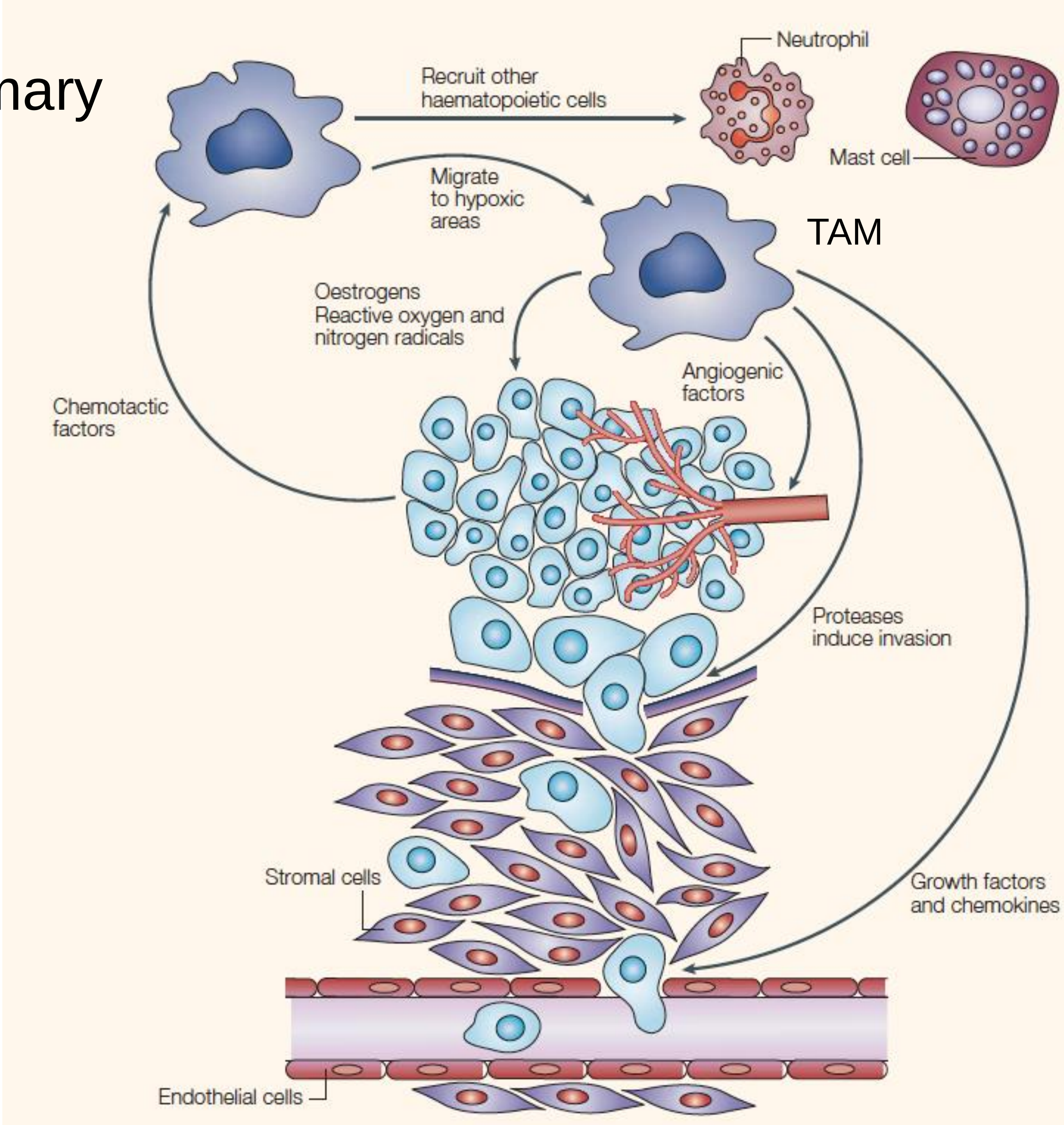
CCL2 is a chemokine that binds CCR2 on monocytes, the precursors of TAMs. Expression of CCL2 in the lung tissue promotes recruitment of CCR2+ monocytes that differentiate into TAMs and promote lung metastasis of breast cancer





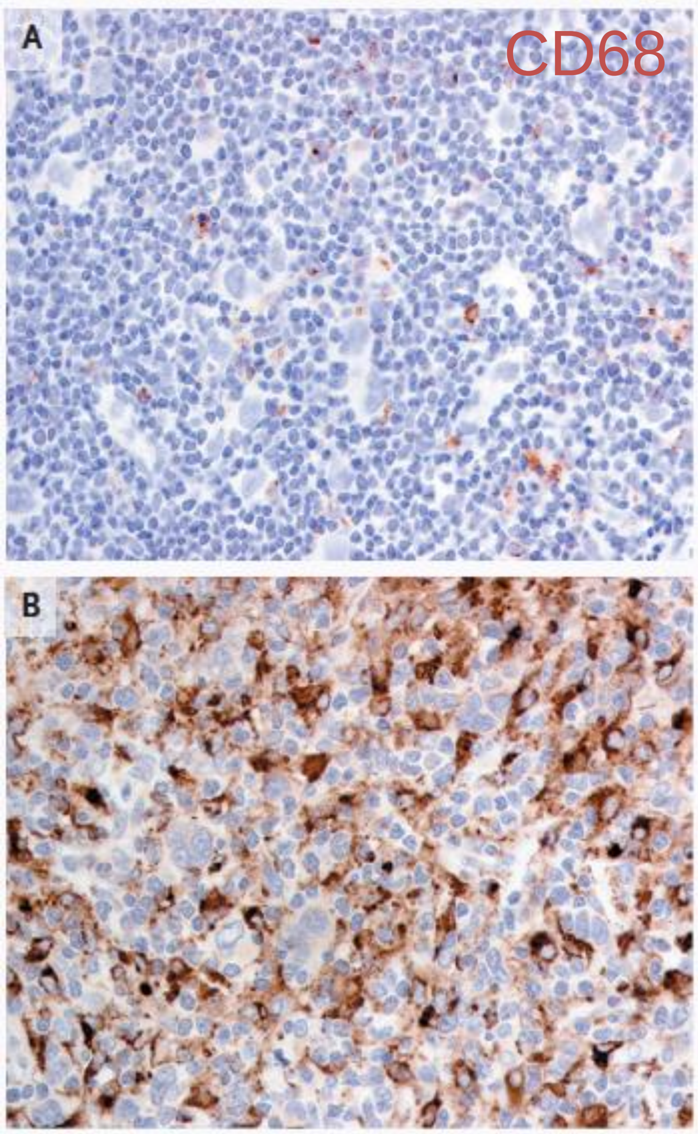
Qian *et al*., *Nature* 2011

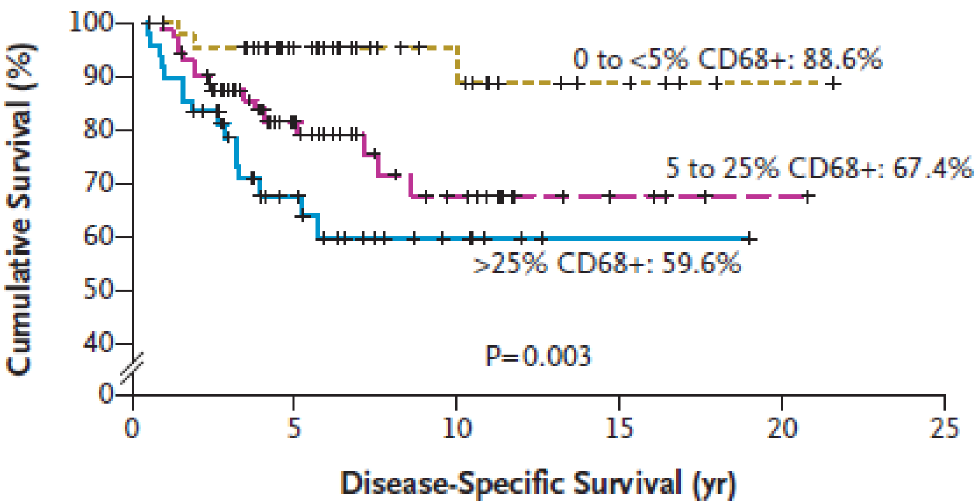
Summary



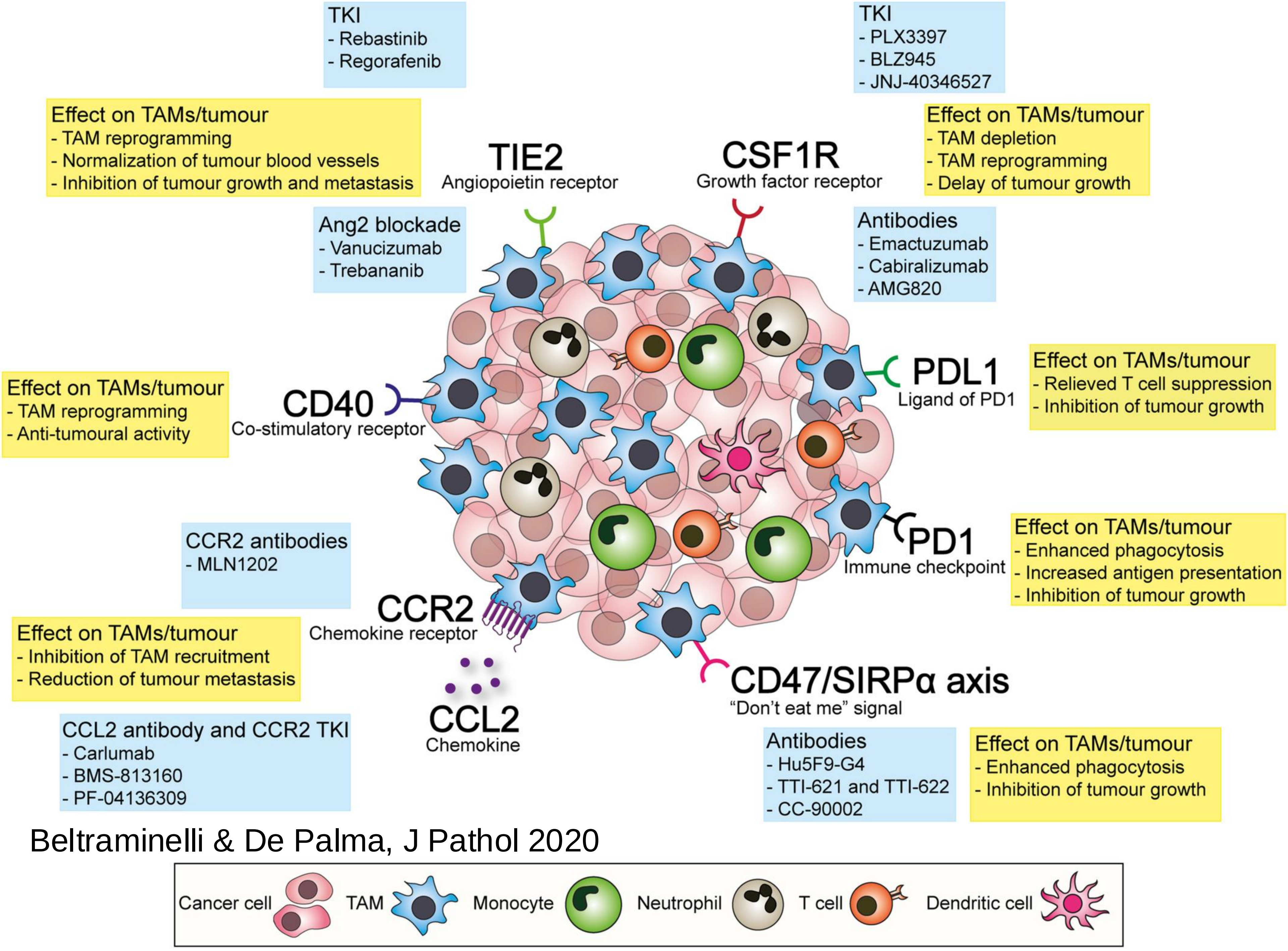
Neutrophil
Recruit other haematopoietic cells
Mast cell
Migrate to hypoxic areas
TAM
Oestrogens
Reactive oxygen and nitrogen radicals
Angiogenic factors
Chemotactic factors
Proteases induce invasion
Stromal cells
Growth factors and chemokines
Endothelial cells

High TAM infiltration correlates with poor survival in Hodgkin's lymphoma patients





Adapted from Steidl *et al*., *New Engl J Med* 2010



Beltraminelli & De Palma, J Pathol 2020