METASTASIS

Learning objectives

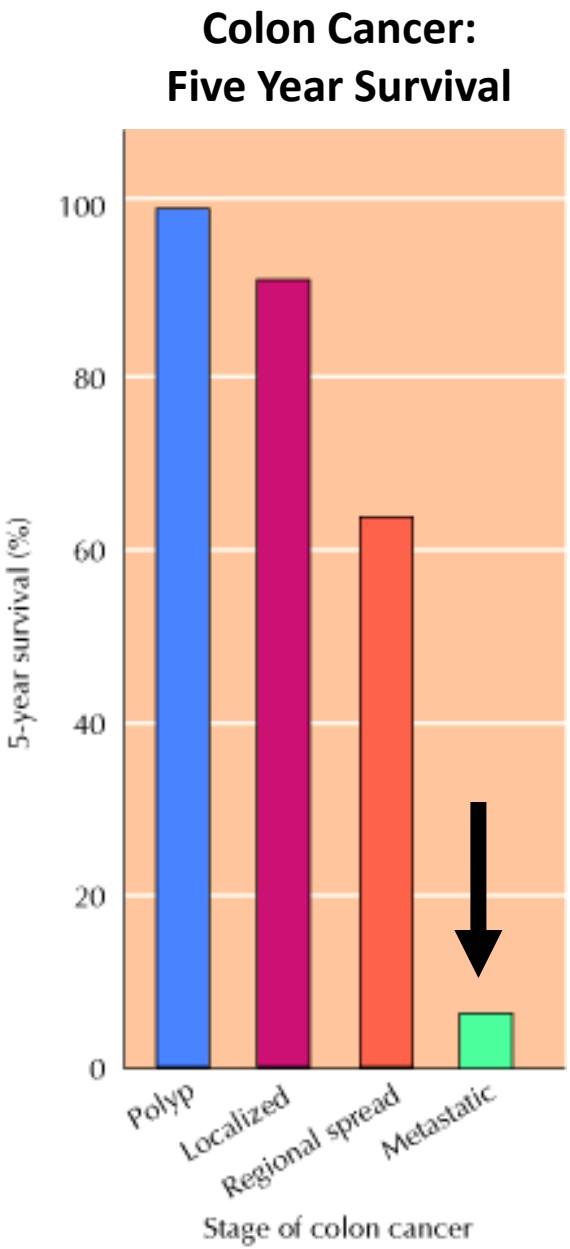
- Metastases Patterns
- Mechanisms of tissue invasion
- Disseminated tumor cells and dormancy
- Parallel evolution of primary and secondary tumors
- Metastasis supporting niches

Spread of Cancer

- **Local Invasion** (direct extension into adjacent tissues)

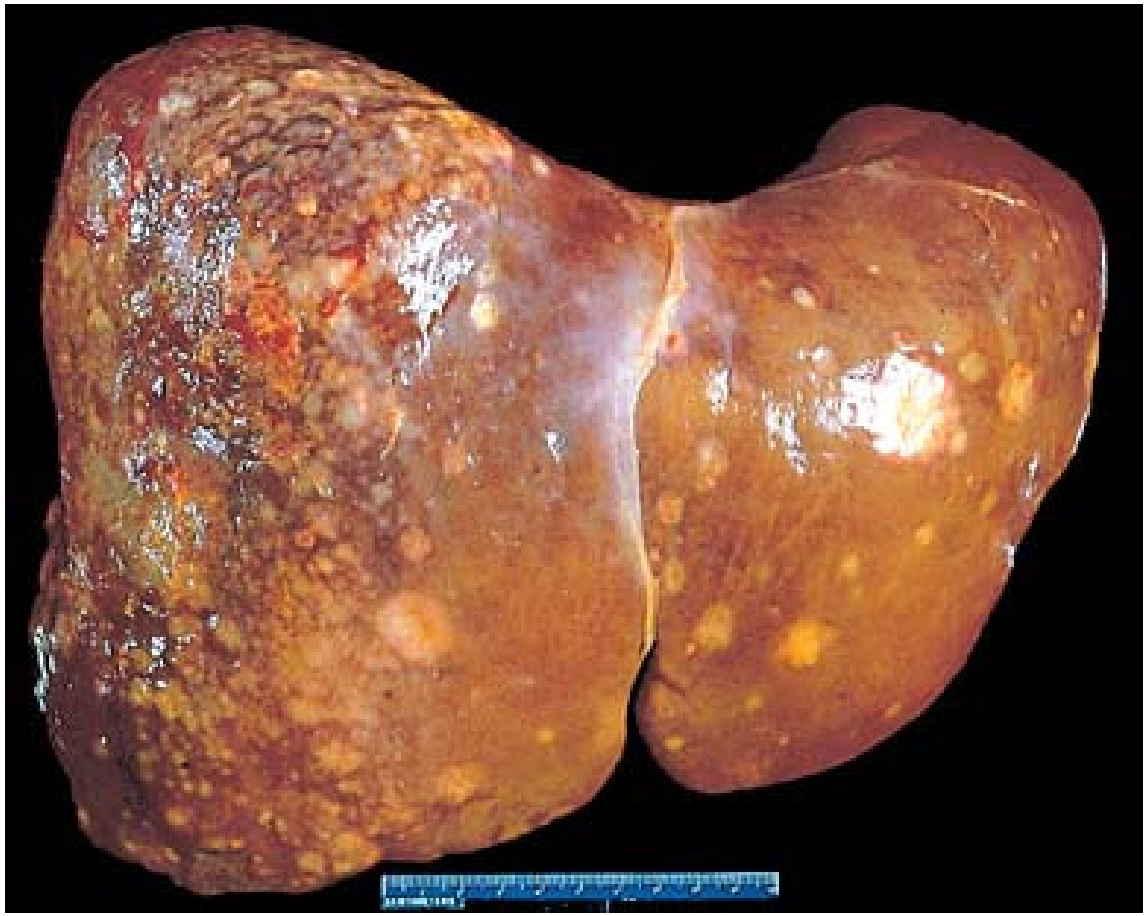
- **Metastasis** (spread at a distance)
  - Hematogenous (via blood vessels)
  - Lymphatic (via lymph vessels and nodes)
  - Body cavity seeding (pleural and peritoneal)
  - **about 90% of cancer deaths are due to metastasis**

- **Adverse Consequences**
  - Impingement on essential structures
  - Ulceration into blood vessels
  - Ulceration through barriers to infection
  - **Cachexia** (wasting): caused by factors secreted by cancer cells and perhaps by inflammatory cells (e.g. TNF)



from Cooper

Colon CA: Metastasis to Liver

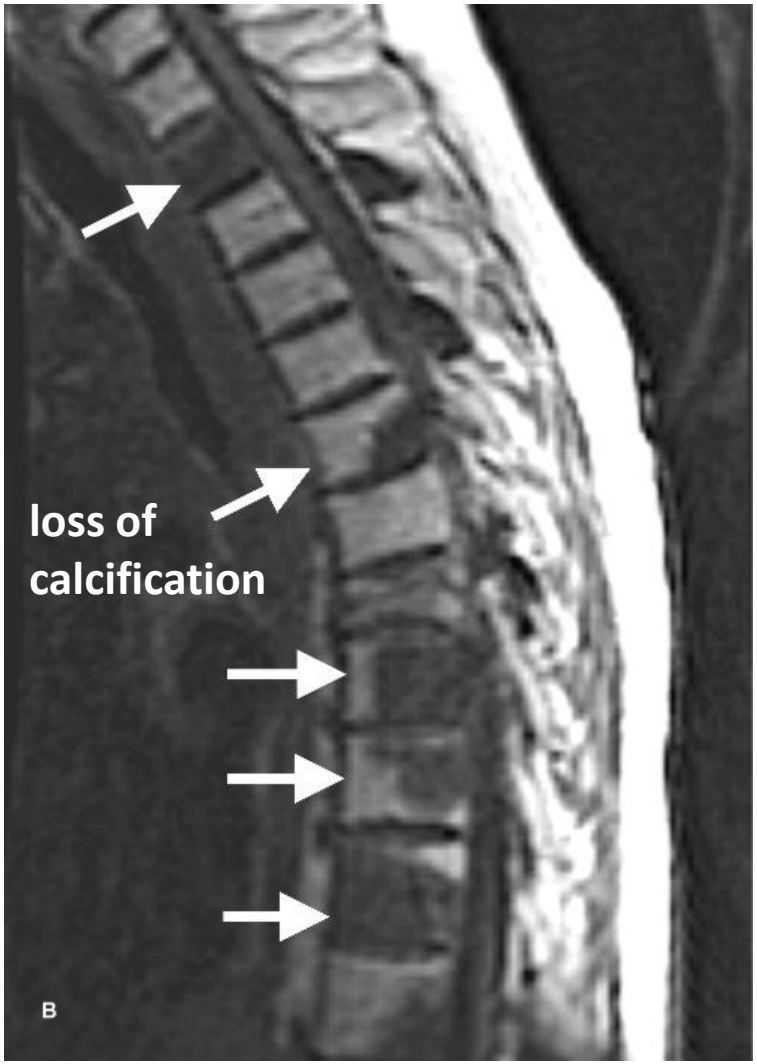


from Robbins

Renal CA: Metastasis to Lung

from Robbins

Lung CA: Metastasis to Spine



from Kufe

Metastases Patterns

Preferential metastatic sites

Primary tumour	Common distant site(s)
Breast adenocarcinoma	Bone, liver, lung
Prostate adenocarcinoma	Bone
Lung small cell carcinoma	Bone, brain
Skin cutaneous melanoma	Brain, liver, bowel
Thyroid adenocarcinoma	Bone
Kidney clear cell carcinoma	Lung, bone, liver, thyroid
Testis carcinoma	Liver
Bladder carcinoma	Brain
Neuroblastoma	Liver, adrenal gland

Metastatic target organs: Where and Why?

Stephen Paget (1889): "Seed and Soil" Theory

cancers can initiate metastasis only in supportive microenvironments

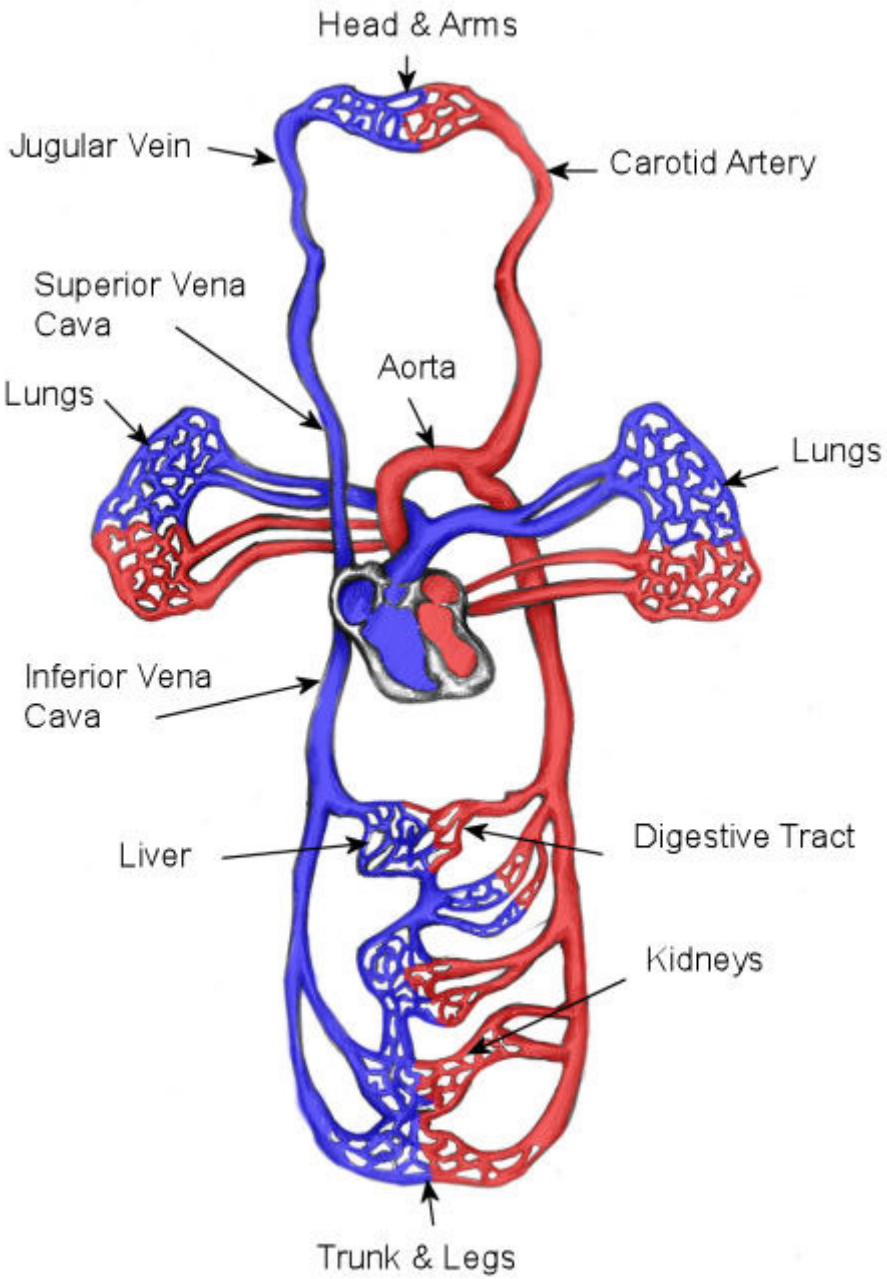
potential molecular mechanisms:

- preferential adhesion in the vessels of the target organ
- selective extravasation
- organ attractants
- organ specific survival and growth signals

James Ewing (1928): Mechanistic theory, hemodynamic patterns

metastatic pattern can be predicted by the venous drainage blood flow

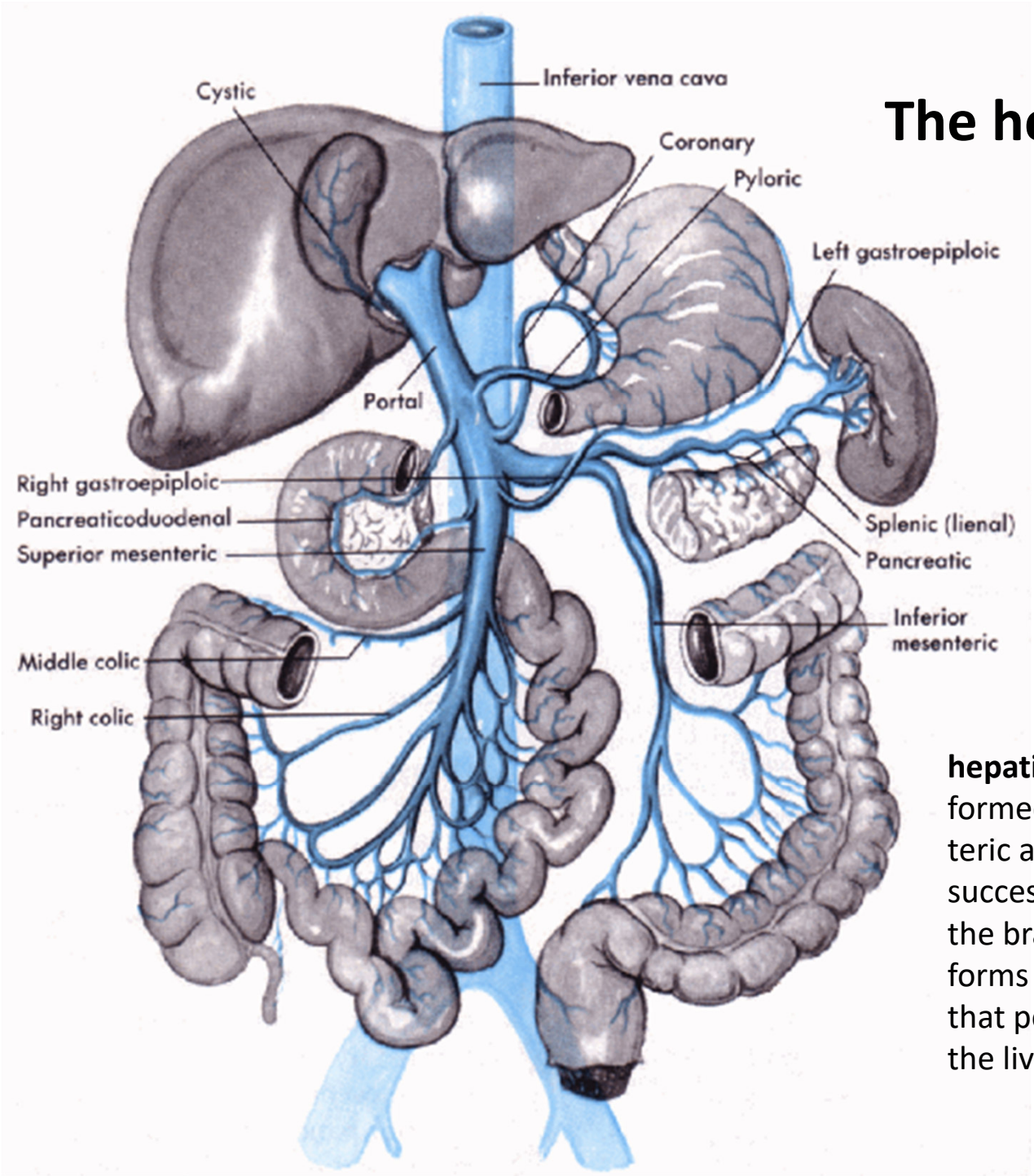
- the organ pattern of metastasis is characteristic of the tumor type and tissue of origin
- 50-70% of the pattern can be predicted by the venous drainage, the remaining 30-50 % may be caused by specific molecular homing mechanisms.



The circulation system

- the lungs are the first organ with a capillary bed which cancer cells encounter after they have accessed the circulation (either via the blood or via lymphatics)
- only for some internal organs is the liver the first capillary bed
- if cancer cells don't get arrested in the lung they should seed with similar frequencies to all organs
- however, in some organs the vessel fenestration/permeability differs e.g. blood-brain barrier vs. bone marrrow

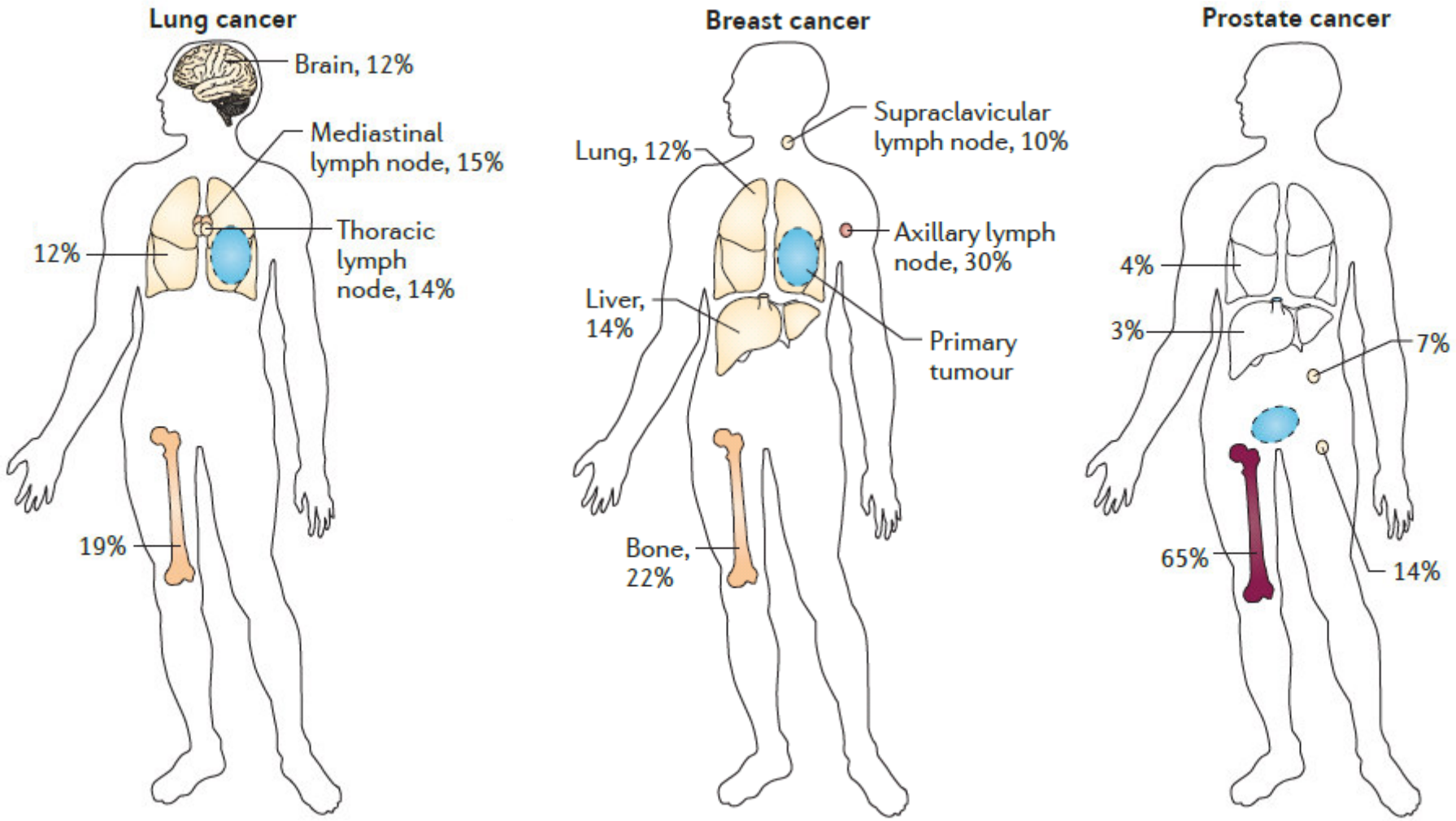
The hepatic portal vein



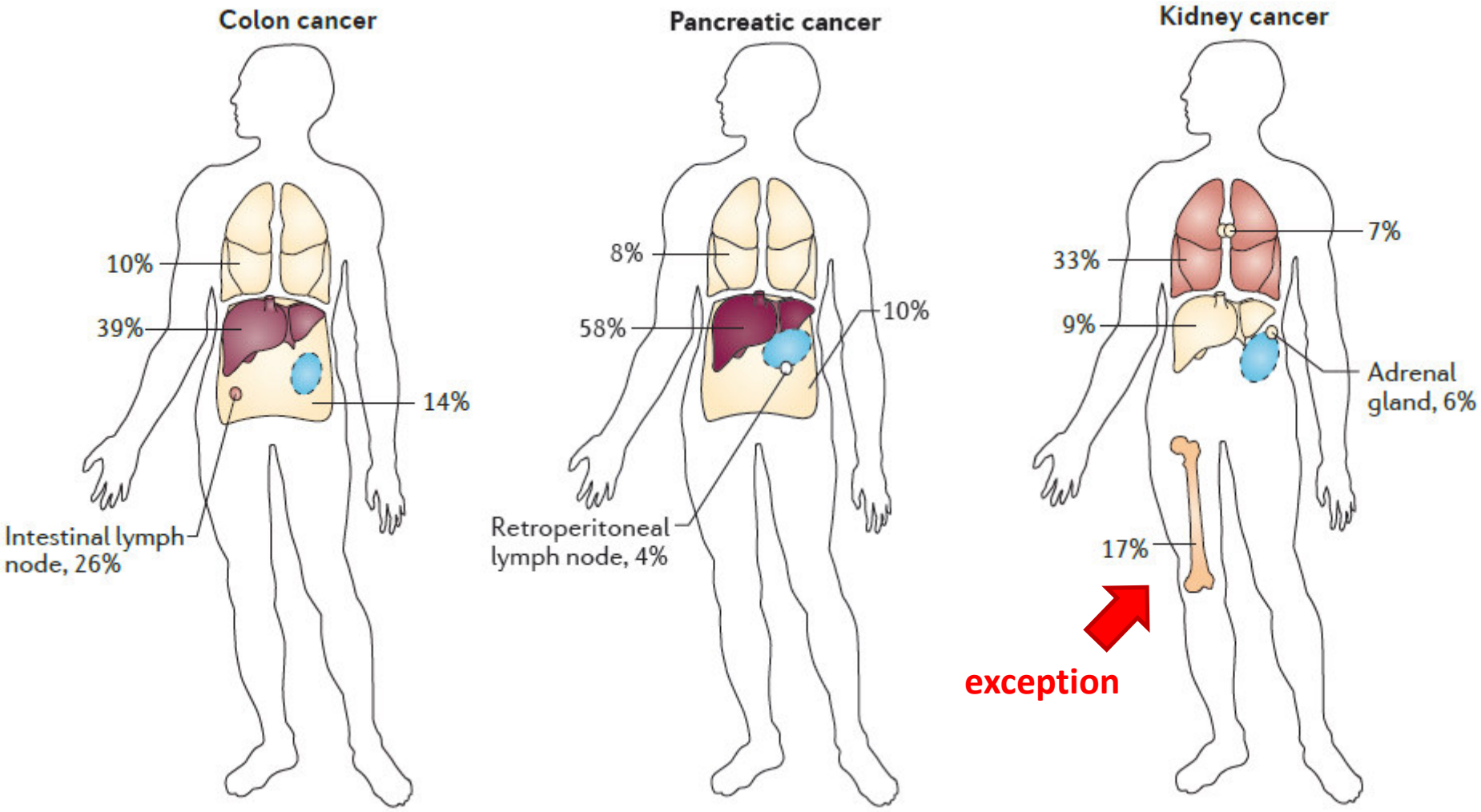
portal system:
an arrangement of vessels whereby blood collected from one set of capillaries passes through a large vessel or vessels and then through a second set of capillaries before it returns to the systemic circulation; such an arrangement occurs in the hypophysis and the liver

hepatic portal vein:
formed by union of the superior mesenteric and the splenic veins, divides into successively smaller branches, following the branches of the hepatic artery, until it forms a capillary-like system of sinusoids that permeates the entire substance of the liver

Cancer metastasis following the seed and soil concept

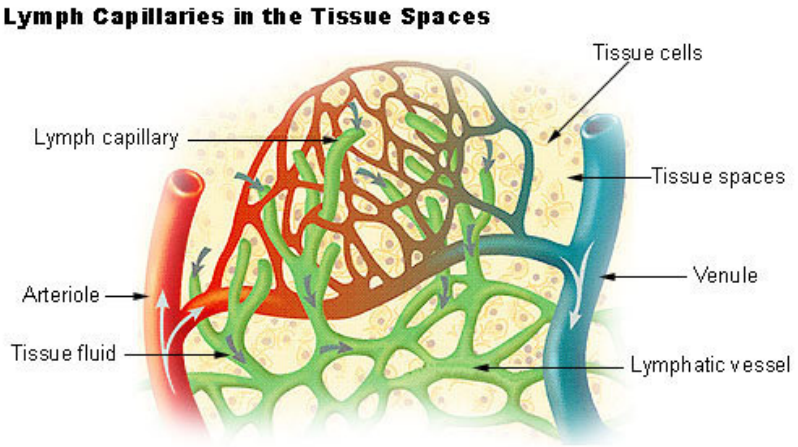


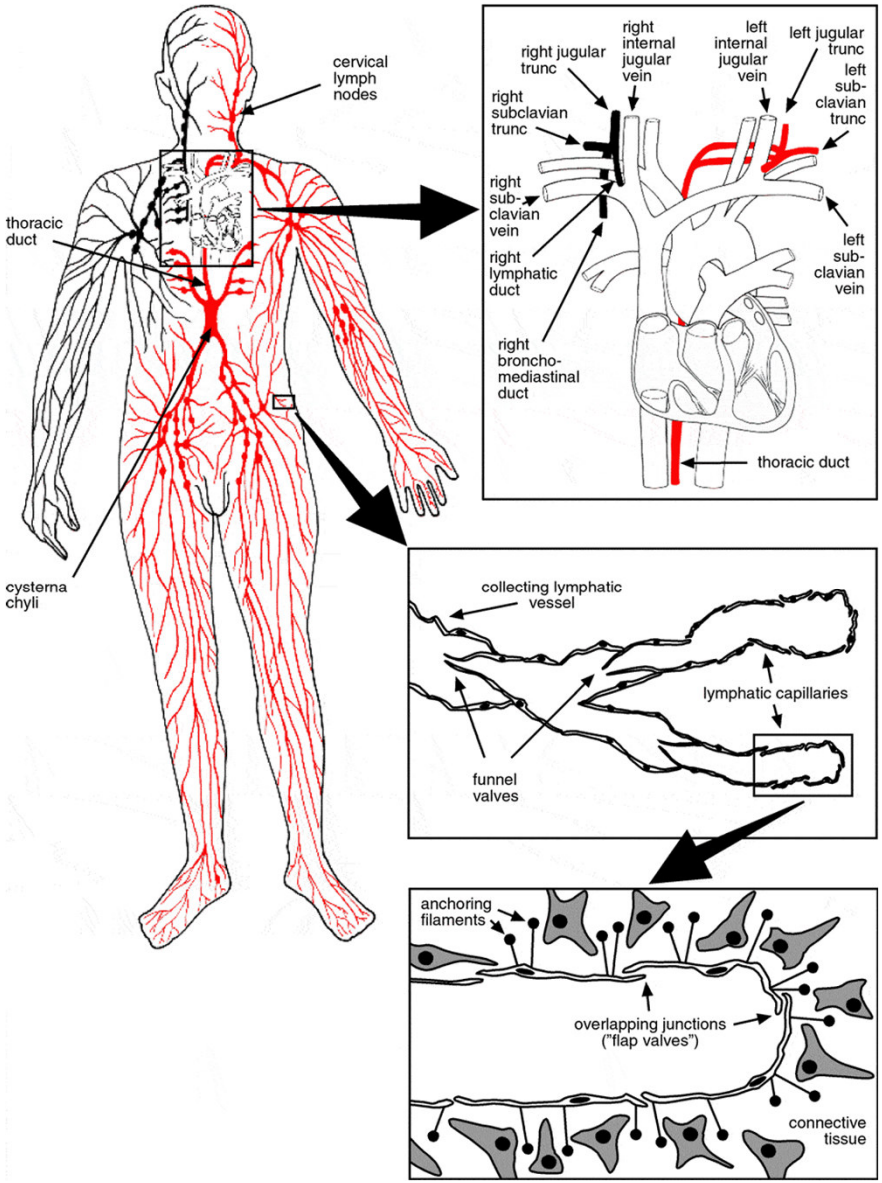
Cancer metastasis following hemodynamic patterns



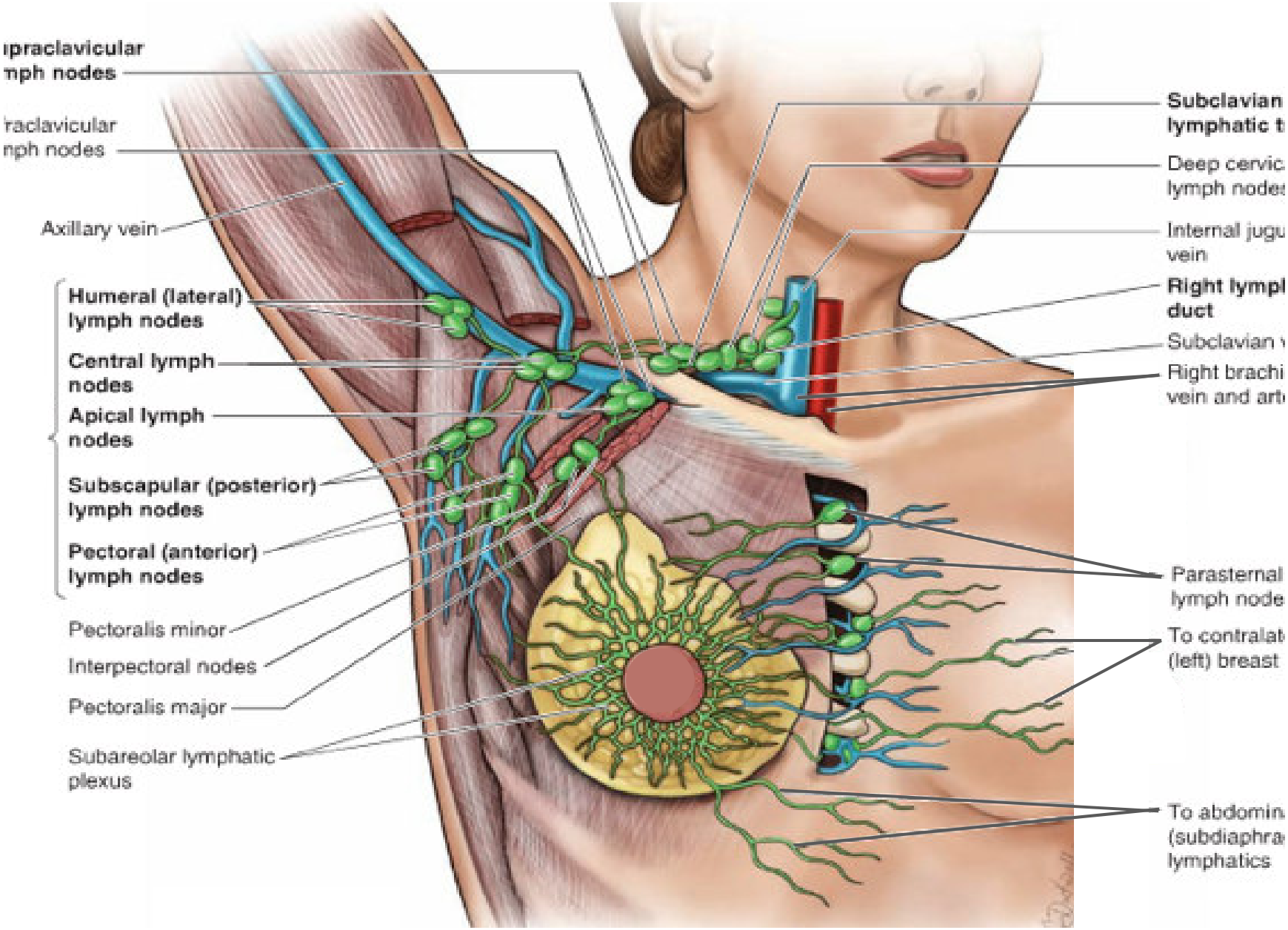
The Lymphatic system facilitates tumor cell spreading

- the lymphatic vascular system serves key physiological functions: it **maintains fluid homeostasis** by absorbing water and macromolecules from the interstitium and serves as a trafficking route for immune cells
- the lymphatic vasculature consists of a highly branched network of capillaries and ducts that is present in most organs with the exception of the central nervous system and avascular tissues, such as cartilage
- unlike the blood vasculature, the lymphatic vasculature is **blind ending**: its small capillaries funnel first into precollecting and larger collecting vessels and then into the thoracic duct or the right lymphatic trunk, which drains lymph into the subclavian veins

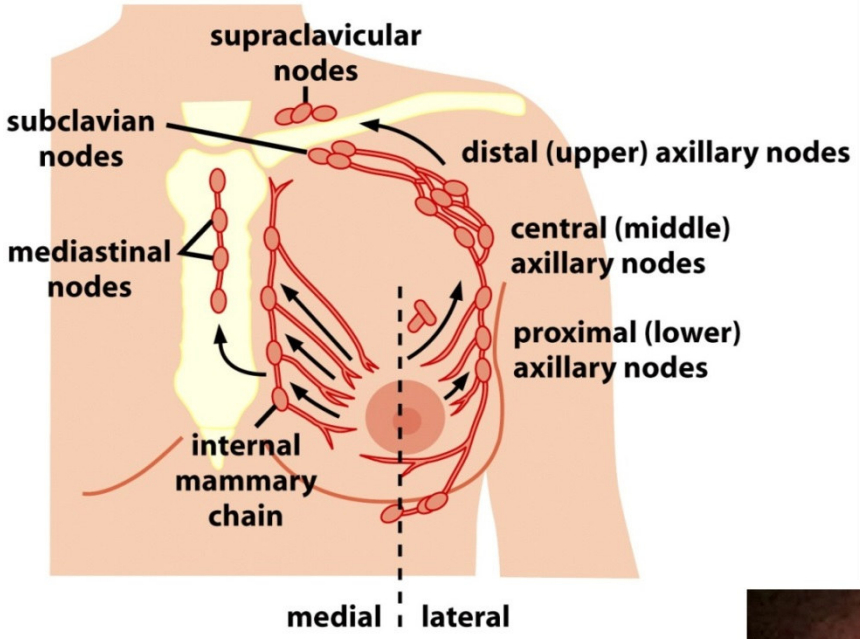




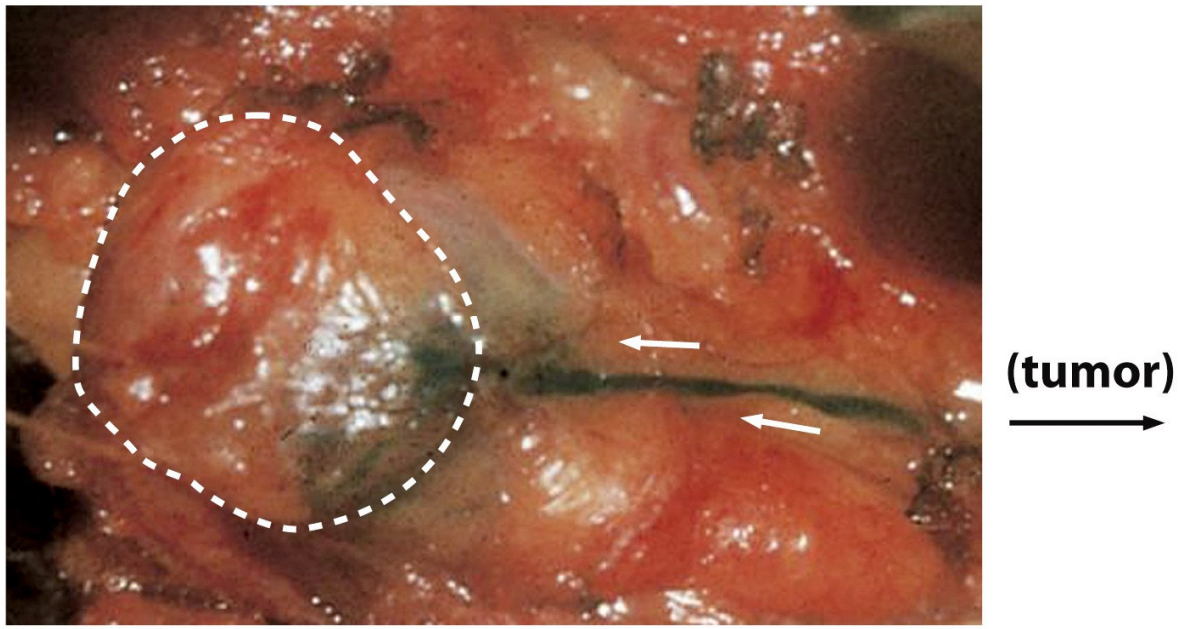
Example for breast cancer: Axillary lymph nodes



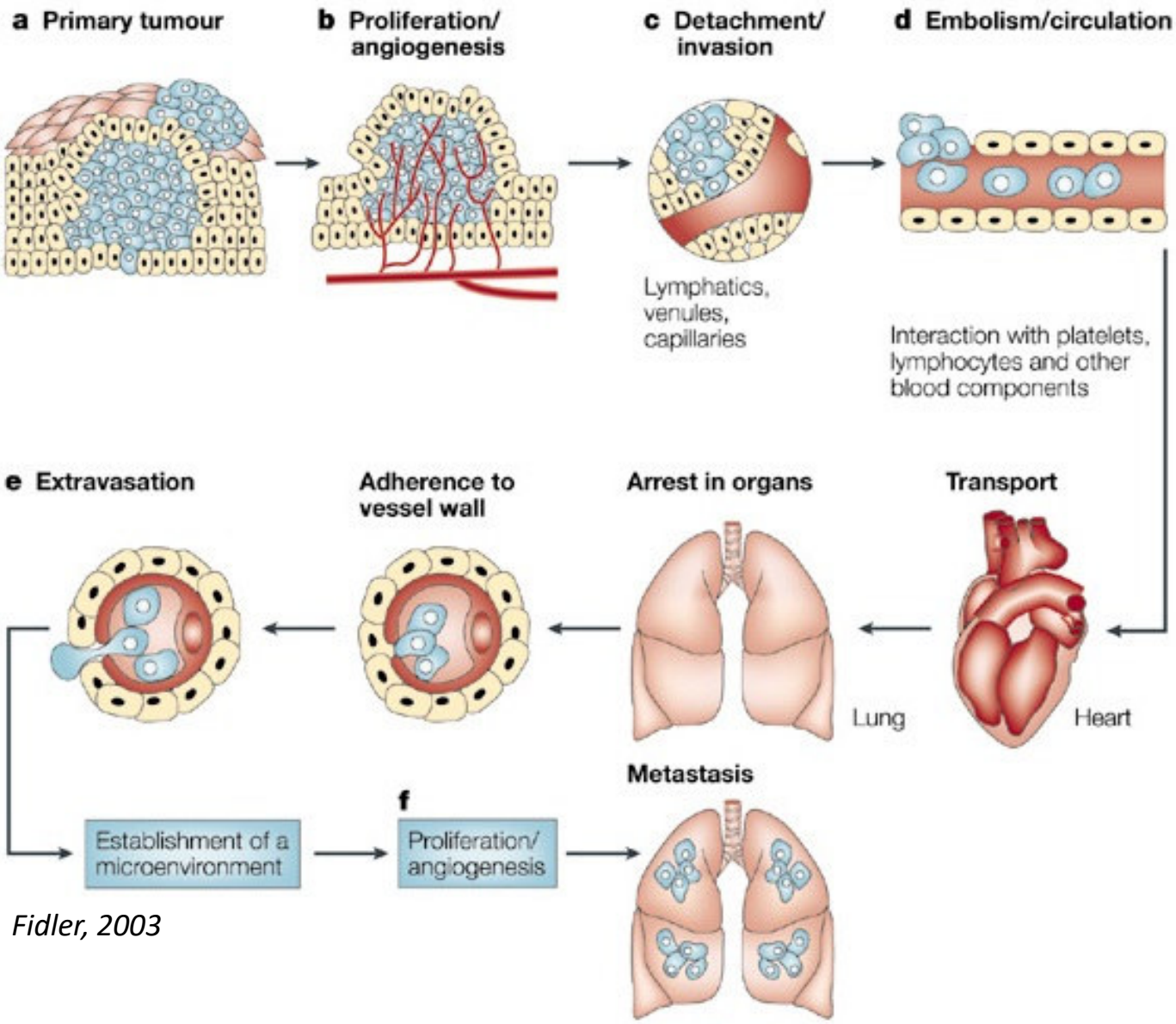
The sentinel lymph node: diagnosis of cell spreading



- ink injection into the tumor helps to identify the individual draining lymph node which is then used for diagnosis to detect tumor cell dissemination
- complete removal of axillary lymph nodes has several side effects and is therefore performed only when the initial diagnosis is positive
- the identity of lymph node metastasis and distant metastasis has not been proven
- it is still unknown whether sentinal lymph node metastasis predicts recurrence or overall survival



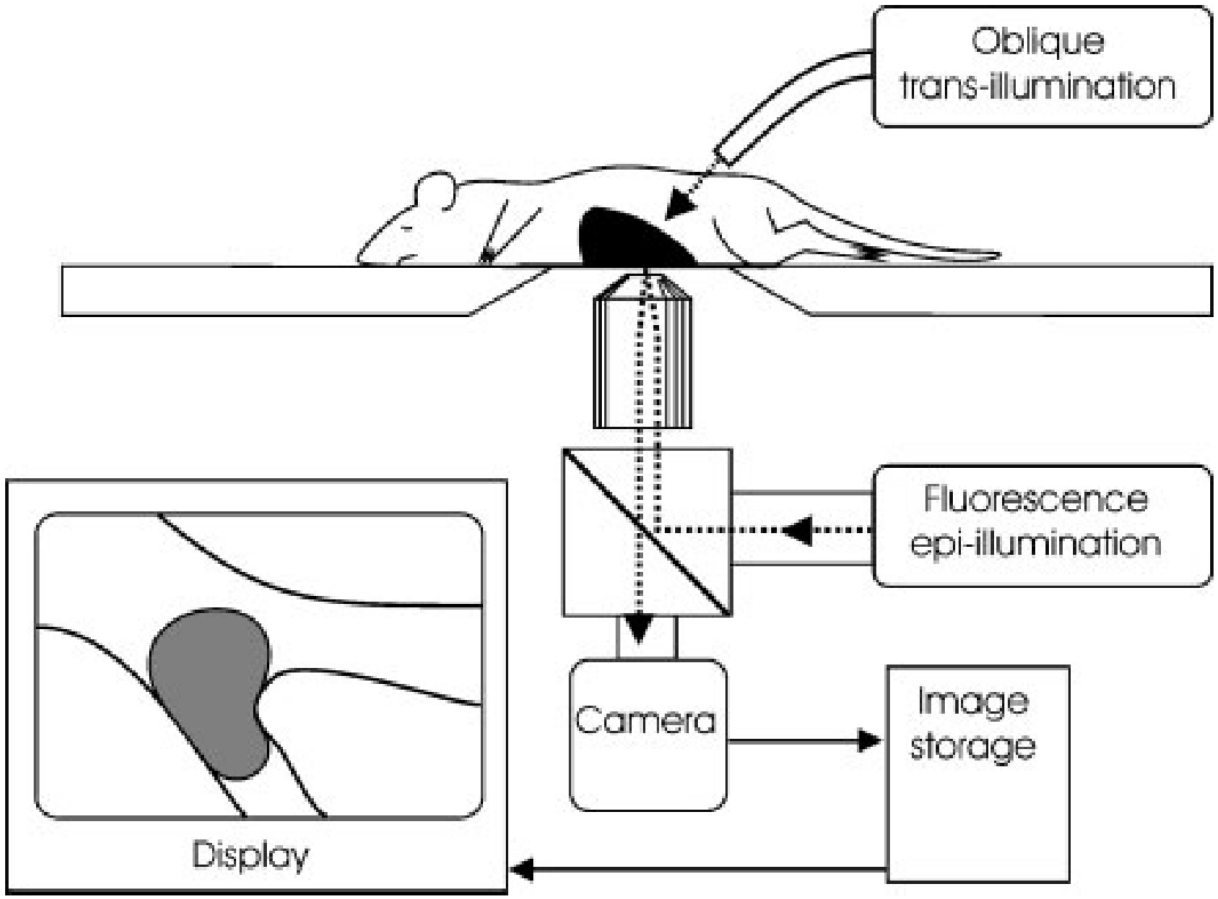
Steps to Metastatic Disease



Fidler, 2003

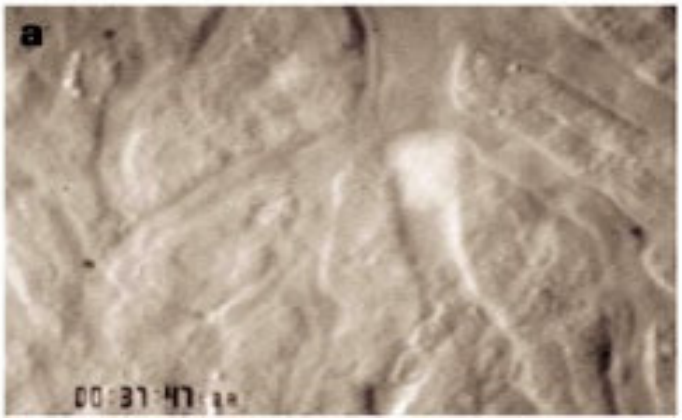
- Motility and tissue invasion
- Intravasation
- Survival in the blood stream (shear stress)
- Arrest at new location
- Extravasation
- Colonization (initiate growth)
- Neoangiogenesis
- Suppression of immune reactions

Accessing the efficiency of metastasis: Intravital Videomicroscopy

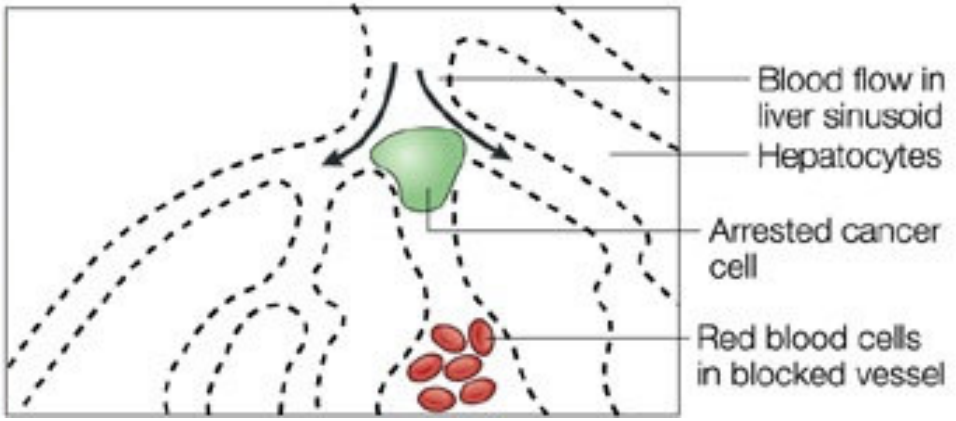


MacDonald, et al, 2002

Intravital Videomicroscopy



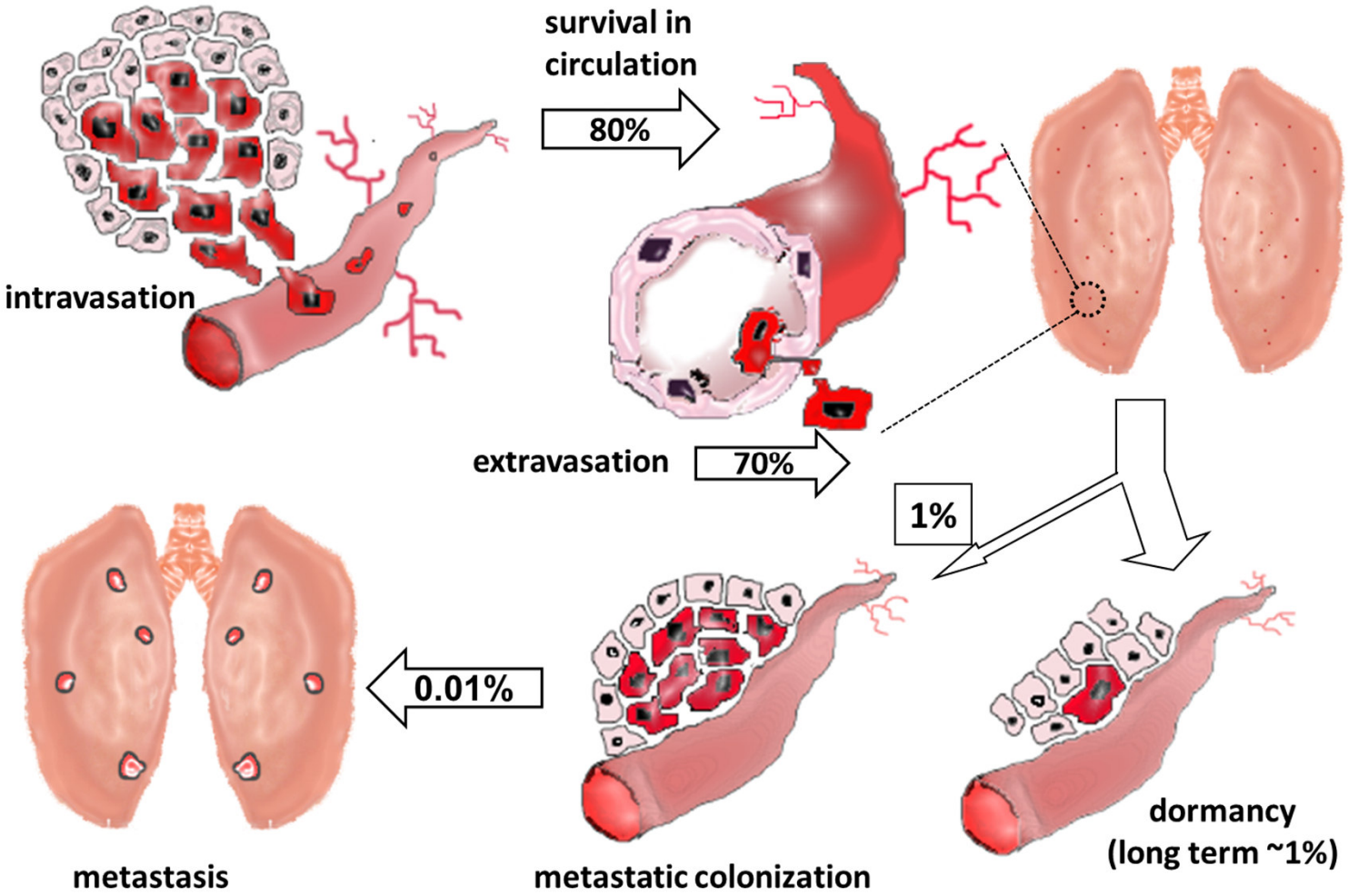
Video Frame



Schematic Diagram

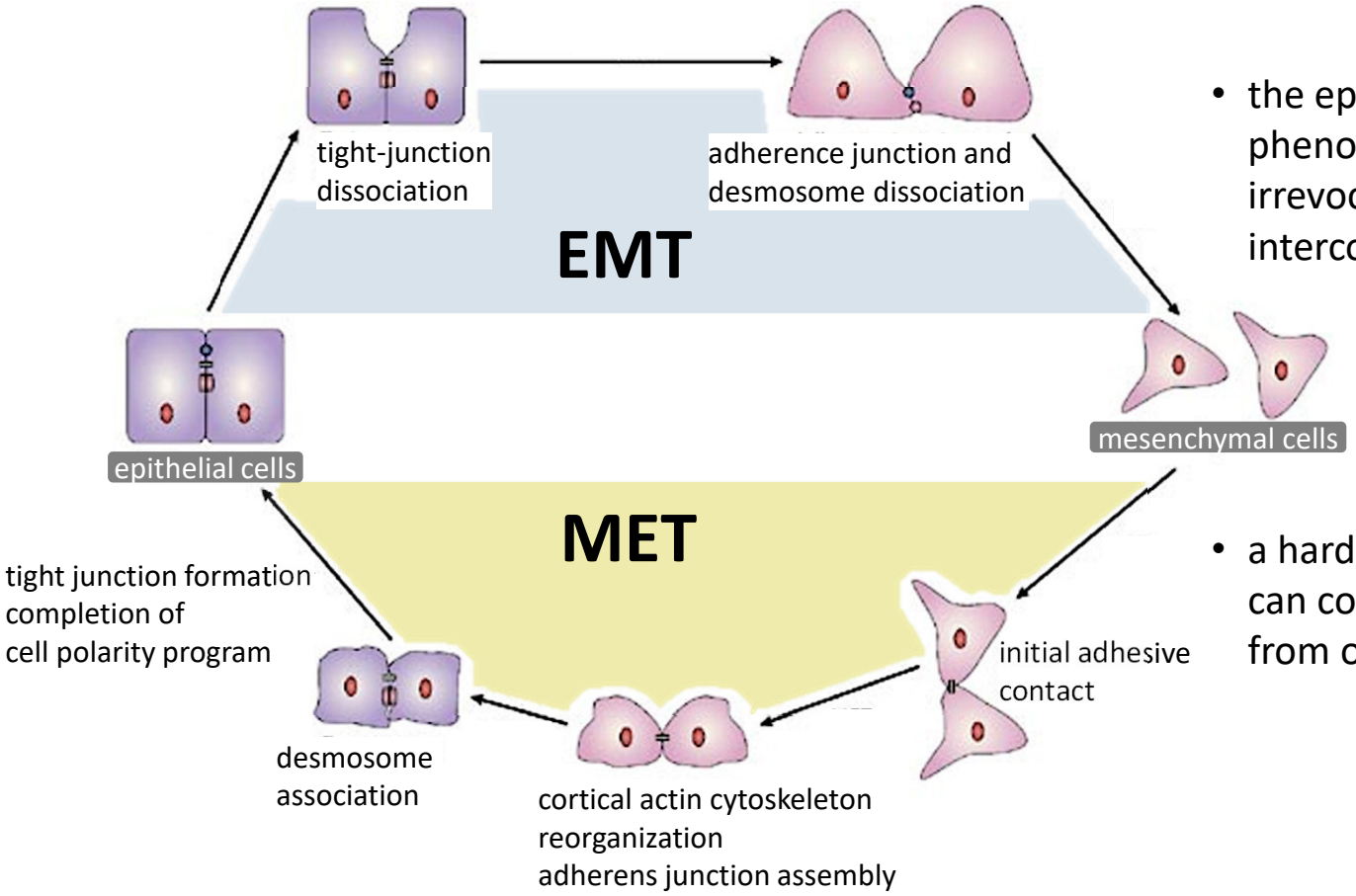
Chambers, et al, 2002

Metastatic progression



Motility and Tissue Invasion

Transitions between epithelial and mesenchymal phenotypes



- the epithelial or mesenchymal phenotype of cells is not irrevocable, but can be interconverted
- a hard-wired program exists which can control a complete switch from one to the other phenotype

such transitions play crucial roles in the formation of the body plan, differentiation of multiple tissues and in tissue repair

Epithelial-to-Mesenchymal Transition = **EMT**

Mesenchymal-to-Epithelial Transition = **MET**

The EMT program

conversion of epithelial cells to mesenchymal cells (and vice versa) is controlled by a group of master regulators and involves profound phenotypic changes that include

loss of:

- epithelial intermediate filaments (keratins)
- loss of cell-cell adhesion (tight junctions, desmosomes, E-cadherin)
- loss of cell polarity

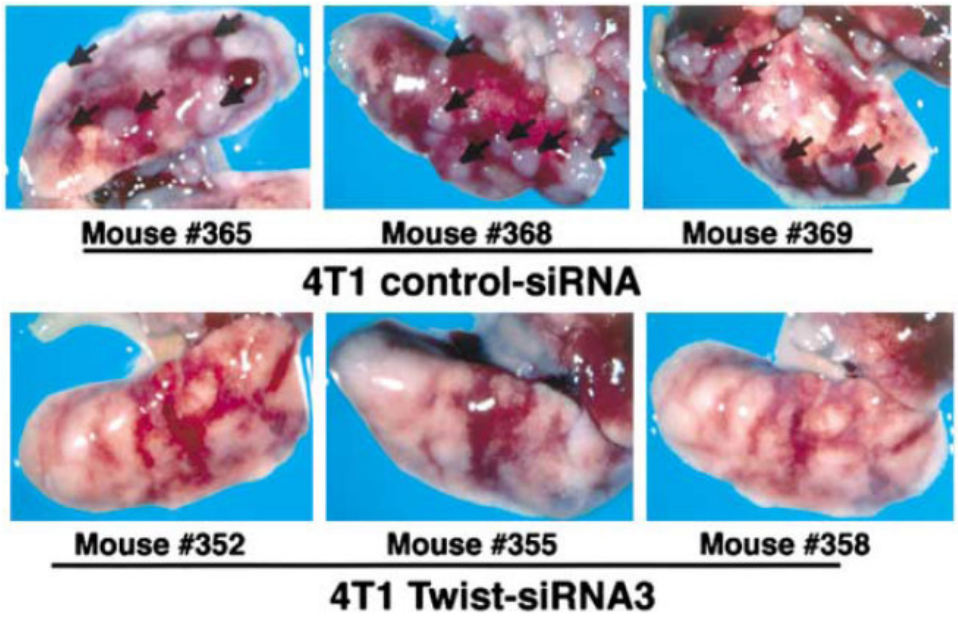
acquisition of:

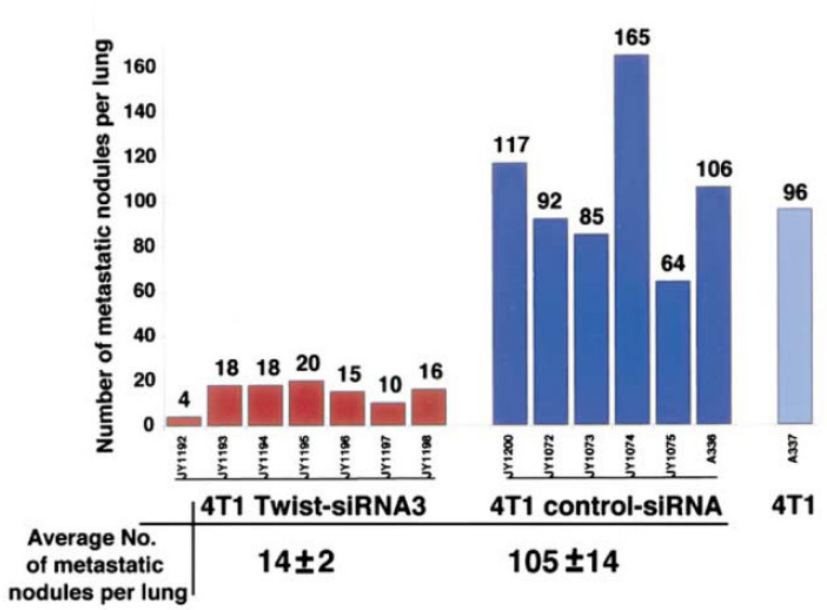
- motility
- invasiveness
- mesenchymal intermediate filaments (vimentin)
- mesenchymal adherens junctions (N-cadherin = weaker adhesion)
- protease production (MMPs, ADAMs)
- secretion of ECM proteins (fibronectin)
- altered integrin expression ($\alpha v \beta 6$ integrin)

KEY TRANSCRIPTION FACTORS:

Name	Where first identified	Type of transcription factor
E47/E2A	associated with E-cadherin promoter	bHLH
FOXC2	mesenchyme formation	winged helix/forkhead
Goosecoid	gastrulation in frog	paired homeodomain
SIP1/ZEB2	neurogenesis	2-handed zinc finger/homeodomain
Slug	delamination of the neural crest and early mesoderm in chicken	C2H2-type zinc finger
Snail	mesoderm induction in *Drosophila;* neural crest migration in vertebrates	C2H2-type zinc finger
Twist	mesoderm induction in *Drosophila;* emigration from neural crest	bHLH

Twist Knock Down and Metastasis





inject murine mammary tumor cell line (4T1) into mammary gland

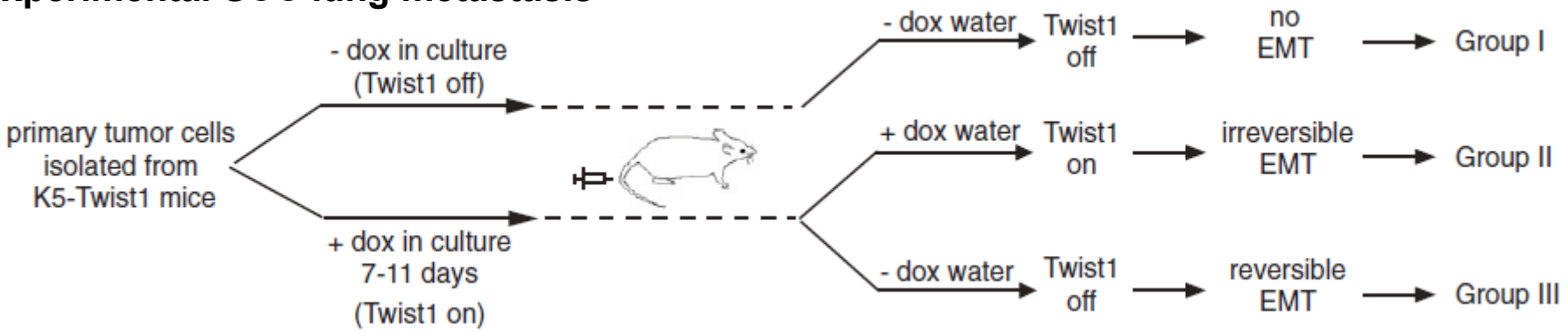
↓

count metastatic nodules in lung

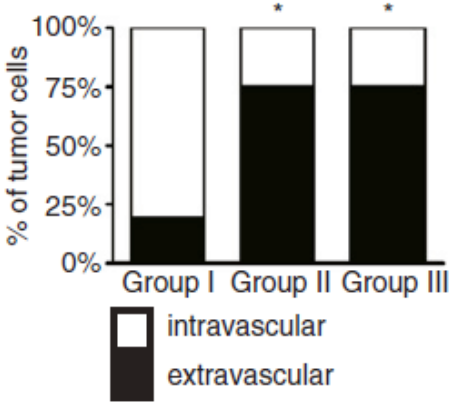
Yang, et al, 2004

Reversibility of EMT Is essential for metastasis

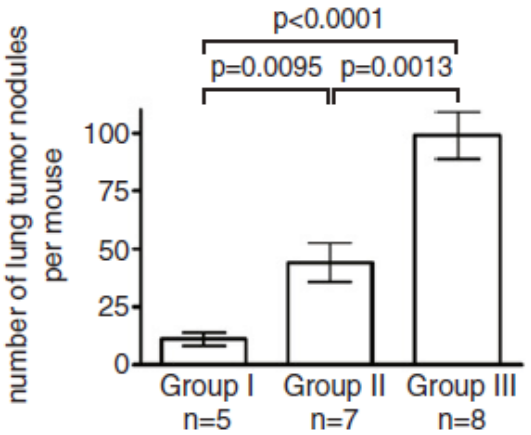
experimental SCC lung metastasis



extravasation *in vivo*



lung metastasis nodules



- reversible EMT represents a key driving force in metastasis
- lack of metastasis could be due to the inability of disseminated tumor cells to revert EMT and proliferate
- blocking EMT reversion may prevent circulating/dormant tumor cells from establishing metastases

Tsai et al., Cancer Cell 2012

Circulating and Disseminated Tumor Cells

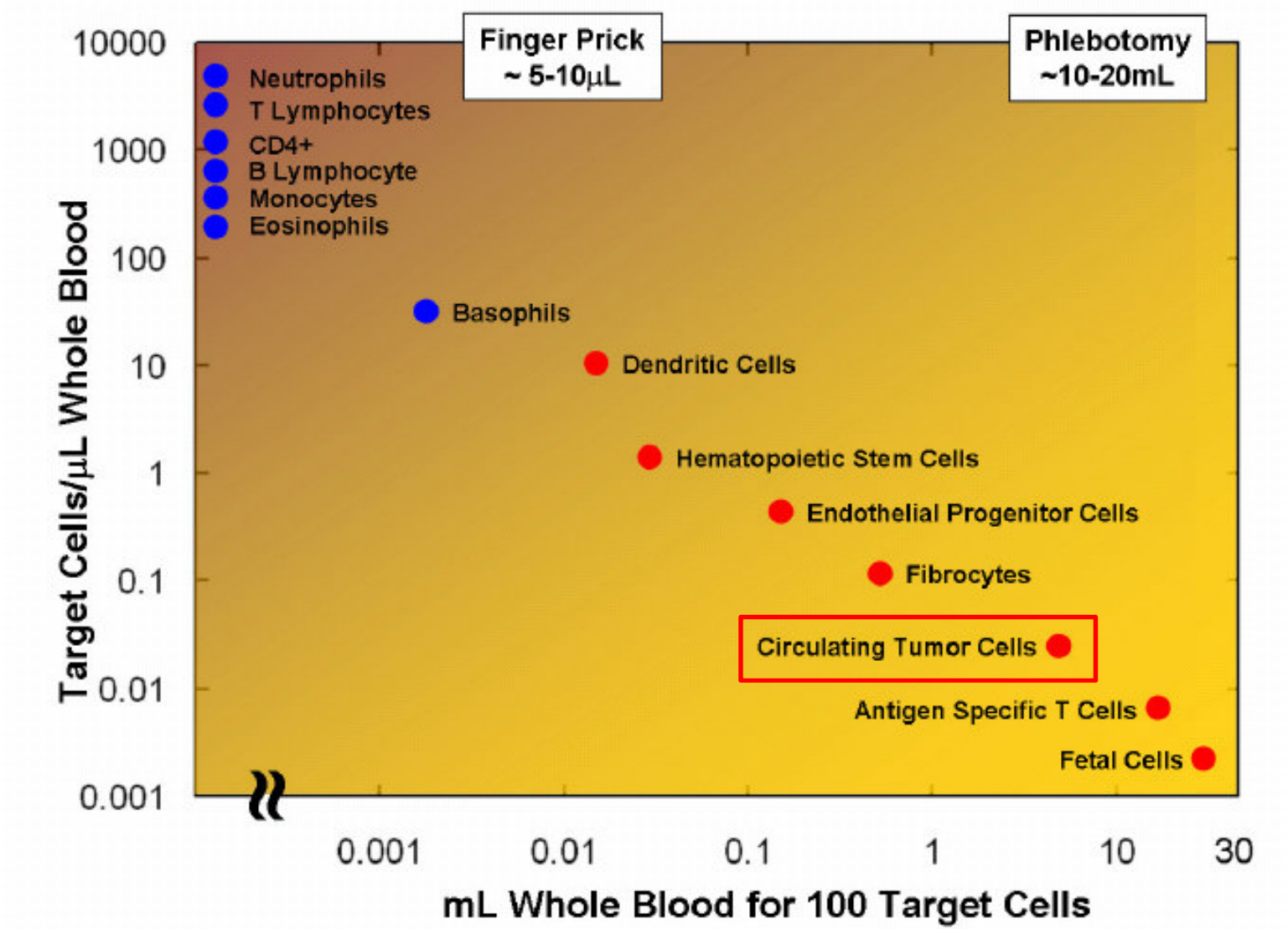
Circulating tumor cells

- Circulating tumor cells (CTCs) were described for the first time more than one century ago
  - Australian Medical Journal (1869):
    A Case of Cancer in which Cells Similar to Those in the Tumors were Seen in the Blood After Death; 14:146
  - Acta Chiurgica Scandinavia (1955):
    Cancer Cells Circulating in the Blood: a Clinical Study on the Occurrence of Cancer Cells in the Peripheral Blood and in Venous Blood Draining the Tumor Area at Operation; 201:1
  - American Journal of Medicine (1976):
    Carcinocythemia: An Acute Leukemia-like Picture Due to Metastatic Carcinoma Cells; 60:273

- currently, their detection may play a pivotal role in the prognosis and prediction of patient outcome, cell dissemination, response to therapy, cancer evolution and drug resistance **=> blood-based biopsy**

(alternatively, detection of cancer-derived DNA in the blood will be used more frequently in the future to detect and monitor disease)

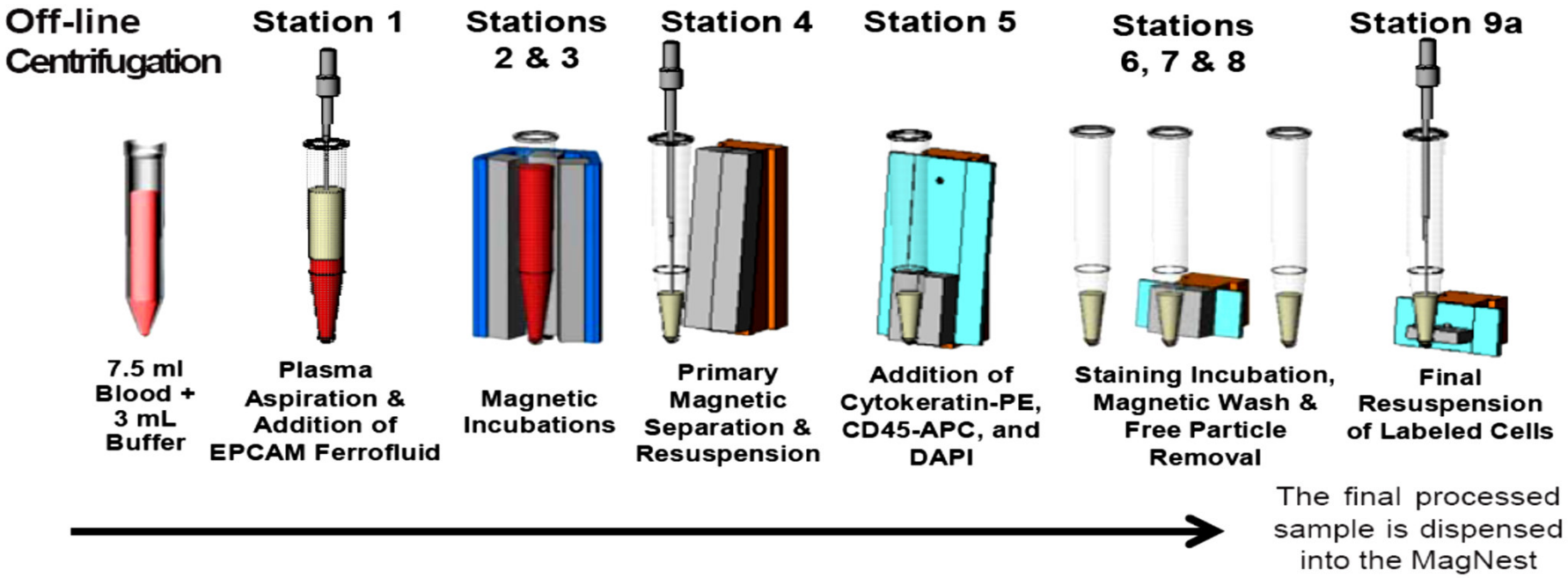
Mining blood for rare cells



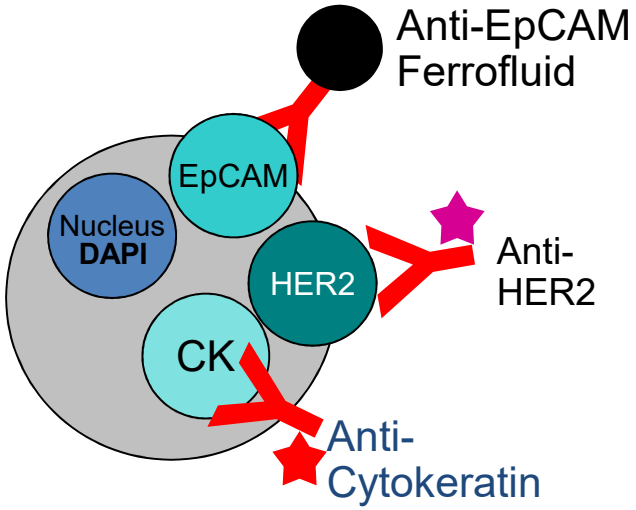
Isolation of CTC from Peripheral Blood

- Two Key Issues:
  - Enrichment of epithelial/tumor cells from RBC & WBC
  - Characterization to distinguish
    - Tumor cells from blood components
    - Tumor cells from normal cells

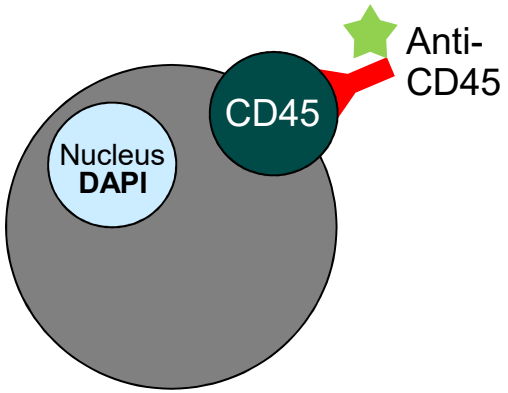
FDA approved **Immunomagnetic Method**: Veridex CellSearch System



Labeling of CTCs and Blood Cells



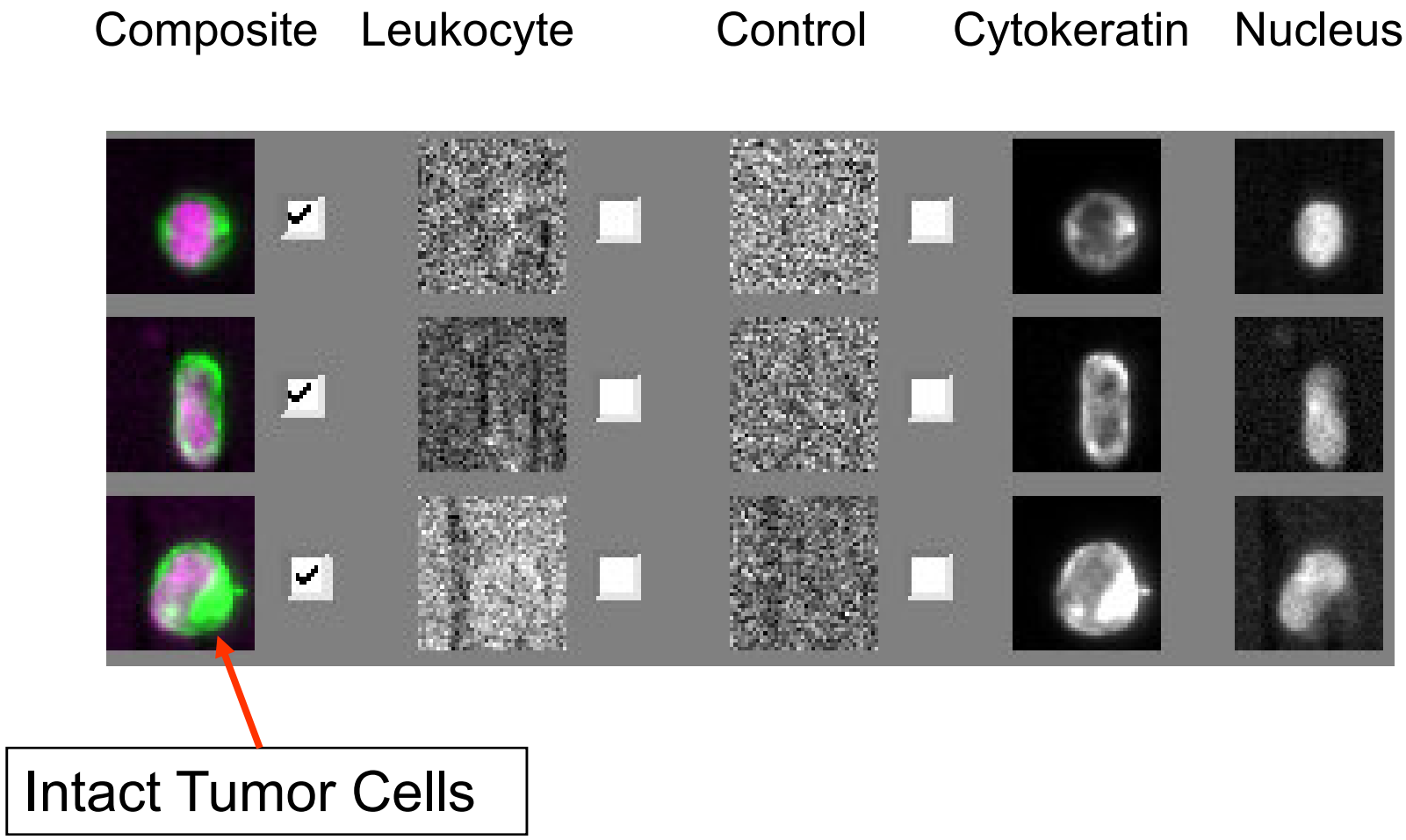
HER2+*
Circulating Tumor Cell



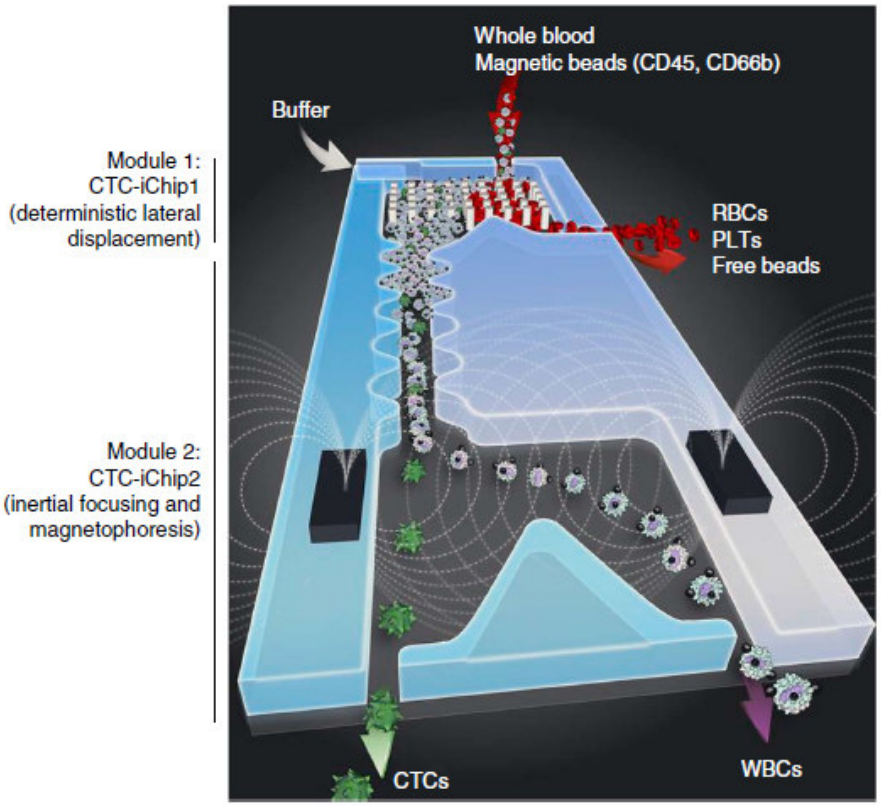
Leukocyte

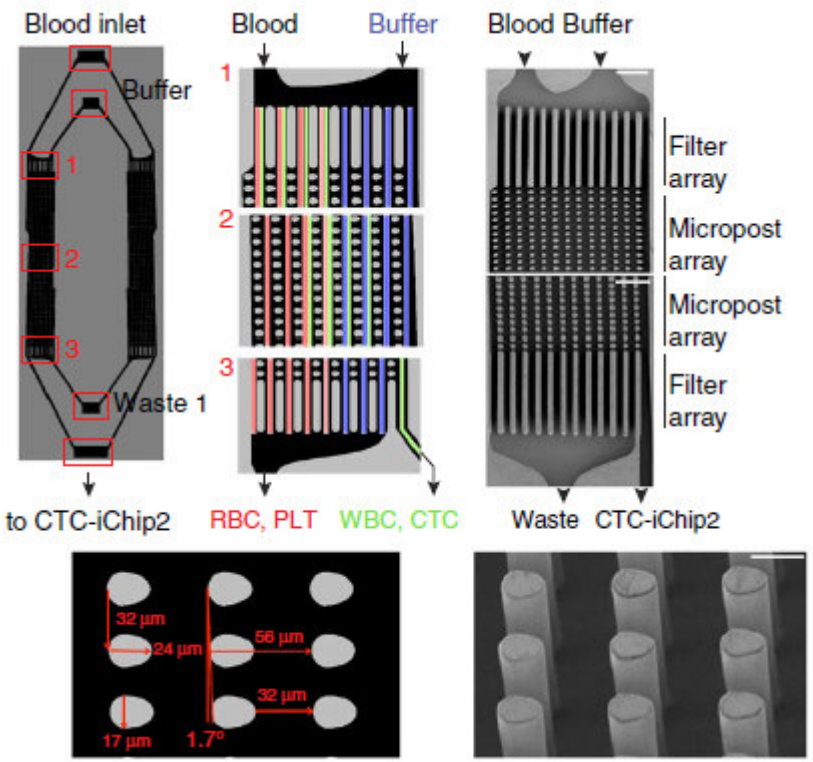
- EpCAM and Cytokeratin are both proteins which are usually not expressed on leukocytes
- in addition, CD45 is used to definitely recognize leukocytes which sometimes can unspecifically bind antibodies
- semi-automated fluorescence microscopy scans a complete reaction cartridge in about 10 minutes and identifies CTC candidates for confirmation by a technician or pathologist

Analysis of Enriched CTC



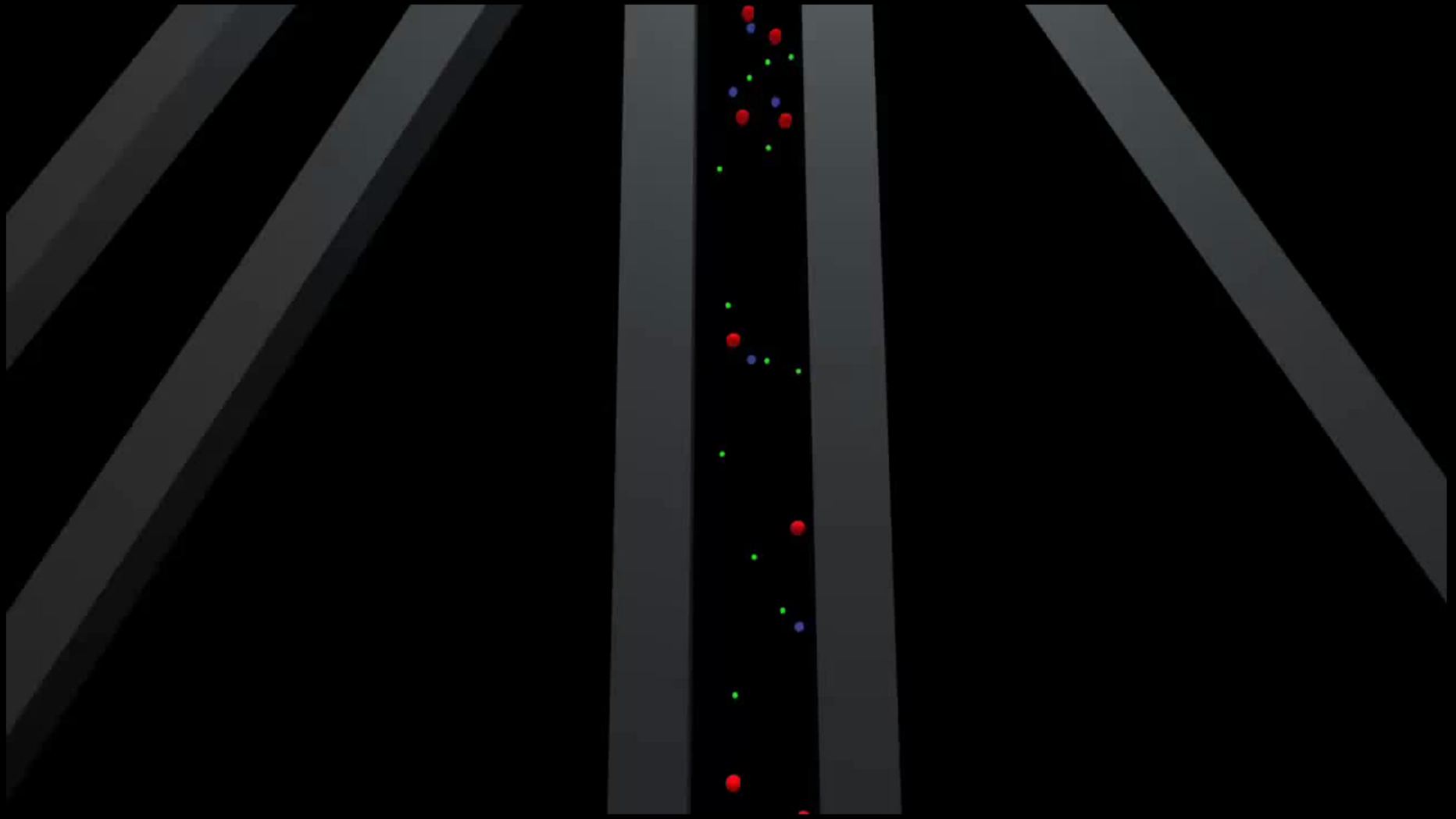
Microfluidics based CTC isolation





Nature Protocols
2014;**9**: 694

Sorting of cells by deterministic lateral displacement



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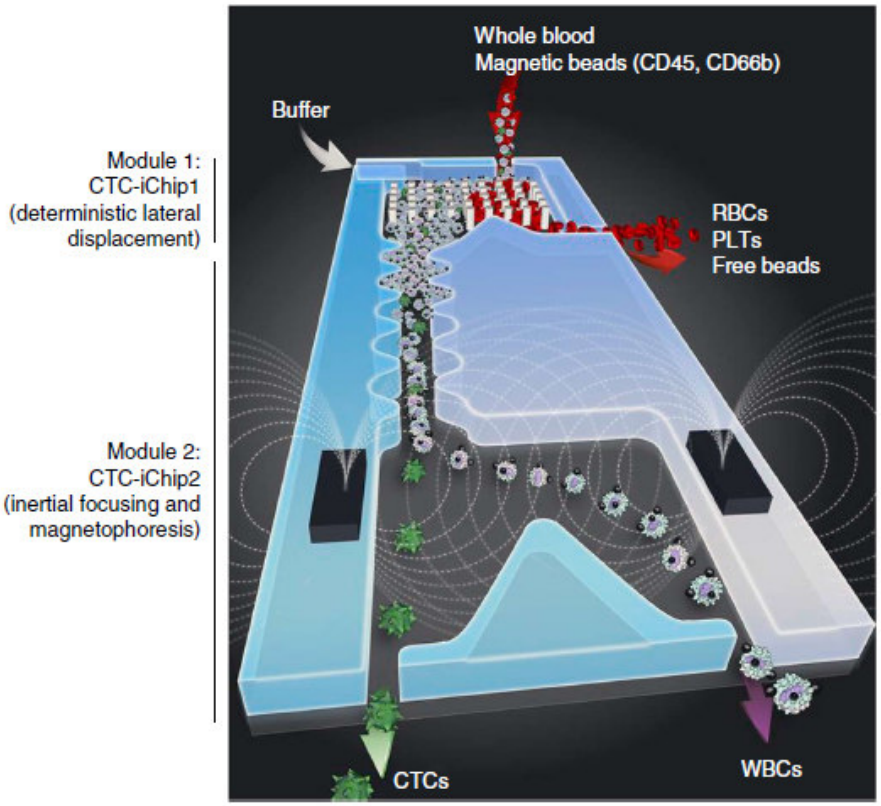

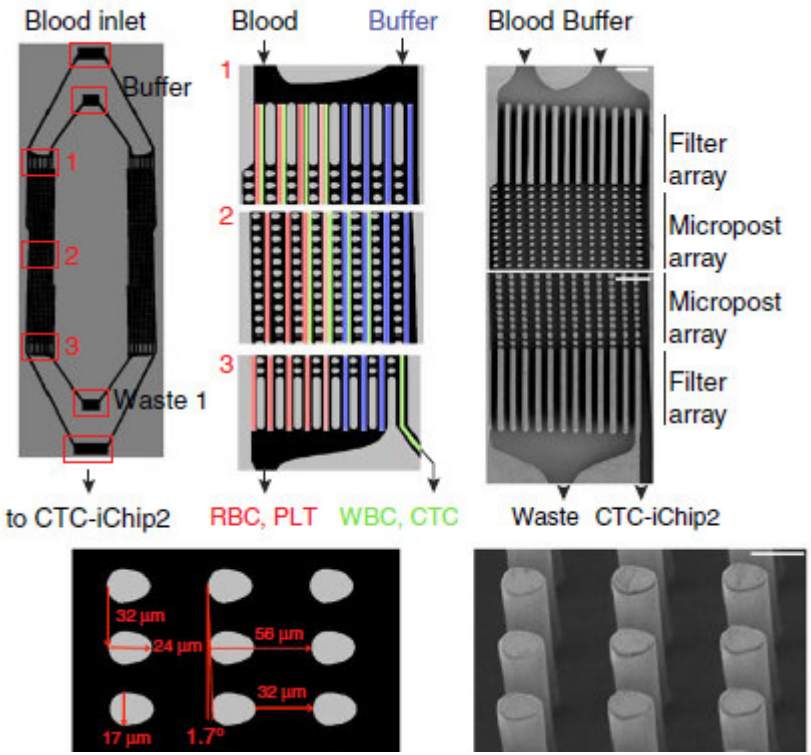

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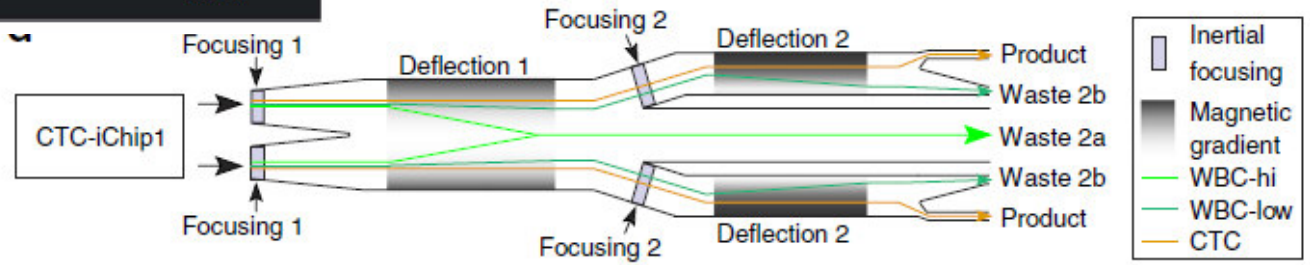
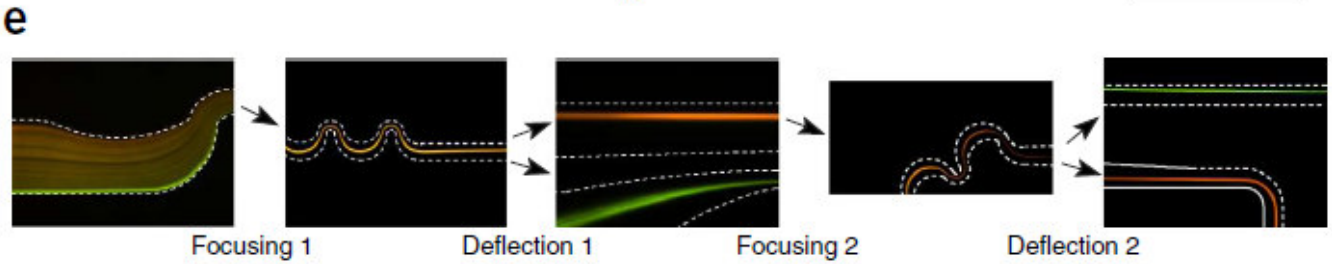
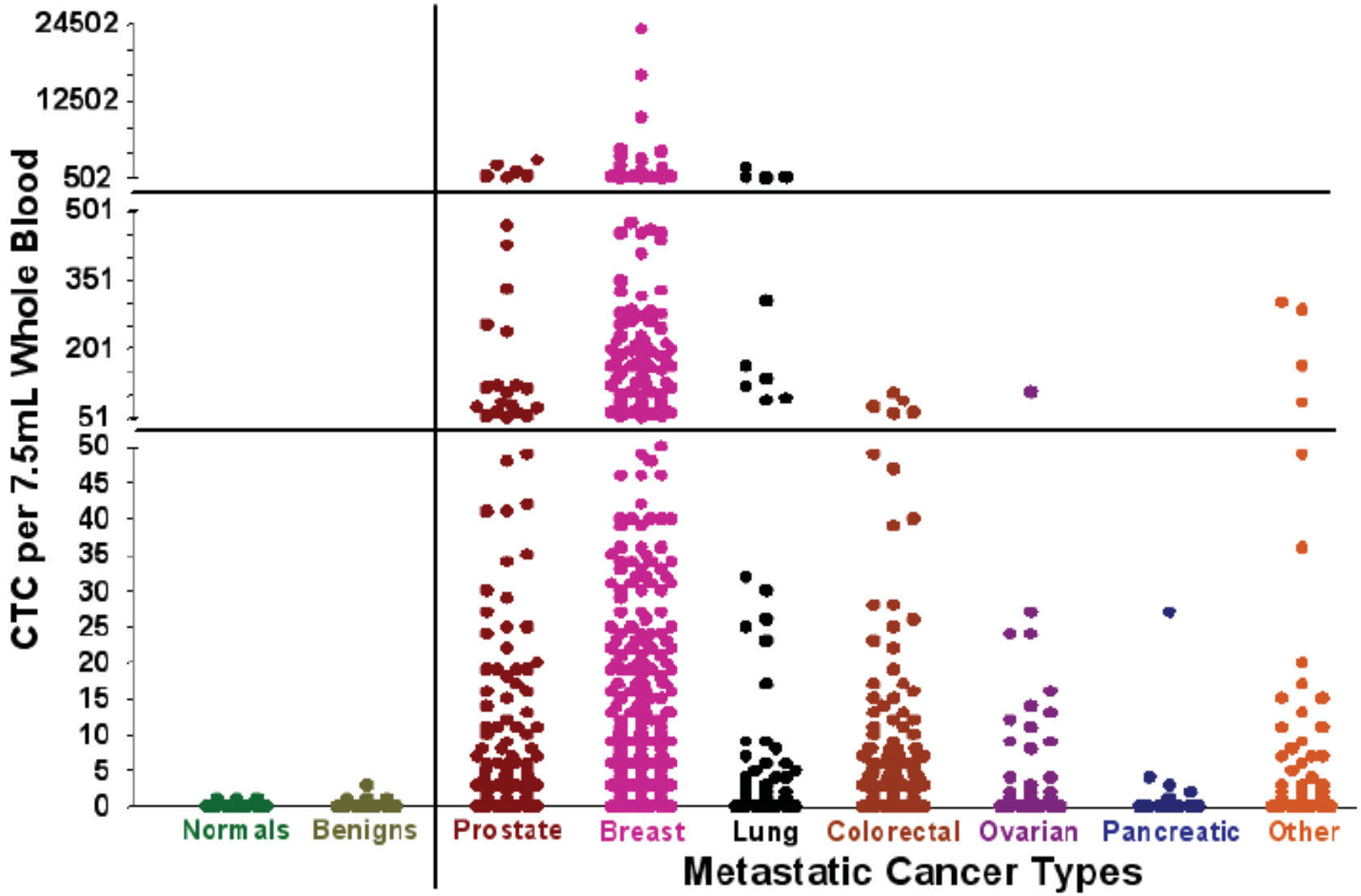




CTC Frequency, by Cancer Type



Allard, Clin Cancer Res, 10:6897, 2004

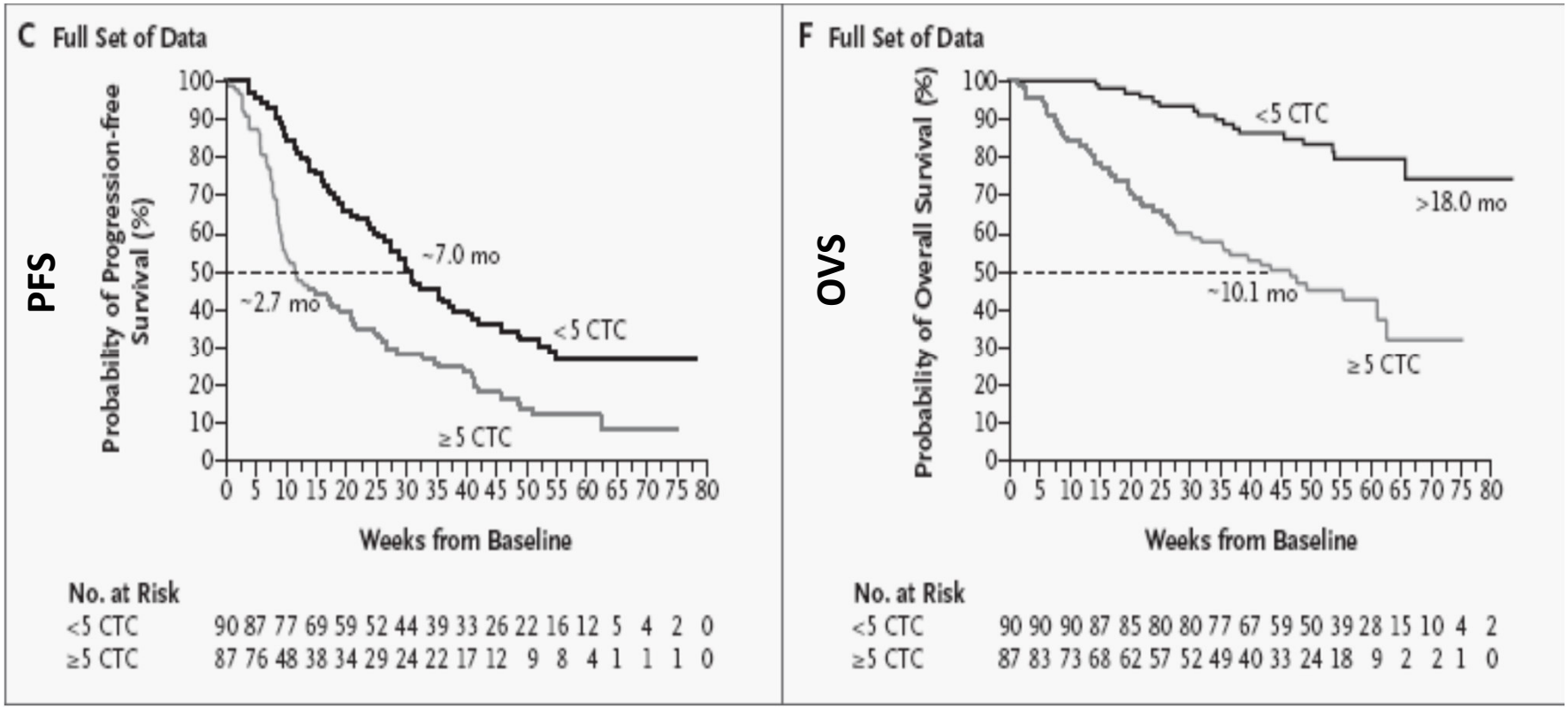
Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer

Massimo Cristofanilli, M.D., G. Thomas Budd, M.D., Matthew J. Ellis, M.B., Ph.D., Alison Stopeck, M.D., Jeri Matera, B.S., R.Ph., M. Craig Miller, B.S., James M. Reuben, Ph.D., Gerald V. Doyle, D.D.S., W. Jeffrey Allard, Ph.D., Leon W.M.M. Terstappen, M.D., Ph.D., and Daniel F. Hayes, M.D.

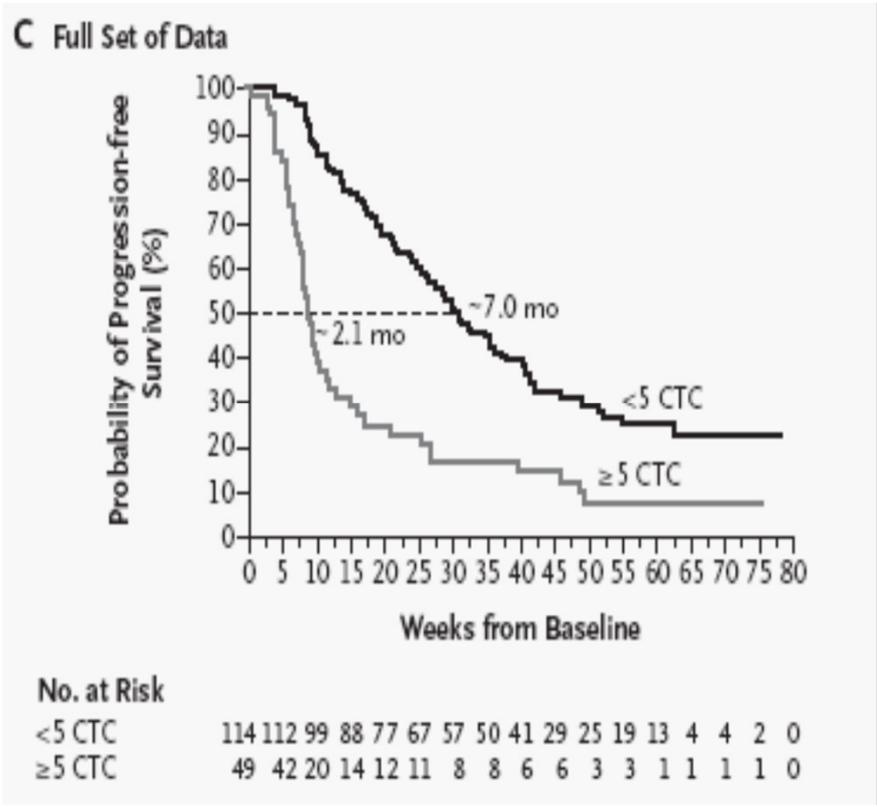
- Multicenter, prospective trial
- Inclusion criteria:
  - Progressive metastatic breast cancer
  - All beginning new, systemic therapy
  - All with measurable disease
  - All with ECOG (Eastern Cooperative Oncology Group) performance status of 0-2 (no to moderate symptoms)

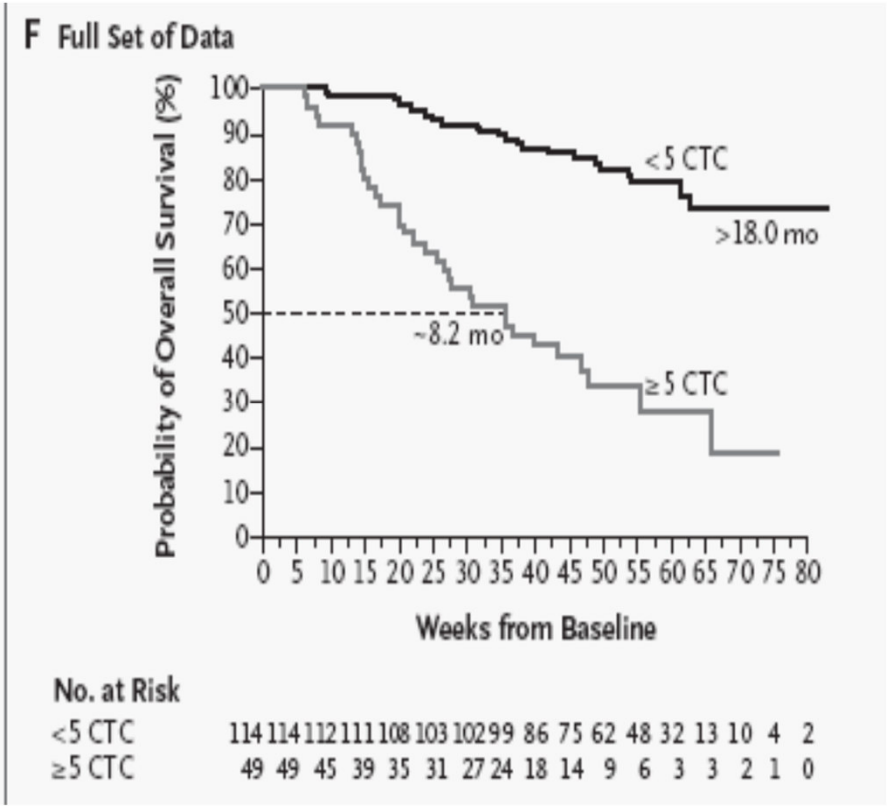
NEJM 351:781, 2004

Number of CTC Before New Therapy Predicts Progression Free Survival and Overall Survival

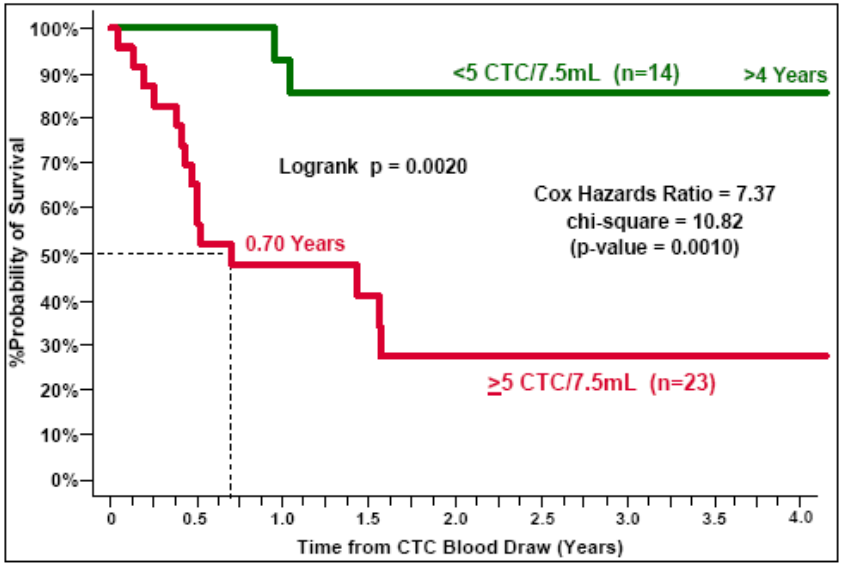


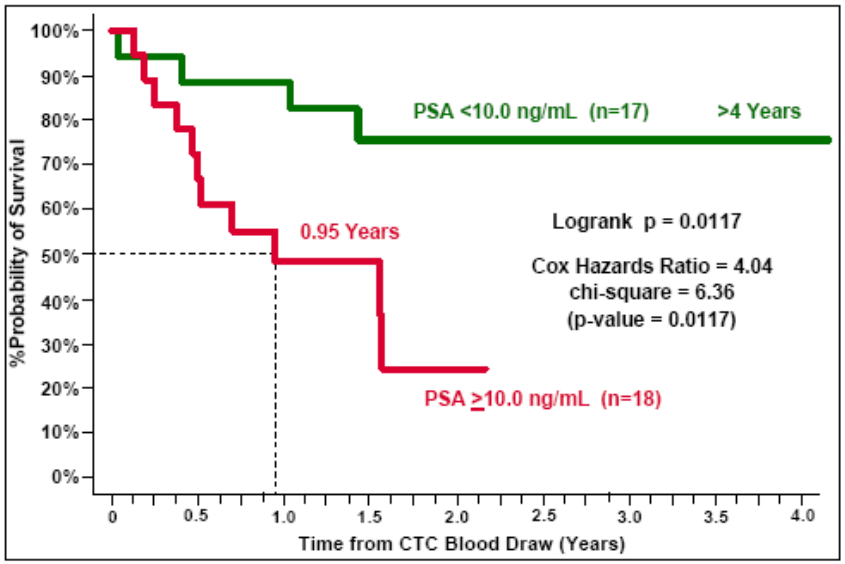
Number of CTC at First Follow Up Predicts Progression Free Survival and Overall Survival

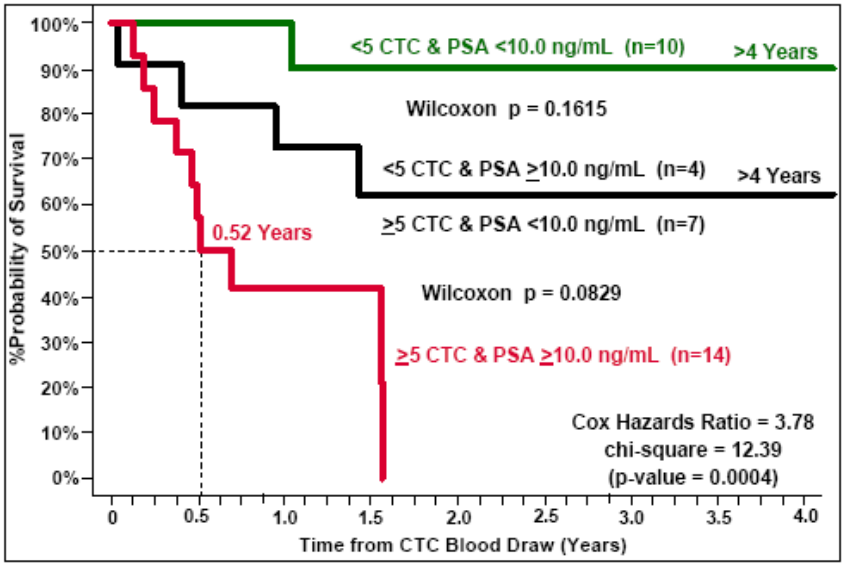




Metastatic Prostate Cancer







Miller, AACR, 2004

Conclusions of the first CTC studies

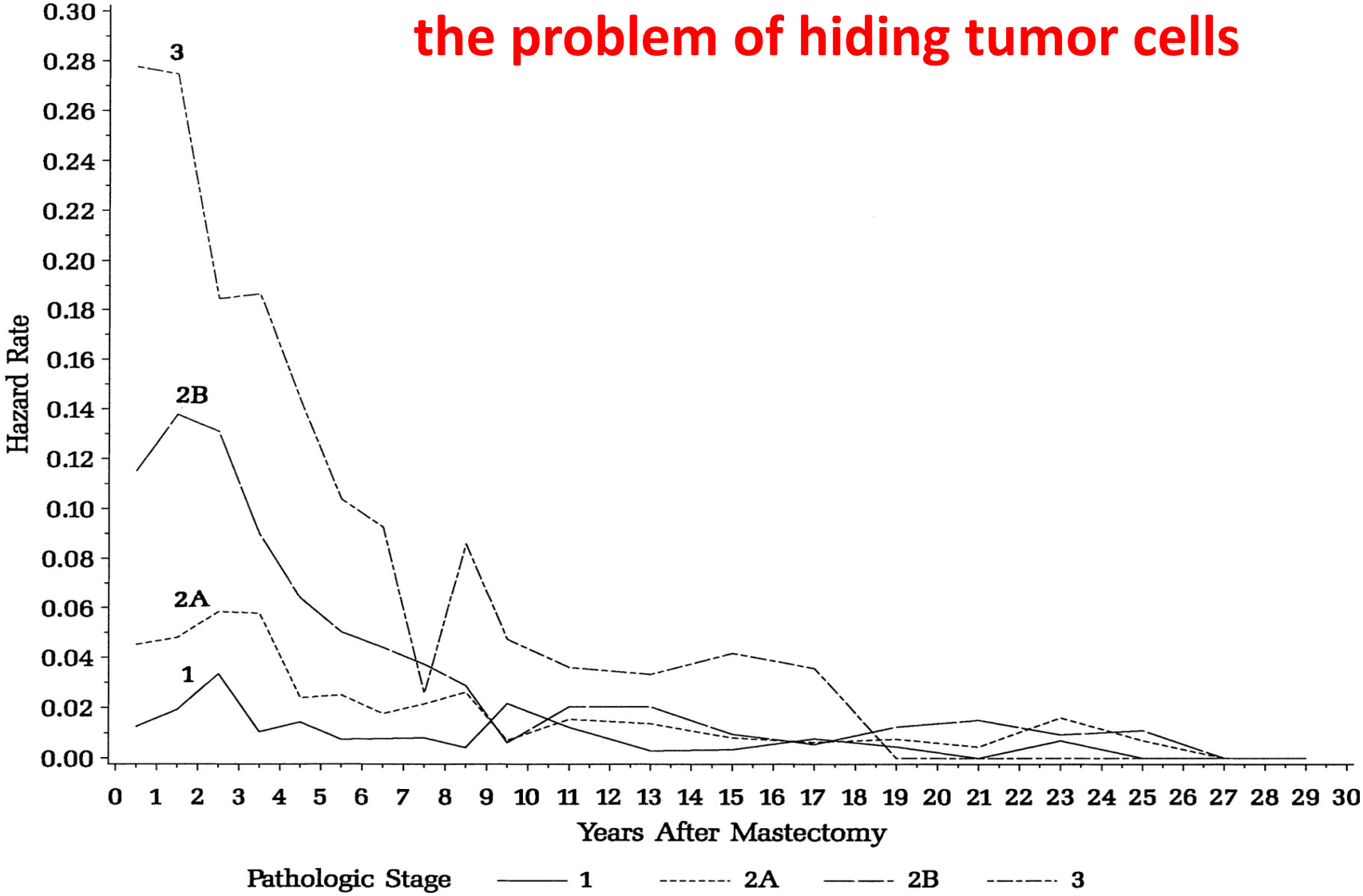
- levels of baseline CTC **are independent prognostic markers of outcomes** (both progression free survival and overall survival) for certain cancer patients
- elevated levels of CTC at First Follow-Up predict both short progression free survival and overall survival—may indicate that patient is receiving futile therapy
- CTC levels give reliable **estimates of disease progression** much earlier than with traditional imaging methods (3-4 weeks vs. 8-12 weeks)
- with a single blood draw, one can
  - confirm oncogene amplification
  - acquire material for direct sequencing of candidate oncogenes
  - enumerate baseline CTC levels to use as monitor for efficacy of selected therapy
  - patients may never need to have a biopsy

- it was however also shown that the PROGNOSTIC VALUE of DTCs (disseminated tumor cells) in the BONE MARROW is HIGHER than CTCs (circulating tumor cells) in the BLOOD **???**

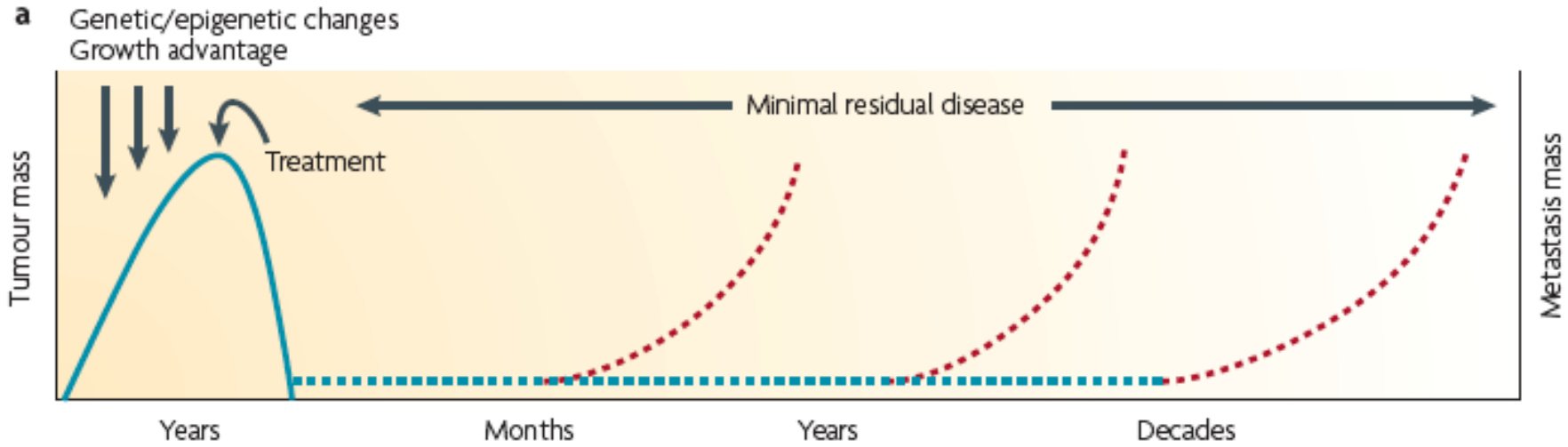
Tumor Dormancy

Late post-mastectomy recurrences of Breast Cancer:

the problem of hiding tumor cells



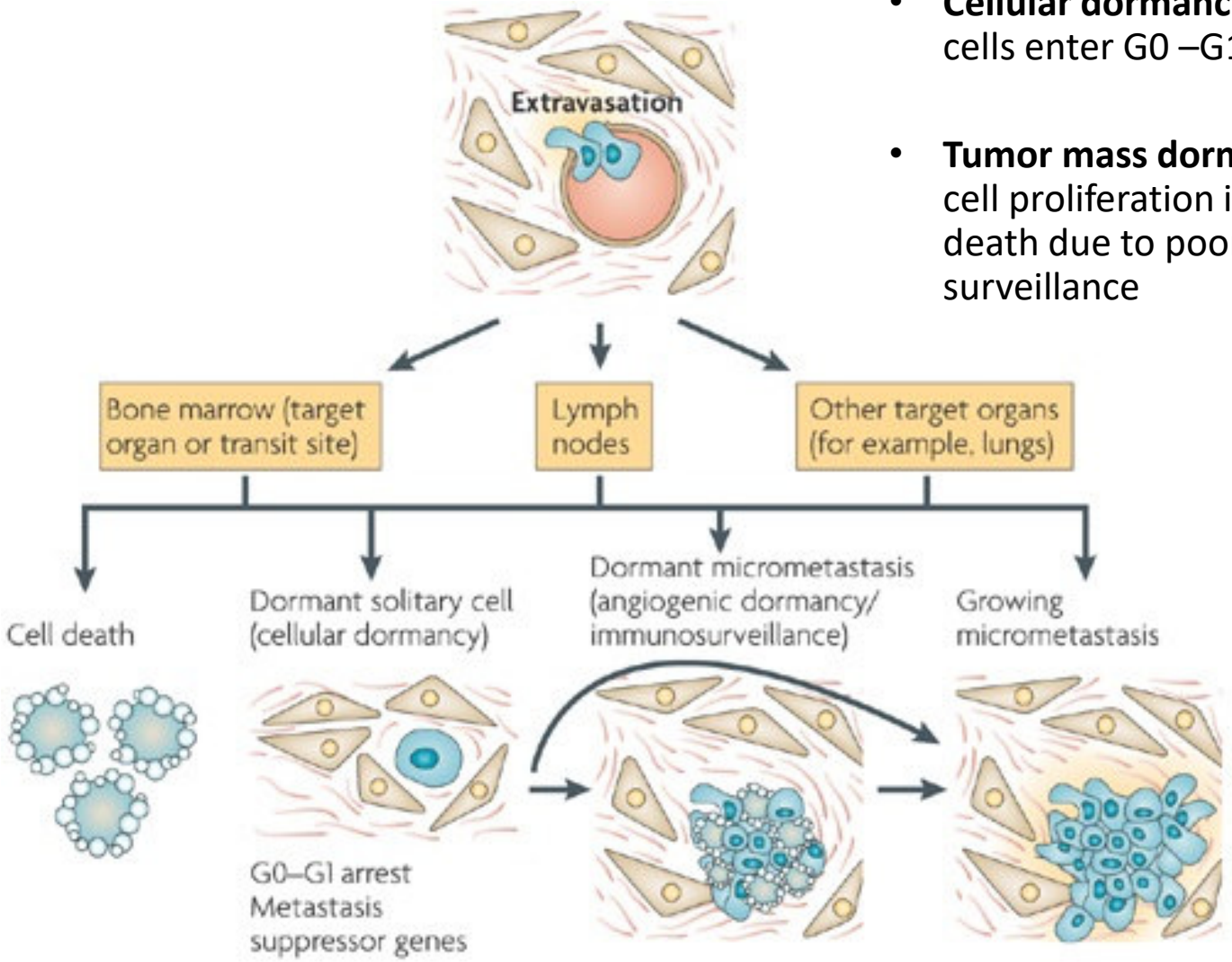
Metastatic dormancy



20-45% of breast cancer and prostate cancer will relapse years or even decades later after initial tumor removal

this cannot be explained by continuous tumor cell proliferation

Tumor dormancy

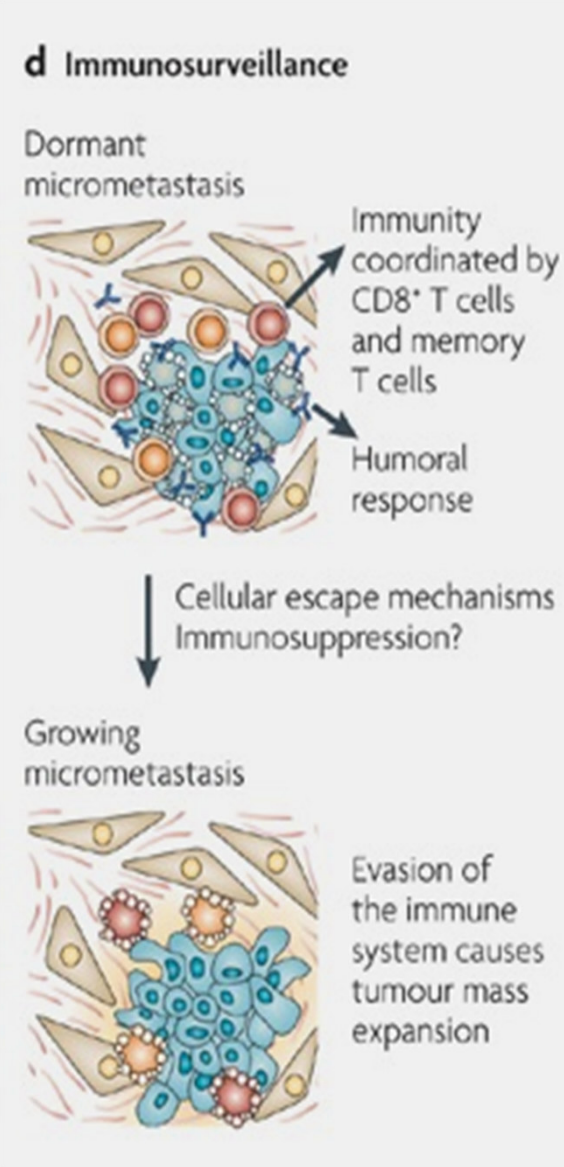


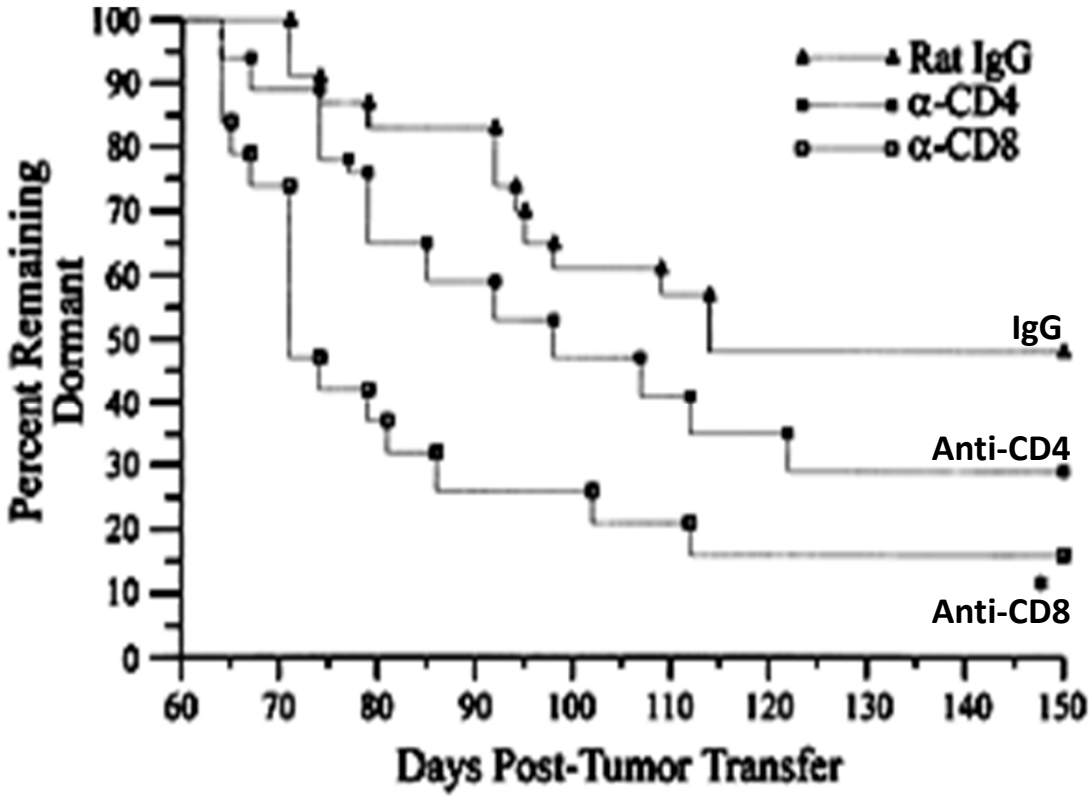
- **Cellular dormancy**:
  cells enter G0 –G1 arrest or senescent state

- **Tumor mass dormancy**:
  cell proliferation is counterbalanced by cell death due to poor vasculature or immune surveillance

Nat. Rev. Canc, 7, 834

Dormancy by immune surveillance

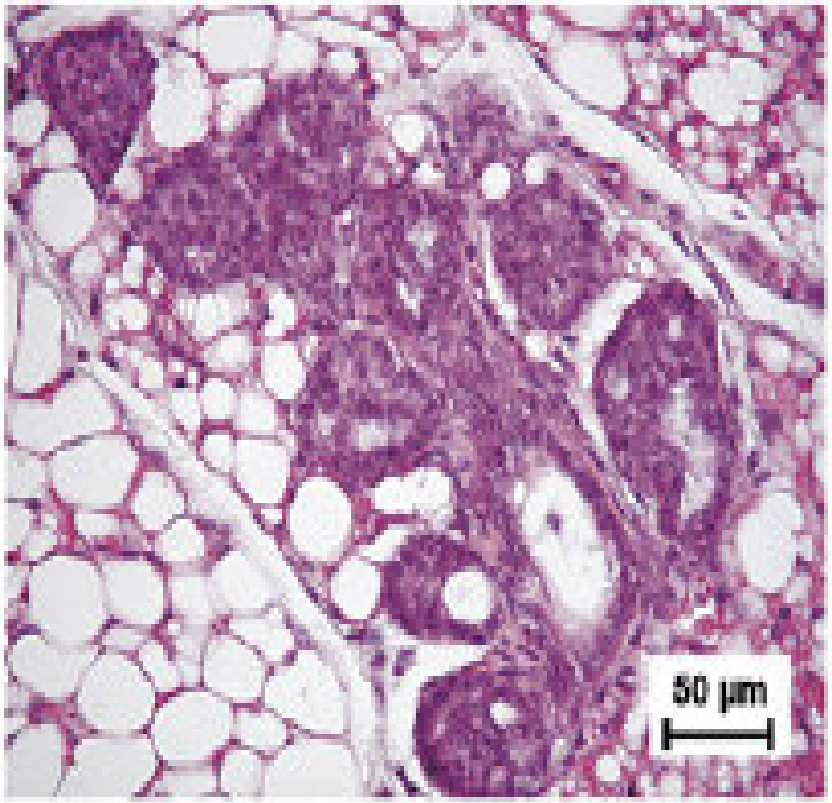




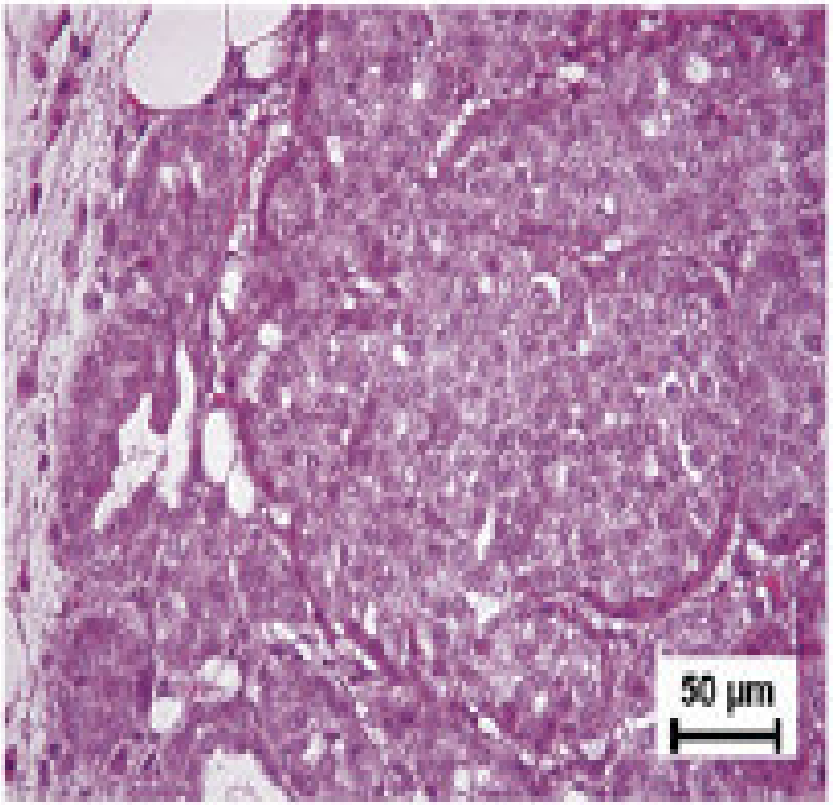
Murine BCL1 B cell lymphoma model (spleen tropic)

Timing: When does metastasis occur?

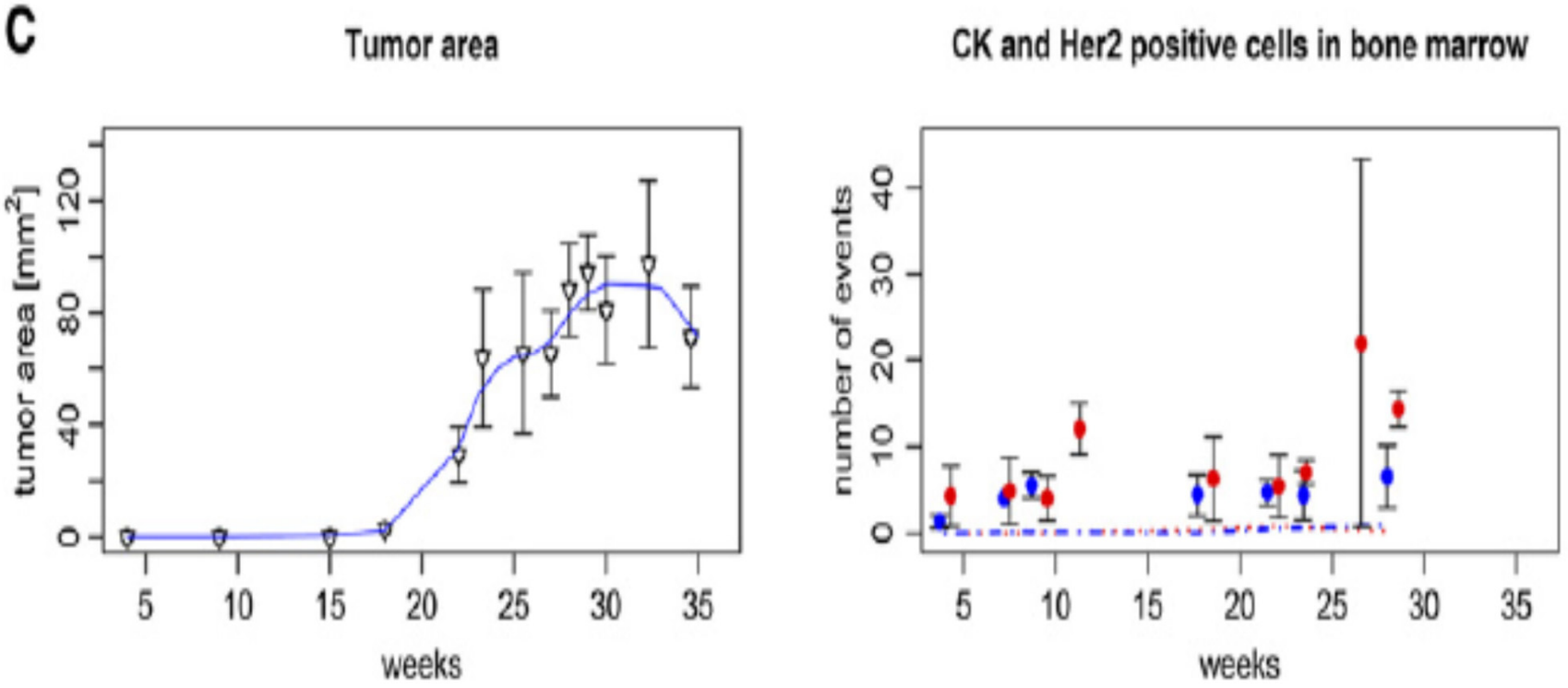
Tumor Progression in transgenic HER-2/neu mice



9 weeks: Atypical Ductal Hyperplasia (ADH)



30 weeks: invasive cancer



- epithelial / tumor cells disseminate in the premalignant phase of murine breast cancers
- DCTs remain dormant at ectopic site

What is the time point of intravasation? Late or ...

- In 2003, Schmidt-Kittler et al., found that DTCs in the bone marrow of breast cancer patients years before metastatic manifestation displayed different and fewer aberrations than their matched primary tumors

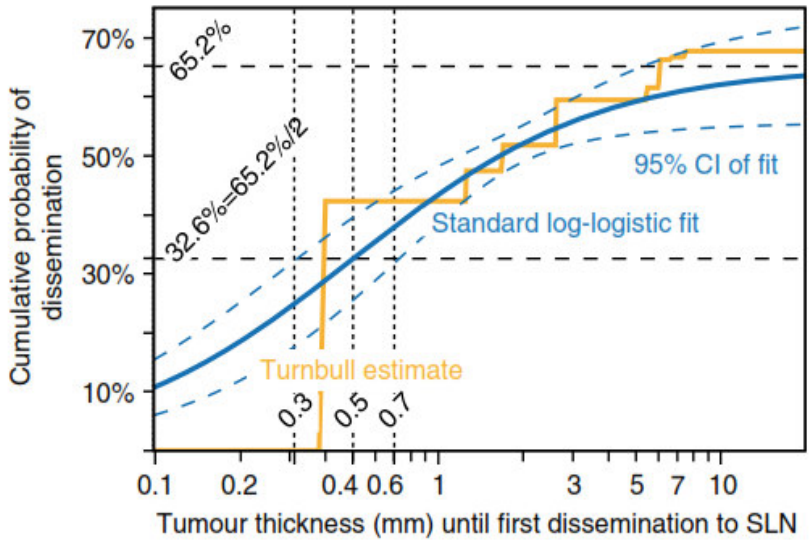
- In 2005, Scharfdt et al., found that breast cancer cells disseminate and survive in the bone marrow before they become chromosomally instable

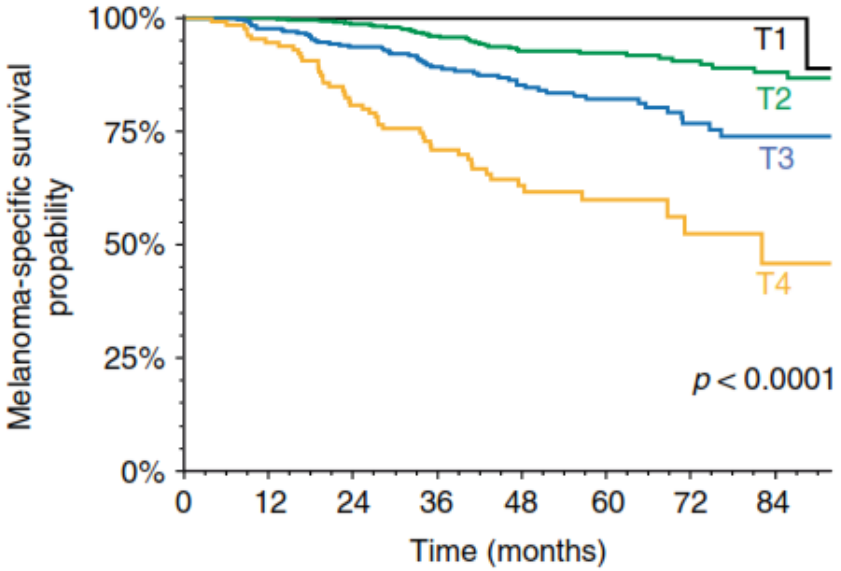
Detection of DTCs at different stages of breast cancer

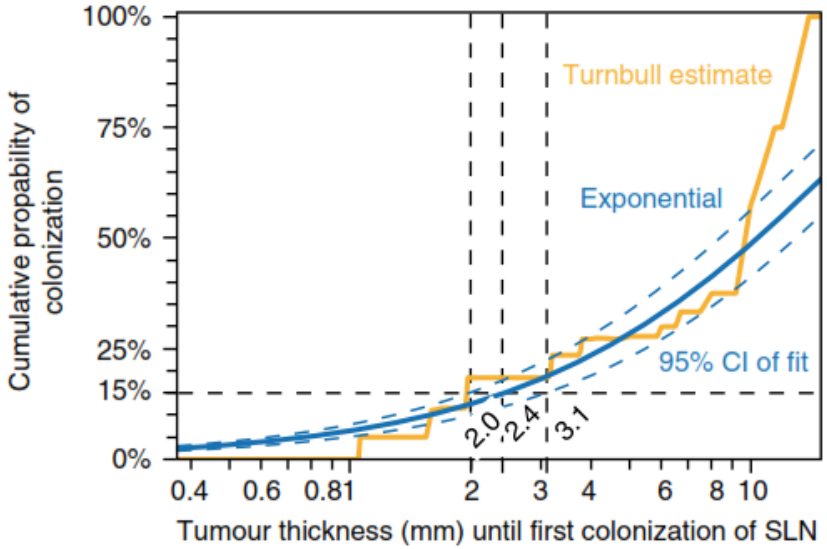
		BM+
Tumour stage	T1	11.2%
	T2	15.0%
	T3	22.6%
Nodal status	N0	9.9%
	N+	20.6%

Naume et al Clin Can Res 2001;7:4122-9

Lymphatic dissemination in melanoma occurs very early and is uncoupled from cancer progression





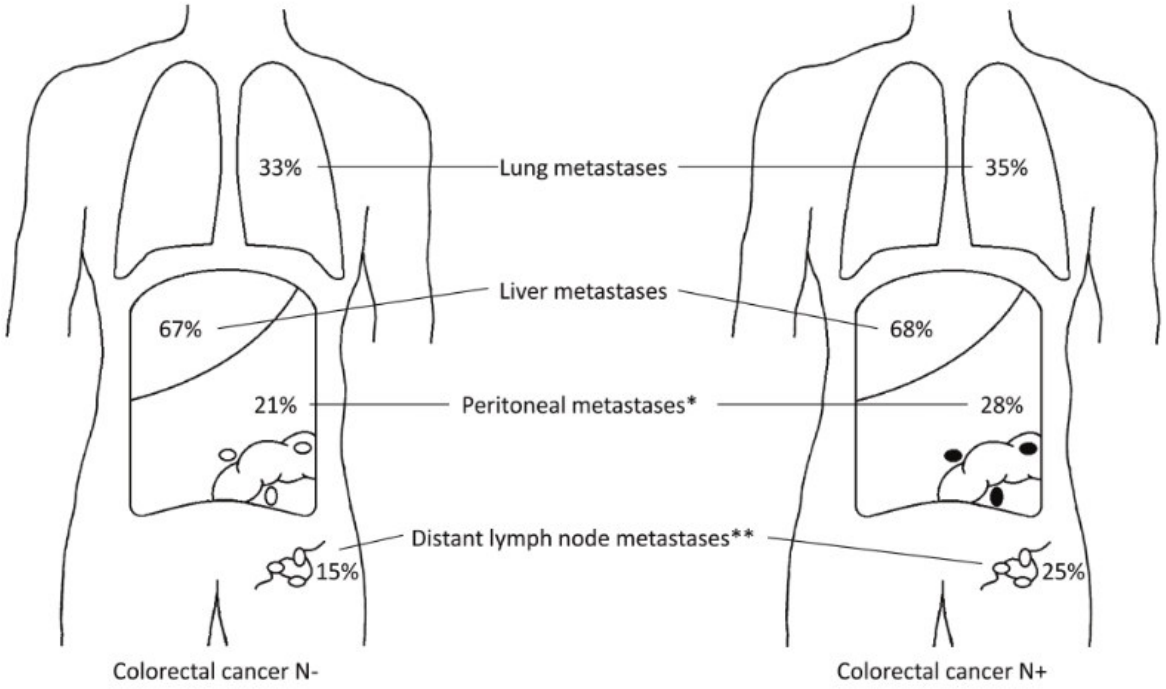


Discrepancy between dissemination and survival

- Patients with T1 and T4 stage melanomas differed only minimal for tumor cell positive sentinel lymph nodes (SLN):
  - 45.8% (38/83) for T1 stage
  - 59.4% (79/133) for T4 stage
- only 1/83 (1.2%) DTC-positive T1 stage melanoma patient died, while 47/133 (35.3%) of T4 stage cases harbouring DTCs died.

Werner-Klein et al., Nat. Comm. 2018

Limited effect of lymph node status on the metastatic pattern in colorectal cancer

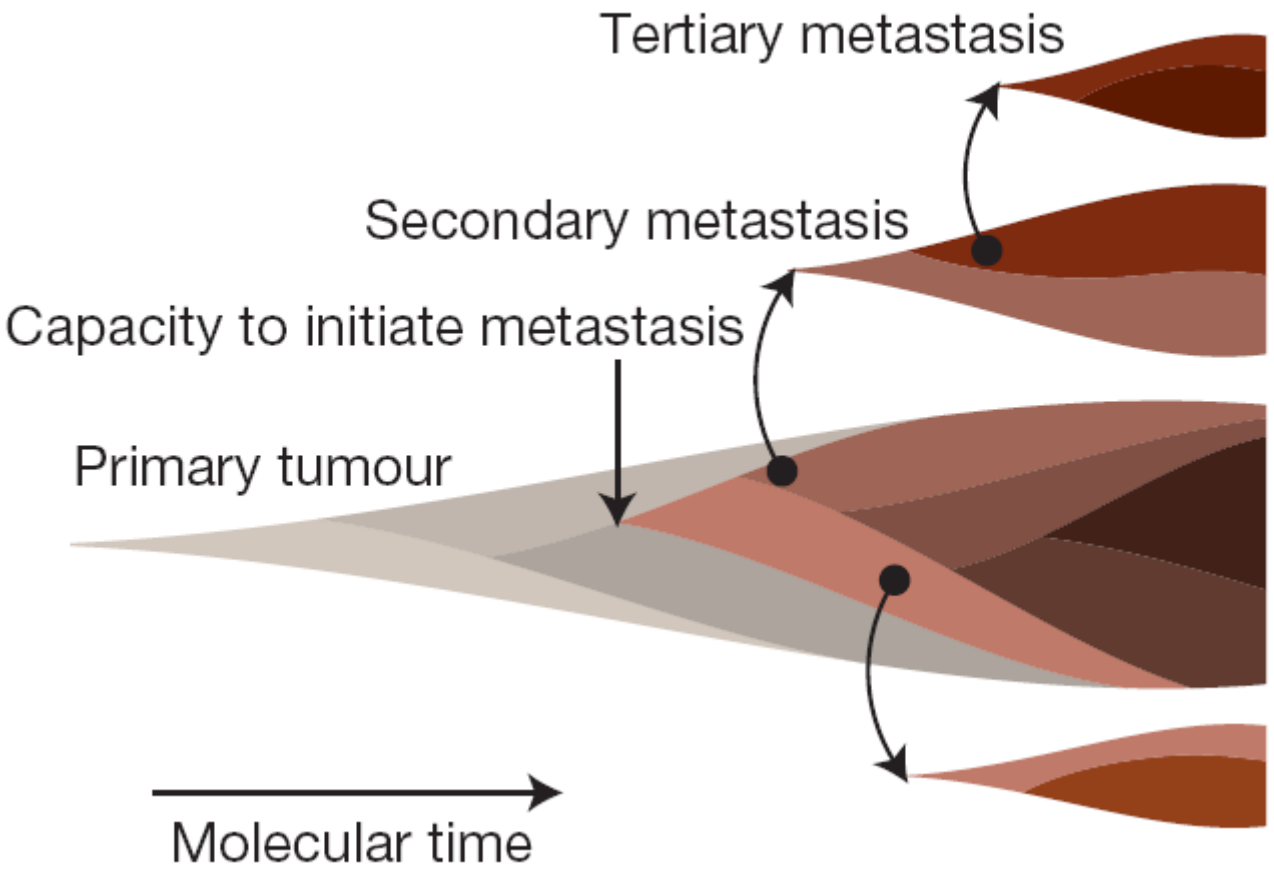


Regional lymph node metastases in colorectal cancer (CRC) decrease outcome. Whether nodal metastases function as a biomarker for advanced disease or are directly involved in the metastatic process is unclear. Evaluation of metastatic patterns of CRC according to lymph node status upon autopsy:

- Regional lymph node (LN) positive CRC more often developed peritoneal metastases (28% vs. 21%, p=0.003) and distant lymph node metastases (25% vs. 15%, p <0.001).
- In contrast, incidences of liver and lung metastases were comparable between regional LN positive and negative patients.
- This supports the hypothesis that dissemination to distant organs occurs independently of lymphatic spread

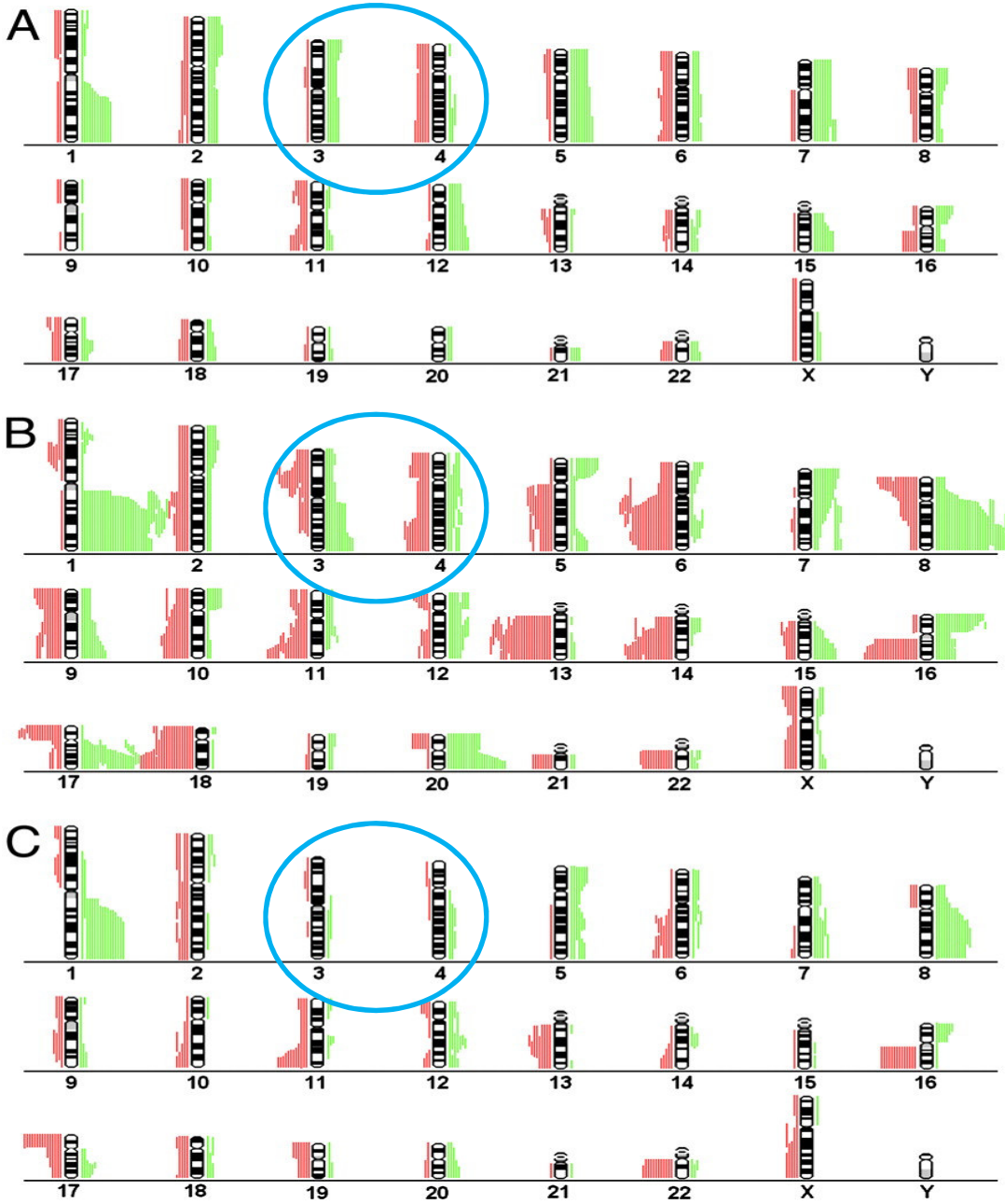
Knijn et al., Oncotarget 2016

Clonal Evolution of primary and secondary tumors



Campbell, et al, Nature 2010

Comparative Genomic Hybridization (CGH)



M0
DTCs BrCA
(minimal residual disease)

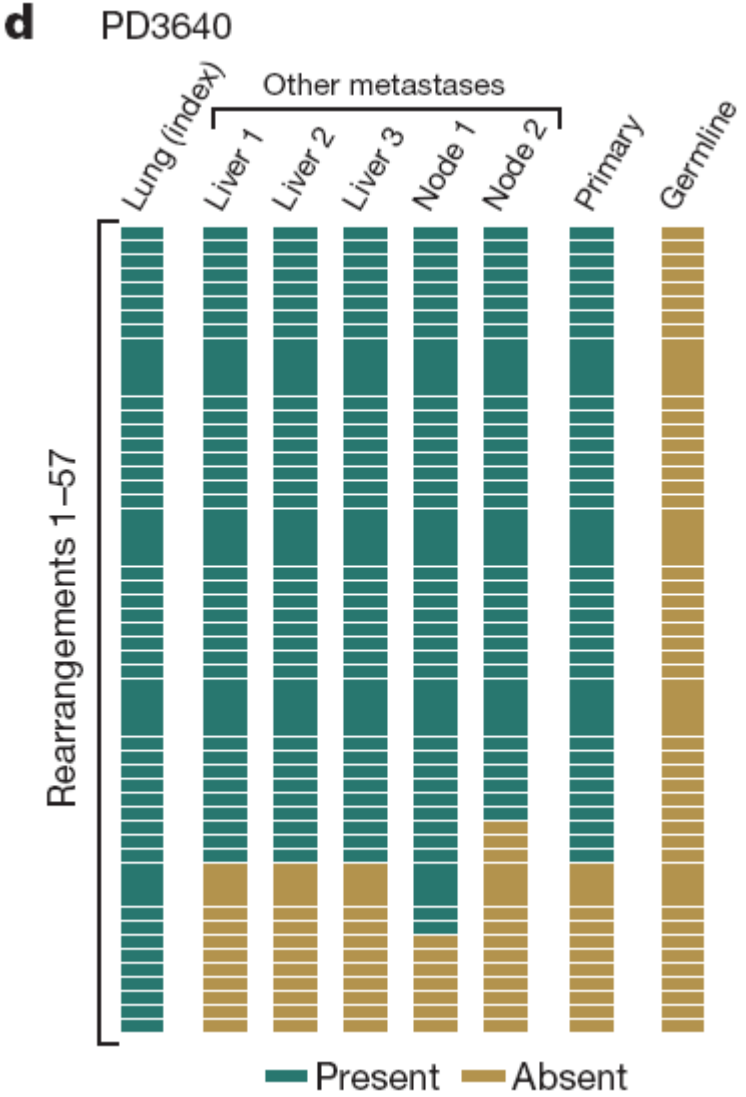
M1
DTC BrCA
(clinical metastasis)

Primary BrCA cells
(samples matching to B)

Early metastasis can occur in less genetically progressed tumor cells

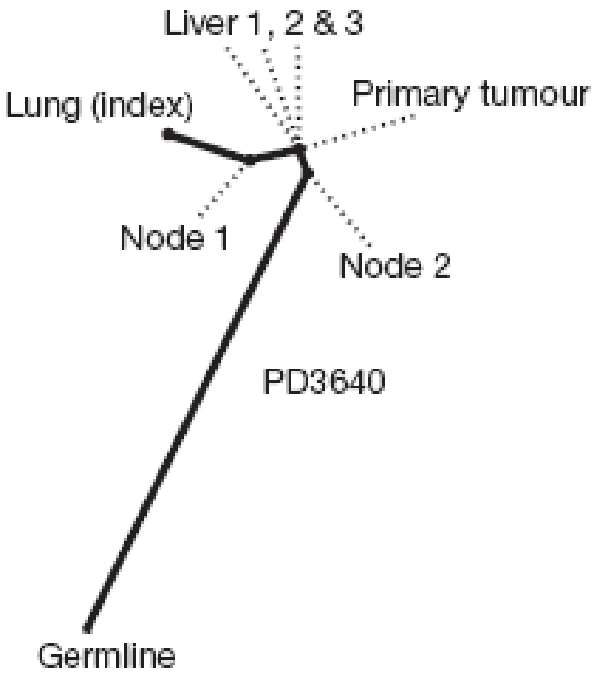
Schmidt-Kittler et al., PNAS 2003

Genomic Alterations During Metastasis in an Individual Pancreatic CA Patient



100 million sequences of paired ends to study chromosomal alterations

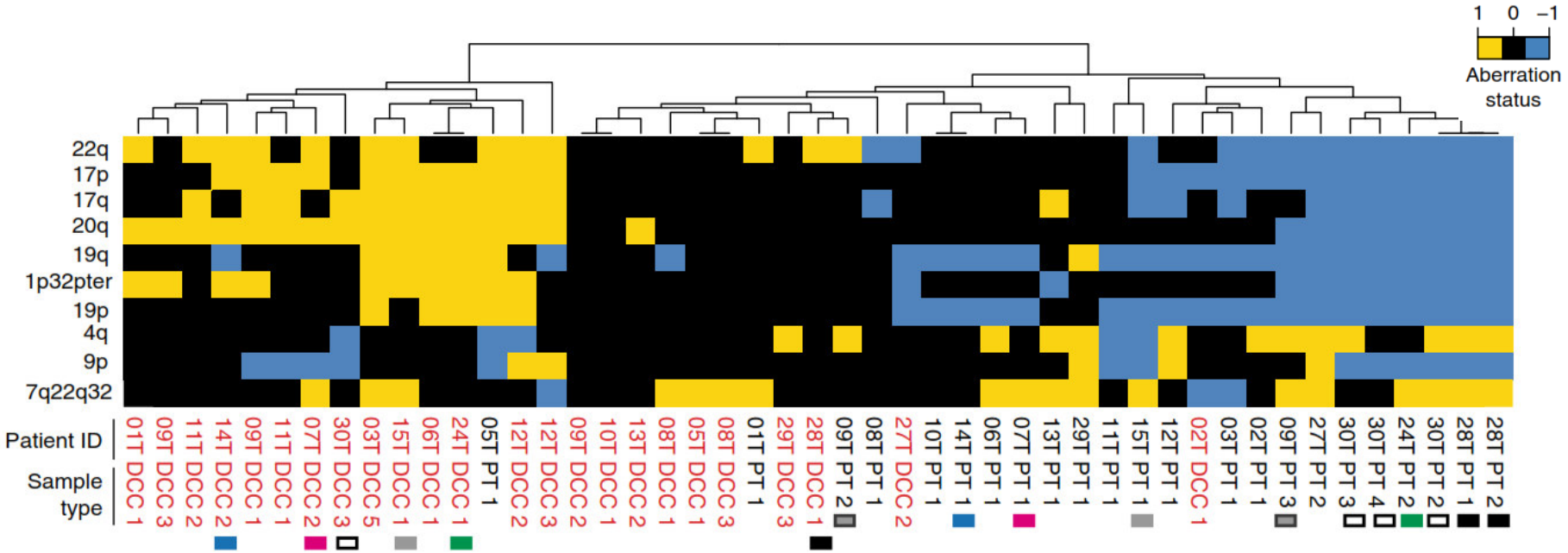
Due to the differential presence of partially shared and private genomic alterations in the primary tumor and its metastases, one can deduce an evolutionary history of a particular tumor.



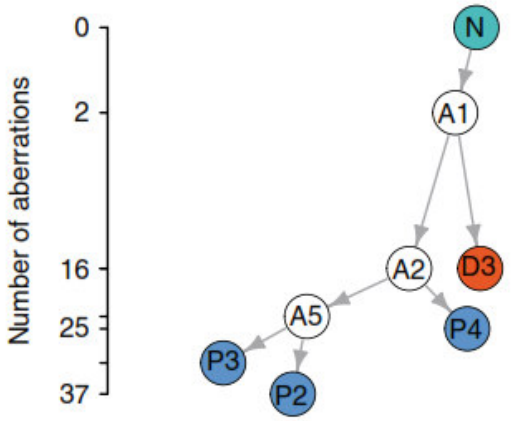
- Metastases to different sites had occurred independently and at different time points.
- The genetic make-up of the primary tumor at diagnosis is not identical with the metastases ancestor.
- Primary and secondary tumors evolve independently.

Campbell, et al, Nature 2010

Genetic alterations driving metastatic colony formation are acquired outside of the primary tumor in melanoma

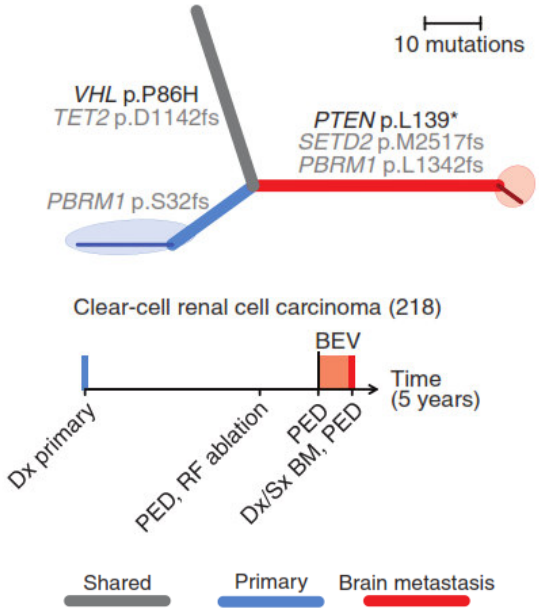


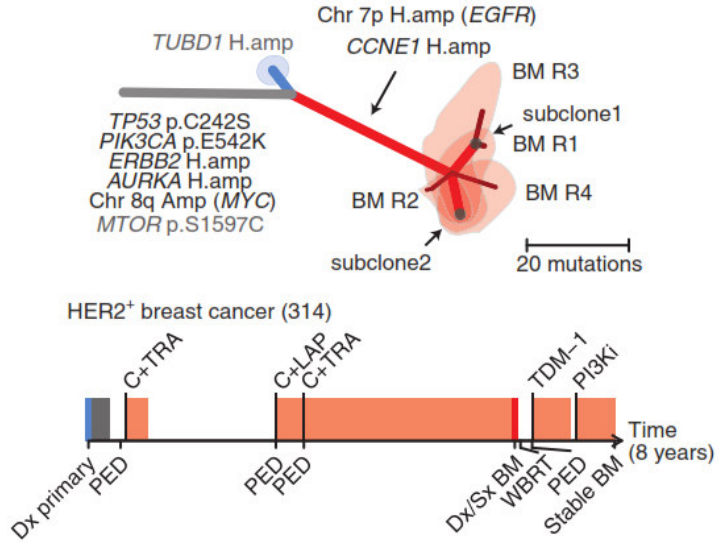
- Typical melanoma driver changes such as BRAF mutation and gained or lost chromosome regions comprising genes like MET or CDKNA2, are acquired within the lymph node at the time of colony formation.
- These changes define a colonisation signature that evolves independently from the primary lesion as soon as cells have left the primary tumour.

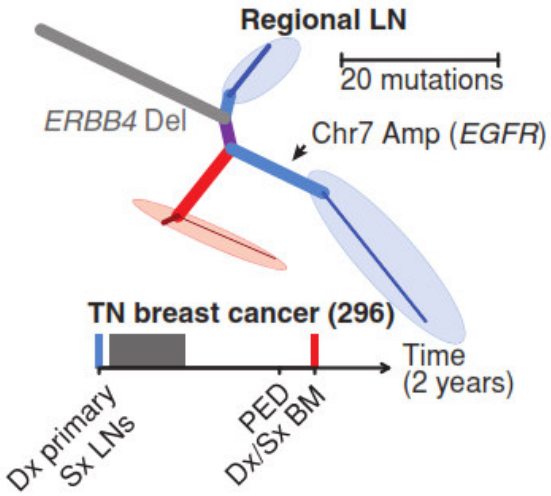


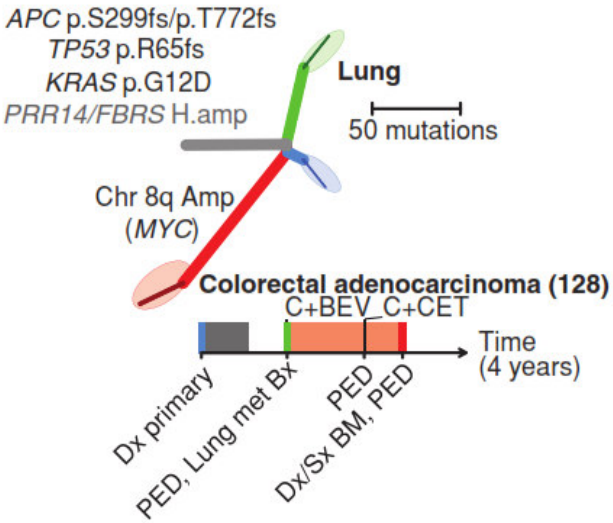
Werner-Klein et al., Nat. Comm. 2018

Clonal Evolution between primary and metastases







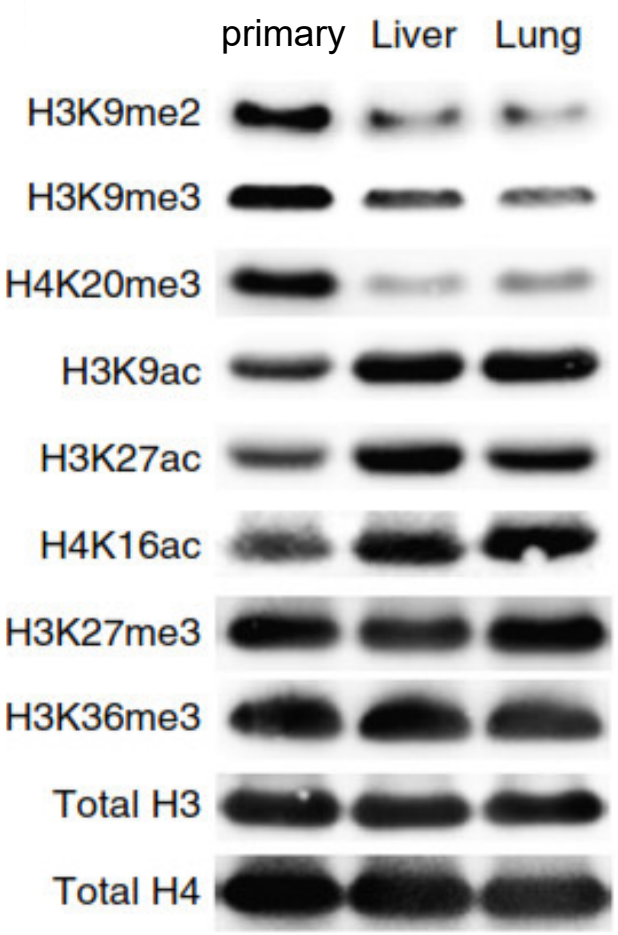


- in all clonally related cancer samples, branched evolution occurred, where all metastatic and primary sites shared a common ancestor yet continued to evolve independently
- in 53% of cases, alterations found in the brain metastases were not detected in the matched primary-tumor sample
- spatially and temporally separated brain metastasis sites were genetically homogenous
- distal extracranial and regional lymph node metastases were highly divergent

Decisions for individualized therapies should be made based on site-specific information

Brastianos, et al, Can. Discov. 2015

Epigenetic reprogramming during metastasis evolution



matched primary and metastatic PDAC lesions collected by rapid autopsy

analysed by whole-exome for coding mutations, paired-end sequencing for rearrangements and whole-genome sequencing for total mutations and copy number alterations

=> no metastasis-specific driver mutations identified

=> ChIP–seq (H3K9me2, H3K9me3, H3K27me3, H3K27ac, H3K36me3) , whole-genome bisulfite sequencing and RNAseq from matched samples

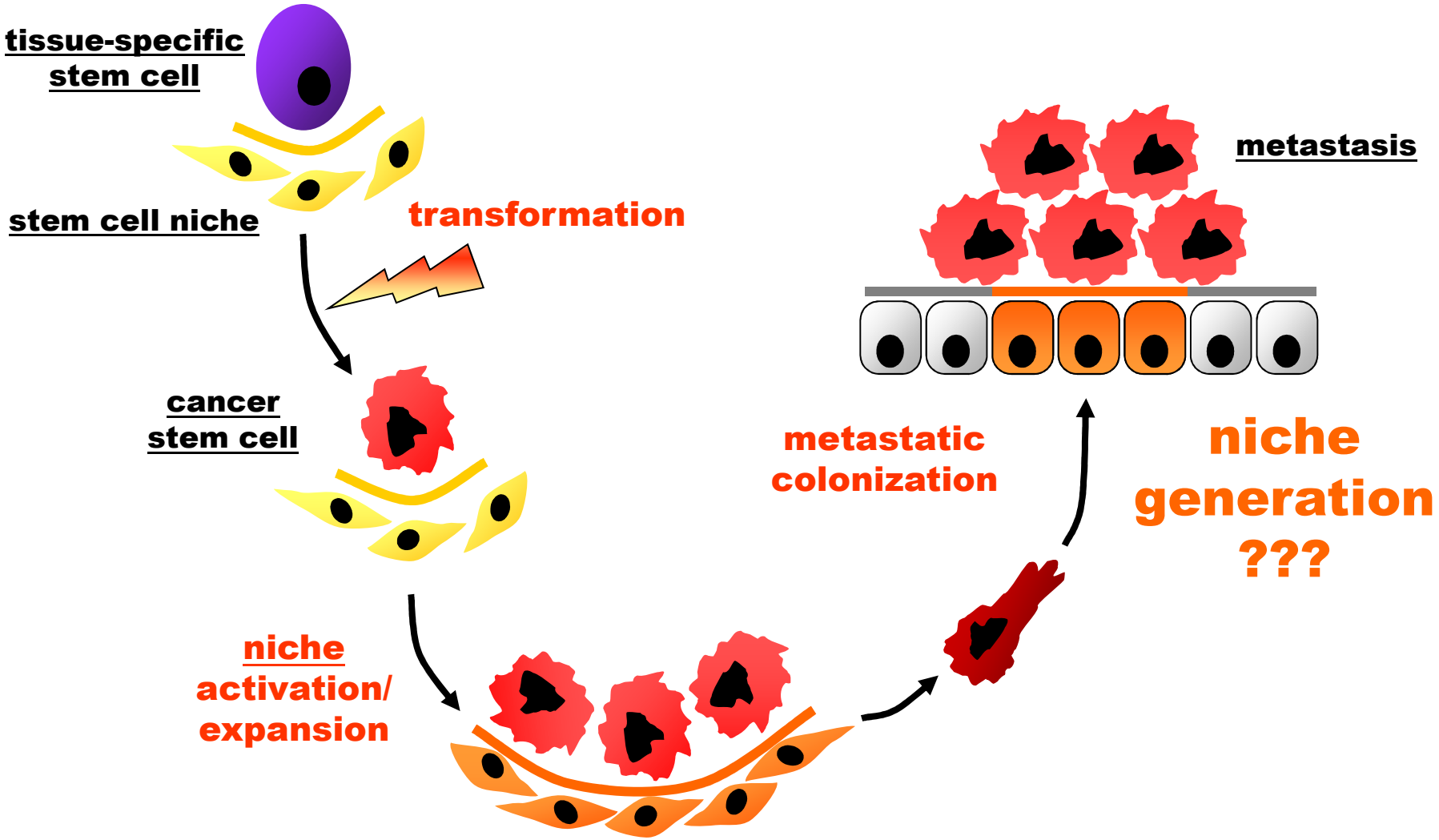
The global epigenetic state was reprogrammed during the evolution of distant metastasis:

- Alteration of **H3K9 methylation**
- Increased **H3K27ac** marks linked to **active gene regulatory elements**

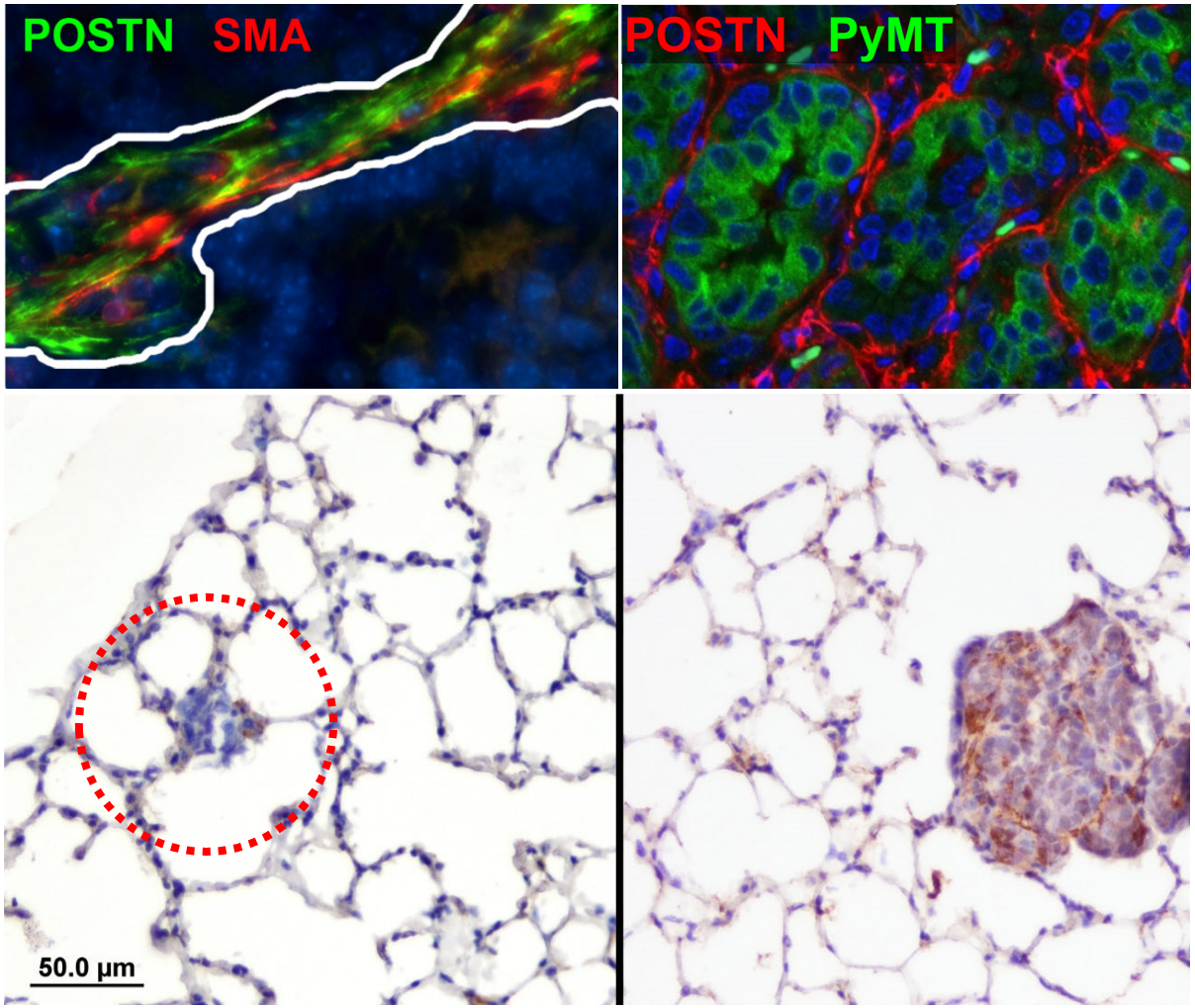
McDonald et al., Nat Gen. 2017

Supportive microenvironments

Niche derived Signals: Role in CSC expansion and metastatic colonization?



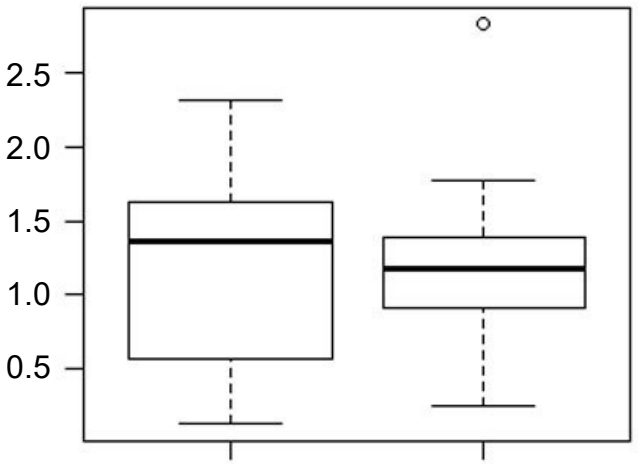
Stromal expression of POSTN in tumor and metastasis



early colony micro metastasis

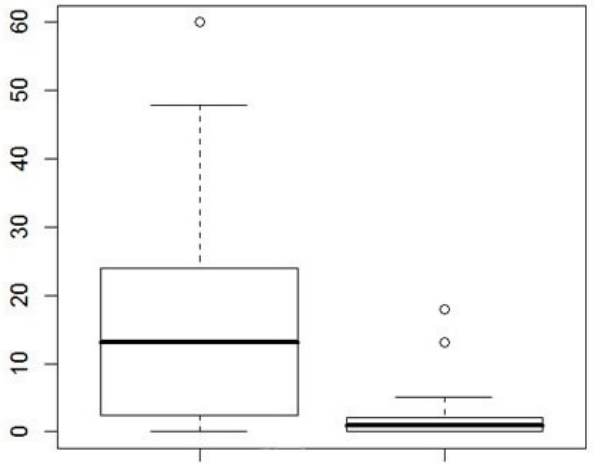
Essential function for POSTN in spontaneous metastasis formation

Primary tumor size

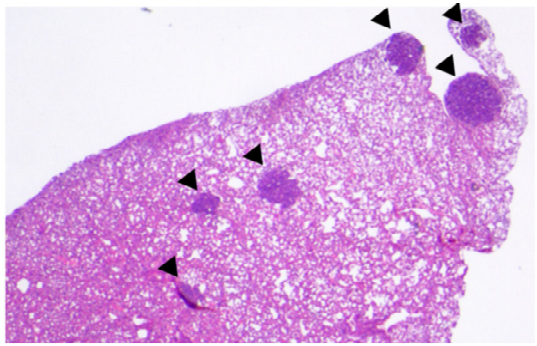


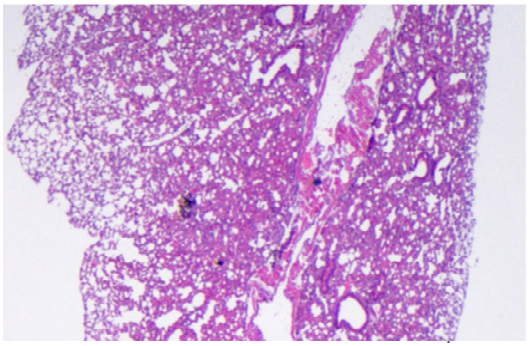
n=21

Number of Lung Metastasis



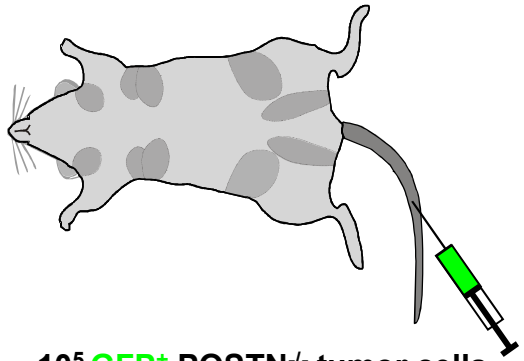
MMTV-PyMT control



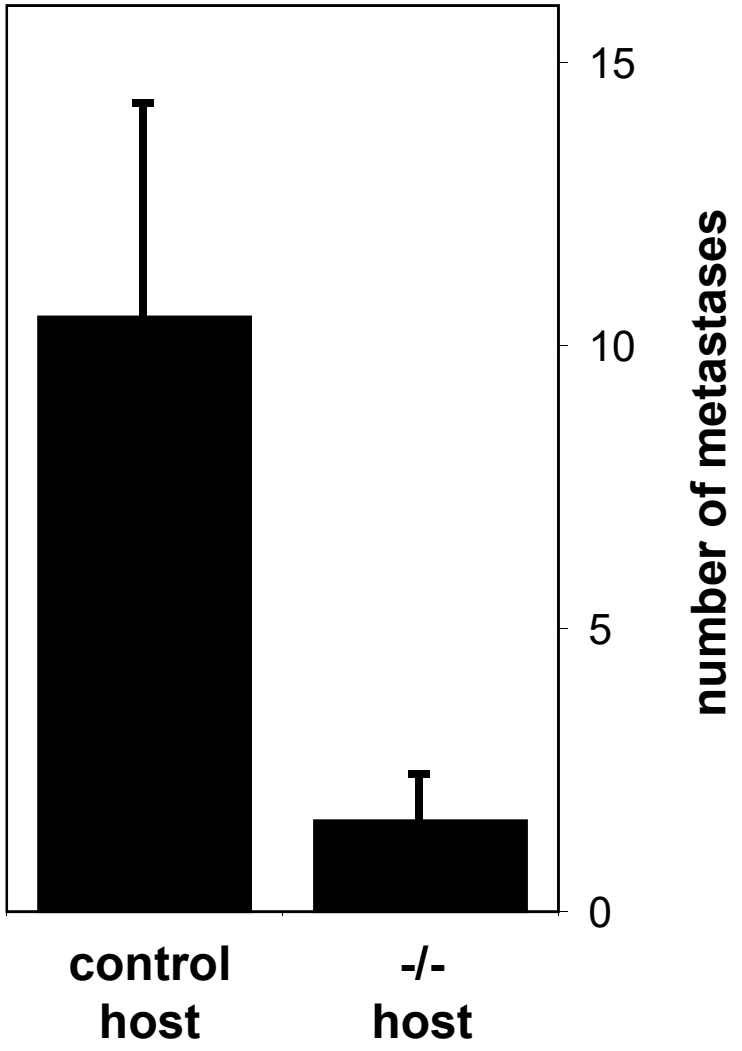


MMTV-PyMT POSTN KO

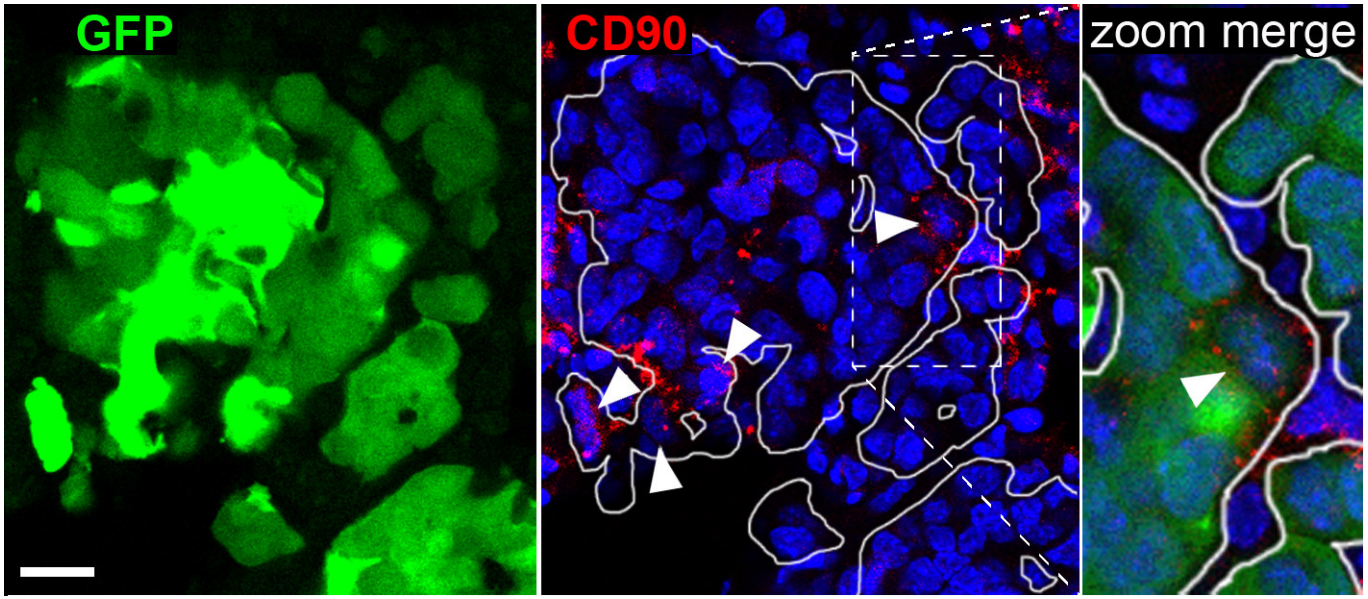
Tail vein injections confirm the requirement for POSTN in the lung stroma

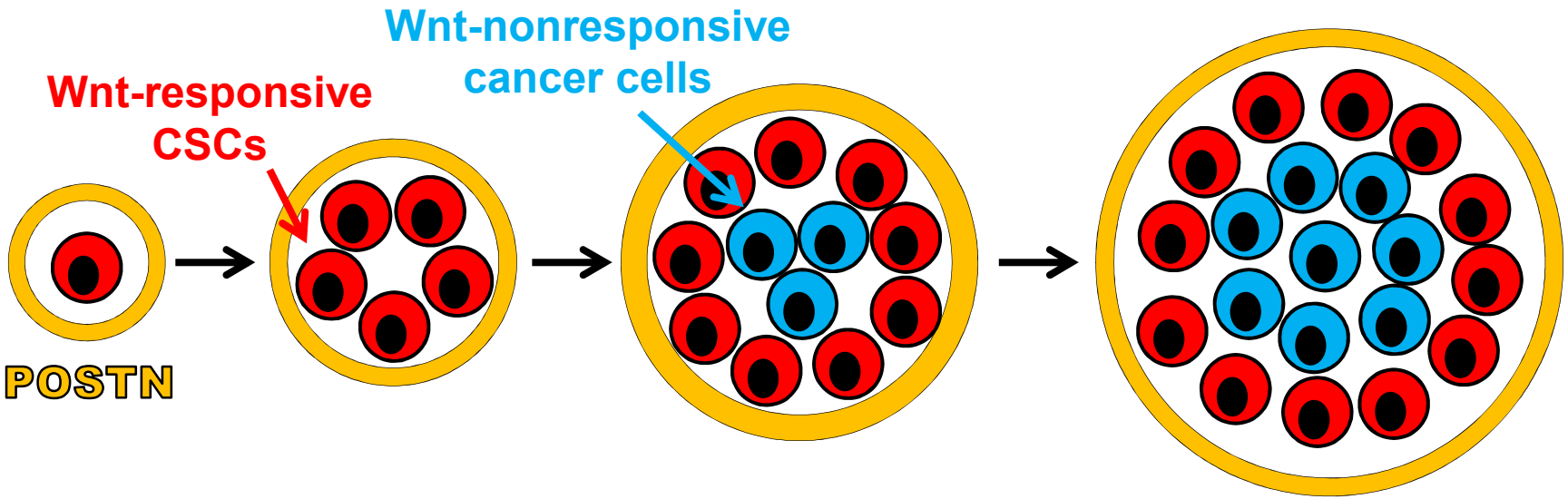


10^5 GFP$^+$ POSTN$^{-/-}$ tumor cells
tail vein injection

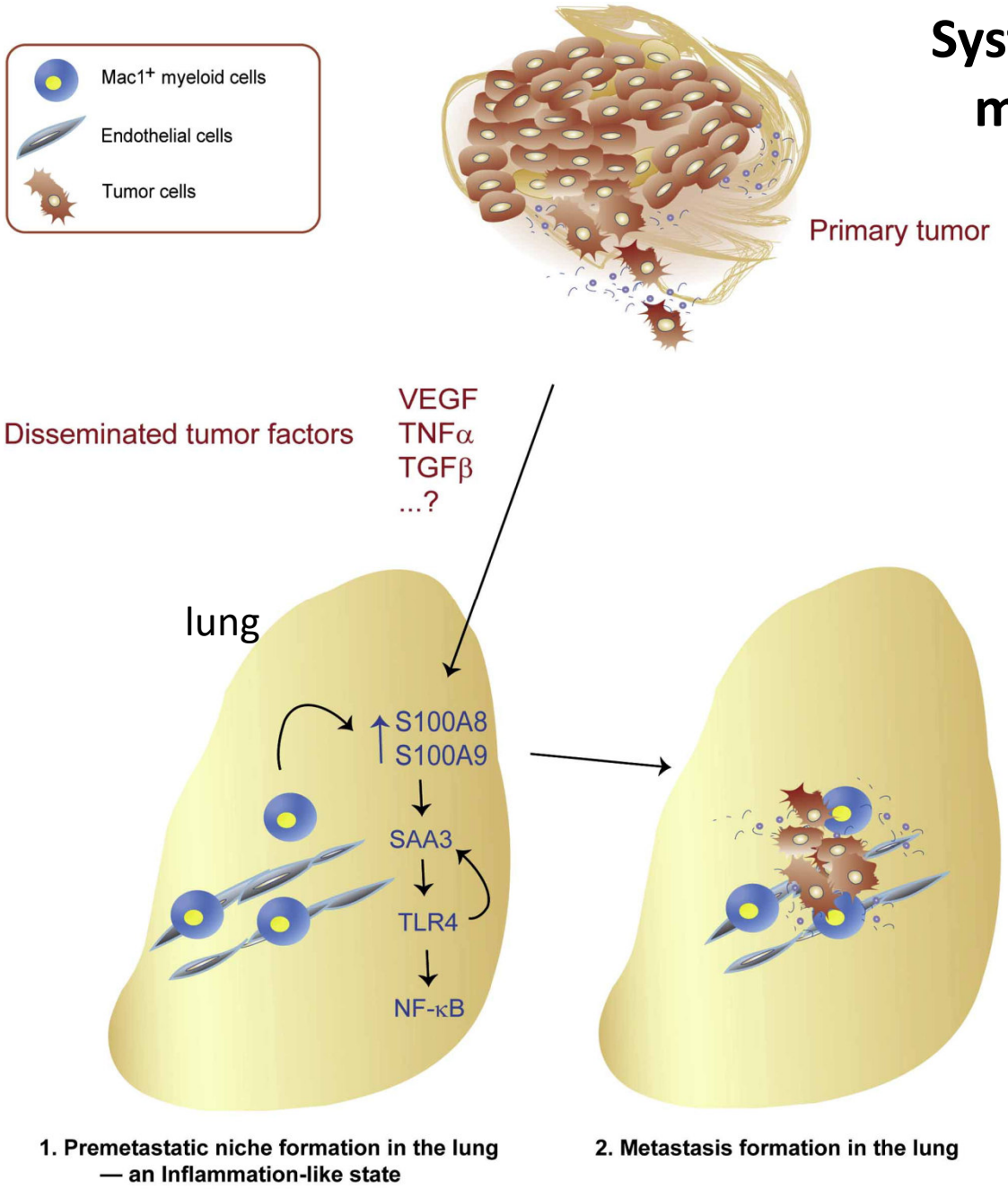


POSTN niches allow Cancer Stem Cell expansion in vivo



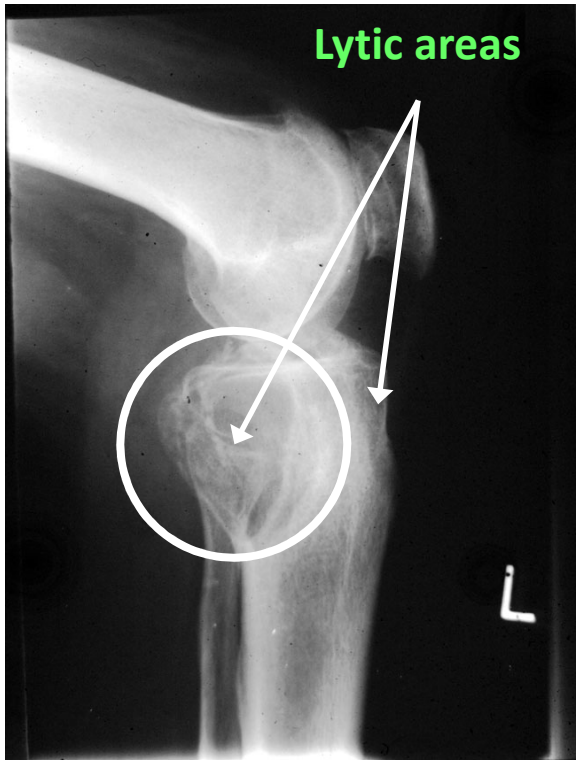


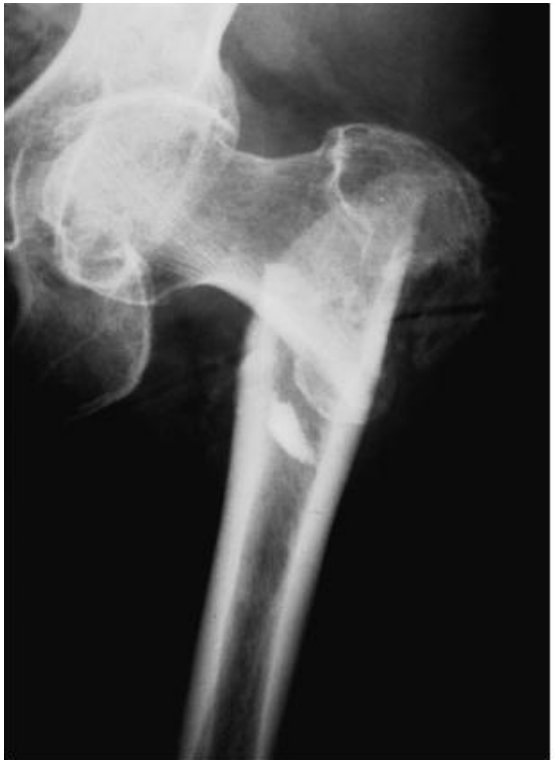
Systemic host conditioning for metastatic niche induction

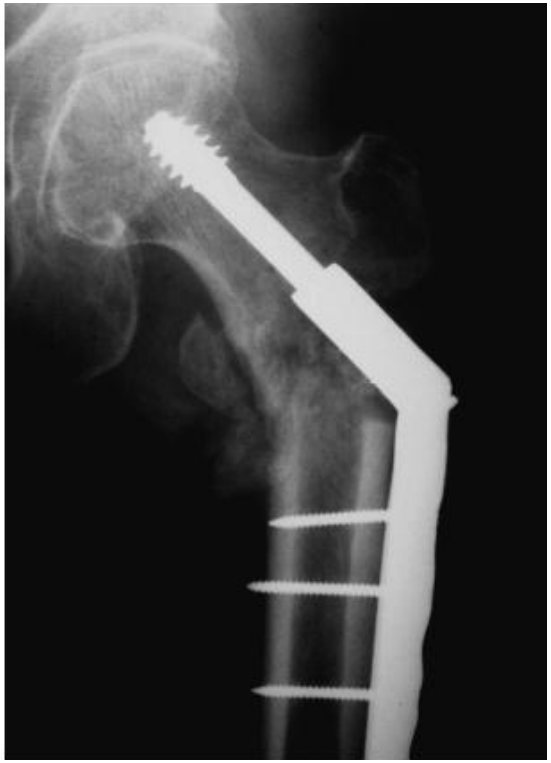


- the **Premetastatic Niche** Is Similar to an Inflammatory Nidus
- both S100A8 and S100A9 induce the expression of SAA3 specifically in future metastasis sites in the lung, promoting $CD11b^+$ myeloid and endothelial cell engraftment
- this inflammation-like state accelerates the recruitment of tumor cells

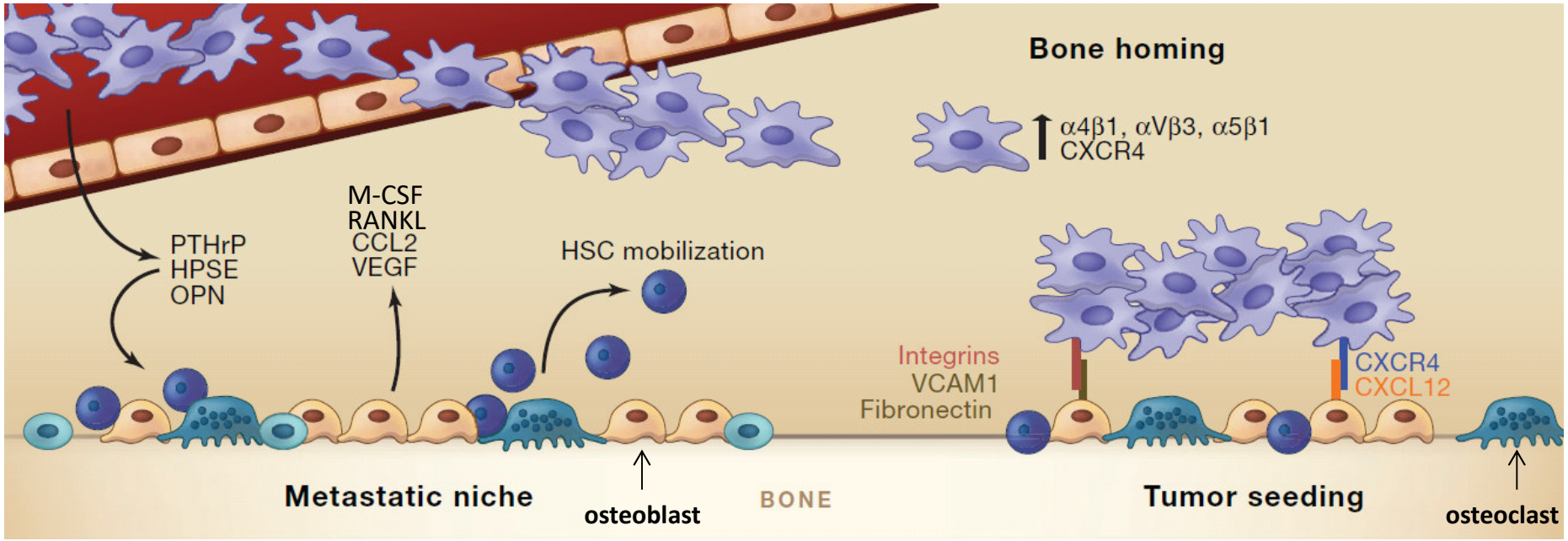
Breast cancer to Bone Metastasis: Osteolysis and Pathologic Fracture





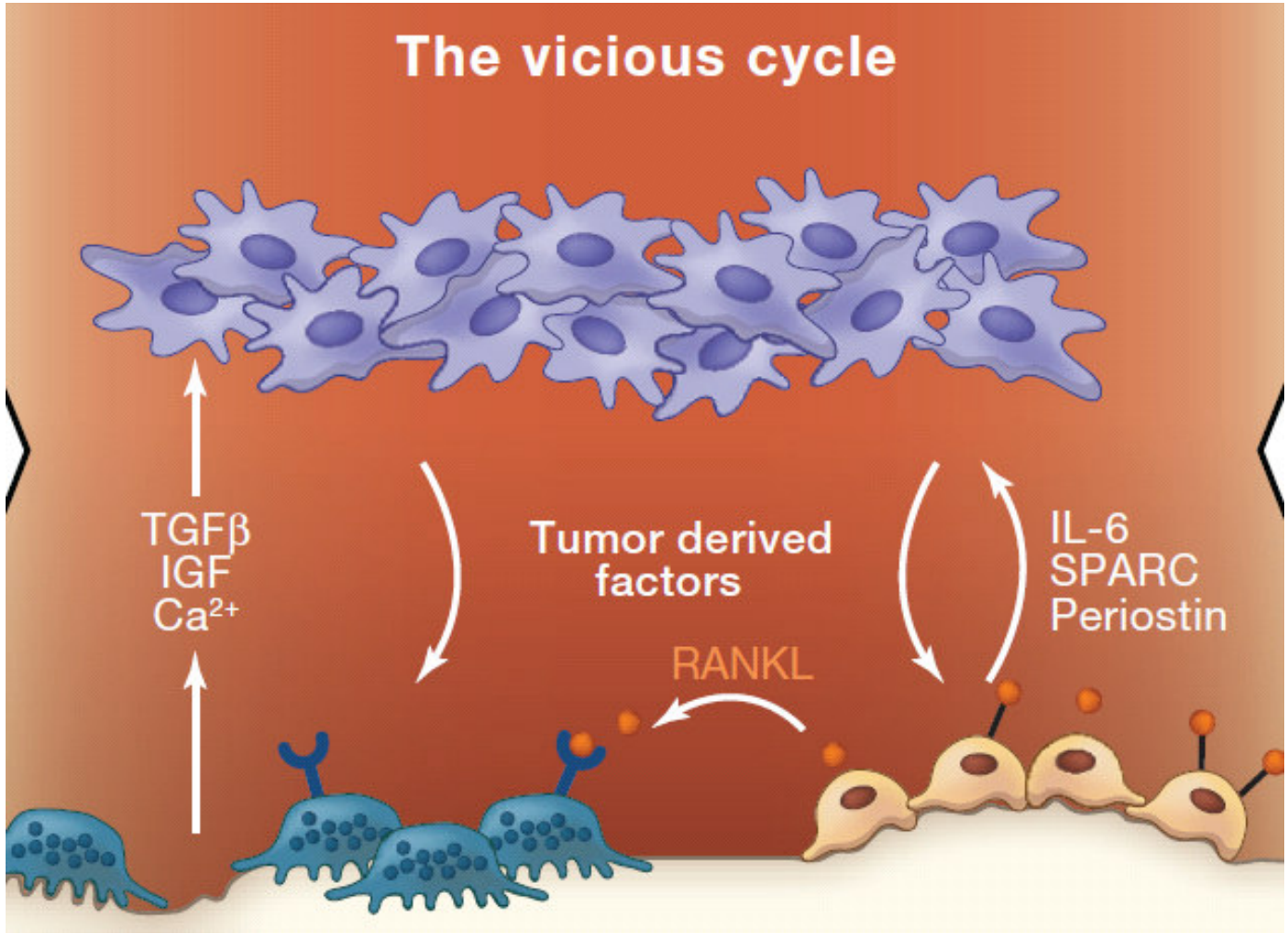


Molecular mechanisms of bone metastasis: seeding



- osteolytic metastases (predominant in breast cancer metastasis) are mediated by interactions of tumor cells with osteoblasts and osteoclasts and involve aberrant bone resorption due to the recruitment and activation of osteoclasts to the tumor-bone interface
- tumor cells secrete parathyroid hormone-related protein, RANKL, heparinase and osteopontin
- PTHrP induces RANKL, M-CSF, CCL2 and VEGF (angiogenesis) production from osteoblasts
- RANKL, M-CSF and HPSE increases osteoclast differentiation, activity and bone resorption
- CCL2 mediates myeloid cell recruitment whereas tumor cells are recruited via CXCR4 and its ligand CXCL12 which is highly expressed in the bone marrow

Molecular mechanisms of bone metastasis: growth phase



TGFβ, IGF, and Ca are released from the bone matrix during lysis, enhancing tumor proliferation and survival whereas IL-6 produced by tumor cells stimuates osteoblasts to express RANKL which in turn differentiates and activates osteoclasts to enhance bone matrix degradation