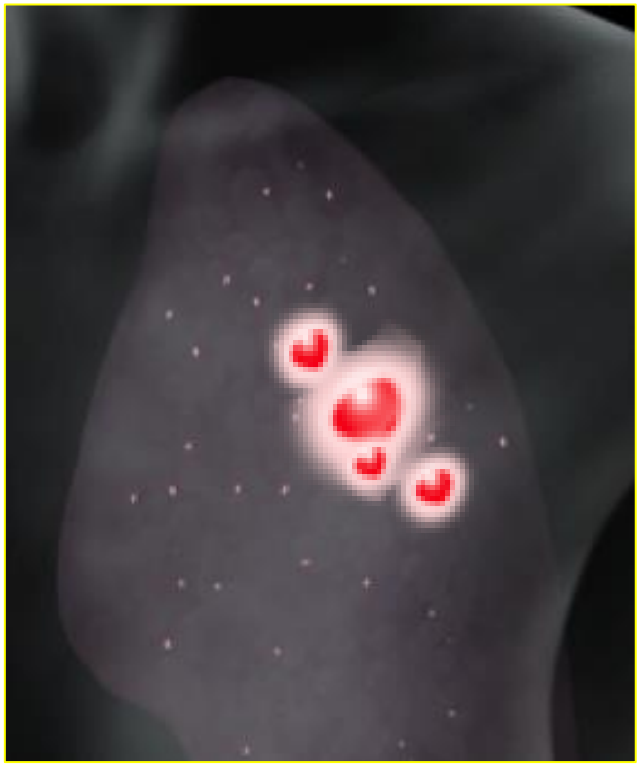


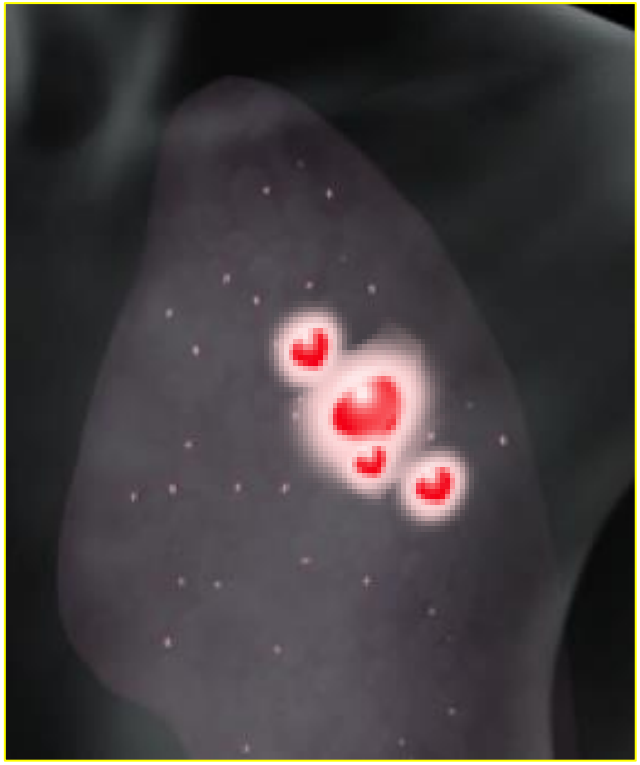
Moving out: Invasion and Metastasis lecture

PATHG4500 Cancer Biology Course 2023

Swarnali Acharyya, Ph.D.,
Associate Professor
Pathology and Cell Biology & ICG,
Columbia University Irving Medical Center

Metastasis Laboratory
https://acharyyalab.wixsite.com/acharyya-lab

October 29th, 2025



Reading material

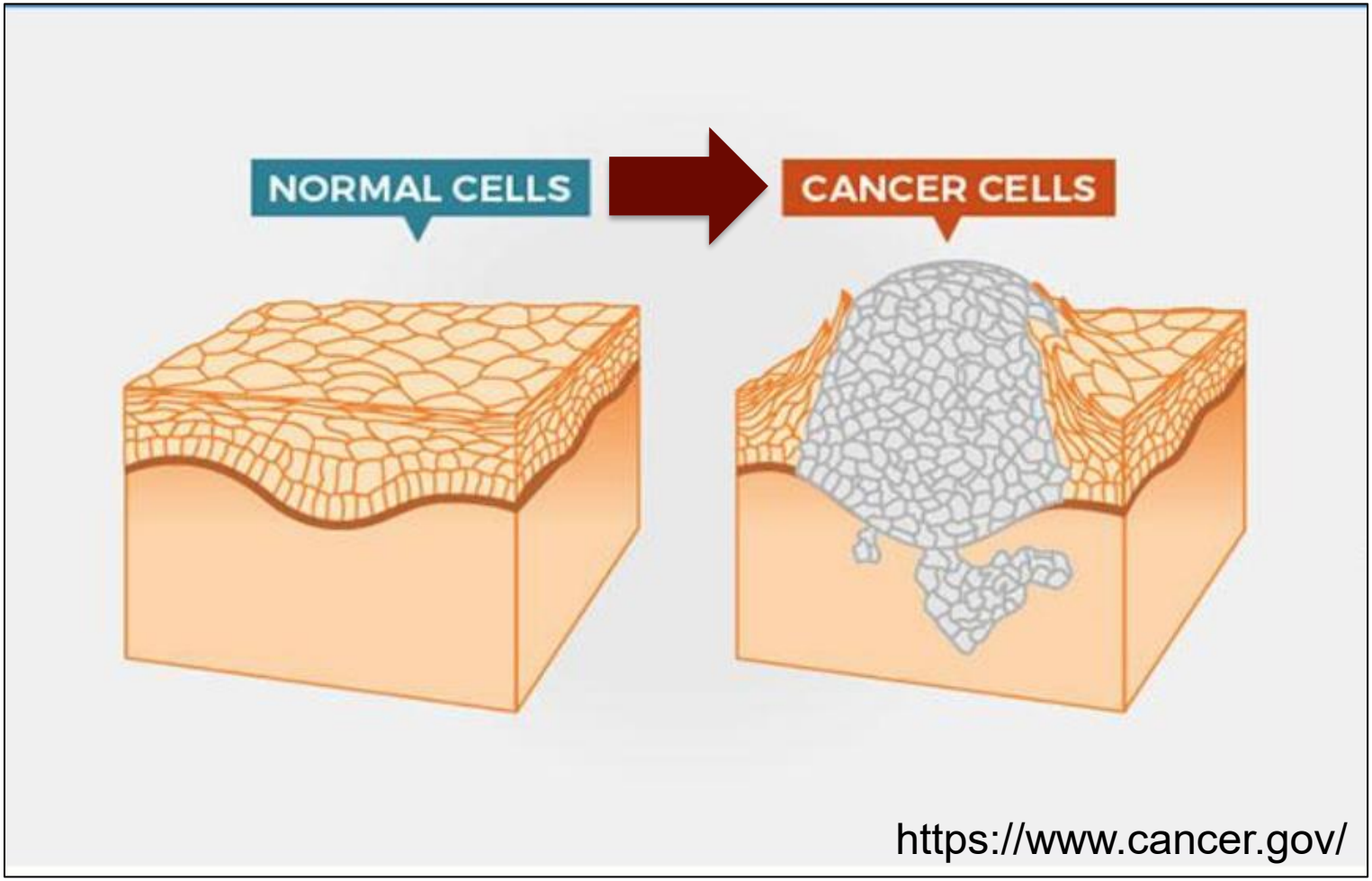
Chapter 14: Invasion and metastasis

The Biology of Cancer by Robert Weinberg (Third Edition) and papers cited throughout the lecture

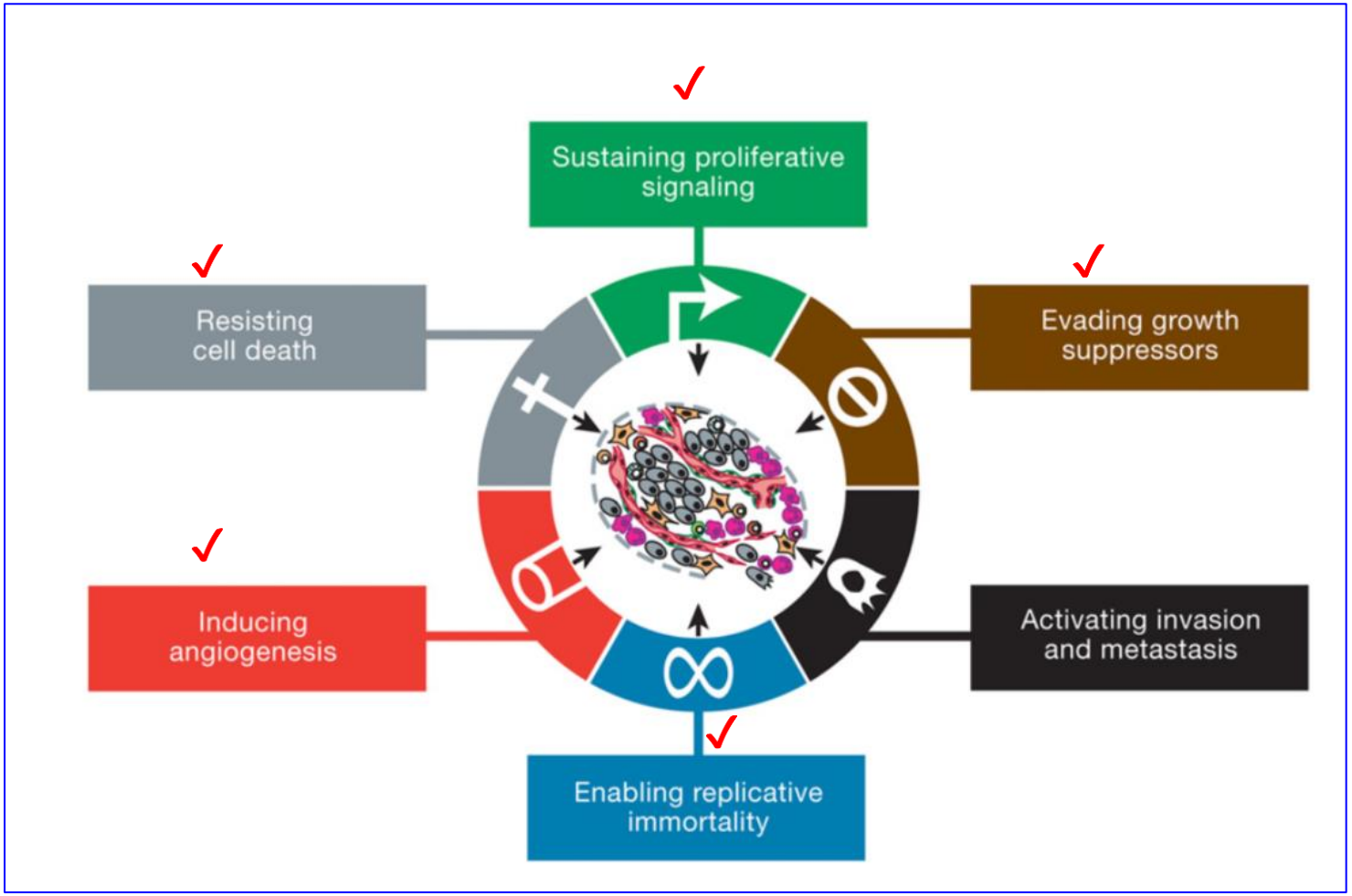
Overview of the lecture

- Introduction to the metastatic cascade
- Historical perspective and foundation of metastasis research
- Studying the journey of a cancer cell from primary tumor to distant sites
- Challenges and therapeutic avenues for targeting metastasis
- Provocative questions in metastasis research

Conversion of a normal cell to a cancer cell



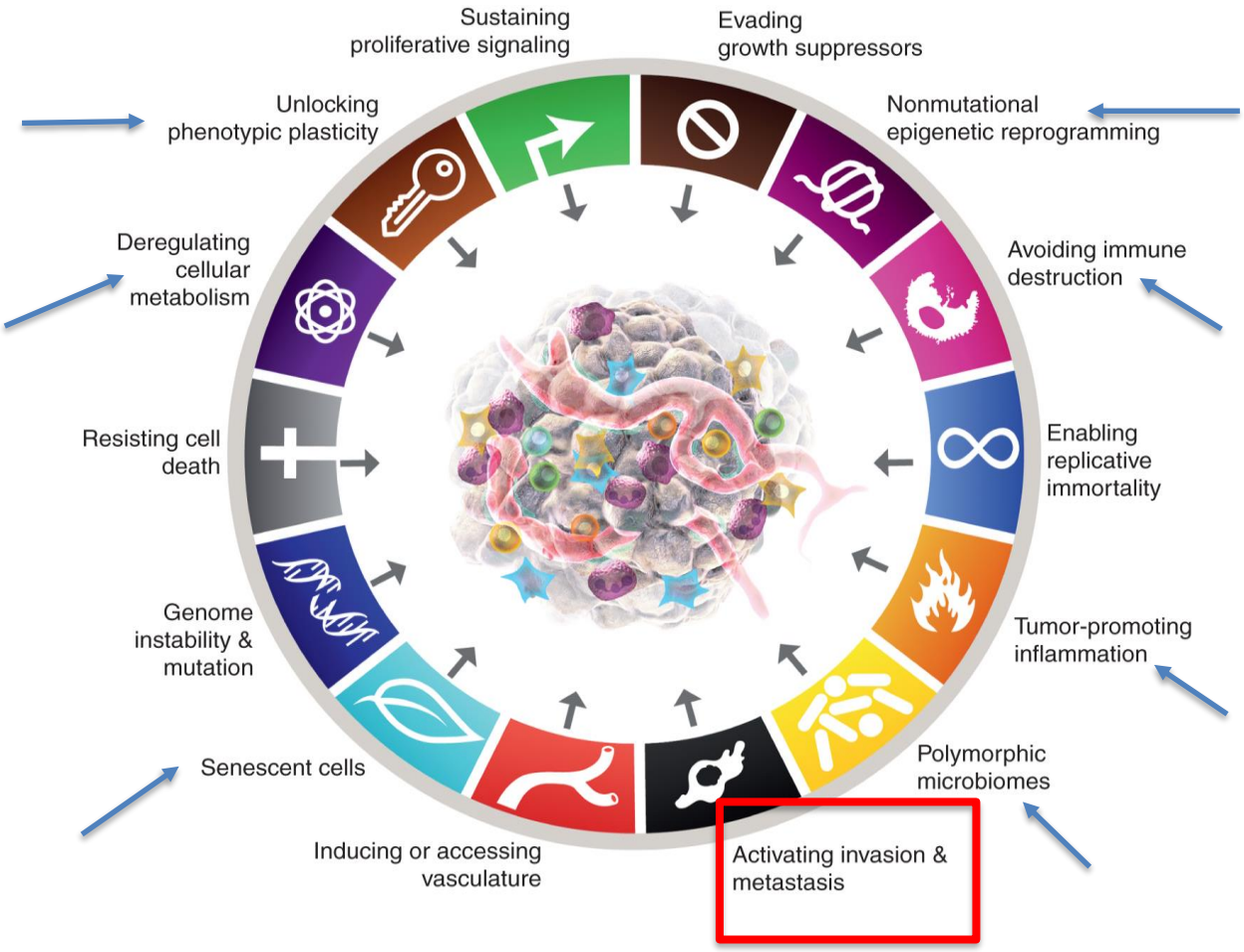
Hallmarks of cancer



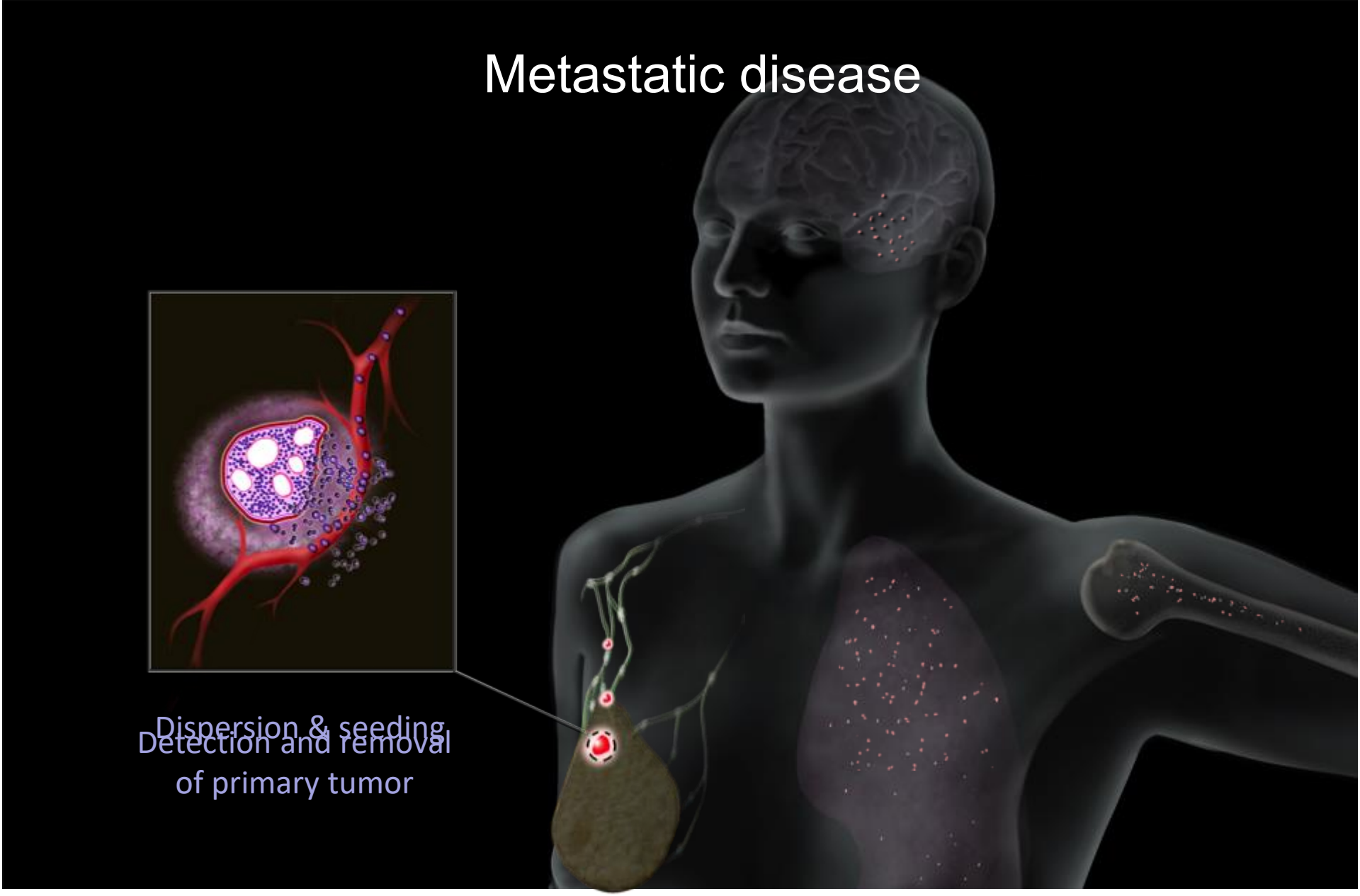
Reference: Hallmarks of Cancer: The next generation, Hanahan and Weinberg, *Cell*, 144, 2011

Hallmarks of Cancer: New Dimensions

Cancer Discov. 2022;12(1):31-46. doi:10.1158/2159-8290.CD-21-1059



Metastatic disease



Dispersion & seeding

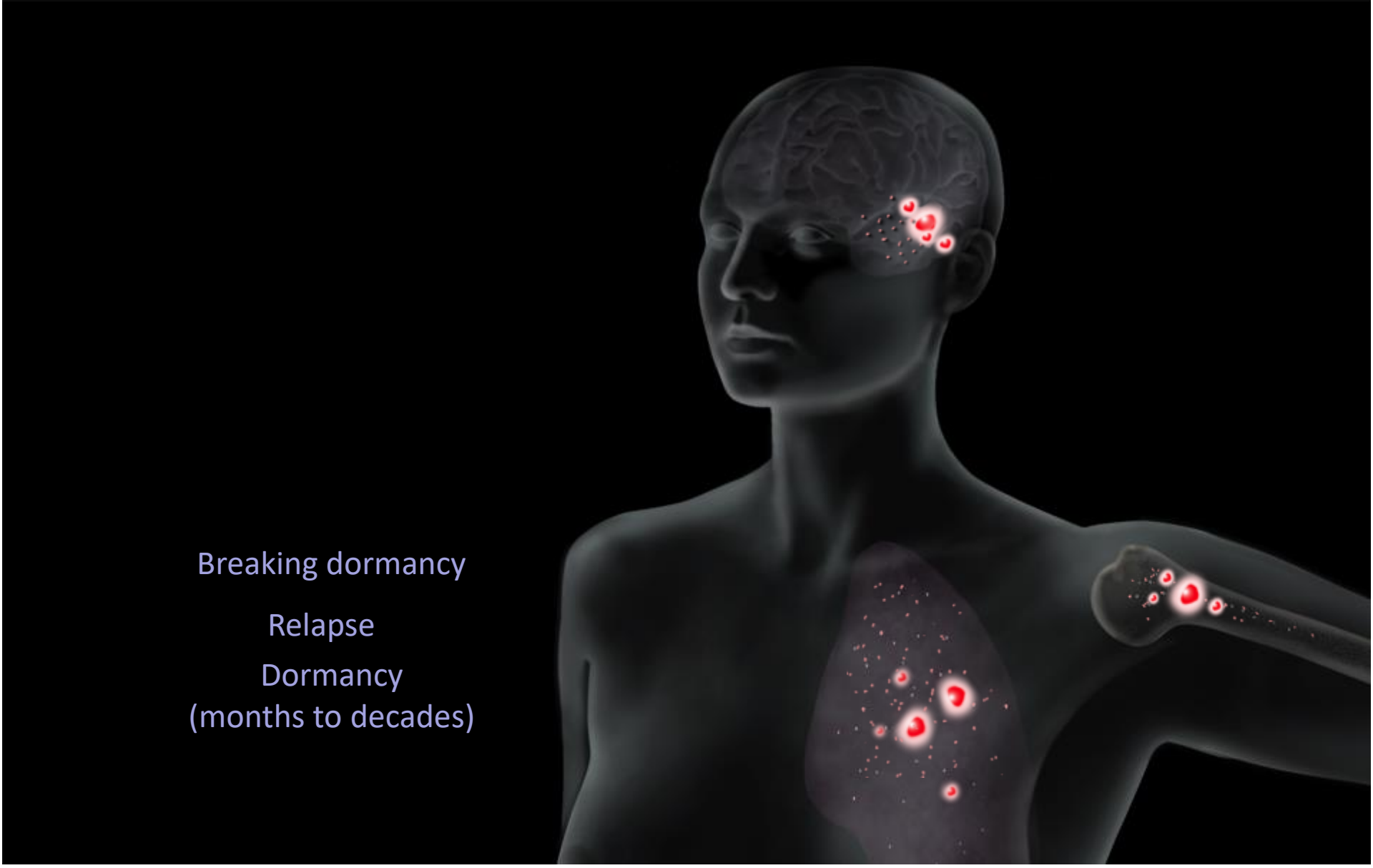
Detection and removal of primary tumor

Vanharanta and Massague, Cancer Cell, 2013

Breaking dormancy

Relapse

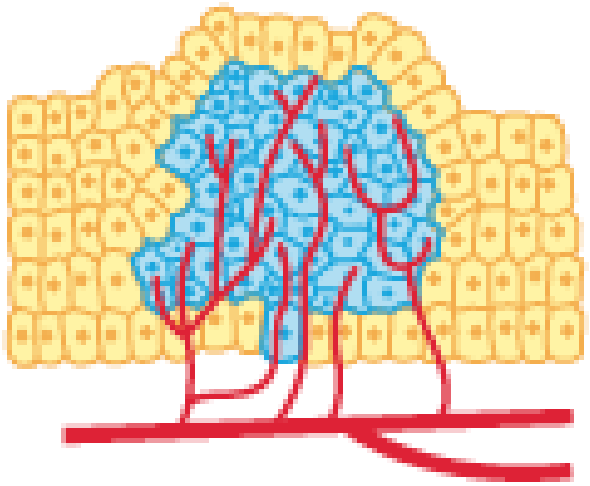
Dormancy
(months to decades)



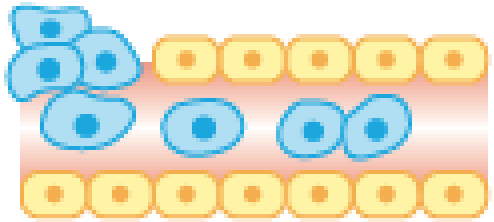
What is metastasis?

In Greek, **methistanai = Removal or change**
In Latin, rapid transition from one point to another

Primary tumor



Escape into circulation



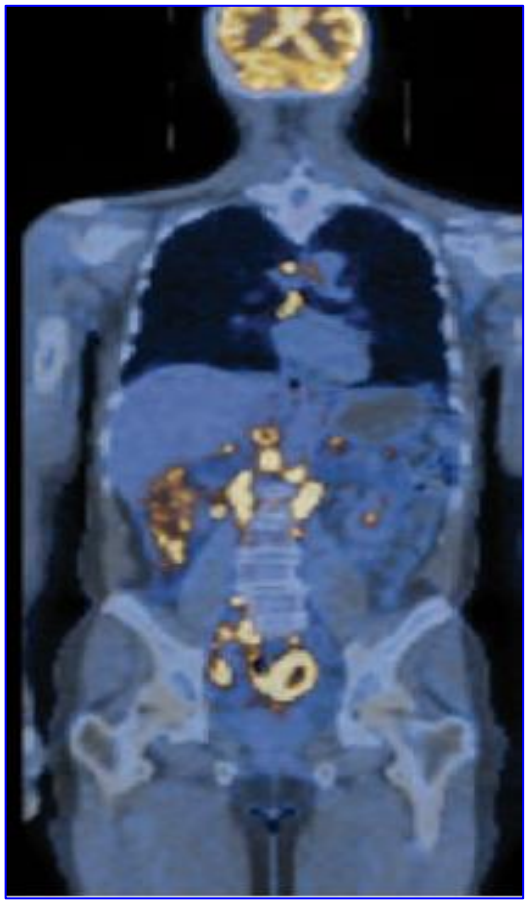
Secondary tumors in distant sites = metastases

Distant organs

See section 14.1 The Biology of Cancer text

Primary tumors account for 10% of cancer-related deaths

Primary tumor

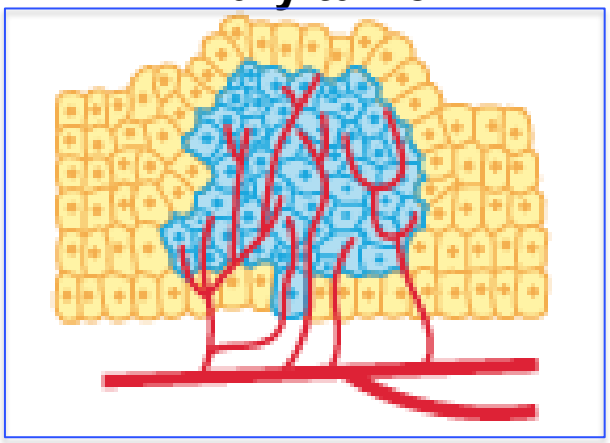


Metastatic disease

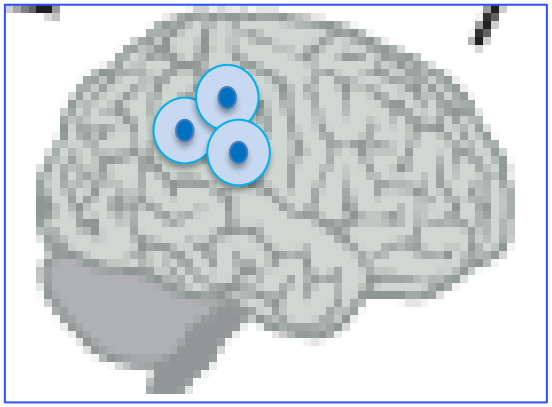
Responsible for 90% of cancer deaths

Why is the mortality different between primary tumors and metastasis?

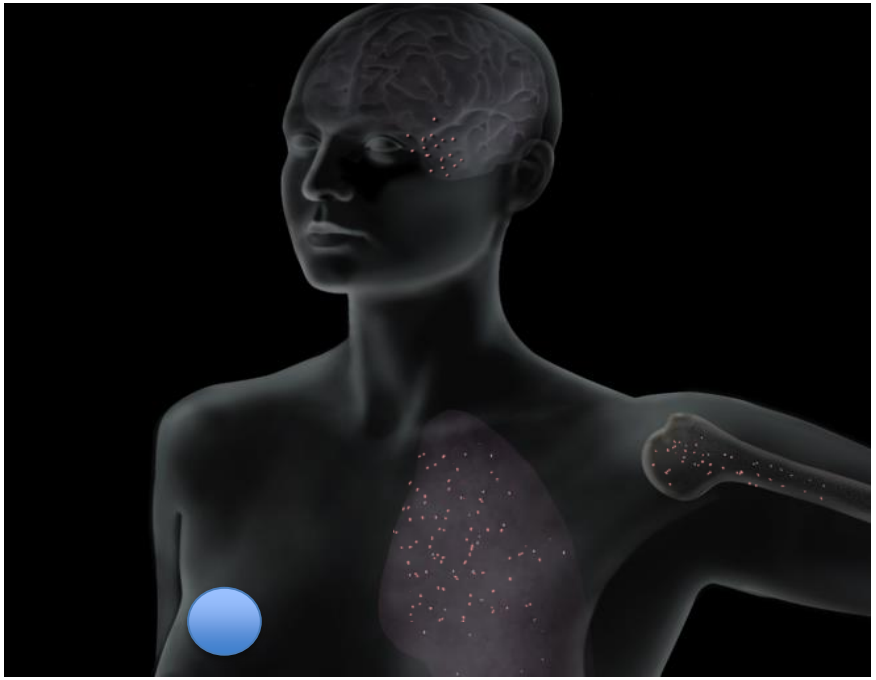
Primary tumor



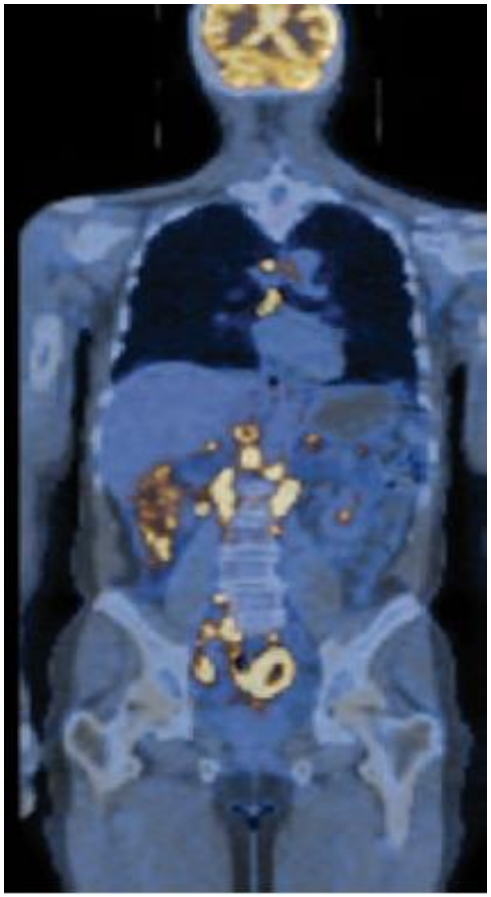
Metastasis



1. Surgical options better for primary tumors?

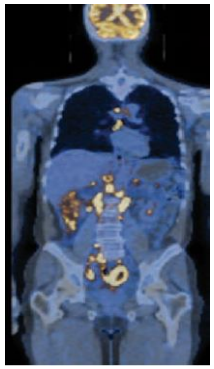


vs

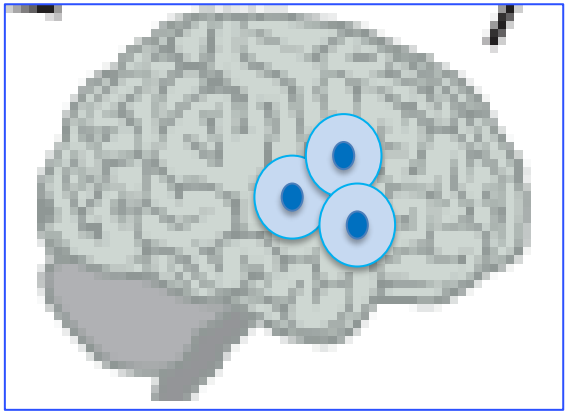


Over 90% cure rate if localized tumor can be removed surgically. Example- breast cancer

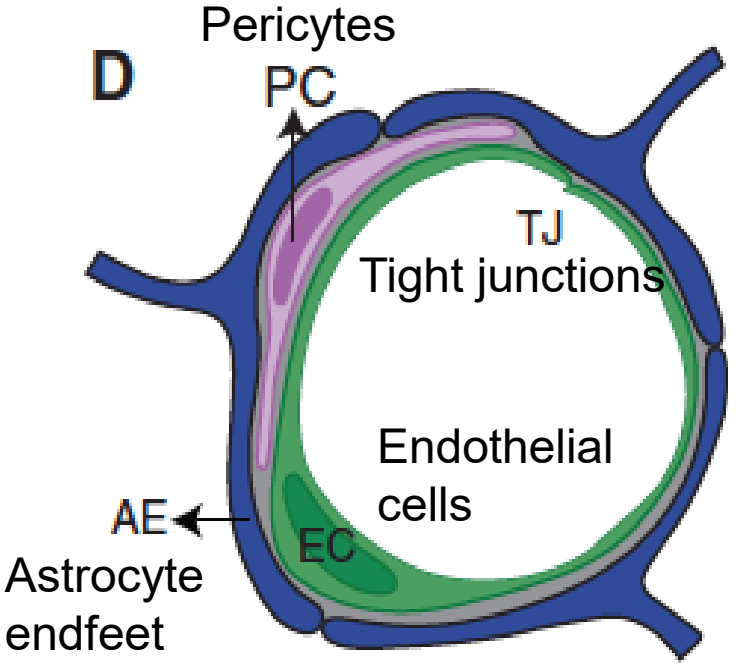
Disseminated disease= Surgical options limited



2. Drug bioavailability differences?



Brain metastases
Drug delivery issues

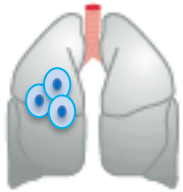


Blood-brain barrier (Selective permeability)

Reference: The blood-brain barrier, Daneman and Prat, Cold Spring Harbor Laboratory Press, 2015

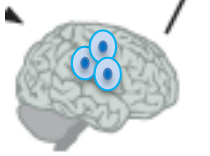
3. Vital organ dysfunction in metastatic disease

Common vital organs of metastasis (from tumors of epithelial origin)

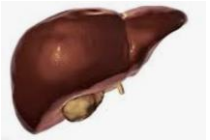


Lung — Respiratory function compromised

Bone — Skeletal collapse



Brain — Compromised central nervous system functions



Liver — Altered systemic metabolism

See introduction section of Chapter 14 in The Biology of Cancer textbook

4. Have the cancer cells changed?

Primary vs metastatic cancer cells: Are they different?

Are there new mutations that were not present in the primary tumor that drive metastasis?

Common tumor suppressor genes

Gene	Type of cancer
APC	Colon/rectum carcinoma
BRCA1	Breast and ovarian carcinomas
BRCA2	Breast carcinoma
DPC4	Pancreatic carcinoma
INK4	Melanoma, lung carcinoma, brain tumors, leukemias, lymphomas
MADR2	Colon/rectum carcinoma
NF1	Neurofibrosarcoma
NF2	Meningioma
p53	Brain tumors; breast, colon/rectum, esophageal, liver, and lung carcinomas; sarcomas; leukemias and lymphomas
PTC	Basal cell carcinoma
PTEN	Brain tumors; melanoma; prostate, endometrial, kidney, and lung carcinomas
Rb	Retinoblastoma; sarcomas; bladder, breast, and lung carcinomas
VHL	Renal cell carcinoma
WT1	Wilms' tumor

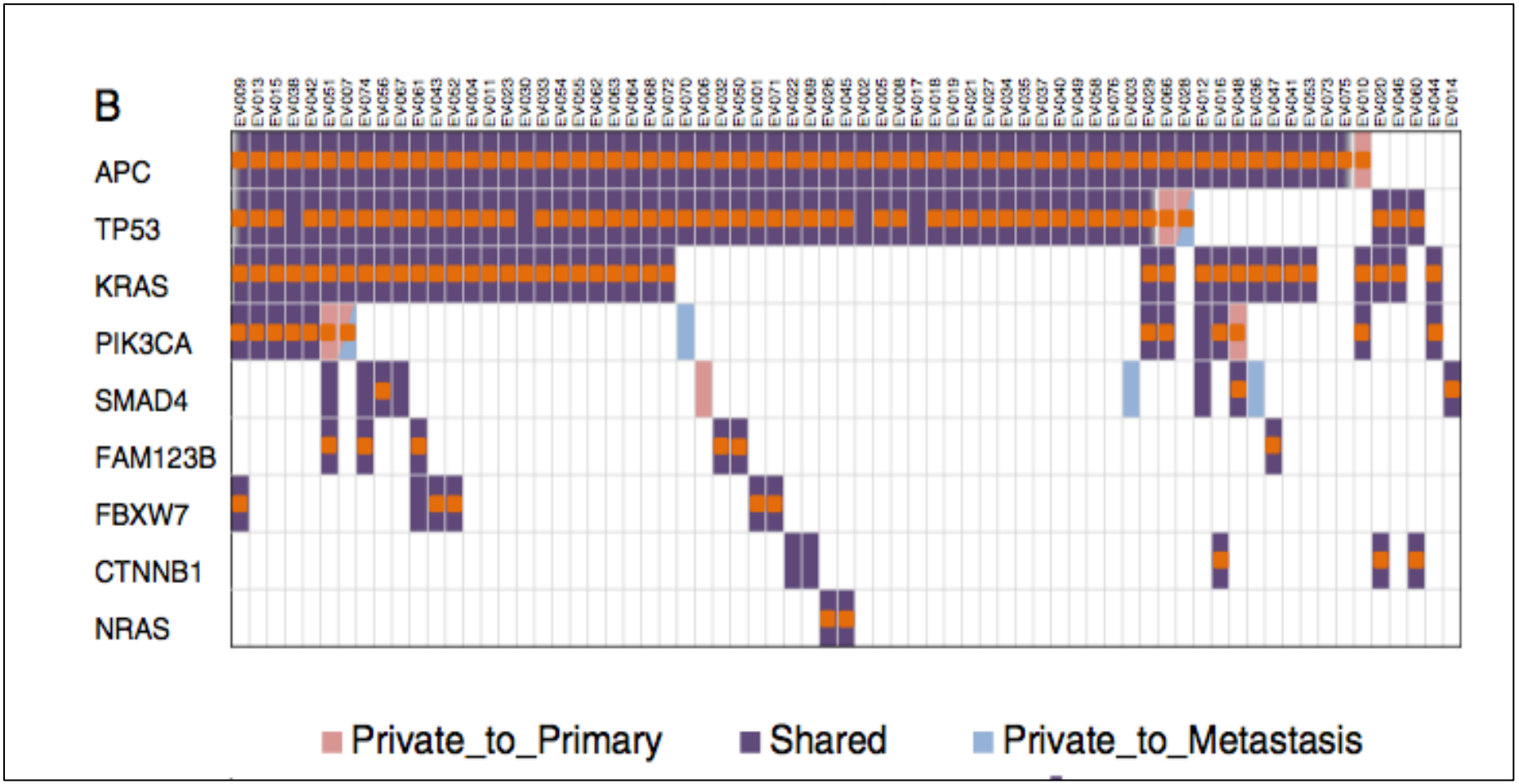
The Cell: A Molecular Approach (2nd edition) Cooper GM.

Common oncogenes that drive cancer

Table 4.3 Some frequently amplified chromosomal regions and the genes they are known to carry

Name of oncogene[a]	Human chromosomal location	Human cancers	Nature of protein[b]
MDM4/MDMX	1q32	breast, colon, lung, pre-B leukemias	p53 inhibitor
PIK3CA	3q26.3	lung SCC, ovarian, breast	PI kinase
erbB1/EGFR	7q12–13	glioblastomas (50%); squamous cell carcinomas (10–20%)	RTK
cab1–erbB2–grb7	17q12	gastric, ovarian, breast carcinomas (10–25%)	RTK, adaptor protein
k-sam	7q26	gastric, breast carcinomas (10–20%)	RTK
FGF-R1	8p12	breast carcinomas (10%)	RTK
met	7q31	gastric carcinomas (20%)	RTK
K-*ras*	12p12.1	lung, ovarian, colorectal, bladder carcinomas (5–20%)	small G protein
N-*ras*	1p13	head and neck cancers (30%)	small G protein
H-*ras*	11p15	colorectal carcinomas (30%)	small G protein
c-*myc*	8q24	various leukemias, carcinomas (10–50%)	TF
L-*myc*	1p32	lung carcinomas (10%)	TF
N-*myc–DDX1*	2p24–25	neuroblastomas, lung carcinomas (30%)	TF
akt-1	14q32–33	gastric cancers (20%)	ser/thr kinase
akt-2	19q13	ovarian carcinomas	ser/thr kinase
cyclin D1–exp1–hst1–ems1	(11q13)	breast and squamous cell carcinomas (25–50%)	G1 cyclin
cdk4–mdm2–sas–gli	12q13	sarcomas (10–30%), HNSCC (40%), B-cell lymphomas (25%)	CDK, p53 antagonist
cyclin E	19q12	gastric cancers (15%)	cyclin
akt2	(19q13)	pancreatic, ovarian cancers (30%)	ser/thr kinase
AIB1, BTAK	(20q12–13)	breast cancers (15%)	receptor co-activator
cdk6	(19q21–22)	gliomas (5%)	CDK
myb	6q23–24	colon carcinoma (5–20%), leukemias	TF
ets-1	11q23	lymphoma	TF
gli	12q13	glioblastomas	TF

Mutations are highly concordant between primary tumor and metastasis



Ref: Colon cancer, Brannon et al., Genome Biology, 2014

DNA sequencing of primary breast cancer and metastases from the same patient

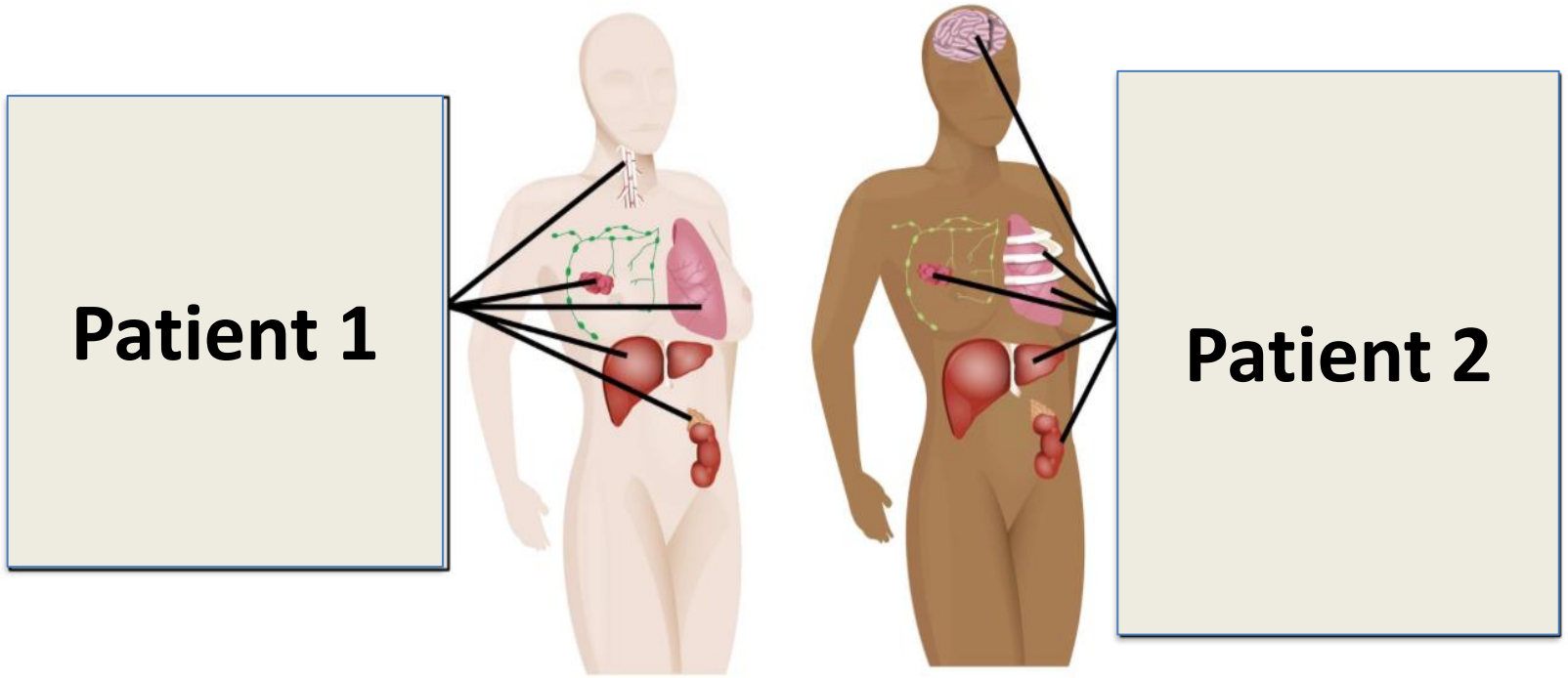


Fig 1. Clinical history and distribution of metastases from patients A1 and A7, who both had clinically triple-negative and basal-like breast cancer.

Majority of the functional mutations in metastases were already present in the primary tumors

Ref: Hoadley et al., PLOS Medicine, 2016

4. Cancer cells have changed?

Primary vs metastatic cancer cells: are they different?

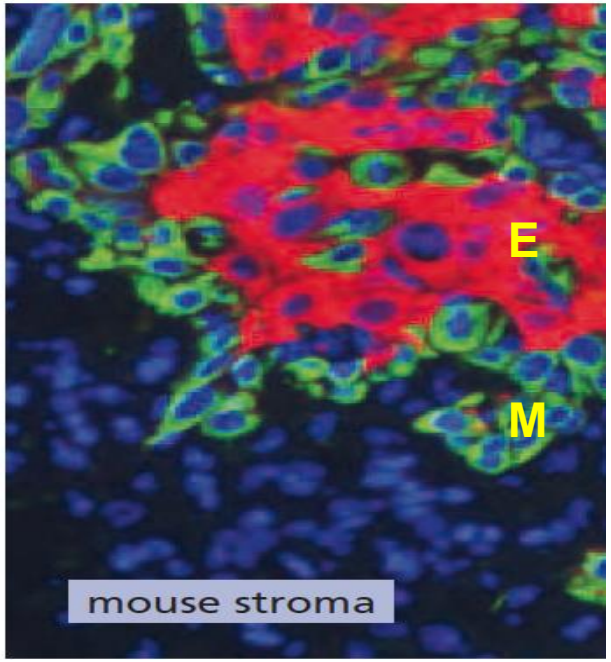
Genetic differences such as acquiring new mutations in the metastases- most still detected in the primary tumors

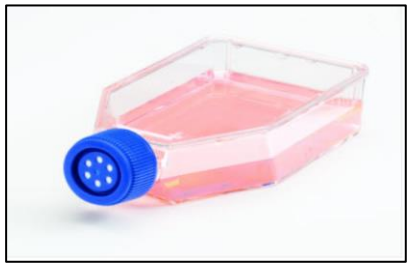
Epigenetic changes? Gene expression changes that drive phenotype, often involved in signaling with the tumor microenvironment.

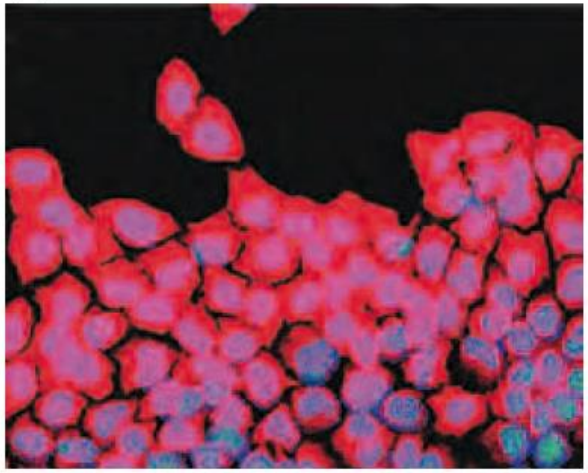
Example- EMT and cancer cell migration

Epithelial-mesenchymal transition: example of epigenetic changes that promote invasion

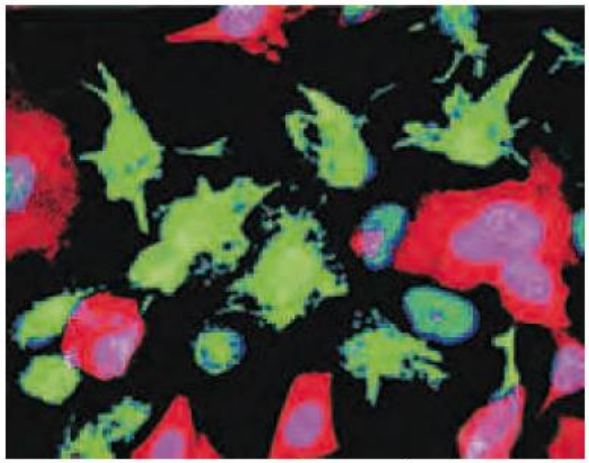
Mammary carcinoma







EMT



Overview of the lecture

- Introduction to the metastasis cascade
- **Historical perspective and foundation of metastasis research**
- Studying the journey of a cancer cell from primary tumor to distant sites
- Challenges and therapeutic avenues for targeting metastasis

Historical perspective of metastasis

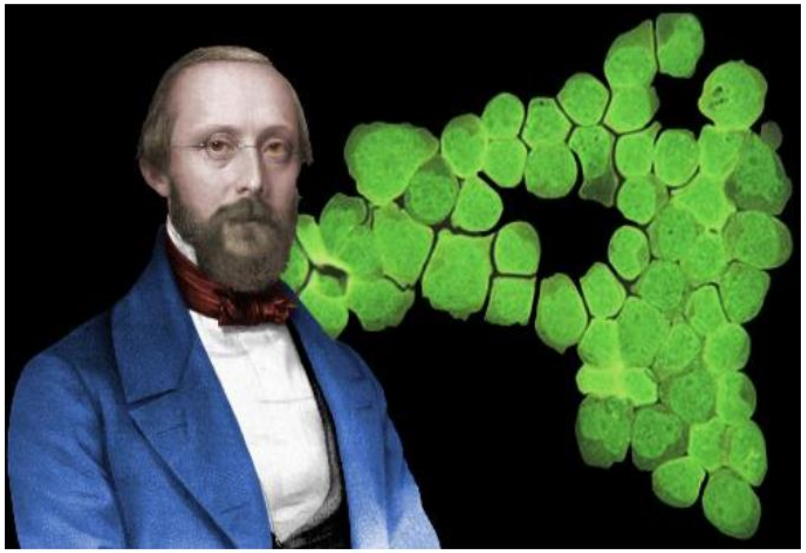
1829
First recorded definition of metastasis by physician, Recamier, summarized as the transfer of disease from one organ or part to another not directly connected to it.

1898
Gould and Pyle in '*Anomalies and Curiosities of Medicine*' describes metastasis of milk referring to product of breast cancer metastasizing in lactating women.

Reference: Landmark discoveries in metastasis research, Metastasis Research Society and Talmadge and Fidler, Cancer Research, 2010, 70(14): 5649–5669 .

Rudolf Virchow

Rudolf Virchow

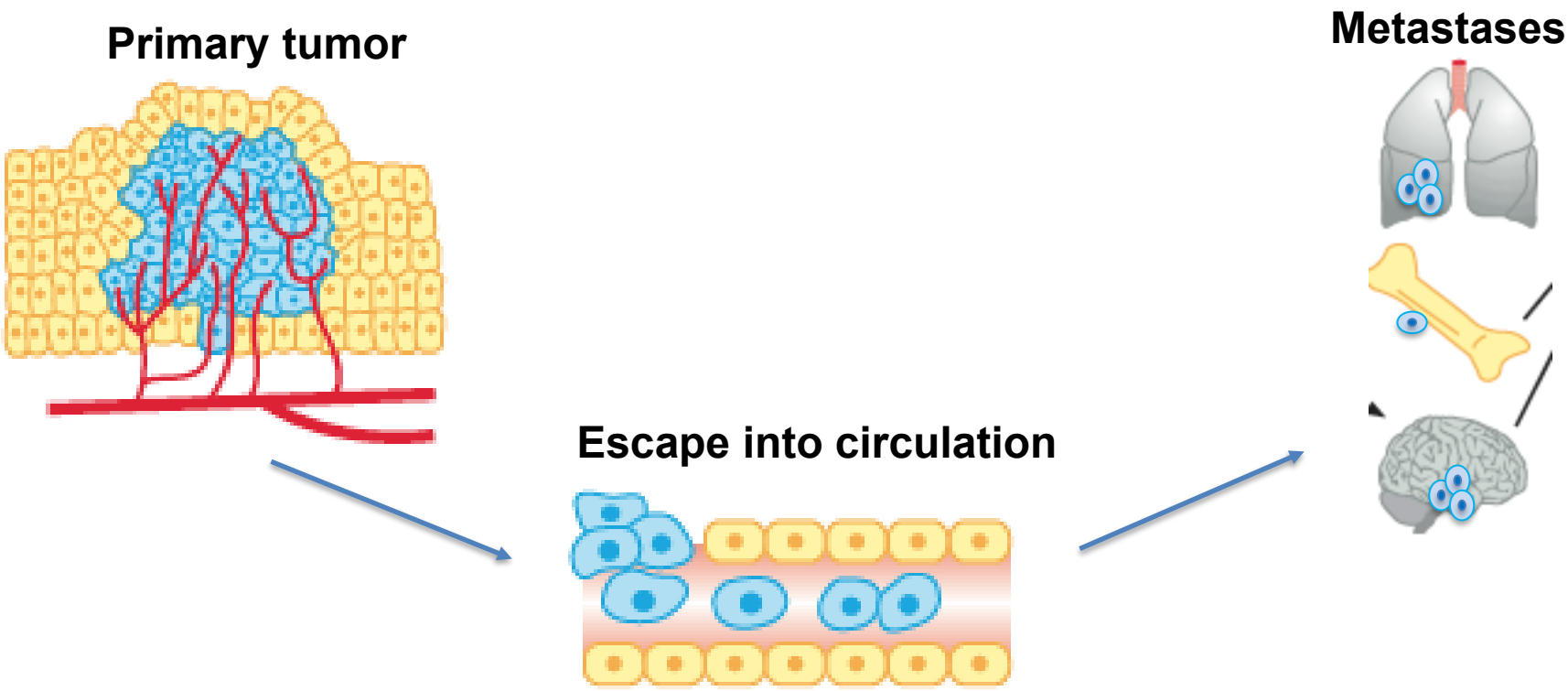


Lived 1821 – 1902.

- Founding father of pathology and social medicine
- Scientist, politician and anthropologist
- Seminal contributions in cell biology, cancer and parasitology
- **Early ideas on organ-tropism for metastatic cells.**

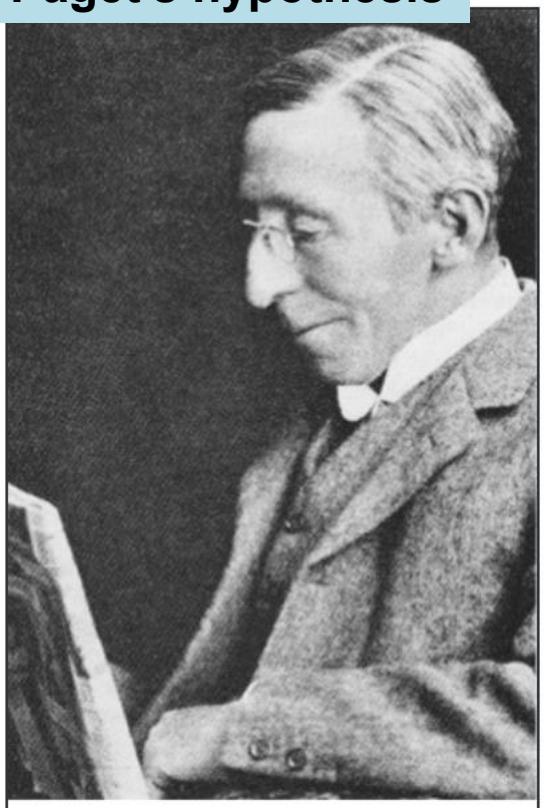
1. Virchow's hypothesis

What determines where the cancer cells will spread and grow?



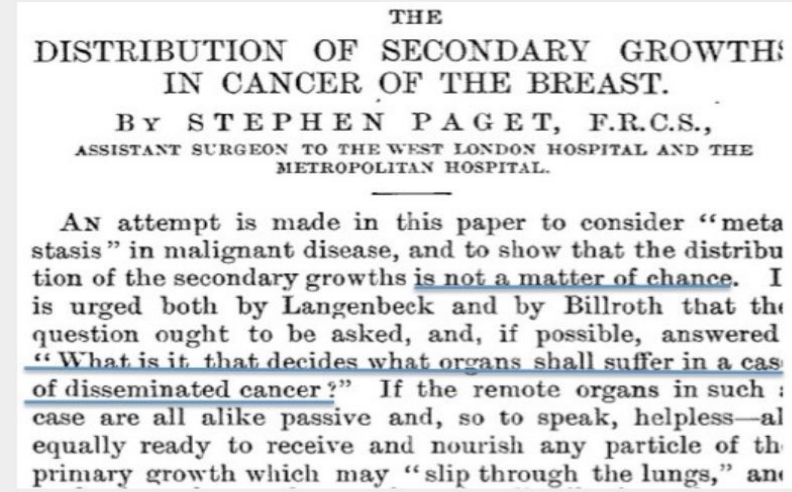
1858: Pathologist Rudolf Virchow suggested that *metastatic dissemination was determined by mechanical factors- from the arrest of tumor-cell emboli in the vasculature* i.e. metastatic dissemination is random.

2. Paget's hypothesis



Stephen Paget (1855-1926)

Stephen Paget



THE
DISTRIBUTION OF SECONDARY GROWTHS
IN CANCER OF THE BREAST.

By STEPHEN PAGET, F.R.C.S.,
ASSISTANT SURGEON TO THE WEST LONDON HOSPITAL AND THE METROPOLITAN HOSPITAL.

An attempt is made in this paper to consider "meta stasis" in malignant disease, and to show that the distribu tion of the secondary growths is not a matter of chance. I is urged both by Langenbeck and by Billroth that the question ought to be asked, and, if possible, answered "What is it that decides what organs shall suffer in a cas of disseminated cancer?" If the remote organs in such a case are all alike passive and, so to speak, helpless—al equally ready to receive and nourish any particle of th primary growth which may "slip through the lungs," an

Figure 4. The first section from Dr. Paget's 1889 Lancet paper on metastasis in which he introduces the concept that metastatic sites are not random, but are congenial soil for the metastatic "seeds."

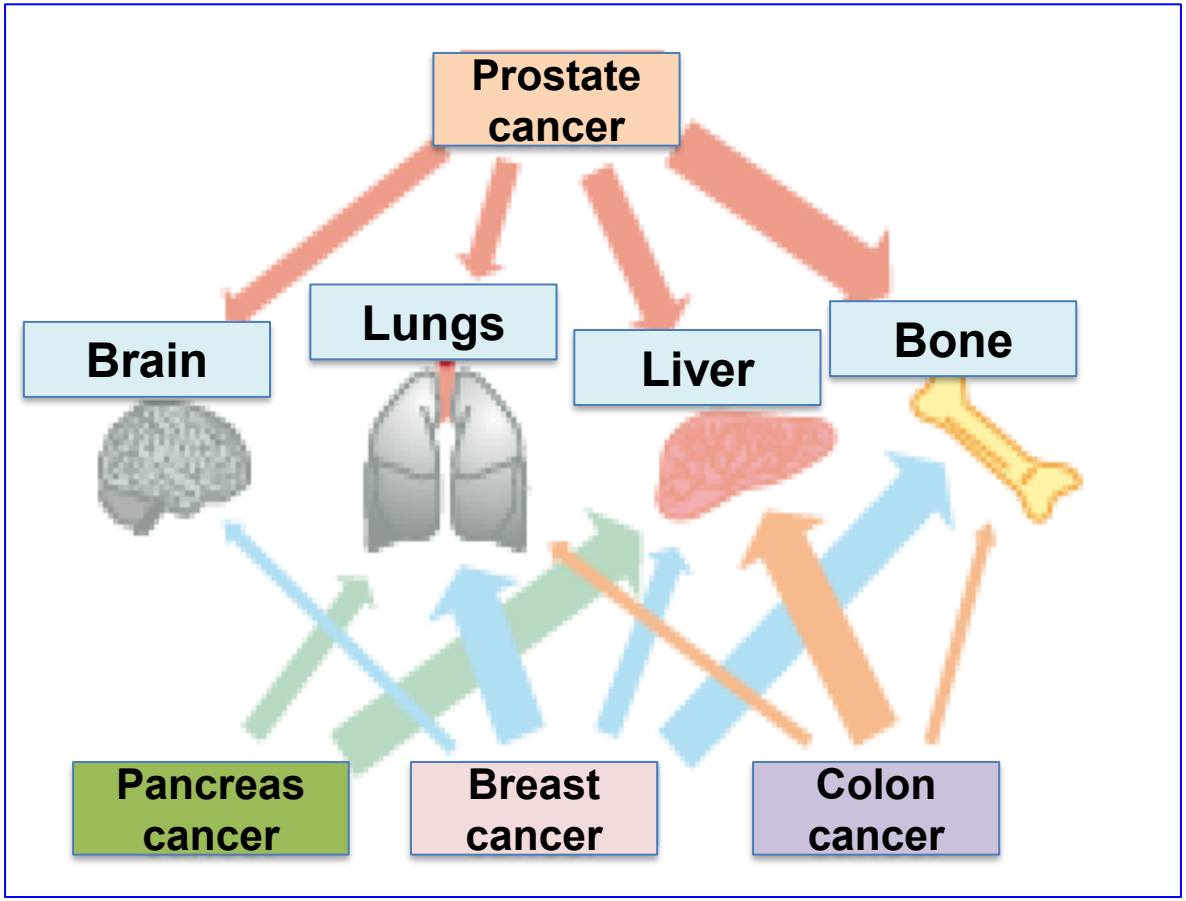
1889: Surgeon Stephen Paget after reviewing autopsy records of 735 women with fatal breast cancer suggested that metastatic dissemination is not random and proposed the '*seed and soil hypothesis*'.

When a plant goes to seed, the seeds are carried in all directions, but they can live and grow only in congenial soil.

Seed= Cancer cell and Soil= Tissue microenvironment in this context.

Stephen Paget's hypothesis

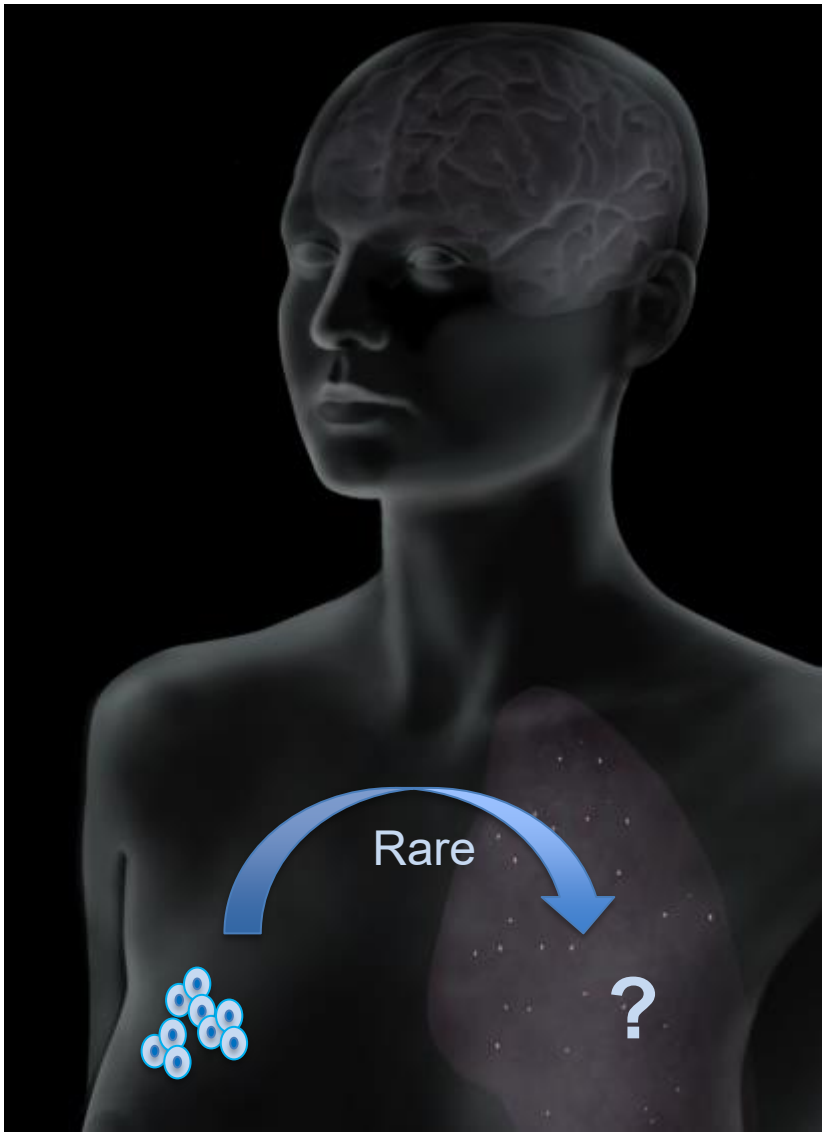
Certain tumor cells 'seeds' have specific affinity for certain microenvironment 'soil' and only when seed and soil are compatible, there is metastasis.



Specific metastatic patterns of different cancers

See Fig. 14.43
The Biology of Cancer text

Can seed and soil explain all metastatic dissemination?



Limitations of Paget's seed and soil hypothesis.

E.g. for breast cancer, contralateral or opposite side of the breast should be one of the most congenial soils for metastasis. However, this is rarely observed and happens in only 2% of cases.

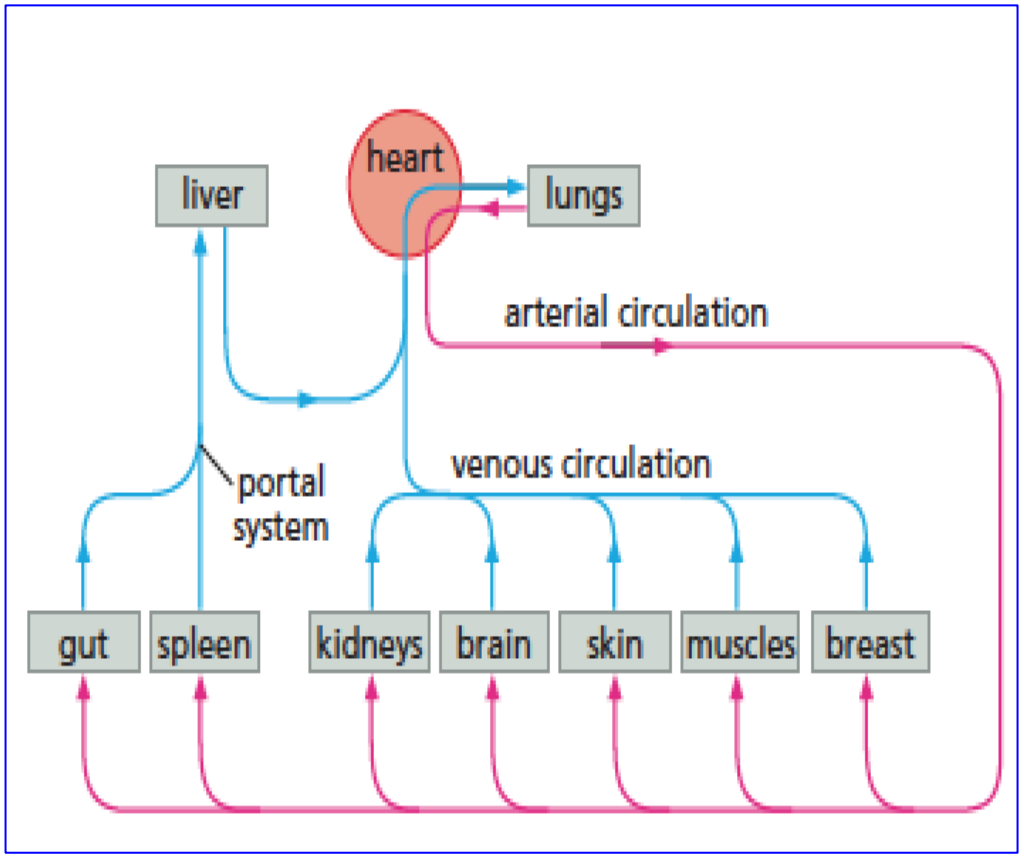
See Sidebar 14.5
The Biology of Cancer text

3. Ewing's hypothesis

Challenge from James Ewing

James Ewing - American pathologist (1866-1943)
- Discovered Ewing's sarcoma

James Ewing's observations



Mechanical forces and circulatory patterns between the primary tumor and the secondary site accounts for organ specificity (1928) for most tumors

Explains

(1) High number of colon cancer cells metastasizing to the liver.

(2) High number of prostate cancer cells to the lumbar vertebrae via Batson's plexus of draining lymph nodes.

See Fig. 14.45
The biology of cancer

4. Fidler's decision to test these hypotheses.

What determines where the cancer cells will spread and grow?

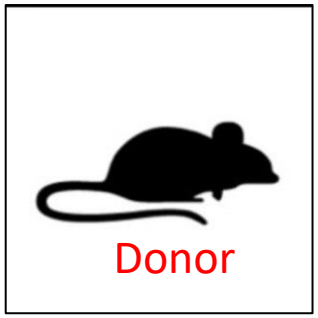
Late 1970s: Ian Hart and Isaiah Fidler provided the first experimental evidence and introduced new concepts of metastasis selection, which have now been verified and validated.

Option I:
Cancer cells grow in organs after getting arrested where capillaries are narrow and is *not selective*

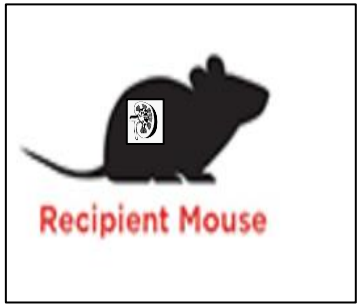
Option II:
Cancer cells get distributed to multiple organs but can only grow in particular ones, and is a *selective process*.

Ref: Hart and Fidler, Cancer Research, 40(7): 2281-7

Q. Is there preferential growth of tumor cells?



Organ isolation from **donor mice** (for grafting of kidney, lung or ovary ectopically e.g. below the skin)



Successful engraftment (transplant of organs)

Inject melanoma tumor cells into the recipient mice in the circulation

If blood flow is the sole driver, **equal likelihood** of tumor growth in different organ grafts. However, if preferential, then **unequal numbers** can be expected.

Implanted organ	Number of mice with tumor growing in transplant site
Kidney	4/28
Lung	20/28
Ovary	7/10

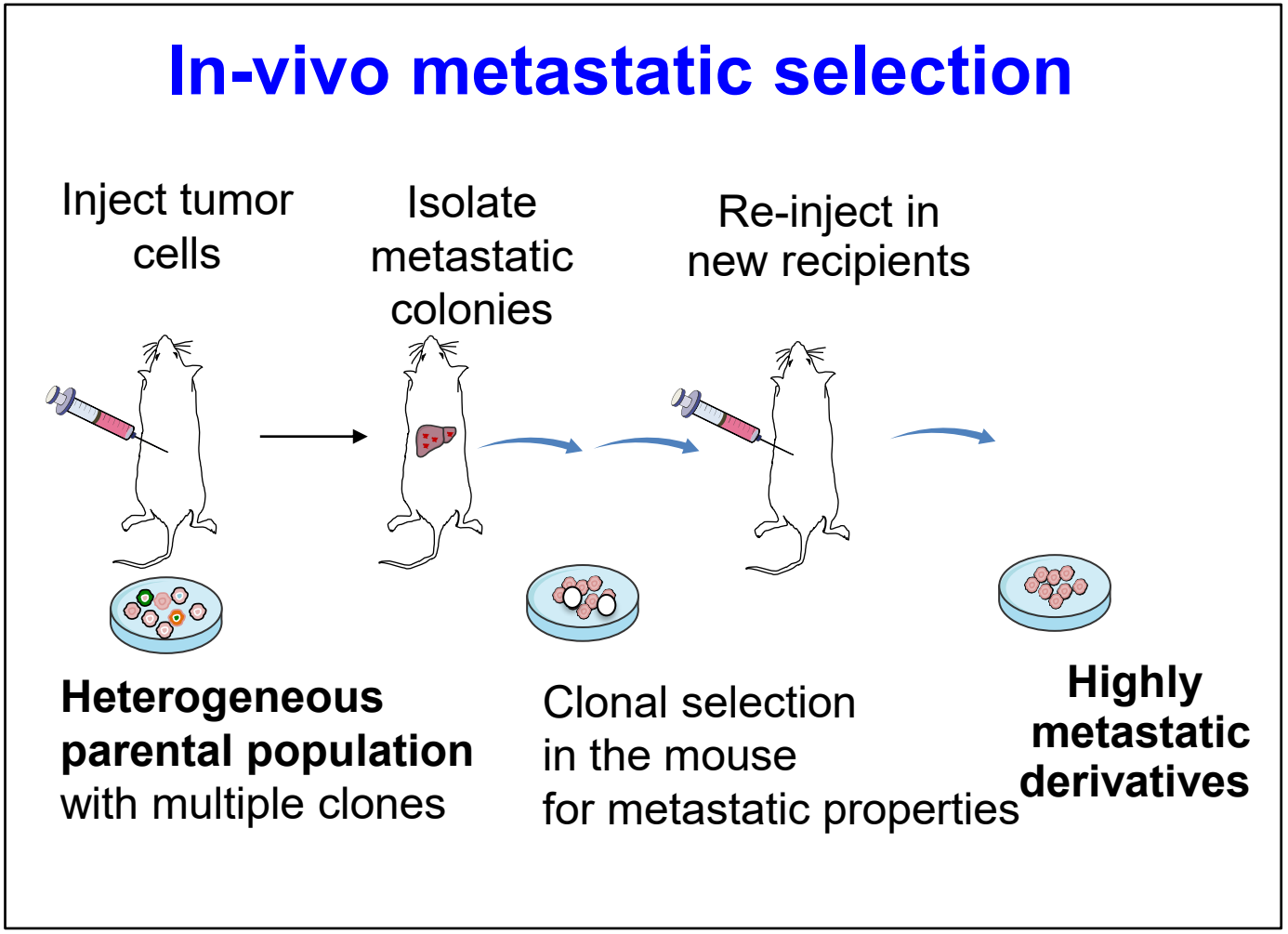
- Initial arrest of the cells were similar in different implanted organs.

- Repeated multiple times with large sample number with similar results.

Conclusions from Fidler's landmark studies

Tumor cells traffic through the vasculature of all organs, however, metastases selectively develop in congenial organs. Supported the "seed and soil hypothesis"

Highly metastatic variants can be selected (Fidler)



Method 1: Highly metastatic variants can be selected by in-vivo selection

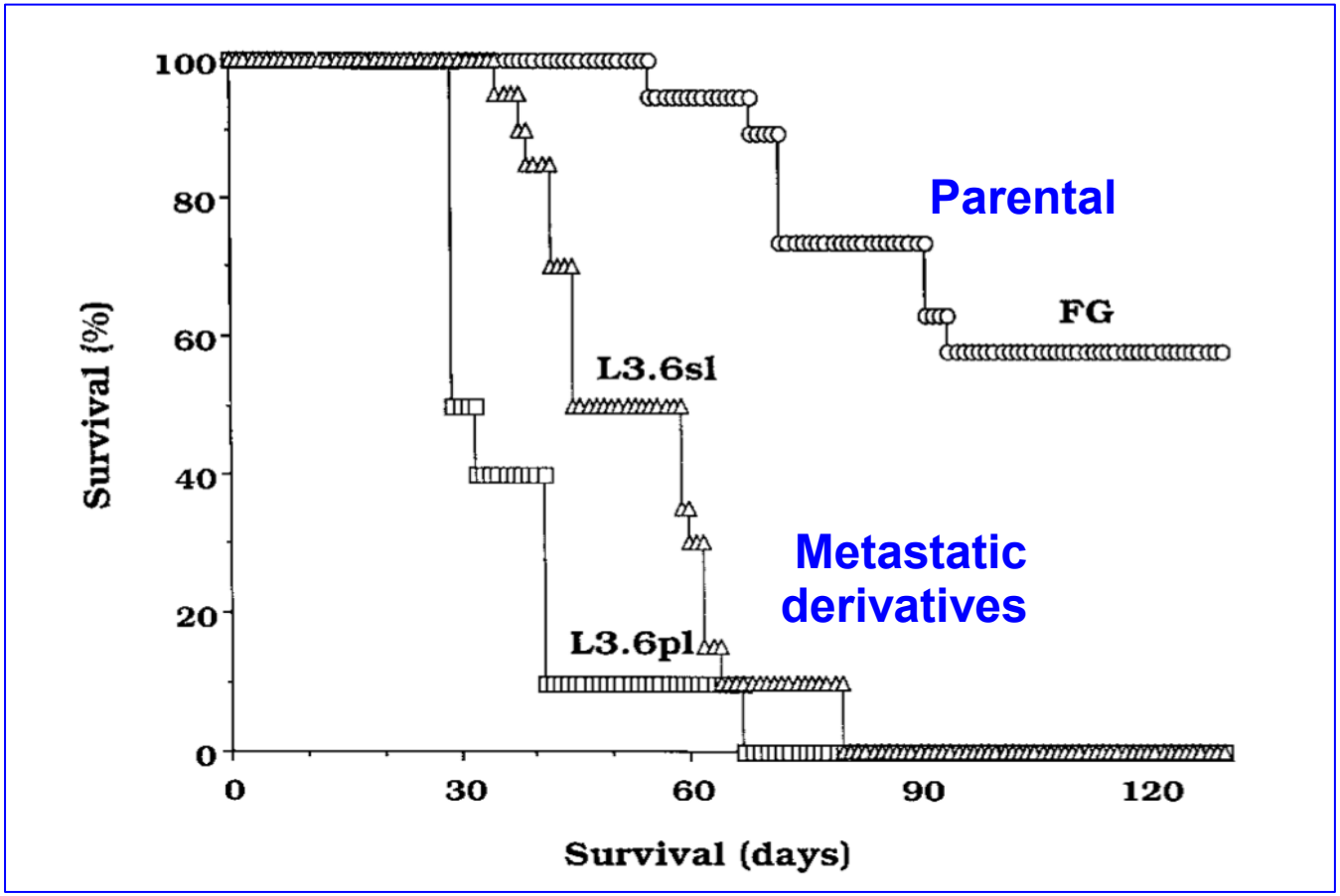
Table 1. Tumorigenicity and Production of Metastasis by Human Pancreatic Cancer Cells Subsequent to Implantation in Athymic Nude Mice.

Site of Implantation*	Cell Line	Median Survival (days)	Tumorigenicity	Liver Metastasis		Lymph Node Metastasis‡
				Incidence†	Median (range)	
Spleen	FG	92	5/20	4/20	0 (0–2)	2/10
	L3.6sl	71	19/20‖	10/19	3 (0–10)	7/19§
	L3.6pl	34	20/20‖	13/20	10 (0–75)	3/20

Ref: Bruns et al, Neoplasia, 1(1): 50-62

Supportive data:
Highly metastatic variants can be selected

Shorter survival time with rounds of metastatic selection



Conclusions from Fidler's landmark studies on selection

Metastasis is a highly selective process that favors the growth and survival of unique subpopulations of metastatic cells that preexist in the heterogeneous parental population.

Method 2: Single cell clones can be derived from heterogeneous parental population of tumor cells by limiting dilution method (isolating single cell clones)

Single cell suspension

Trypsinize cells and resuspend in cell culture media

Count cells

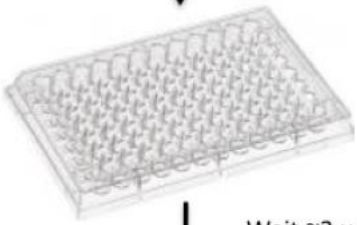
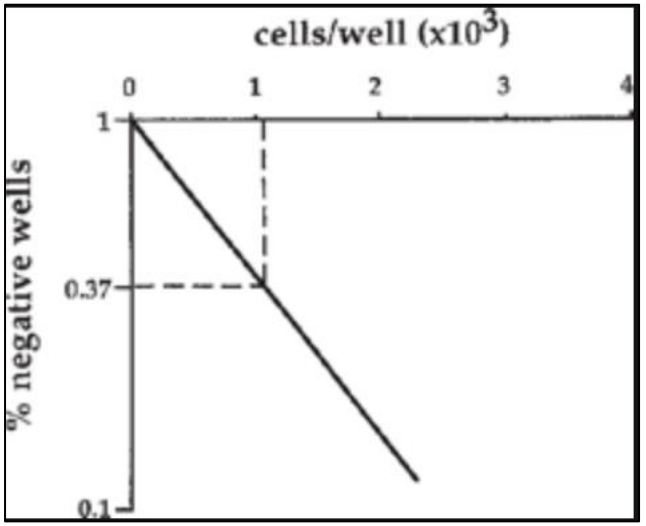
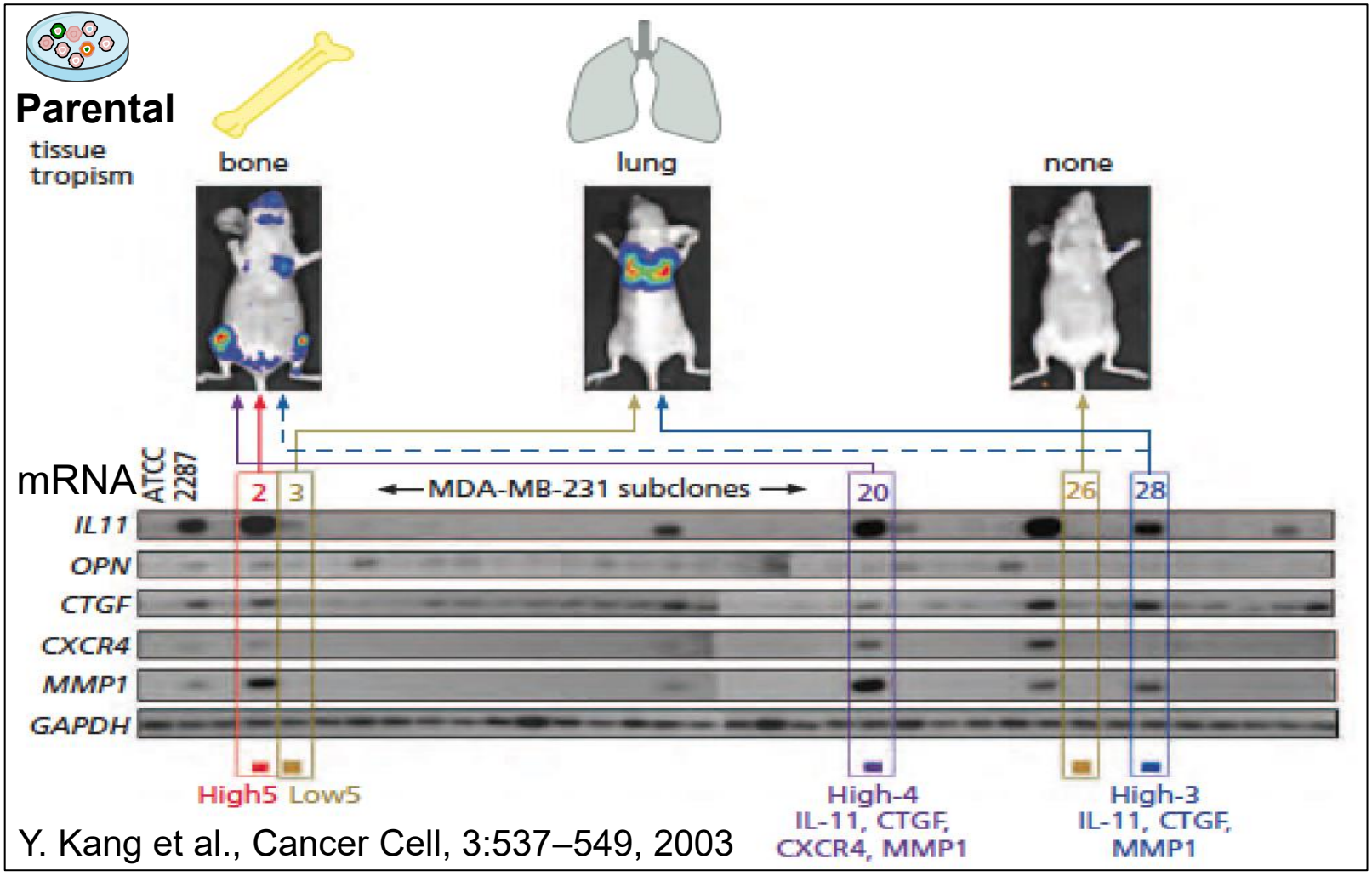


Plate cells in limiting dilution

Wait ~3 weeks



Method 2: Single cell clones can be derived from heterogeneous parental population of tumor cells

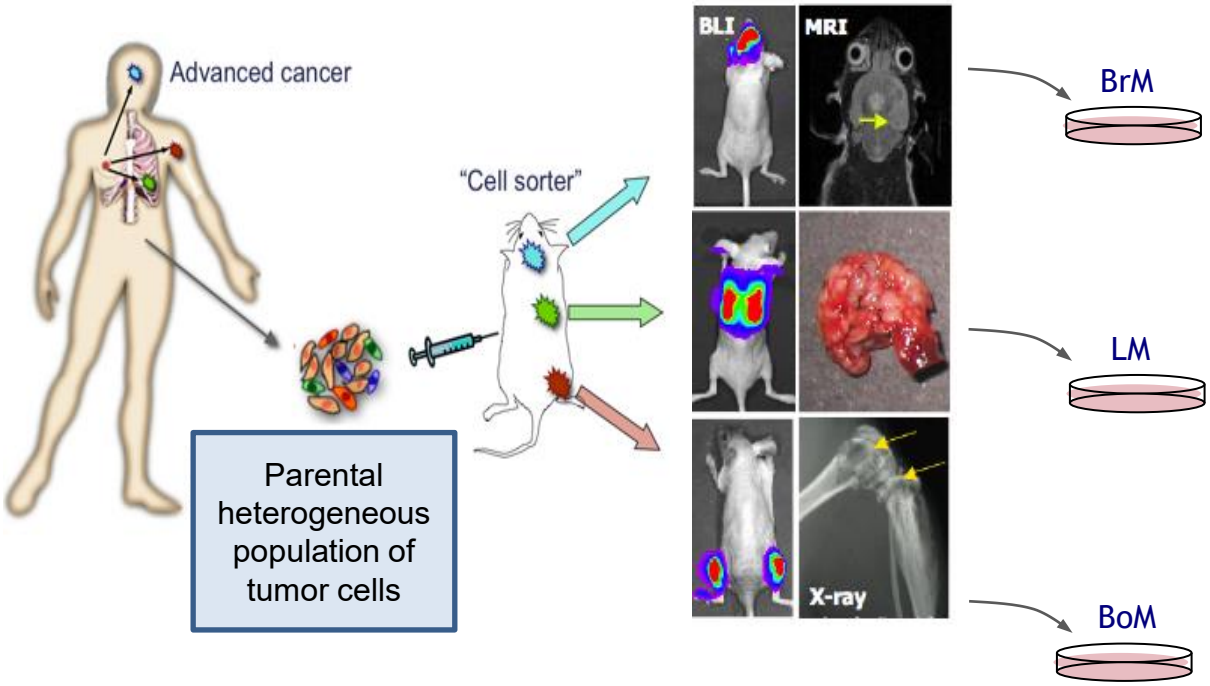


Y. Kang et al., Cancer Cell, 3:537–549, 2003

See Fig. 14.53
The biology of cancer

Mouse models of metastasis

Unique gene expression changes in selected metastatic clones in different organs

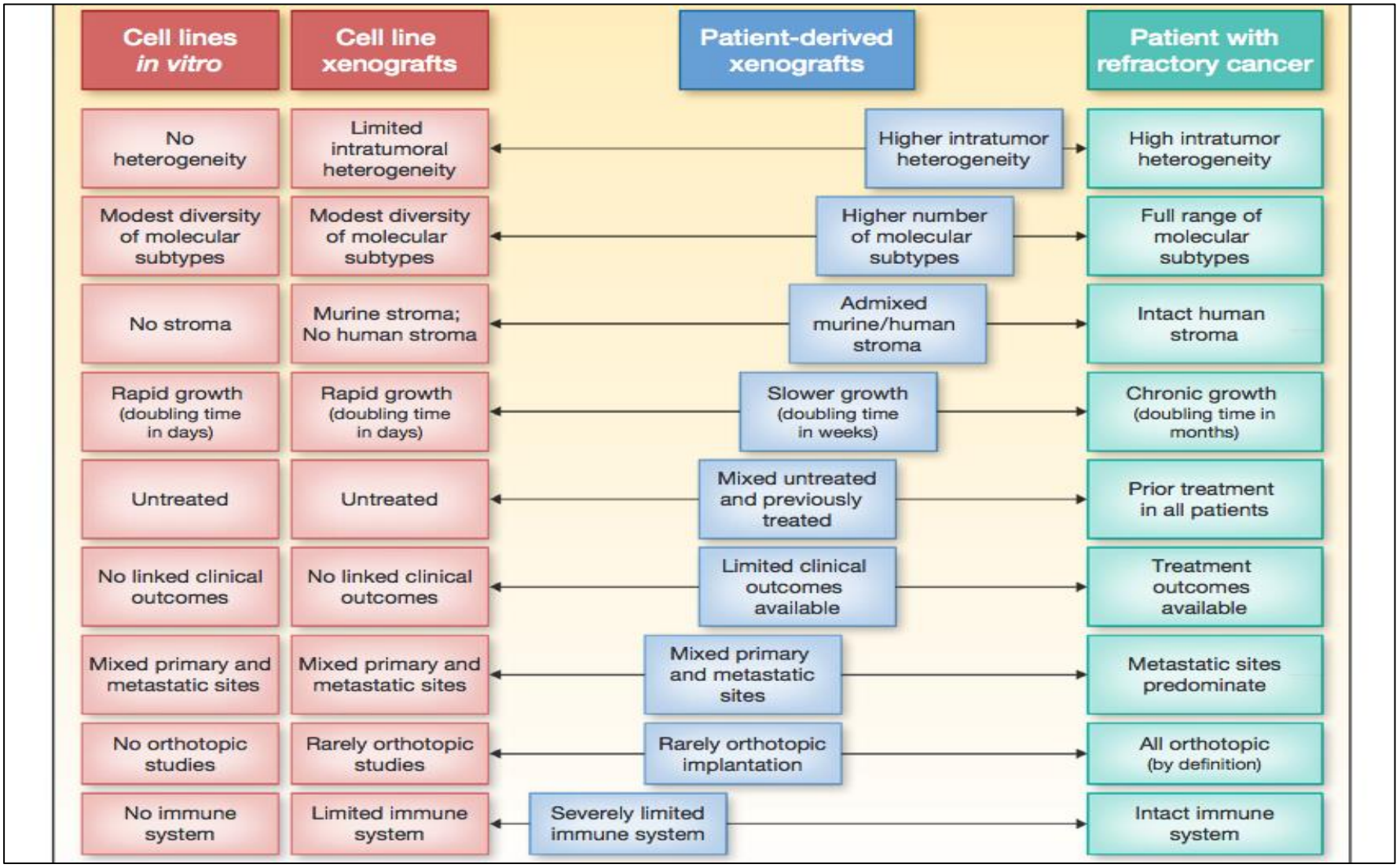


Reviewed in Gupta and Massague, Cell, 127(4):679-695, 2006

Transgenic mouse models of breast cancer

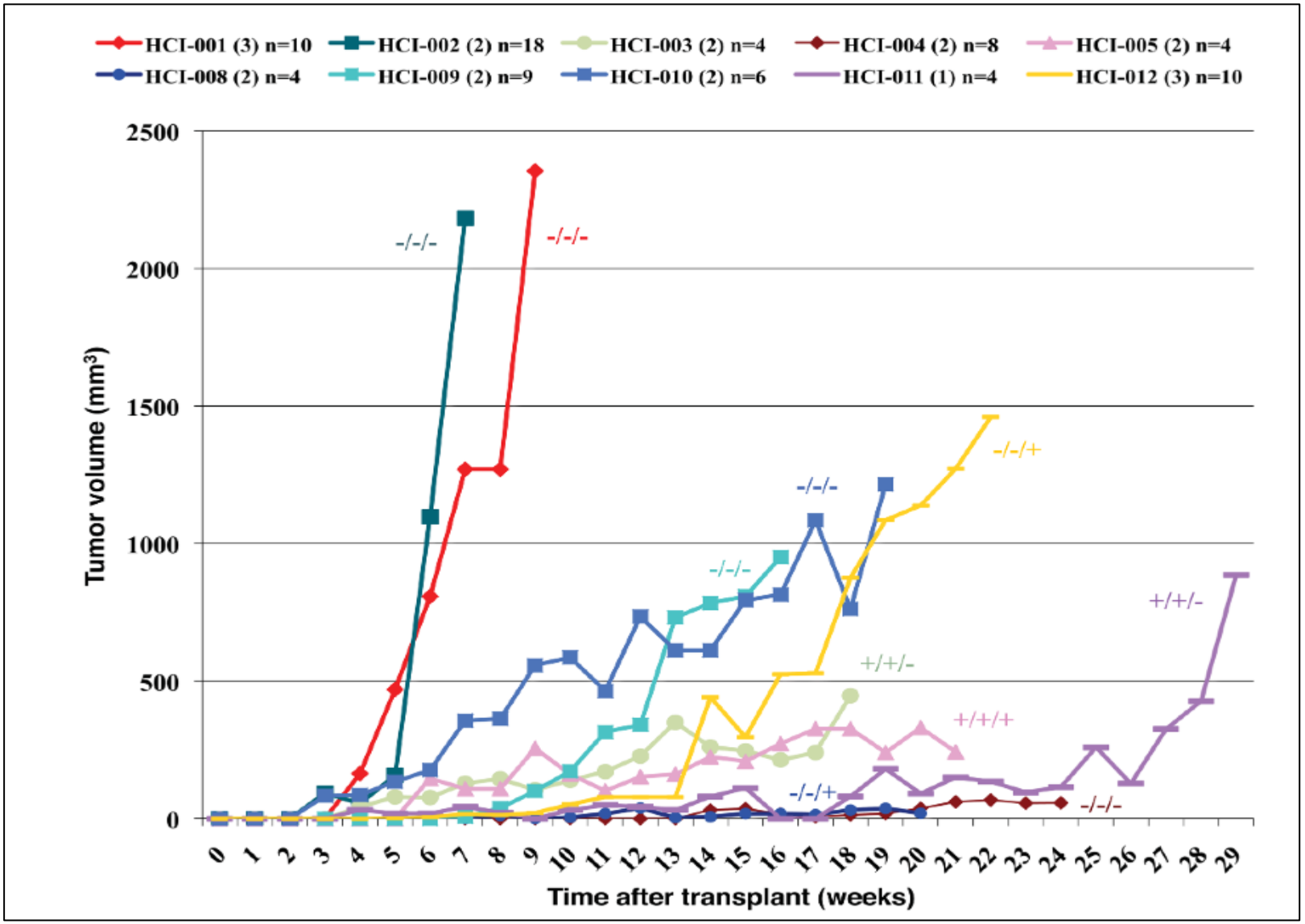
Model	Pr. Tumor site				Metastatic site		
MMTV-Wnt1	Mammary gland	60	8	b	Lung, LN		[7,16,77]
MMTV-Neu	Mammary gland	100	6.8[a]	72	Lung	8	[16,102]
MMTV-Neu activated	Mammary gland	100	3[a]-5	20	Lung	3.5	[42,44]
MMTV-Neu (YB)	Mammary gland	100	6[a]	65	Lung	2	[44,67]
MMTV-Neu (YD)	Mammary gland	100	3.6[a]	44	Lung	2	
MMTV-PyMT	Mammary gland	100	1–6	>85; 51	Lung; LN	3.5	[16,45,51]
MTB-TAN	Mammary gland	100	-	92	Lung		[16,103]
MT-Met	Mammary gland	b	10	b	Lung; LN; kidney; heart; cecum		[16,104]
C3(1)-Tag	Mammary gland	100	3–6	b	Lung		[16,105]

Development of Patient derived xenografts

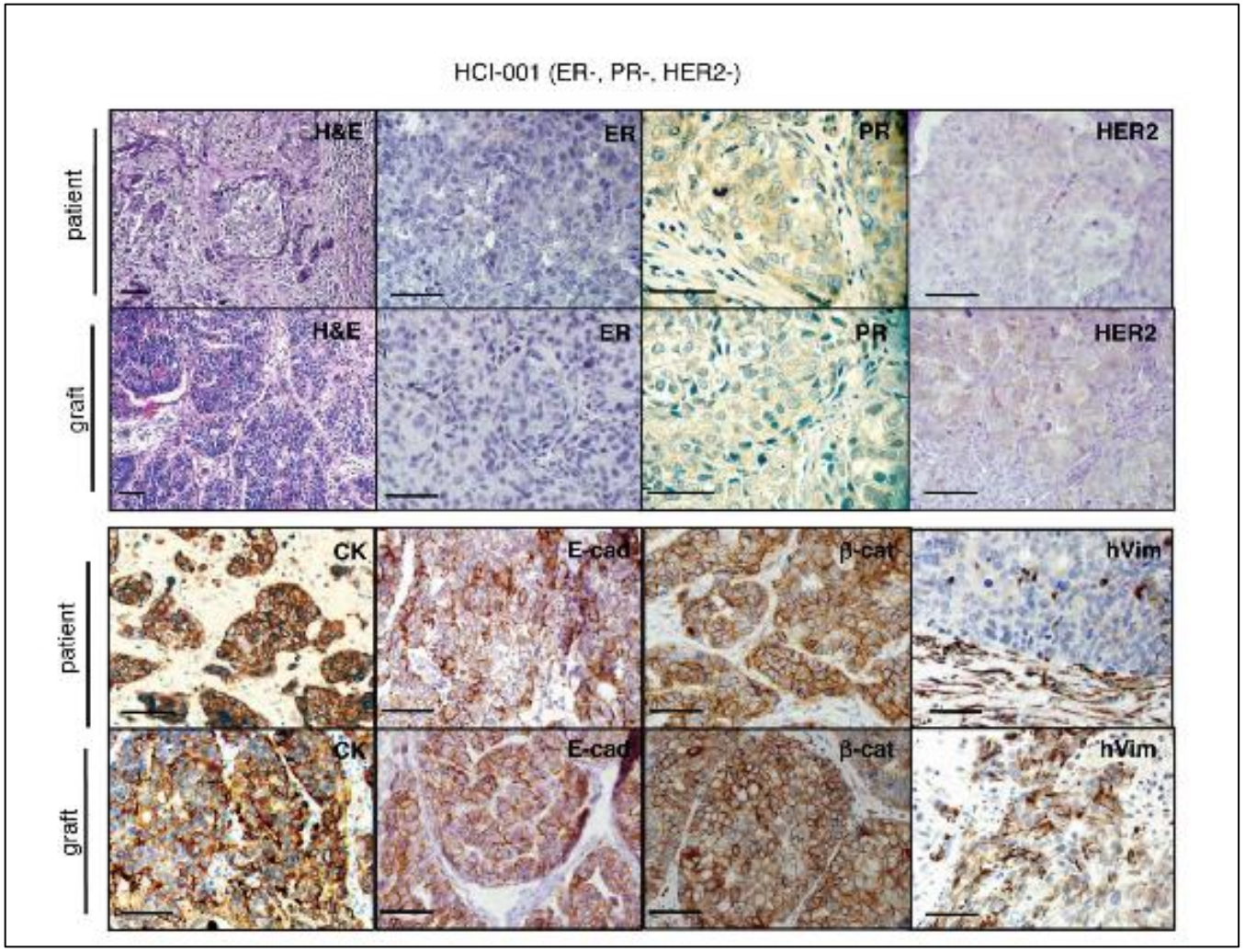


Kopetz et al., Clinical Cancer research, 2012

Fresh tumor implantation in immunocompromised mice



Tumor grafts in mice resemble the donor patient tumors by histology, genomics, growth



Sites in patients

METASTASIS SITES

Sites in mice

	Xenograft Information [1,2]				
Clinical metastasis[5]	ER status[6]	PR status[6]	HER2 status[6]	Estrogen Dependence[7]	Metastasis[8]
Lung	neg	neg	neg	n/a	Lung, LN
LN	neg	neg	neg	n/a	LN
LN	pos	pos	neg	Yes	Lung, LN
Not detected	neg	neg	neg	n/a	Not detected
Lung, bone	pos	pos	pos	Yes	Lung, LN, peritoneum
	pos	pos	not tested	not tested	Lung, peritoneum LN,
	pos	pos	not tested	not tested	Lung, LN, bone
Skin, lung	neg	neg	pos	n/a	Lung, LN
LN, pancreas bone, peritoneum	neg	neg	neg	n/a	Lung, peritoneum LN
Lung	neg	borderline	neg	n/a	Lung, LN
LN, pleura	pos	pos	neg	no, but estrogen stimulated	Lung, LN, bone
LN, pericardium	neg	neg	pos	n/a	LN, thymus

PDX for studying metastasis: Challenges

PLOS | ONE

RESEARCH ARTICLE

Development of Patient Derived Xenograft Models of Overt Spontaneous Breast Cancer Metastasis: A Cautionary Note

Marta Paez-Ribes[1], Shan Man[1], Ping Xu[1], Robert S. Kerbel[1,2]*

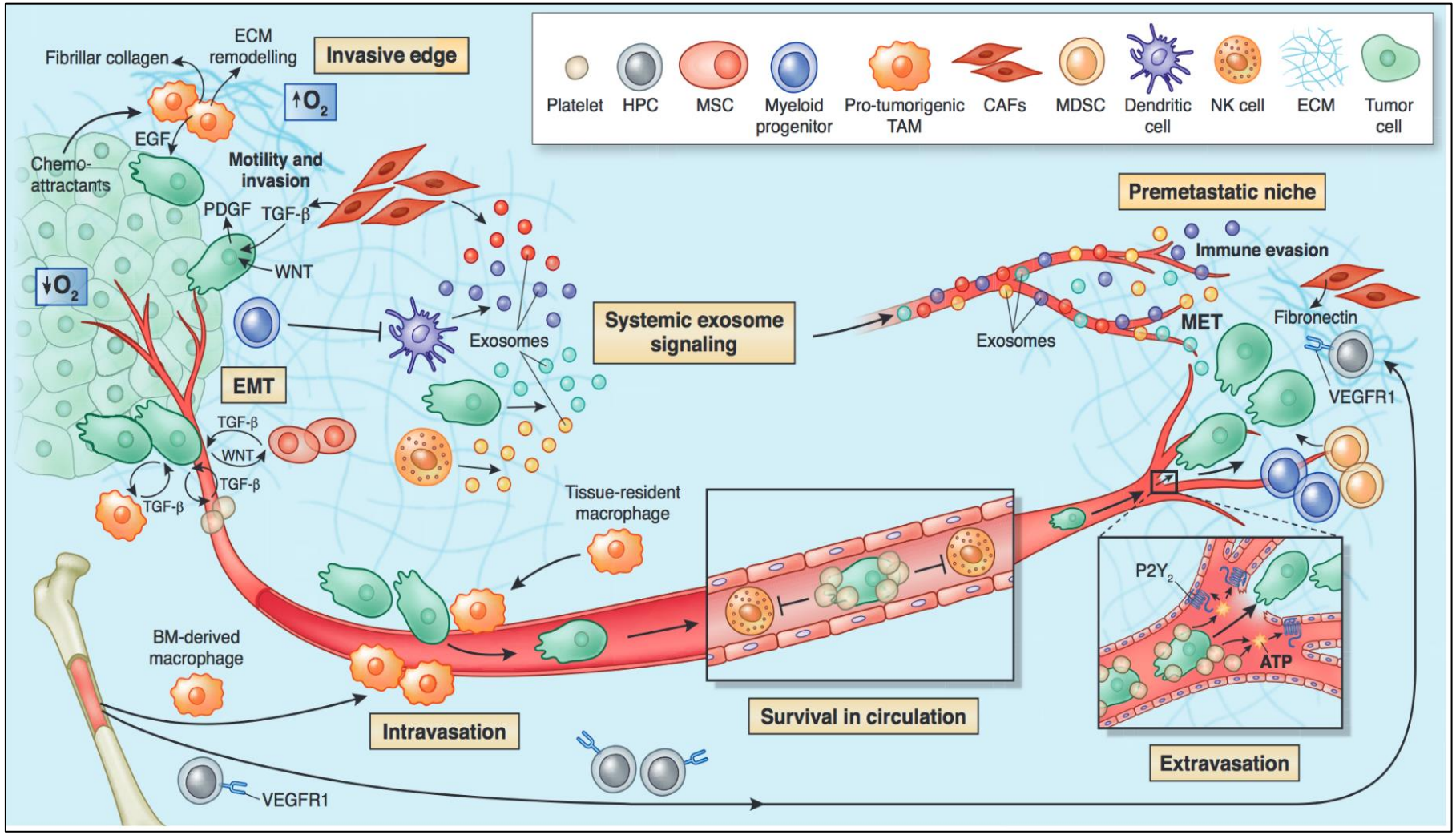
Tumor type	Metastases of human origin	Incidence of mouse thymomas (%)	Total mice implanted
HCI-001	1	6 (15.3%)	39
HCI-002	2	9 (15%)	60
HCI-004	0	2 (13.3%)	15
HCI-008	0	3 (20%)	15
HCI-009	0	3 (20%)	15
All tumors	3	23 (15.9%)	144

- Lack of spontaneous metastasis from many PDX primary tumors (3 in 144 observed by the Kerbel group).
- Mouse thymomas arising in aged mice.
- Tumor heterogeneity selects for limited clones in mice- Mouse PDX/Avatars
- not representative of human tumors with passaging in mice.

Overview of the lecture

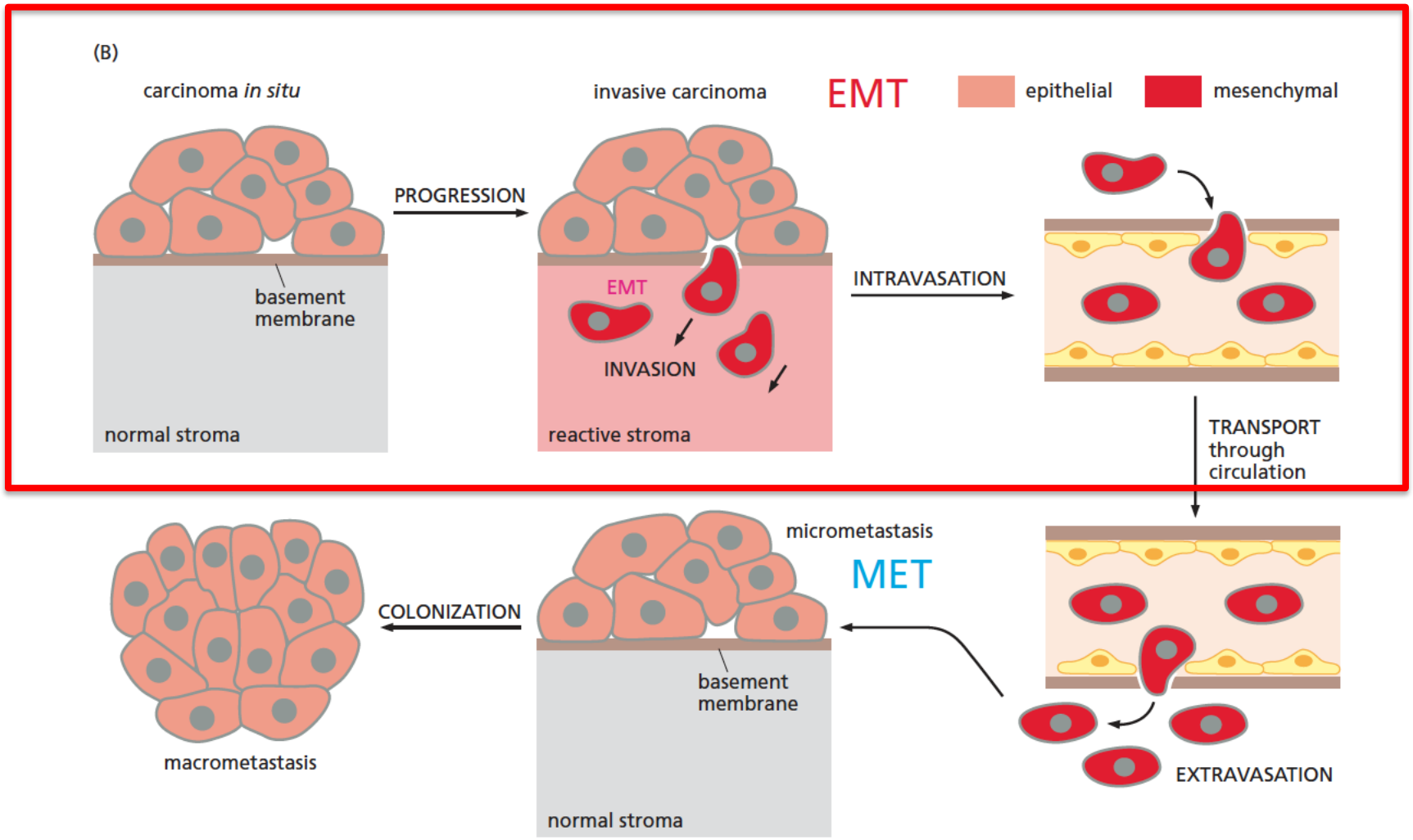
- Introduction to the metastasis cascade
- Historical perspective and foundation of metastasis research
- **Studying the journey of a cancer cell from primary tumor to distant sites**
- Challenges and therapeutic avenues for targeting metastasis

The steps of the metastatic cascade



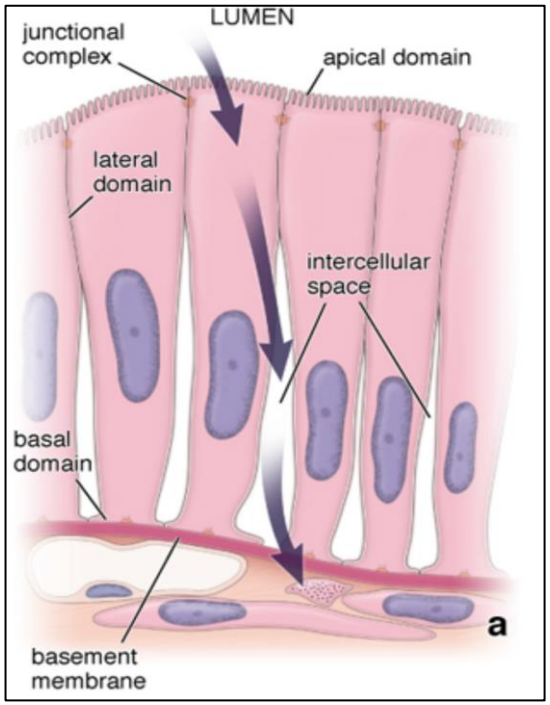
Quail and Joyce, Nat Med, 2013

Local invasion, intravasation and transit



Epithelial tissue

- One of the four tissues in our body besides connective, muscular and nervous tissue.
- >80% of life-threatening cancers occurs in epithelial tissues.
- Common characteristics:

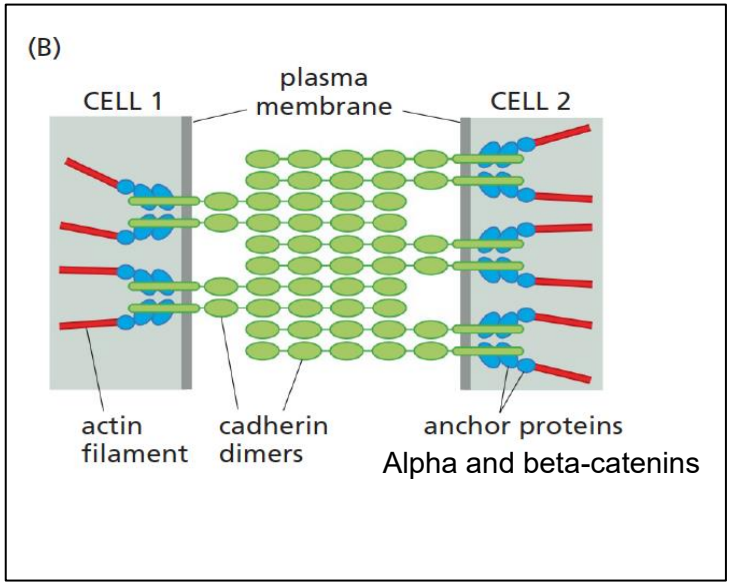


Free surface; With apical/free edge

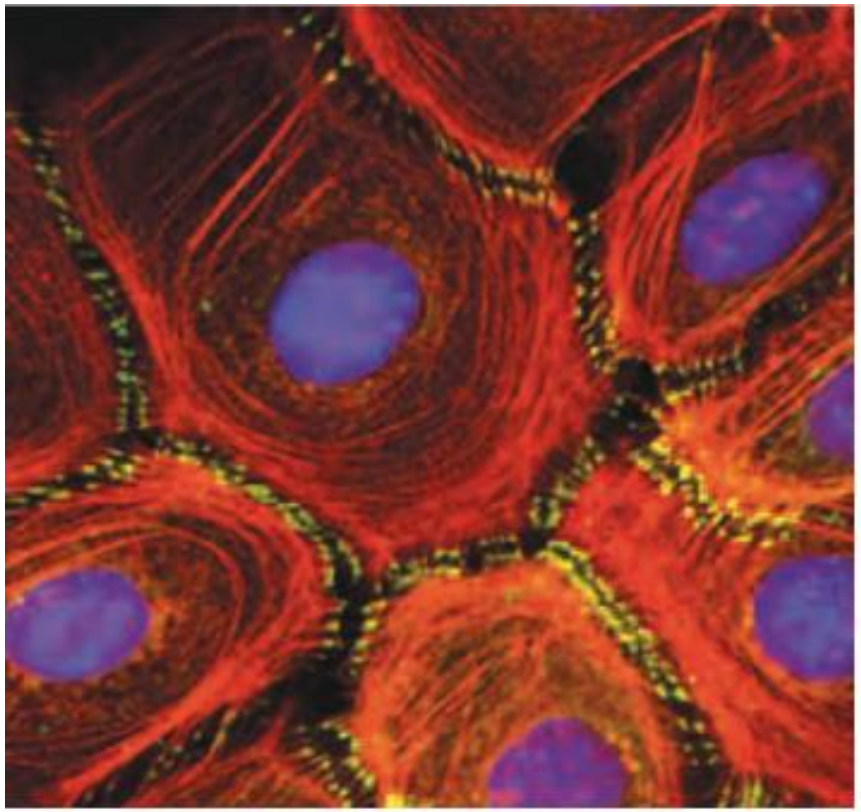
Cells bound closely together to form specialized cell junctions

Firmly anchored by basal lamina

Pre-requisites for EMT in epithelial tumor cells



Attachments of epithelial cells to their neighbors and the basement membrane



E-Cadherin (yellow) adherens junction
Actin cytoskeleton (red)
Nuclei in blue

EMT in epithelial tumor cells

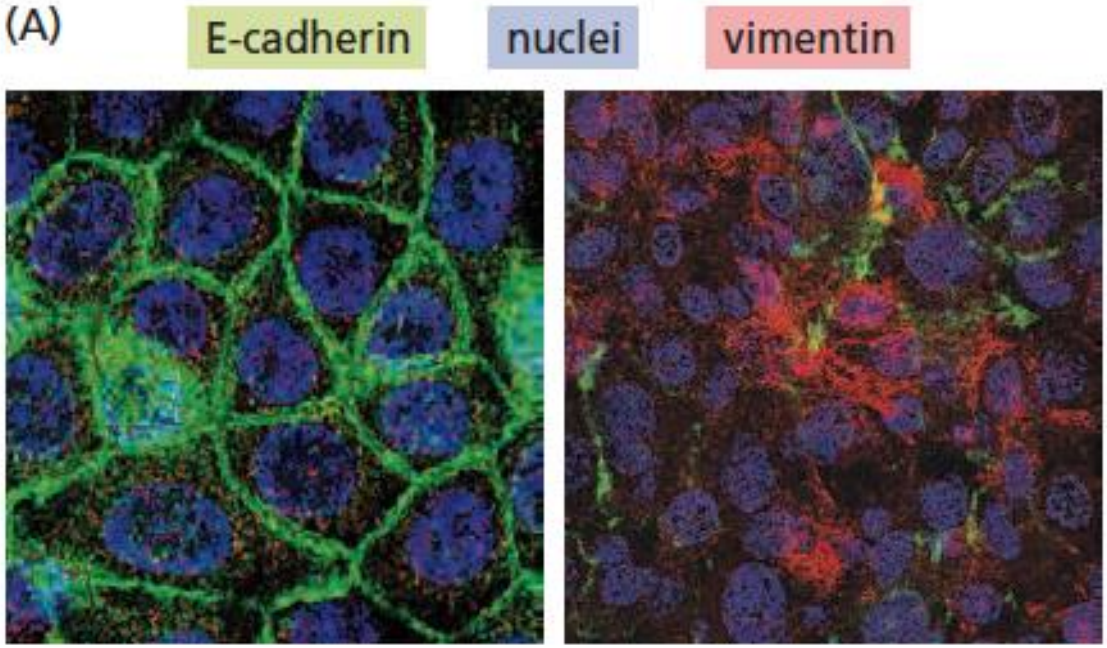


Table 14.2 Cellular changes associated with an epithelial–mesenchymal transition

Loss of
Cytokeratin (intermediate filament) expression
Tight junctions and epithelial adherens junctions involving E-cadherin
Epithelial cell polarity
Epithelial gene expression program
Acquisition of
Fibroblast-like shape
Motility
Invasiveness
Increased resistance to apoptosis
Mesenchymal gene expression program including EMT-inducing transcription factors
Mesenchymal adherens junction protein (N-cadherin)
Protease secretion (MMP-2, MMP-9)
Vimentin (intermediate filament) expression
Fibronectin secretion
PDGF receptor expression
$\alpha_v\beta_6$ integrin expression
Stem cell-like traits

Breaching of the basement membrane

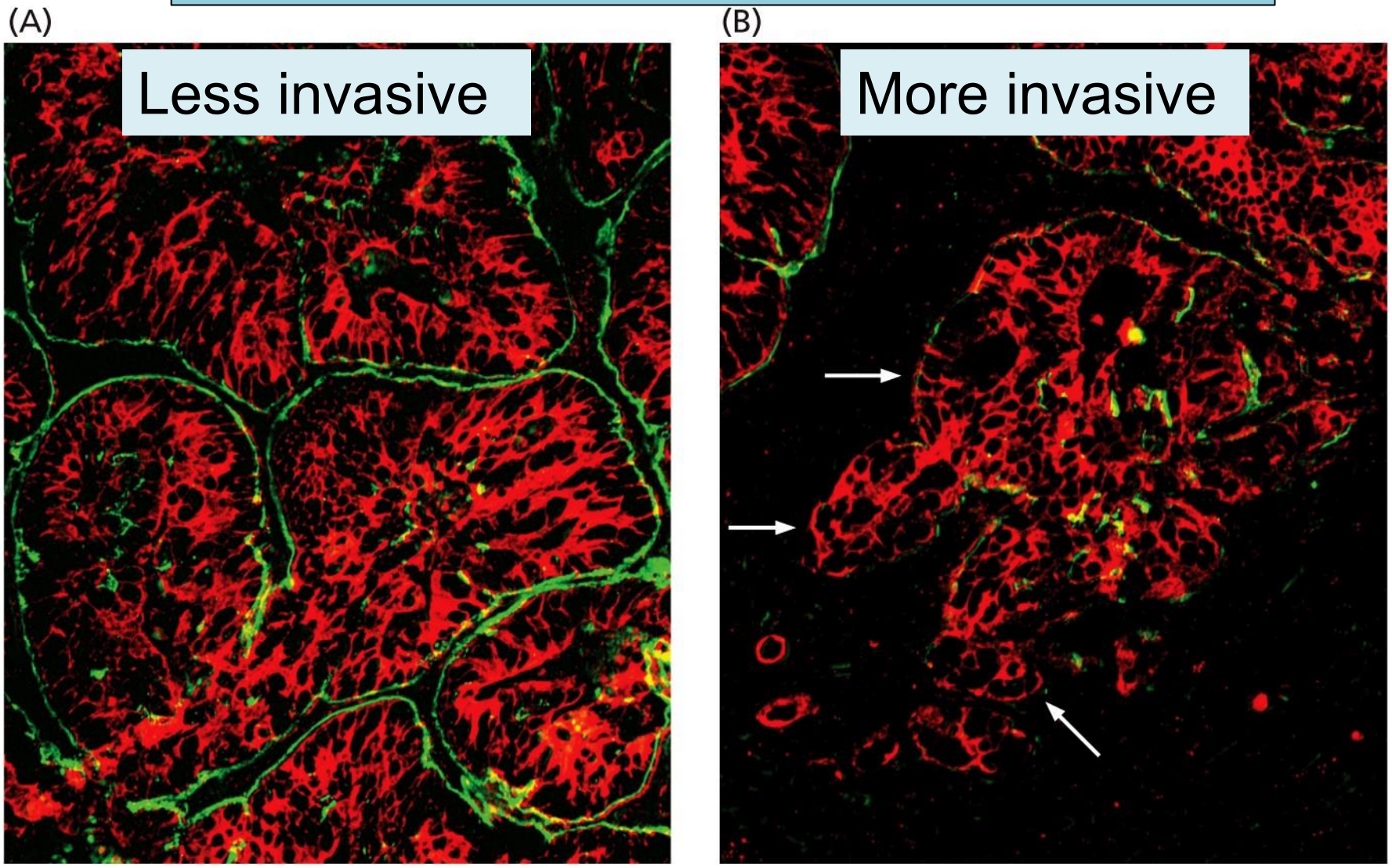


Figure 14.4ab The Biology of Cancer (© Garland Science 2014)

Anti-cytokeratin (**red**) for epithelial tumor (carcinoma)
Anti-laminin alpha-3 (**green**) for basement membrane

See Fig. 14.4
The Biology of Cancer

Partial or complete loss of BM within tumors shows strong likelihood of metastases development

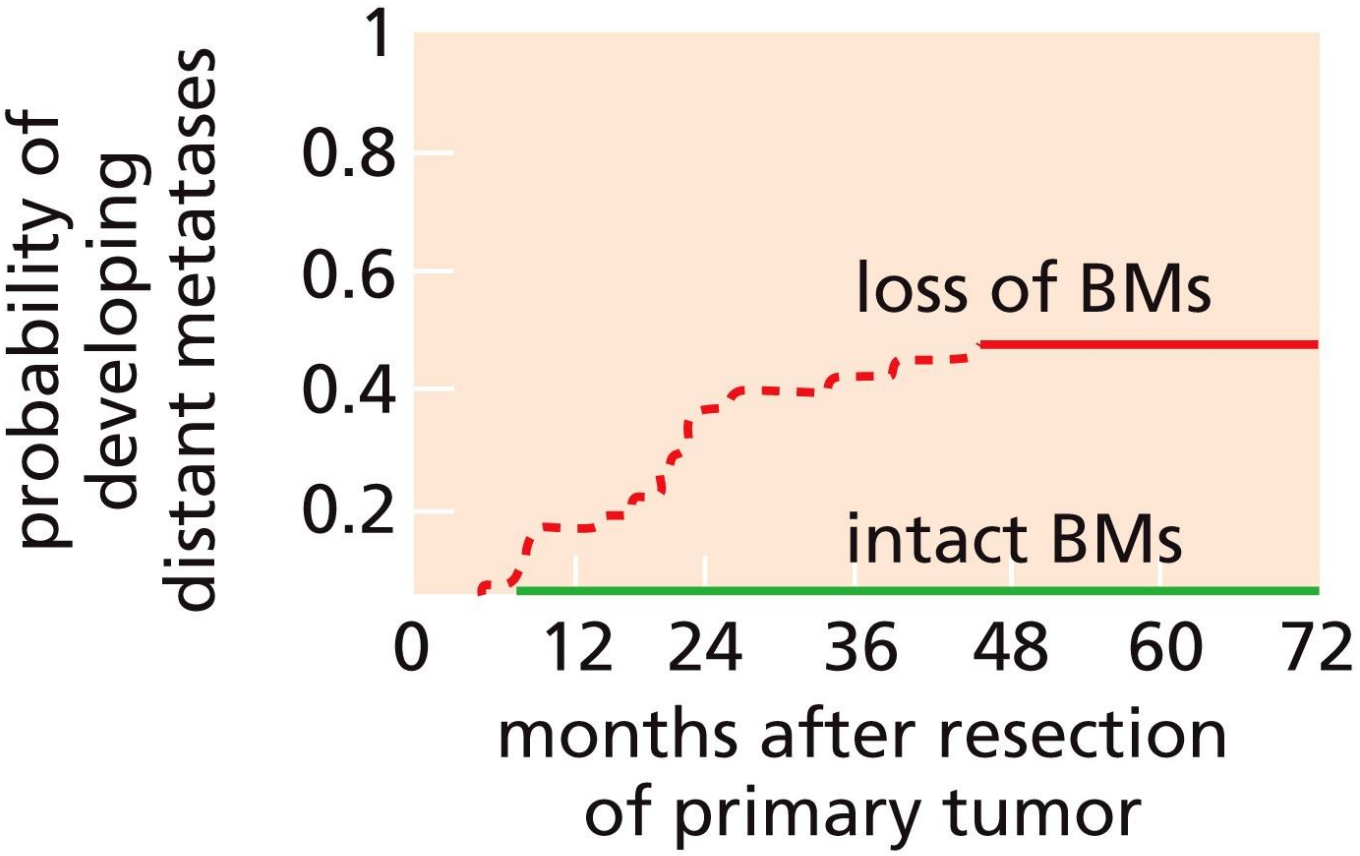
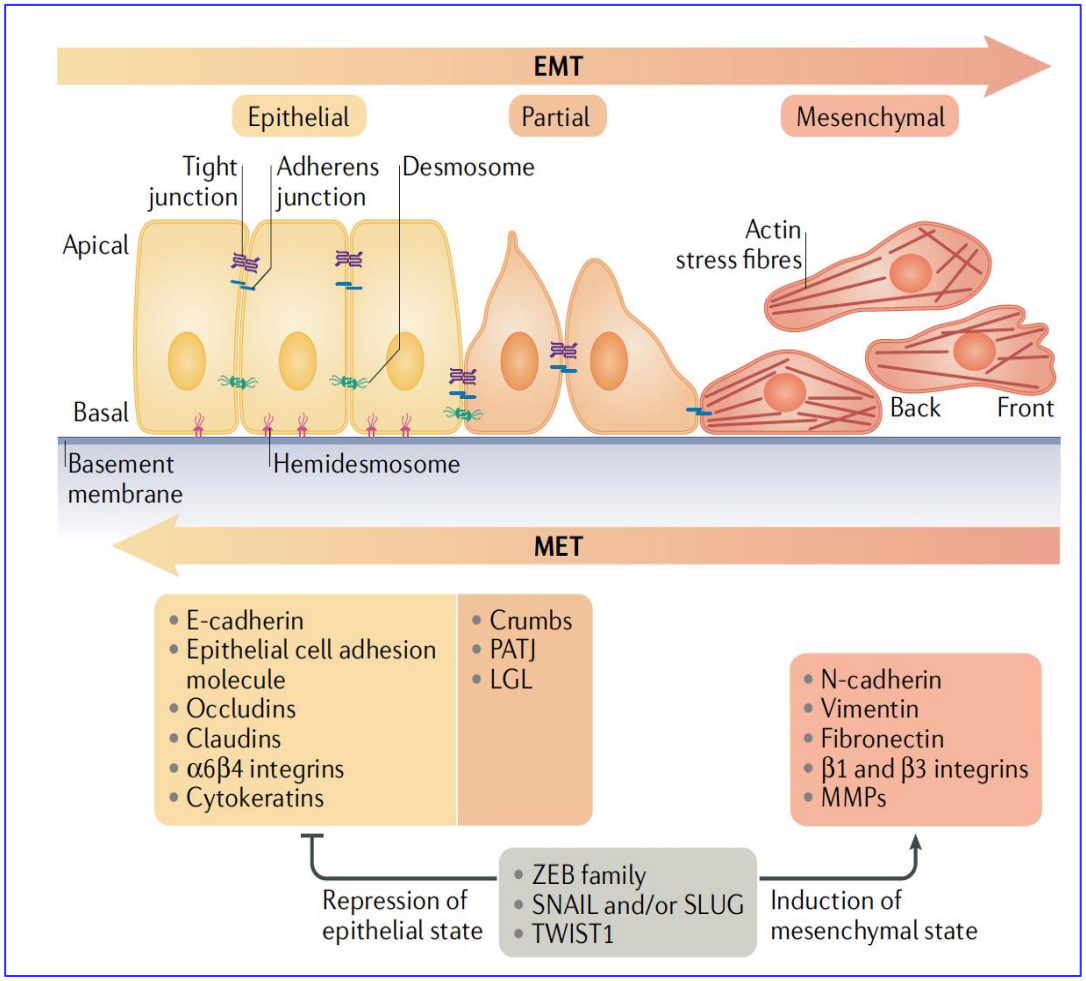


Figure 14.4c The Biology of Cancer (© Garland Science 2014)

See Fig. 14.4 (C)
The Biology of Cancer

Epithelial-mesenchymal transition (EMT)

EMT= Reversible cellular program that transiently converts epithelial cells into mesenchymal-like (quasi-mesenchymal) cell states.



- Epigenetic process.
- Induced by stromal signals.
- Orchestrated by EMT-inducing transcription factors (EMT-TFs).
- Aids in multiple steps of the metastatic cascade.

EMT in development

ELSEVIER

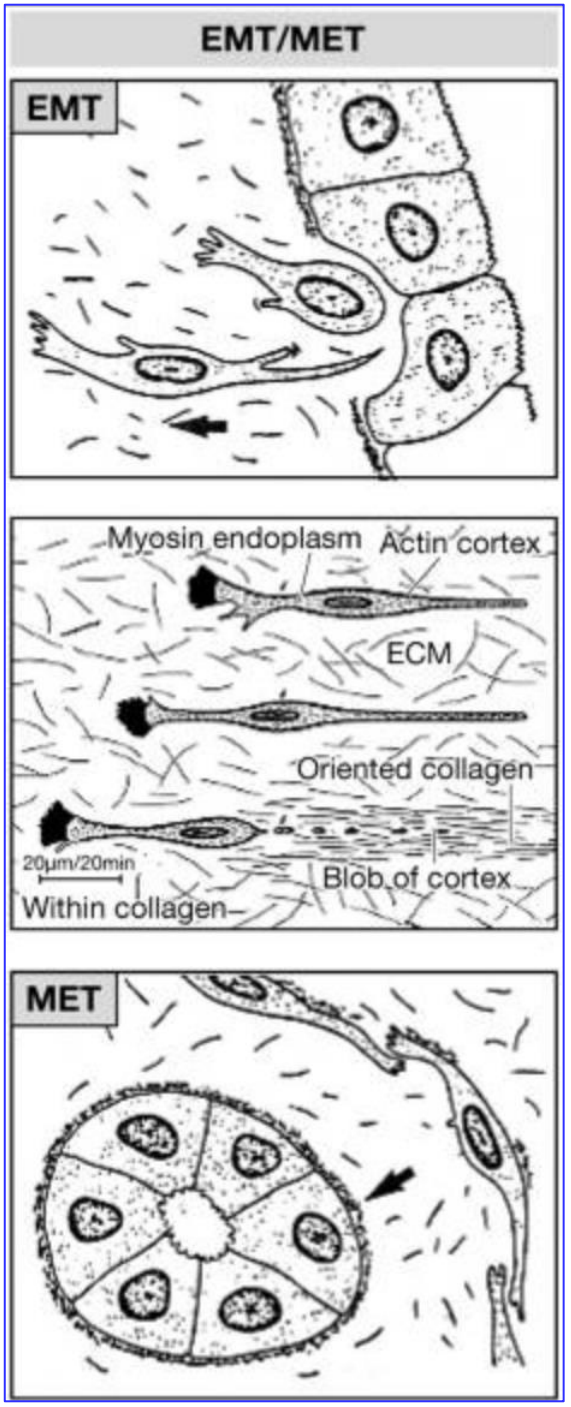
Developmental Biology

Volume 16, Issue 1, July 1967, Pages 78-106

Cell contact during early morphogenesis in the chick embryo ☆

Robert L. Trelstad [2], Elizabeth D. Hay, Jean-Paul Revel

EMT is reversible,
possibly epigenetically regulated



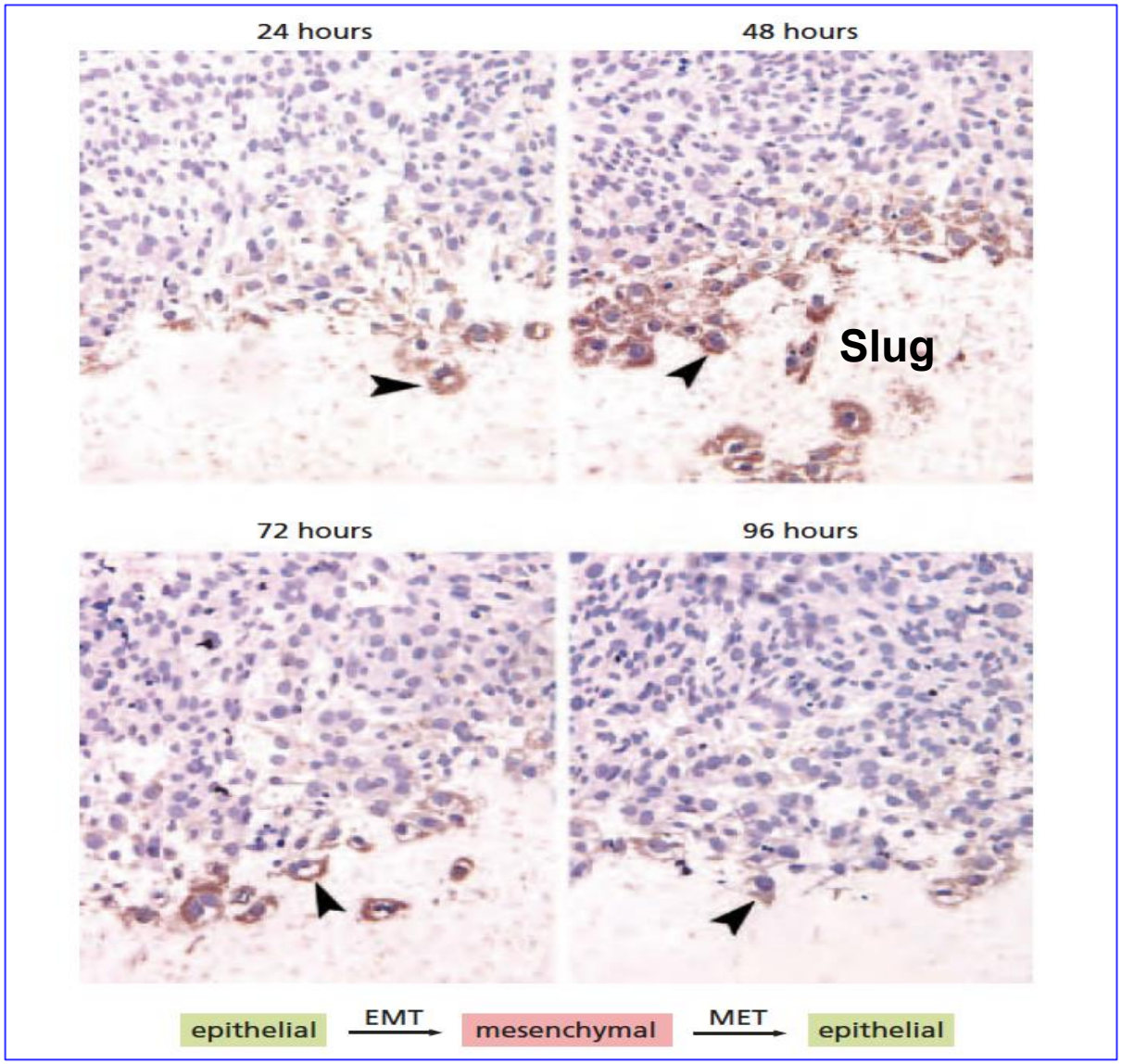
EMT-TFs

Table 14.3 Transcription factors orchestrating an EMT

Regulate each other, redundancy in functions

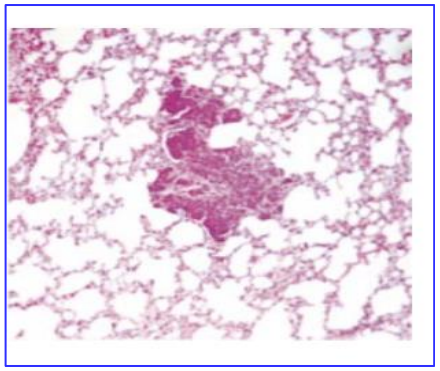
Name	Where first identified	Type of transcription factor	Cancer association
Snail (SNAI1)	mesoderm induction in *Drosophila*; neural crest migration in vertebrates	C2H2-type zinc finger	invasive ductal carcinoma
Slug (SNAI2)	delamination of the neural crest and early mesoderm in chicken	C2H2-type zinc finger	breast cancer cell lines, melanoma
Twist	mesoderm induction in *Drosophila*; emigration from neural crest	bHLH	various carcinomas, high-grade melanoma, neuroblastoma
Goosecoid	gastrulation in frog	paired homeodomain	various carcinomas
FOXC2	mesenchyme formation	winged helix/forkhead	basal-like breast cancer
ZEB1 (δEF1)	postgastrulation mesodermal tissue formation	2-handed zinc finger/ homeodomain	wide variety of cancers
ZEB2 (SIP1)	neurogenesis	2-handed zinc finger/ homeodomain	ovarian, breast, liver carcinomas
E12/E47 (Tcf3)[a]	associated with E-cadherin promoter	bHLH	gastric cancer

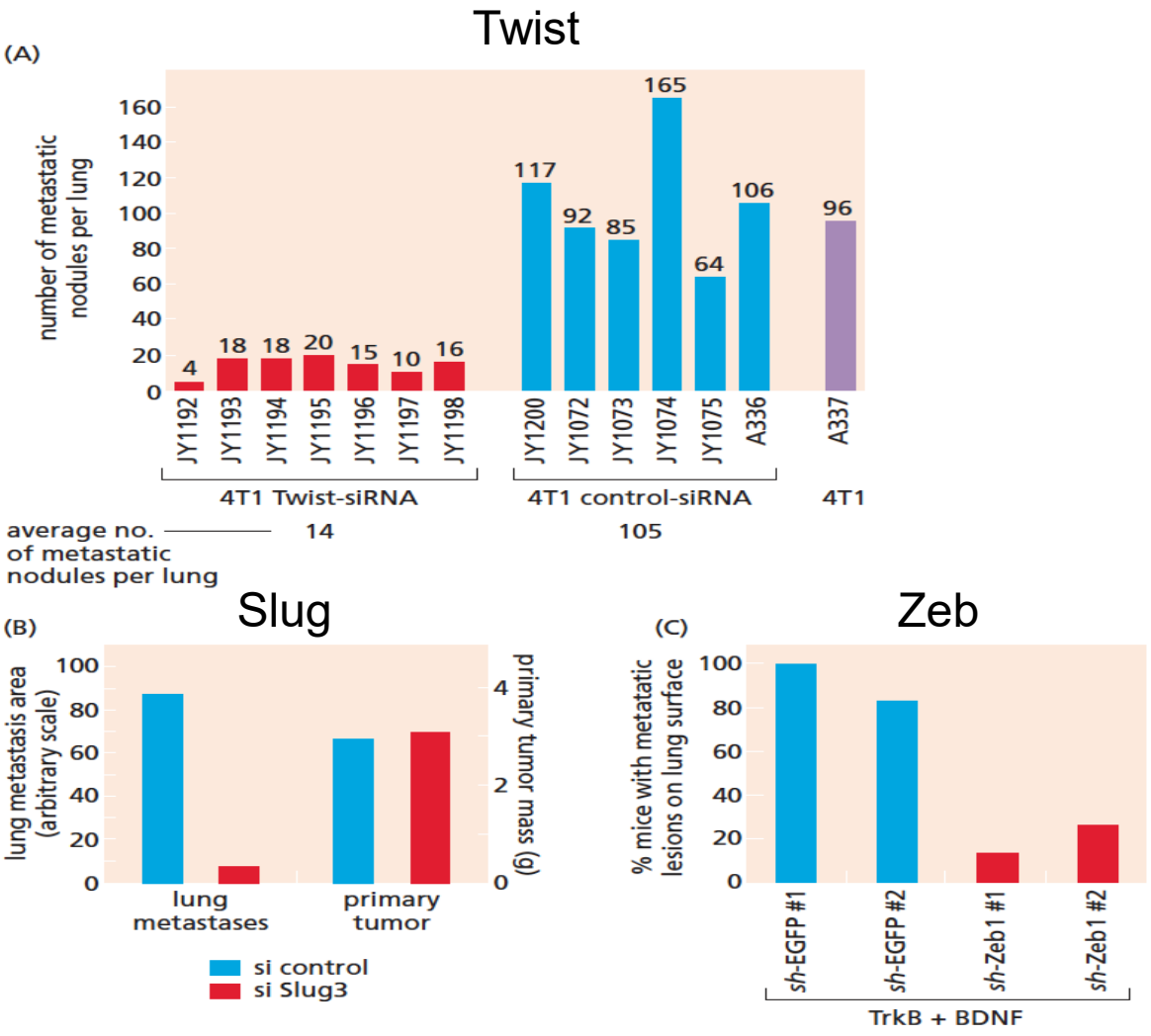
Transient EMT-TF expression in wound healing



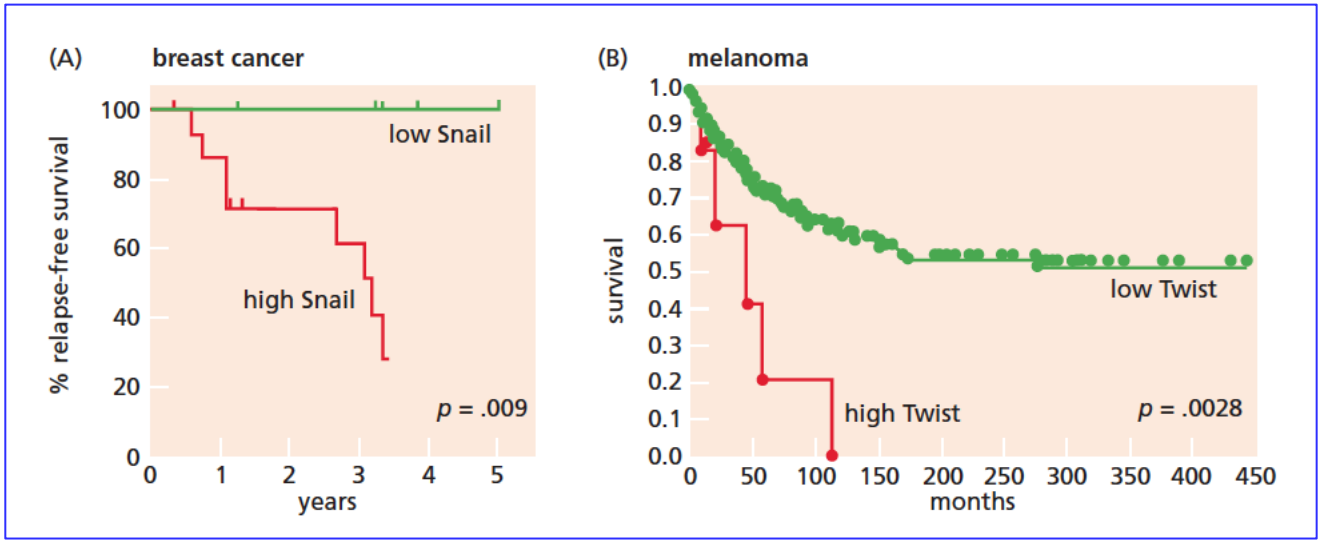
Role of EMT-TFs in promoting metastasis:

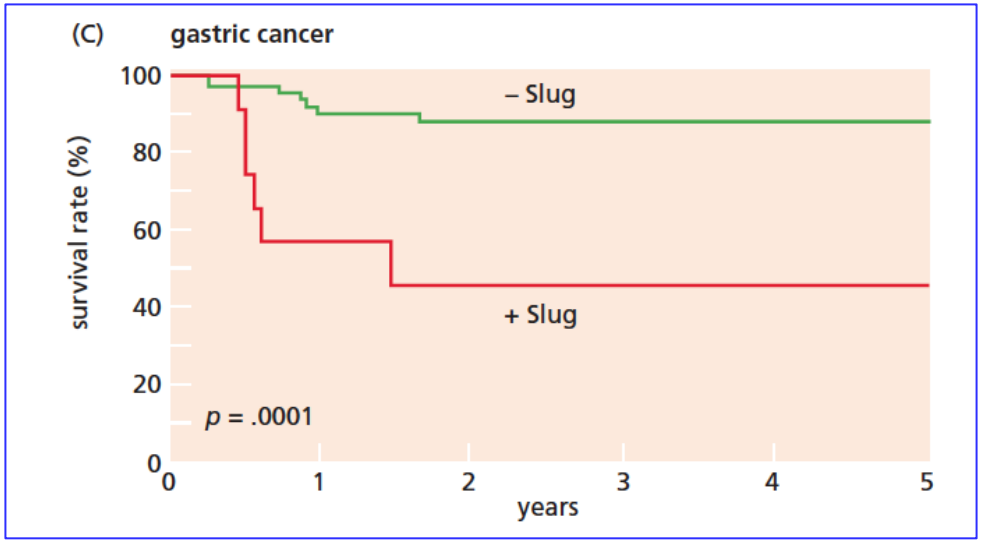
knockdown and suppression experiments





Levels of EMT-inducing factors and correlation with cancer patient survival





EMT-inducing TFs confer stem cell (SC) properties

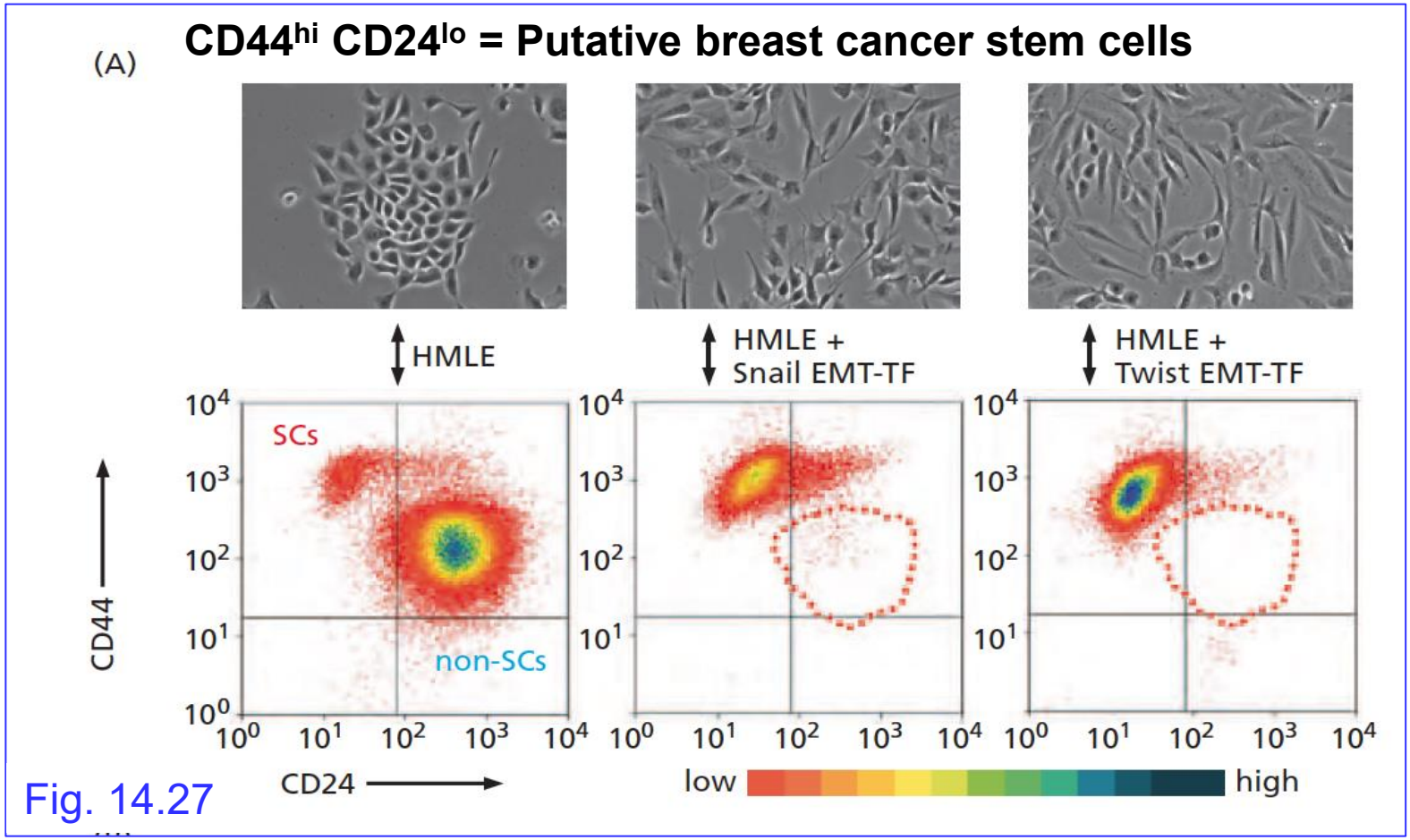
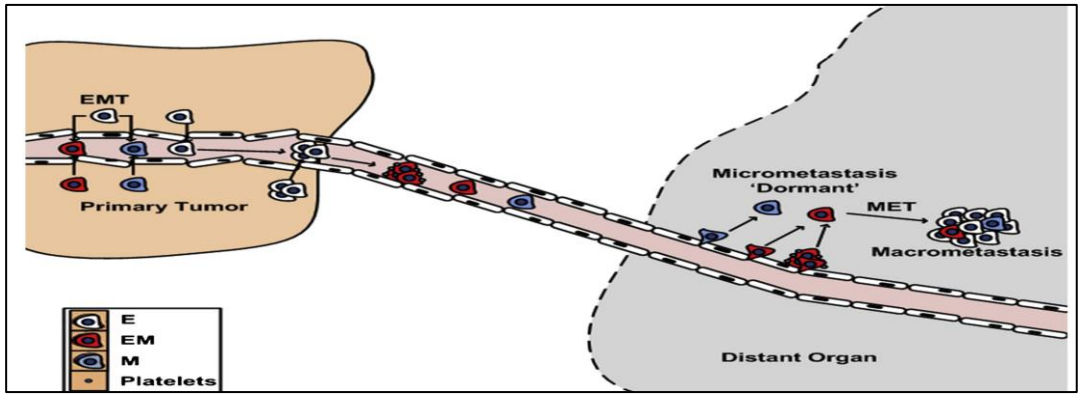


Fig. 14.27

Stem cells persist after anti-cancer treatment

Stem cells undergoing EMT are more invasive and metastatic

EMT: Required or dispensable for metastasis?



nature
International journal of science

Altmetric: 63 Citations: 324 More detail »

Article

Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance

Kari R. Fischer, Anna Durrans, Sharrell Lee, Jianting Sheng, Fuhai Li, Stephen T. C. Wong, Hyejin Choi, Tina El Rayes, Seongho Ryu, Juliane Troeger, Robert F. Schwabe, Linda T. Vahdat, Nasser K. Altorki, Vivek Mittal & Dingcheng Gao

nature
International journal of science

Altmetric: 78 Citations: 328 More detail »

Letter

Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer

Xiaofeng Zheng, Julienne L. Carstens, Jiha Kim, Matthew Scheible, Judith Kaye, Hikaru Sugimoto, Chia-Chin Wu, Valerie S. LeBleu & Raghu Kalluri

Snail or Twist deletion

Thierry and Lim, Cancer Cell Previews, 2013

Nat Cell Biol. 2017 May;19(5):518-529. doi: 10.1038/ncb3513. Epub 2017 Apr 17.

The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer.

Krebs AM[1,2,3,4], Mitschke J[5], Lasierra Losada M[1], Schmalhofer O[5], Boerries M[3,4,6], Busch H[3,4,6], Boettcher M[7], Mougiakakos D[7], Reichardt W[3,4,8], Bronsert P[3,4,9], Brunton VG[10], Pilarsky C[11], Winkler TH[12], Brabletz S[1], Stemmler MP[1], Brabletz T[1].

Brief Communications Arising | Published: 06 July 2017

Upholding a role for EMT in pancreatic cancer metastasis

Nicole M. Aiello, Thomas Brabletz, Yibin Kang, M. Angela Nieto, Robert A. Weinberg & Ben Z. Stanger

Nature **547**, E7–E8(2017) | Cite this article

Signals initiating EMT from the microenvironment

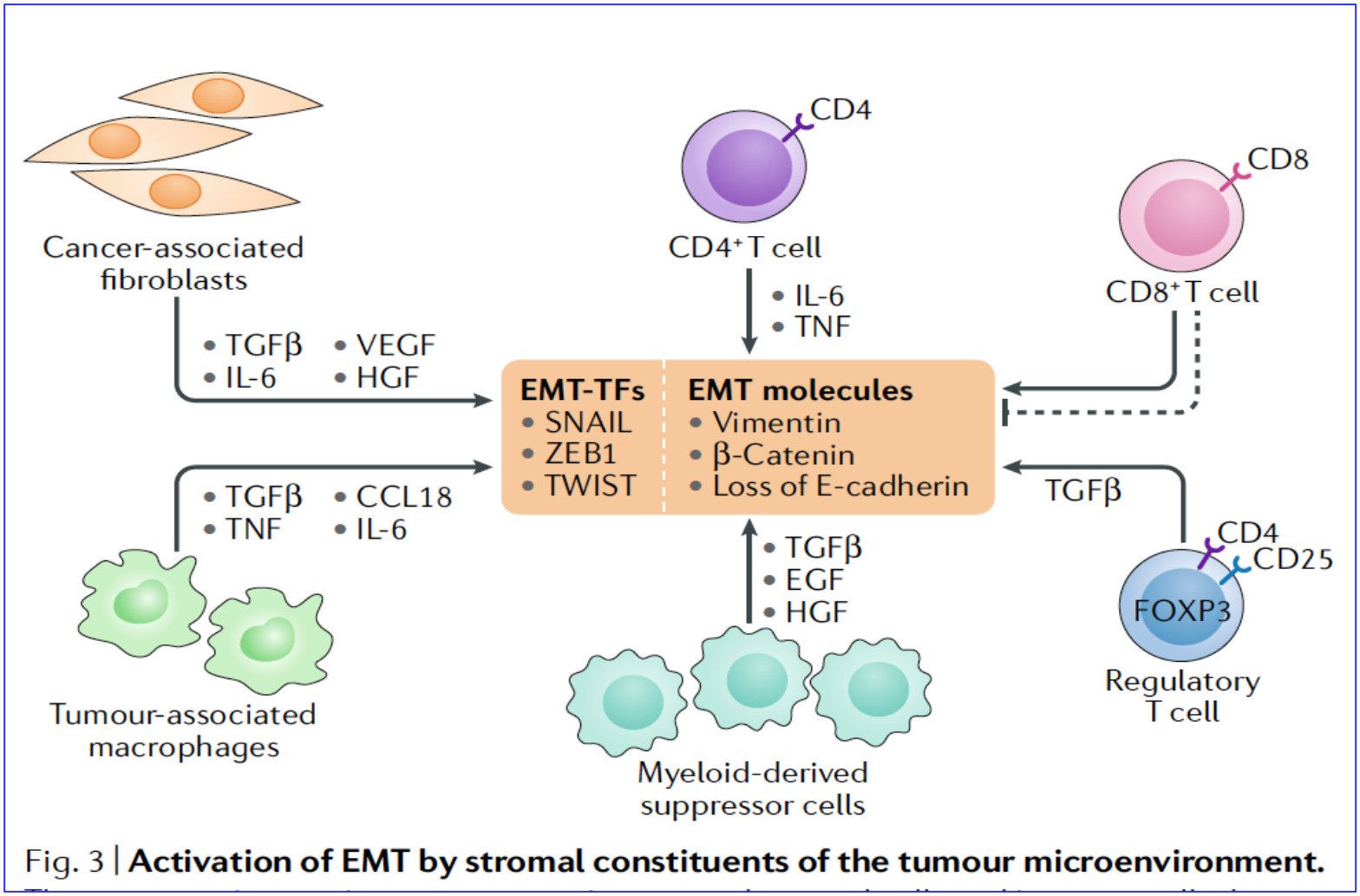
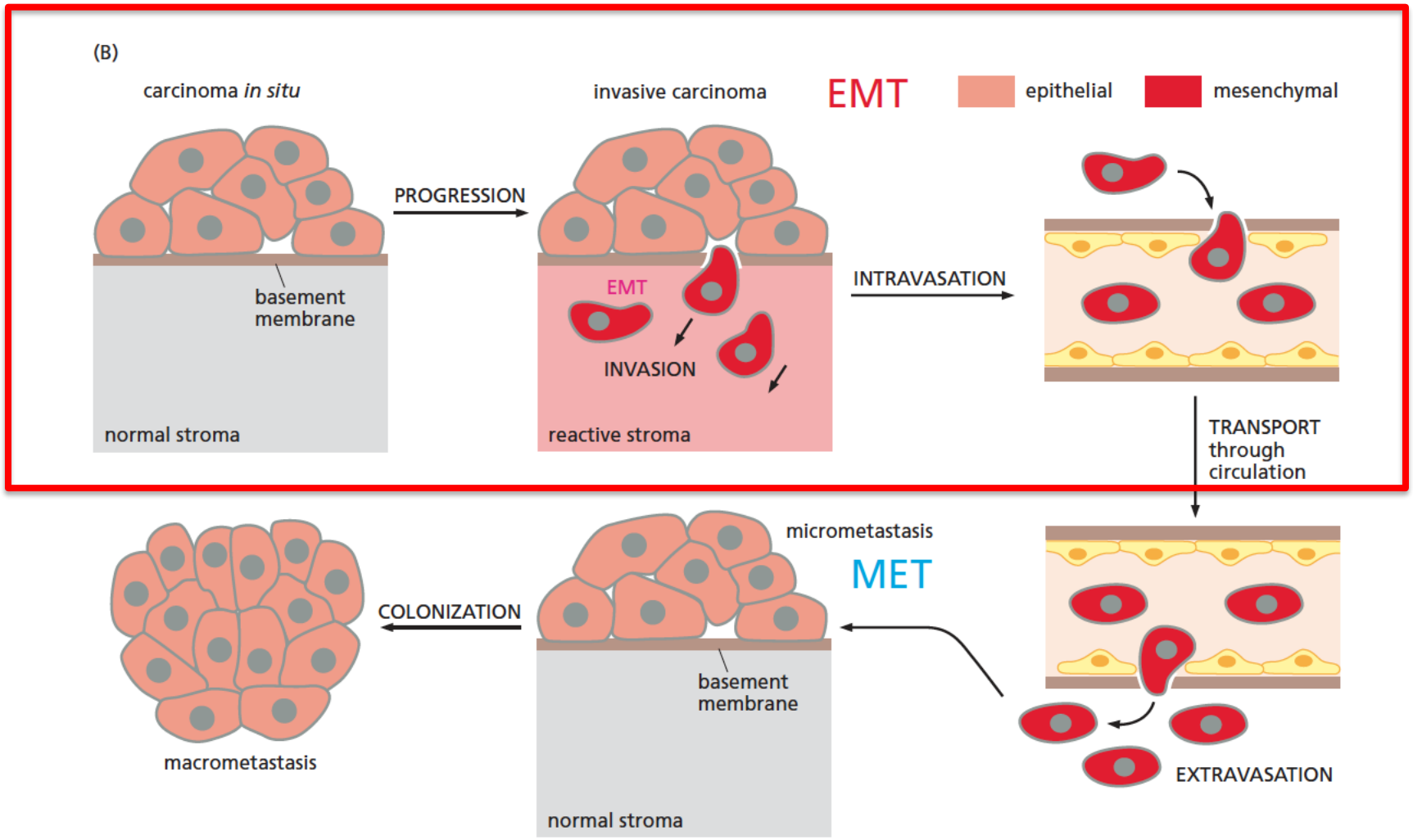


Fig. 3 | **Activation of EMT by stromal constituents of the tumour microenvironment.**

Few examples of paracrine signaling that helps in cancer cell intravasation



Macrophages promote intravasation of tumors

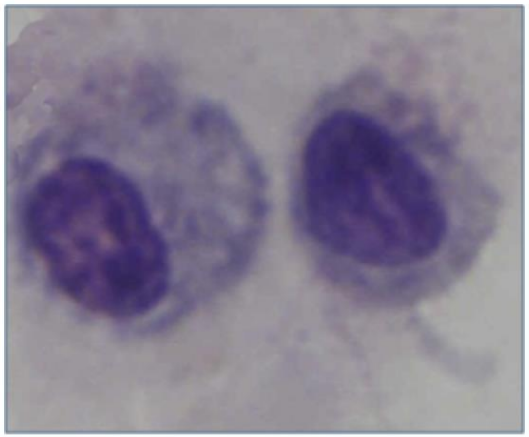
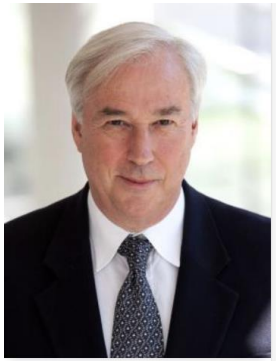


Figure 1. Lung macrophages stained with Wright-Giemsa

Macrophages
Immune cells in our body
that are specialized for detection,
phagocytosis and destruction of bacteria.
Can antigen-present to T-cells and initiate
inflammation.
Also can have tumor-promoting/inhibiting function
depending on their subtypes, location and polarization.

https://www.immunology.org

Macrophages promote intravasation of tumors and lung metastasis



Professor Jeffrey Pollard

Colony-stimulating Factor 1 Promotes Progression of Mammary Tumors to Malignancy

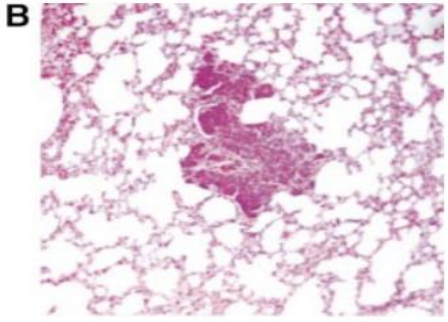
By Elaine Y. Lin,* Andrew V. Nguyen,* Robert G. Russell,‡ and Jeffrey W. Pollard*

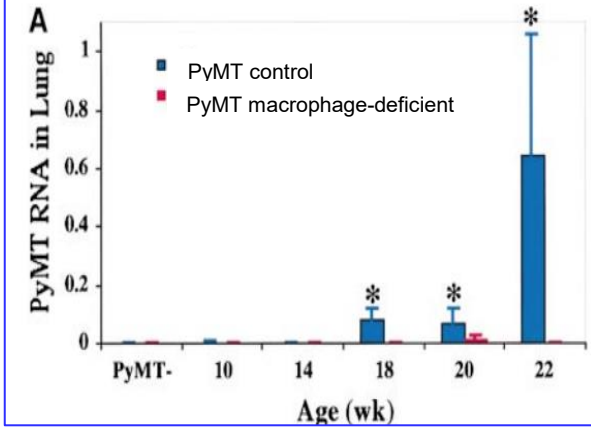
MMTV-PyMT X CSF1 knockout mice =Genetically altered mammary tumor prone mice that lack the ability to generate CSF-1 in their mammary epithelium

→ No mature macrophages →

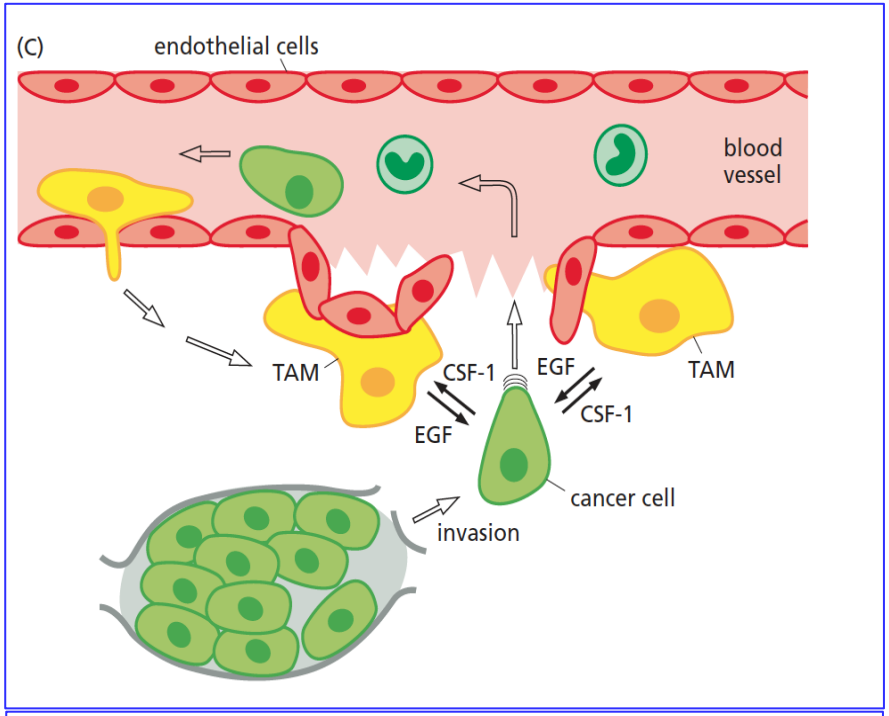
→ No differences in primary tumor growth

Lung metastasis





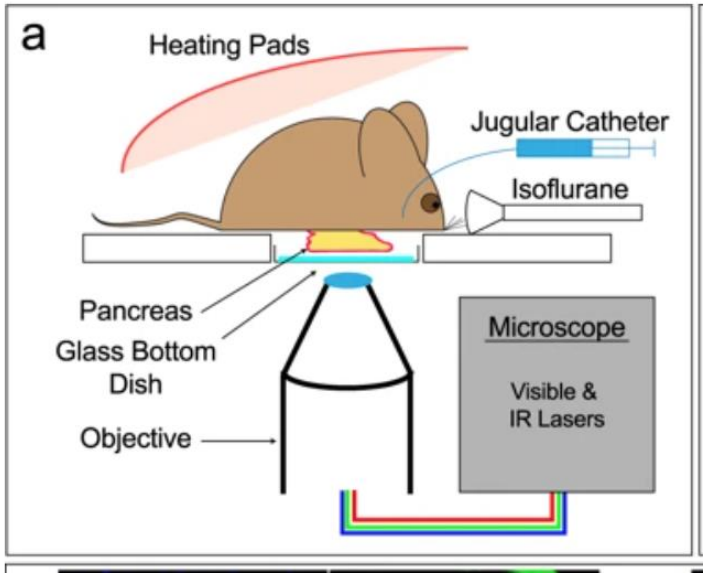
Macrophages promote intravasation of tumors via degradation of E-Cadherin and ECM proteins to increase the invasiveness of cancer cells

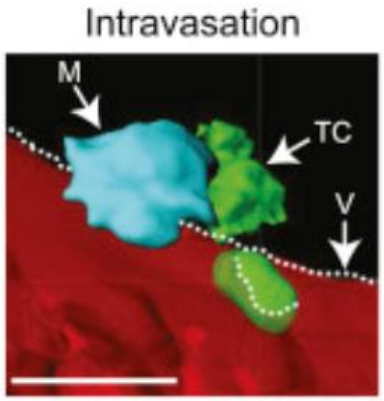


Macrophages secrete cathepsin-proteases that leads to proteolytic degradation of E-Cadherin → Can initiate EMT.

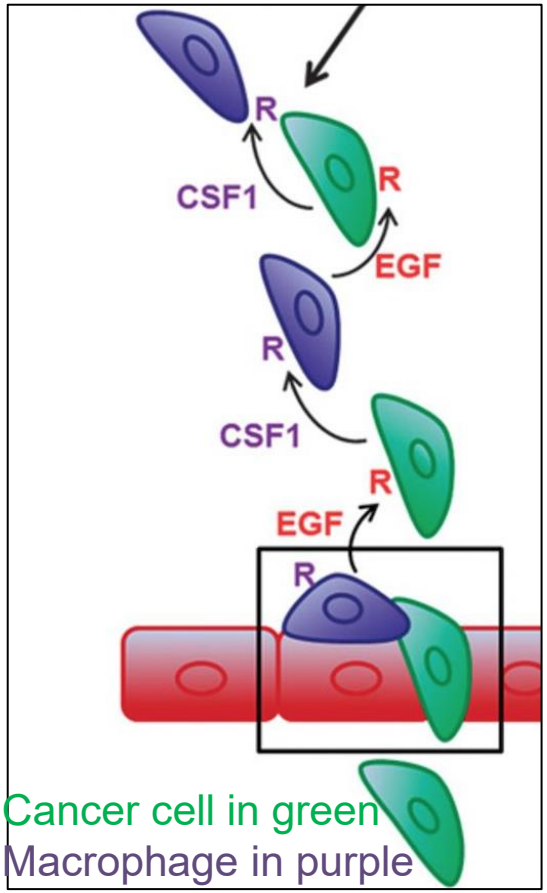
Macrophage-cancer cell interactions aid in intravasation

Intravital microscopy



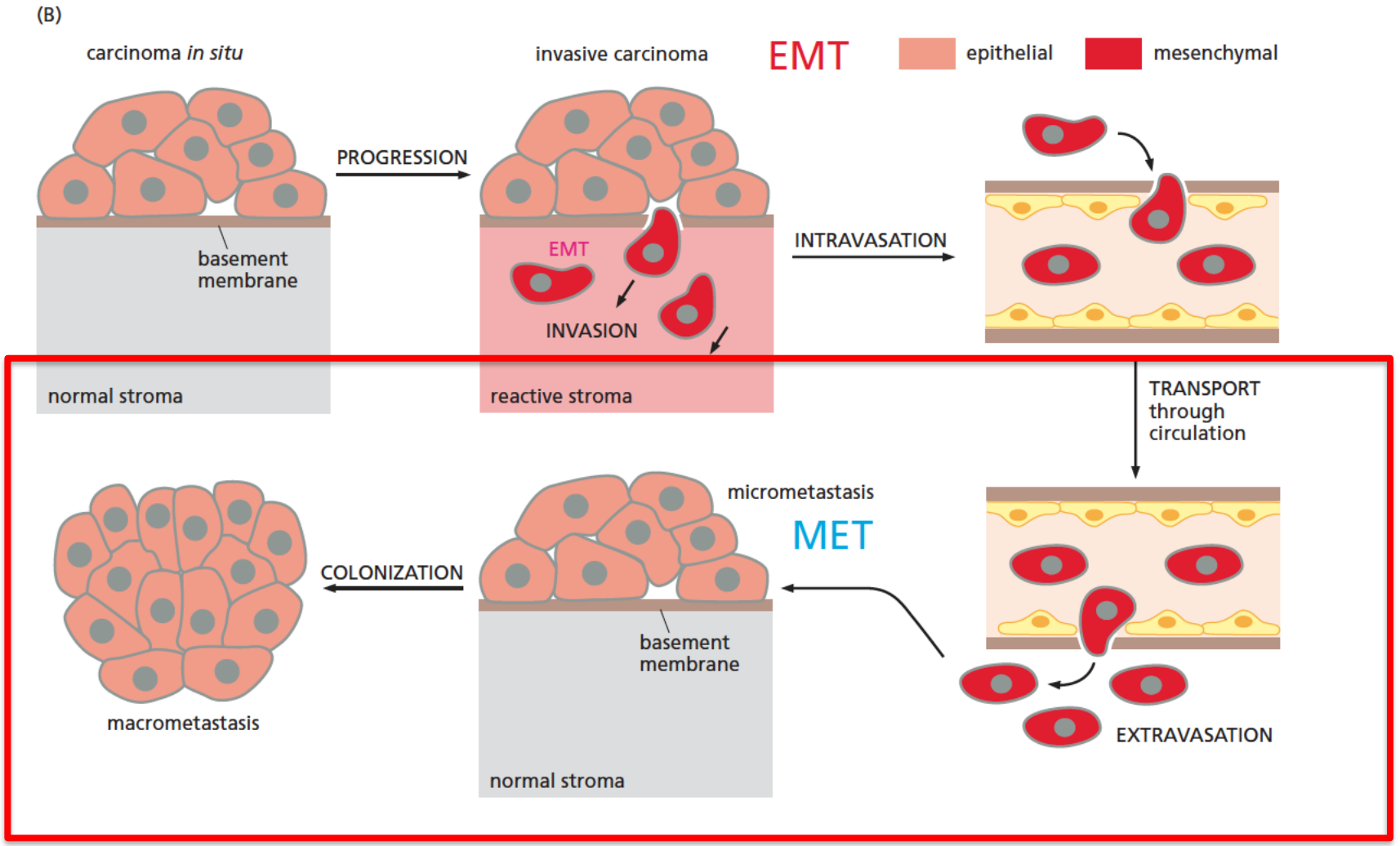


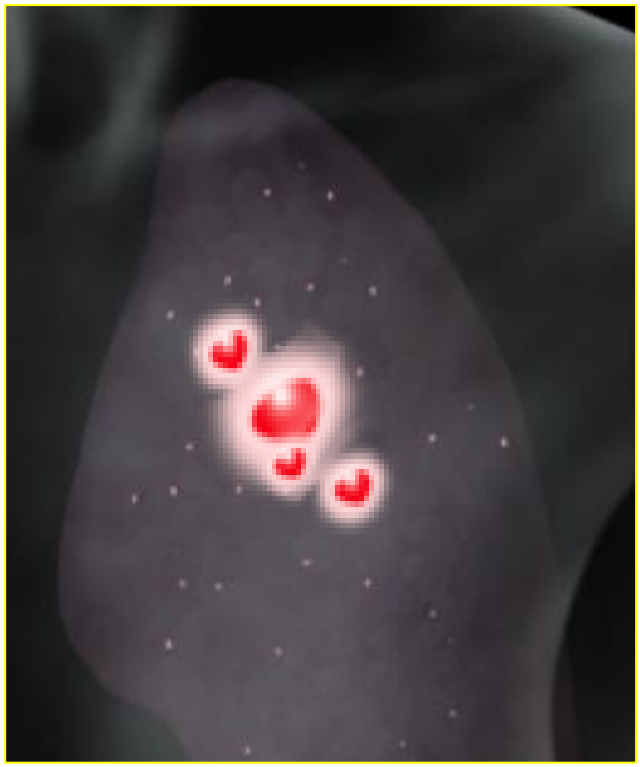
Condeelis laboratory



https://ars.els-cdn.com/content/image/1-s2.0-S0092867406000559-gr3_lrg.jpg

The final steps: extravasation and colonization





Extravasation

Process of leaving a blood or lymphatic vessel and invading the surrounding tissue

Passaging into microvessels in tissues

Easy for leukocytes and red blood cells

but not tumor cells

Intravital microscopy imaging studies (internal capillary diameter 3-8 μm)

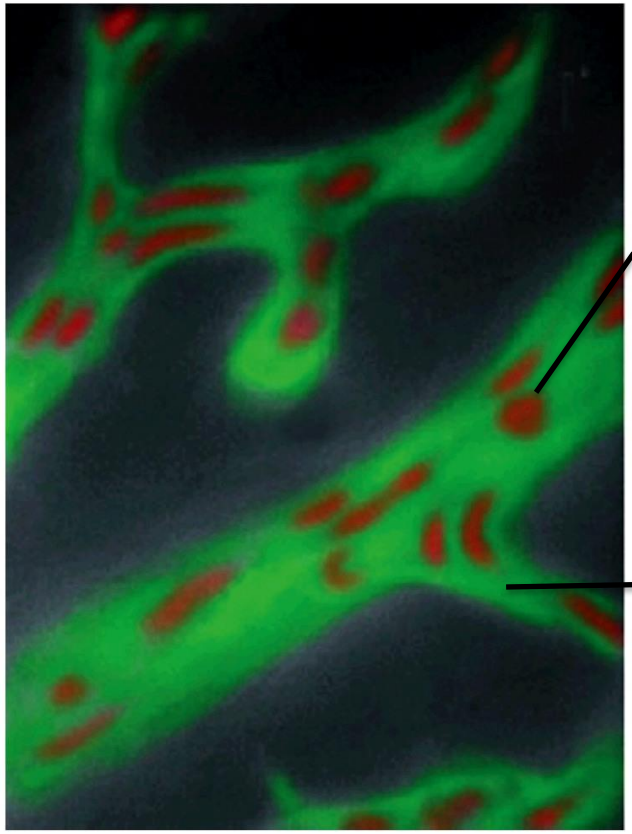


Figure 14.8b The Biology of Cancer (© Garland Science 2014)

Platelets (red)

Red blood cells (red) 7μm
Easily deformable

Plasma (green)

Tumor cells (green) >20μm (not deformable, trapped in larger arterioles)

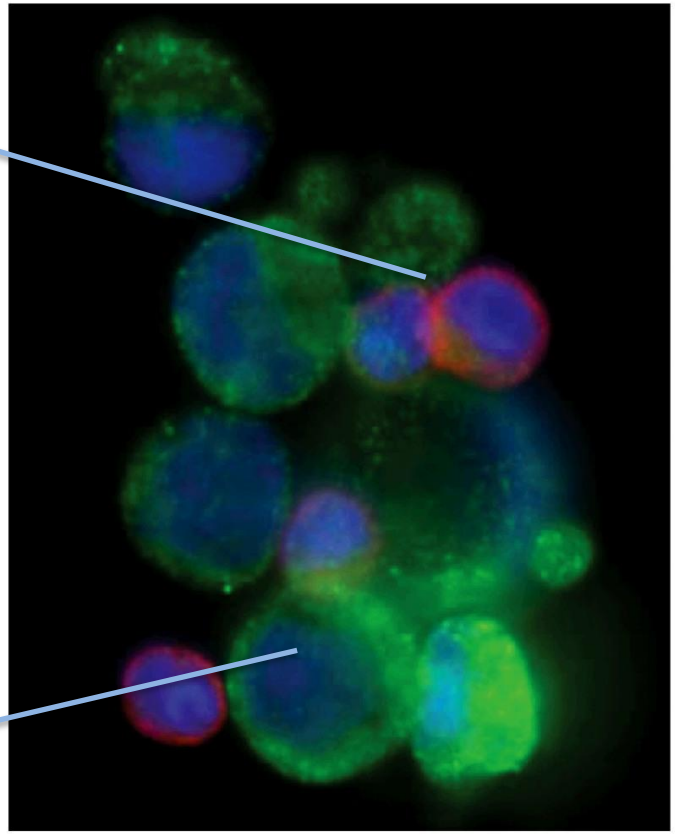


Figure 14.8c The Biology of Cancer (© Garland Science 2014)

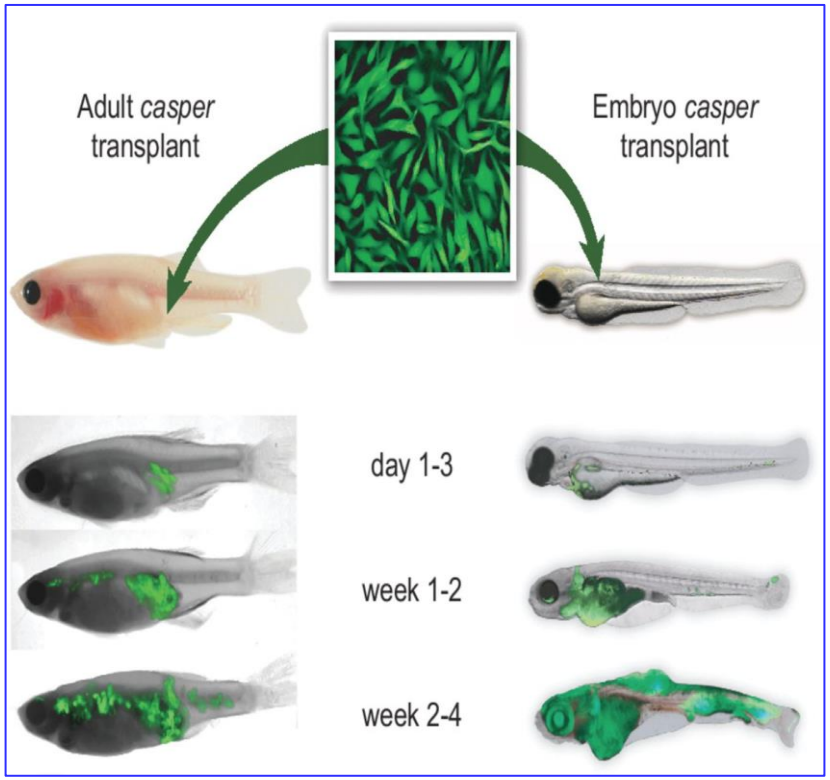
What aids in extravasation?

See Fig. 14.8
The Biology of Cancer

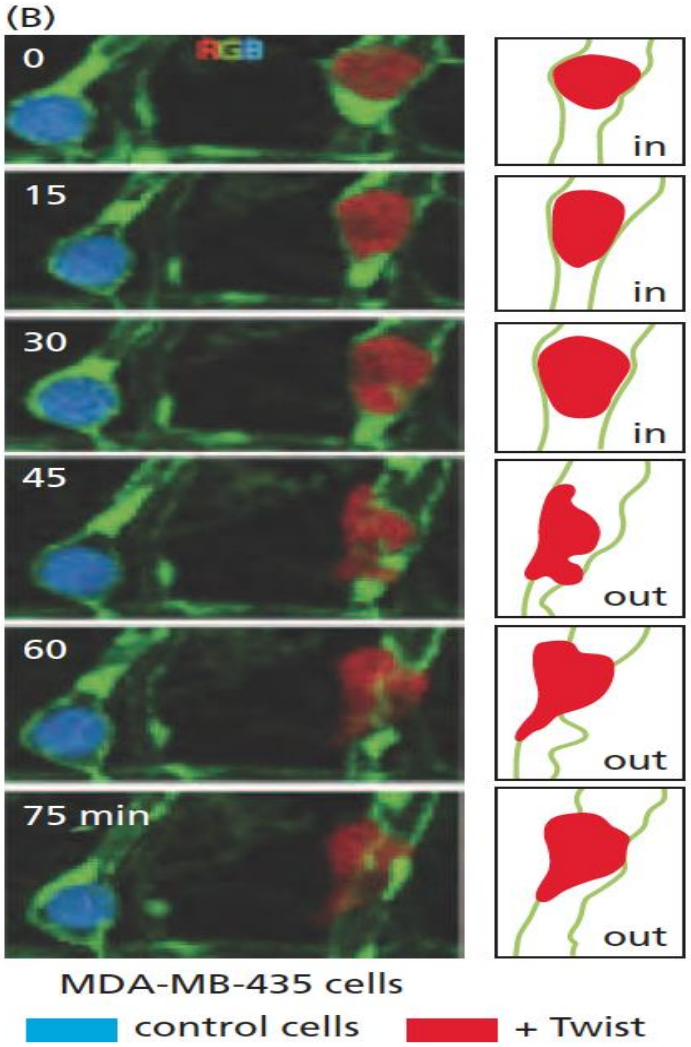
What aids in extravasation?

1) The EMT program promotes cancer cell-extravasation

Transparent Zebrafishes for studying metastasis



Heilmann et al., Cancer Res, 2015



2) Cancer cells secrete factors that create gaps in capillary walls: Modes of escape

Diapedesis= Leukocytes can induce the endothelial cells in post-capillary venules to retract and create a portal into the tissue

Cancer cells lodged in micro-vessels secrete a factor known as Angpl4 that induces endothelial cells to retract from one another

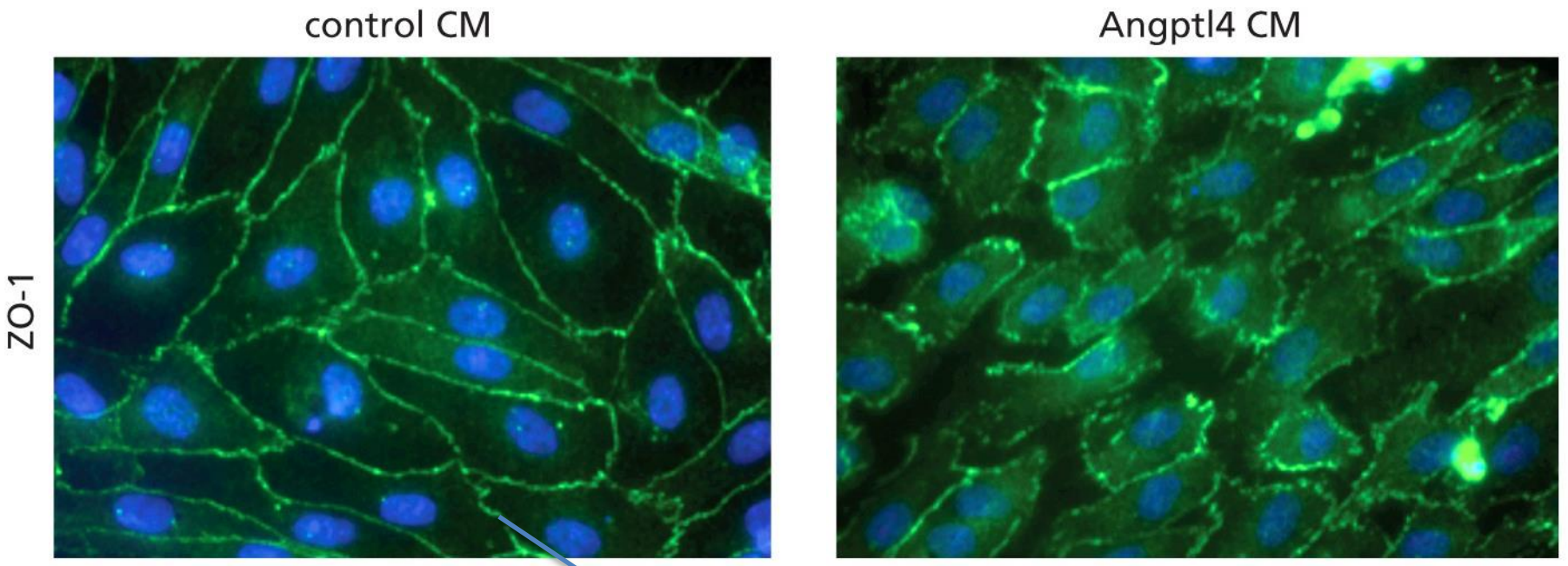


Figure 14.9c The Biology of Cancer (© Garland Science 2014)

Human vascular endothelial cells
with blue nuclei and tight junctions (green)

3) Grow at the site of the lumen of the vessel: Alternate entry point for extravasating cancer cells

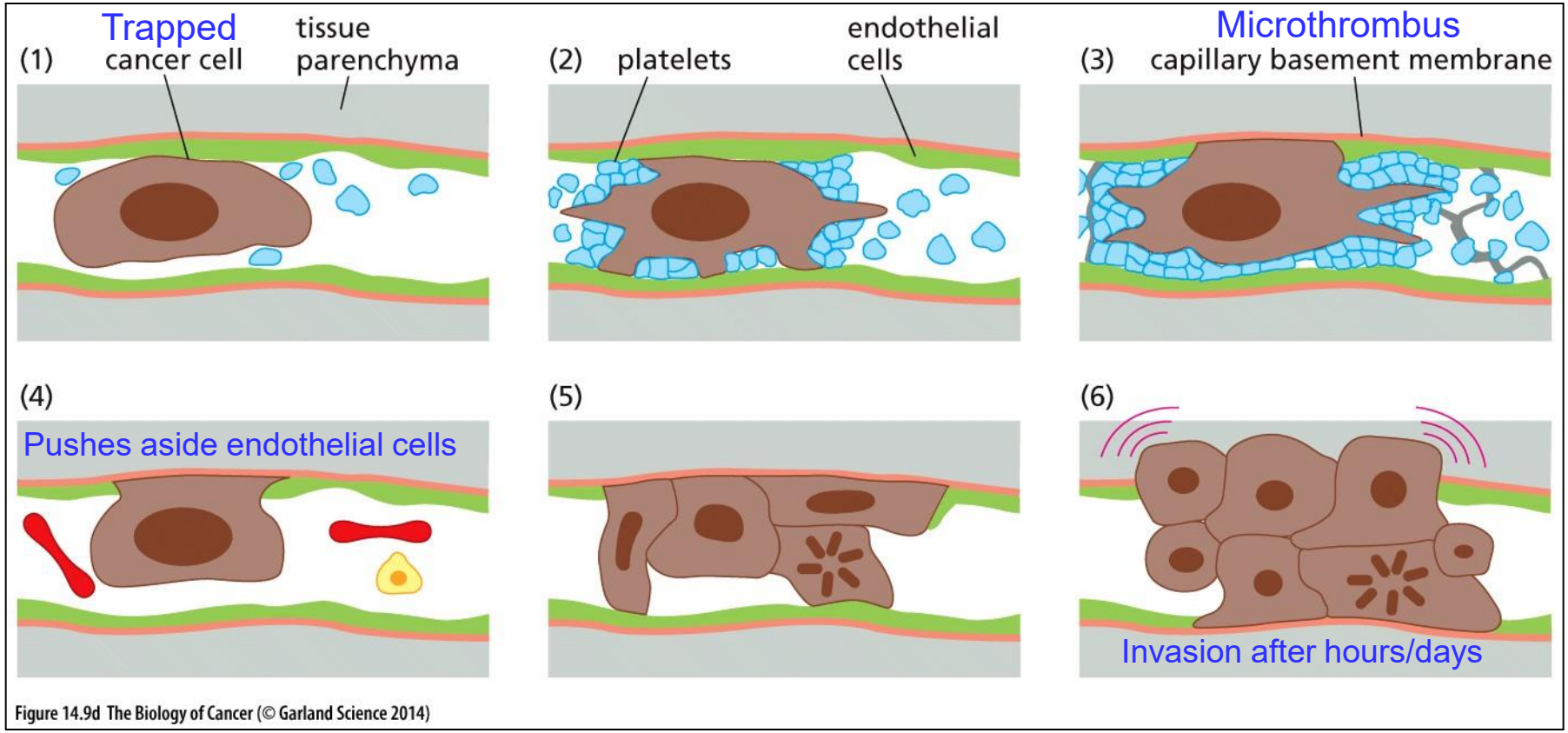


Figure 14.9d The Biology of Cancer (© Garland Science 2014)

See Fig. 14.9
The Biology of Cancer

Common metastasis sites for solid tumors

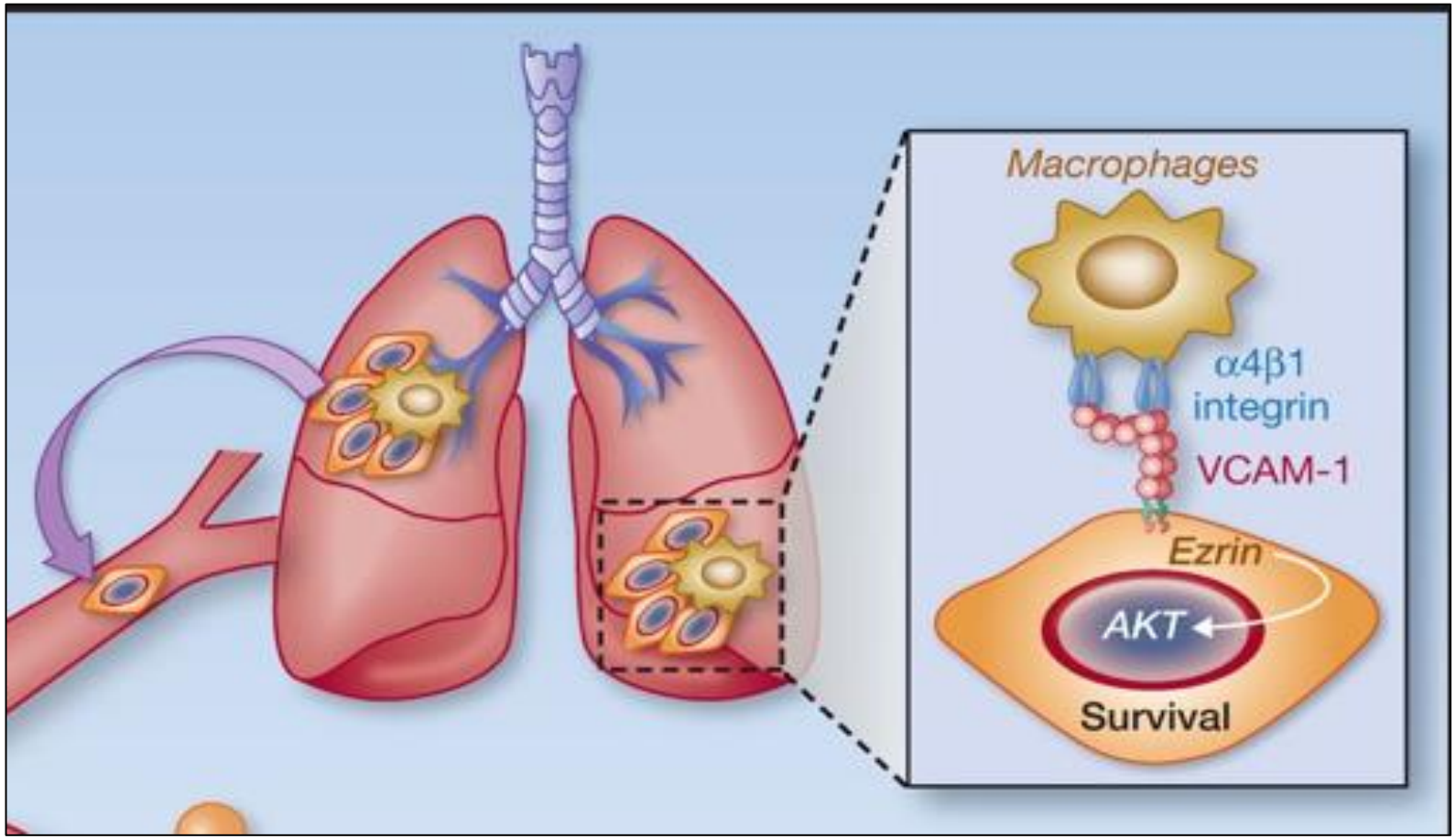
- Lung
- Bone
- Brain

How do cancer cells colonize these organs?

Lung colonization traits

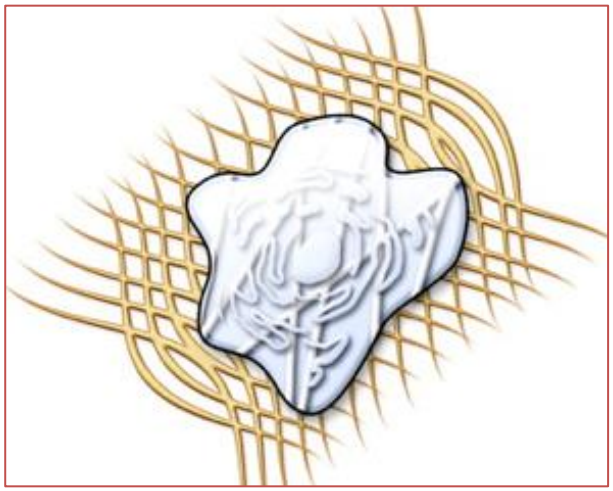
1. Microenvironment dependent
2. Cell-autonomous

Cancer cell-macrophage partnership in lung colonization



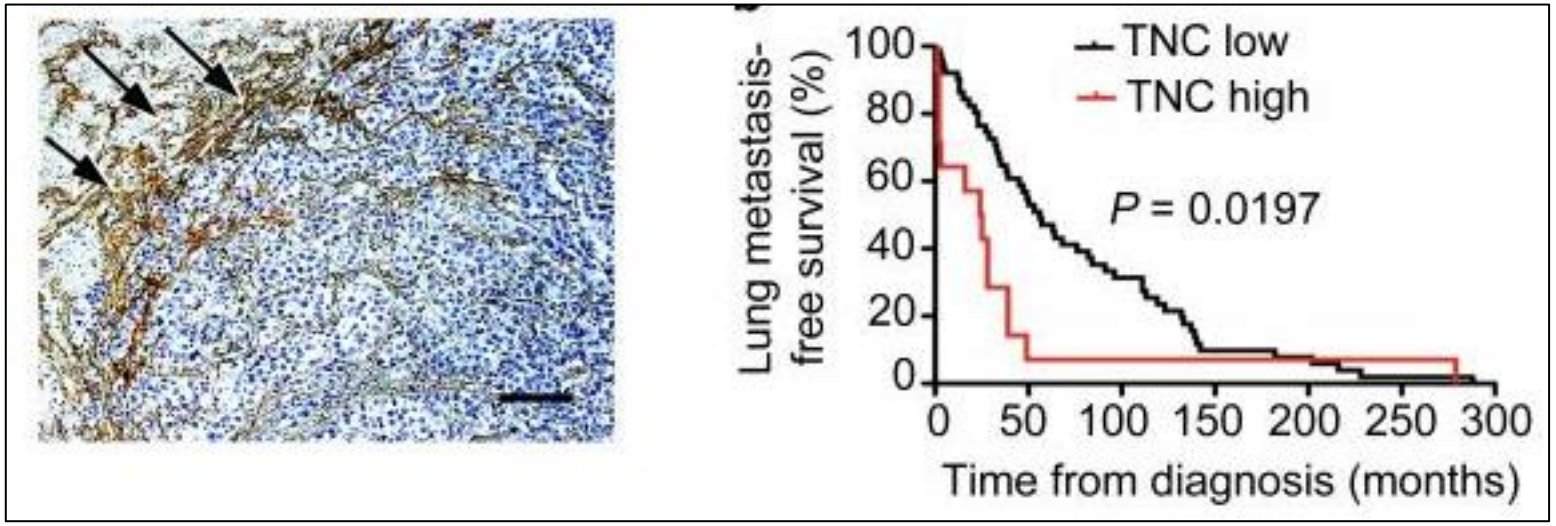
Chen and Massague, Clinical Cancer Research, 2012

Cancer cells secrete their own ECM niche



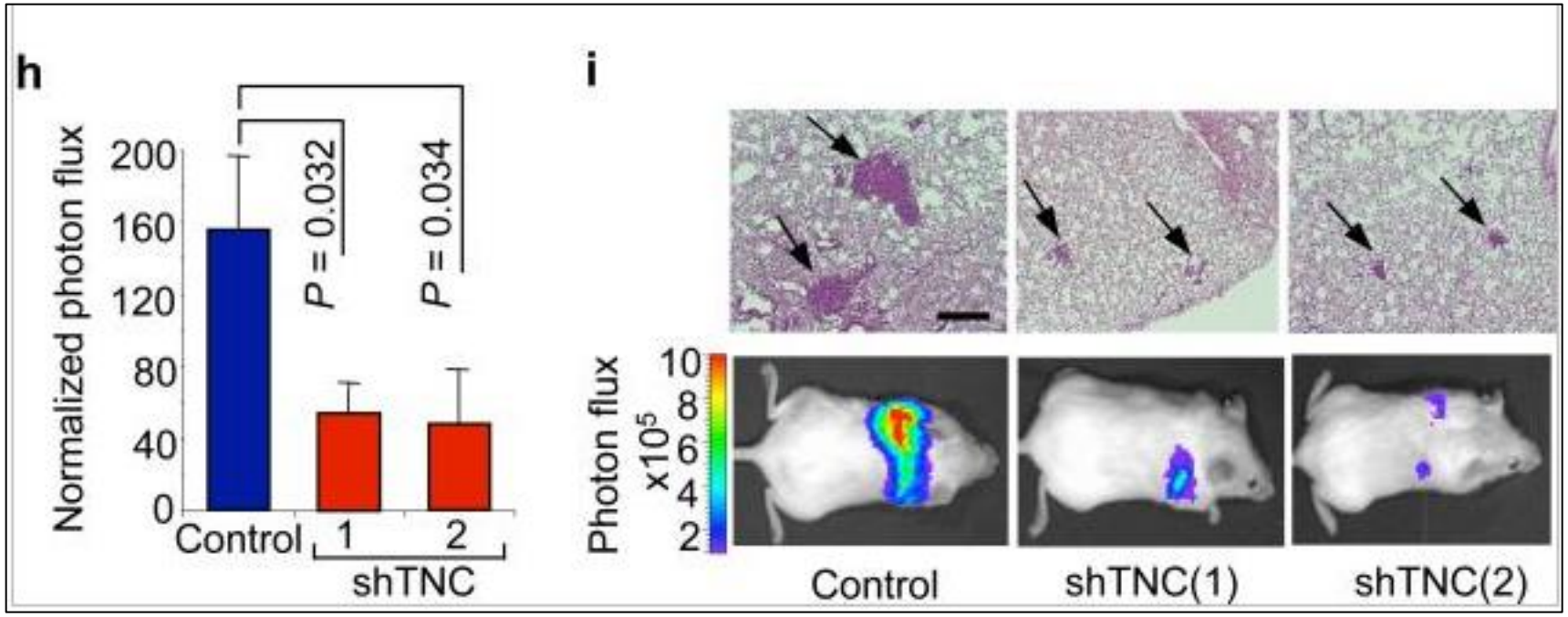
Extracellular matrix
Mesh of secreted proteins, proteoglycans, surrounding most cells within tissues

Tenascin C at the invasive edge of tumors

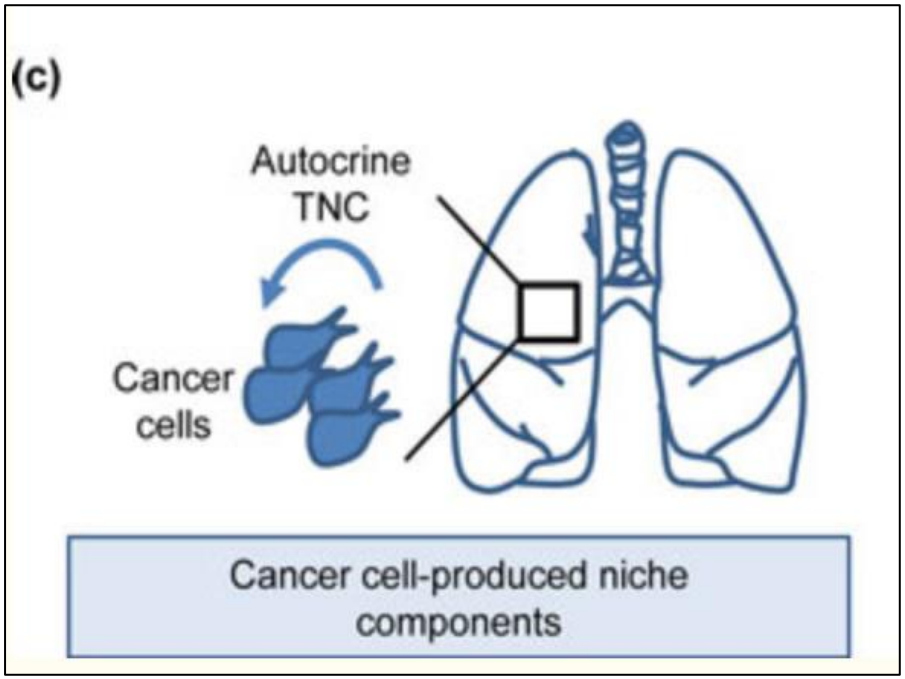


Ref: Oskarsson et al., Nature Medicine, 2011

Depleting Tenascin-C in cancer cells reduces lung metastasis



TNC: Cancer cell secreted-ECM molecule turns on stem cell program

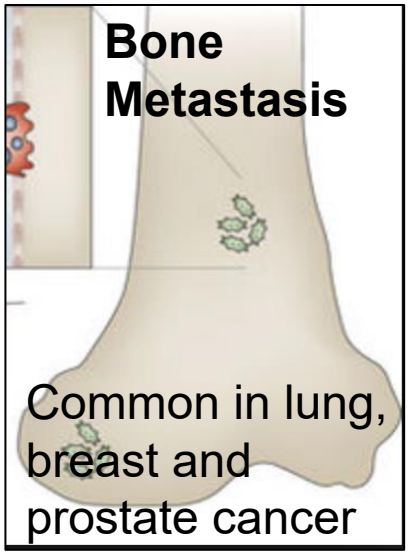


TNC interaction with cancer cells at the invasive front enhances **NOTCH and WNT** signaling

↓

Enhances fitness of metastasis-initiating cells

Bone colonization traits



The biology of bone metastases

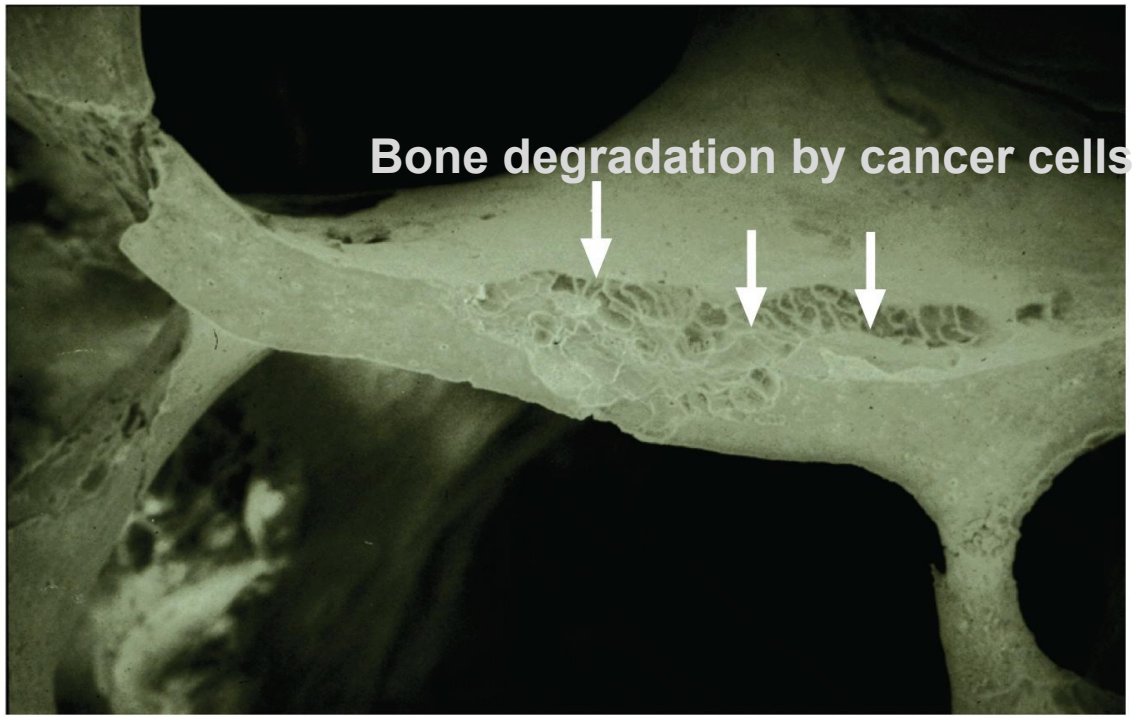


Figure 14.46d The Biology of Cancer (© Garland Science 2014)

Normal turnover of 10% of our skeletal bone mass.

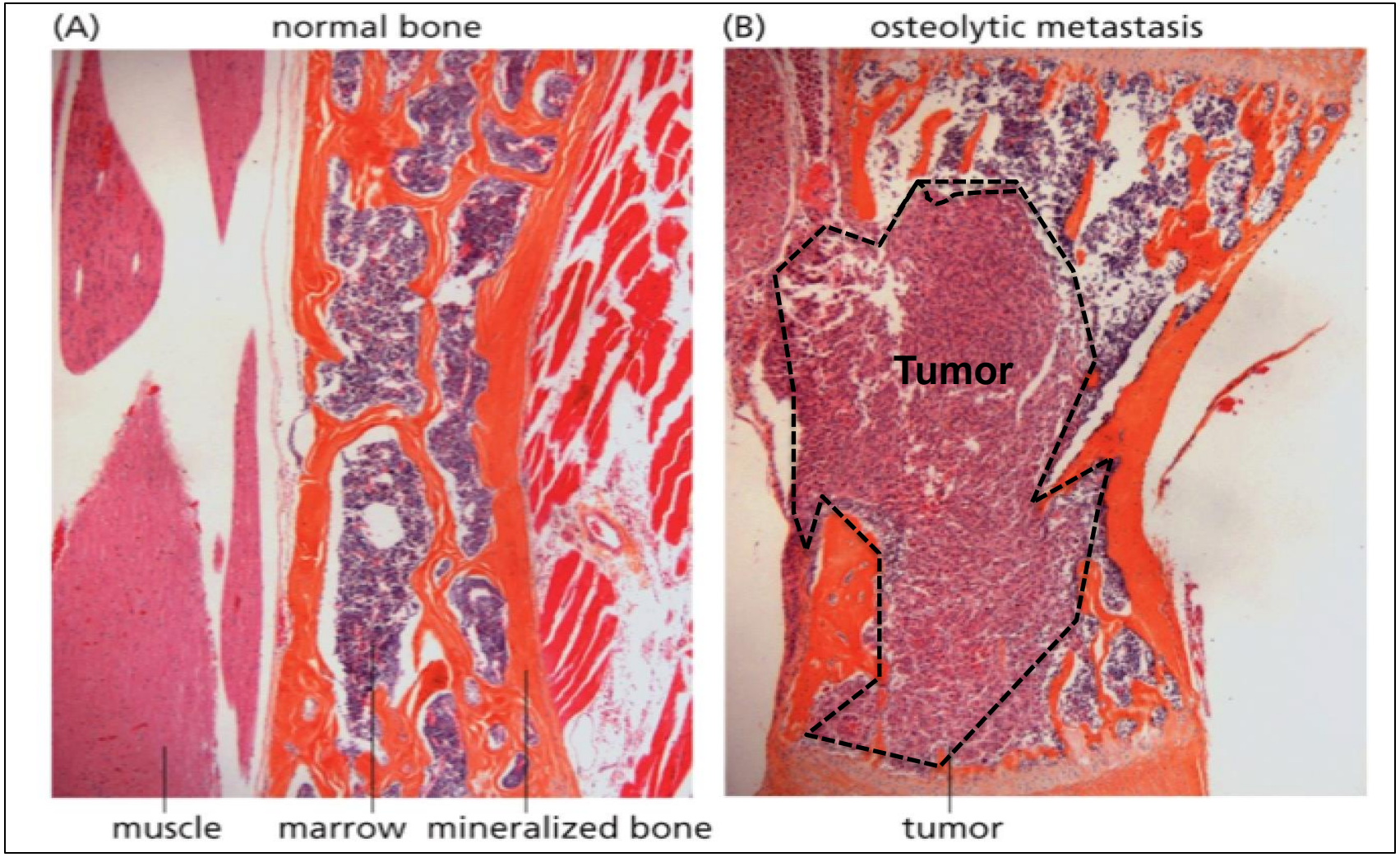
Bone turnover is coordinated by the action of

(1) osteoclasts which break down mineralized bone. These cells demineralize the bone by dissolving its calcium phosphate crystals and then degrade the now-exposed extracellular matrix, a process known as resorption (secretes protons to dissolve the mineral components and acid proteases to degrade collagen-rich extracellular matrix).

(2) Reconstruction by osteoblasts. These cells assemble new ECM, deposit calcium phosphate crystals in the matrix.

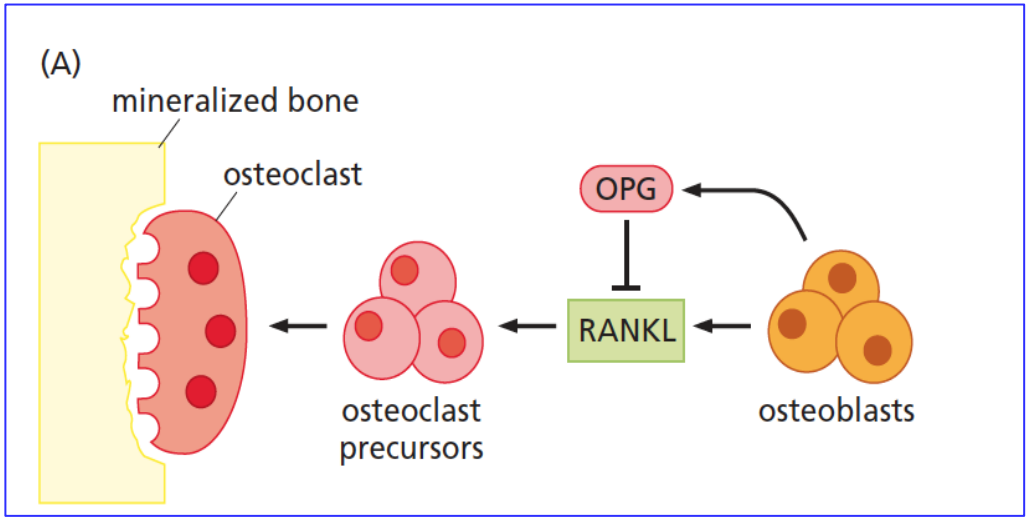
See Fig. 14.46
The Biology of Cancer

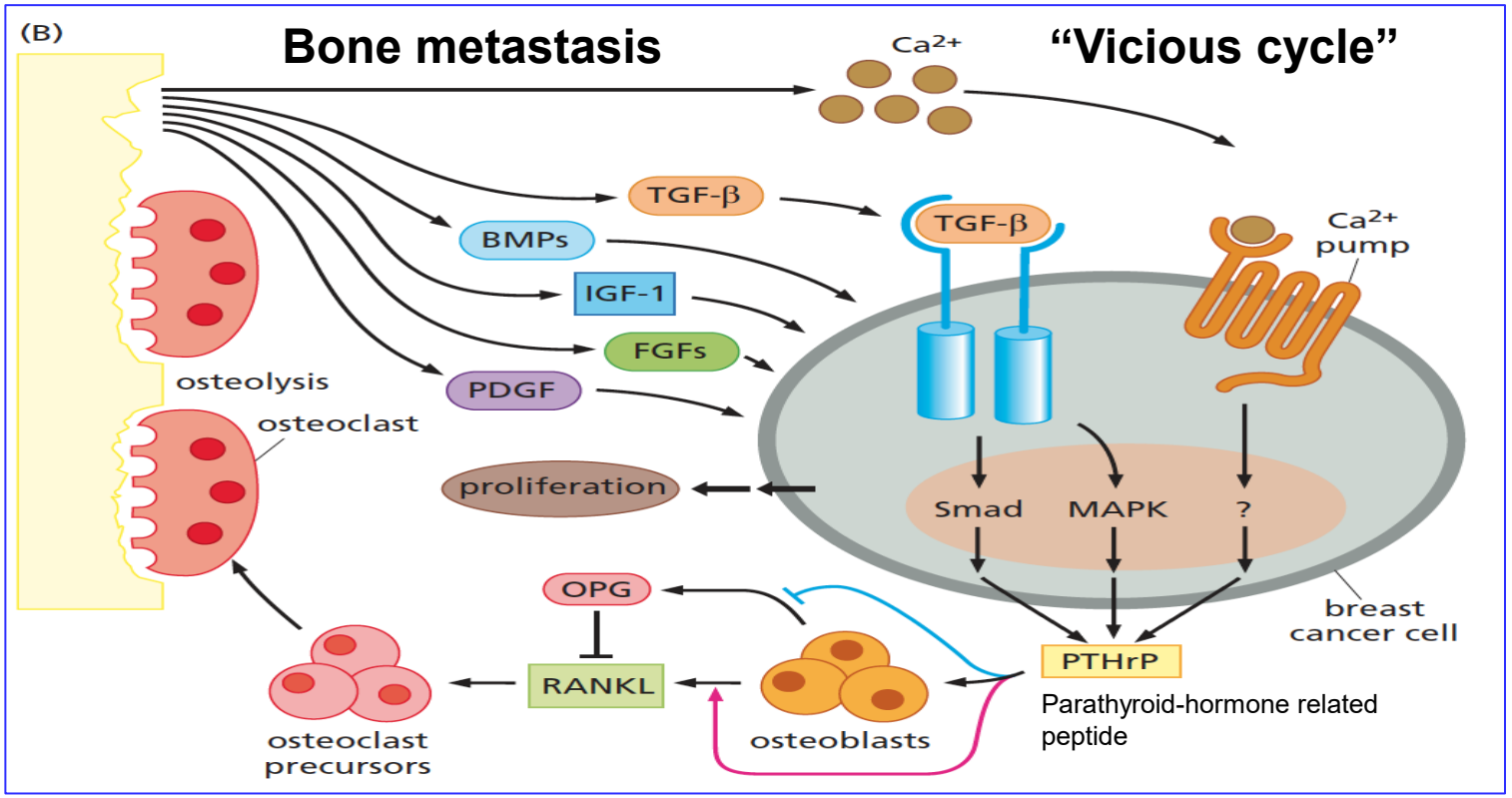
Osteolytic bone metastases = Metastases that can dissolve bone e.g. breast cancer



Cancer cells can activate osteoclasts and start this process

See Fig. 14.47
The Biology of Cancer


(A)
mineralized bone
osteoclast
OPG
RANKL
osteoclast precursors
osteoblasts

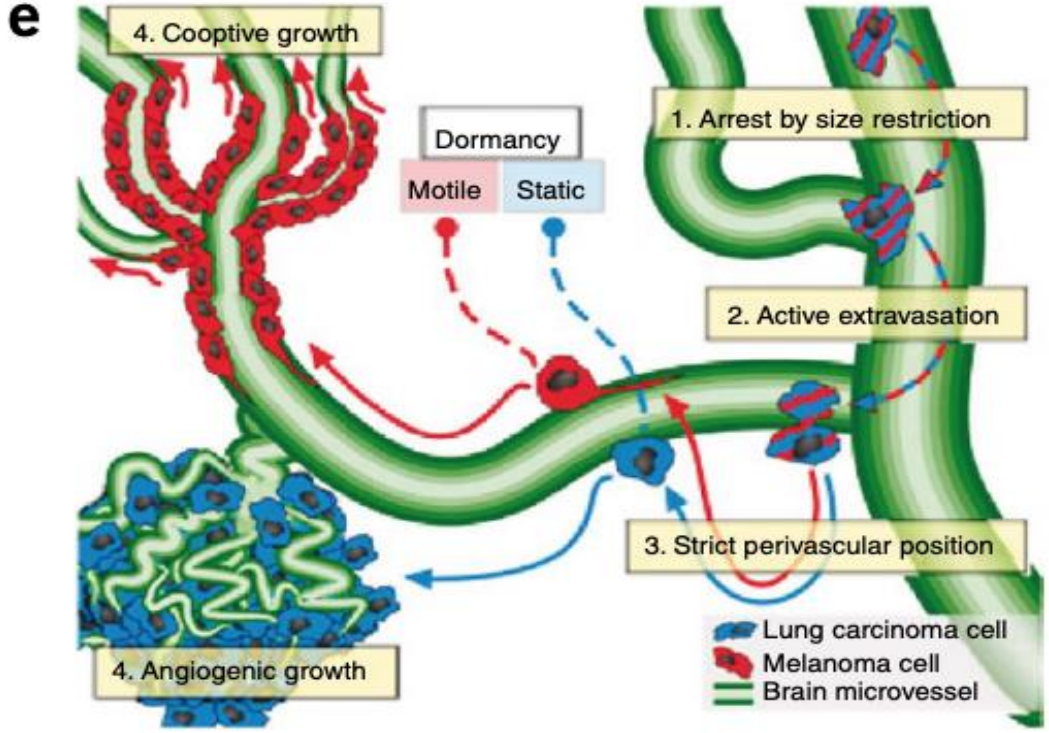

(B)
Bone metastasis
"Vicious cycle"
Ca2+
TGF-β
BMPs
IGF-1
FGFs
PDGF
TGF-β
Ca2+ pump
osteolysis
osteoclast
proliferation
Smad
MAPK
?
breast cancer cell
OPG
RANKL
PTHrP
Parathyroid-hormone related peptide
osteoclast precursors
osteoblasts

Brain colonization traits

Real-time imaging reveals the single steps of brain metastasis formation

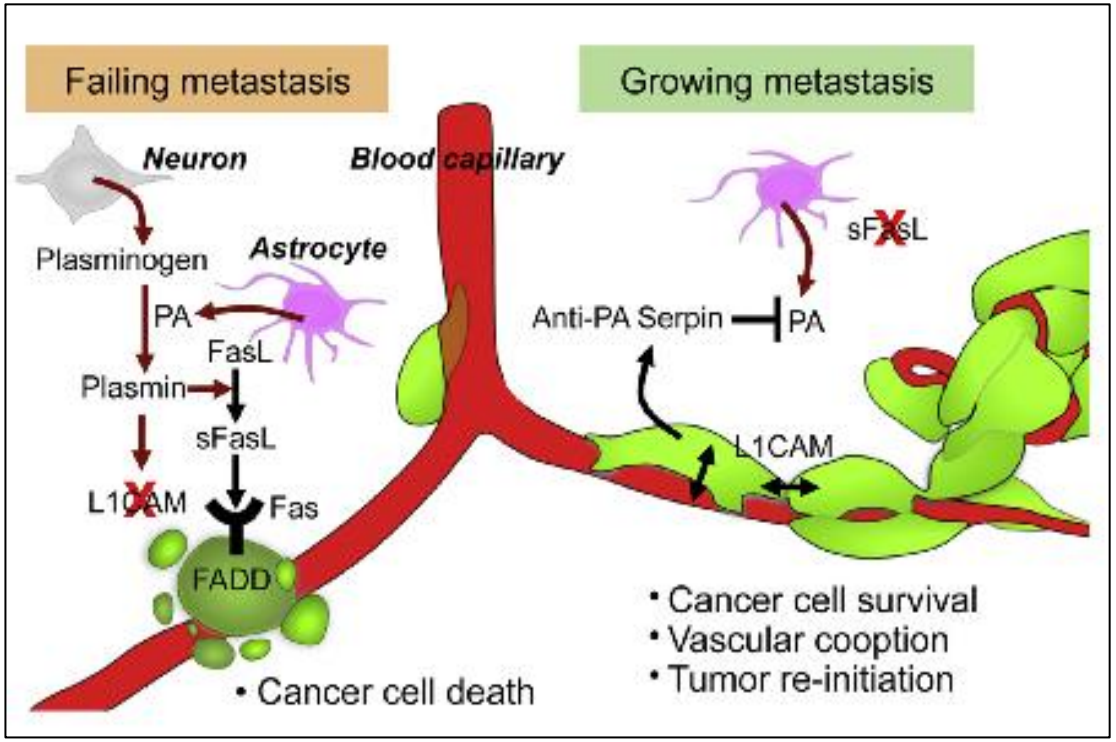
Yvonne Kienast[1,2], Louisa von Baumgarten[1], Martin Fuhrmann[2], Wolfgang E F Klinkert[4], Roland Goldbrunner[3], Jochen Herms[2,5] & Frank Winkler[1,5]

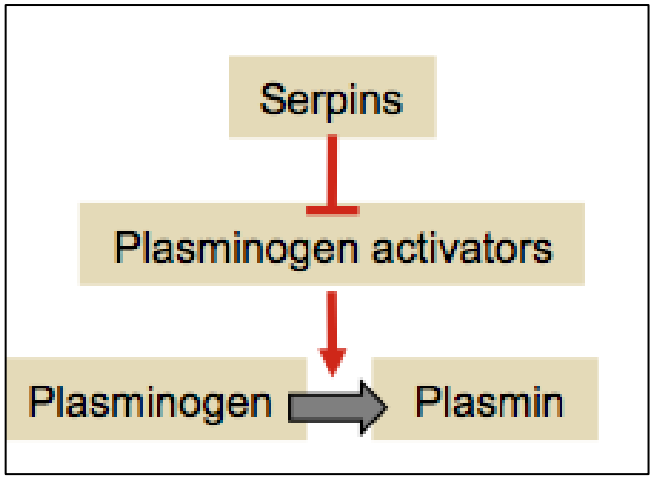
Cancer cells migrate along the blood vessels (**melanoma**)



New blood vessel formation (**lung cancer**)

Serpin-shield protects brain metastatic cancer cells during colonization





Neurons and cancer cells secrete serpins to protect themselves from Plasminogen-plasmin-soluble-Fas-Ligand induced killing.

Astrocytes- source of both Plasminogen activator and FasL. Plasmin makes membrane-FasL → soluble FasL.

Plasminogen-activators (PA) from reactive astrocytes activates cell death (Fas) in invaders
Neurons express neuro-serpins (PA inhibitors)
Cancer cells successfully colonizing in the brain secrete a form of serpins that protect them from killing

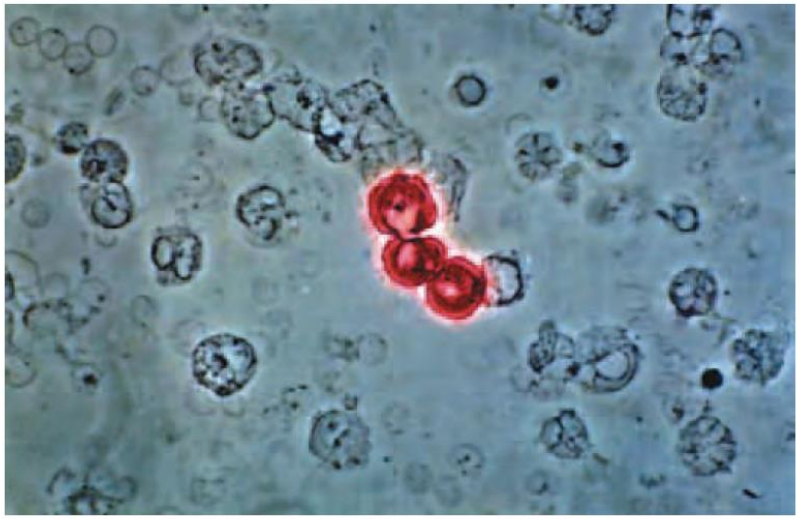
Valiente et al., Cell, 2014

Overview of the lecture

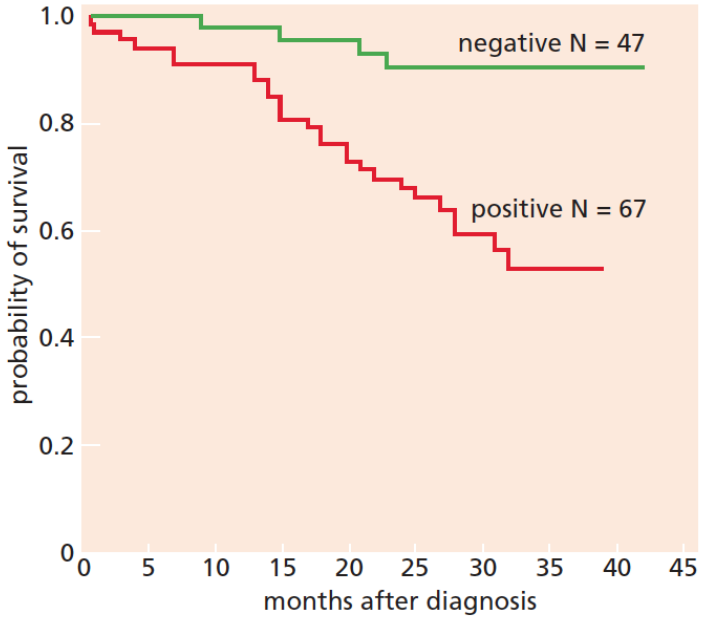
- Introduction to the metastasis cascade
- Historical perspective and foundation of metastasis research
- Studying the journey of a cancer cell from primary tumor to distant sites
- **Challenges and therapeutic avenues for targeting metastasis**

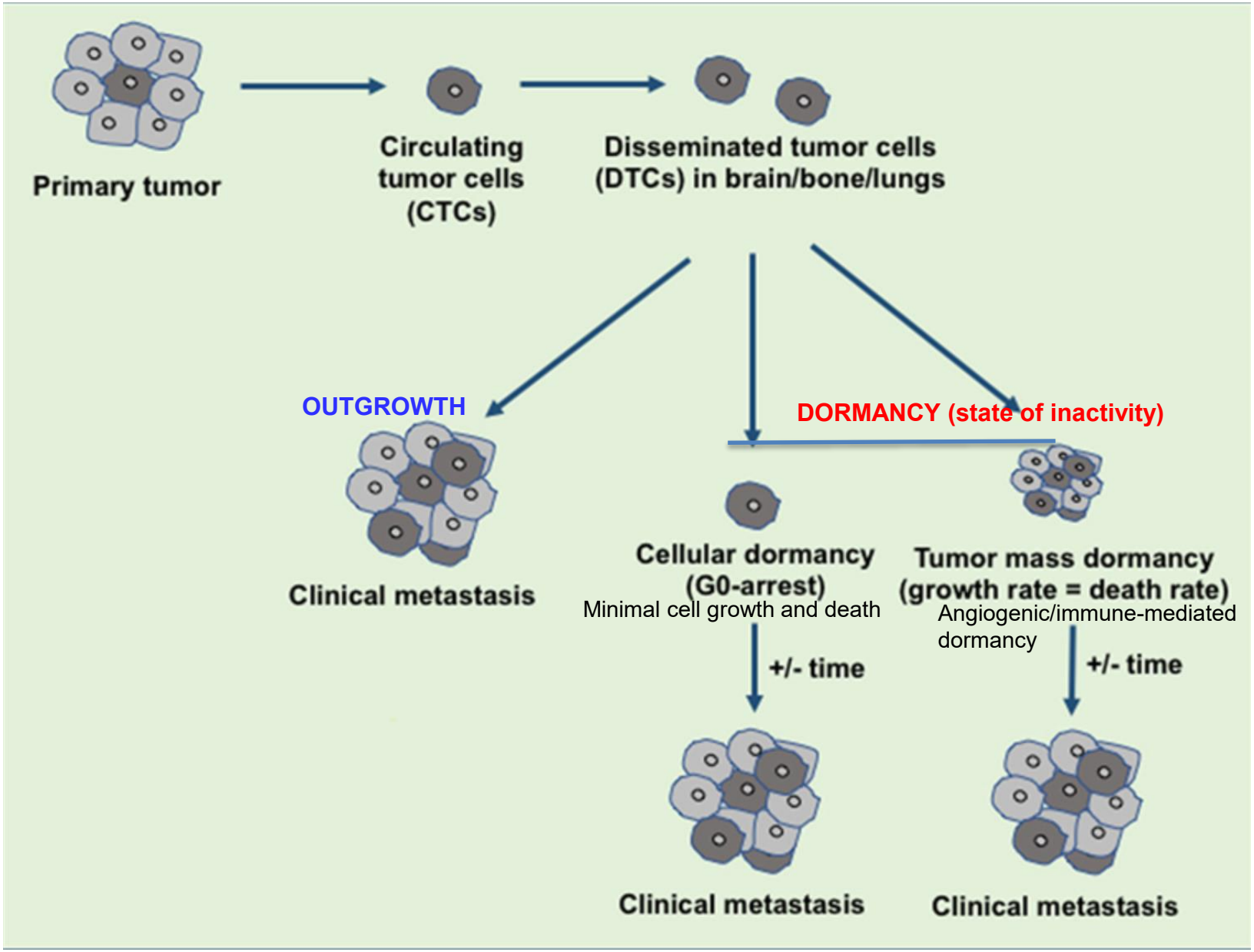
Disseminated cancer cells in human bone marrow: seeds of relapse?

(A)



(D)





https://www.frontiersin.org/articles/10.3389/fonc.2018.00072/full

Targeting the metastatic cascade

Treatments that reduces risk of metastatic relapse

Hormone receptor (ER)-positive breast cancers ---> treated with ER antagonist (Tamoxifen) for 5 years → risk of relapse halved; for 10 years → further reduced (ATLAS trial)

Adjuvant chemotherapy round 2 → for breast cancer patients with disseminated cancer cells (DTCs) in BM → 79% showed DTC elimination and prolonged survival.

Targeting bone metastasis (block osteoclast function or activation) → Reduces skeletal related effects. Ongoing trials to test the magnitude of survival benefits.

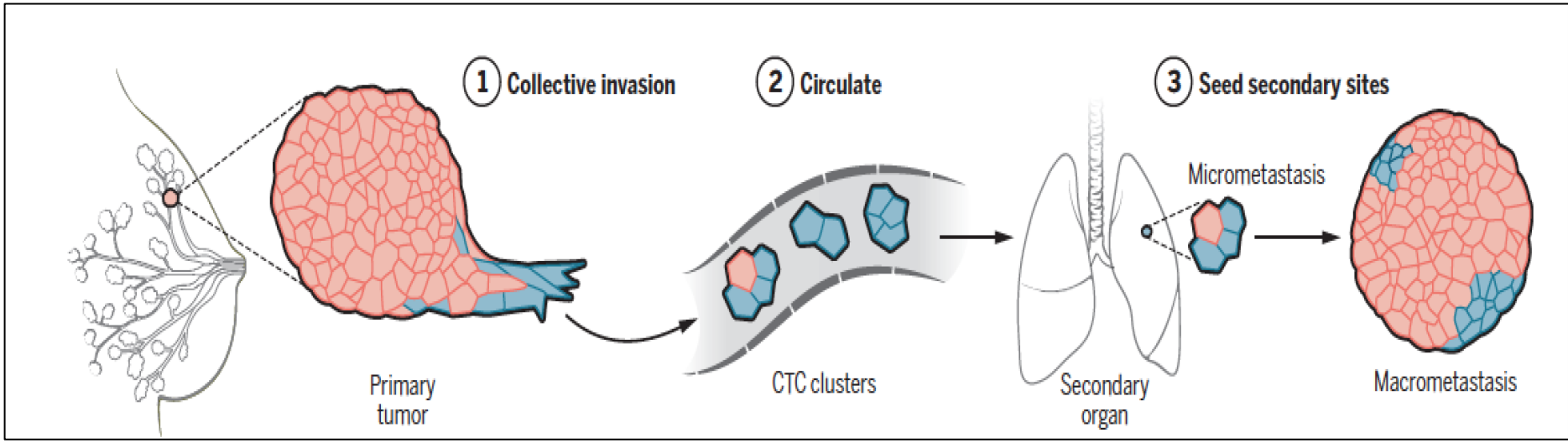
Targeted therapies for brain metastasis for lung cancer has been successful, although are not completely durable.

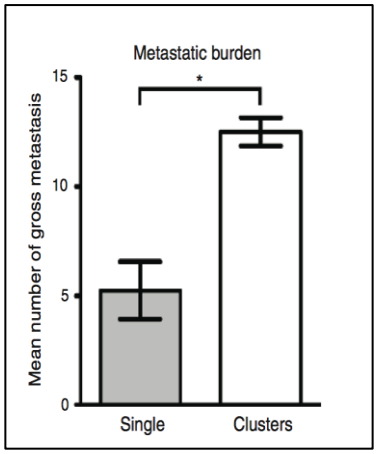
Provocative questions in metastasis research today

Q1. How do cancer cells travel in circulation? Single cancer cells or in clusters?

Collective invasion of tumor cell clusters

Examples from breast and pancreatic cancer models



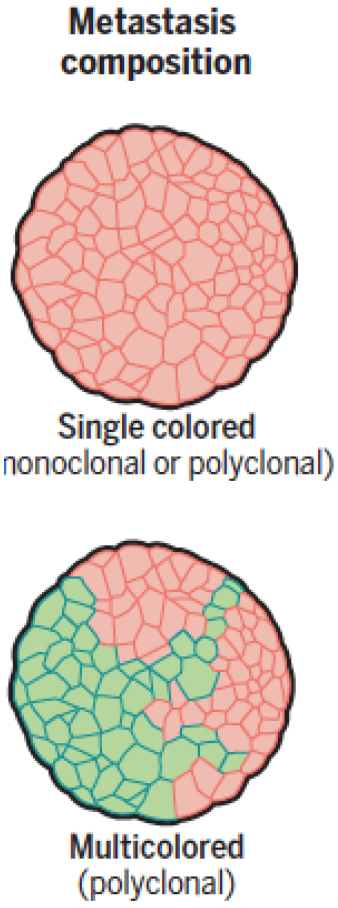


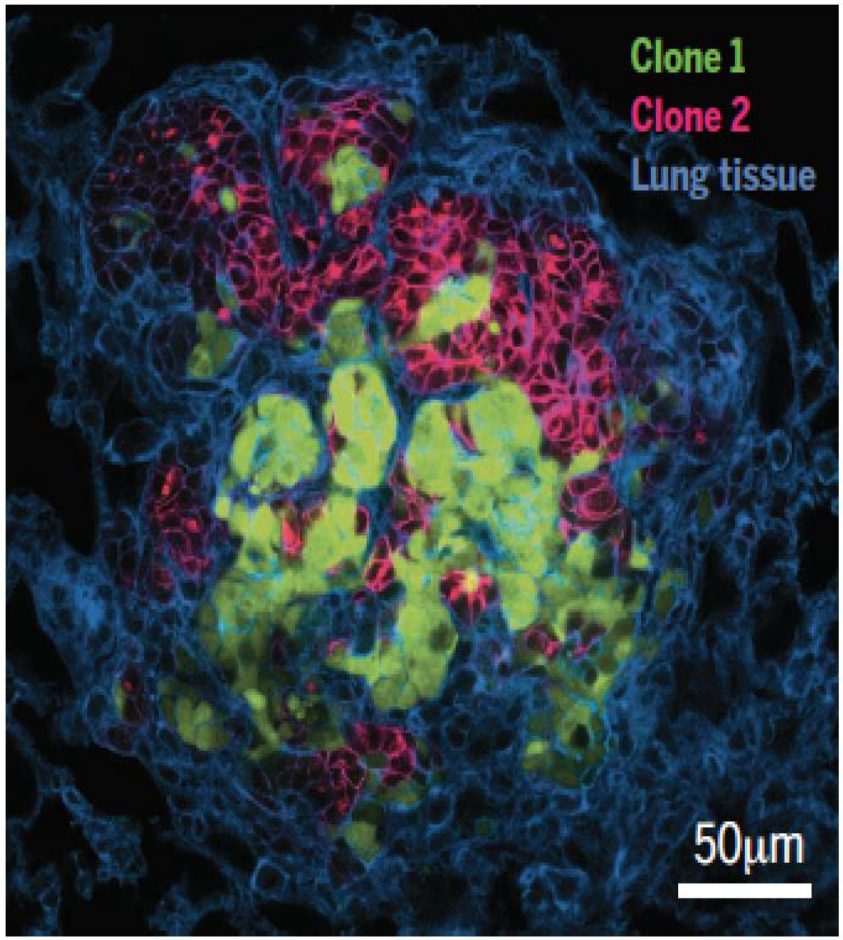
PMID: 26209539

Q2. Seeding of metastasis: monoclonal or polyclonal?

Clone 1 Tumor cells

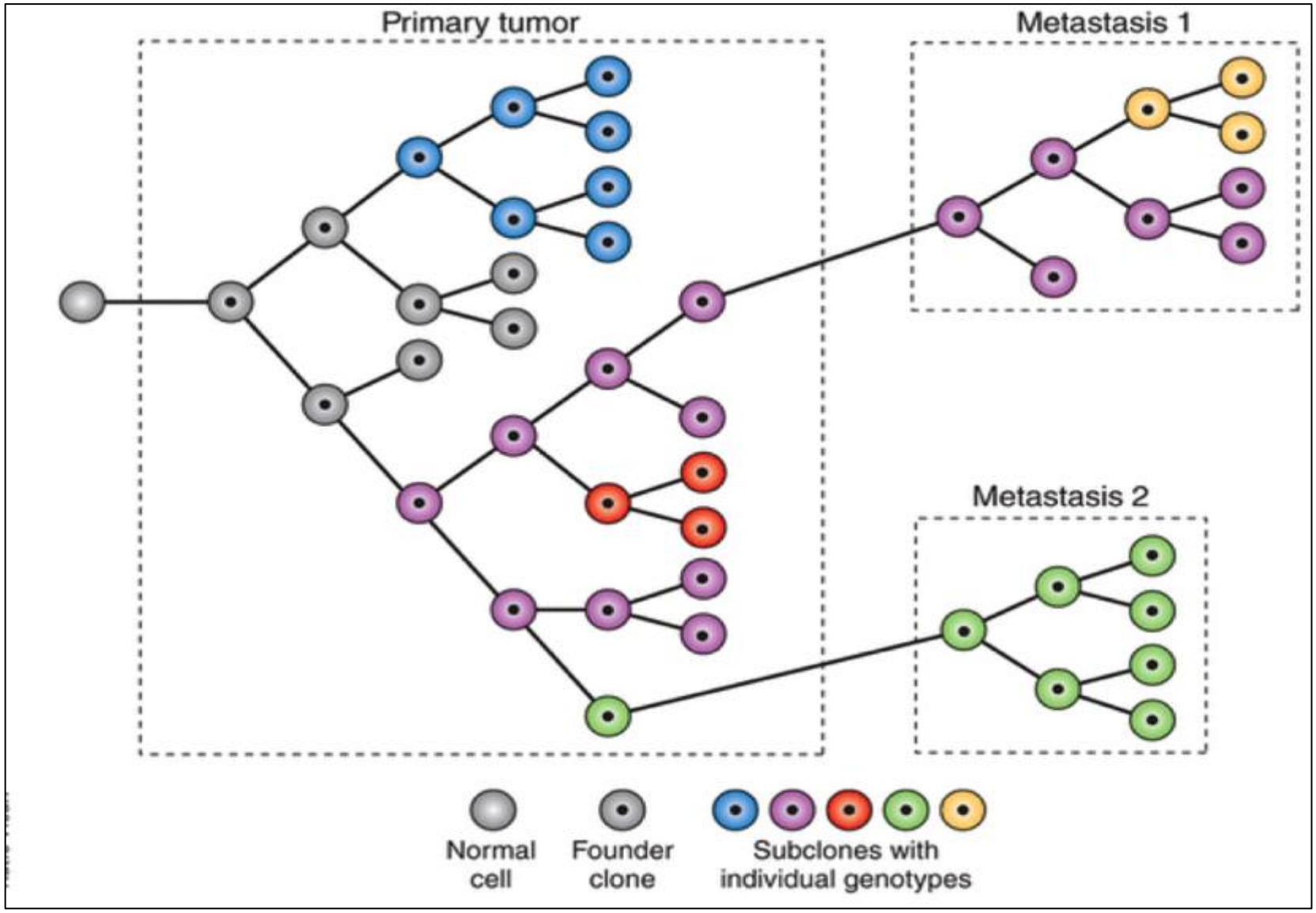
Clone 2 Tumor cells





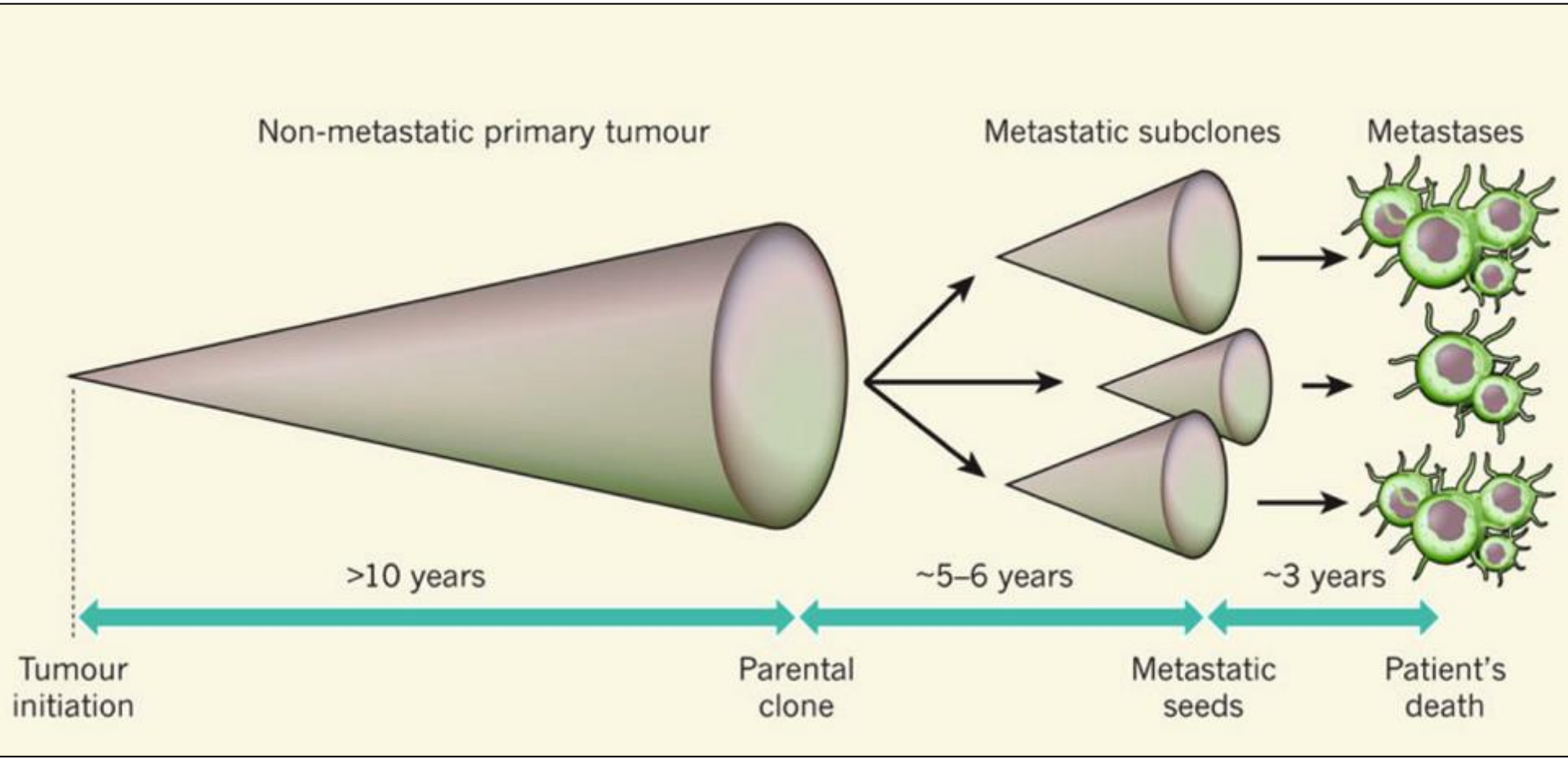
Lineage tracing studies by Andrew Ewald's group
Reviewed in Science, 352, 6282, 2016

Q3. When do cancer cells disseminate- stepwise evolution vs early dissemination?



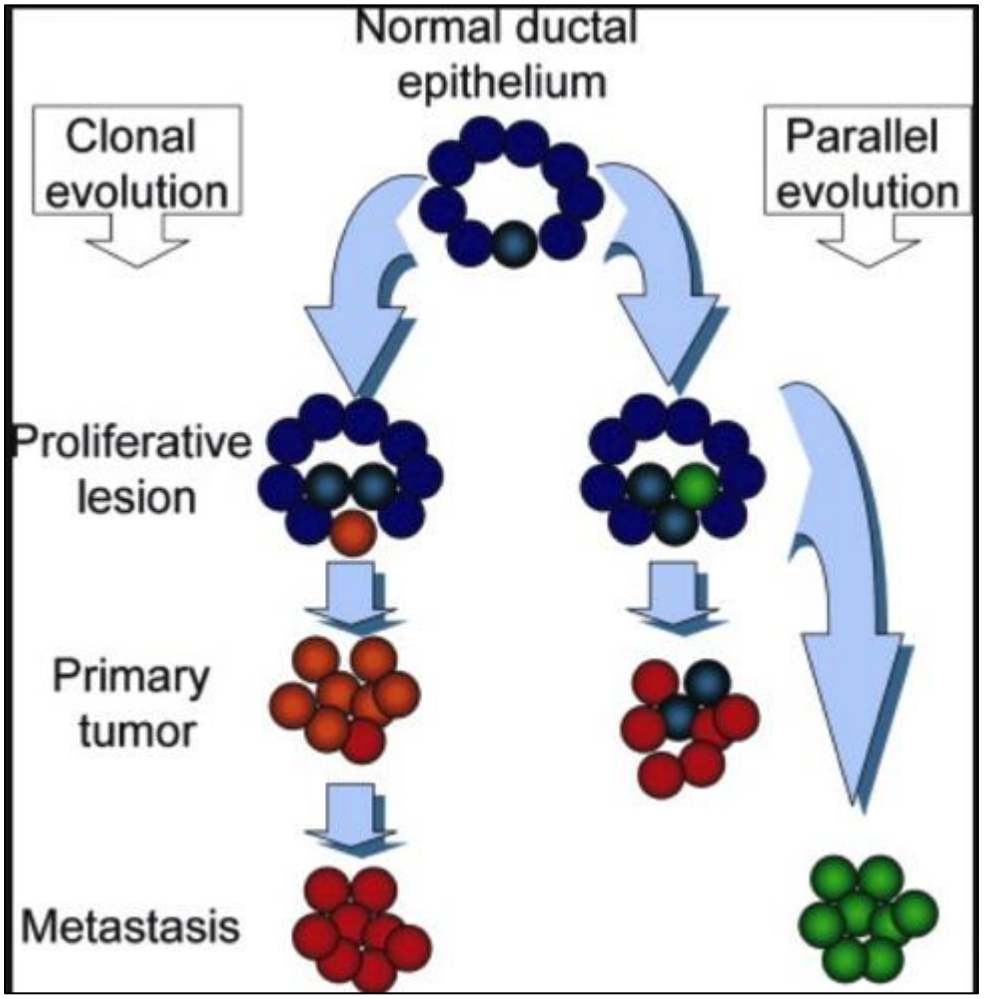
Clonal Evolution of heterogeneous primary tumors giving rise to metastasis

Stepwise evolution



Luebeck, Nature, 2010

Alternative hypothesis: Parallel evolution of metastasis (Christoph Klein)



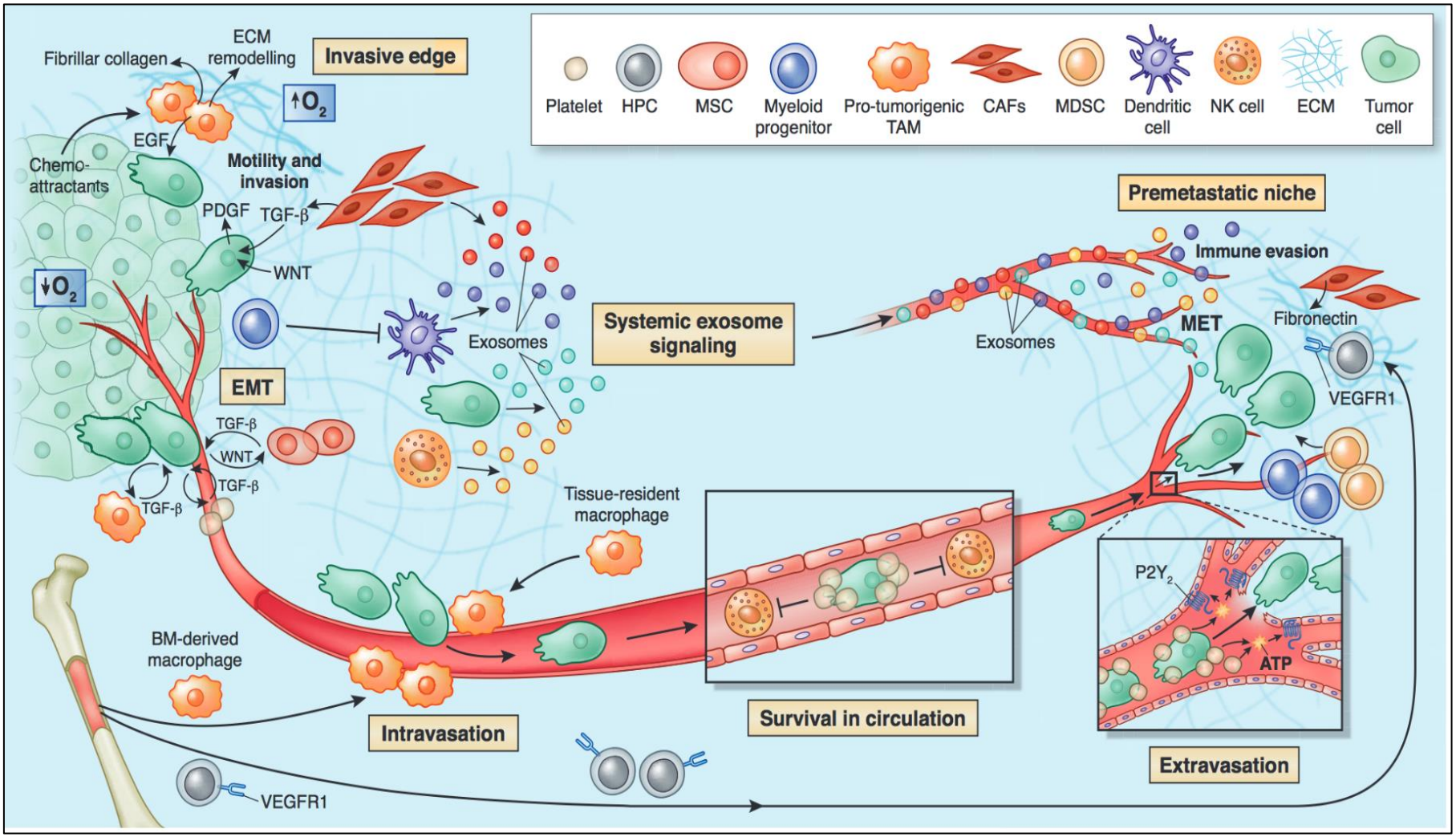
Bone marrow cancer cells genomically different from primary tumor mix

Spread early and evolve

Clinically- optimal to decide treatments based on DTCs or primary tumors?

Reference: Gray, Cancer Cell, 2003

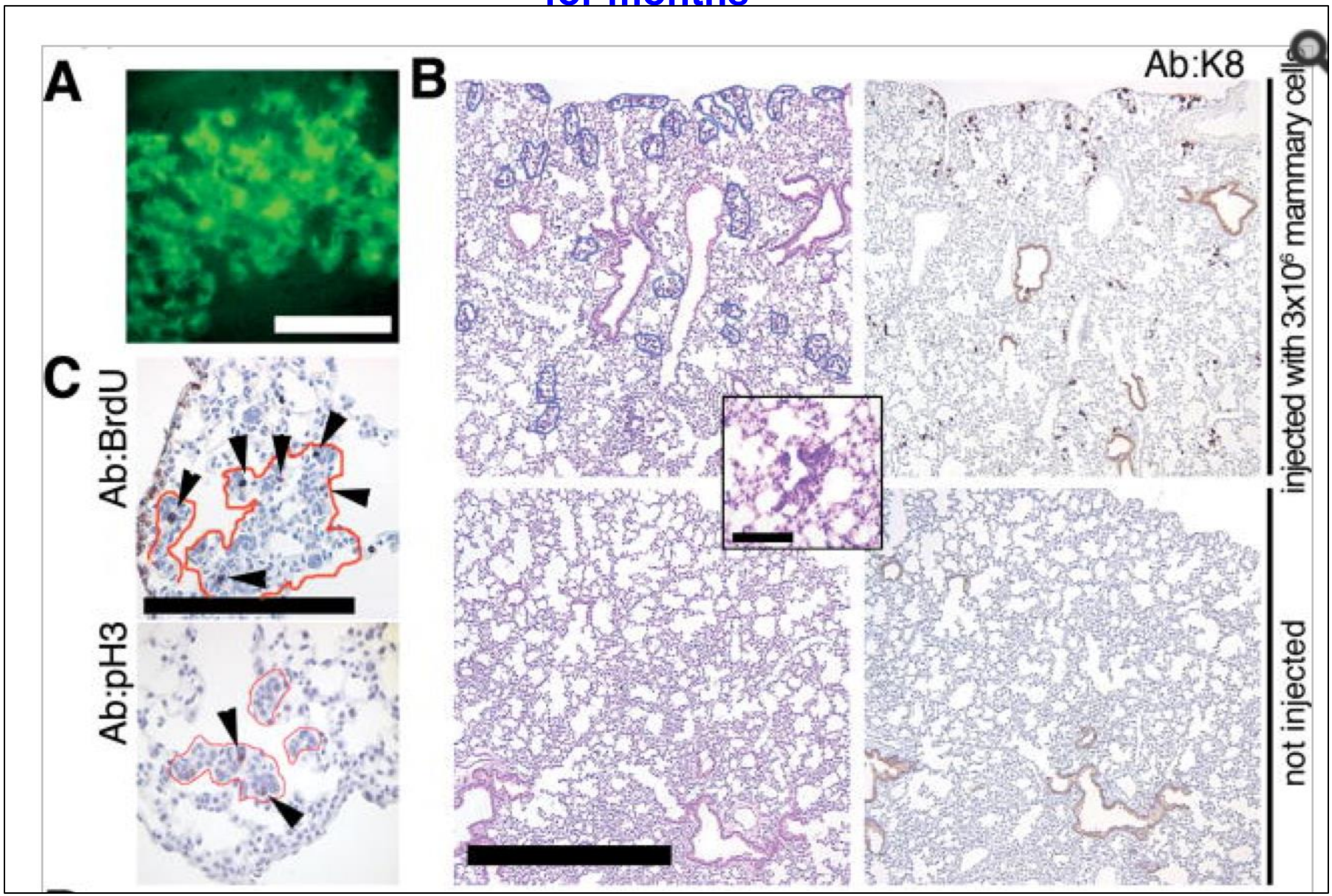
Can normal cells from one organ colonize other organs?



Normal GFP-labeled untransformed
mammary epithelial cells injected in circulation

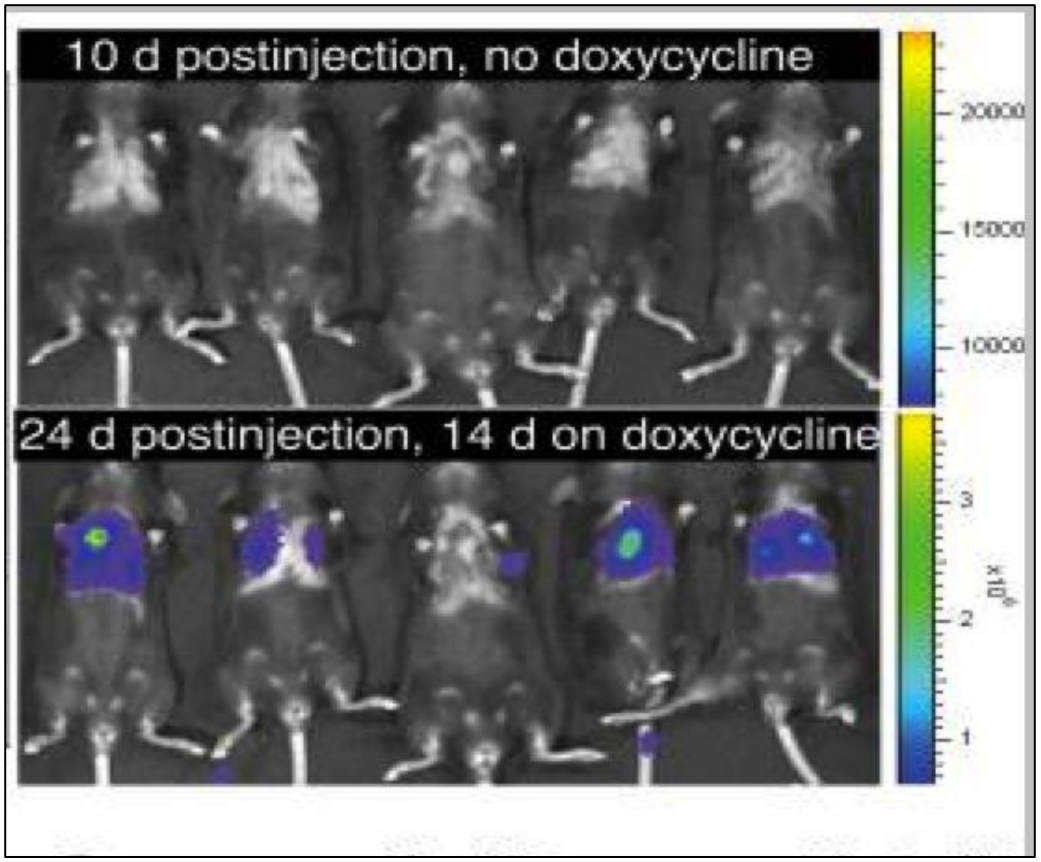
Quail and Joyce, Nat Med, 2013

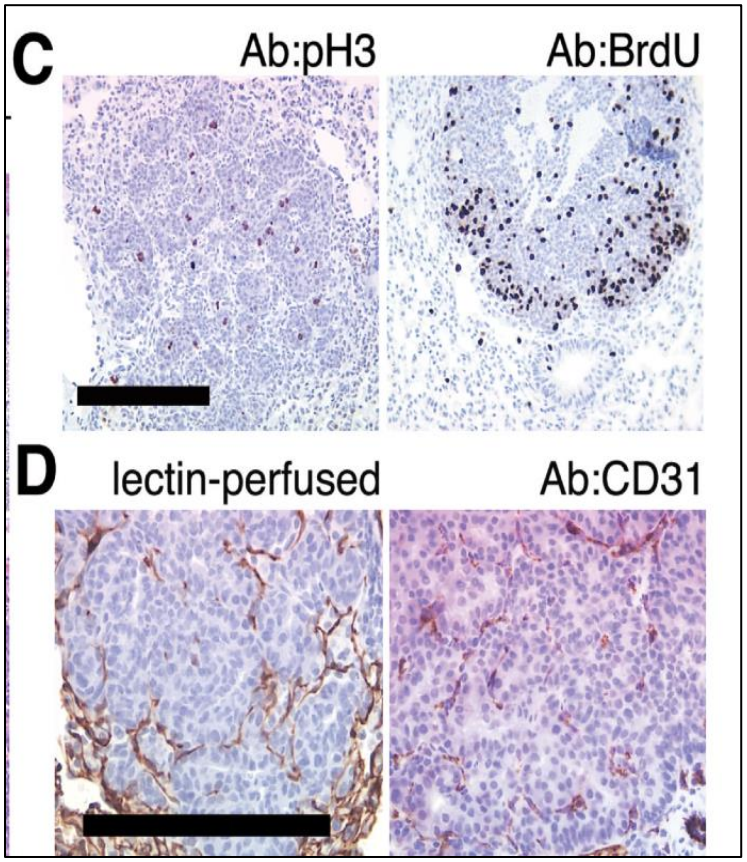
Phenotypically normal untransformed cells can persist in the lungs for months



Podsypanina et al., Science, 2008

Delayed oncogene induction still forms tumors





When normal mammary cells have reached the distant site in the absence of transformation at the primary site.

Questions?