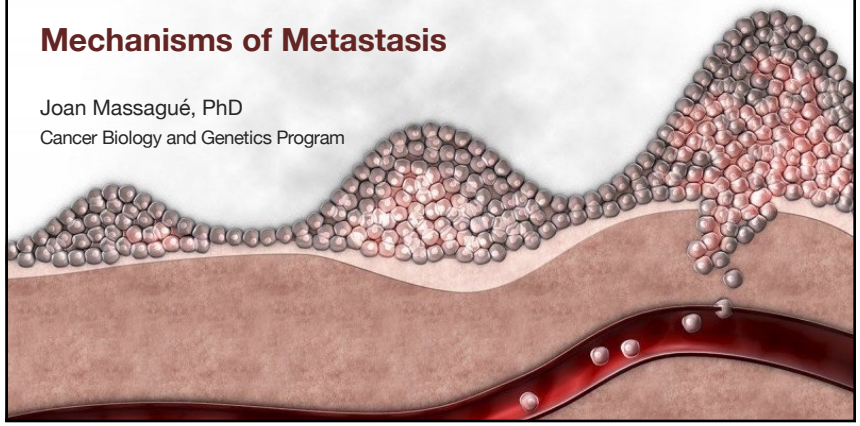
GSK Course Lecture, April 2023

Mechanisms of Metastasis

Joan Massagué, PhD
Cancer Biology and Genetics Program



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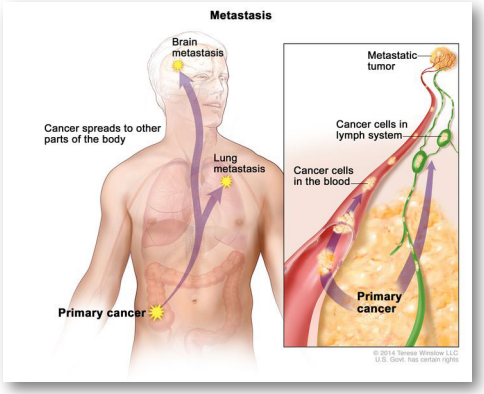
Mechanisms of Metastasis

- **Metastasis as a medical problem**
- Phases of the metastatic process
  - Metastatic dissemination
  - Dormancy and immune evasion
  - Organ colonization and metastatic tropism
- Metastasis initiating cells
- Tumor evolution and metastatic progression
- Metastasis as a systemic disease
- Treating metastasis

2

Metastasis as a clinical problem

- Metastasis causes the vast majority of deaths from cancer
- Current treatments can inhibit but rarely cure metastasis.
- Limited predictive capacity to identify tumors that will metastasize
- Latency: metastasis may appear years after treatment of the primary tumor
- Understanding and targeting the basis for metastasis is a major goal of current research.



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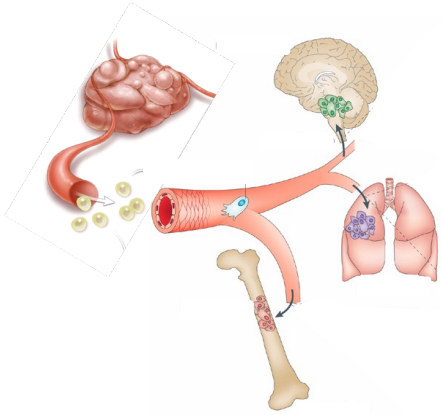
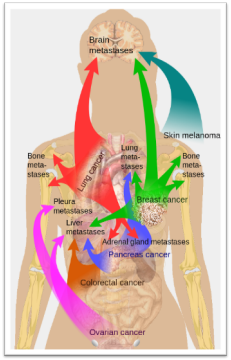
Each cancer has a stereotypical pattern of metastatic relapse

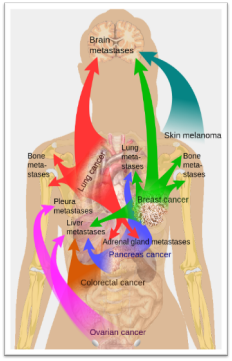




4

Why is it so difficult to cure metastasis?

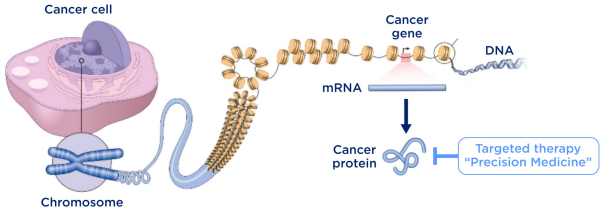
- High tumoral load (10^{12} cells)
- Tumor heterogeneity
- Host organ heterogeneity
- Rapid development of resistance to therapy



5

A turn of the century view

- Look for driver mutations in cancer genes
- Then develop drugs to target the gene products



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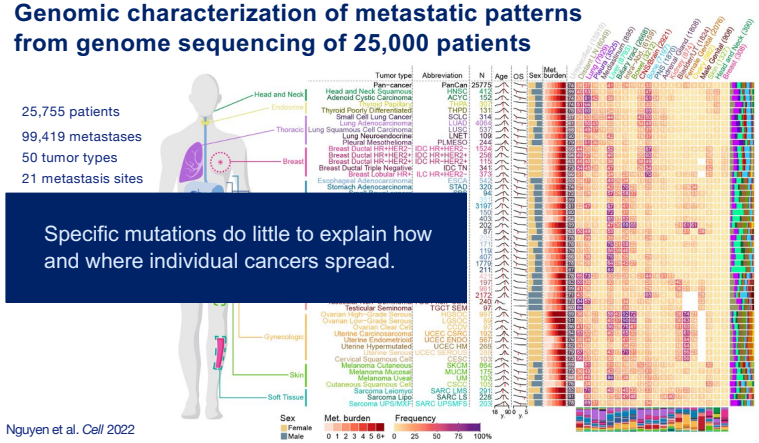
Genomic characterization of metastatic patterns from genome sequencing of 25,000 patients

25,755 patients
99,419 metastases
50 tumor types
21 metastasis sites

Specific mutations do little to explain how and where individual cancers spread.



Nguyen et al. *Cell* 2022

7

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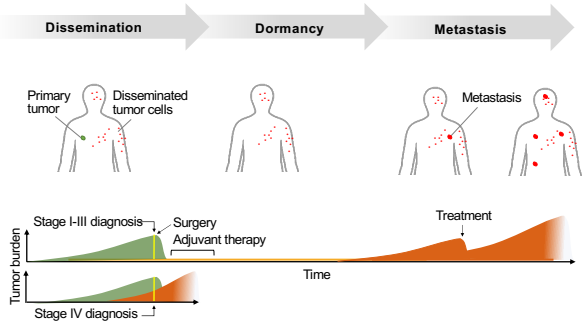
8

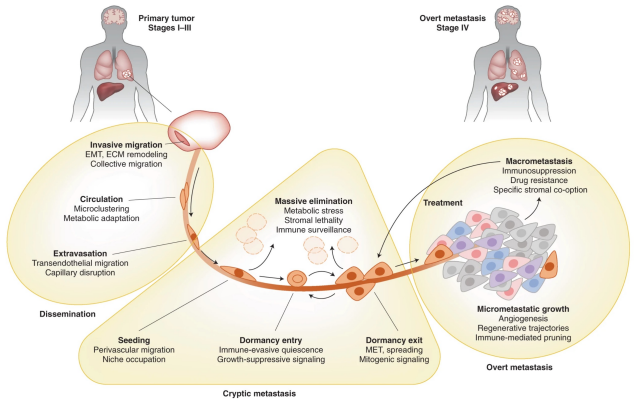
Phases of metastasis



Massagué & Ganesh *Cancer Disc.* 2021

9

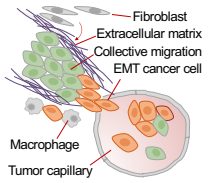
Metastasis steps and vulnerabilities



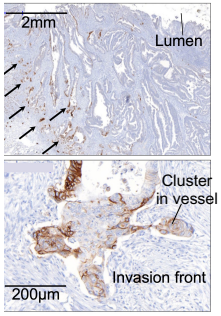
Ganesh & Massagué *Nat Med.* 2021

10

Metastatic dissemination: Intravasation



Primary colorectal cancer

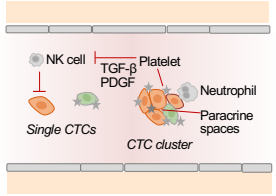


11

Metastatic dissemination: Circulation

Barriers
- Shear stress
- Oxidative stress
- NK cell attack

Adaptations
- MIC Clustering
- Metabolic adaptation
- Platelet coating
- Neutrophil interaction



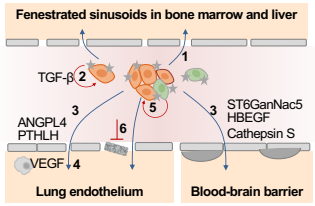
12

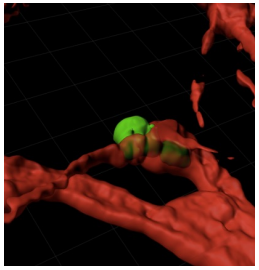
Metastatic dissemination: Extravasation

c. Extravasation

Barriers
- Endothelial junctions
- Shear stress

Adaptations
1. Fenestrated endothelia
2. Platelet TGF-β for EMT
3. CTC endothelial disjunction and motility factors
4. Monocyte endothelial permeability factors
5. Cooperation within clusters
6. Endothelial necroptosis

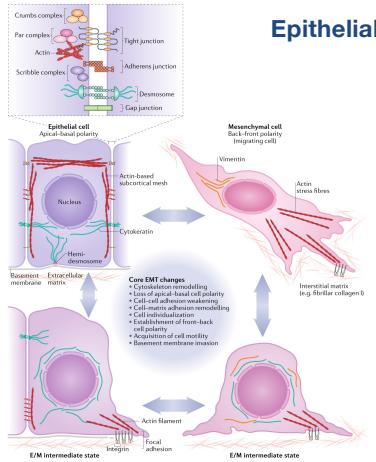




Lung cancer cells extravasating the the brain of a mouse.
Valiente et al *Cell* 2014

13

Epithelial-mesenchymal transitions (EMTs)



- Phenotypic plasticity processes in which epithelial cells lose apicobasal polarity and adhesion, and gain migration
- Key in gastrulation, morphogenesis, wound healing
- EMT promotes tumor invasion and metastasis.
- EMT is coupled with differentiation, apoptosis or fibrosis depending on context.
- TGF-β and other cytokines drive EMTs by inducing the expression of EMT transcription factors: Snail, Slug, ZEB1, ZEB2, Twist1, Twist2
- EMT TFs repress epithelial genes and enable the expression of mesenchymal genes.

Yang et al. *Nat Revs.* 2020

14

Metastatic dissemination: Alternative routes

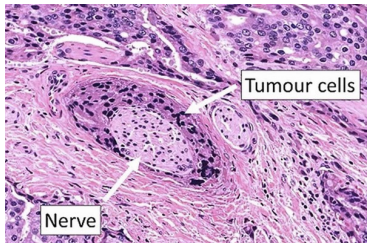
Perineural
(e.g. head & neck, pancreatic cancers)

Perivascular
(e.g. melanoma)

Lymphatics

Intraperitoneal
(e.g.ovarian cancer)

Spread through air
spaces (e.g. lung cancer)

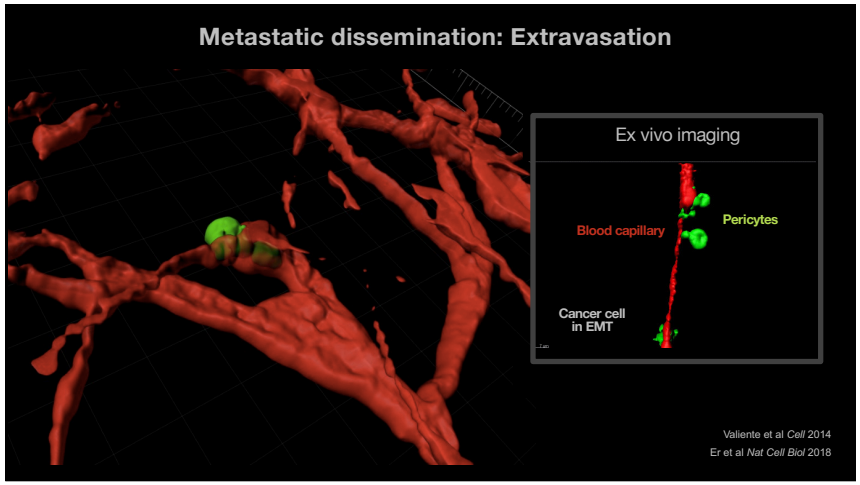


15

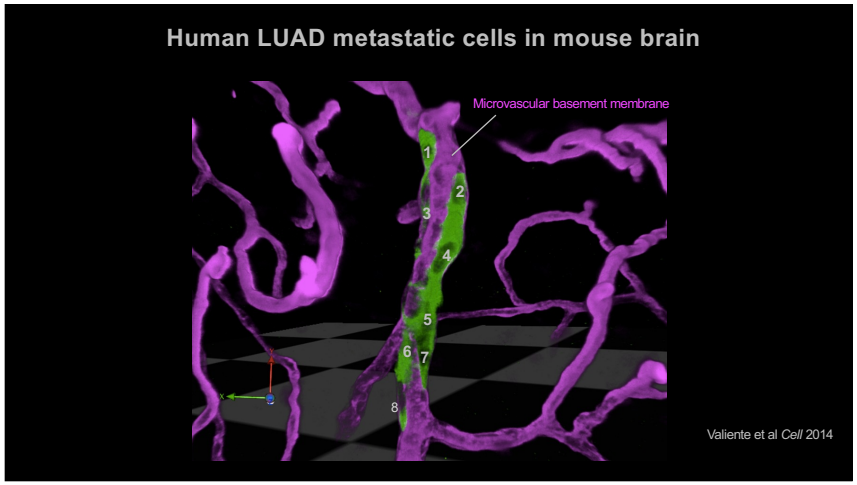
Metastatic colonization

- How does metastatic colonization start?
- How does it enter and exit dormancy?
- How does it evade immune surveillance?
- How does it adapt to different organs?
- How can it be prevented and treated?

16



17



18

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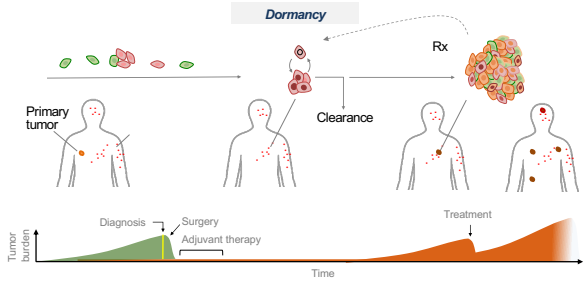
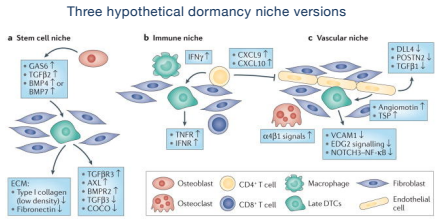
19



20

Dormancy: Growth inhibitory niches

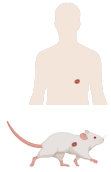
- Growth suppressive signals inhibit the proliferation of disseminated cancer cells.
- Growth supportive signals reawaken dormant cancer cells at some point for progression to overt metastasis.



Sosa et al. *Nat Rev, Cancer* 2014

21

Immune surveillance prevents exit from dormancy

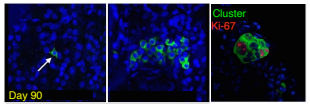


Dormancy models

H2030-LCC model
Human stage-I LUAD
(athymic host)

KPad1 model
Adenoma stage LUAD
(immunocompetent host)

H2087-LCC model
human stage I Ras-mutant LUAD



Properties of dormant metastasis cells

- Early-stage regenerative progenitors (SOX2+)
- Occupy perivascular niches
- Enter quiescence in response to TGF-β, WNT inhibitors
- Immune evasive during quiescence
- Proliferative clusters regress; infrequent outbreaks
- Progression upon depletion of NK and T cells

Malladi et al., *Cell* 2016
Laughney et al *Nature Genet.* 2020
Hu et al *Nature* 2023

22

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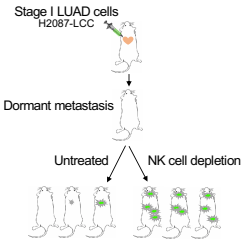

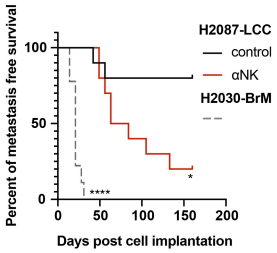

Malladi et al., *Cell.* 2016

23

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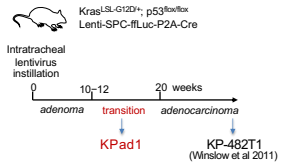
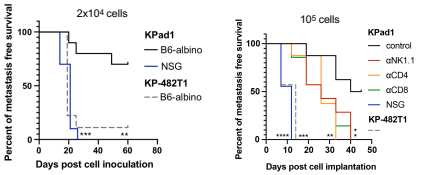
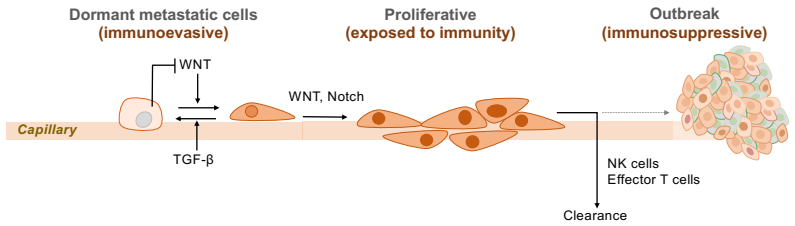




Hu et al *Nature* 2023

24

Immune surveillance prevents exit from dormancy



25

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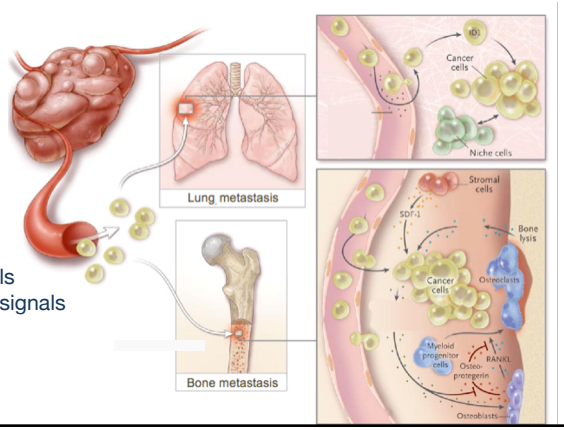
26

Each organ presents distinct determinants of metastatic colonization

Barriers:
- Vascular walls
- Resident immunity
- Metabolic stress

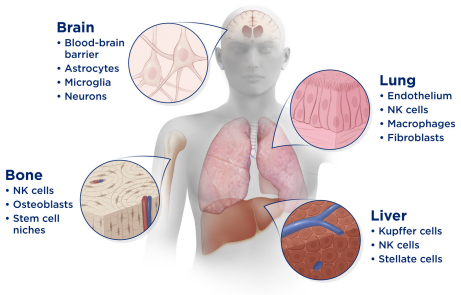
Opportunities:
- Coopted stromal cells
- Microenvironmental signals



27

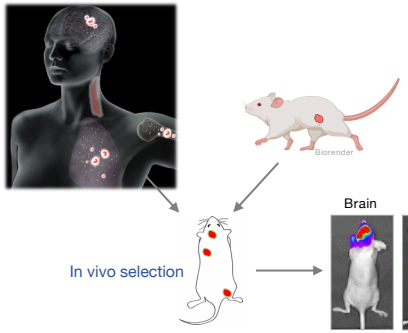
Metastasis and local tissue ecosystems

- A highly inefficient process under strong selective pressures.
- Different organs – different barriers and opportunities for metastasis
- Metastatic cells adapt to different degrees in different organs depending on the tumor type.



28

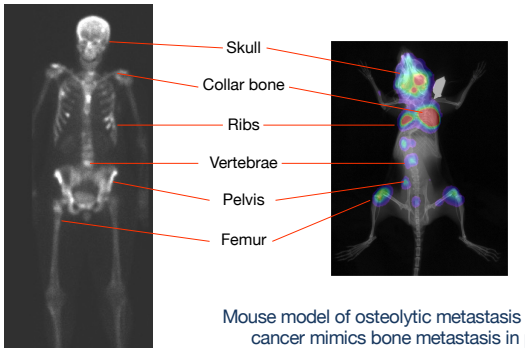
Models to study organ selective metastasis



1. Comparative omics
2. Candidate genes
3. Functional validation
4. Clinical validation

29

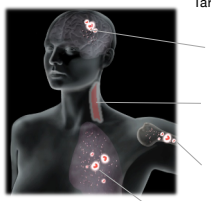
Models to study organ selective metastasis



Mouse model of osteolytic metastasis from breast cancer mimics bone metastasis in patients

30

Mediators of organ-selective metastasis



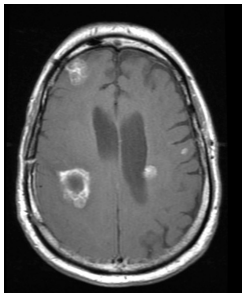
Target organ	Extravasation	Seeding	Colonization
Brain	COX2 MMP1 HB-EGF ST6GALNAC5	SERPINs L1CAM	PCDH7 Conexin 43
Meninges			Complement C3
Bone		SRC CXCR4 CXCL1 L1CAM	IL11 MMP1 Osteopontin Jagged 1 CXCR4
Lung	ANGPTL4 Fascin1 COX2 MMP1 Epiregulin	Tenascin C CXCL1/2 L1CAM miR-216* miR-335*	VCAM-1

Kang et al *Cancer Cell* 2003
Minn et al *Nature* 2005
Gupta et al *Nature* 2007
Tavazoie et al *Nature* 2008
Padua et al *Cell* 2008
Zhang et al *Cancer Cell* 2009
Kim et al *Cell* 2009
Bos et al, *Nature* 2009
Nguyen et al *Cell* 2009
Oskarsson et al *Nat. Med.* 2011
Chen et al *Cancer Cell* 2011
Acharyya et al *Cell* 2012
Vanharanta *Nat. Med.* 2013
Zhang et al *Cell* 2013
Valiente et al *Cell* 2014
Obenauf et al *Nature* 2015
Malladi et al *Cell* 2016
Chen et al *Nature* 2016
Boire et al *Cell* 2017
Er et al *Nat Cell Biol* 2018

31

Example: Brain metastasis

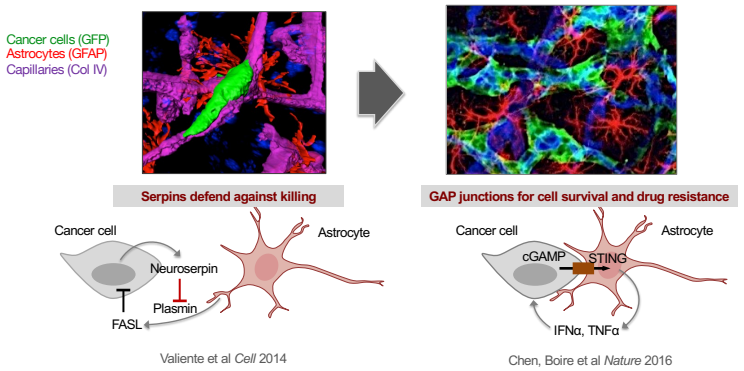
- Common (>200,000/yr in US)
  - Lung cancer
  - Breast cancer
  - Melanoma
  - Colorectal cancer
  - Renal carcinoma
- Highly lethal
- Chemoresistant



48 yr old with
non-small cell lung carcinoma,
K-Ras mutant.

32

Carcinoma–astrocyte interactions in brain metastasis from LUAD and triple-negative breast cancer

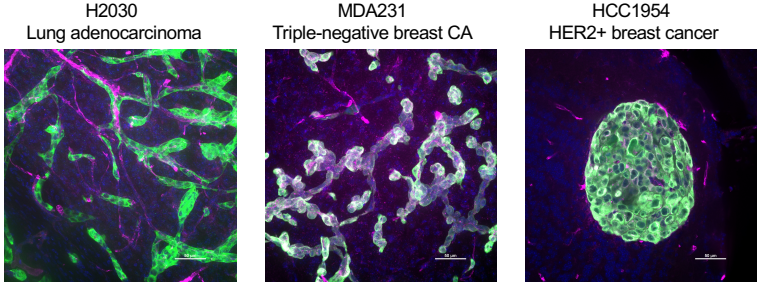


Valiente et al *Cell* 2014

Chen, Boire et al *Nature* 2016

33

Different tumor types interact differently with the metastatic microenvironment



34

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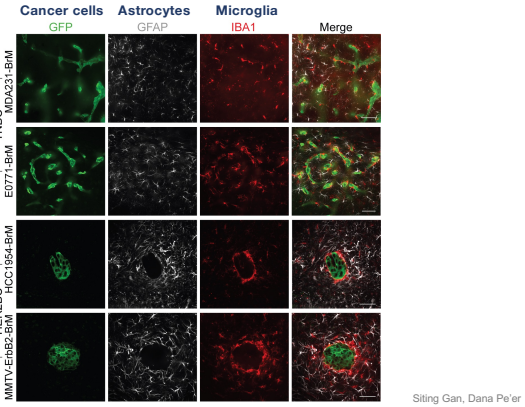
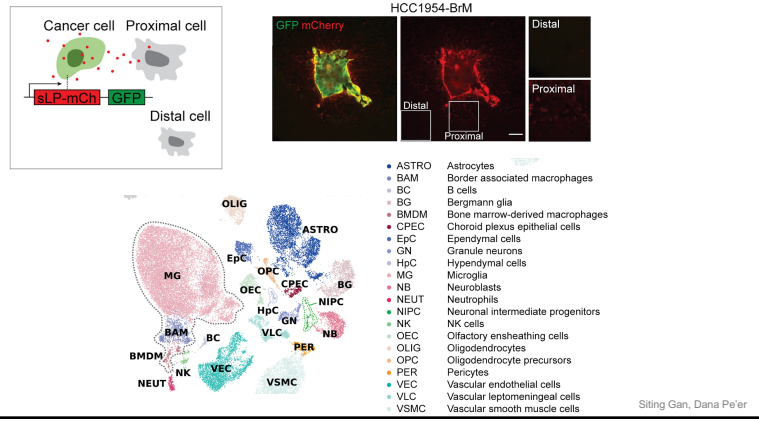


Siting Gan, Dana Pe'er

35

Approach to interrogate the metastasis microenvironment



Siting Gan, Dana Pe'er

36

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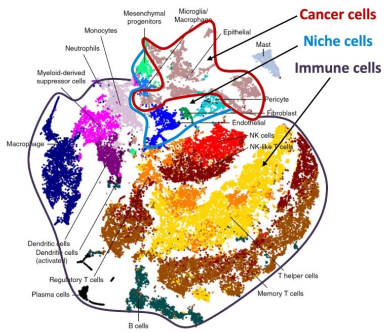
37

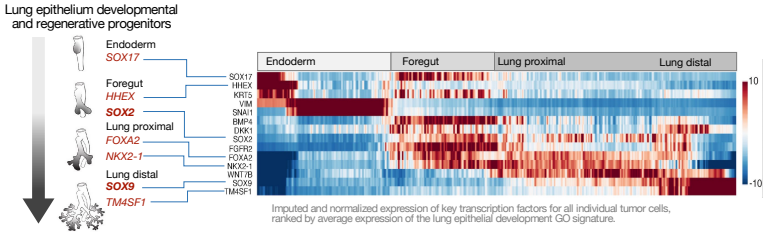
Single-cell transcriptional landscape of primary and metastatic human lung adenocarcinoma (LUAD)



Laughney et al., *Nat. Med.* 2020

38

Metastasis: a continuum of developmental and regenerative phenotypes under selective immune pruning

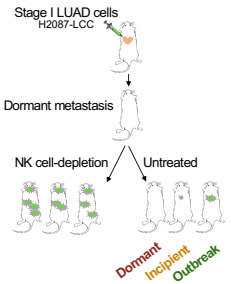


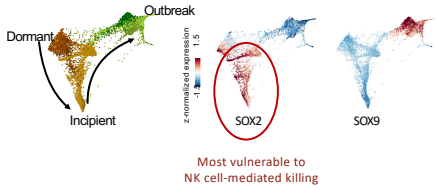
The continued development of metastatic progenitors is a major source of **intra-tumoral heterogeneity**

Laughney et al., *Nat. Med.* 2020

39

Immune surveillance prevents exit from dormancy





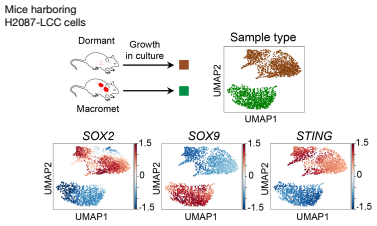
Most vulnerable to NK cell-mediated killing

Single-cell RNA-seq: Force-directed layout and PhenoGraph clusters of 8,748 metastatic cells isolated from six mice at different outbreak stages

Laughney et al., *Nat. Med.* 2020

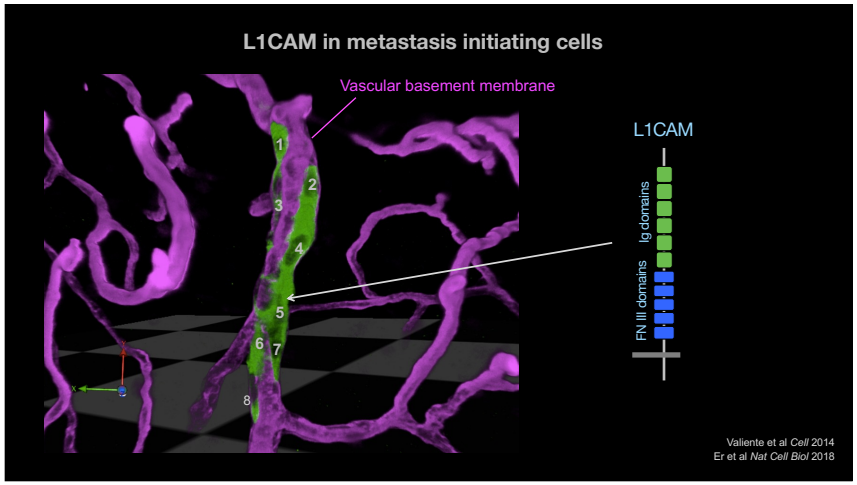
40

STING expression in dormant SOX2 progenitors reentering the cell cycle



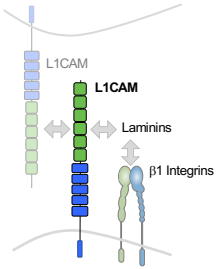
Hu et al *Nature* 2023

41



42

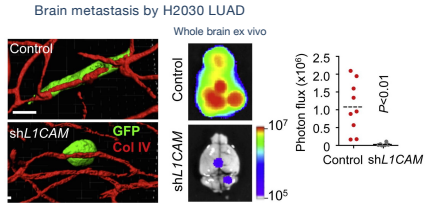
L1 Cell Adhesion Molecule (L1CAM)



- Cell-basement membrane and cell-cell adhesion molecule
- Expression: neurons, oligodendrocytes, some monocytes, kidney distal ducts. Absent in most other tissues.
- Expression in primary tumors >> poor prognosis
  Lung, breast, colorectal, gastric, renal, ovarian, pancreatic, and endometrial carcinomas; melanomas; sarcomas; gliomas.

43

L1CAM mediates metastatic colonization



L1CAM is required for metastasis:

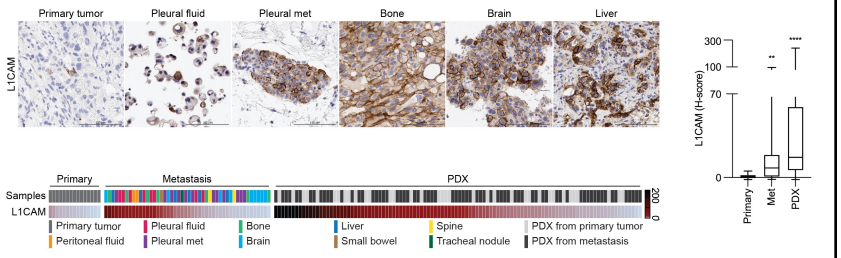
- by **different tumor types** (breast, lung, colorectal, renal)
- in **multiple organ sites** (brain, lung, bone, liver)

Valiente et al *Cell* 2014
Er et al *Nat Cell Biol* 2018
Ganesh et al *Nature Cancer* 2020

44

L1CAM+ cells appear upon loss of epithelial integrity

- L1CAM expression in human LUAD primary tumors and metastases

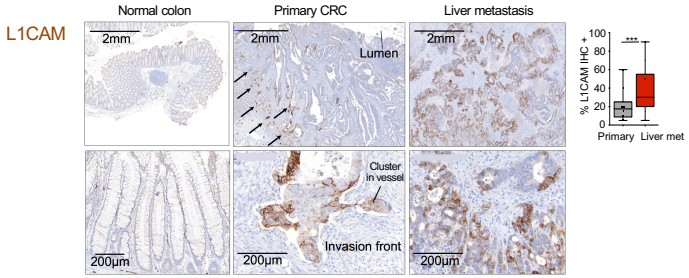


Kaygusuz*, Park* et al, in progress

45

L1CAM+ cells appear upon loss of epithelial integrity

- L1CAM expression in human LUAD primary tumors and metastases

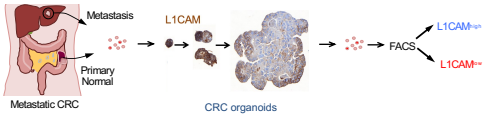


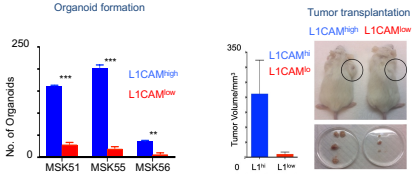
Rare L1CAM+ cells in the tumor invasion front; enriched in metastases

Ganesh et al, *Nature Cancer* 2020

46

CRC L1CAM+ cells regenerate organoids and tumors



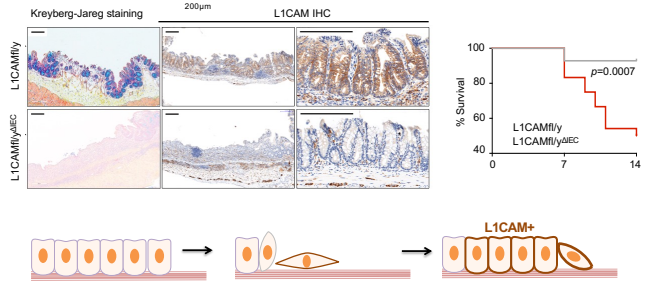


Ganesh et al, *Nature Cancer* 2020

47

L1CAM is expressed and required in normal progenitors during epithelial regeneration

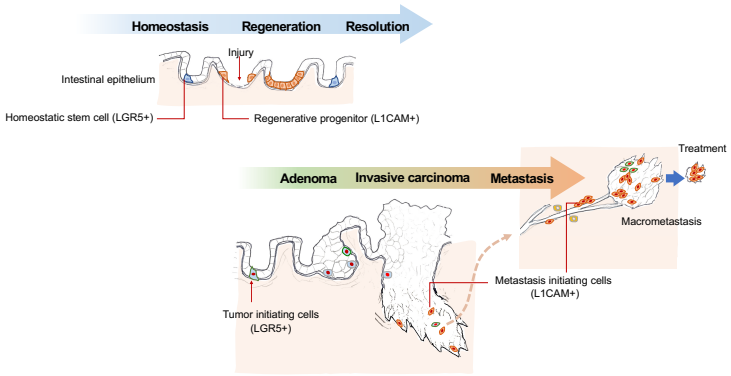
VillinCre;*L1CAM*$^{fl/y}$ (L1CAMfl/y$^{\Delta IEC}$)
Tamoxifen + Dextran Sulfonate Sodium → monitor DSS colitis and recovery



Ganesh et al, *Nature Cancer* 2020

48

L1CAM+ progenitors in epithelial regeneration and cancer



Massagué & Ganesh *Cancer Disc.* 2021

49

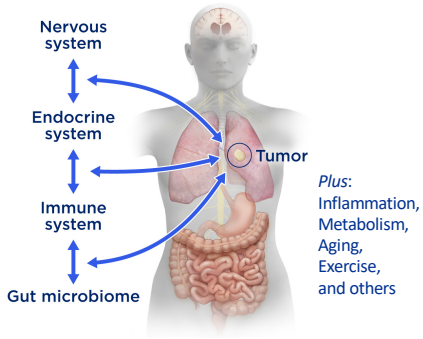
Mechanisms of Metastasis

- Metastasis as a medical problem
- Phases of the metastatic process
  - Metastatic dissemination
  - Dormancy and immune evasion
  - Organ colonization and metastatic tropism
- Metastasis initiating cells
- Tumor evolution and metastatic progression
- **Metastasis as a systemic disease**
- **Treating metastasis**

50

Metastasis and the whole-body ecosystem

- Local ecosystems and whole-body physiology determine metastatic progression and its response to therapy.



Plus: Inflammation, Metabolism, Aging, Exercise, and others

Massagué & Ganesh *Cancer Disc.* 2021

51

Treating metastasis

Pillars of current therapy
- Chemotherapy
- Hormone therapy
- Radiotherapy
- Surgery
- Immunotherapy

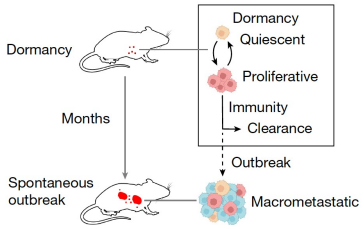
Treatments have been palliative in most cases, suppressing metastasis but not eliminating it.

Advances in targeted therapy and immunotherapy are beginning to provide long-term cures.

Clinical trials for early intervention are challenging
- Regulatory environment
- "Business as usual" mindset

52

Immune surveillance prevents exit from dormancy

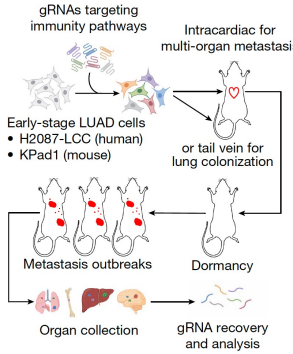


What cell-intrinsic factors trigger the elimination of dormant metastatic cells that reenter the cell cycle?

Hu et al *Nature* 2023

53

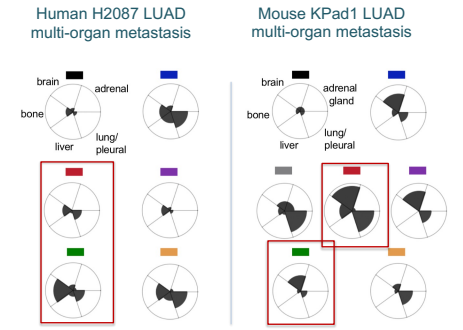
In vivo CRISPR screen for suppressors of LUAD metastatic outbreak



Hu et al *Nature* 2023

54

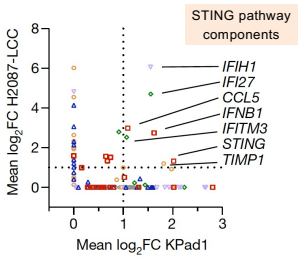
Suppressors of metastatic outbreak: MHC class I, NK ligands, and STING



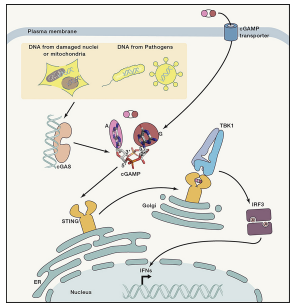
Hu et al *Nature* 2023

55

STING pathway scores as suppressor of LUAD metastatic outbreaks



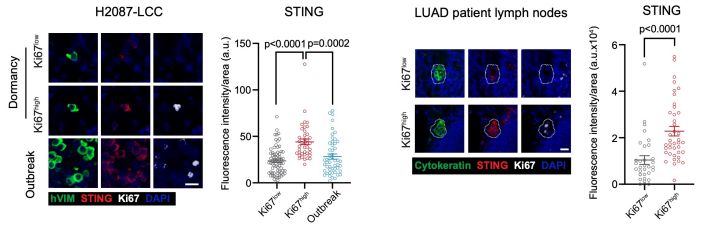
Hu et al *Nature* 2023



Zhang et al *Immunity* 2020

56

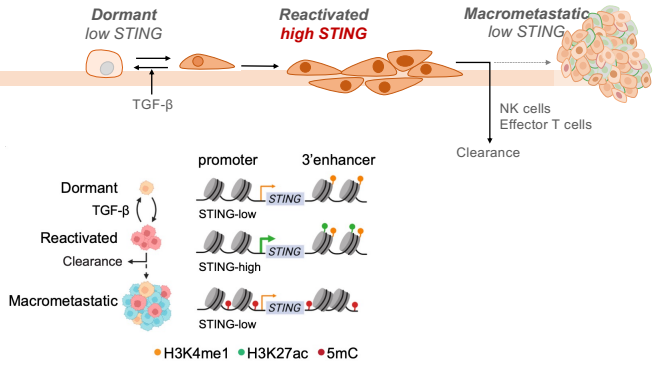
Heightened STING expression in dormant LUAD cells reentering the cell cycle



Hu et al *Nature* 2023

57

Immune surveillance prevents exit from dormancy

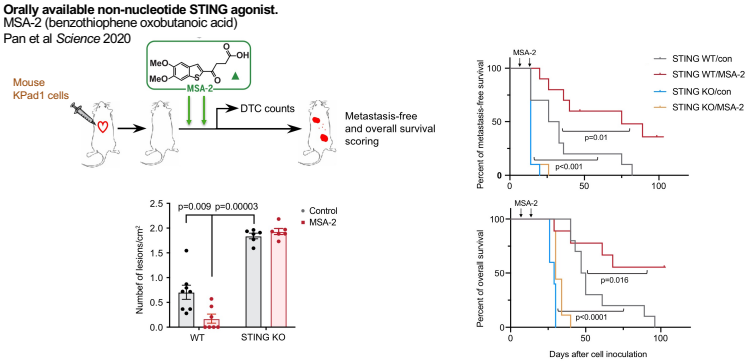


Hu et al *Nature* 2023

58

STING agonist eliminates dormant metastasis and prevents relapse

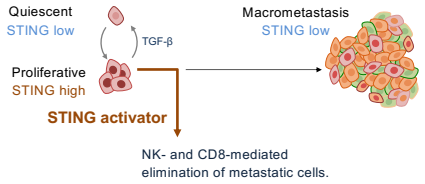
Orally available non-nucleotide STING agonist.
MSA-2 (benzothiophene oxobutanoic acid)
Pan et al *Science* 2020



Hu et al *Nature* 2023

59

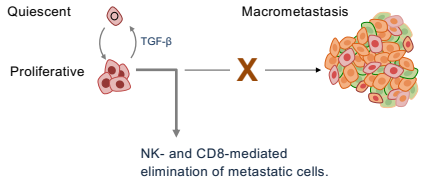
Leveraging STING to eliminate dormant metastasis



- **Approach 1: Promote clearing of dormant metastasis**
- Approach 2: target mediators of metastatic outbreak

60

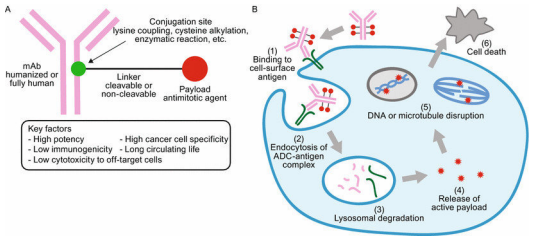
Preventing relapse by targeting metastasis initiating cells



- Approach 1: Promote clearing of dormant metastasis
- **Approach 2: target mediators of metastatic outbreak**

61

Antibody-drug conjugates



62

Mechanisms of Metastasis

- Metastasis as a medical problem: *remains the main cause of death from cancer*
- Phases of the metastatic process
  - Metastatic dissemination: *well understood; many mediators identified; more to do*
  - Dormancy and immune evasion: *not a passive process; experimentally tractable now*
  - Organ colonization and tropism: *well understood; many mediators identified; more to do*
- Metastasis initiating cells: *emerging insights; regenerative progenitors with high plasticity*
- Tumor evolution and metastatic progression: *metastases as a developmental continuum*
- Metastasis as a systemic disease: *knowledge at a very early stage*
- Treating metastasis: *new approaches and a paradigm-shift are requited*

63