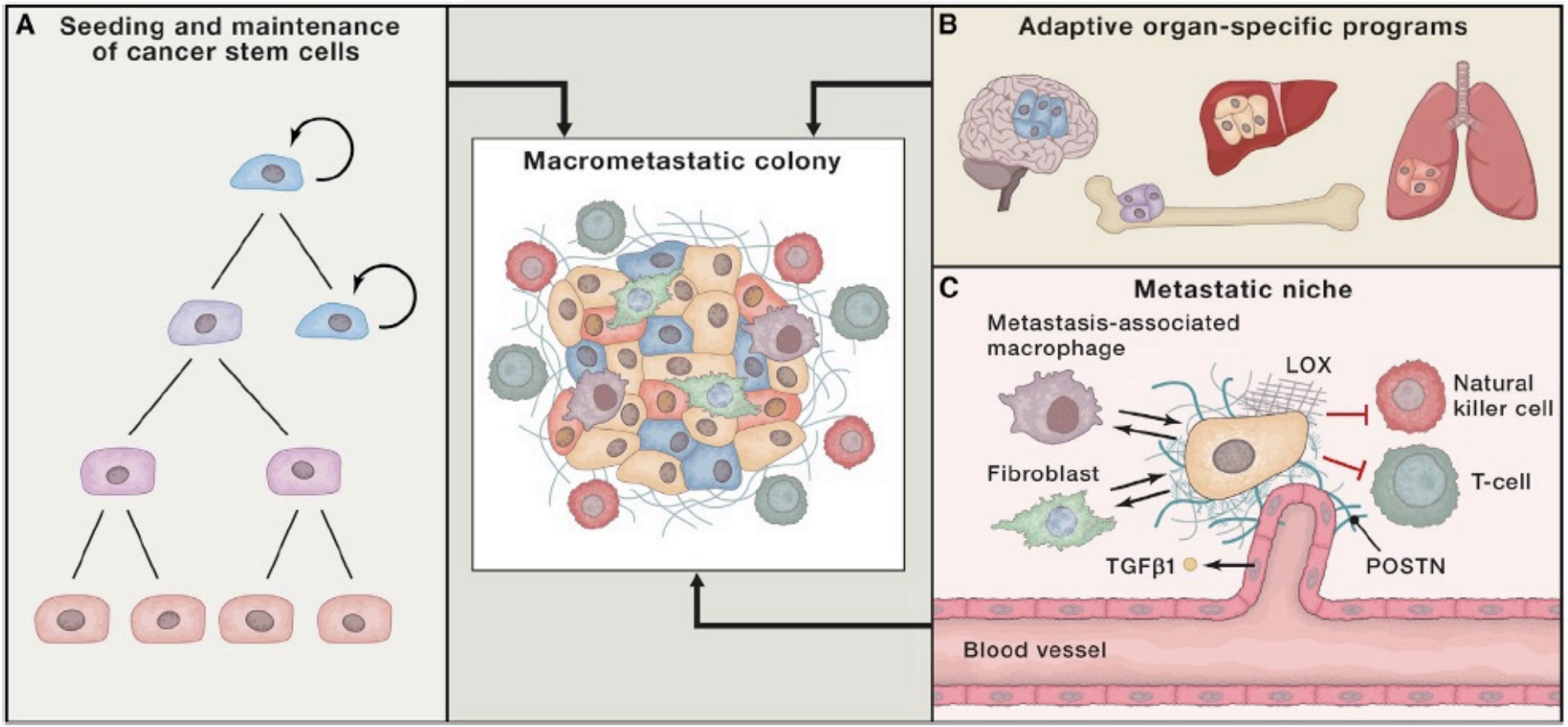
Corso di Biologia Cellulare del Cancro AA 2020-2021

Dormienza e colonizzazione metastatica

Requisiti per la colonizzazione metastatica



The seed and soil hypothesis

"What is it that decides what organs shall suffer a case of disseminated cancer?"

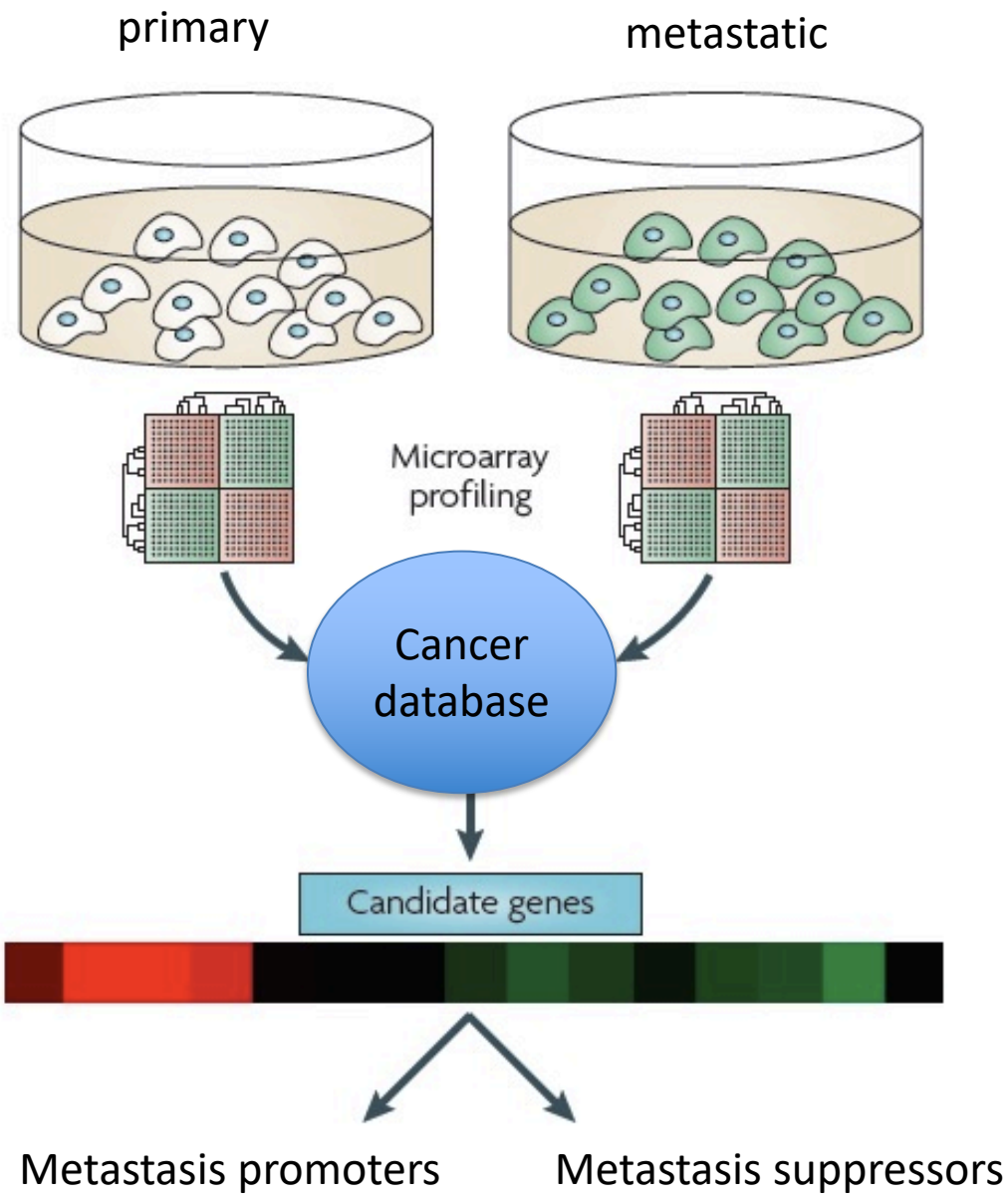
"When a plant goes to seed, its **seeds** are carried in all directions….,but they can only live and grow if they fall on congenial **soil**."
-Stephen Paget 1889-

Seed: i geni della metastasi

Metastasis develops through genetic and epigenetic changes and the subsequent selection for favourable traits under the pressure of successive bottlenecks.

Anna C. Obenauf and Joan Massagué
Trends Cancer. 2015

Identificazione di geni drivers della metastasi



Origine della competenza metastatica

Genomic comparisons show **close clonal relationships between primary tumours and their metastases**.
Specific ancestors of metastatic clones can often be identified in the primary tumour, supporting the **hypothesis that late a clonal expansion in the primary tumour gives rise to metastasis competent clones**.
These studies also provide evidence for **metastases seeding new metastases**.

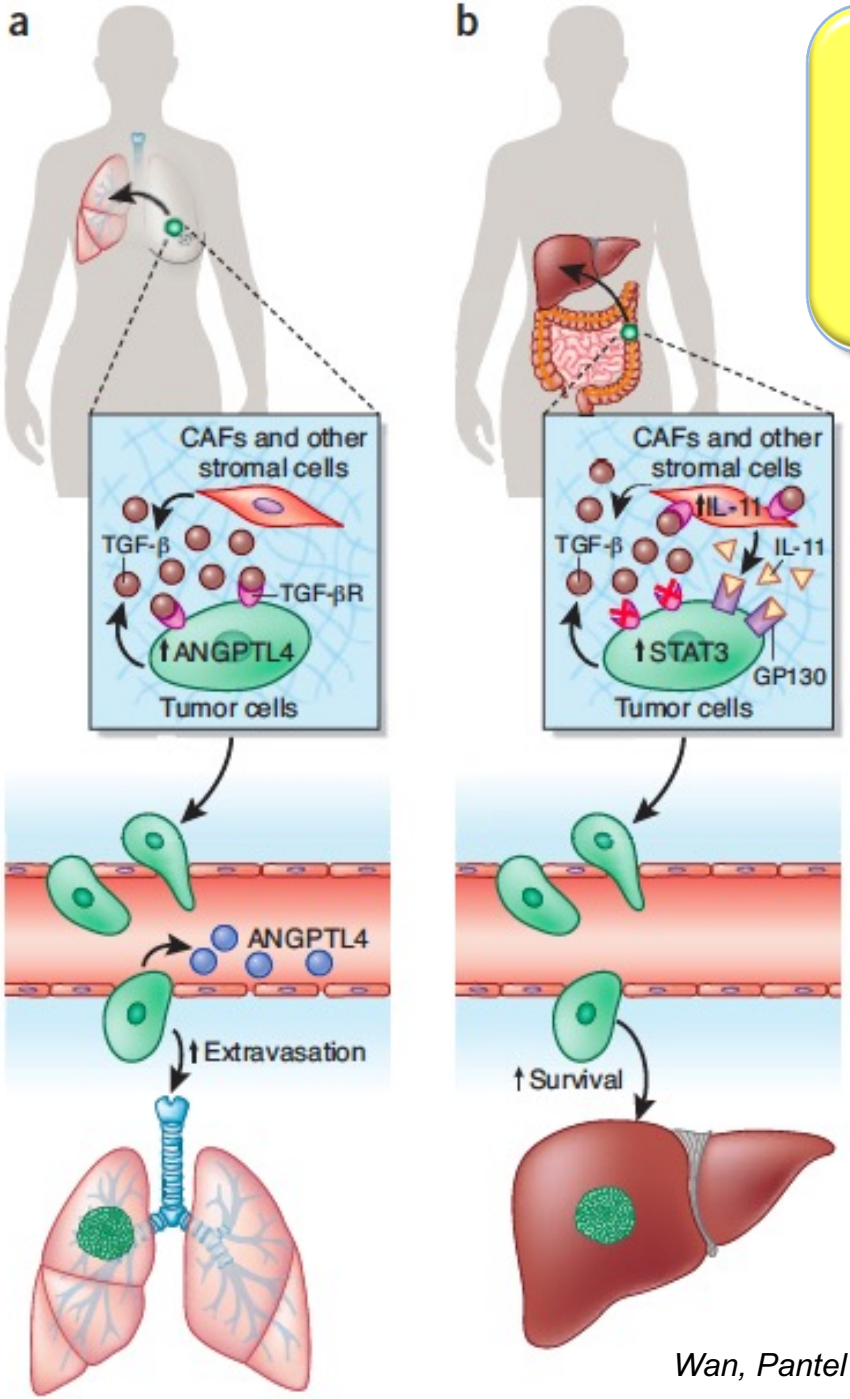
Disseminated cancer cells remain dependent on the oncogenic mutations that underlie the primary tumour, providing a basis for **treating metastasis** with drugs that target these oncogenic drivers.
In line with this observation, **gains in oncogenic mutant alleles occur in metastases**, including gains in mutant **KRAS** in pancreatic cancer metastasis, and **TP53** and androgen receptor mutations in prostate cancer metastasis.

General mediators of metastasis, such as those **supporting invasion, ability to amplify survival pathways, or immune evasion** **increase the probability** of cancer cells to adapt and, consequently, survive through multiple specific challenges in multiple organs.

In contrast, **certain genes and pathways enable passage through critical organ-specific barriers**, such as crossing the blood-brain barrier, or mediate beneficial interactions with organ-specific cell types, such as the osteoclasts in the bone marrow.

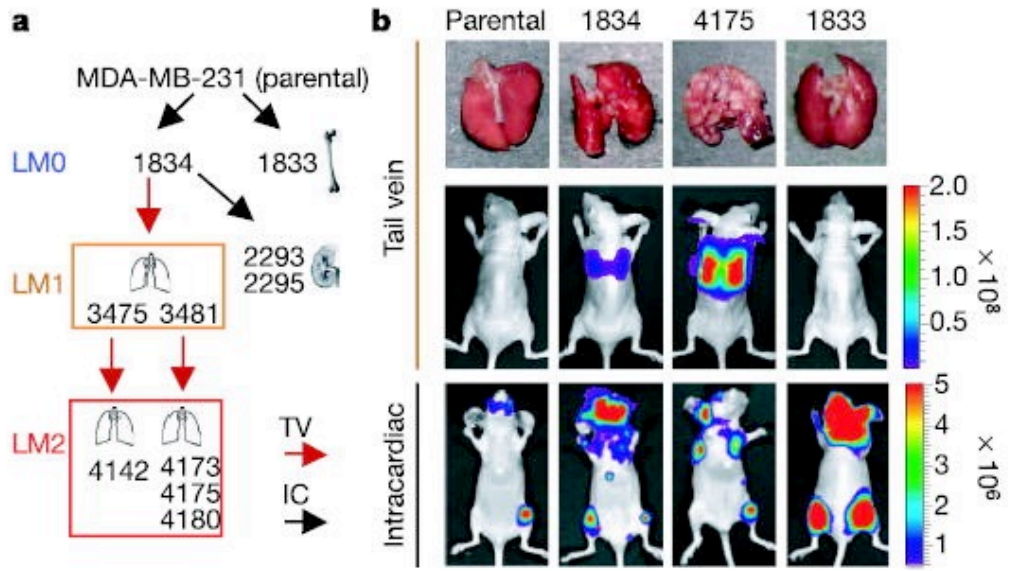
Anna C. Obenauf and Joan Massagué
Trends Cancer. 2015

L'interazione con il microambiente del tumore primario influenza la successiva metastasi

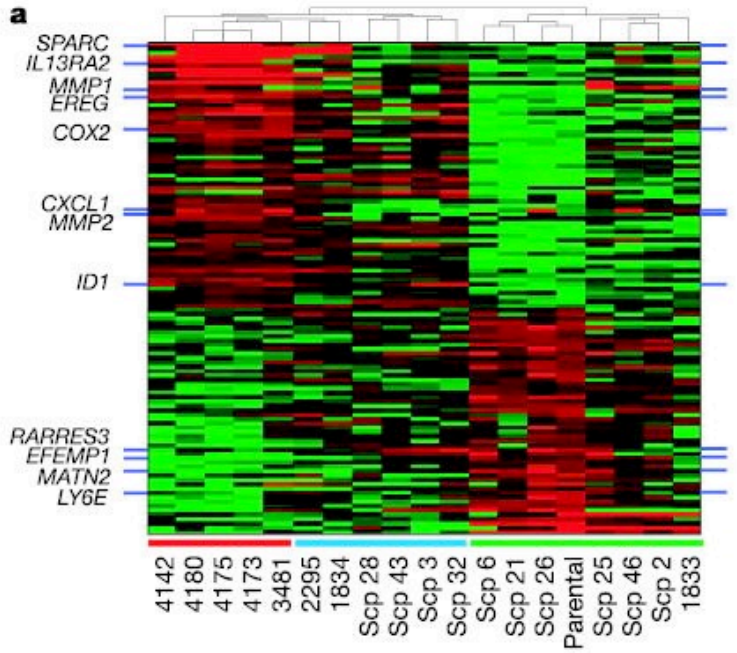


Wan, Pantel & Kang, Nat Med 2013

Identificazione di geni del tropismo metastatico



Flow chart of the selection of organ-specific metastatic subpopulations in vivo, indicating the organs from which these subpopulations were isolated. Each **subsequent lung-metastatic generation** is designated LM0, LM1 and LM2. The LM2 cells were further analysed for metastasis by either tail-vein (TV) or intracardiac (IC) xenografting.



Gene-expression signature associated with lung metastasis

a, Comparison of gene expression profiles of LM2 populations with parental cells identifies 113 probe sets that are correlated with lung metastatic activity.

Vol 459|18 June 2009|doi:10.1038/nature08021 nature

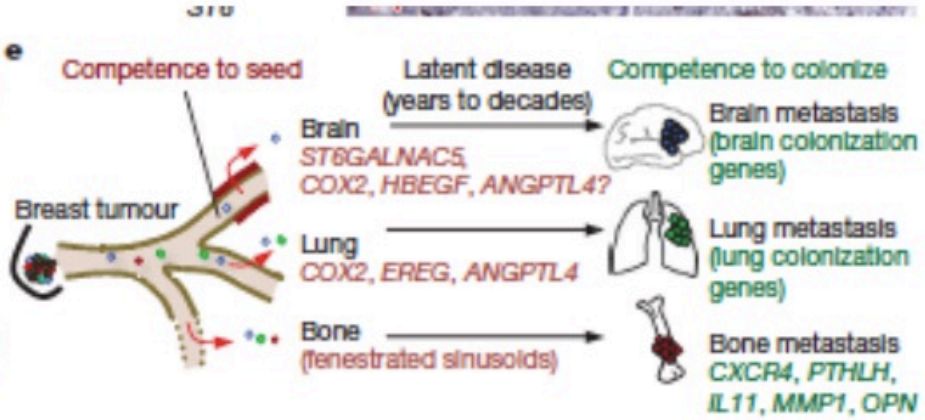
LETTERS

Genes that mediate breast cancer metastasis to the brain

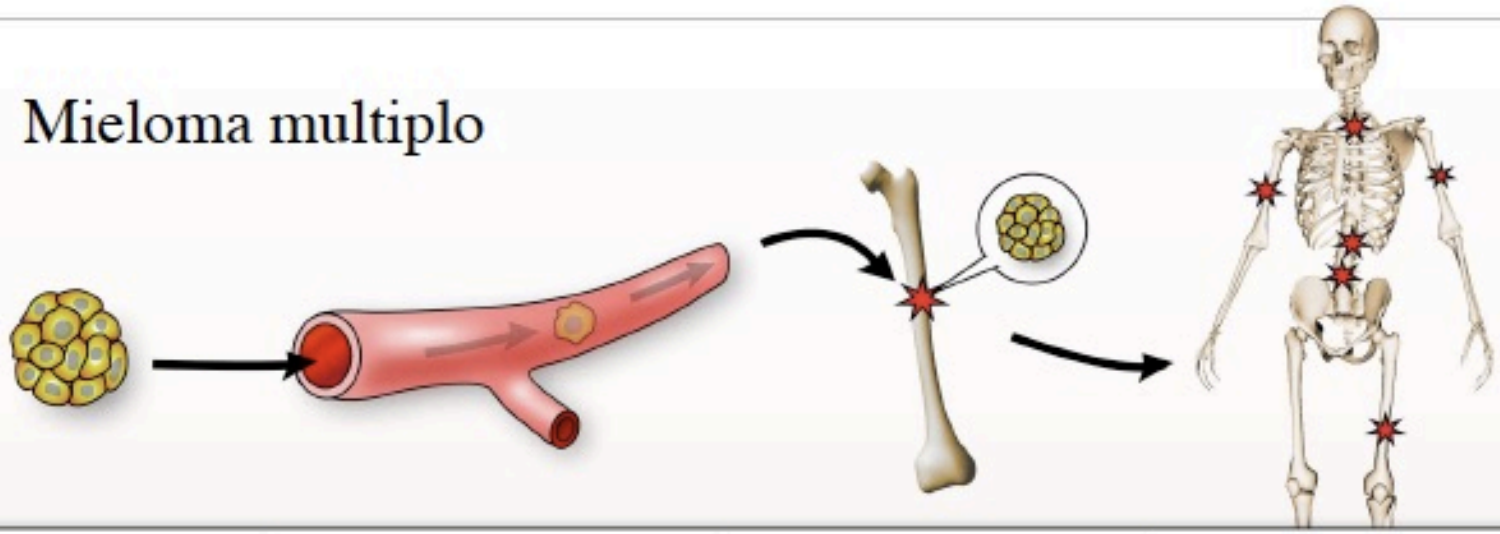
Paula D. Bos[1], Xiang H.-F. Zhang[1], Cristina Nadal[1]†, Weiping Shu[1], Roger R. Gomis[1]†, Don X. Nguyen[1], Andy J. Minn[2], Marc J. van de Vijver[3], William L. Gerald[4], John A. Foekens[5] & Joan Massagué[1,6]

***in-vivo*-selected brain metastatic derivatives**

Gene expression analysis of these cells and of clinical samples+ functional studies:
cyclooxygenase COX2 (al known as PTGS2), the epidermal growth factor receptor (EGFR) ligand HBEGF, and the a2,6-sialyltransferase ST6GALNAC5 **as mediators of cancer cell passage through the blood–brain barrier**



Mieloma multiplo



Primary tumor; Local invasion; Plasmacytoma	Circulation	Micrometastasis; MGUS	Colonization; Multiple lytic lesion-symptomatic disease

Genes regulating tumor initiating function:
Cyclin D1/D3, FGFR3, MMSET, c-maf, del 13q

Metastasis initiating function: Angiogenesis, invasion and circulation; EMT- transcription factors, hypoxia-regulated genes, CXCR4

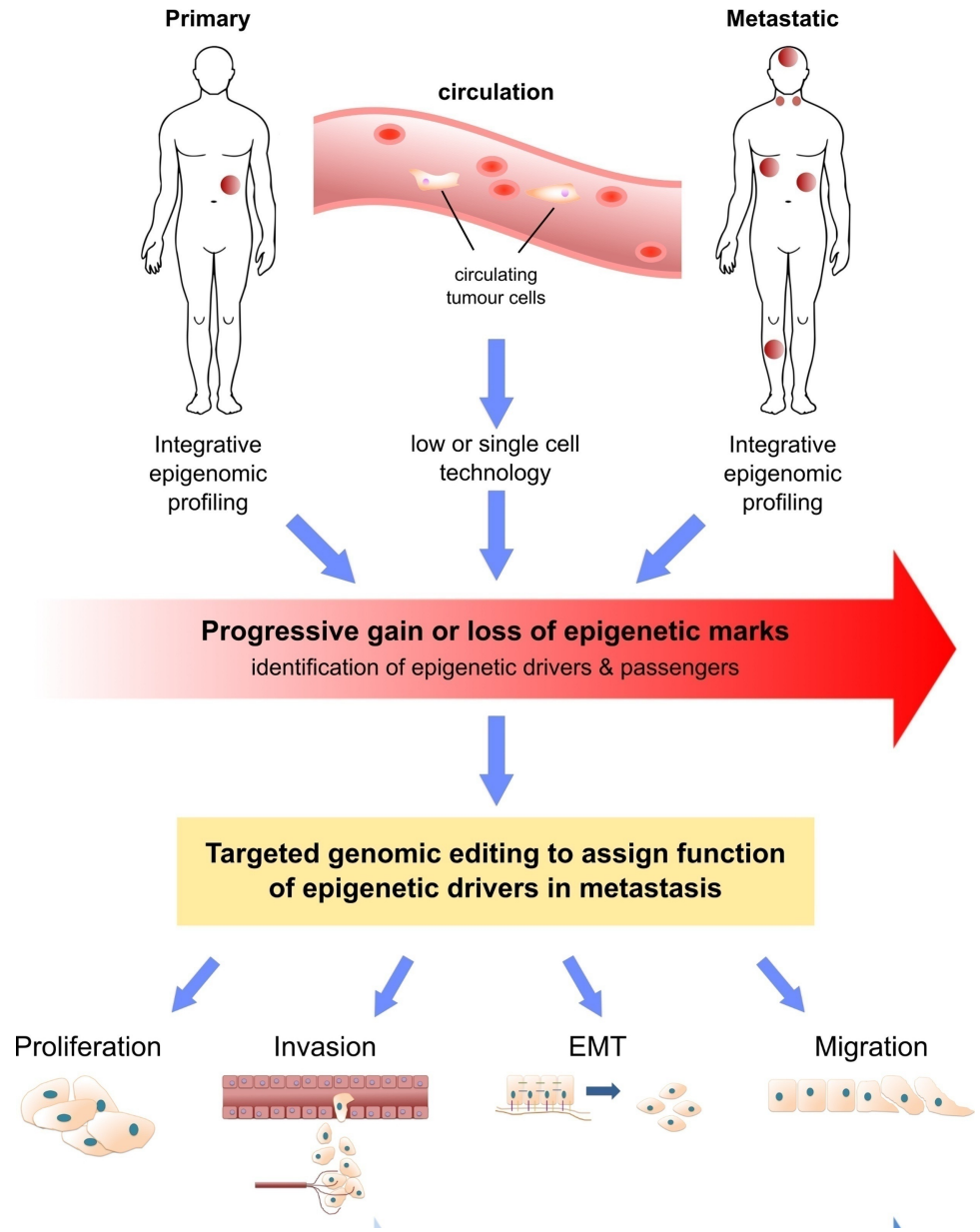
Metastasis progression:
Activation of NFκB, N-ras, K-ras, FGFR3

Metastasis virulence:
PTHRP, IL-6, TNFα, VEGF, RANKL

Seed: i geni della metastasi

To date, **no recurrent metastasis-specific mutations have been identified**, suggesting that **epigenetic** alterations and other sources of **modified gene expression** are the predominant **source of selectable pro-metastatic traits** during clonal evolution in metastasis.

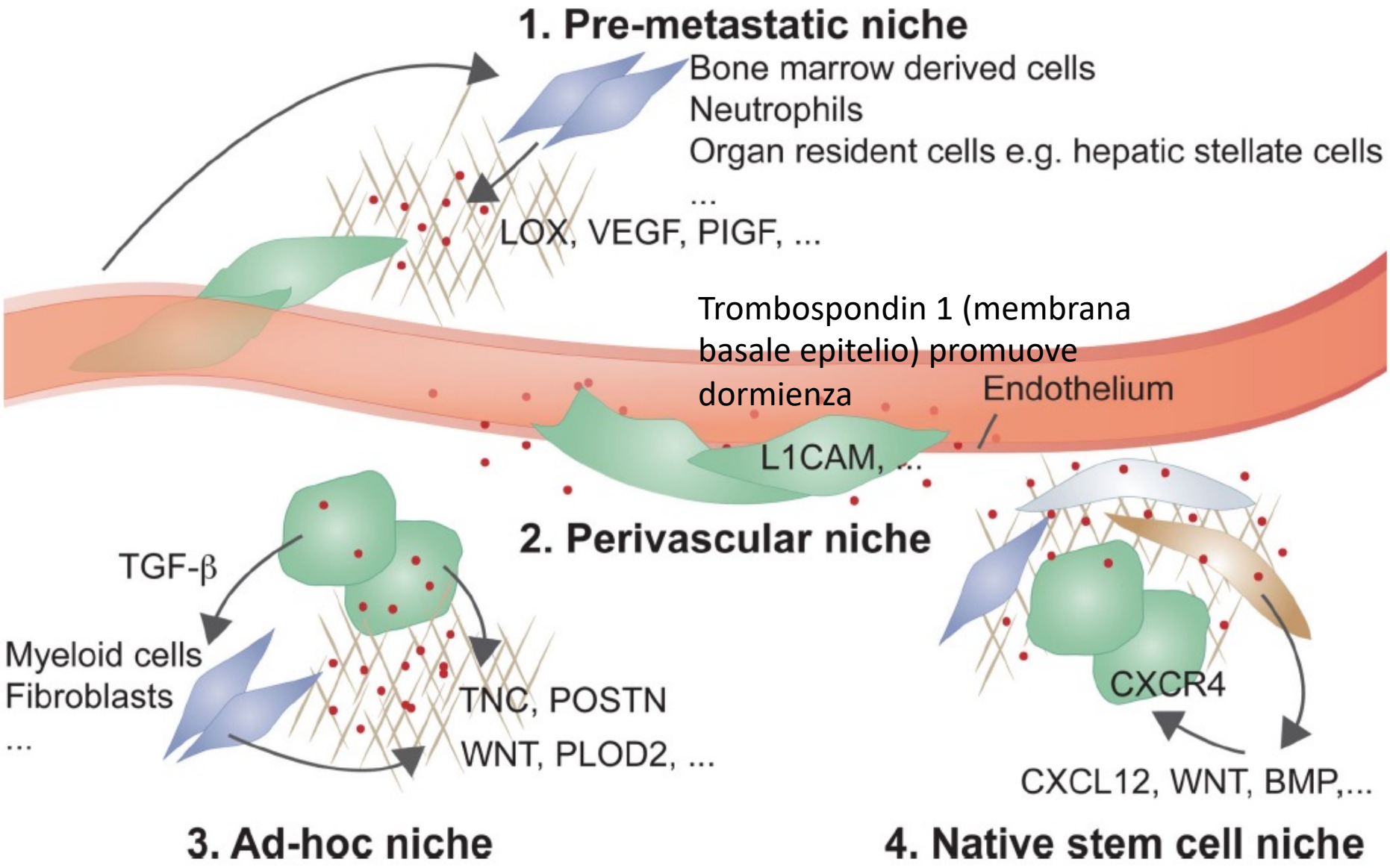
Identificazione di drivers epigenetici



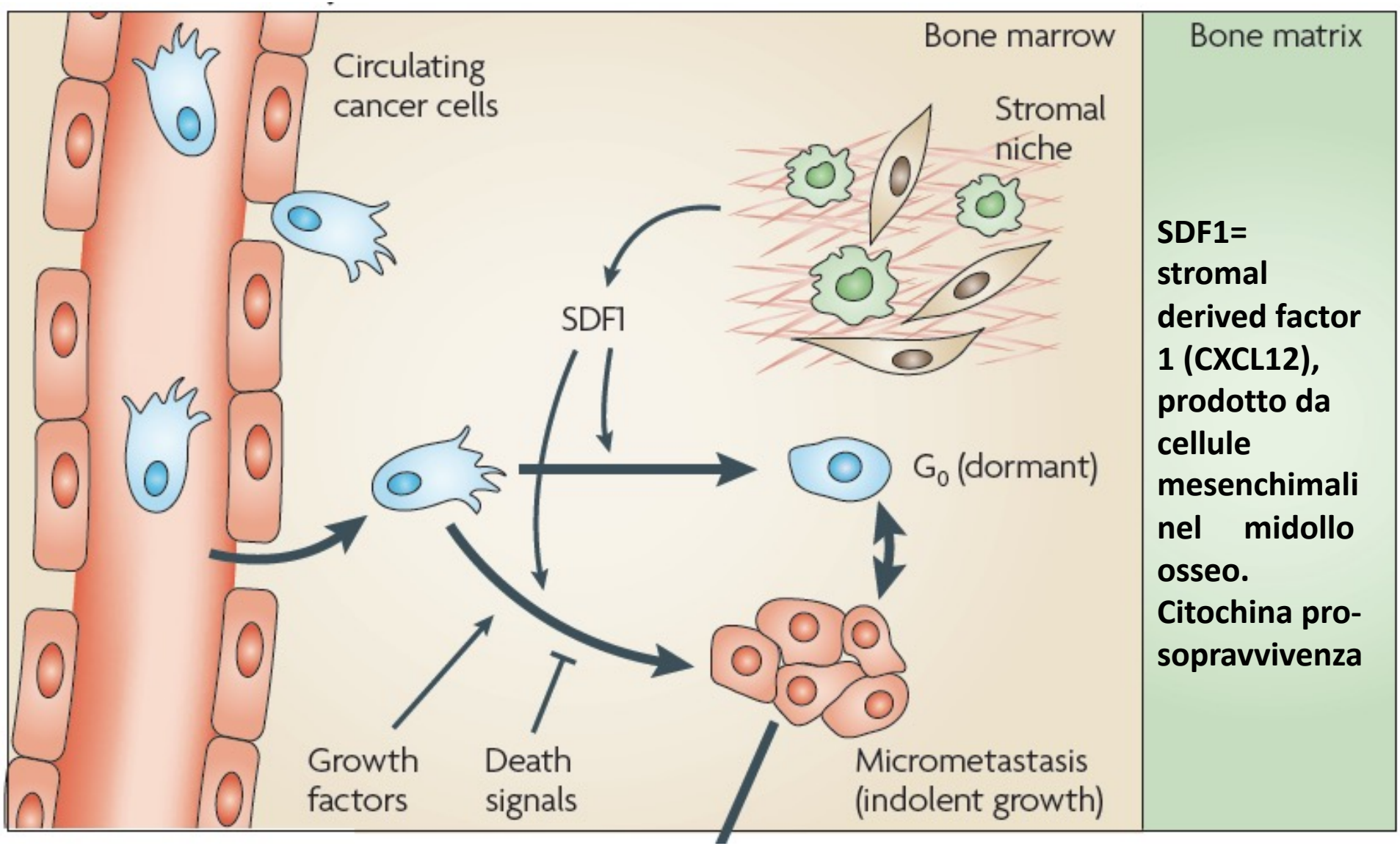
Paired **genomic/transcriptomic/epigenetic analysis** of a metastatic tumour and corresponding primary tumour from the same patient provides a **powerful approach to identify putative metastasis-drivers.** Simultaneous study of **circulating tumour cells** provides a comprehensive model for studying progressive changes during the metastatic cascade.

Functional studies and sophisticated editing experiments could then be used to understand the cellular consequences of these changes.
If the identified drivers are causal, they will have **significant translational implications** in regards to early diagnosis of cancer and potentially in developing new therapeutic strategies.

SOIL: la NICCHIA metastatica



Dormienza metastatica



SDF1= stromal derived factor 1 (CXCL12), prodotto da cellule mesenchimali nel midollo osseo. Citochina pro-sopravvivenza

Nguyen & Massagué Nat Rev Cancer 2009

Dormienza metastatica

Tumour dormancy is thought to occur in two modes.
Cellular dormancy involves isolated DTCs that enter a state of proliferative quiescence. Indeed, in patient bone marrow samples most DTCs are found as quiescent single cells.
In contrast, tumour mass dormancy involves micrometastases that cease to grow due to insufficient vascularization or to constant culling by immune defences.

Tumor mass dormancy depends on the balance between proliferation and apoptosis due to :

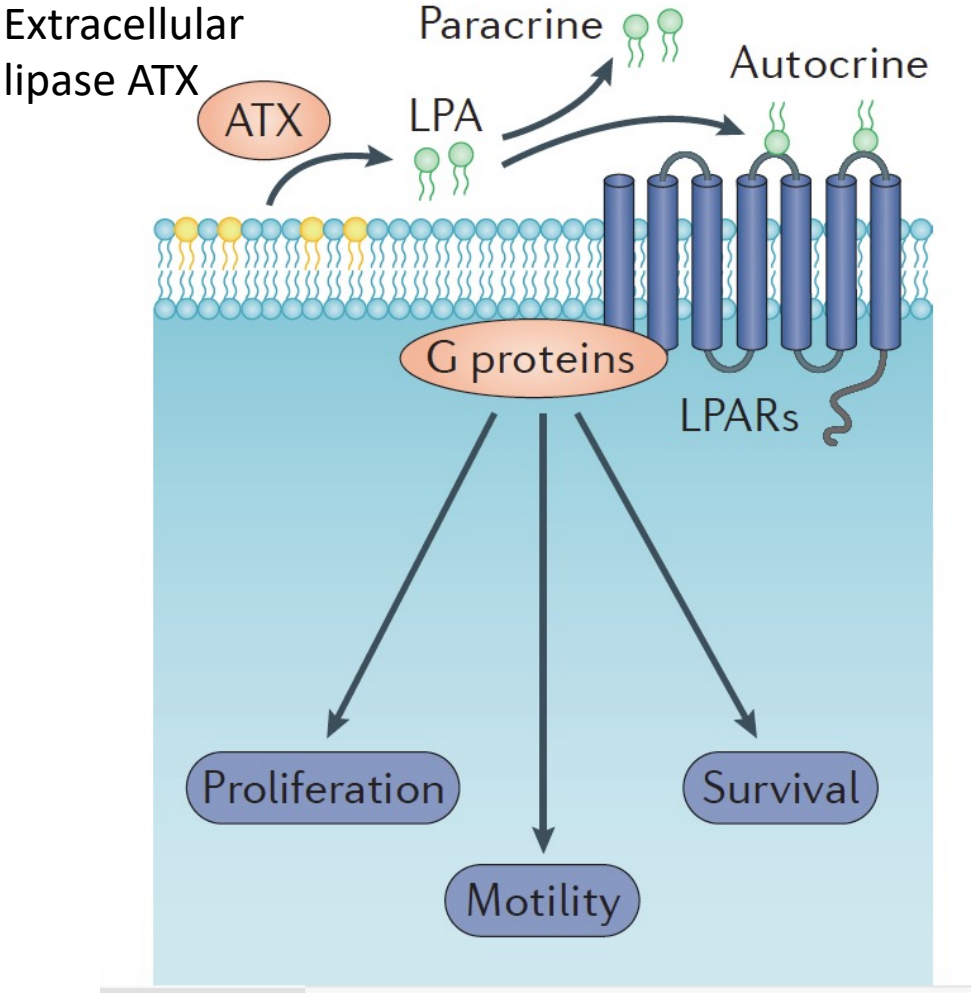
- anti-growth signals embedded in the extracellular matrix of the normal tissue;
- tumor suppressing actions of the immune system
- maladaptation of cancer cells to surrounding stroma
- Niche competition

Escape from dormancy: dormant tumor cells may exit this state and resume active growth and proliferation when changes in tissue microenvironment, such as access to more nutrients (e.g. vessel formation) occur.

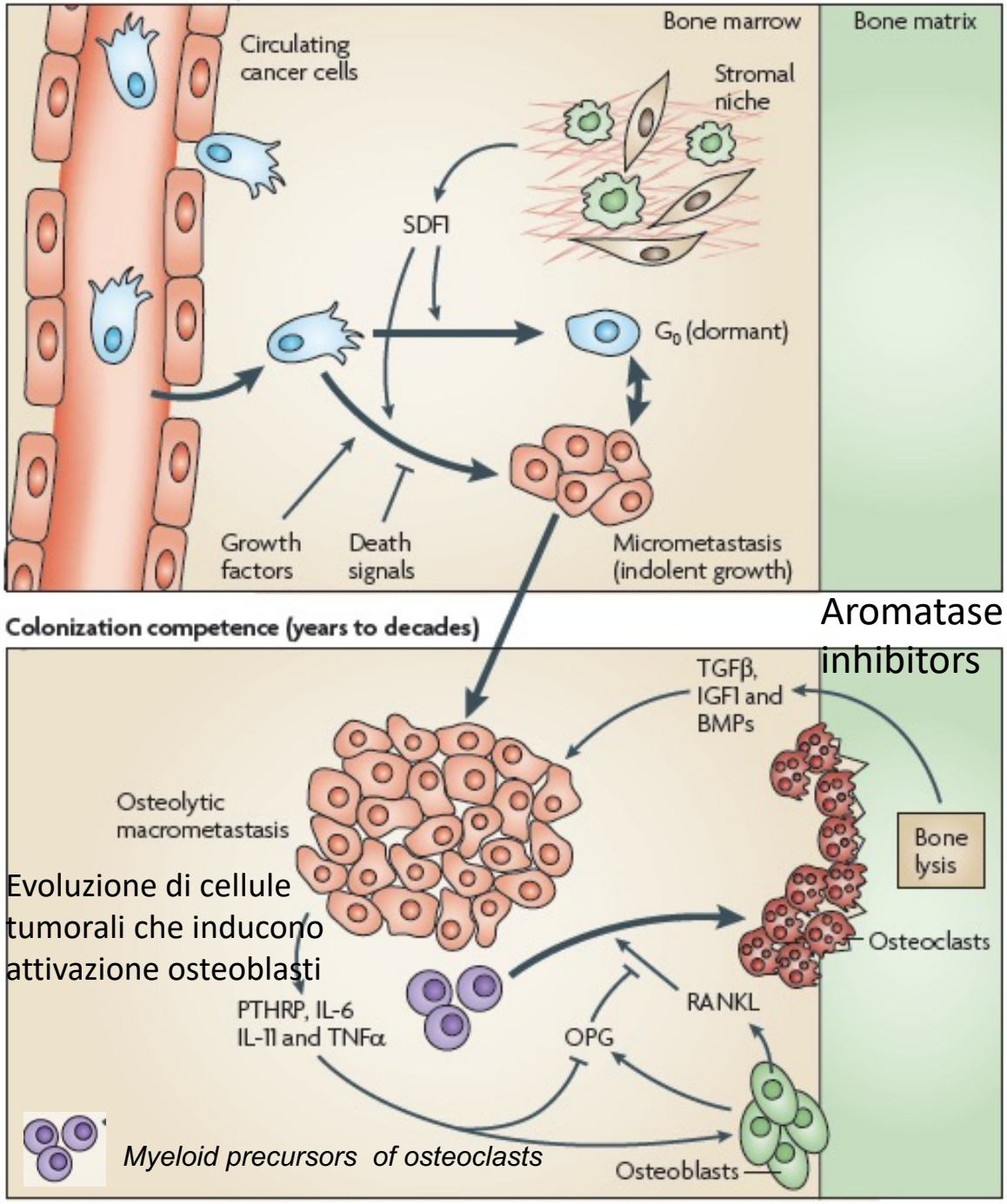
Nuovi targets: recettore del LPA

La finestra terapeutica più attraente è quella della colonizzazione

Uno small-molecule inhibitor del recettore 1 dell'acido lisofosfatidico (LPAR1) previene la crescita metastatica e induce l'entrata delle DTCs in uno stato di dormienza (studi preclinici)



Il rimodellamento della nicchia e l'uscita dalla dormienza: metastasi ossea

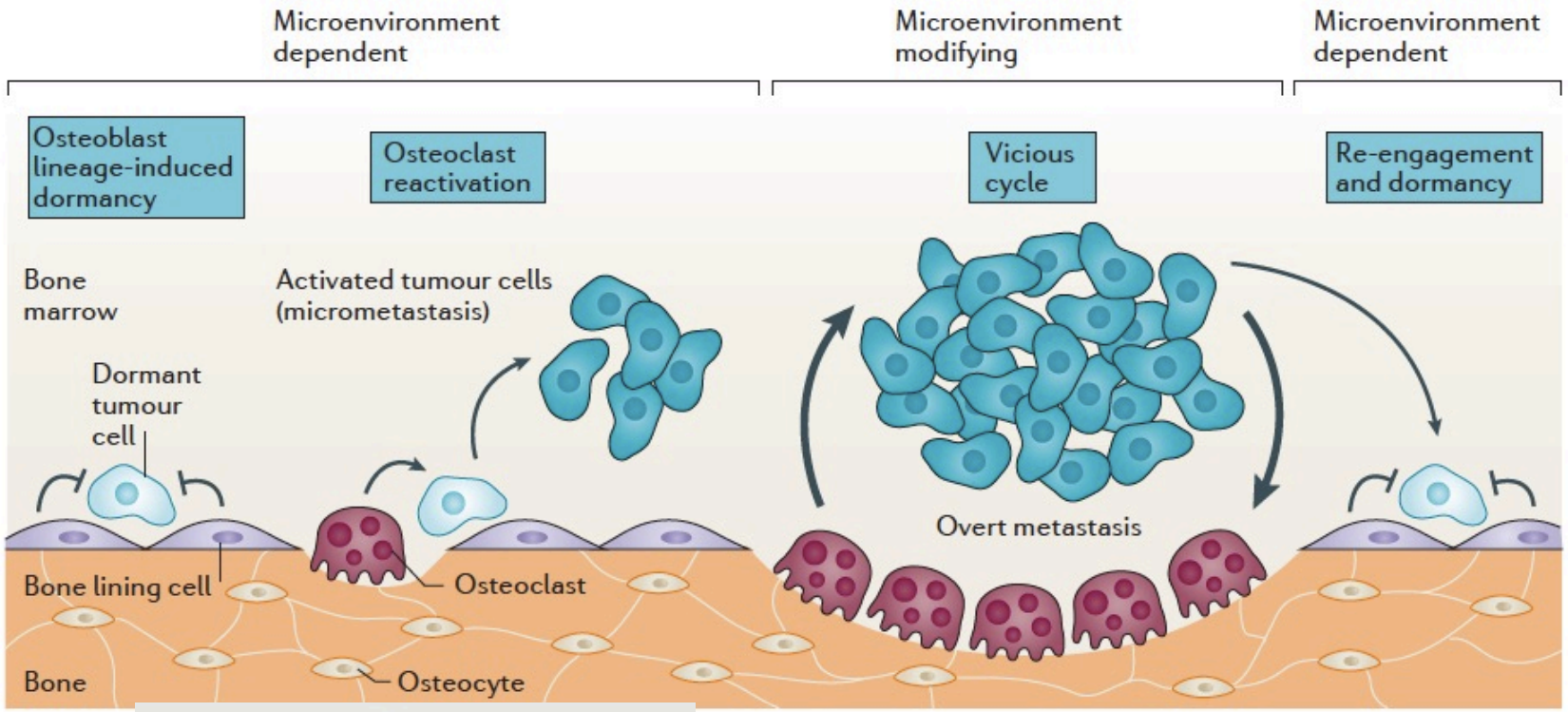


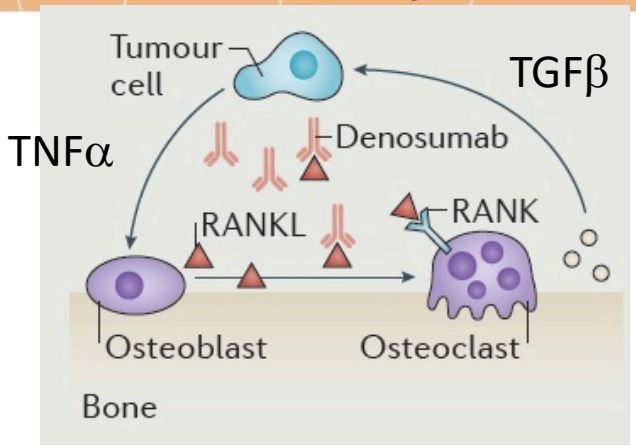
Evoluzione di cellule tumorali che inducono attivazione osteoblasti

Rimodellamento della nicchia endostale: induzione di osteoblasti a secernere **RANKL** che **attiva gli osteoclasti** che liberano nuove citochine pro-tumorali (TGFbeta) digerendo la matrice dell'osso.

Nguyen & Massagué Nat Rev Cancer 2009

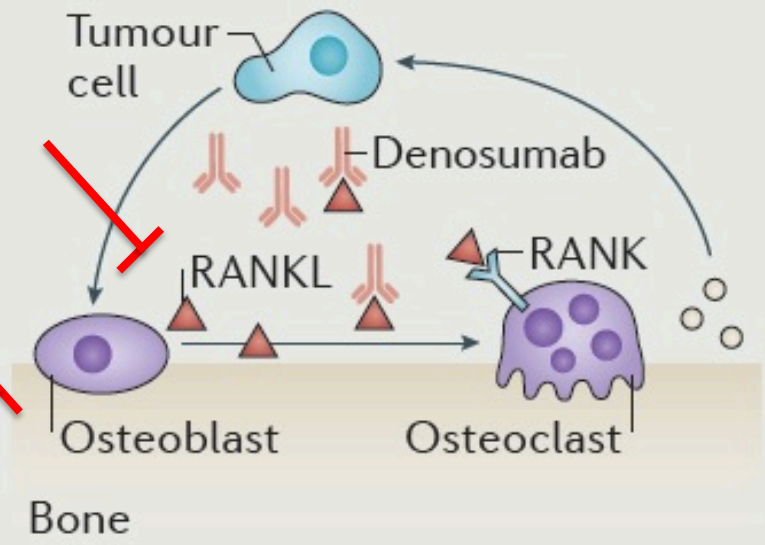
Colonizzazione della nicchia endostale: il circolo vizioso delle mestastasi osteolitiche nel BC





RANKL activates osteoclasts and promotes bone destruction;

Terapie contro la nicchia endostale

Description	Pathway	Preclinical validation
Denosumab		
Monoclonal antibody to RANKL		RANKL activates osteoclasts and promotes bone destruction; denosumab reduced bone resorption in mice expressing human RANKL[29]

BIFOSFONATI

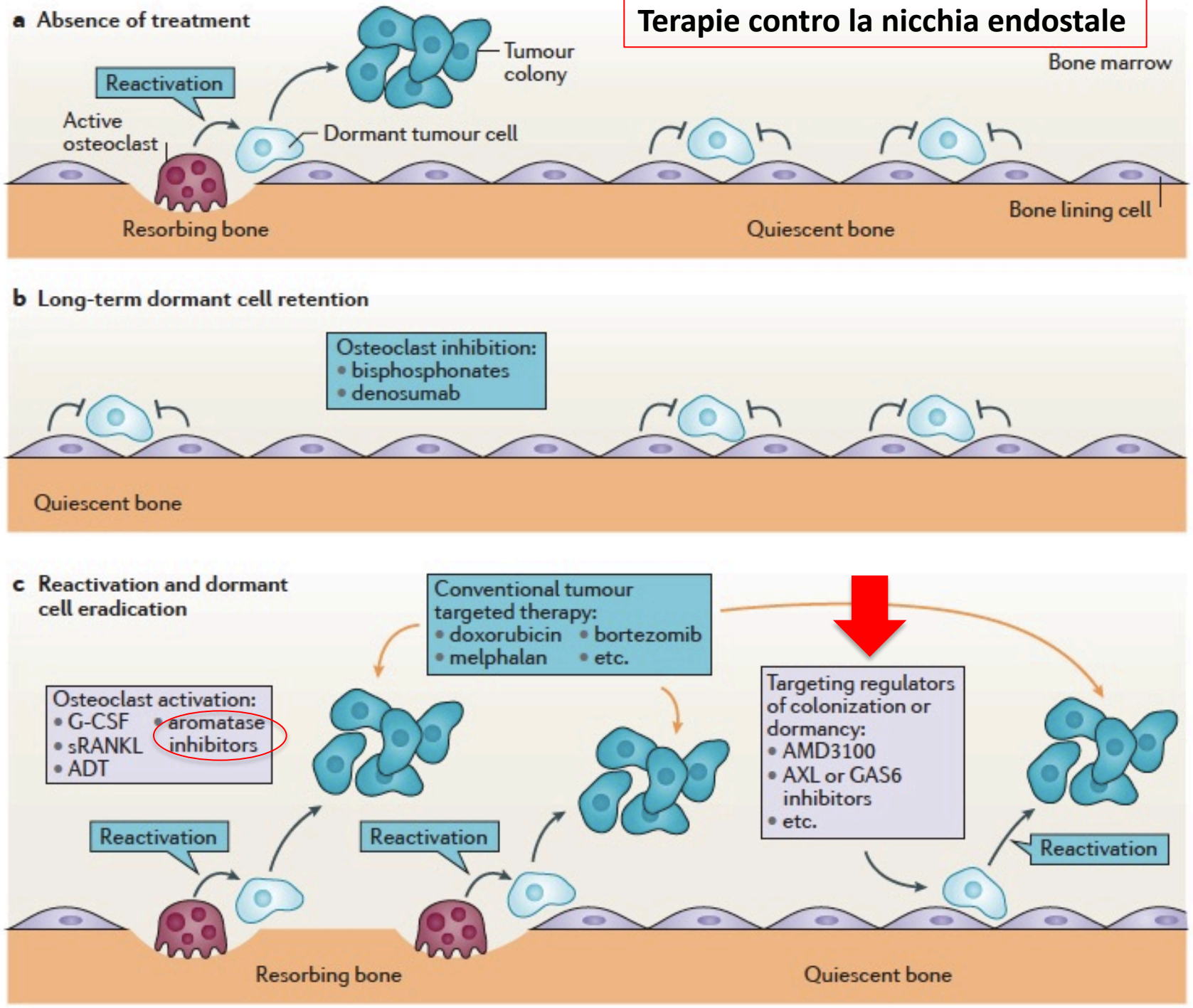
Rallenta la comparsa di metastasi ossee

Pivotal trials and end points	Outcomes
SREs* in metastatic setting; adjuvant trials used time to first bone metastasis or fracture[30–33]	FDA approved for prevention of SREs in solid tumours; approved as adjuvant therapy in prostate cancer

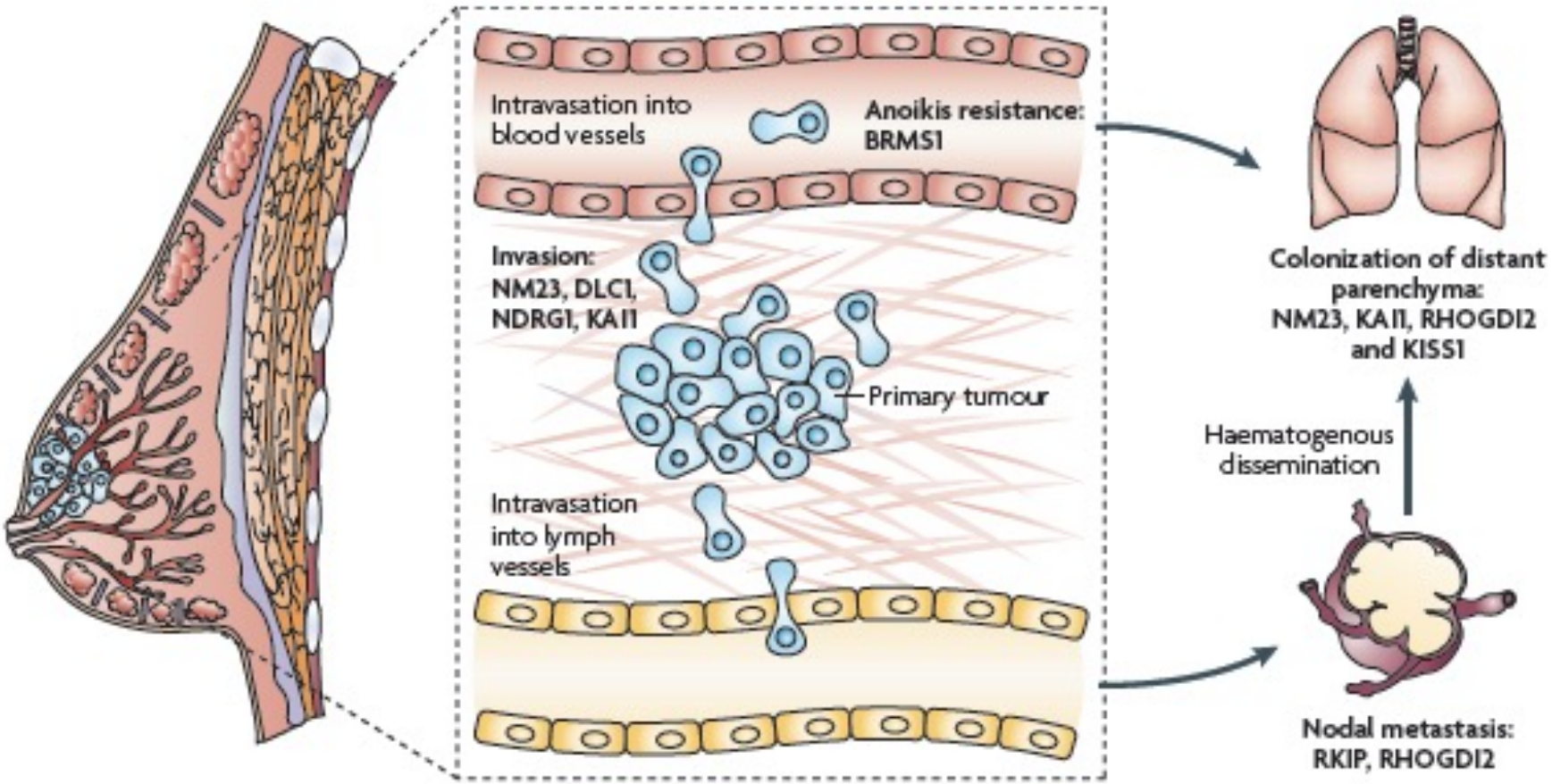
RANKL = receptor activator of NF-κB ligand

SRE = skeletal-related event

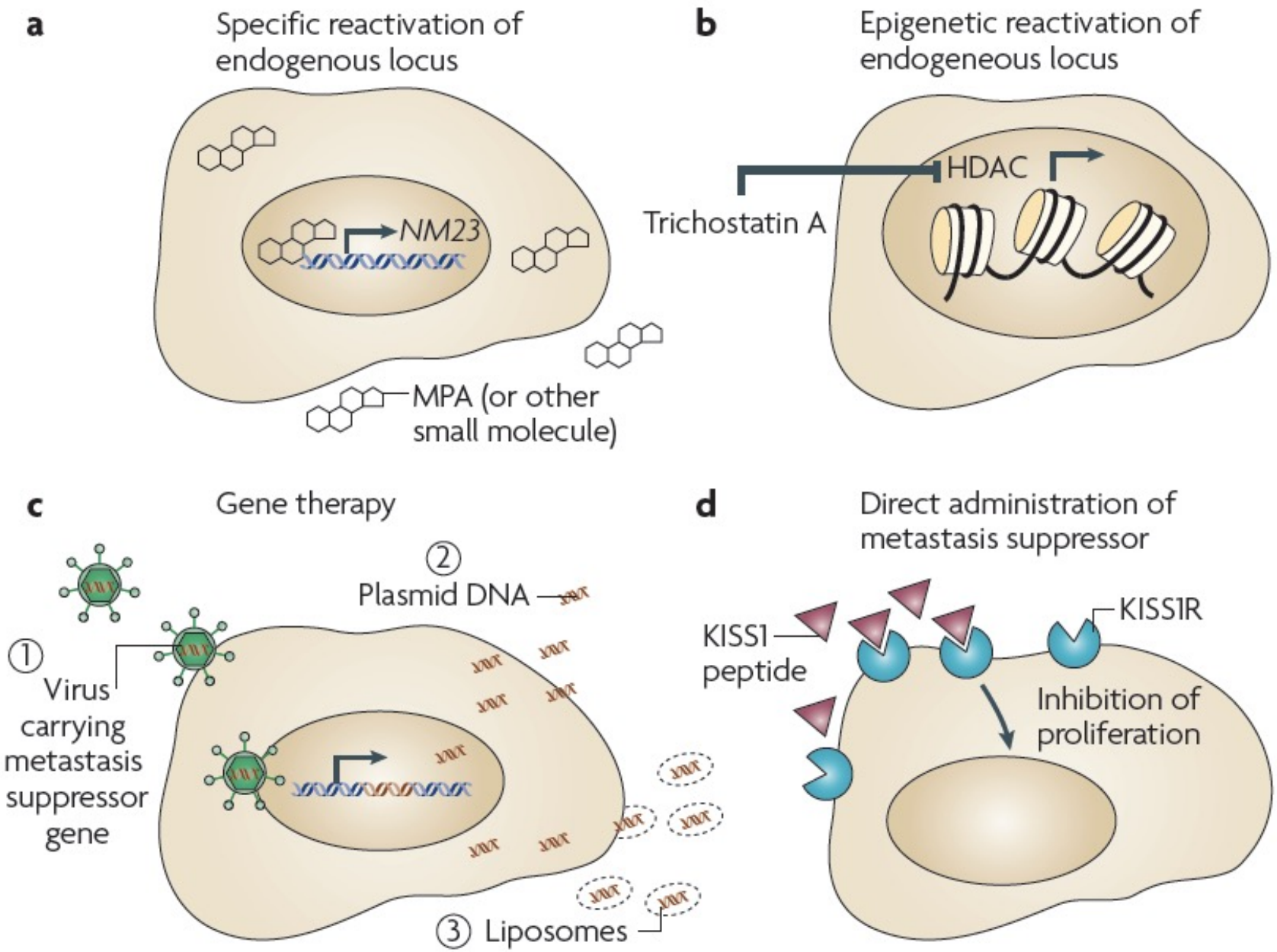
Terapie contro la nicchia endostale



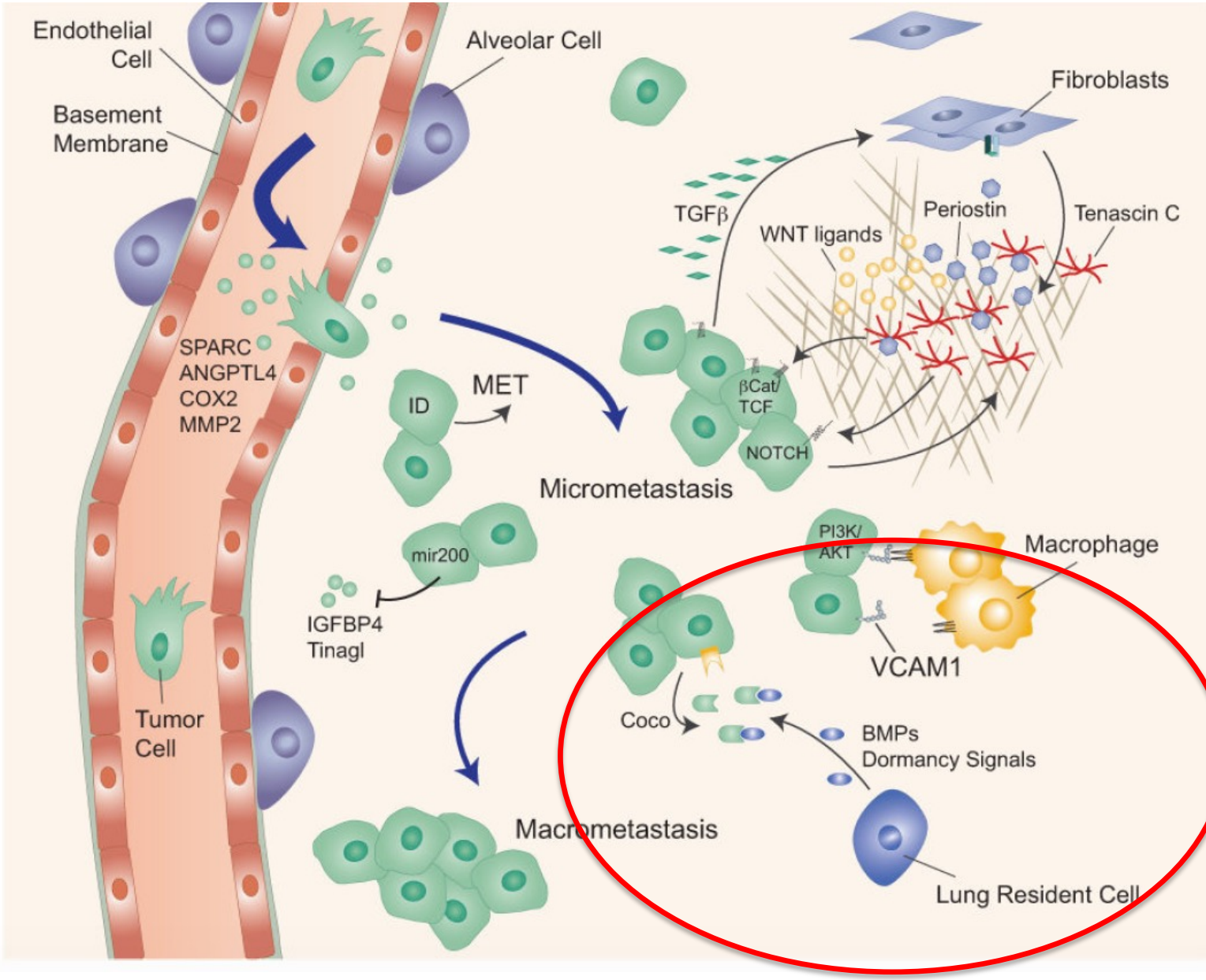
Geni soppressori delle metastasi



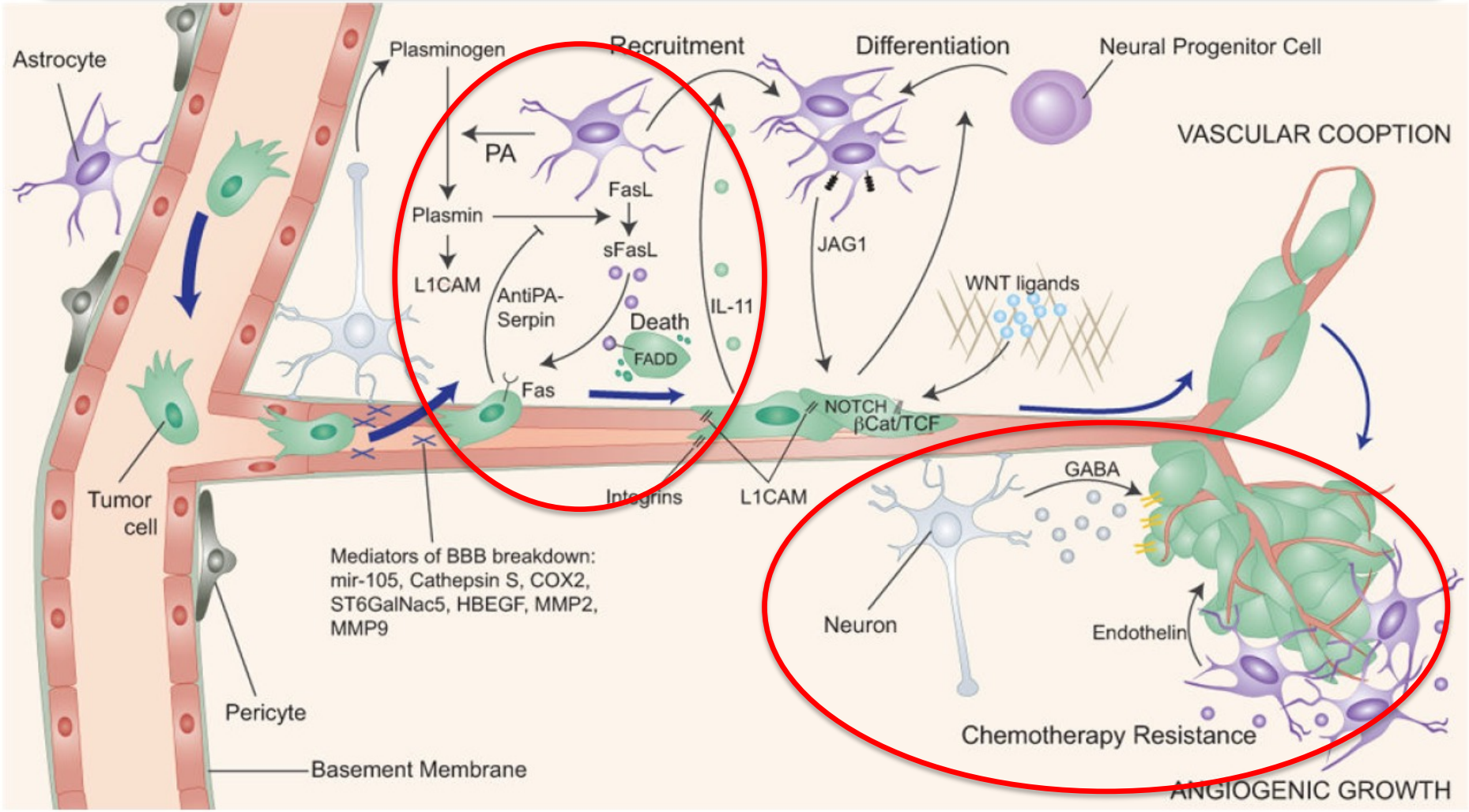
Strategie basate sulla riattivazione dei MS genes



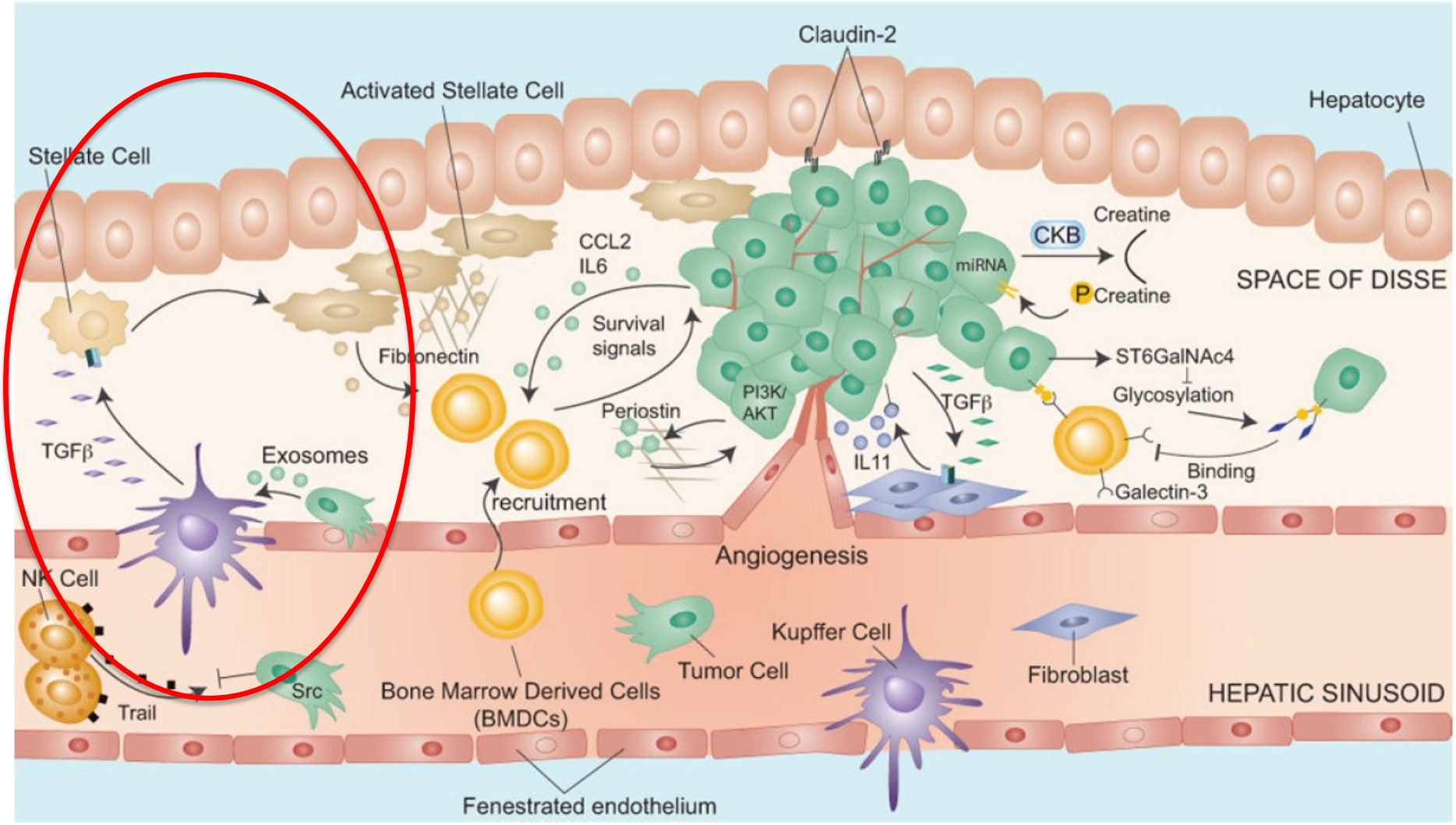
Colonizzazione metastatica del polmone



Colonizzazione metastatica del cervello



Colonizzazione metastatica del fegato



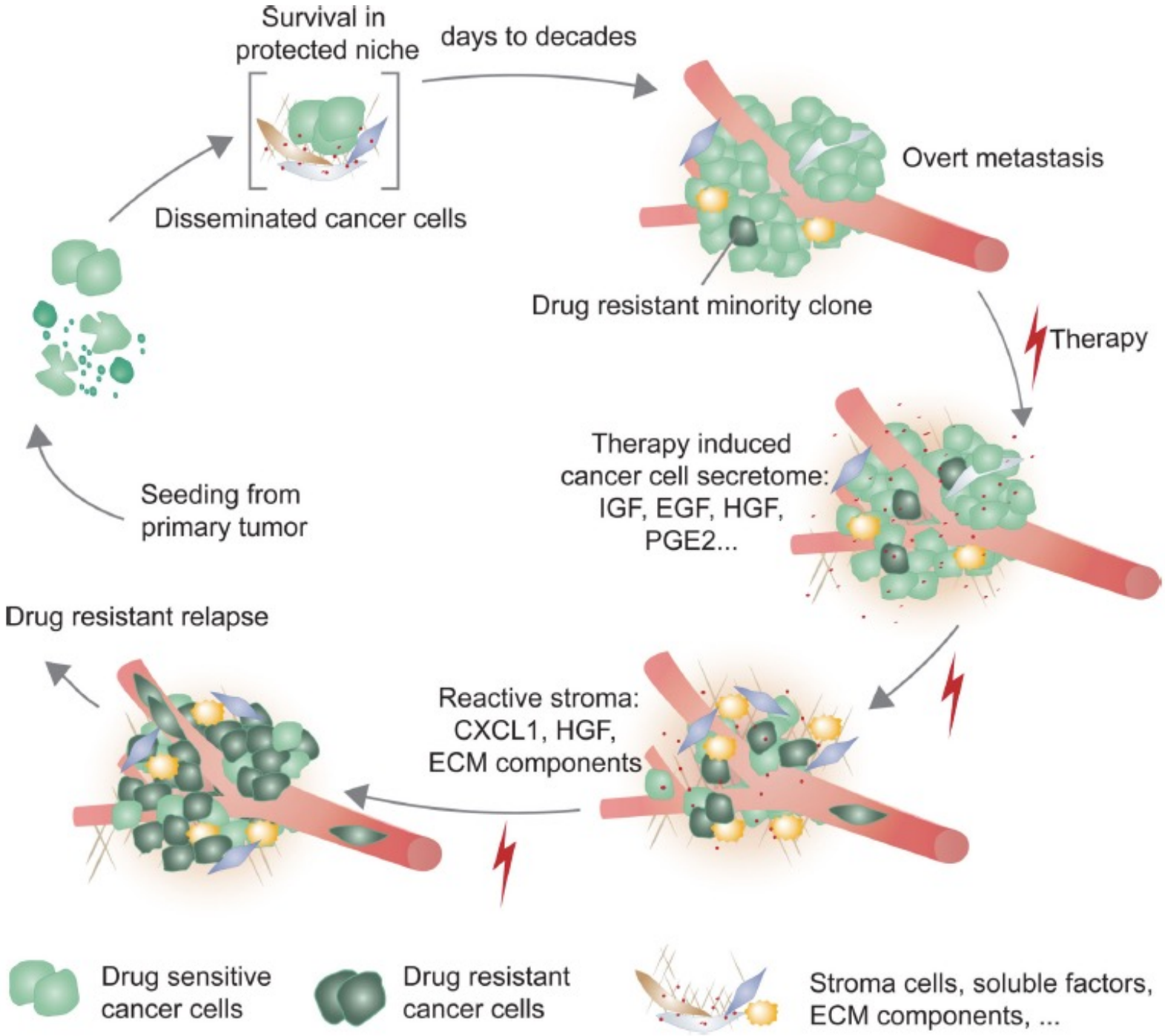
Kupffer cells = liver macrophages Stellate cells = fibrogenic liver-specific mesenchymal cells

MECCANISMI DI CHEMIORESISTENZA NEL PROCESSO METASTATICO

Gli effetti delle terapie sulla Minimal Residual Disease

The surgical removal of a malignant tumour is often complemented with **radiotherapy** and systemic adjuvant chemotherapy to suppress relapse. Unfortunately, **almost all currently deployed cytotoxic therapies preferentially kill proliferating cells** rather than those that have exited the active cell cycle, rendering **dormant cells intrinsically more resistant** to almost all currently available therapies.
Latent metastasis results from conditions that preserve the survival and tumour-initiating ability of disseminated cancer cells. **Eliminating latent metastasis by targeting these survival mechanisms would prevent metastasis.**

If metastasis becomes **clinically manifest**, most systemic treatments target metastasis irrespective of organ site. Treatments include classical chemotherapy, targeted therapy against oncogenic drivers, immunotherapeutic agents that leverage the antitumour power of the immune system, and increasingly, a combination of all of the above.
The treatment may dramatically reduce the metastatic burden, but **tumour elimination is frequently incomplete.**


Survival in protected niche
days to decades
Overt metastasis
Drug resistant minority clone
Therapy
Therapy induced cancer cell secretome: IGF, EGF, HGF, PGE2...
Reactive stroma: CXCL1, HGF, ECM components
Drug resistant relapse
Seeding from primary tumor
Disseminated cancer cells
Drug sensitive cancer cells
Drug resistant cancer cells
Stroma cells, soluble factors, ECM components, ...

Meccanismi paracrini di chemioresistenza

- **Under the stress of targeted therapy, drug-sensitive cancer cells express a large number of secreted factors (therapy-induced secretome) that salvage drug-sensitive cells and accelerate the growth of minority drug-resistant clones.**
- DNA damaging agents induce the secretion of trophic factors including IL-6 and Timp-1 in normal cells of the thymus, creating a **chemoprotective niche** for the survival of residual cancer cells and eventual relapse. In BRAF-mutant melanomas treated with RAF inhibitors, tumor-associated **macrophages** secrete TNF-α and VEGF and tumor-associated **fibroblasts** secrete HGF, which protect the cancer cells and limit the effectiveness of therapy.
- The accelerated growth leads drug-resistant clones to drive relapse as a drug-resistant tumor. The growth and survival mechanisms utilized by residual cancer cells under treatment **might resemble those utilized by their predecessors during the latent phase** before overt colonization in this model.

Effetti pleiotropici dei farmaci antitumorali

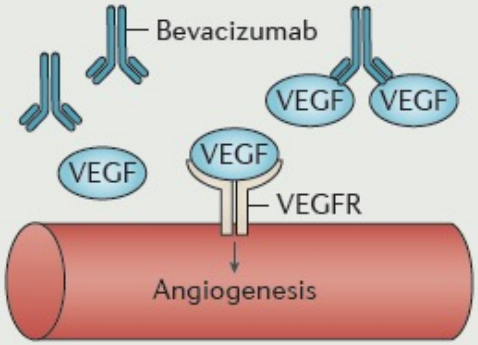
La maggior parte dei farmaci antitumorali approvati dalle agenzie del farmaco (es. EMA, FDA) sono diretti contro alterazioni coinvolte nel processo di tumorigenesi (tumore primario), e vengono testate in sperimentazioni precliniche che valutano l'effetto sul tumore primario e frequentemente anche sulla metastasi.
Nei trials clinici vengono spesso reclutati pazienti con malattia metastatica, valutando la risposta (riduzione del tumore primario), OS e PFS. Questo permette di comprendere (almeno in parte) l'effetto della terapia sulle metastasi.

Molti farmaci antitumorali già approvati hanno mostrato una attività inibitoria sul tumore primario, **ma azione stimolatoria nei confronti della metastasi** (mutant BRAF inhibitors, **paclitaxel**, cisplatin, **anti-androgens**, everolimus e sunitinib). Lo stesso vale per farmaci utilizzati per lenire gli effetti secondari delle terapie, come I **glucocorticoidi**.

È quindi necessario che il processo per l'identificazione e validazione di nuovi farmaci anti-tumorali si basi su **opportuni modelli preclinici** di metastasi e su un **accurato design dei clinical trials** (scelta opportuna dell'endpoint).

Limitazioni del processo di drug development

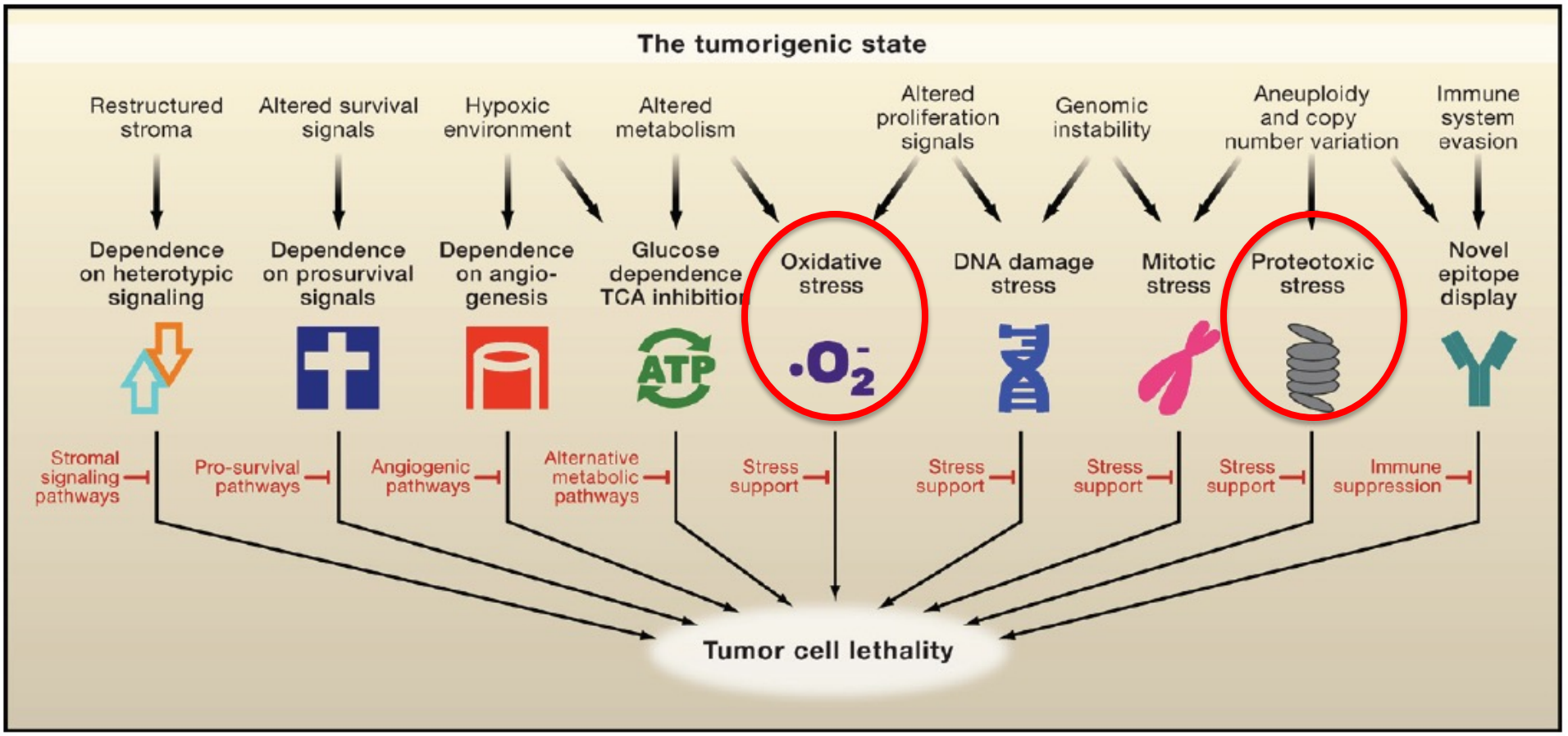
Table 1 | **Preclinical and clinical history of four metastasis-directed drug development efforts**

Description	Pathway	Preclinical validation	Pivotal trials and end points	Outcomes
Bevacizumab				
Monoclonal antibody to VEGF		• Bevacizumab inhibited corneal angiogenesis and lymphangiogenesis[244] • In multiple cancer xenograft models, bevacizumab reduced primary tumour growth rates and, in some studies, enhanced survival. Reduced angiogenesis and vessel normalization was observed[245] • Prevention or, less frequently, abrogation of metastasis[246,247]	• Recurrent ovarian cancer, PFS[35,36] • Metastatic colorectal cancer, OS[260,261] • Metastatic or resistant HER2+ breast cancer, PFS[38] • Metastatic renal cancer, PFS[262] • Glioblastoma, OS, PFS[263] • Advanced lung cancer, OS[37] • Adjuvant therapy in triple-negative breast cancer, DFS[41]	• FDA approved for resistant ovarian, cervical and colorectal cancers, glioblastoma, also advanced or metastatic lung, colorectal and renal cancers • Revoked for metastatic breast cancer • Negative trials for first-line treatment of glioblastoma

Limitations identified in other drug development efforts include an inadequate understanding of the molecular pathway in metastatic colonization, **poor drug characteristics**, **overinterpretation** of early-phase trial data, a preclinical focus on effects on the primary tumour and the **wrong trial design**.

Steeg, Nat Rev Cancer 2016

Targeting non-oncogene addiction mechanisms



Screening guidati da un processo, pathway, o bersaglio molecolare con cui si vuole interferire

Recently, new informatic techniques have been developed to extrapolate drug efficacy from cell line model systems to patient outcomes.
Advanced informatic technologies including the Connectivity Map allow for screening an entire gene expression signature against gene expression changes induced by compounds, **systematically identifying molecules capable of inducing non-metastatic gene expression** and phenotype.
The list of hits is then further filtered using (e.g.) the COXEN algorithm, which provides a list of those suppressor signature-inducing agents that additionally have therapeutic efficacy in the NCI-60 cell line system and, most importantly, predicts function in patient tumours.
Together, this integrated approach could deliver therapeutics based on metastasis suppressor biology to the clinic with a high likelihood of efficacy.

Firme molecolari

CMAP

Small-molecule hits

NCI-60 cell line panel

Connectivity map genera una lista di molecole che inducono una firma molecolare (es. di dormienza/regressione metastatica etc.)

Human tumour samples

Algoritmo di predizione

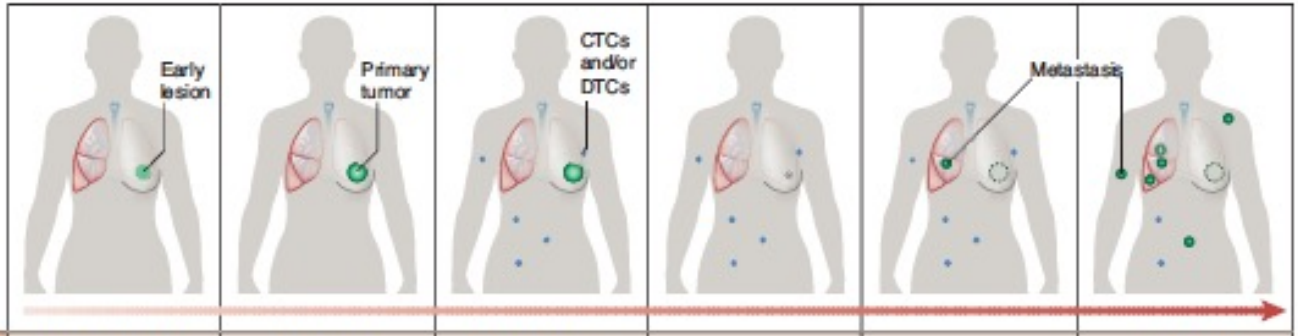
Identification of hits that are likely effective in specific human cancers

Evaluation of hits in cancer cell lines of specific histology

Xenograft experiments to test efficacy and pharmacokinetics

Clinical trials

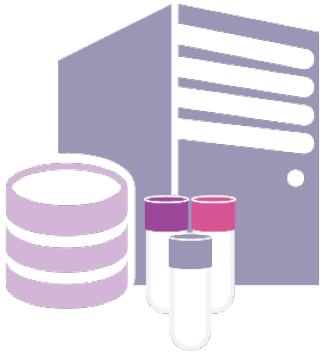
Sfide e strategie per il trattamento dei tumori metastatici



Status	Pre-neoplasm Subclinical	Primary (–) CTCs and/or DTCs	Primary (+) CTCs and/or DTCs	Dormancy	Oligometastases	Systemic metastases
Focus	Management of primary tumor		Prevention of metastasis		Treatment of metastasis	
Challenge	Early detection and prevention Identify high-risk patients		Prevent local and distant relapse Drug resistance of DTCs		Early detection of relapse Heterogeneity and drug resistance	
New tools	Diagnostic markers	Prognostic markers	Profiling of primary tumor, metastases, CTCs and/or DTCs for accurate targeting Biomarkers and imaging technologies for disease monitoring Biomarkers for therapeutic efficacy			
Possible treatment strategies	Prophylactic treatment Vaccination	Surgery, radiotherapy (+) Systemic therapy		Targeted therapy against driver oncogenes and their pathways tailored by genetic makeup of tumor cells		
			Long-term adjuvant treatment (for high-risk patients): • Metronomic chemotherapy and anti-angiogenesis • Targeting common driver oncogenes and pathways • Immunotherapy • Targeting dormancy-related survival and CSC signaling and niche components		Systemic therapy Immunotherapy Stroma-targeting treatments Palliative radiation and/or surgery	
					Surgery stereotactic radiotherapy	
Possible new targets		DTC and/or CTC survival pathways; stem cell features; tumor-stroma crosstalk and niche factors Activation of metastasis-suppressive signaling				

Debbie Maizels

Wan, Pantel & Kang, Nat Med 2013



Raccolta e caratterizzazione molecolare di organoidi tumorali

Approcci innovativi di ricerca di base e traslazionale

Integrare le conoscenze

IDENTIFICARE BERSAGLI TUMORALI PER TERAPIE MIRATE

IDENTIFICARE E VALIDARE NUOVE TERAPIE

IDENTIFICARE BIOMARCATORI PER LA DIAGNOSI E LA PREDIZIONE DELLA RISPOSTA ALLE TERAPIE (companion diagnostic)