Corso di Biologia Cellulare del Cancro AA 2019-2020

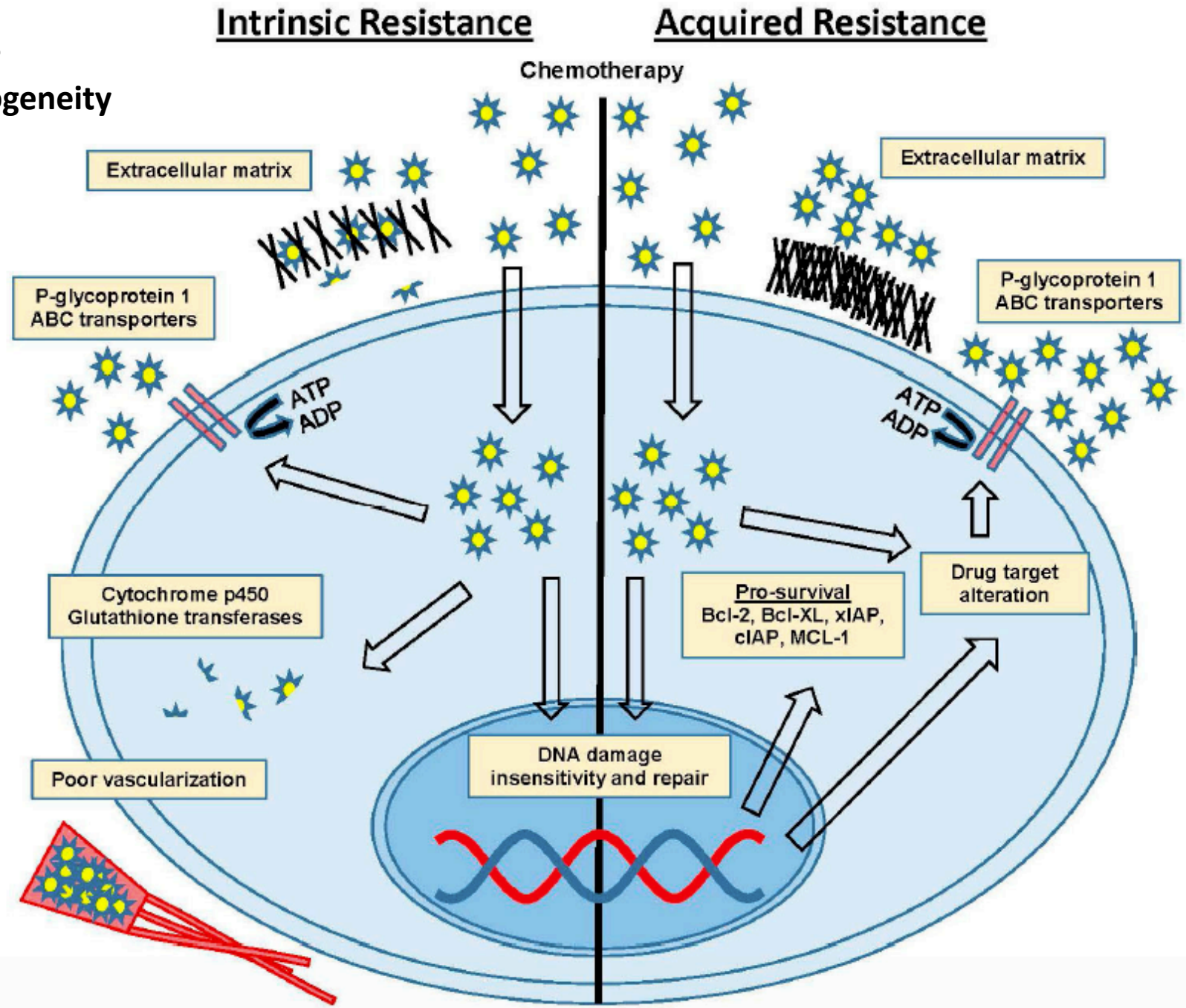
LE TERAPIE ANTITUMORALI NEL PROCESSO METASTATICO

Gli effetti delle terapie sulla Minimal Residual Disease

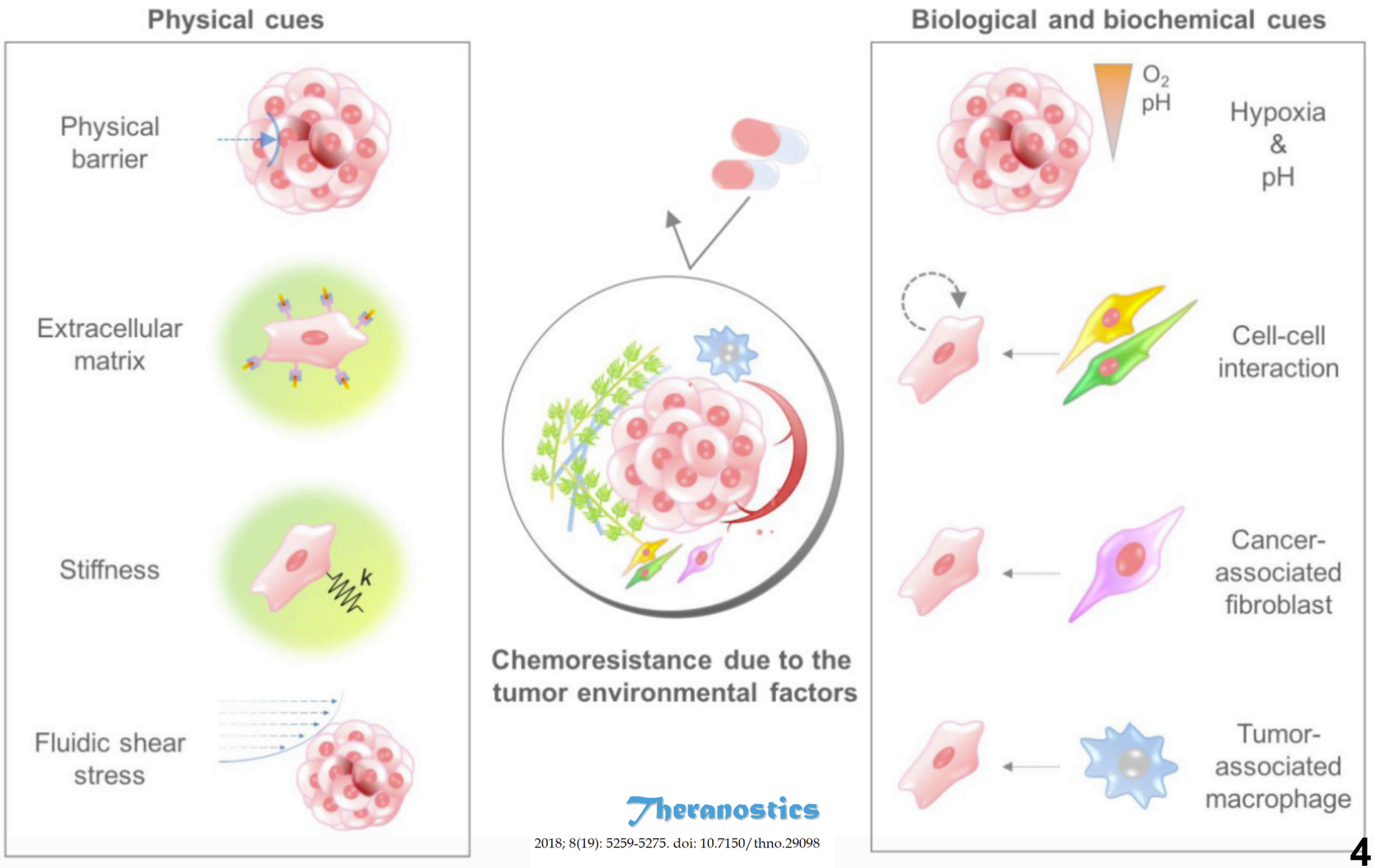
The surgical removal of a malignant tumour is often complemented with **radiotherapy** and systemic adjuvant chemotherapy to suppress relapse. Unfortunately, **almost all currently deployed cytotoxic therapies preferentially kill proliferating cells** rather than those that have exited the active cell 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.**

Tumor heterogeneity



Ruoli del microambiente nella chemioresistenza

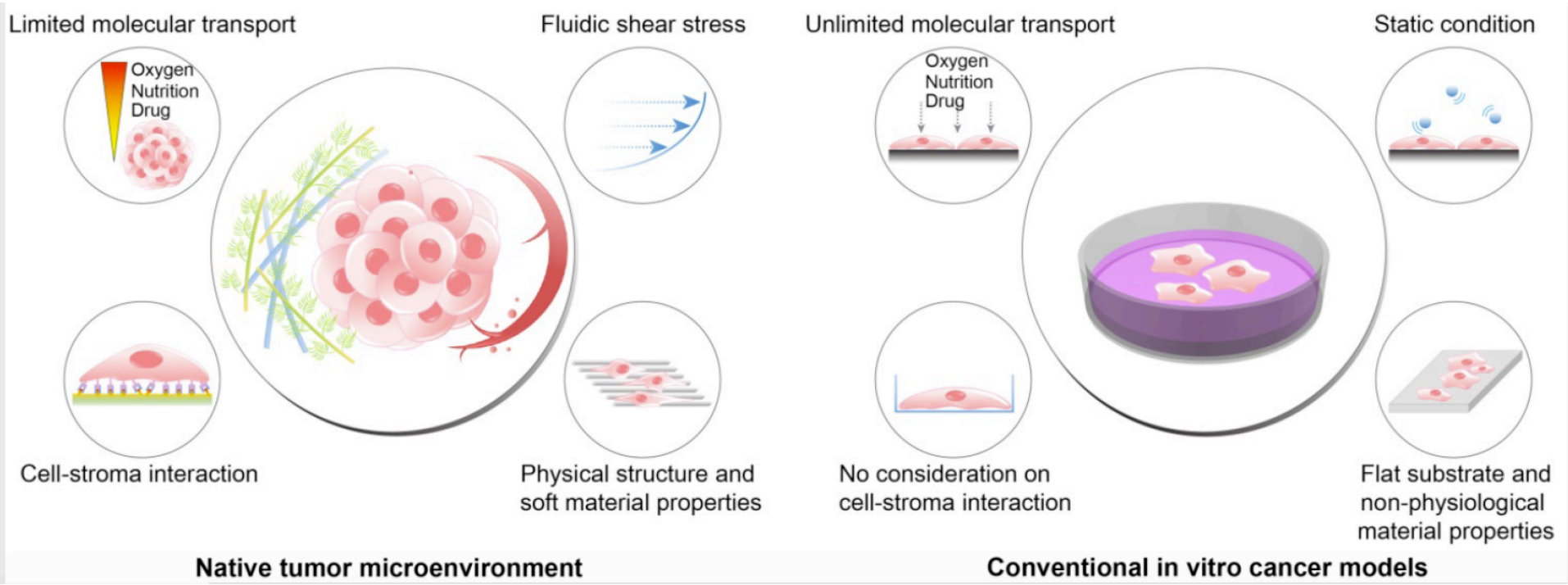


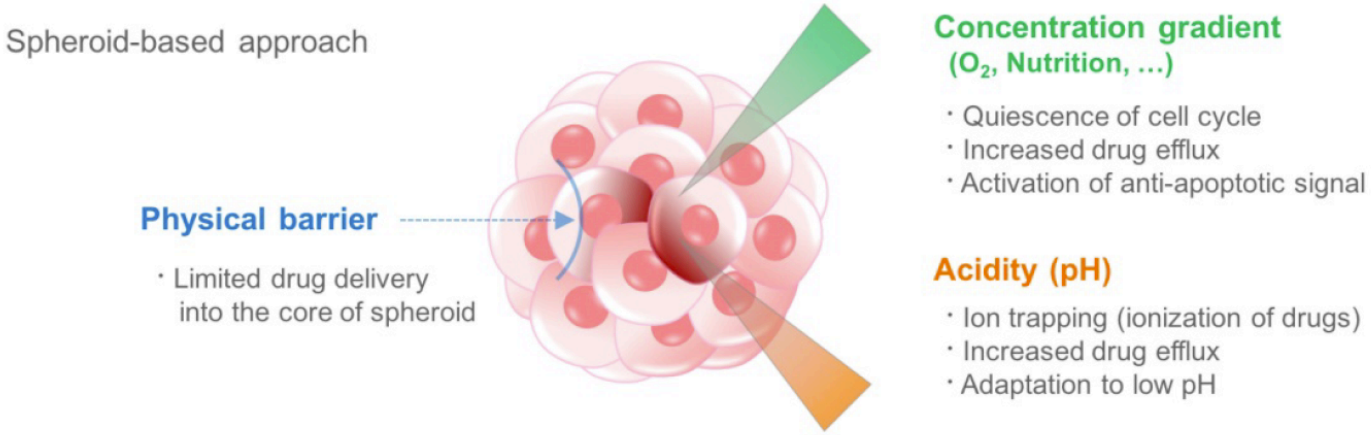
Theranostics
2018; 8(19): 5259-5275. doi: 10.7150/thno.29098

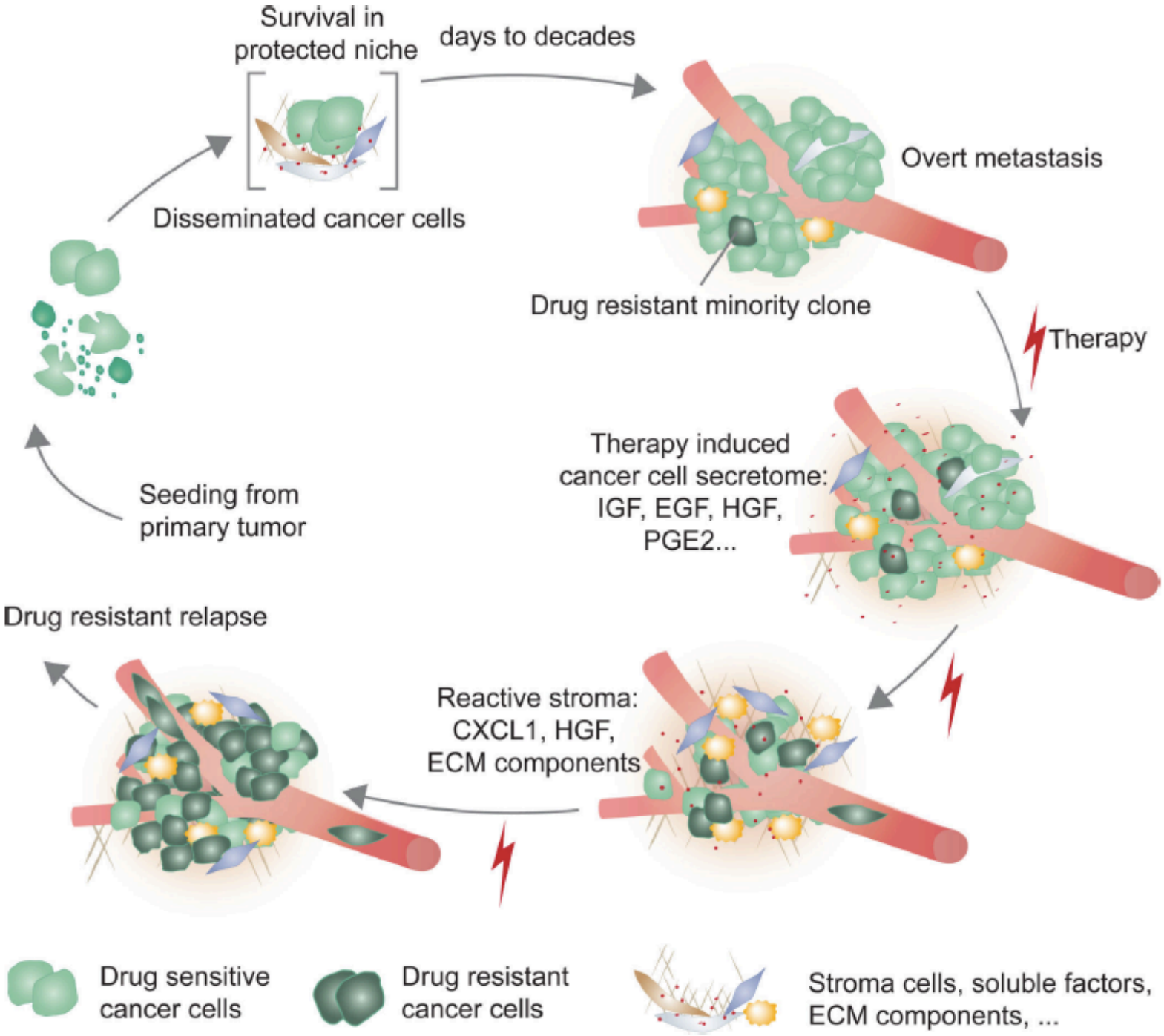
Ruoli del microambiente nella chemioresistenza

Tumor microenvironmental factors	Mechanism	Results
Physical cues		
Physical barrier	Limited penetration of drugs or drug carriers	Unable to deliver drugs into the core of the tumor mass
	Changed interstitial fluid flow	Outward fluid flux from the tumor mass to the surroundings
ECM	Cell adhesion mediated drug resistance (CAM-DR)	Activation of anti-apoptotic signals by integrin-mediated ligand-receptor binding
	Porosity	Diffusion-limited molecular transport
Stiffness	Matrix stiffness-induced mechanotransduction	Matrix stiffness-mediated induction of mechanotransduction pathways such as YAP and TAZ
Fluidic shear stress	Flow-mediated activation of autocrine signaling (IGF-1R pathway)	Increased IGF-1 release in response to increasing fluidic shear stress (feed-forward loop)
	Caspase pathway-dependent receptor-mediated apoptosis (tumor necrosis factor apoptosis-inducing ligand, TRAIL)	TRAIL-induced apoptosis observed only under the fluidic shear stress condition
	PI3K/Akt signaling and microRNA-199-3p	Chemoresistance to cisplatin and paclitaxel under the fluidic shear stress condition
Biological and biochemical cues		
Hypoxia	Quiescence of cancer cells (nonproliferating or slow cell cycle)	Decreased cell death against anti-proliferating agents
	HIF-1 mediated enhancement of drug efflux	Decreased intracellular concentration of drugs
	HIF-1 mediated enhancement of antiapoptotic signals	Avoiding necrotic or apoptotic cell death
pH	P-glycoprotein-mediated drug efflux	Enhanced activity of the drug efflux pump in the acidic microenvironment
	Ion trapping	Reduced cell permeability of positively ionized weak base drugs in the acidic environment
	Chronic exposure to acid pH	Increased expression of heat shock protein HSP27 levels in tumor cells causing chemoresistance to cisplatin
Cell-cell interaction	Cytokines secreted by nearby cells	Autocrine and paracrine-mediated activation of antiapoptosis signaling
	Heterocellular interaction (stromal cell-cancer cell)	Trogocytosis-mediated chemoresistance
Cancer-associated fibroblast (CAF)	Cytokines secreted by CAFs	Chemoresistance of cancer cells by CAF-secreted cytokines such as interleukins, CCL1, and SDF-1
	Exosome-mediated miRNA delivery from CAFs to cancer cells	Acquired chemoresistance via transferred miRNA such as miR-155, 100, 222, 30a, and 146a
	Changed metabolism of CAFs by effector T-cells	Abrogated stroma-mediated chemoresistance in cancer cells
Tumor-associated macrophage (TAM)	Secretion of cytokines by TAM in an M2 polarization state	Activation of anti-apoptotic signals in the cancer cells

Modelli cellulari 3D per lo screening di farmaci






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

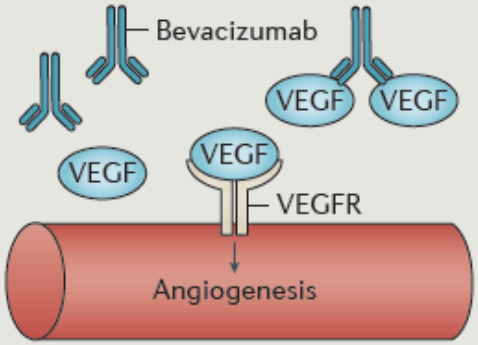
La maggior parte dei farmaci antitumorali approvati dalle agenzie del farmaco (es. EMA, FDA) sono diretti contro alterazioni coinvolte nel processo di tumorigenesi (tumore primario), e vengono testate in sperimentazioni precliniche che valutano l'effetto sul tumore primario e frequentemente anche sulla 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

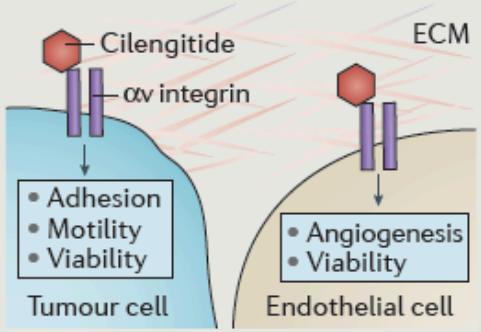
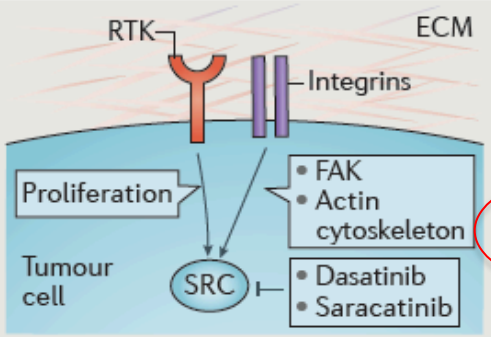
Table 1 | **Preclinical and clinical history of four metastasis-directed drug development efforts**

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

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

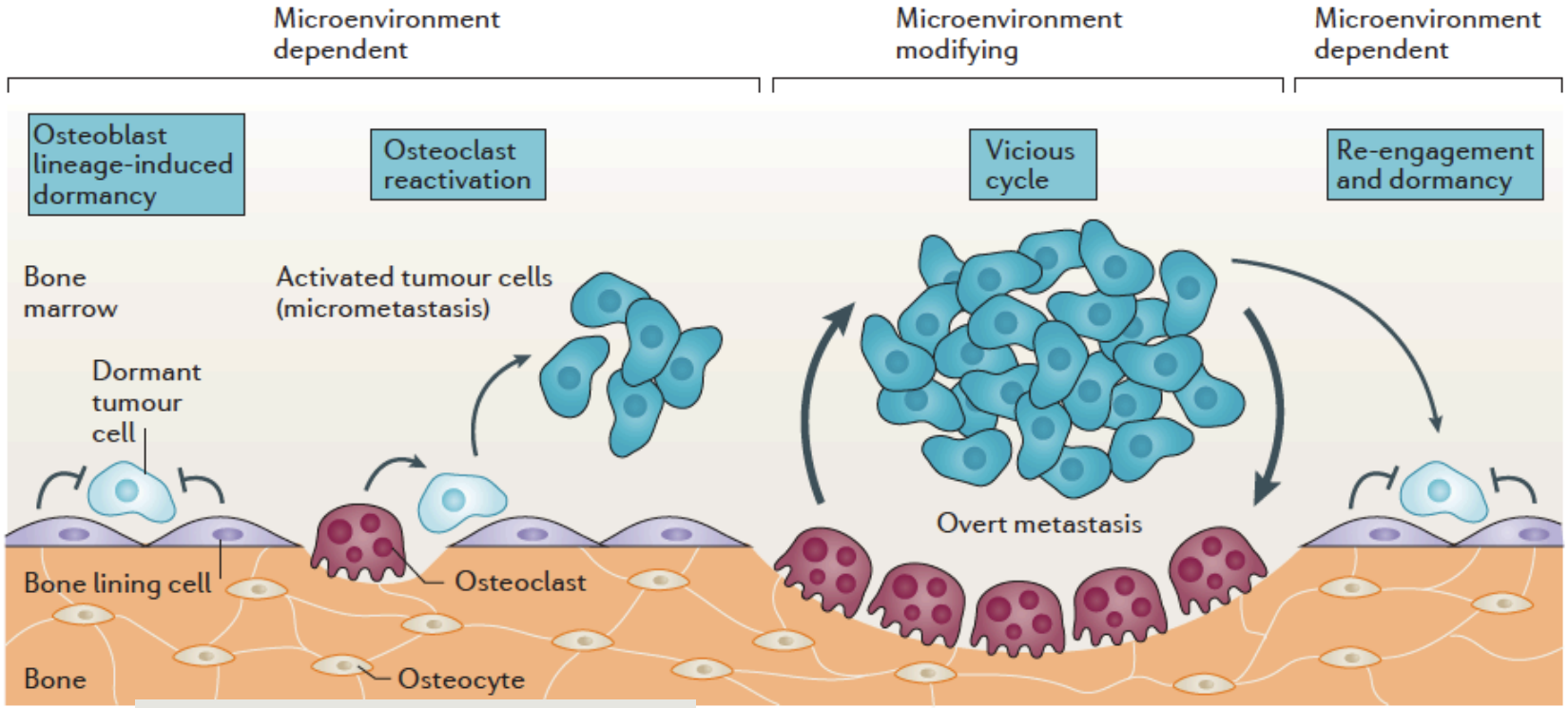
Description	Pathway	Preclinical validation	Pivotal trials and end points	Outcomes
Cilengitide				
αvβ3 and αvβ5 integrin peptide inhibitor		• Stabilization of glioma growth and angiogenesis. Synergistic inhibition of glioma with TMZ[61–64] • Synergy with therapeutics in melanoma primary tumour growth[63], synergy with radio-immunotherapy in breast cancer tumour growth[248] • Inhibition of metastasis[62] • Synergy with verapamil increased angiogenesis and reduced metastasis[249]	• Phase III CENTRIC EORTC, with radiation therapy and TMZ, for glioma, OS. Newly diagnosed glioma, same combination, recurrence[65] • Phase II trials in melanoma and lung and prostate cancers, PFS[66–68]	All advanced trials were negative
Dasatinib and saracatenib				
SRC kinase and BCR–ABL kinase inhibitor		• Inhibition of CML models[250] • Inhibition of primary tumour growth in multiple model systems, as monotherapy or in combination[251–253] • Prevention of metastasis in multiple cancer model systems[254–258], but not osteosarcoma[259] • Inhibition of prostate cancer growing in bone and bone remodelling[82,83]	• Cytogenetic response end points for CML • Response for advanced solid tumours[71–80] • OS in Phase III prostate cancer[87]	• FDA approved for CML and resistant ALL • Discontinued in advanced lung, ovarian, colorectal and breast cancers • Negative in prostate cancer Phase III trial with docetaxel • Multiple adjuvant trials terminated

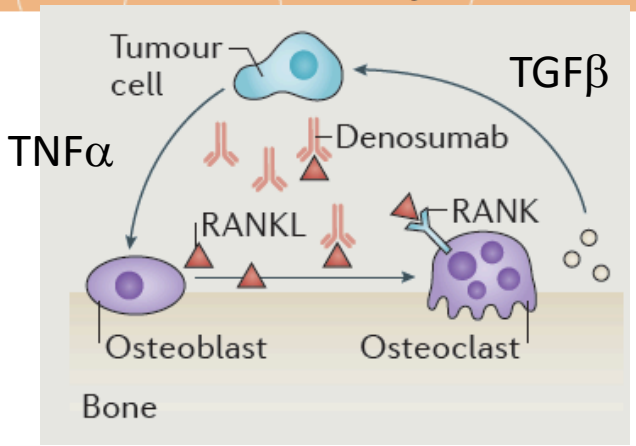
Dovuto a caratteristiche farmacologiche!

the overwhelming majority of the preclinical data indicated a **prevention** of metastasis, **not a shrinkage** of overt lesions.
This would be tested in an adjuvant trial.

Steeg, Nat Rev Cancer 2016

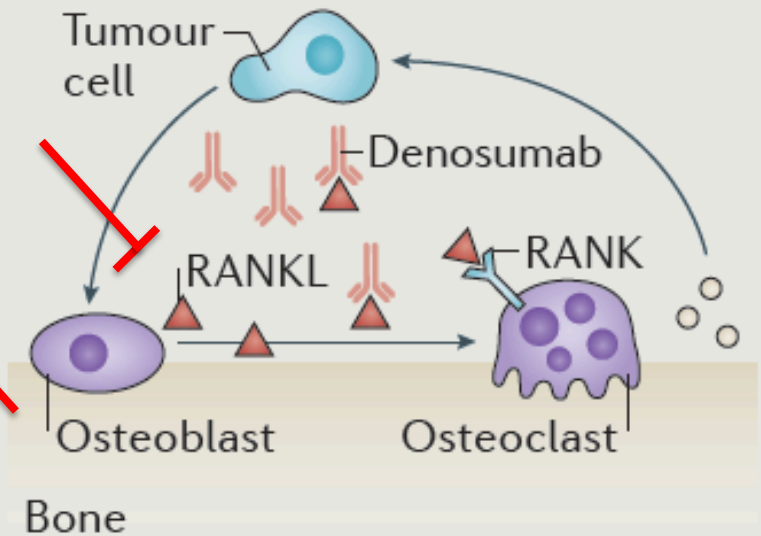
Colonizzazione della nicchia endostale: il circolo vizioso delle mestastasi osteolitiche nel BC





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

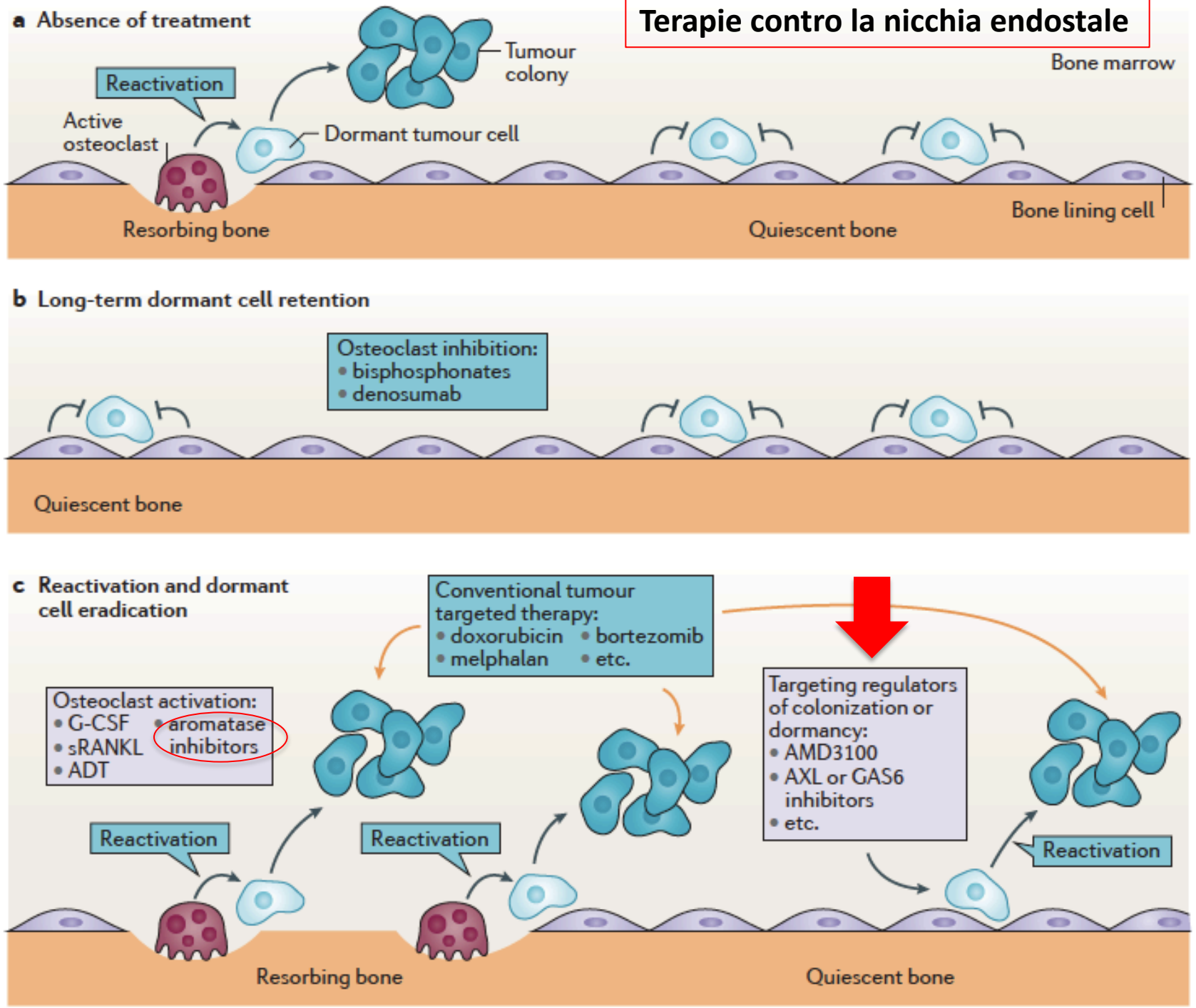
Rallenta la comparsa di metastasi ossee

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

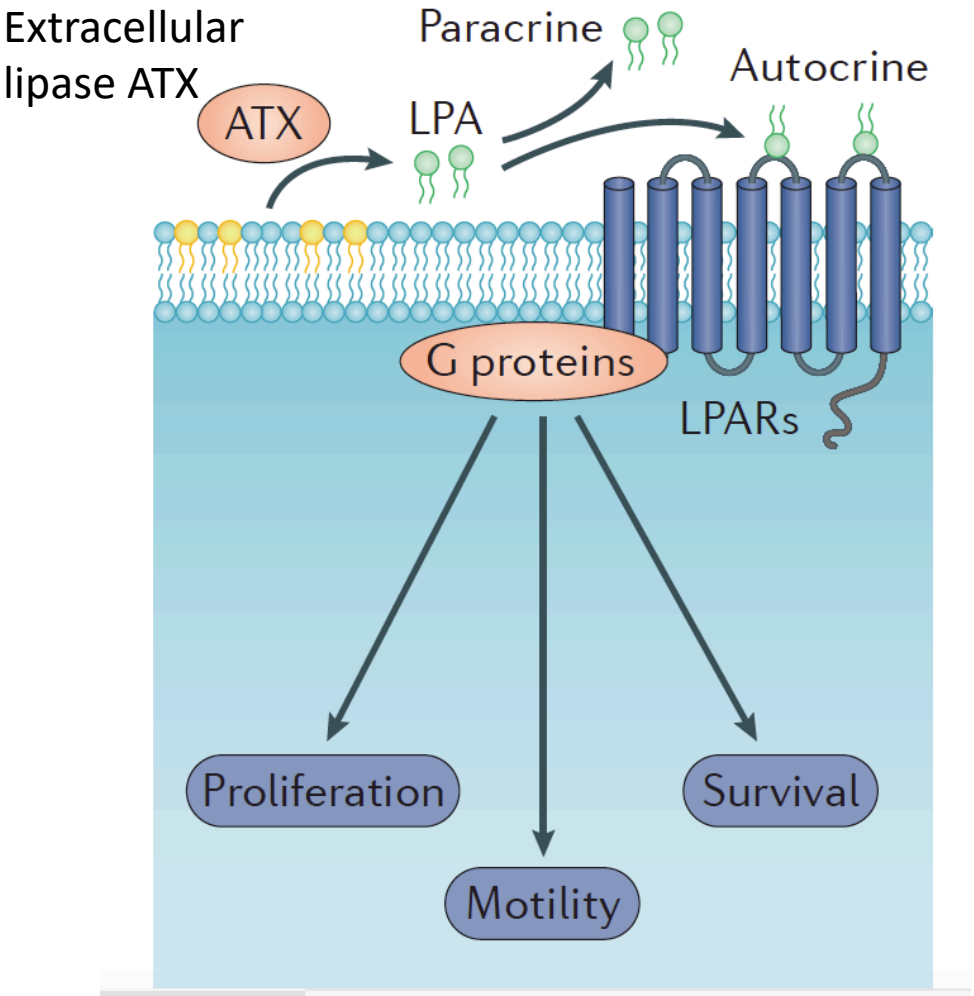


Nuovi targets

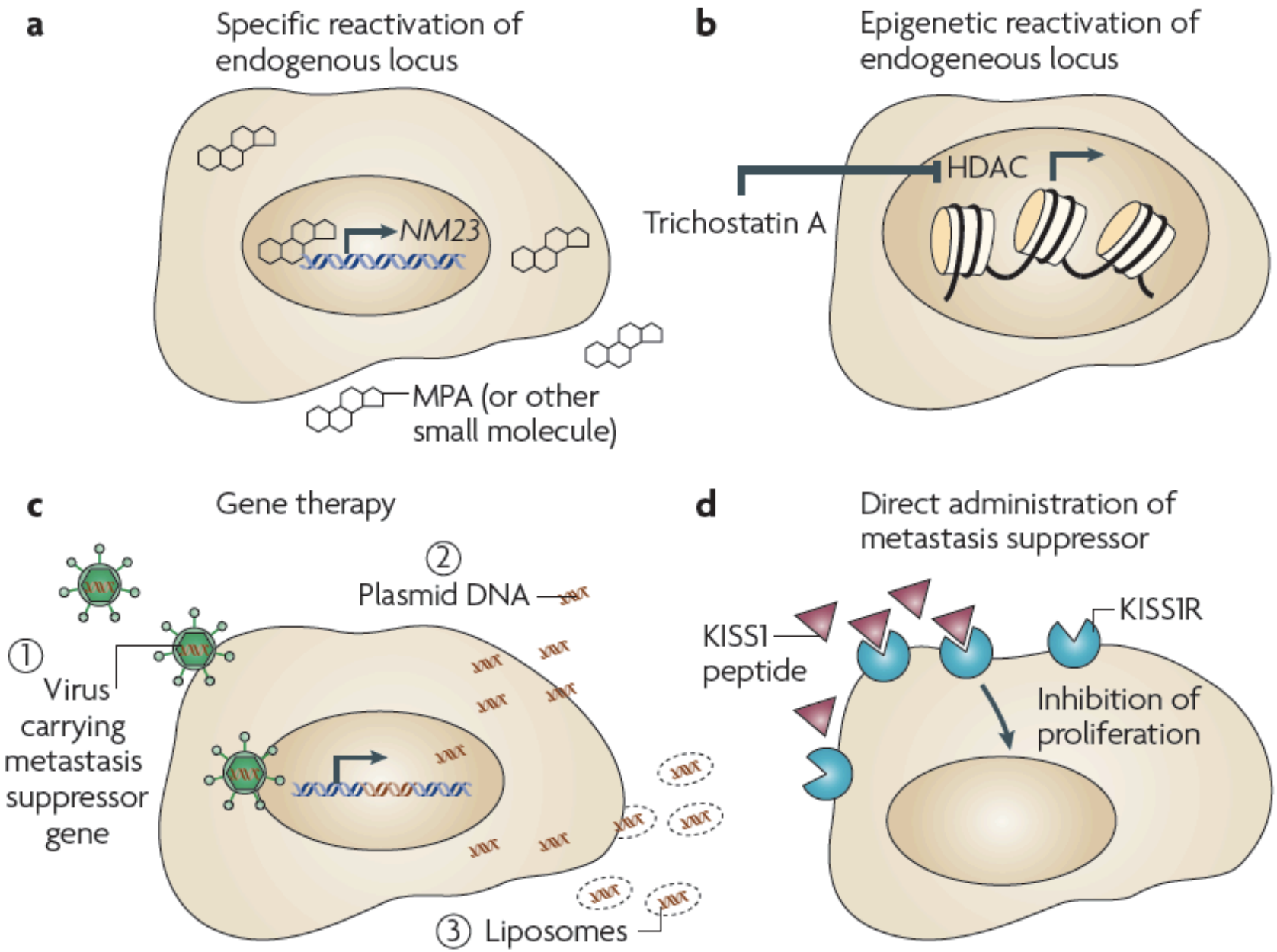
La finestra terapeutica più attraente è quella della colonizzazione

DORMANCY INDUCERS:

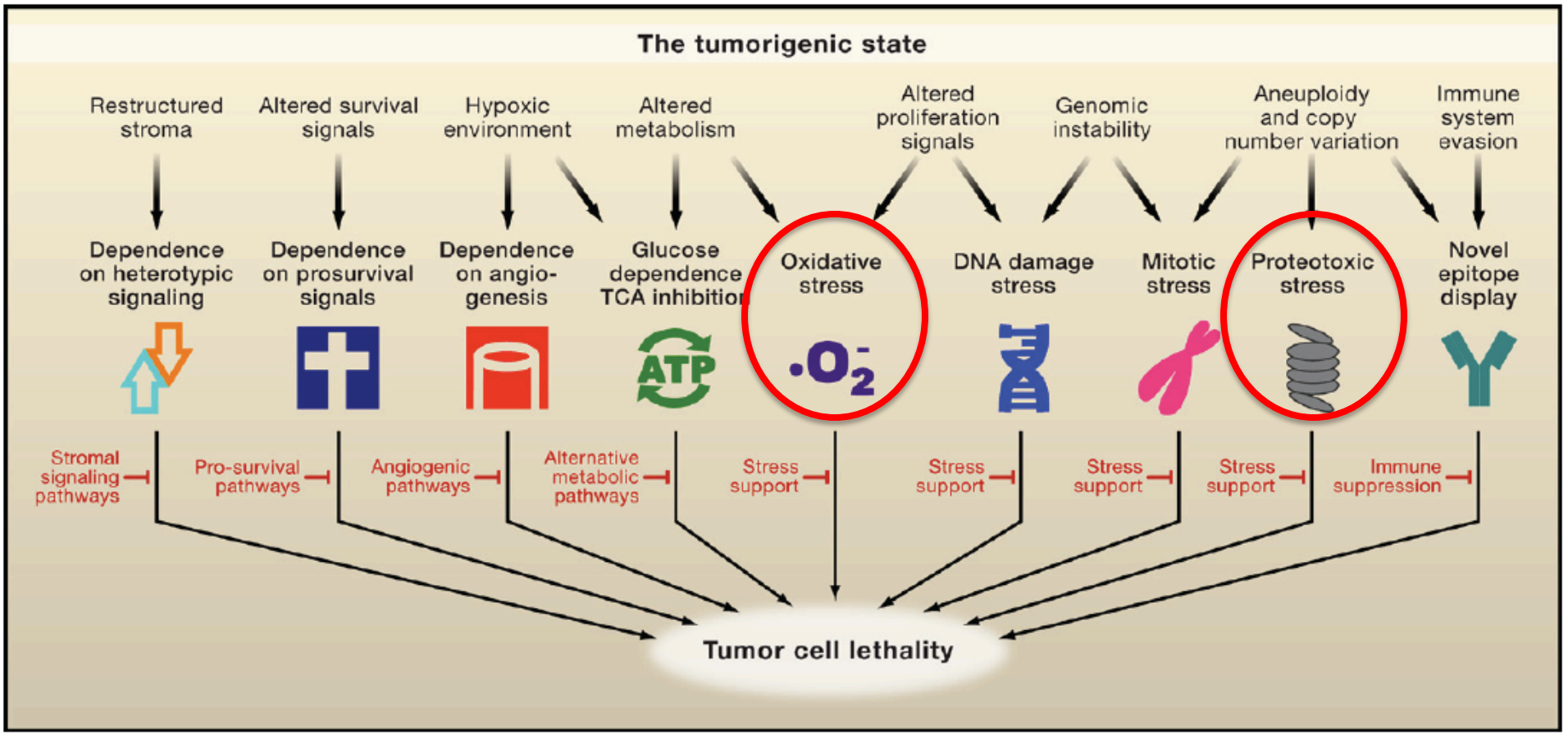
small-molecule inhibitor del recettore 1 dell'acido lisofosfatidico (LPAR1) previene la crescita metastatica e induce l'entrata delle DTCs in uno stato di dormienza (studi preclinici)



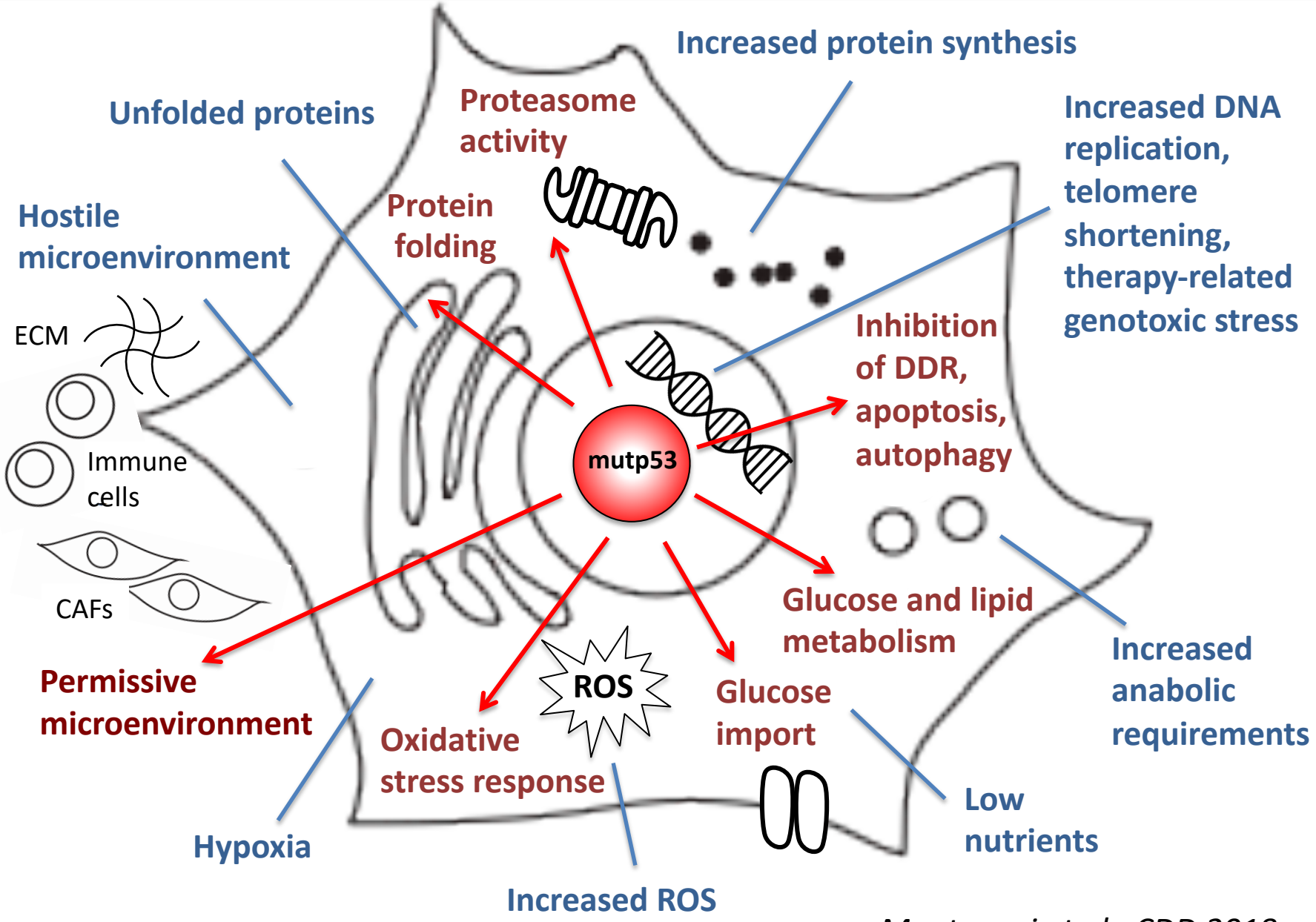
Strategie basate sulla riattivazione dei MS genes



Targeting non-oncogene addiction mechanisms

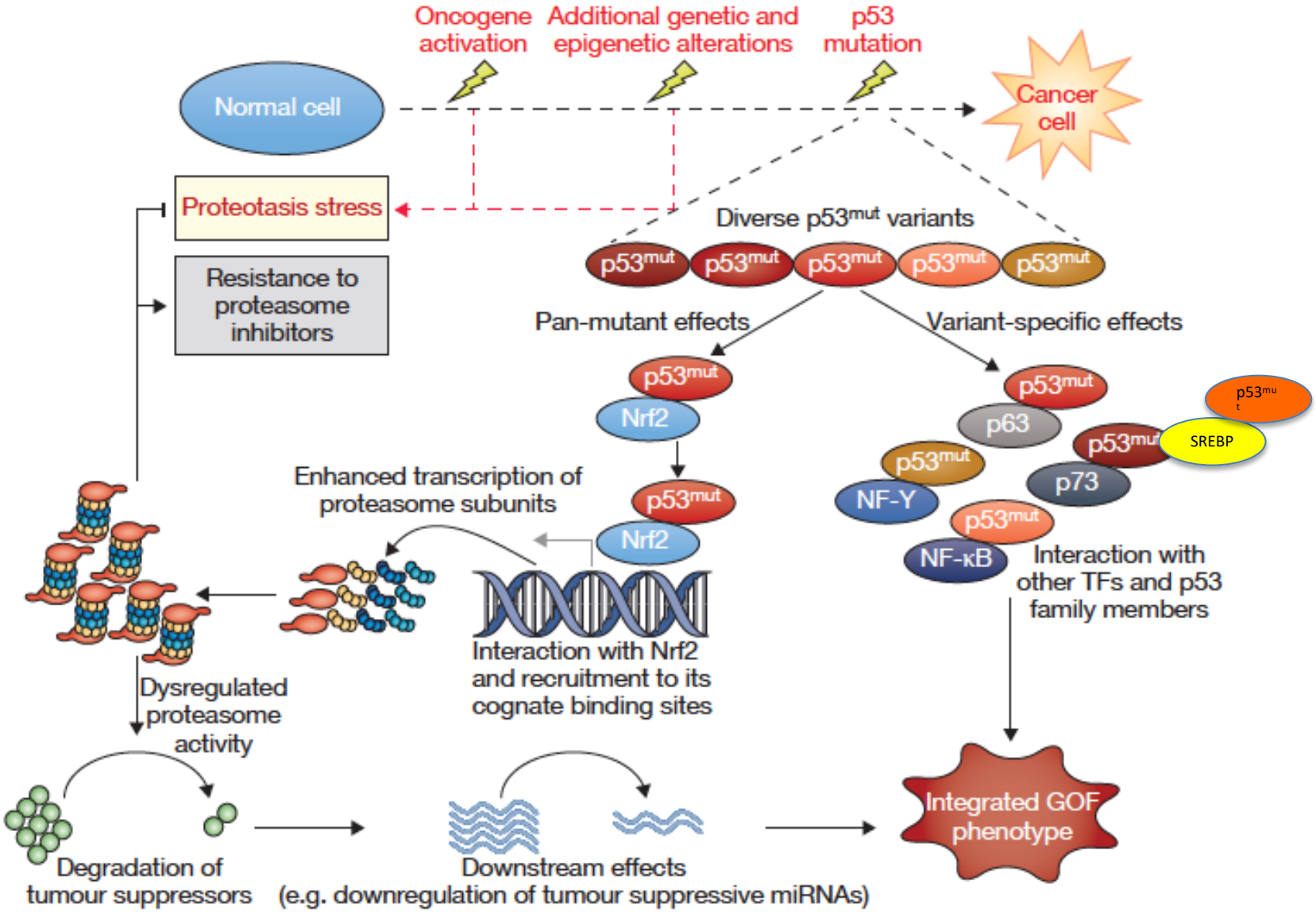


Adaptive responses induced by mutant p53 support tumor progression.



Mantovani et al., CDD 2018

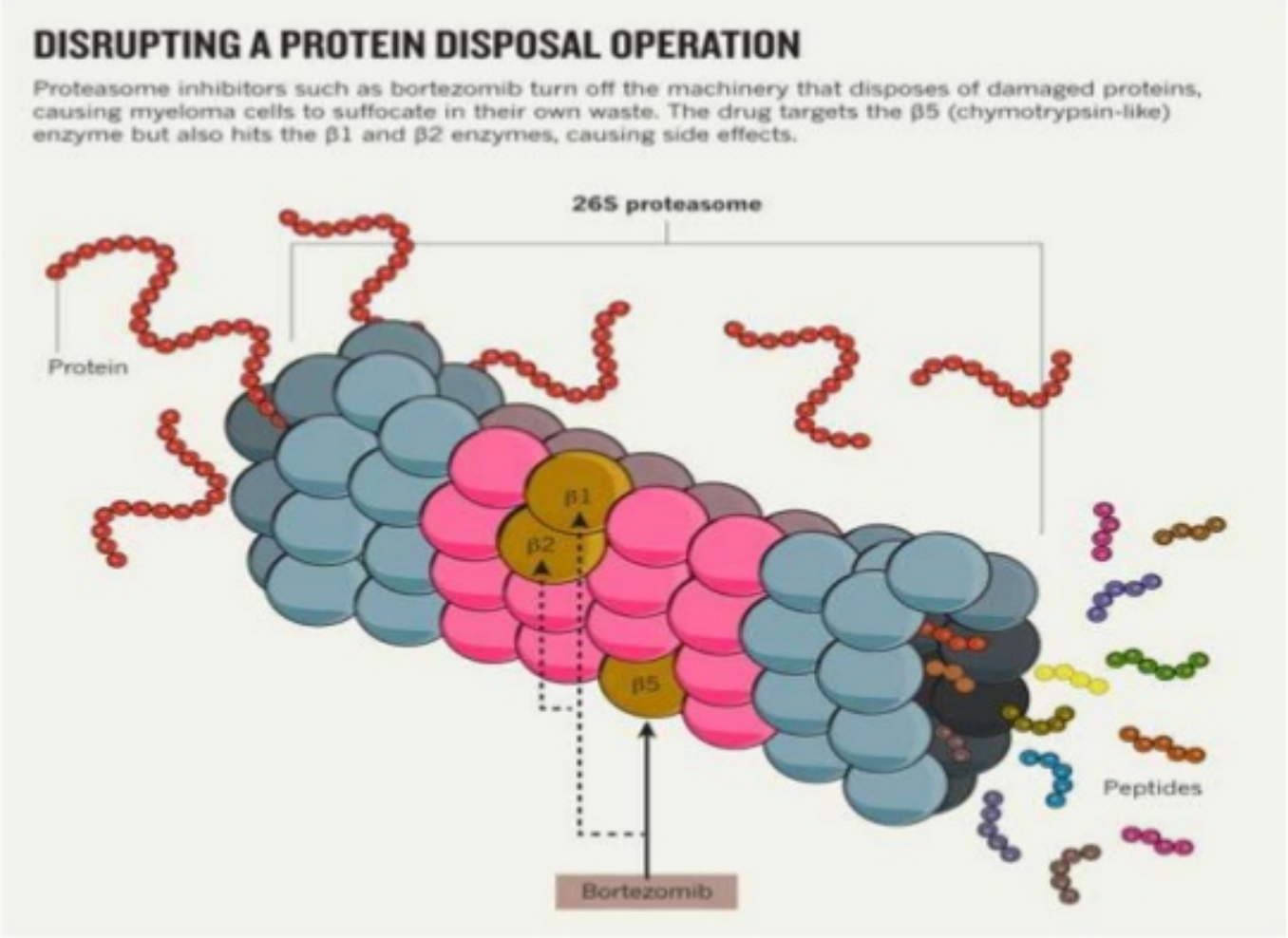
Mutp53 enhances proteasome activity to cope with proteotoxic stress



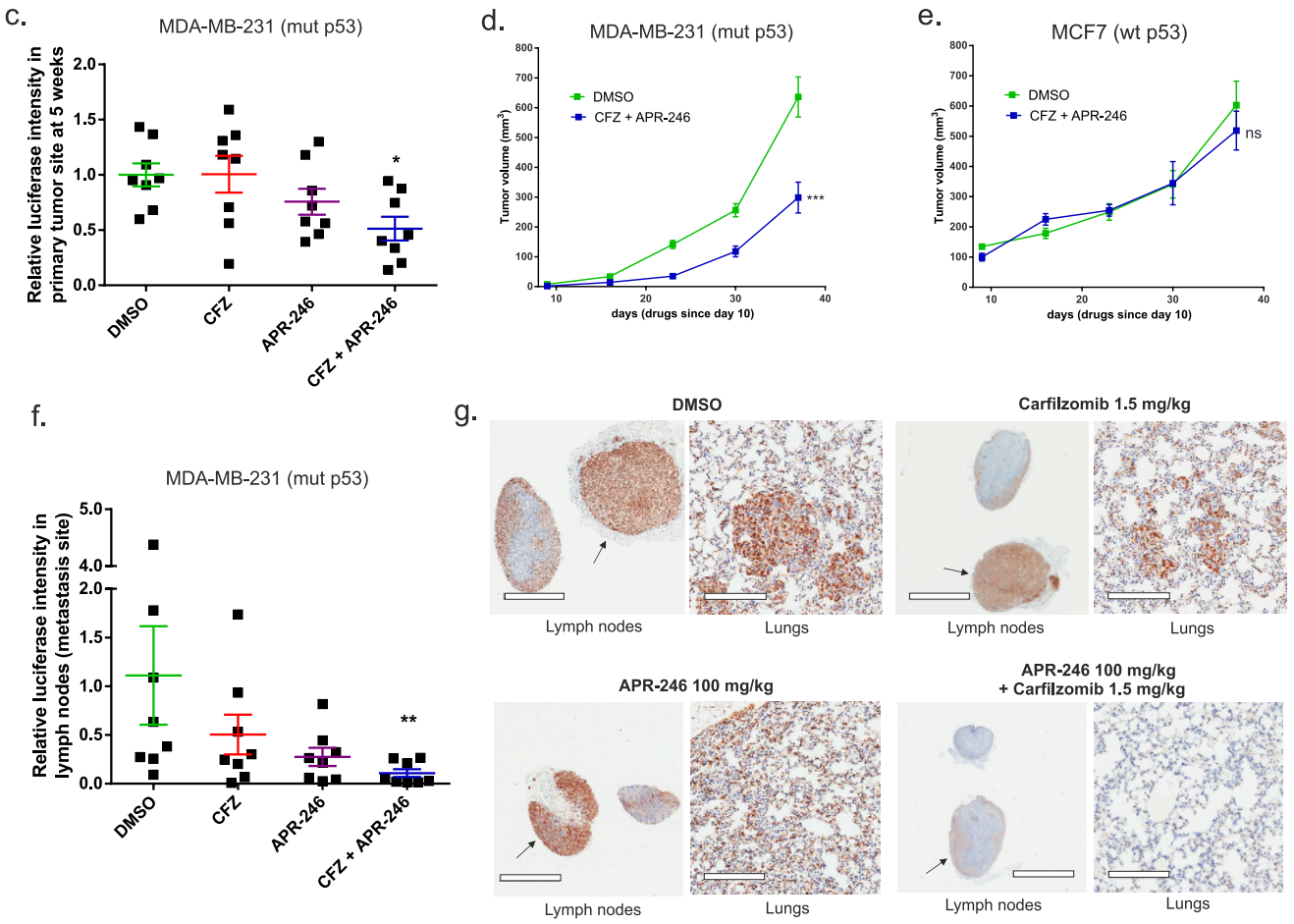
Oren and Kotler, *Nat Cell Biol,* 2016

Gli inibitori del proteasoma sono utilizzati nella terapia antitumorale

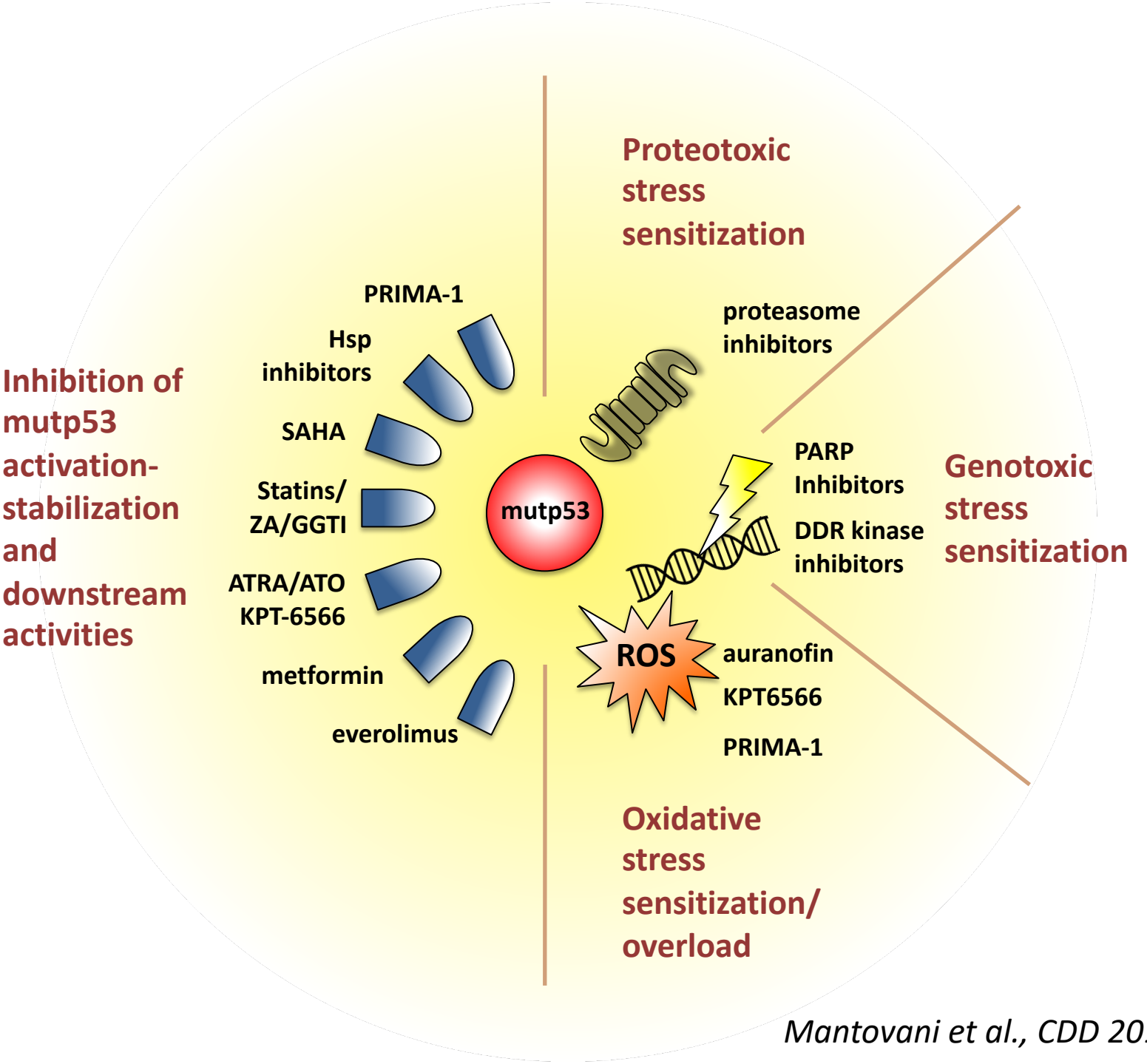
Bortezomib



Combination of mutp53 and proteasome inhibitors in BC treatment



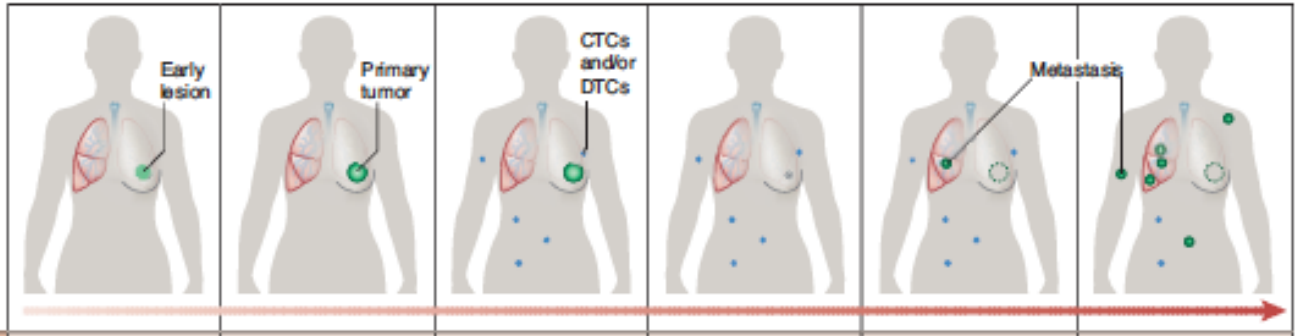
Walerych et al, *Nat Cell Biol,* 2016



Mantovani et al., CDD 2018

Approcci di ricerca e drug development per una medicina personalizzata

Sfide e strategie per il trattamento dei tumori metastatici

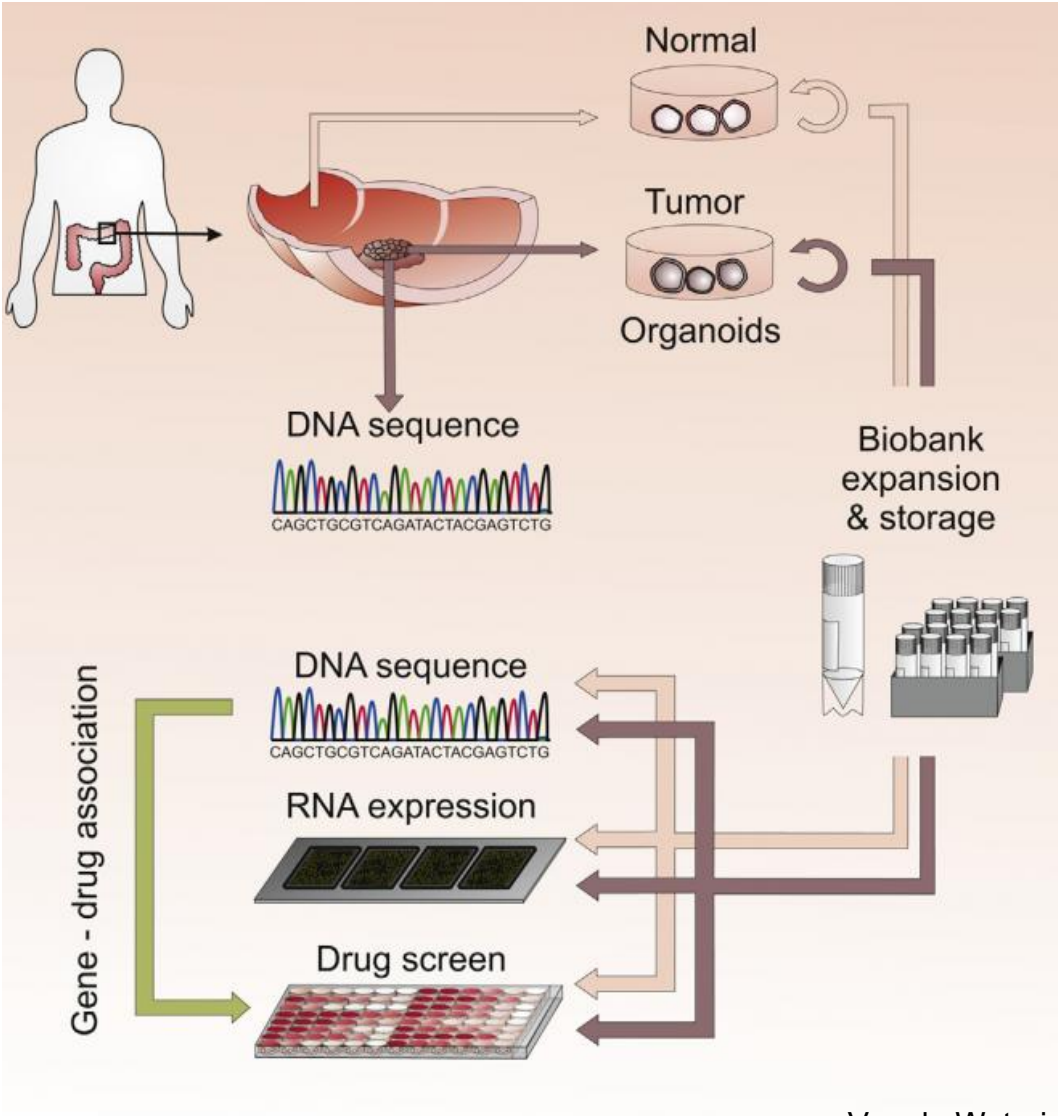


Status	Pre-neoplasm Subclinical	Primary (–) CTCs and/or DTCs	Primary (+) CTCs and/or DTCs	Dormancy	Oligometastases	Systemic metastases
Focus	Management of primary tumor		Prevention of metastasis		Treatment of metastasis	
Challenge	Early detection and prevention Identify high-risk patients		Prevent local 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

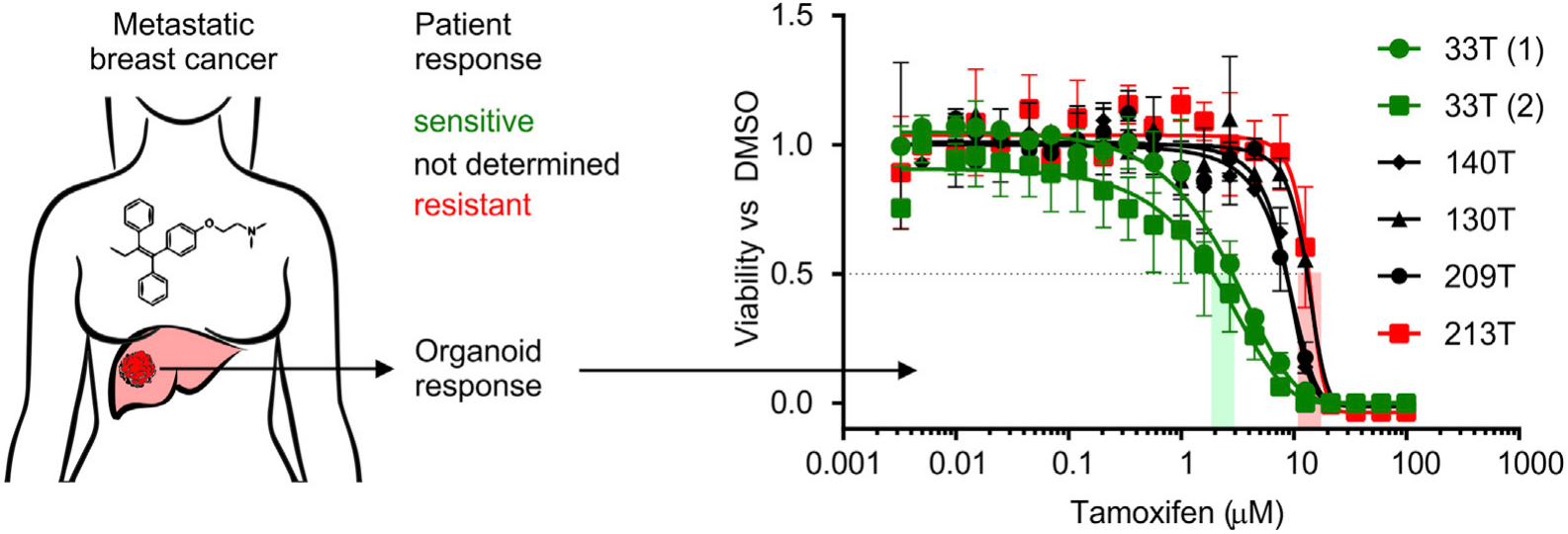
Generazione di organoidi tumorali da pazienti



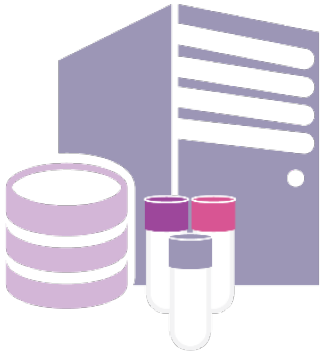
Van de Wetering et al., Cell 2015

Organoidi tumorali derivati da pazienti per il design di terapie personalizzate

Gli organoidi tumorali ricapitolano le caratteristiche del tumore in vitro: possono essere utilizzati per testare la risposta alle terapie e per drug screening



The response to tamoxifen of 10 metastasis-derived BC organoids matched that of the respective patients indicating the potential use of BC organoids as predictive in vitro surrogates for BC in vivo.



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)

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

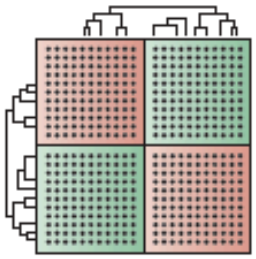
CMAP

Small-molecule hits

NCI-60 cell line panel

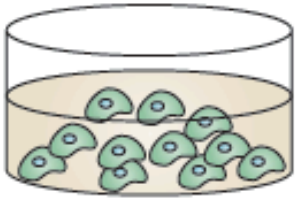
Connectivity map genera una lista di molecole che inducono una firma molecolare (es. di dormienza/regressione metastatica etc.)

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