Cds in Scienze e Tecnologie Biologiche

AA 2018-2019

Corso di Laboratorio di Biologia Cellulare

Lezione 11

APPLICAZIONI IN MEDICINA MOLECOLARE

LA MEDICINA MOLECOLARE

Identificare
geni, proteine e processi
responsabili
delle patologie

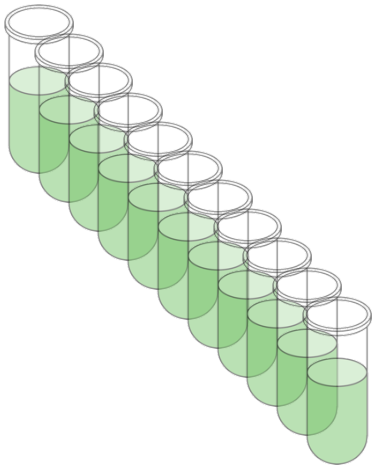
Diagnosi

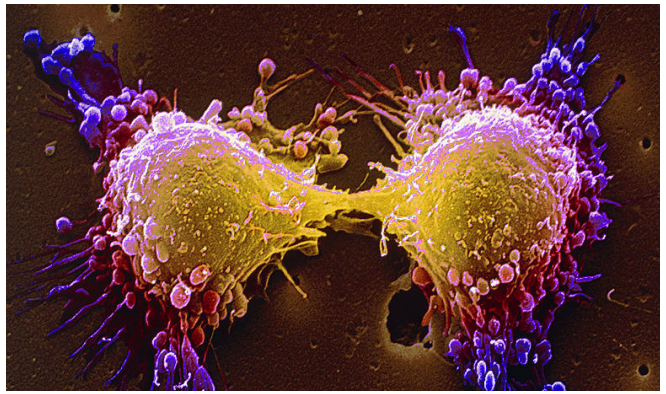
Risposta
alle terapie già in uso

Nuove
terapie mirate

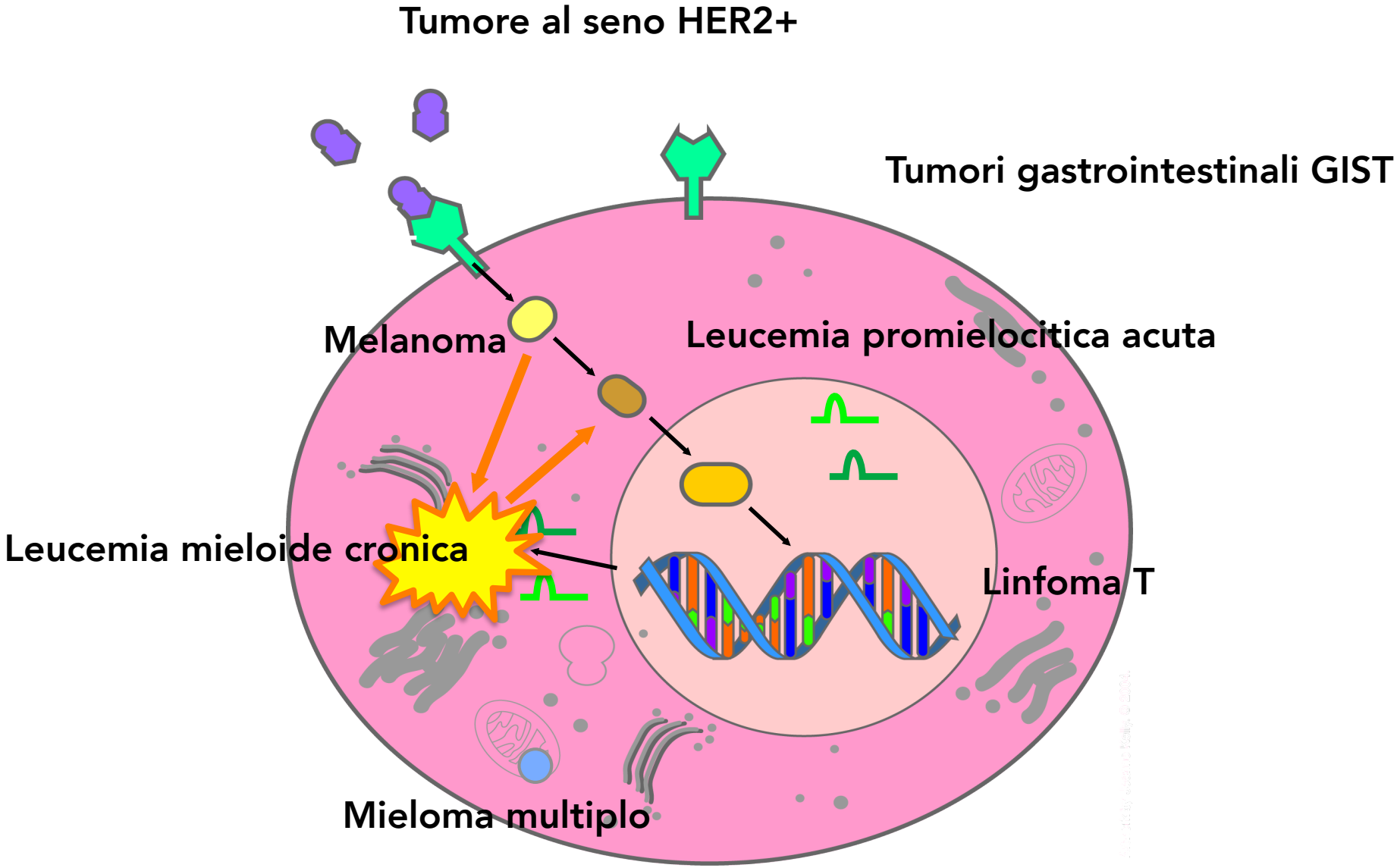
LA MEDICINA DI PRECISIONE

Molecola o processo bersaglio → Selezione o disegno di farmaci a bersaglio molecolare → Effetti su specifici processi biologici alterati





FARMACI DI PRECISIONE nelle terapie antitumorali



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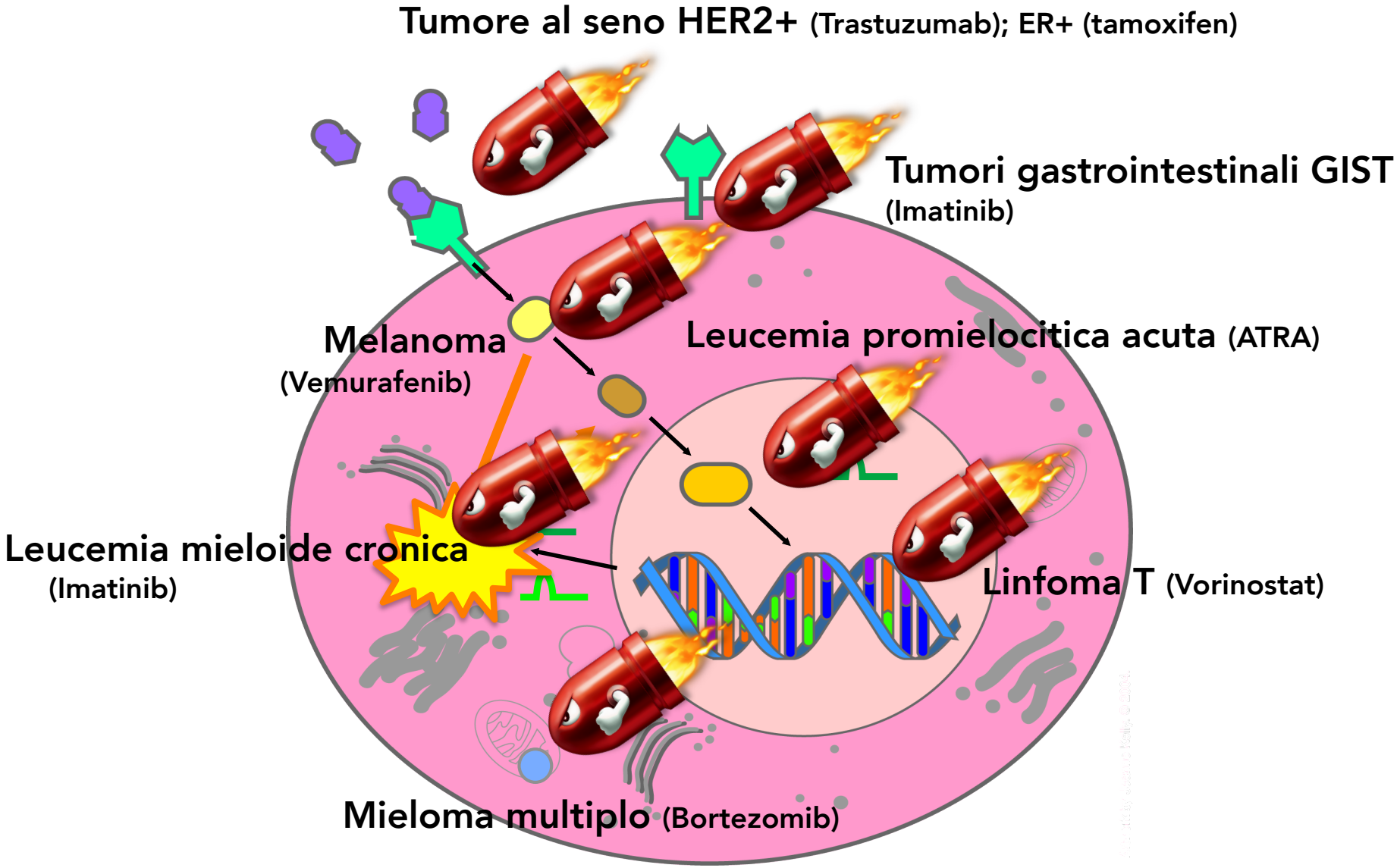
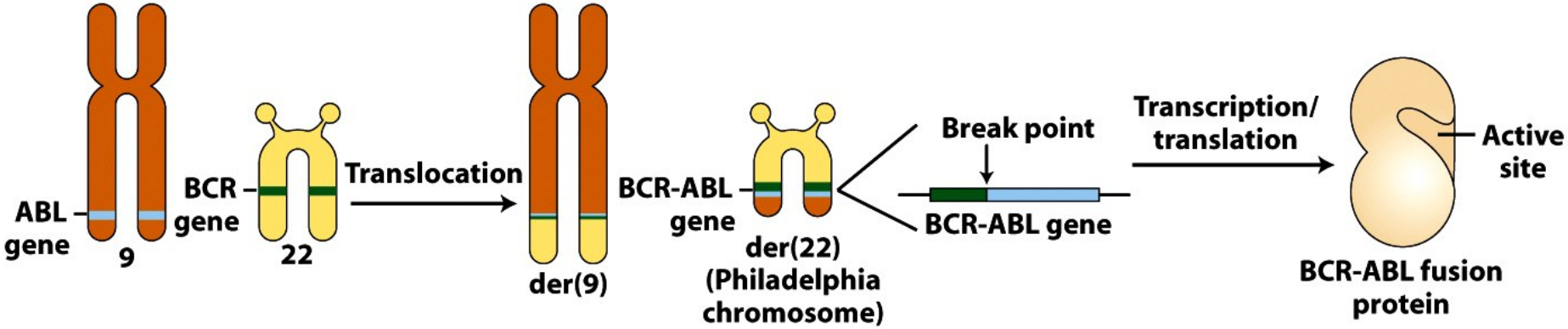
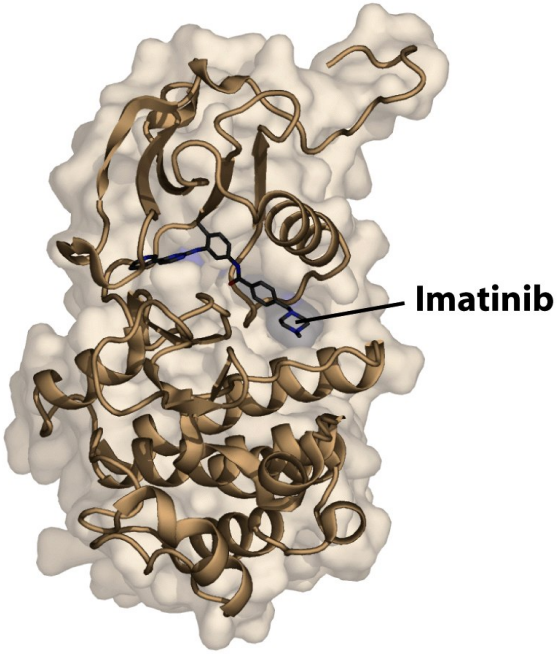


IMATINIB – un farmaco "disegnato" in base alla conoscenza del meccanismo molecolare



BCR-ABL fusion protein



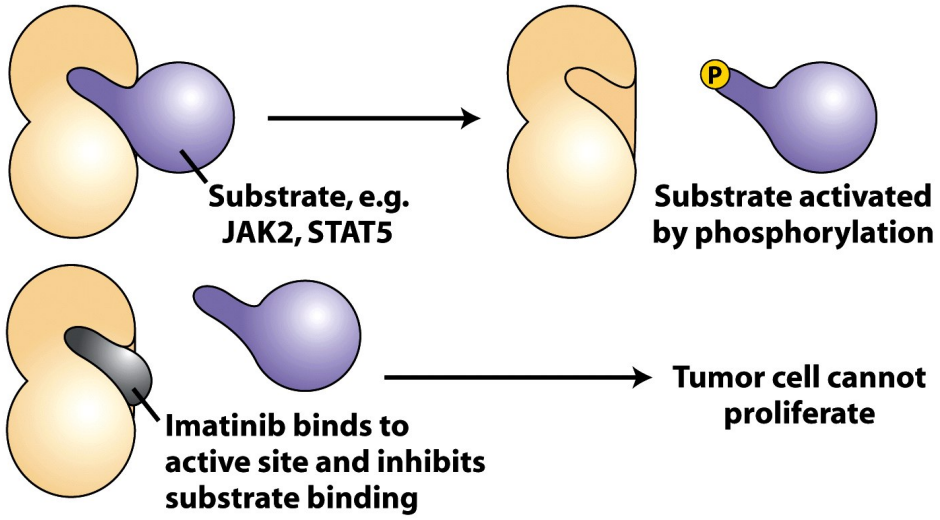
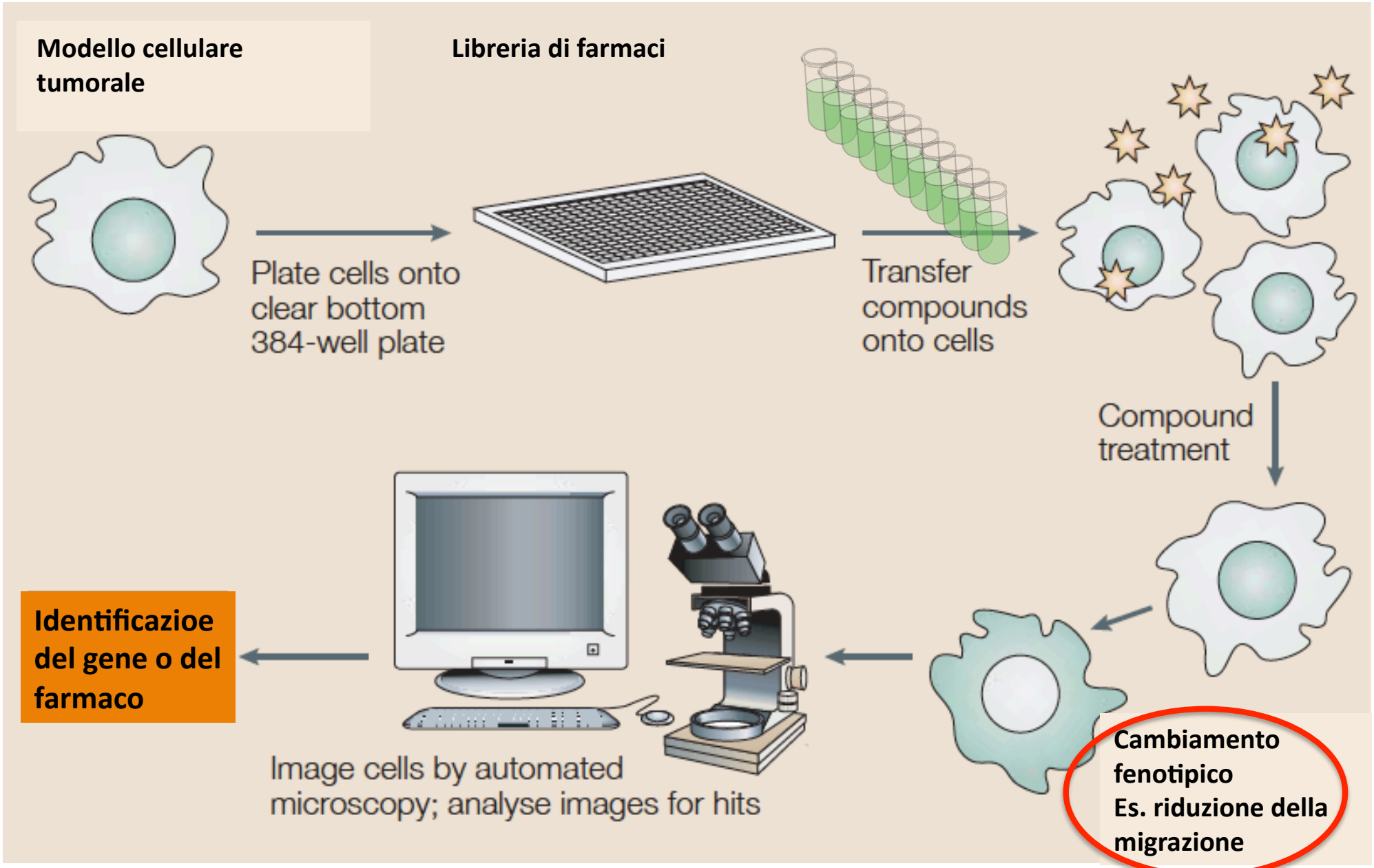
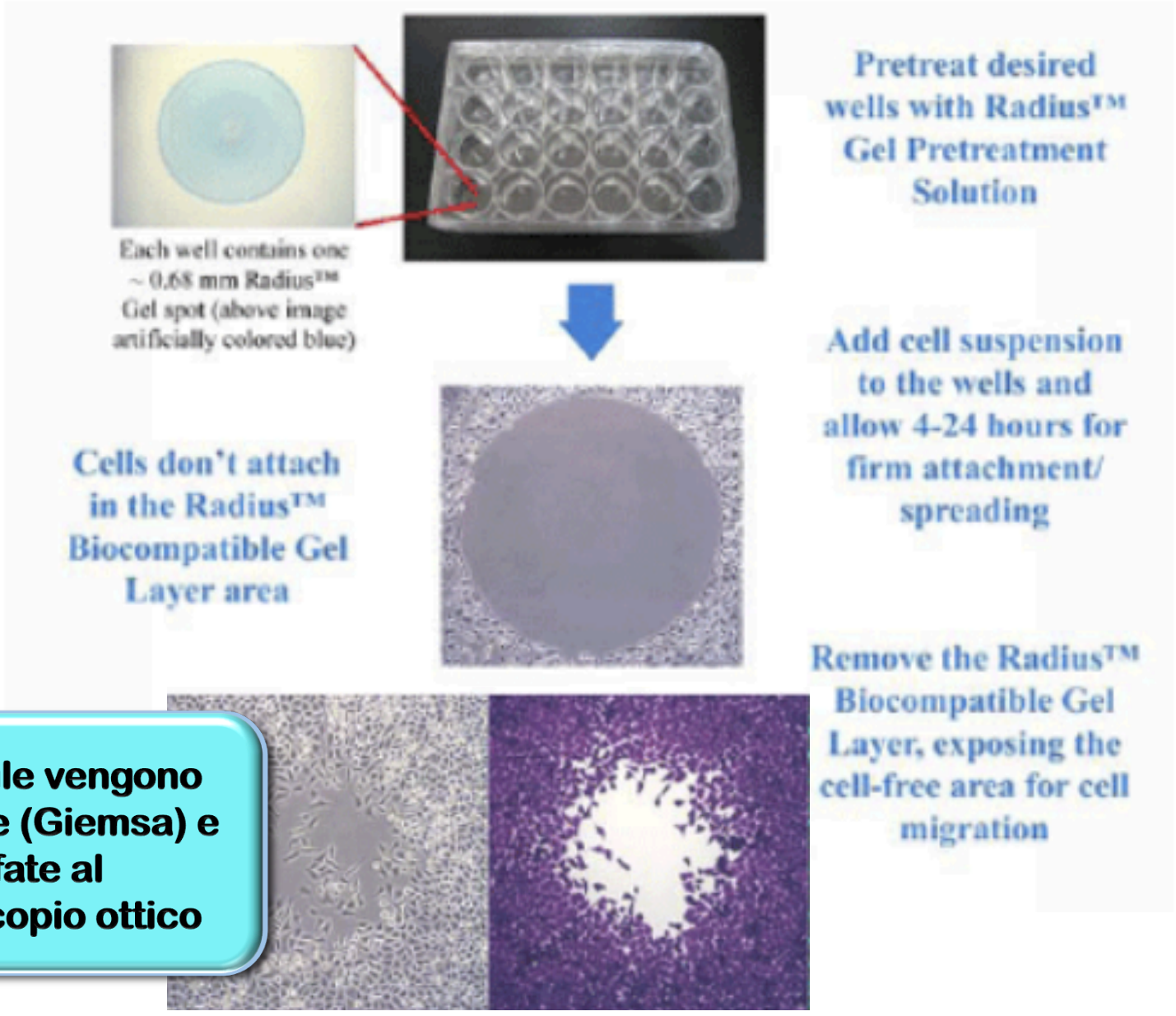


Figure 25-20b
Molecular Cell Biology, Sixth Edition

Screening FUNZIONALI high-throughput per nuovi farmaci

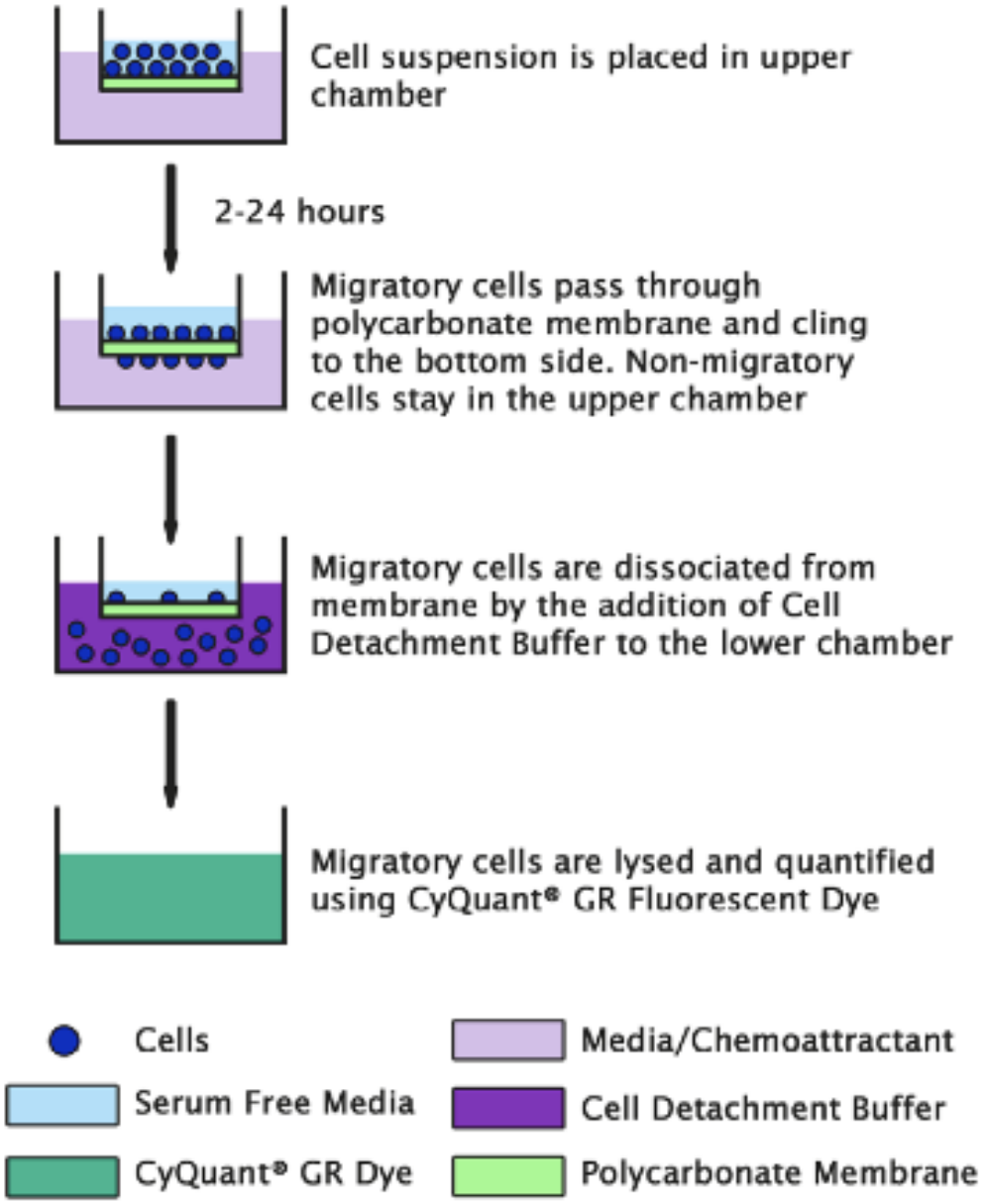


Saggi di motilità in piastra multipozzetto con lettura automatizzata al microscopio ottico



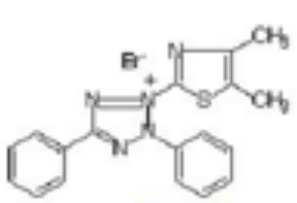
Le cellule vengono colorate (Giemsa) e fotografate al microscopio ottico

Saggi di migrazione (Boyden) in piastra multipozzetto con lettura fluorimetrica



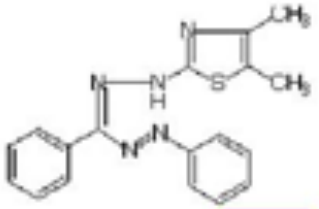
Saggi di vitalità cellulare MTT assay

MTT: (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide



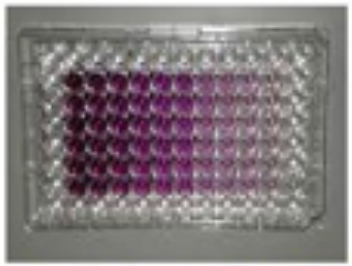
MTT **yellow**

Live cells → Mitochondrial reductase present → converts



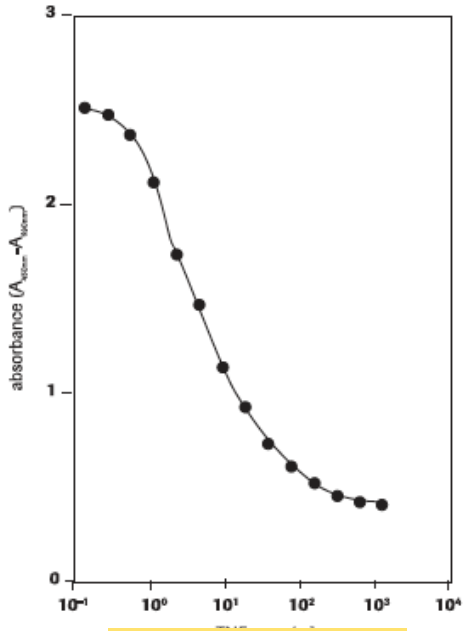
MTT formazan **violet**

Absorbance read at 690 nm and subtract background at 570 nm.



http://en.wikipedia.org/wiki/File:MTT_Plate.jpg

MTT test at different concentrations

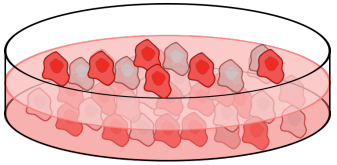


Farmaco X

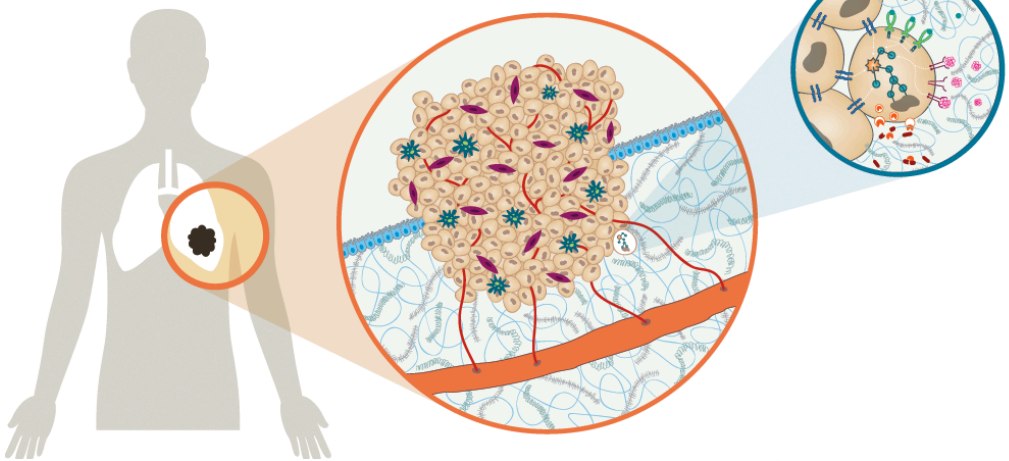
Il riposizionamento dei farmaci

Farmaci approvati da EMA/FDA e commercializzati per qualsiasi malattia

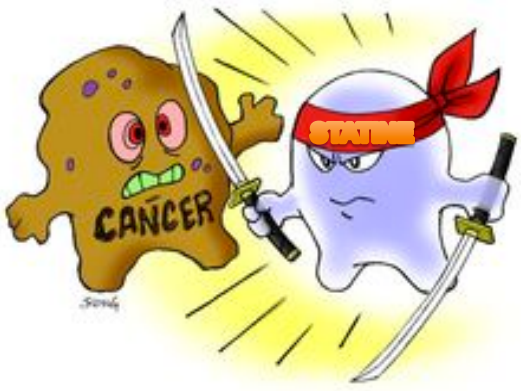
Testare in vitro l'efficacia antitumorale dei farmaci già approvati

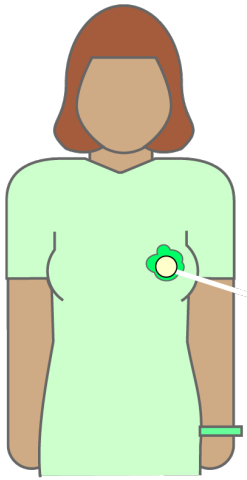


Paziente con tumore alla mammella



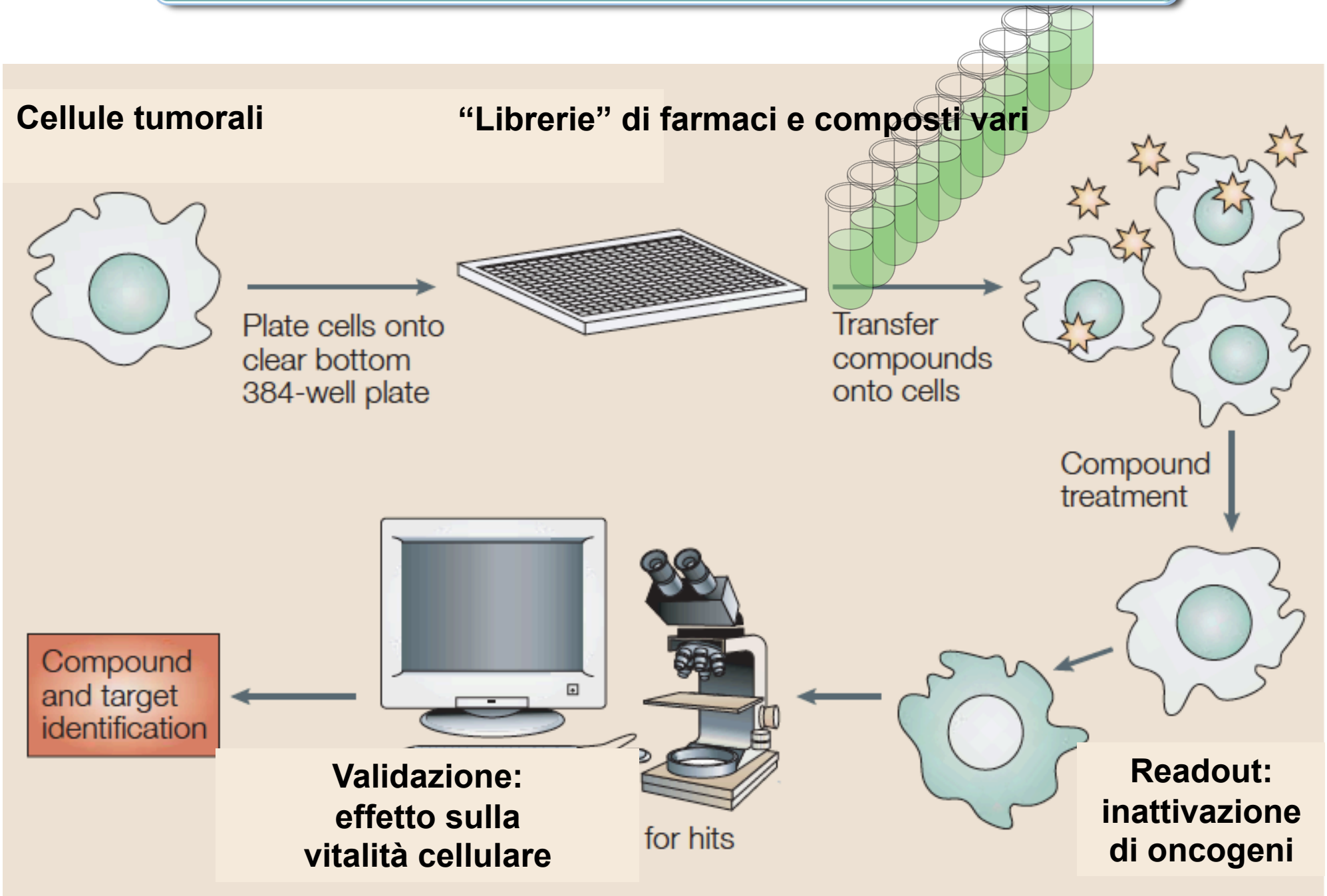
Es. Identificazione delle statine (anticolesterolemici) come potenziali farmaci antitumorali

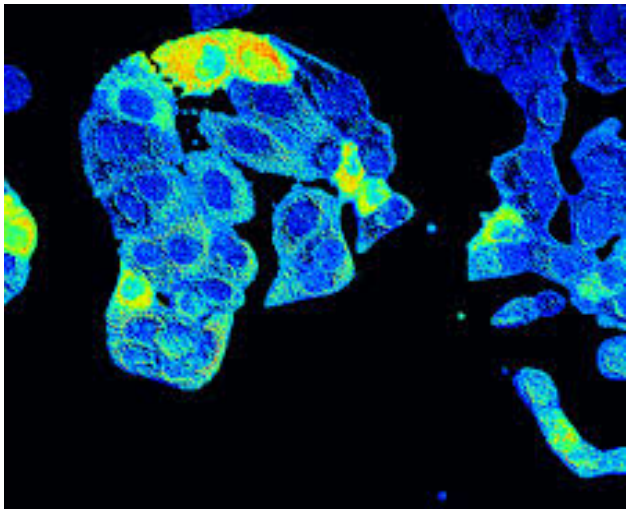




Trial clinico per l'efficacia delle statine in pazienti con tumore al seno in combinazione con le terapie standard

Screening per il riposizionamento di farmaci

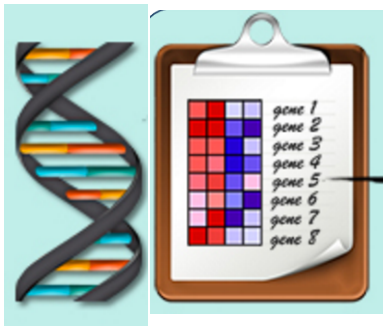




Next generation confocal high content screening system, designed to reliably discriminate phenotypes of complex cellular models, such as primary cells and 3D microtissue, integrated with automated microplate loader and liquid handling robot station for automated transfection of cells in 96- and 384-well microplates and assay preparation

Risorse bioinformatiche per il riposizionamento di farmaci

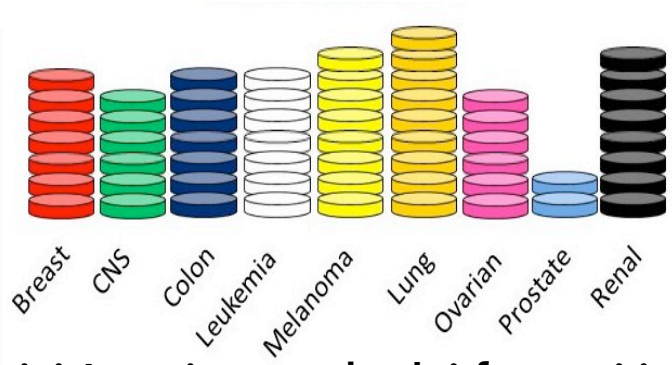
Dati genetici



Profili molecolari di cellule tumorali

+

Dati farmacologici



Attività antitumorale dei farmaci in vitro

↓

MDP DATABASE

IDENTIFICAZIONE DELLA SENSIBILITÀ AI FARMACI in base al profilo molecolare (mutazionale o di espressione genica)

Saggi di tumorigenicità in vivo

Scopi:

i. **Comprendere il contributo di un gene/processo/condizione alla progressione tumorale nel contesto di un organismo complesso**

ii. **Verificare l'efficacia di una terapia in vivo in diversi stadi dell'evoluzione tumorale (bersaglio- farmaco)**

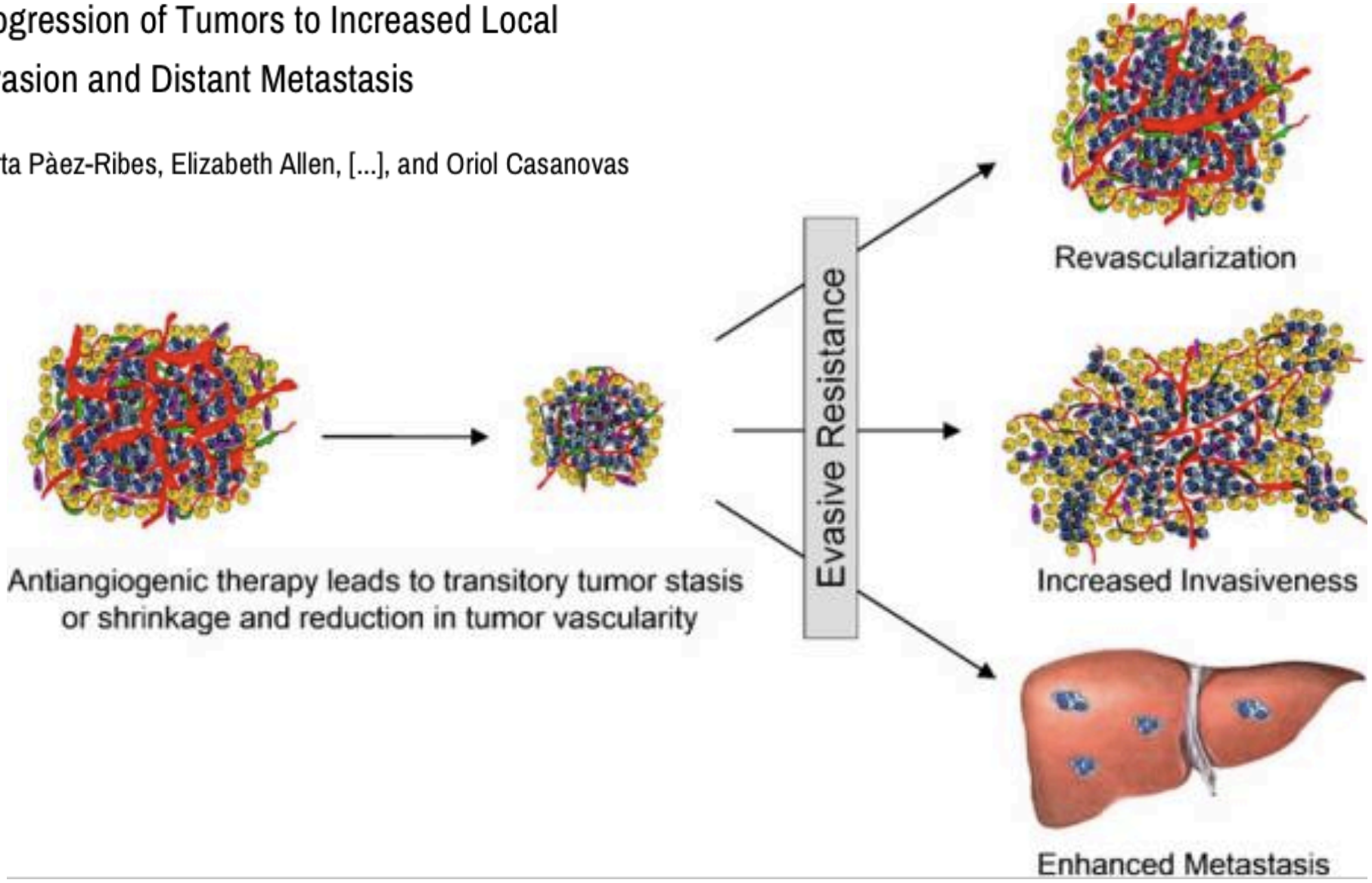
iii. **Sperimentazione preclinica di farmaci:**
- **tossicità,**
- **formulazione-somministrazione,**
- **immunogenicità,**
- **farmacocinetica (assorbimento, biodistribuzione, metabolismo),**
- **farmacodinamica (curve dose-risposta farmacologica),**
- **scaling interspecie.**

Cancer cell
Author Manuscript
NIH Public Access

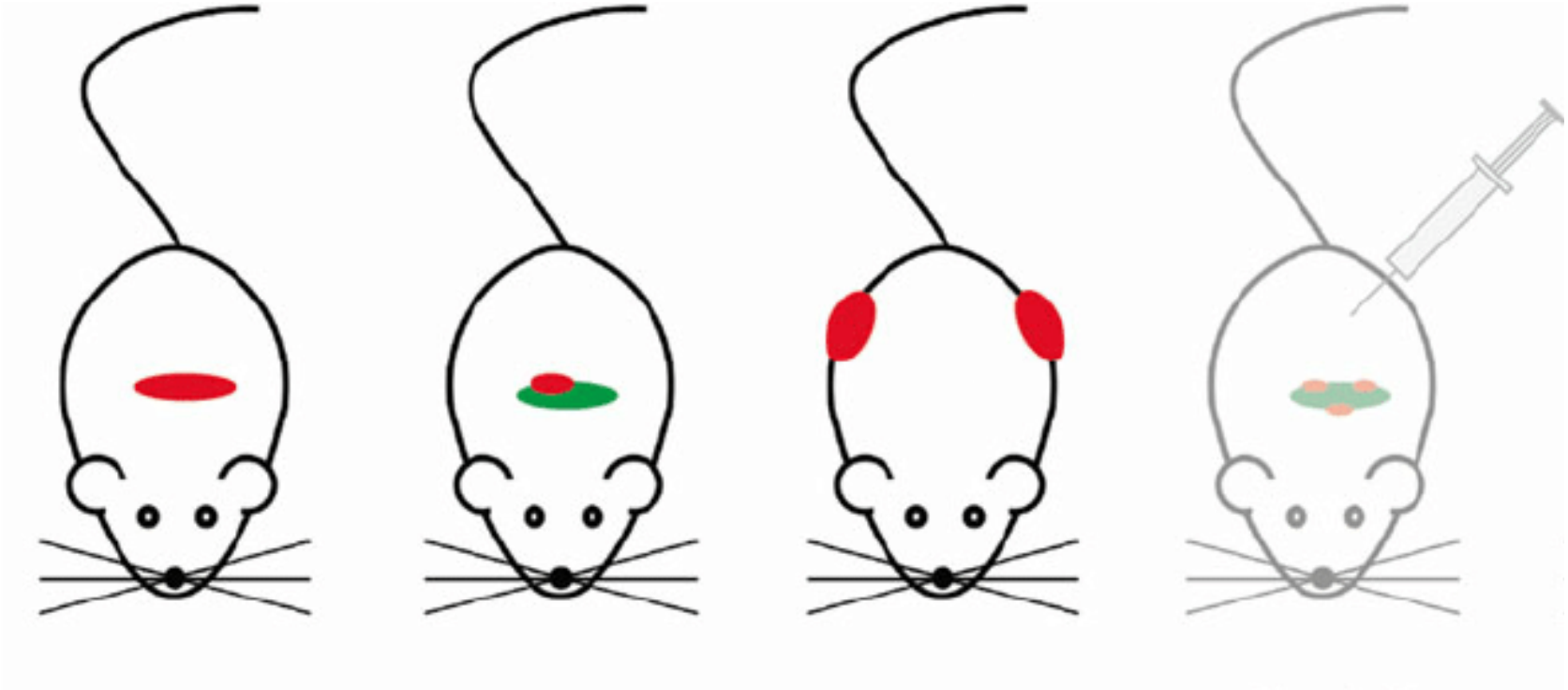
Cancer Cell 2009

Antiangiogenic Therapy Elicits Malignant Progression of Tumors to Increased Local Invasion and Distant Metastasis

Marta Pàez-Ribes, Elizabeth Allen, [...], and Oriol Casanovas



Modelli murini di tumorigenesi



Genetic Engineered Mouse (GEM)

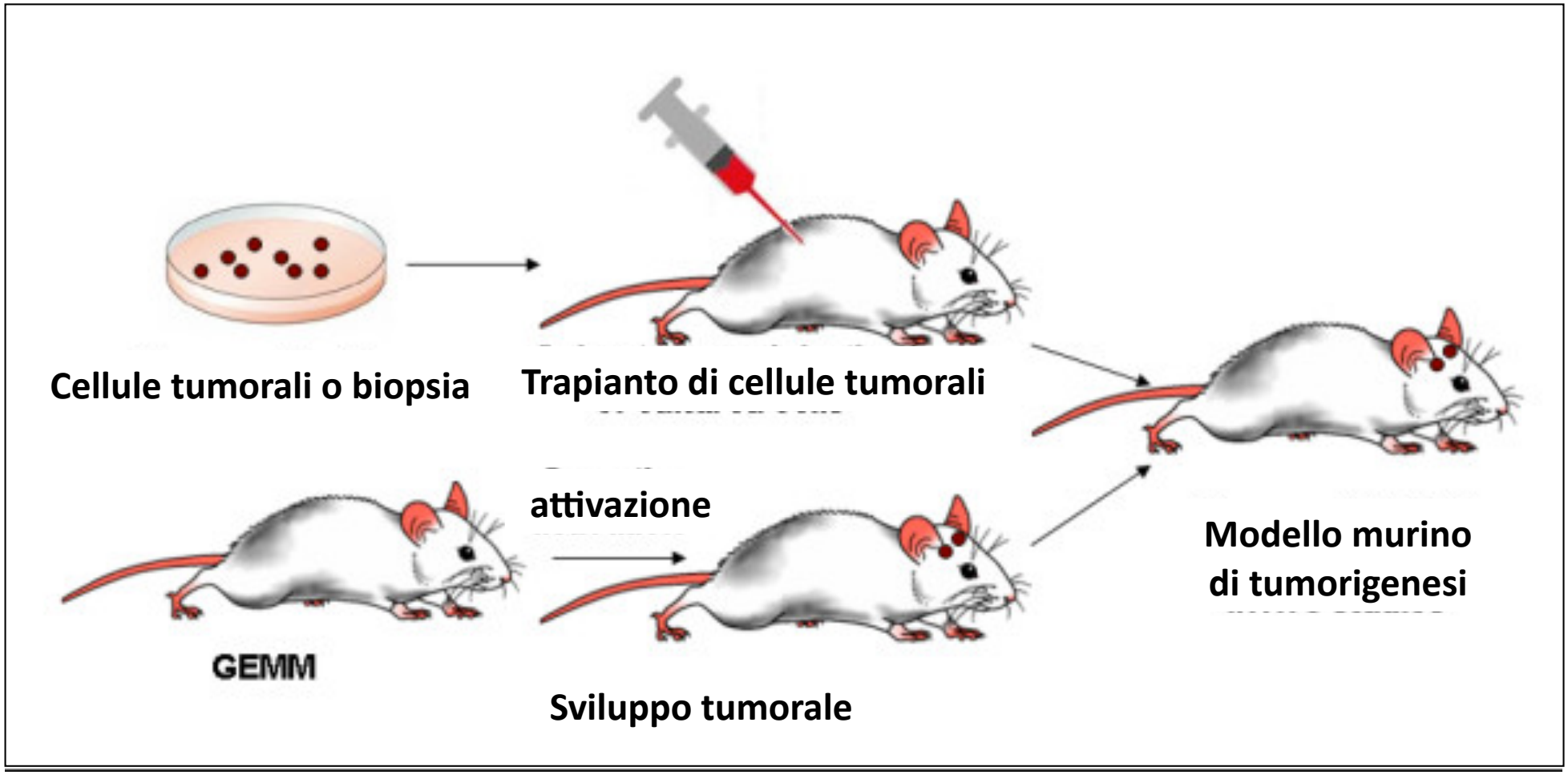
Orthotopic

Subcutaneous

Trapianto di cellule/biopsie tumorali

Disseminazione metastatica
Iniezione iv

Saggi di tumorigenicità in vivo



Regolamentazione della sperimentazione animale

1) È effettuata solo **quando** rappresenta il modello più appropriato per confermare ipotesi formulate in base a sperimentazioni in vitro/ex vivo.

2) Lo stesso modello potrà essere utilizzato successivamente per testare **terapie** basate sui risultati ottenuti.

Legislazione EU

Declaration:

I have obtained the **clearance from the Ministry of Health** to carry out the described animal experimentation.

All experiments with mice will be conducted in accordance with **laws and regulations that control experiments and procedures in Italy,** following the Directive 2010/63/EU actualized by the Italian EU member state starting from the Italian DL 26/2014.

The experiments described in the proposal will be performed following the **guidelines** described in: Wolfensohn S, Lloyd M: 'Handbook of Laboratory Animal Management and Welfare, 4th Edition' (Wiley-Blackwell, 2013)

Il principio delle 3R

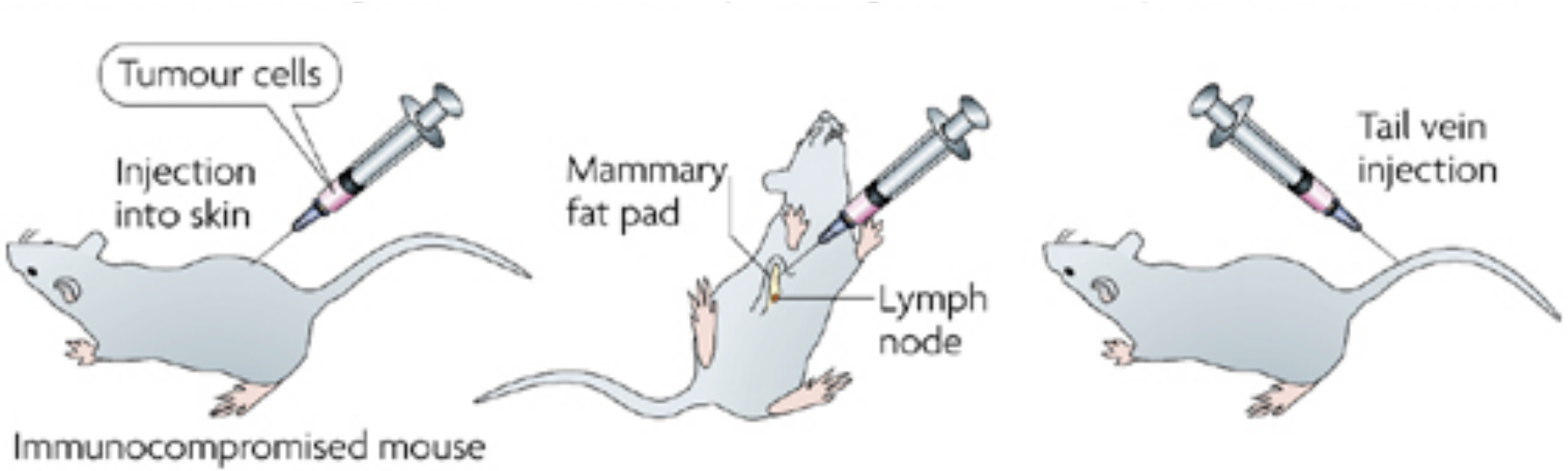
Replacement:
ove possibile, la sperimentazione animale va **sostituita** con la sperimentazione in vitro (colture cellulari), ex-vivo (es. organoidi) o al limite in vivo su altra specie con SN meno complesso.

Reduction:
mediante un uso accurato della statistica, si mira a **ridurre** il numero di **soggetti** utilizzati in un determinato protocollo sperimentale in modo da ottenere comunque una quantità di dati significativi evitando ripetizioni non necessarie.

Refinement:
gli animali vanno manipolati da personale esperto sotto controllo veterinario e devono essere attuate tutte le procedure che possono migliorare il **benessere animale** e ridurre la sofferenza (anestesia, analgesia, eutanasia).

Xenotrapianti di cellule tumorali (xenografts, PDX)

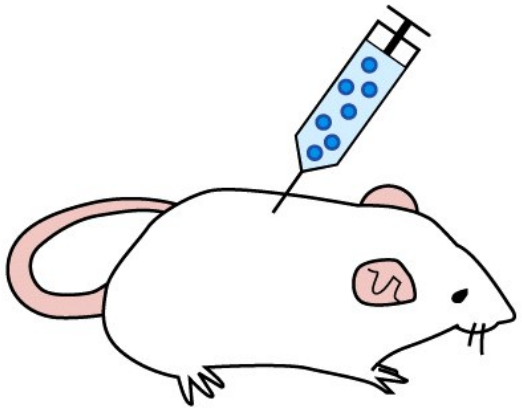
Trapianto sottocutaneo **Trapianto ortotopico** **Iniezione iv**



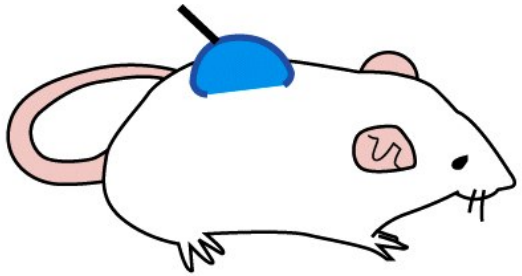
Nature Reviews | Cancer

Possono essere trapiantati sia cellule che frammenti di tessuto, tumori sperimentali o da pazienti (PDX: patient-derived xenografts).

Xenotrapianti di cellule tumorali (xenografts, PDX)



Formazione di un tumore



Modelli per il trapianto:

- animali immunodeficienti

- animali singenici

Ceppi murini immunodeficienti

Esiste una varietà di ceppi murini immunodeficienti che appartengono a 4 categorie principali:

- **“Nude” mice**
- **“Scid” mice**
- **“Rag-deficient” mice**
- **“Higher-order, multigenic” immunodeficient mice**

Nude mice: topi atimici = T-cell deficient

“nude” mice are homozygous for the Foxn1nu, or “nude,” mutation. Foxn1 encodes a transcription factor required for both hair follicle and thymic development. In its absence, mice are both hairless and **athymic**.

Because the thymus fails to form, there is no place for CD4+ and CD8+ T cells to differentiate and mature, making nude homozygotes **T cell-deficient.**

SCID e RAG-deficient mice: mancano della maturazione dei linfociti B e T

“Scid” mice are homozygous for the Prkdcscid mutation. The gene Prkdc encodes the catalytic subunit of DNA-dependent protein kinase that is required for DNA repair and for sealing the double-stranded DNA breaks that occur during somatic recombination of T cell receptor (TCR) and immunoglobulin (Ig) genes. In the absence of Prkdc protein, TCR and Ig genes cannot rearrange, resulting in mice that are **both T and B cell deficient**.

“Rag-deficient” mice are mice that fail to express functional Rag1 or Rag2 proteins. Like the Prkdc gene, both Rag1 and Rag2 are required for somatic recombination of TCR and Ig genes, and the absence of either gene results in **T and B cell deficiency**.

Finally, “higher-order, multigenic” immunodeficient mice are constructed from either Prkdcscid or Rag-deficient mice, and carry additional immunodeficiency-enhancing mutations. These mice are **B, T and NK cell deficient**. Additionally, they are hemolytic **complement-deficient** and carry alleles that adversely affect **macrophage and dendritic cell** functions.