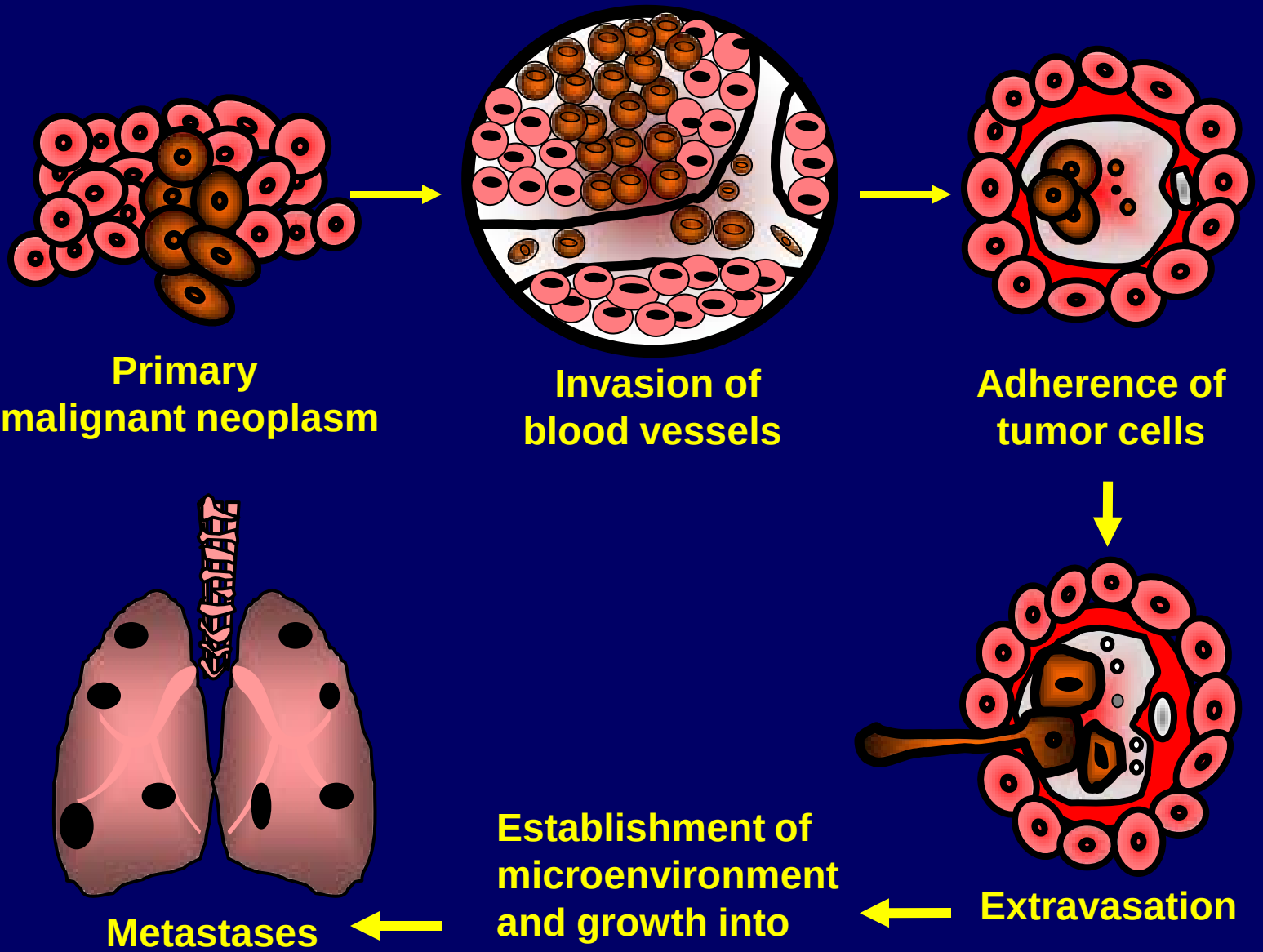
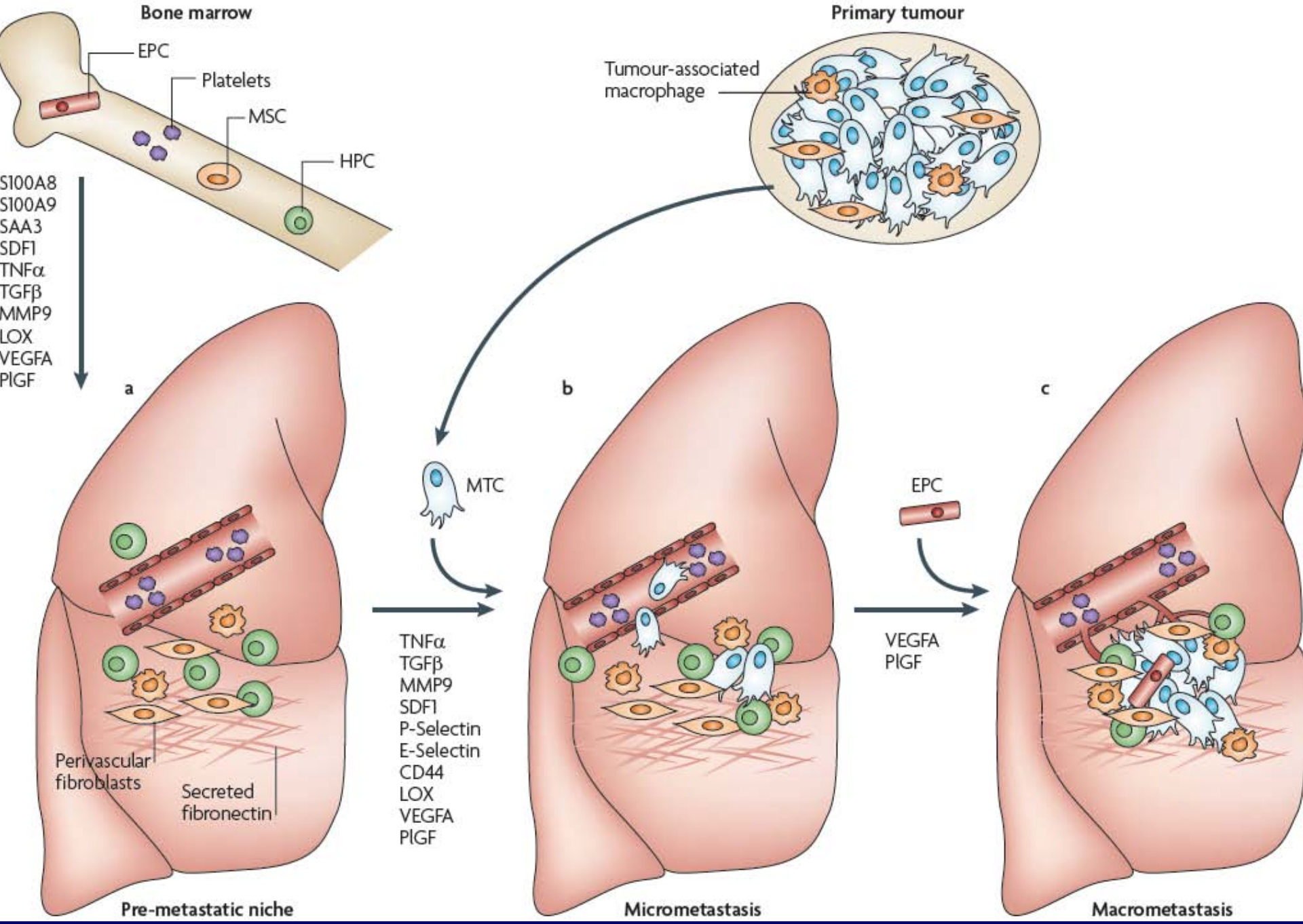
The Pathogenesis of a Metastasis



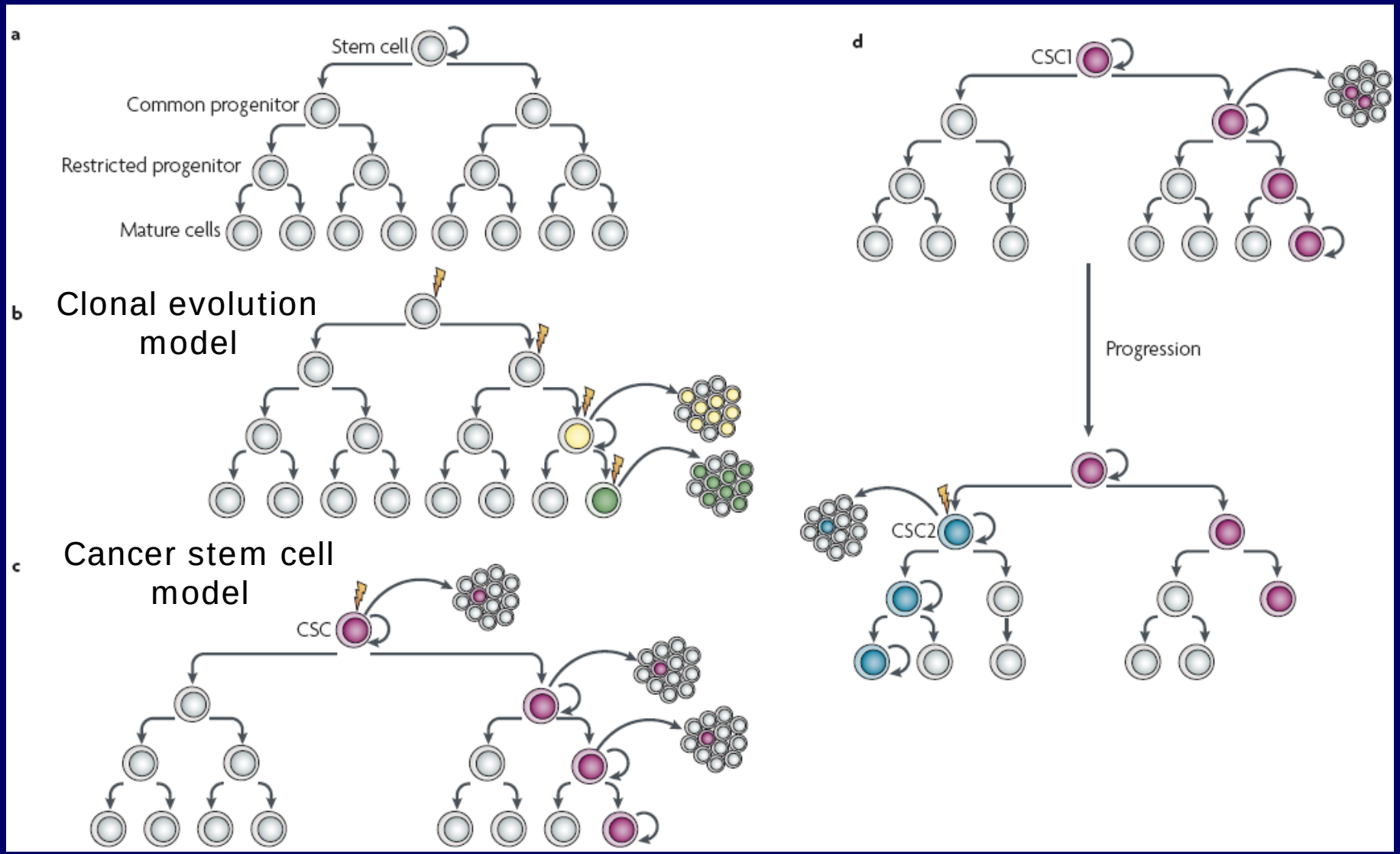
Cancer metastasis-
Seed and Soil

Soil


Bone marrow
EPC
Platelets
MSC
HPC
S100A8
S100A9
SAA3
SDF1
TNFα
TGFβ
MMP9
LOX
VEGFA
PlGF
Primary tumour
Tumour-associated macrophage
a
b
c
MTC
EPC
TNFα
TGFβ
MMP9
SDF1
P-Selectin
E-Selectin
CD44
LOX
VEGFA
PlGF
VEGFA
PlGF
Perivascular fibroblasts
Secreted fibronectin
Pre-metastatic niche
Micrometastasis
Macrometastasis

Seed

Models for tumor hererogeneity and propagation



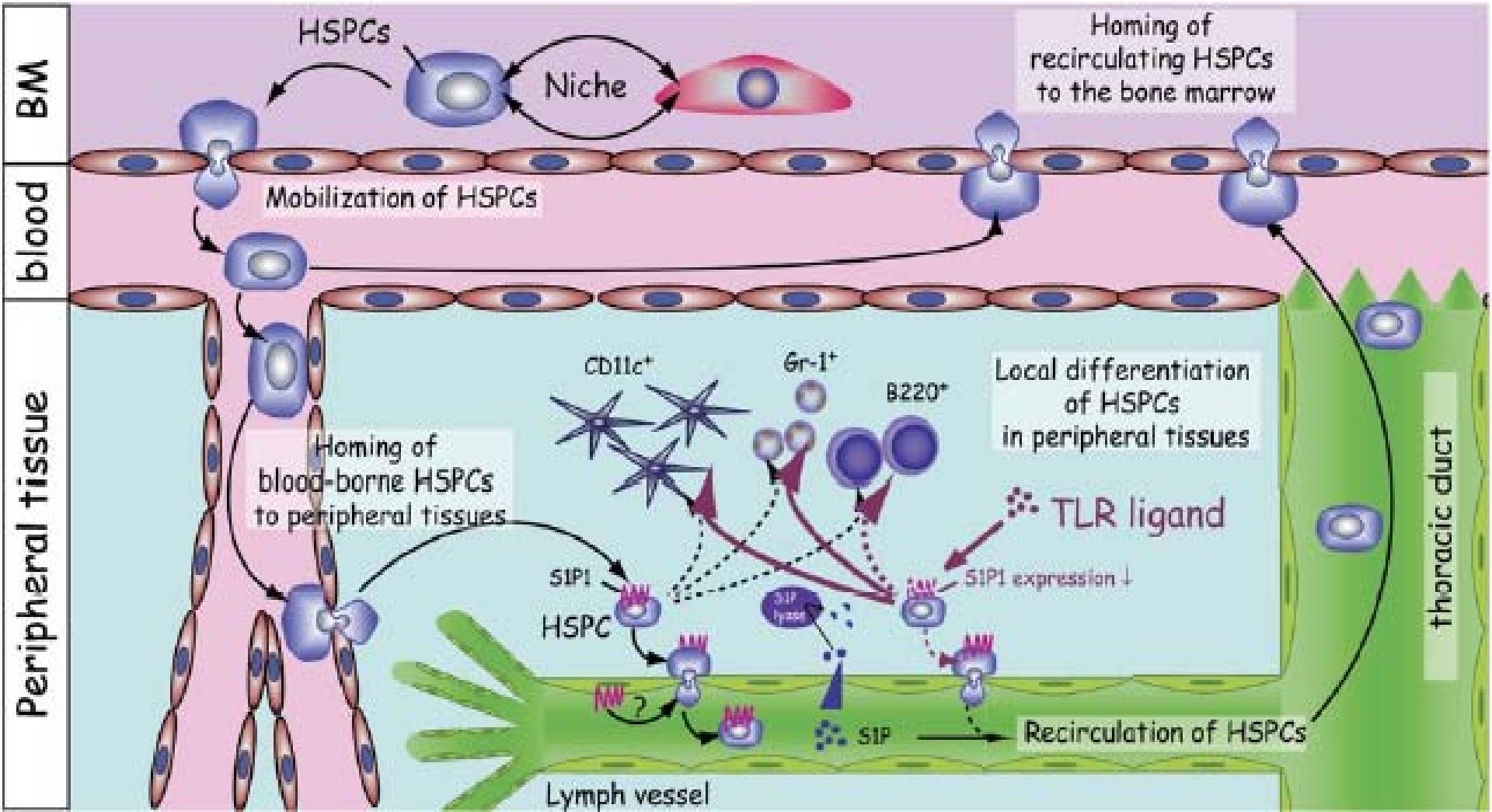
Cancer Stem Cells

- Tumor originates in tissue stem cells or their immediate progeny
- Tumor contains a cellular component that retains stem cell properties

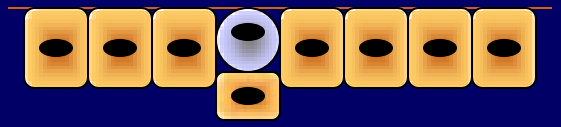
Stem cells are the only cells capable of undergoing self-renewal divisions

In bone marow transplantation models, a single **hematopoetic stem cell** introduced into a lethally irradiated mouse is able to reconstitute the entire hematopoetic system

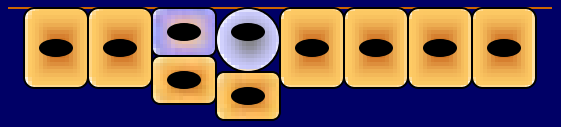
Trafficking of Hematopoietic Stem and Progenitor Cells



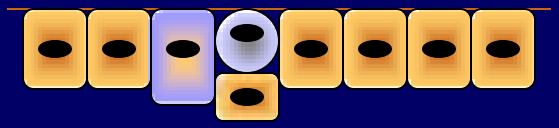
Tissue Homeostasis



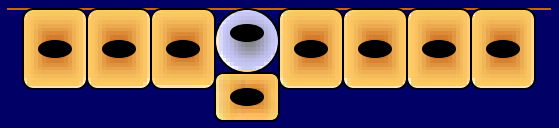
Asymetric self-renewal
Stem cell maintenance



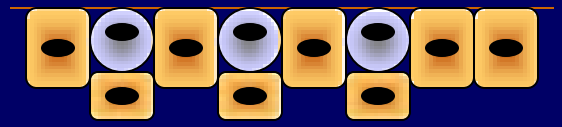
Proliferation
Transient amplifying population



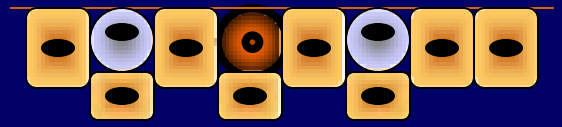
Terminal differentiation



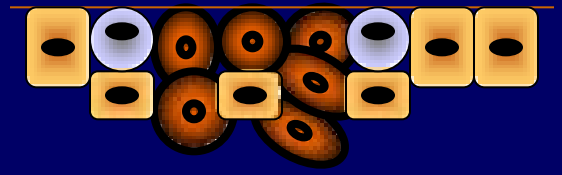
Carcinogenesis



Initiation



Symmetric self-renewal
Cancer stem cell expansion



Normal stem cells and cancer cells share several properties

- The capacity for self-renewal
- The ability to differentiate
- Active telomerase expression
- Activation of antiapoptotic pathways

Markers of CSC in solid tumors from patients

ABCG5
Member of the ATP binding cassette family, involved in transport of sterol and other lipids. ABCG2 (also known as breast cancer resistance protein) is a multi-drug transporter (see Hoechst SP below). ABCG5 confers doxorubicin resistance.

ALDH1
The ubiquitous aldehyde dehydrogenase (ALDH) family of enzymes catalyse the oxidation of aliphatic and aromatic aldehydes to carboxylic acids. ALDH1 has a role in the conversion of retinol to retinoic acid, which is important for proliferation, differentiation and survival.

CD24 (HSA)
A heavily glycosylated glycosylphosphatidylinositol-anchored adhesion molecule, which has a co-stimulatory role in B and T cells. The only known ligand for P-selectin. Although CD24 is not a specific marker of cancer stem cells, low levels can characterize breast tumour-initiating cells.

CD44 (PGP1)
An adhesion molecule with multiple isoforms that has pleiotropic roles in signalling, migration and homing. The standard form CD44H exhibits a high affinity for hyaluronate; CD44V confers metastatic properties.

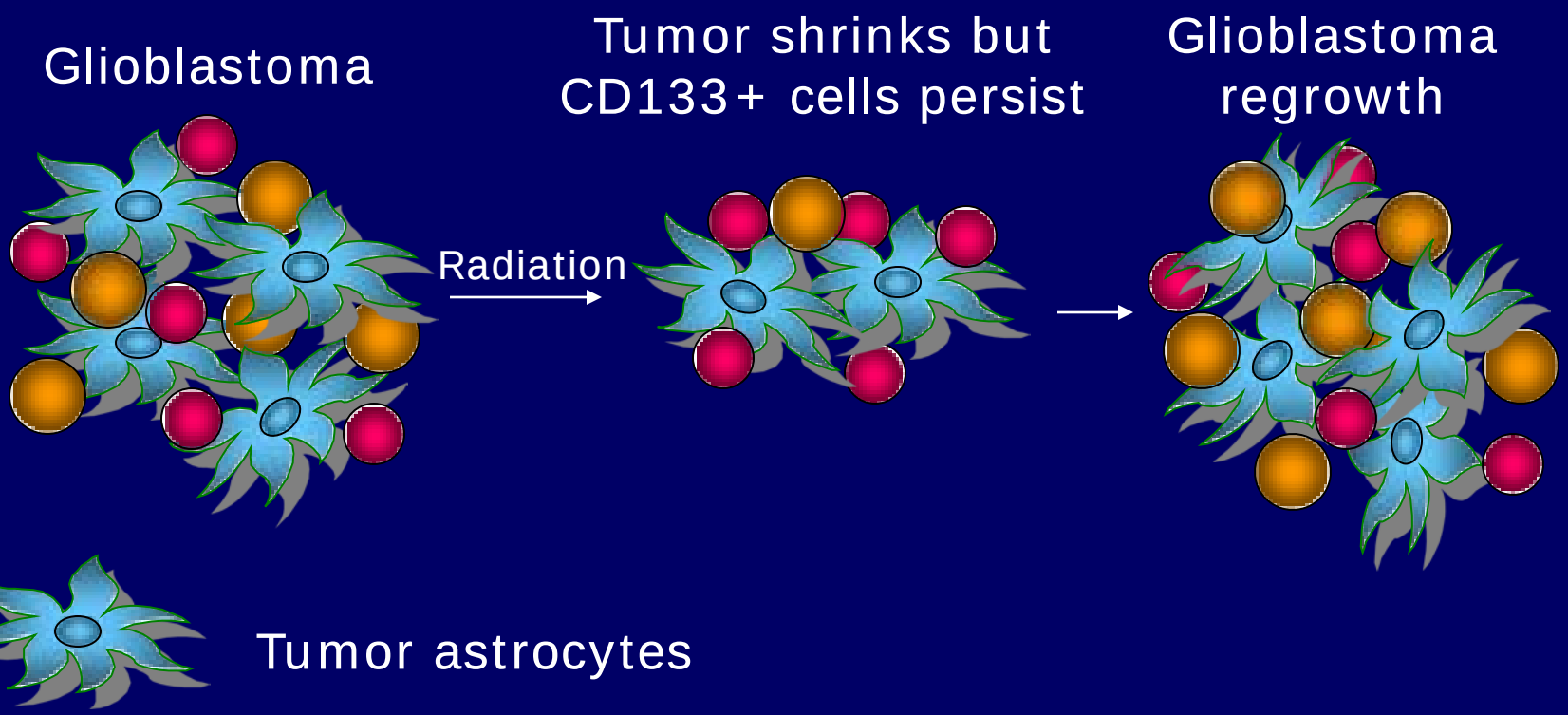
CD90 (THY1)
A glycosylphosphatidylinositol-anchored membrane glycoprotein involved in signal transduction that has a potential role in stem cell differentiation. It may mediate the adhesion of thymocytes to the thymic stroma.

CD133 (prominin 1)
Five-transmembrane domain glycoprotein with a potential role in the organization of plasma membrane topology. Expressed on $CD34^+$ stem and progenitor cells in fetal liver, endothelial precursors, fetal neural stem cells and developing epithelium, CD133 has been detected by its glycosylated epitope in the majority of studies. Thus, AC133 may be a more reliable cancer stem cell marker[116].

EpCAM (epithelial cell adhesion molecule; ESA, TROP1)
Homophillic Ca^{2+}-independent cell adhesion molecule expressed on the basolateral surfaces of most epithelial cells.

Hoechst SP
Side population (SP) phenotype due to the $Hoechst_{33342}$ efflux pump present on the plasma membrane in diverse cell types. Activity conferred by the ABC transporter ABCG2. Inhibited by verapamil.

Response of glioblastomas to ionizing radiation



Animal models of tumorigenicity

- Spontaneous tumors that arise in rodents
- Syngeneic transplantation of spontaneously occurring rodent cancers
- Chemical induction of cancers in selected strains of rats and mice
- Transgenic mouse models
- Xenografts of human tumors into immunodeficient rodents

Transplantable tumor models-drawbacks

- Today's syngeneic mouse strains may no longer be fully syngeneic with transplantable tumors, which were derived many years ago
- T. tumors usually grow in the anatomically inappropriate site
- T. tumors generally progress very rapidly following inoculation
- Most t. tumors are not spontaneously metastatic

Mouse models to study metastasis of allogenic/xenogenic tumor cells

- Nude absence of the thymus=a lack of functional T cells
- SCID DNA repair defect and a defect in the rearrangement of genes that code for An-specific receptors on lymphocytes=an absence of functional T cells and B cells. APC, myeloid and NK cell functions are strain dependent
- RAG-1, RAG-2 mutations causing a failure to rearrange the An receptor genes=a lack of functional T cells and B cells

SV40-driven transgenic models

- Some transgenic mice have been generated by placing the transforming genes of the SV40 virus early regions under the control of a tissue-specific promoter. These mice spontaneously develop tumors in the targeted tissue
- The SV40 early region contains both large T and small t antigens (T ag inactivates p53 and Rb genes, t ag activates cyclin Dp, which alteres MAPK and SAPK pathways)

Organ-specific oncogene-driven transgenic models

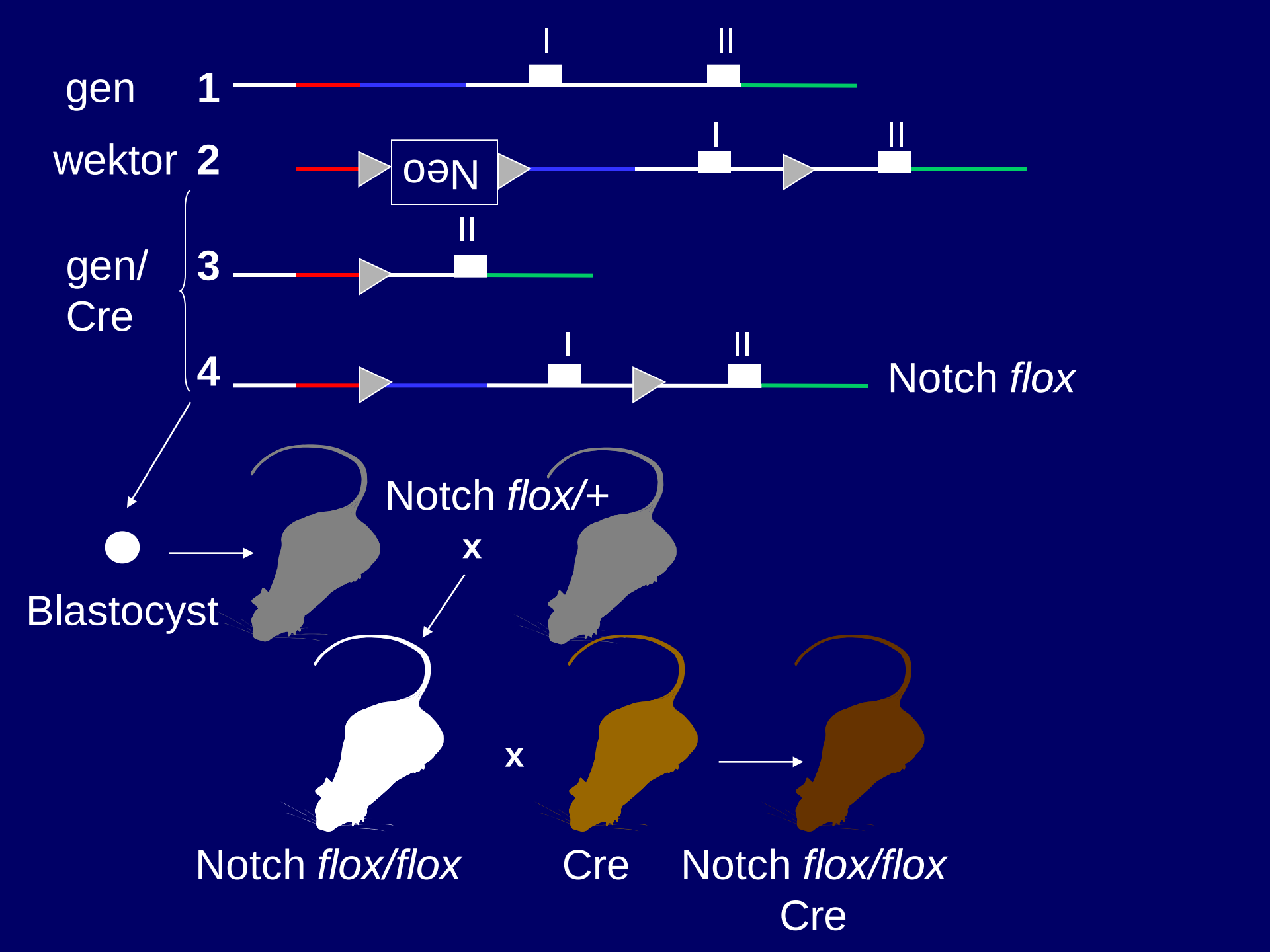
- These models utilize a cell or tissue-specific promoter driving an oncogene that plays a key role in tumorigenesis (Her2/neu driven by mammary tissue-specific promoters such as the Her2/neu endogenous promoter or mouse mammary tumor virus (MMTV) promoter)

Tumor-suppressor-gene KO models

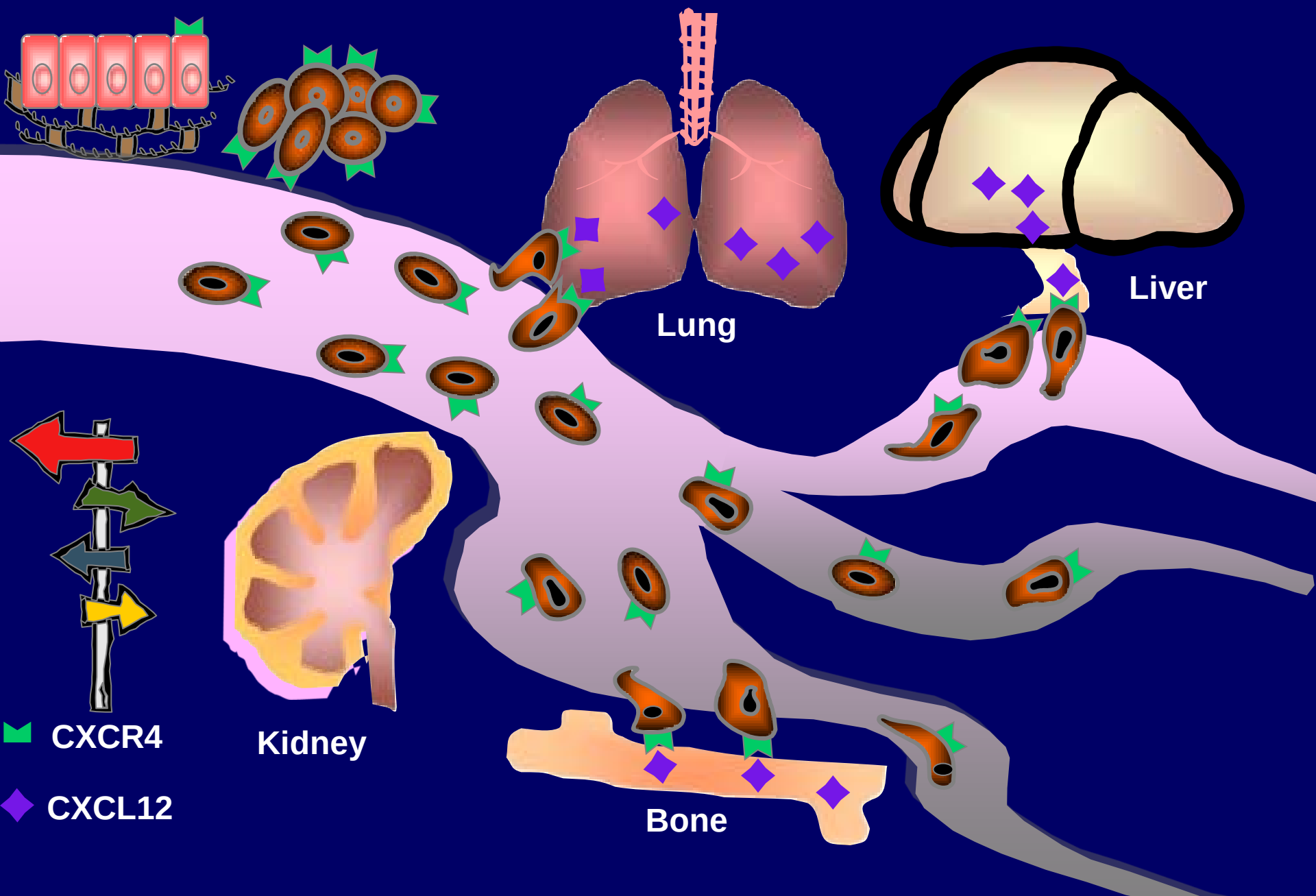
Loss of function of p53 and Pten (tumor suppressor gene that has anti-apoptotic activity)

Cre-lox conditional models

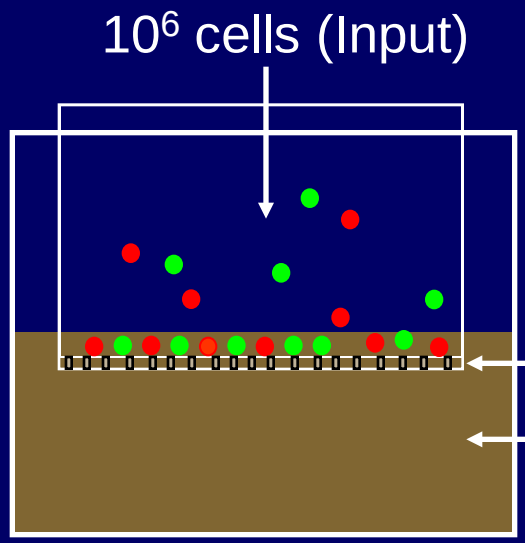
- Unlike human malignancies, which usually develop after birth, the transgenes in KO mouse models are altered during embryonic development
- To overcome this problem, mouse models are being developed based on the ability of the bacterial recombinase Cre to remove genes that are flanked by LoxP sites


gen
1
I
II
wektor
2
Neo
I
II
gen/
Cre
3
II
4
I
II
Notch *flox*
Blastocyst
Notch *flox*/+
x
x
Notch *flox/flox*
Cre
Notch *flox/flox*
Cre

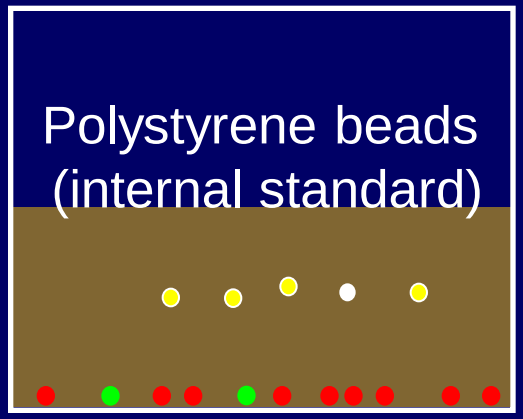
Targeted metastasis of breast cancer cells



Transwell Chemotaxis Assay



2 hr migration at 37°C



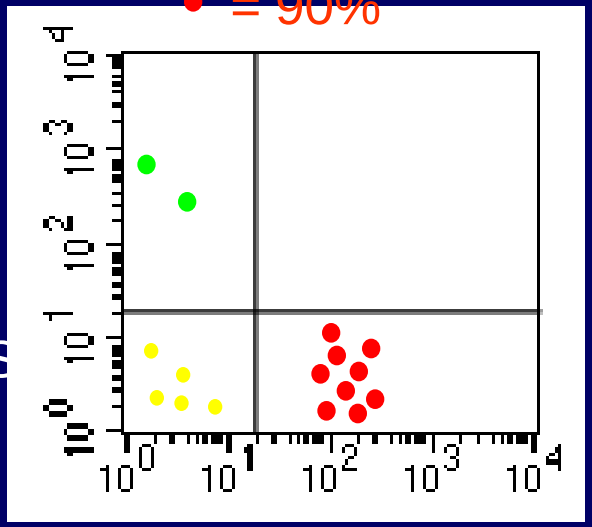
Stain cell subsets mAbs

Acquire samples by FACS

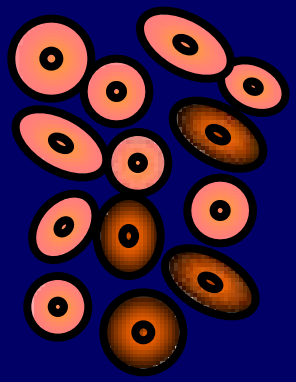
Calculate % chemotaxis of input:

● = 20%

● = 90%

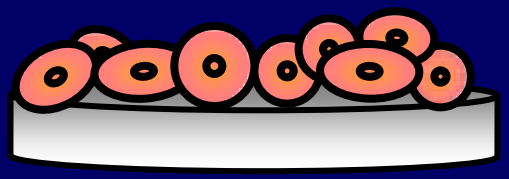


In vivo selection of metastatic cells

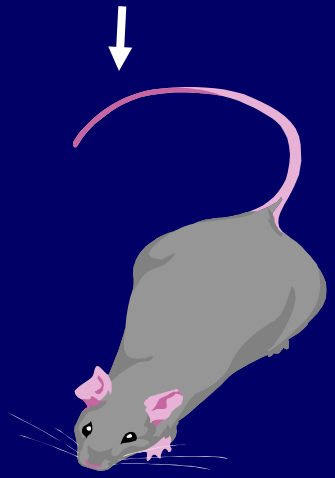


Tumour cells
some metastatic

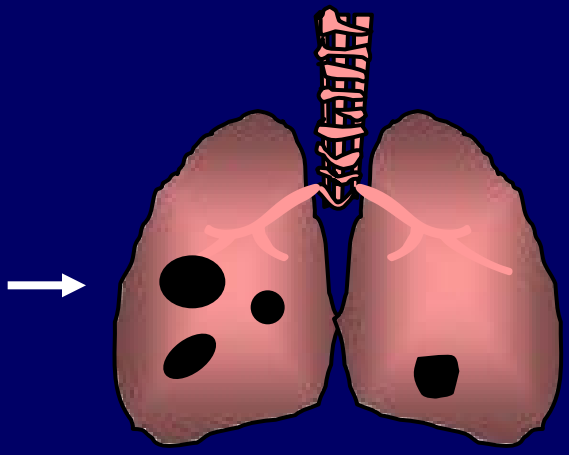
Tissue culture



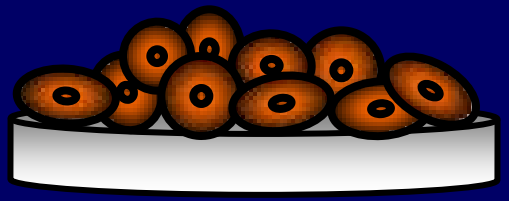
Tissue culture



Tail vein injection

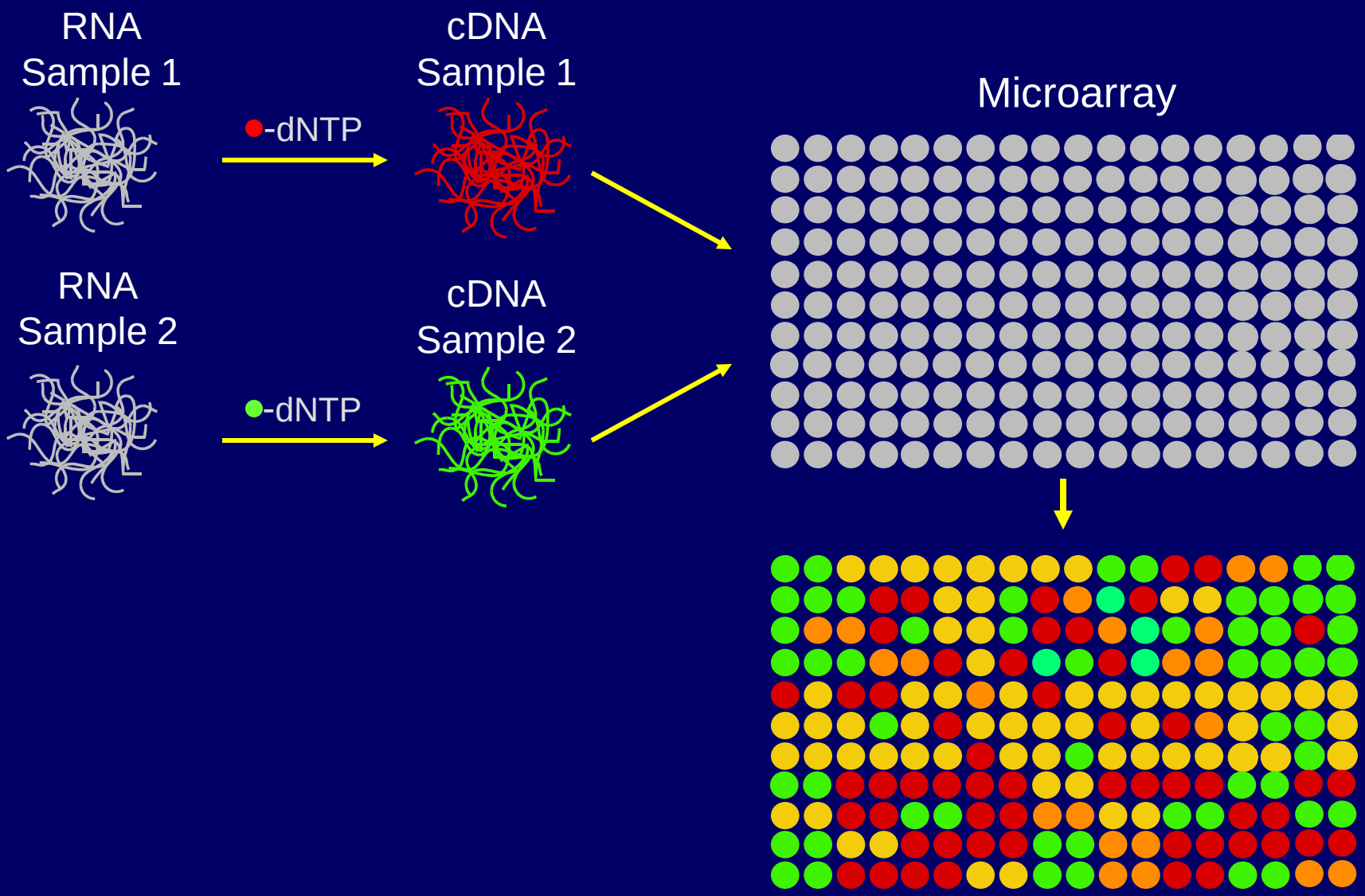


Pulmonary metastasis

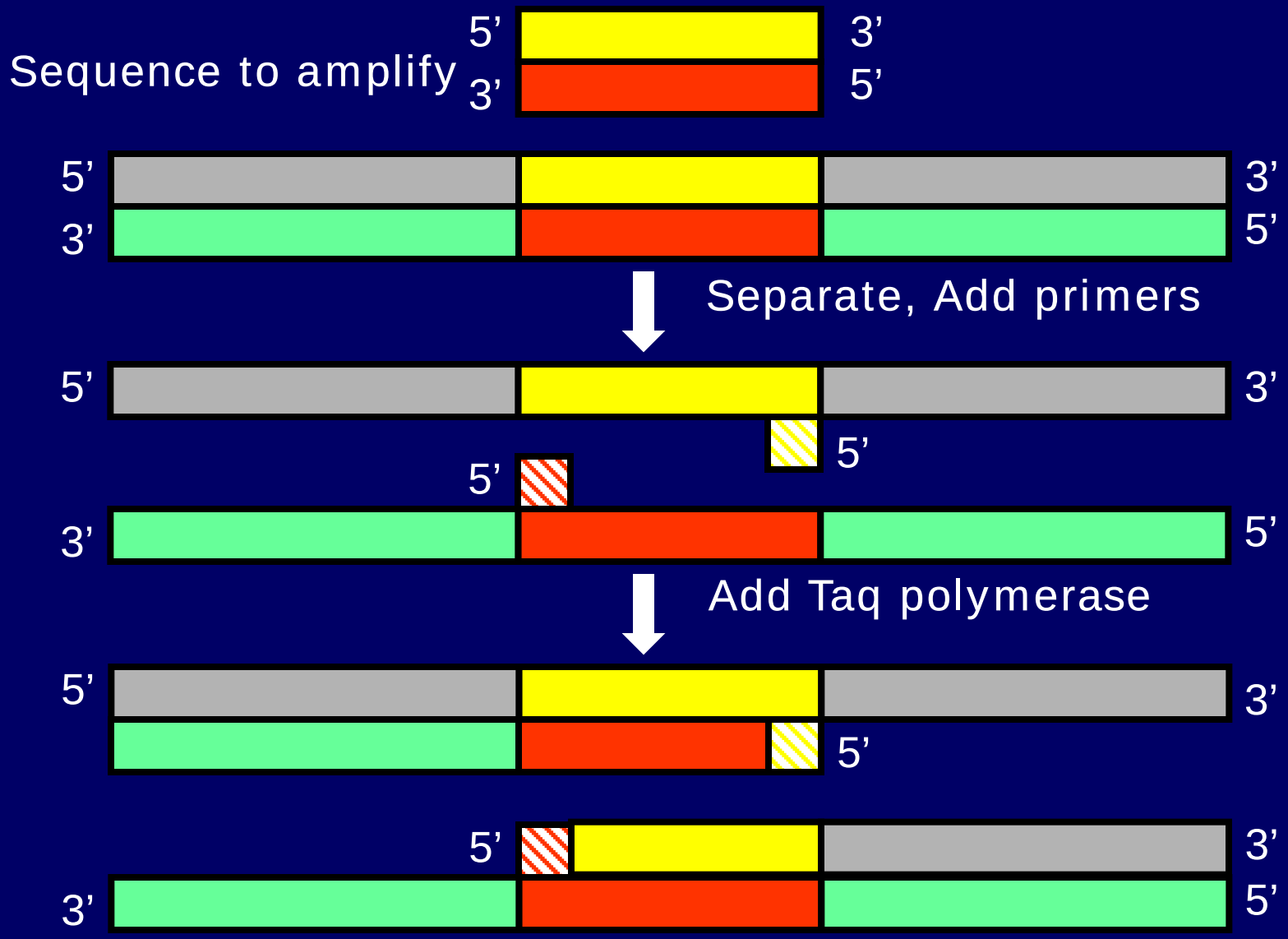


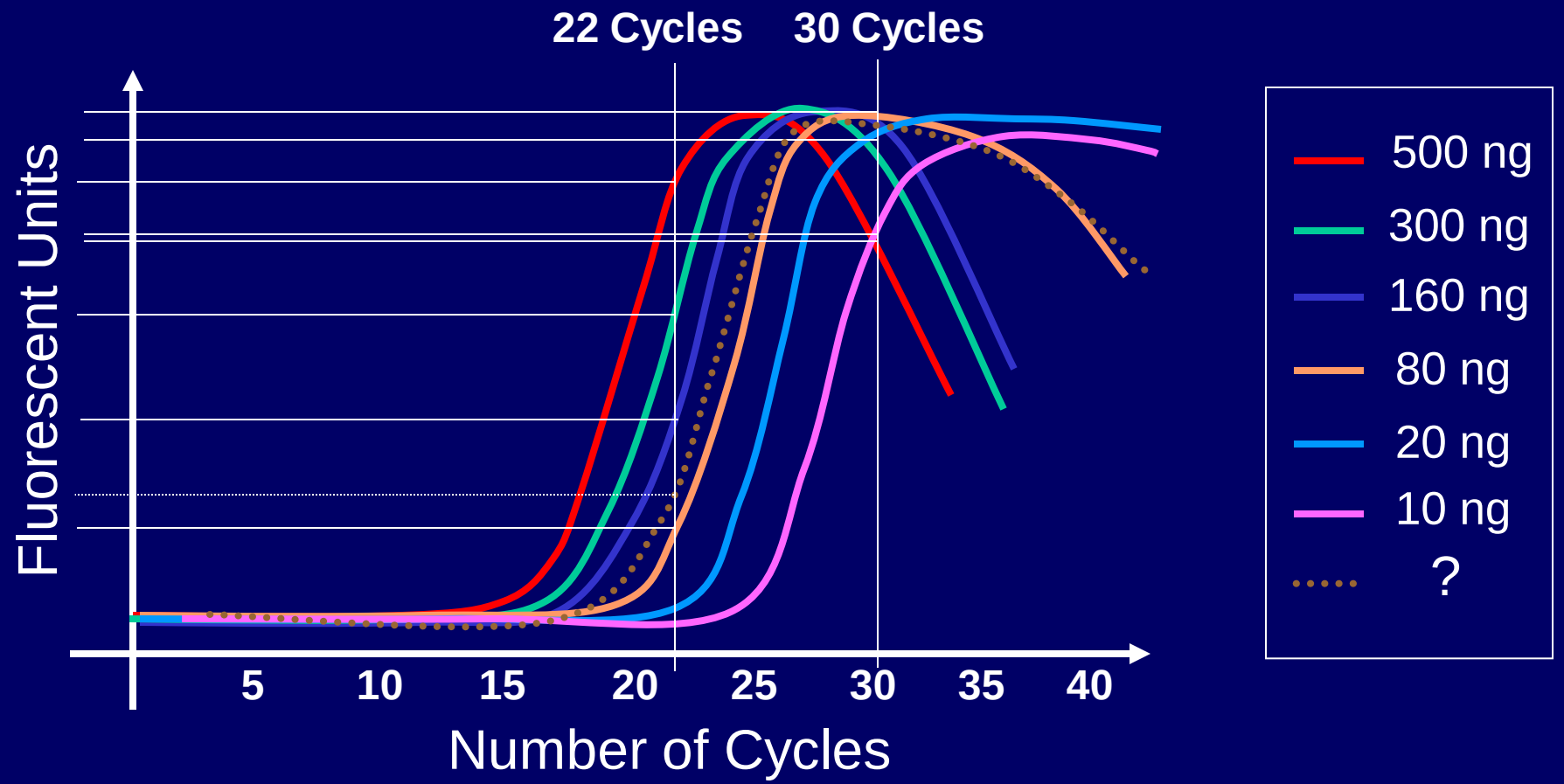
Tissue culture

Microarray analysis

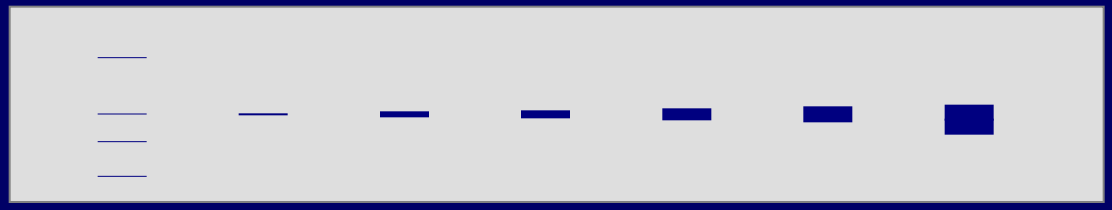


PCR


Sequence to amplify
5’
3’
3’
5’
5’
3’
3’
5’
Separate, Add primers
5’
3’
5’
5’
3’
5’
Add Taq polymerase
5’
3’
5’
5’
3’
3’
5’



Gel after
22 cycles



Intravasation assay