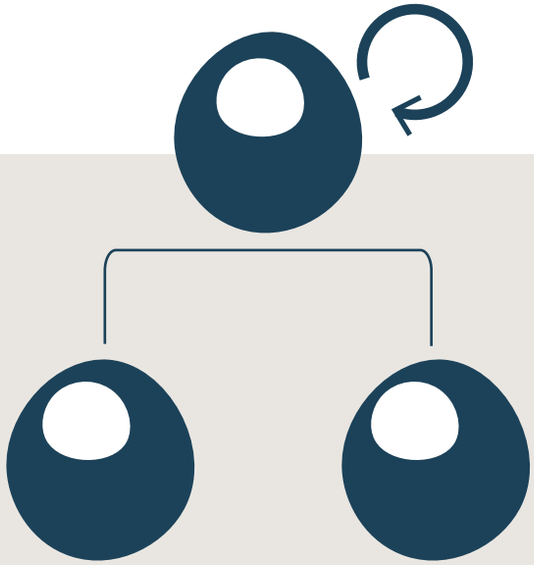
# Cancer Stem Cells:

*new perspectives versus old definitions*

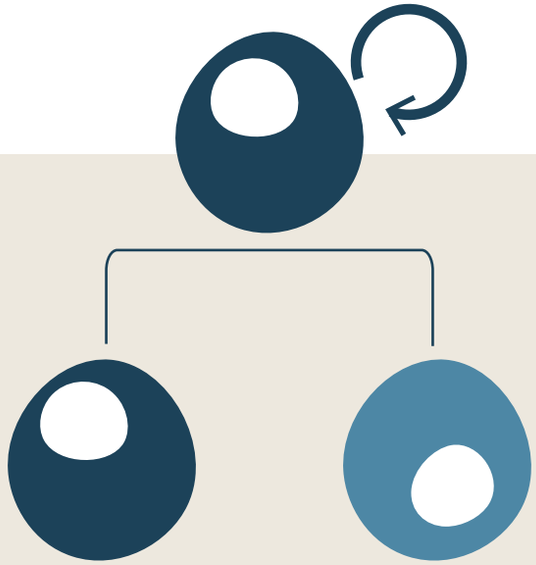



María Salazar Roa

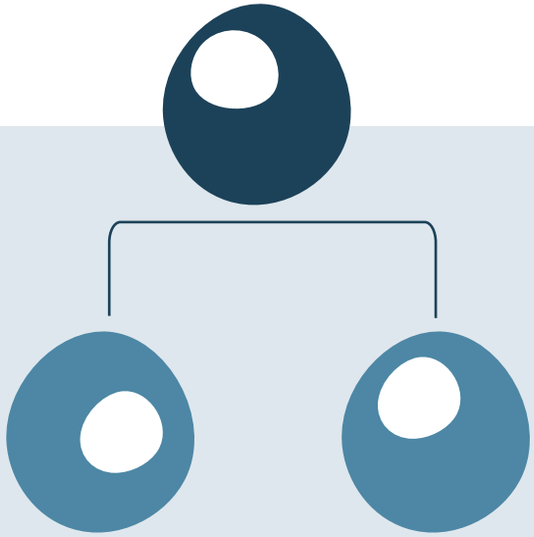
# How do stem cells divide?



Symmetric Self Renewal



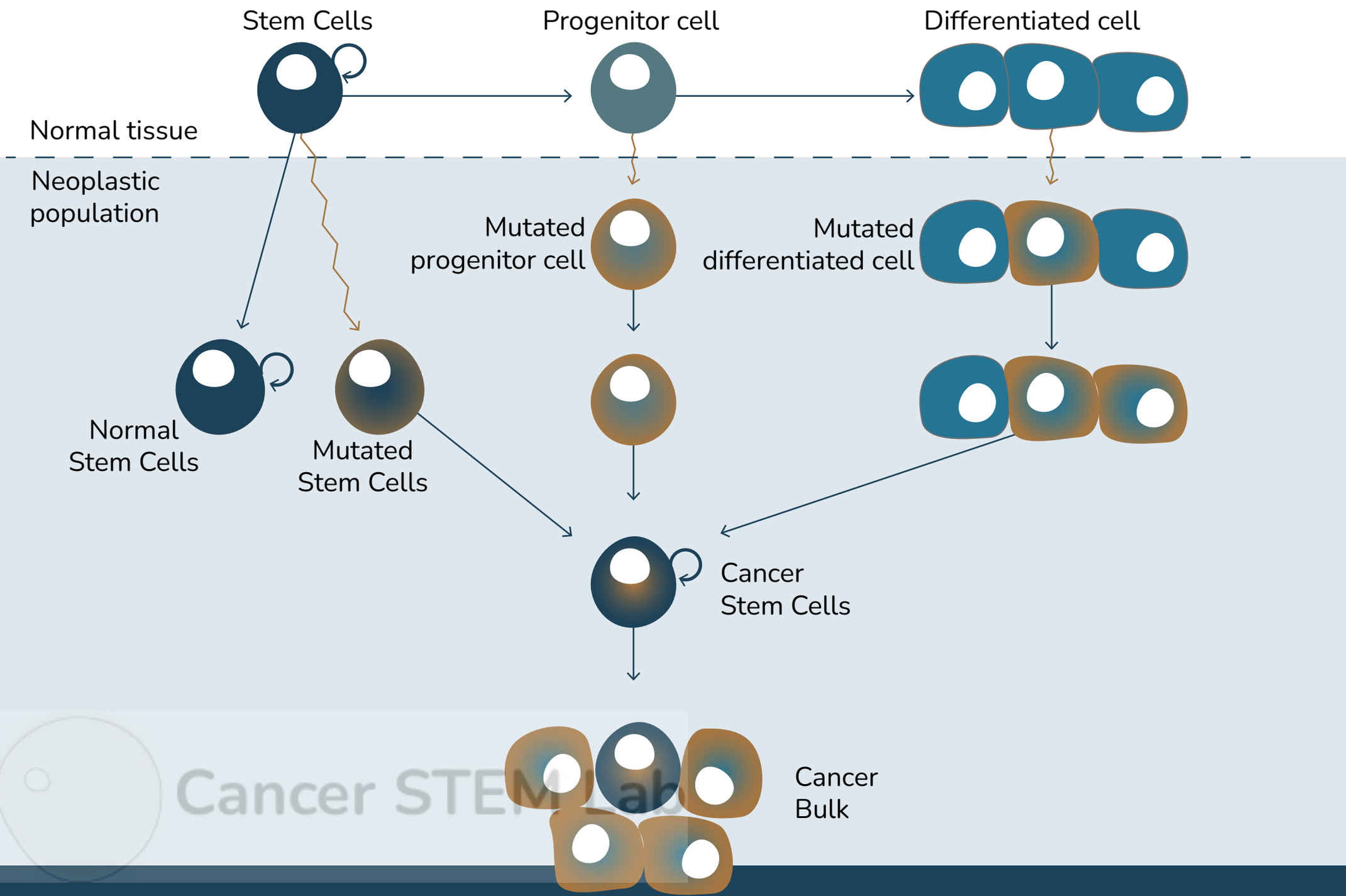
Asymmetric Self Renewal



Symmetric Differentiation

Cancer STEM Lab

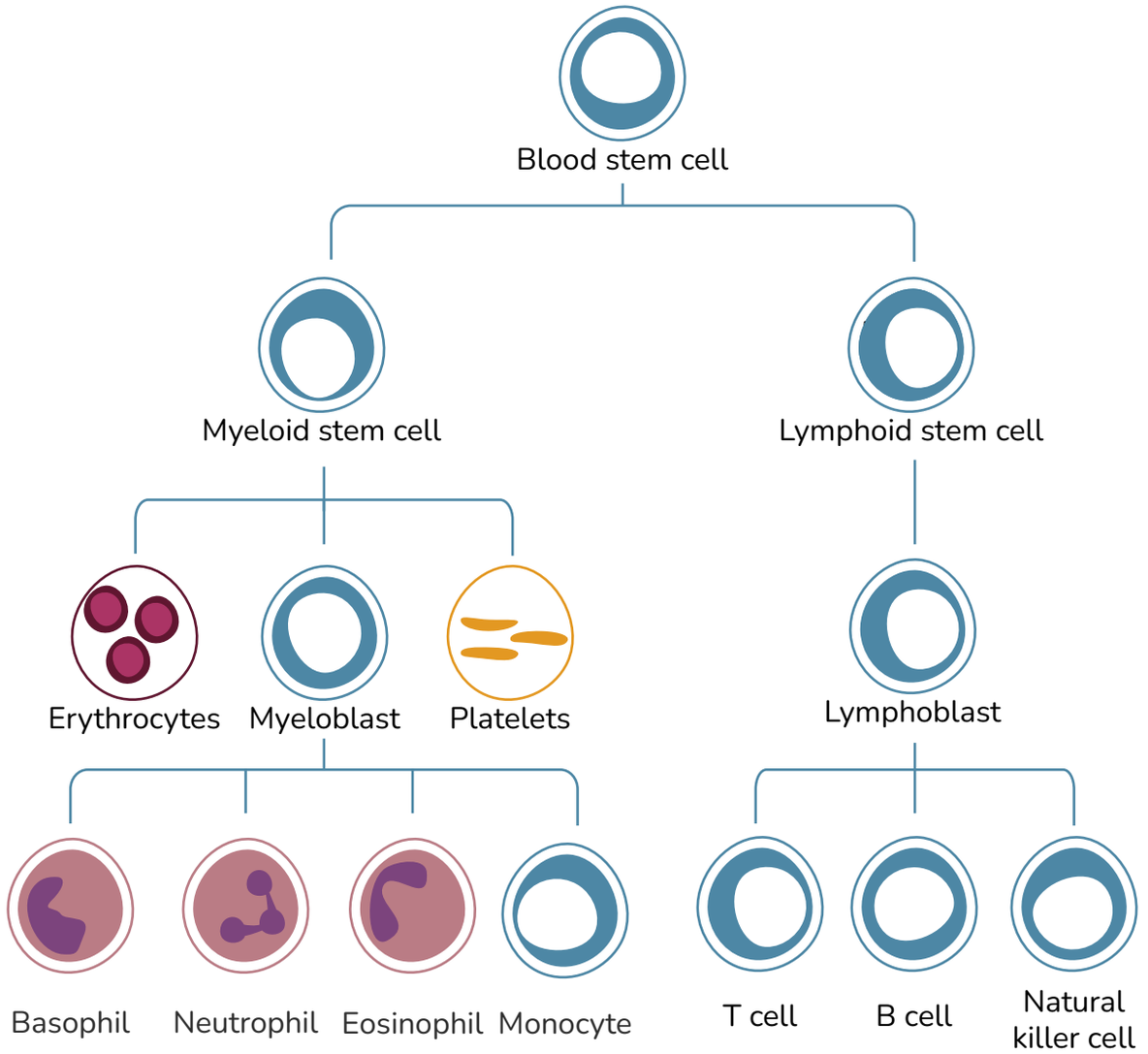
# Models for cancer stem cell formation: different theories




Cancer STEM Lab

# The origins of the CSC concept: hematopoietic stem cells

- HSCs were first identified 60 years ago.
- HSC is at the top of the **hematopoietic hierarchy**.
- HSC is a **rare** cell type and **divides infrequently**, i.e., it is quiescent.
- When HSC divides, it does so in an **asymetric** fashion and its lineage is long-lived.
- Individual HSCs can generate all blood lineages, they are **multipotent**.
- As daughter cells continue to divide, **they migrate down in the hierarchy and become progressively lineage restricted**.
- In homeostasis, the cell hierarchy is **rigid**.

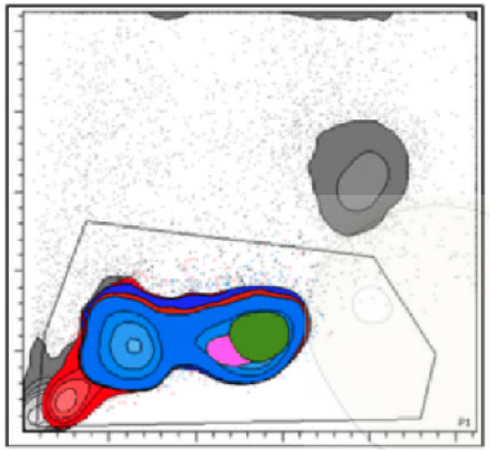



Cancer STEM Lab

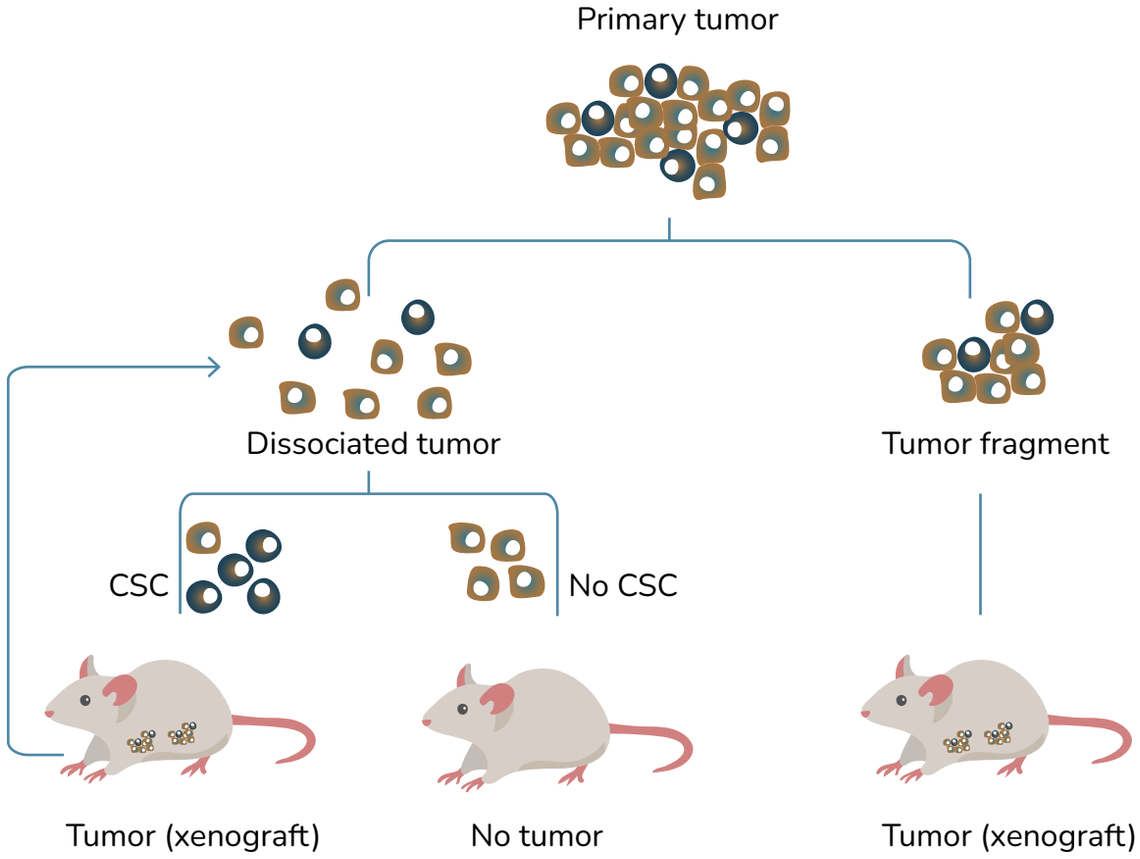
# The origins of the CSC concept: cell sorting and xenografting approaches

- **Transplantation assays involve dissociation of the tumor mass. Yet, cells in solid tumors rely heavily on cell-to-cell contacts, attachment to the extracelular matrix, and signals from the microenvironment.**

- Tumor-initiating potential in transplantation assays may serve as a surrogate for CSC-autonomous properties or even simply reflect the adaptation of particular tumor-cell populations to the experimental conditions.



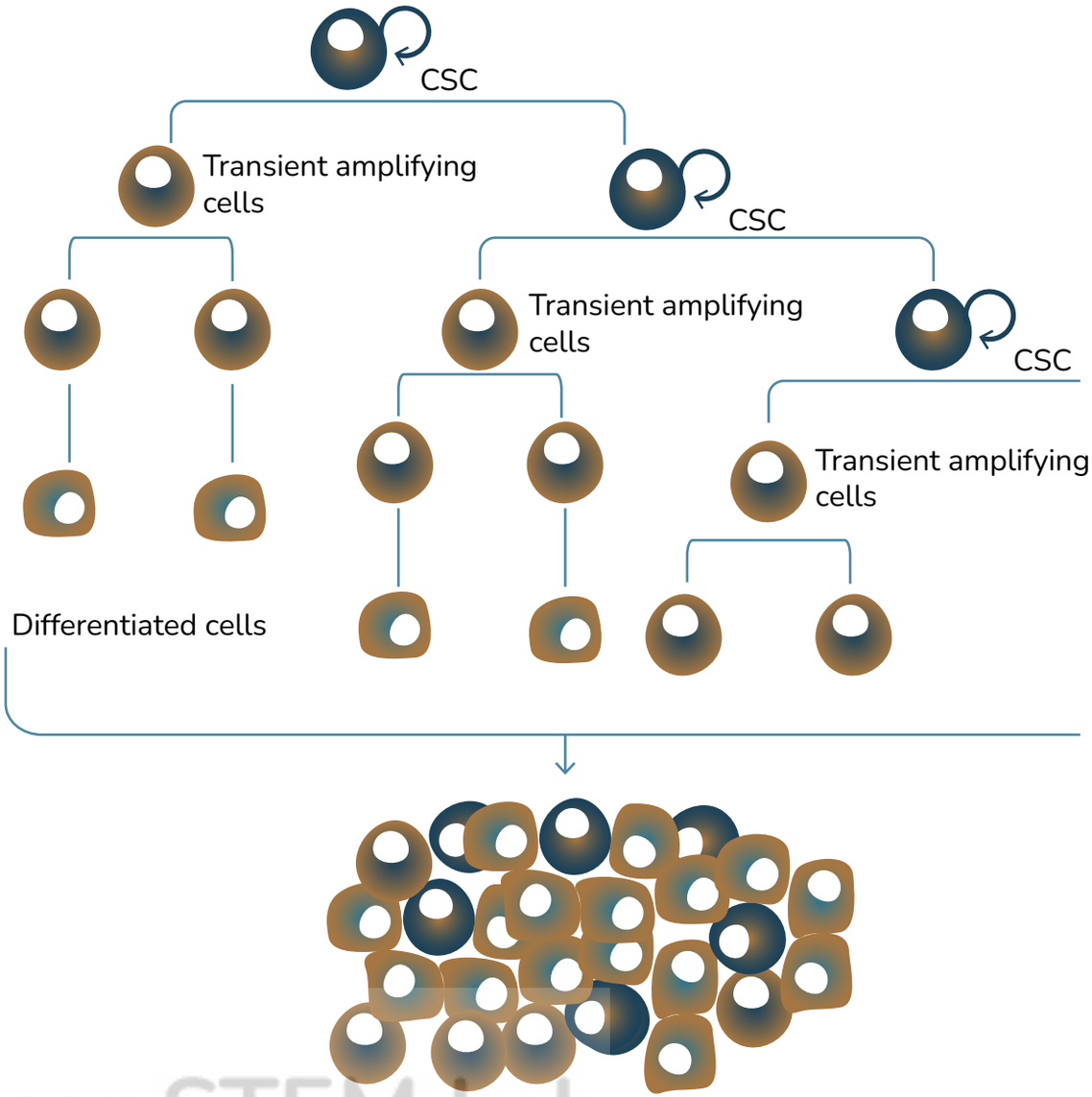
Immunophenotyping by Flow Cytometry




Cancer STEM Lab

# The standard cancer stem cell model: four premises

- 1.- A substantial fraction of celular **heterogeneity observed in tumors results from its hierarchical organization**, which is often reminiscent of the hierarchy in the tissue of origin.
- 2.- Tumor hierarchies are fueled by **rare self-renewing—typically quiescent— CSCs**, whereas the bulk of the tumor is composed of non-CSCs, which are capable only of transient proliferation and therefore do not contribute to long-term growth.
- 3.- **CSC identity is hardwired**, as illustrated by the fact that non-CSCs rarely initiate tumors in xenograft assays. Thus, **there is limited plasticity in the tumor hierarchy**.
- 4.- **CSCs are resistant to standard chemotherapy and radiation treatment**, which preferentially target non-CSCs—a phenomenon that explains relapse after treatment.



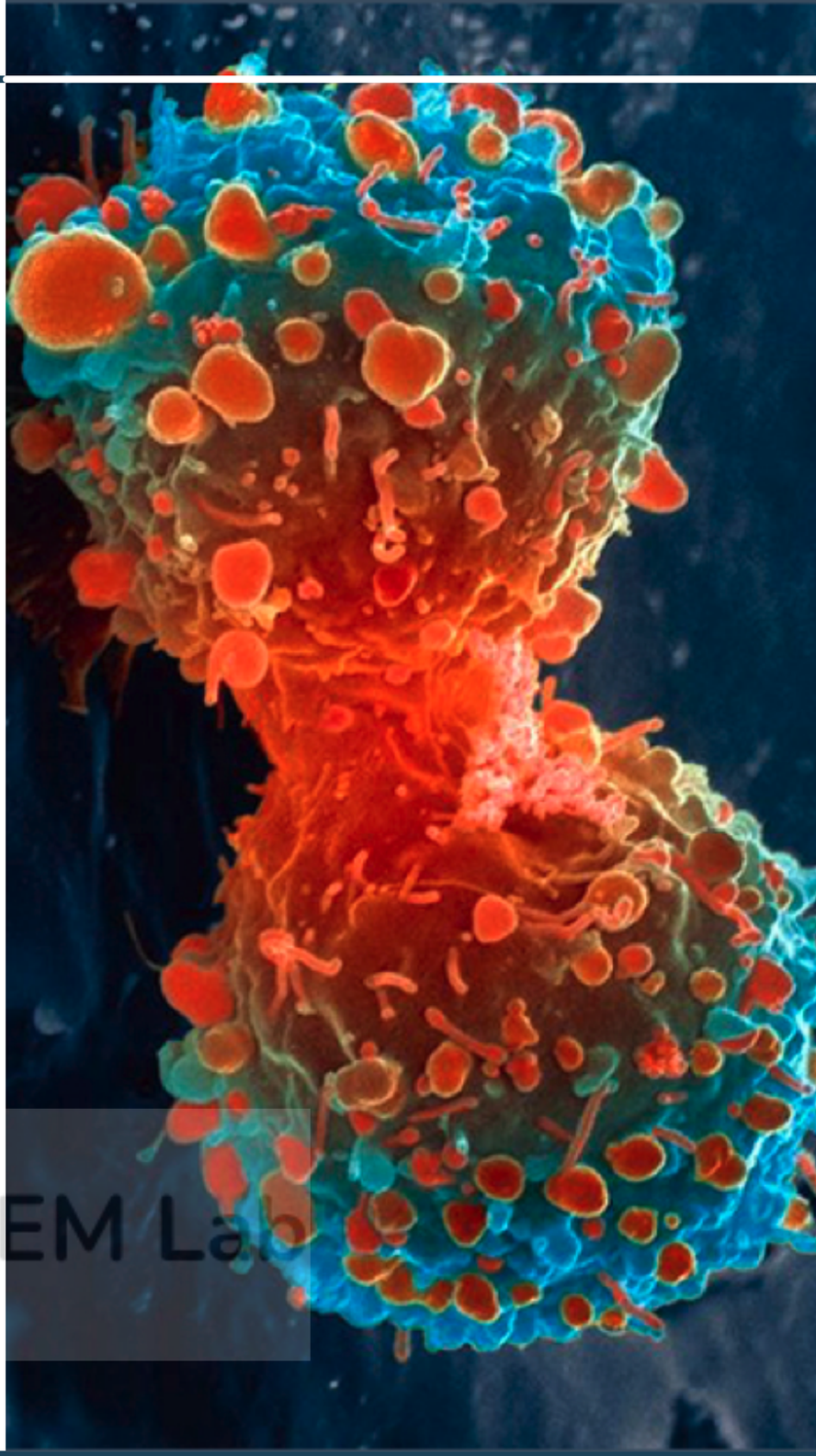

Cancer STEM Lab

# The shaping of the cancer stem cell concept

*“The CSC concept was proposed four decades ago and states that tumor growth, analogous to the renewal of healthy tissues, is fueled by small numbers of dedicated stem cells.*

*However, CSCs seem to have a particular plasticity, renewal and therapeutic response”*

Hans Clevers

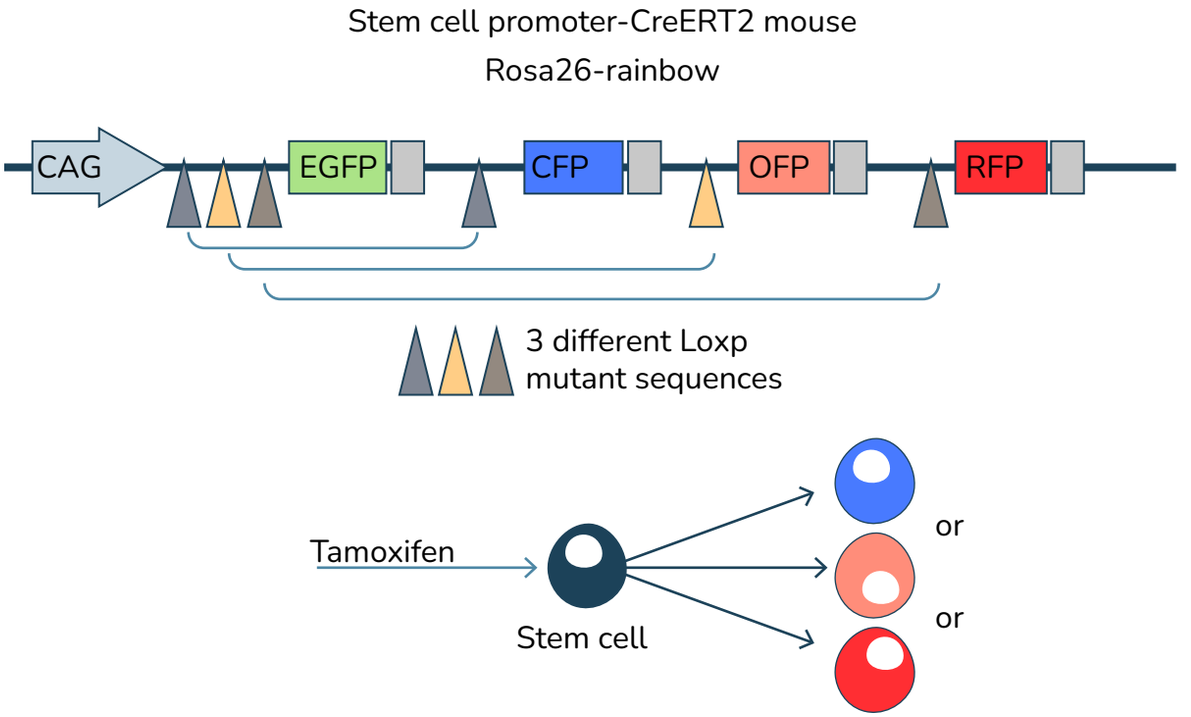


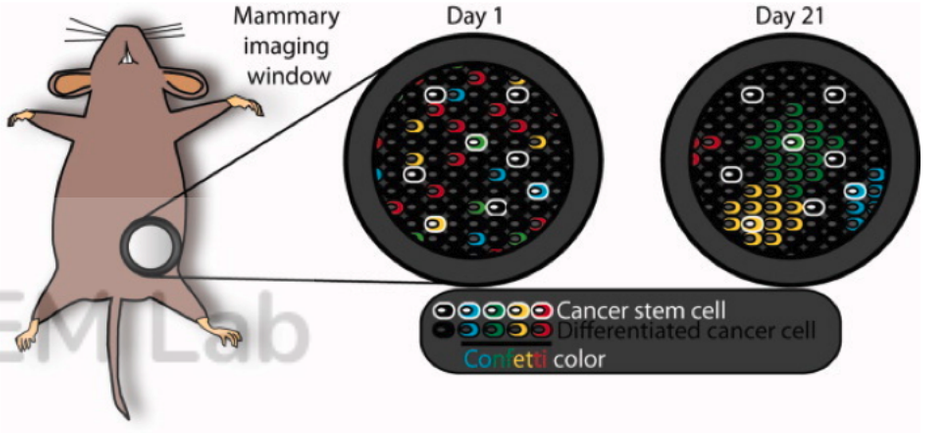
Cancer STEM Lab

# In search for more direct evidences: Lineage-tracing studies in tumors
# Or…How to study cancer stem cells in intact tumors *in situ*

- **Genetic-lineage tracing enables the identification and study of stem cells in solid tissues *in situ*, while <u>avoiding mechanical perturbation</u>.**

- Importantly, stable reporter expression is maintained in all daughter cells of the marked cell.
- **Persistence, size, and composition of cell clones** generated over time are used to evaluate stemness potential.

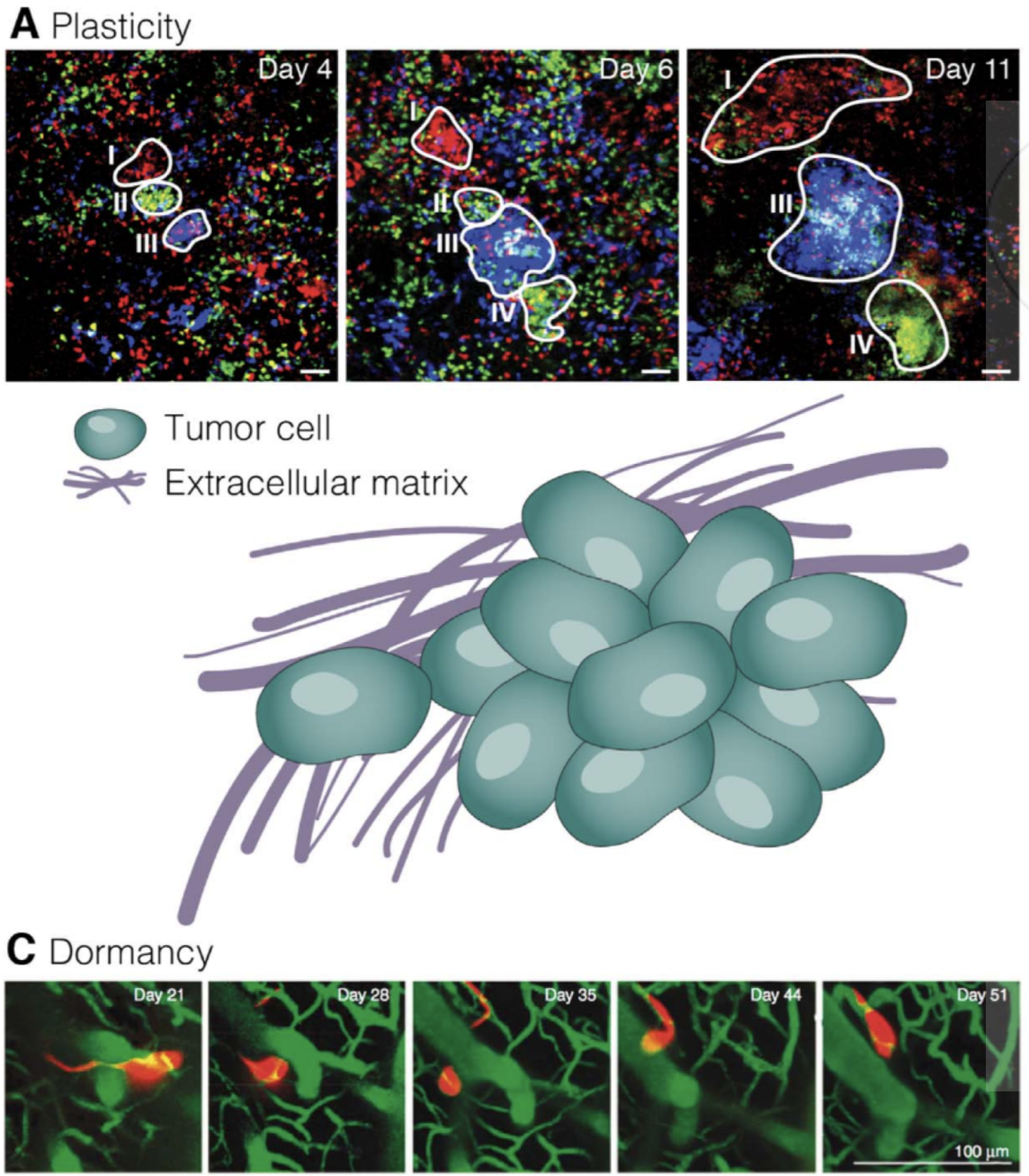



Cancer STEM Lab

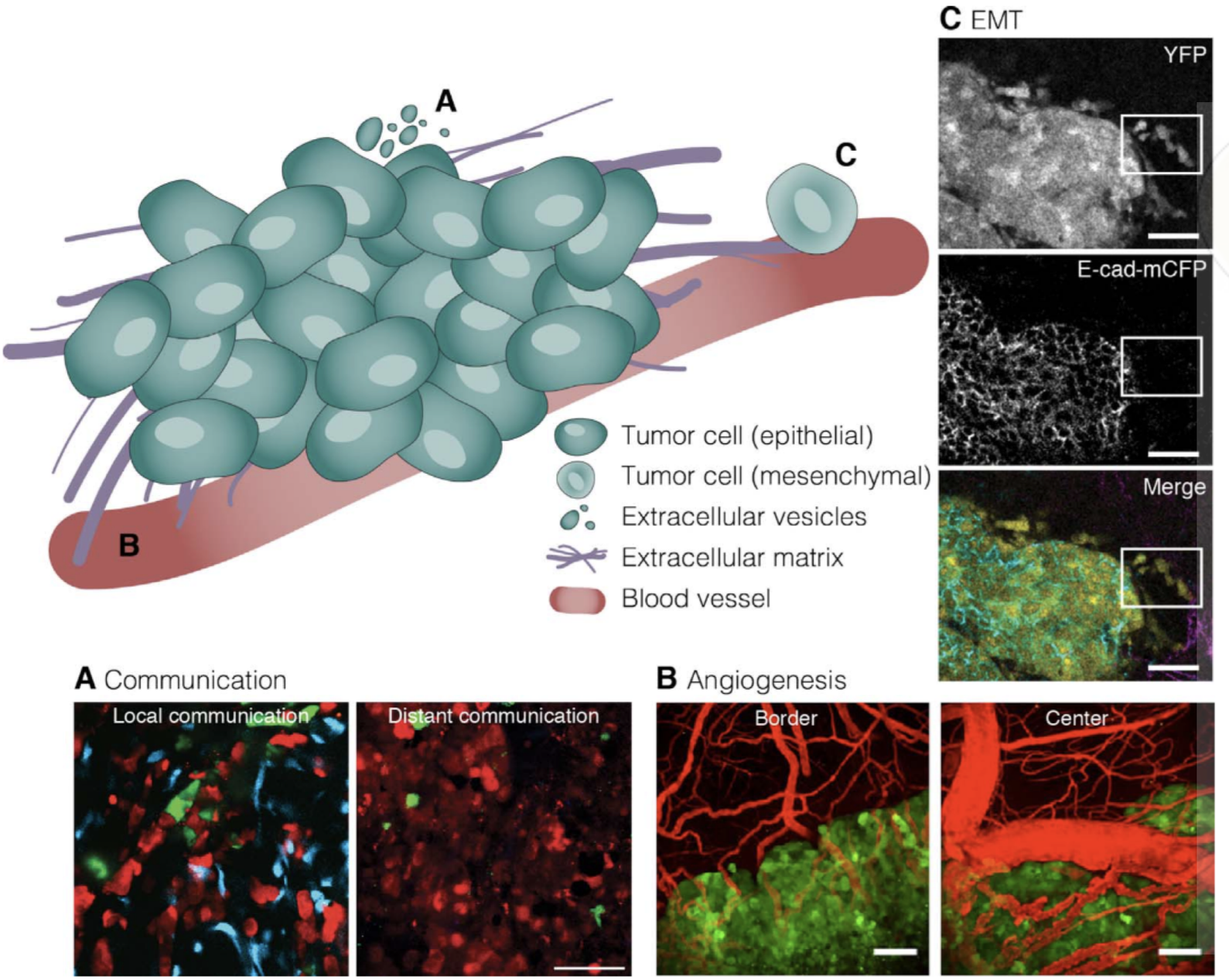
# In search for more direct evidences: Intravital imaging

## Or…How to observe and analyze cancer stem cells in intact tumors *in situ*




Cancer STEM Lab

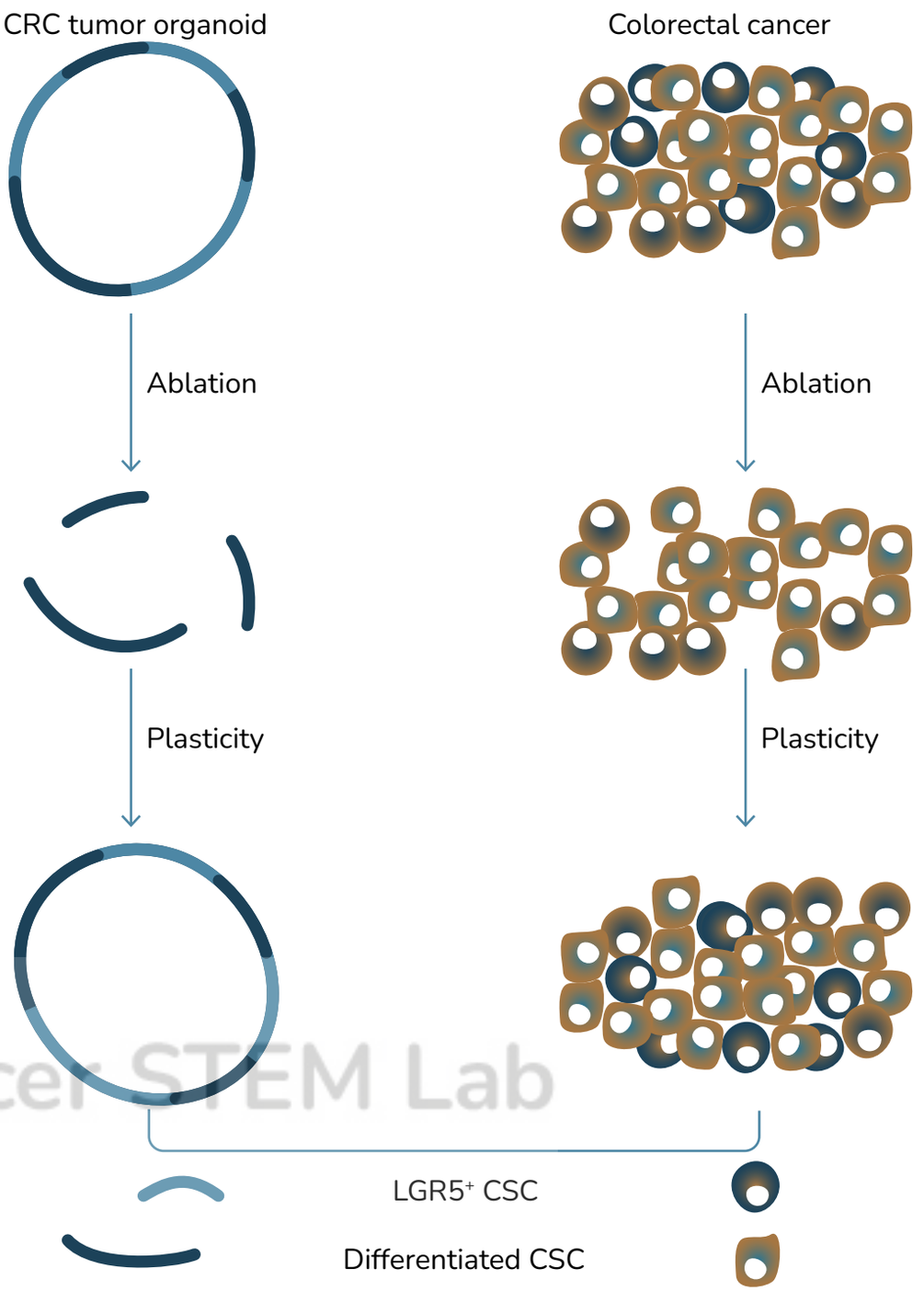
# In search for more direct evidences: Intravital imaging
## Or…How to observe and analyze cancer stem cells in intact tumors *in situ*




Cancer STEM Lab

# More evidences of plasticity on cancer stem cells

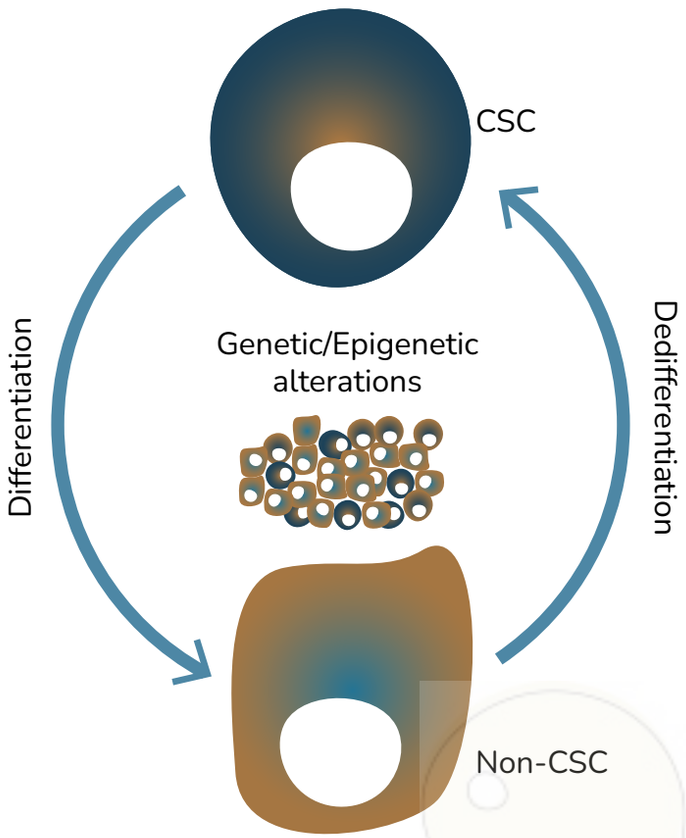
- CSC plasticity has recently been investigated through **cell ablation experiments** in xenografted human cancers.
- **In those experiments, cancer stem cell ablation was accompanied by a re-emergence of the cell population, indicative of plasticity.**

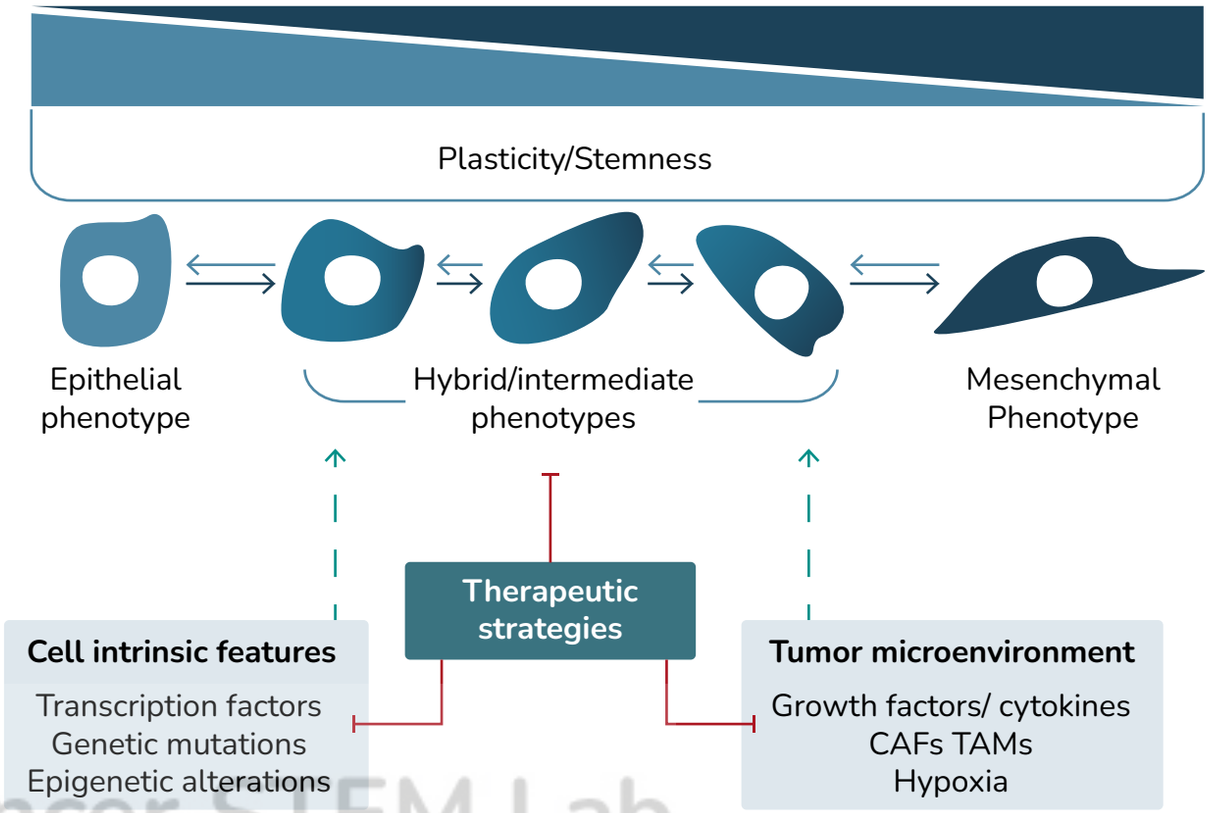



Cancer STEM Lab

# The plasticity of cancer stem cells: epithelial-to-mesenchymal transition

- Epithelial cancer cells can acquire a mesenchymal gene program that facilitates migration and invasion, a process known as **epithelial-to-mesenchymal transition** (EMT). Intravital imaging of breast cancer xenografts revealed that **migratory cells, having undergone EMT, immediately reverted to an epithelial state upon reaching the metastatic site.**
- **EMT in cancer cells may be transient** because epithelial tumor cells can adopt—depending on environmental cues—intermediate mesenchymal states that are reversible.




Cancer STEM Lab

# Novel views of adult stem cells: new perspectives, new definitions

Adult stem cells can be abundant in their niches, such as in the epidermis and intestinal crypts—up to 10% of crypt cells are intestinal stem cells expressing Lgr5. **Adult stem cells are not obligatorily quiescent**, and can actively divide throughout life, as seen in the stomach pylorus and intestinal crypts.

It has been found that **stem cell daughters do not display intrinsically divergent fates**, but, rather, that a stem cell division can result in zero, one, or two new stem cells, **depending on the available niche space**. In other words, **the stem cell progeny compete to occupy the niche**, a process known as **neutral competition**. This mechanism contrasts sharply with the 'classical' model of the existence of a stem cell–intrinsic, asymmetric mitotic process, in which every stem cell division invariably creates one new stem cell and one daughter cell.

**Stem cell hierarchies can be extensively plastic, meaning that daughter cells—and even fully differentiated cells—can re-enter the niche and dedifferentiate to replace lost stem cells.**

Finally, fully differentiated cells (such as hepatocytes) can enter the cell cycle to replace lost tissue upon hemi-hepatectomy, without ever reverting to a distinct stem cell phenotype. In sum, with some notable exceptions, such as muscle satellite cells and hair-follicle stem cells, **the search for rare, quiescent, hardwired professional stem cells in solid tissues has been less than fruitful**.

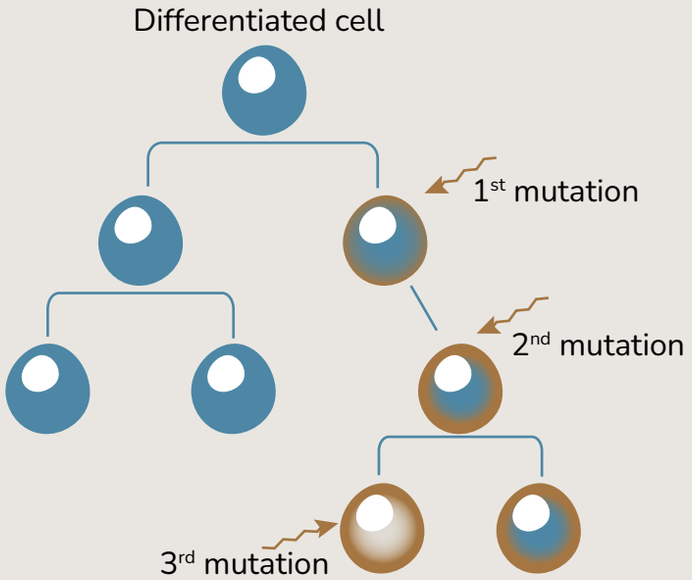
***"Apparently, evolution has resulted in many different ways of maintaining and repairing our organs"***

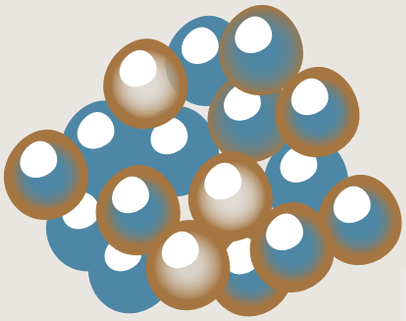
***(H. Clevers)***

Cancer STEM Lab

# Novel views of adult stem cells: new perspectives, new definitions

## Clonal evolution model

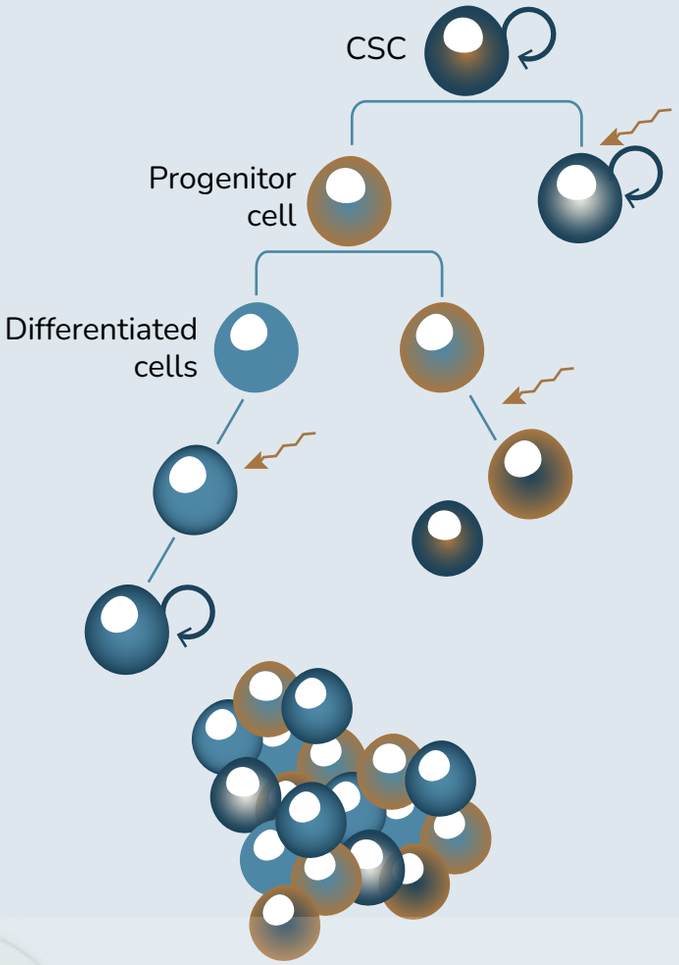


Tumor

Selective pressure and mutations contribute to tumor formation & tumor heterogeneity

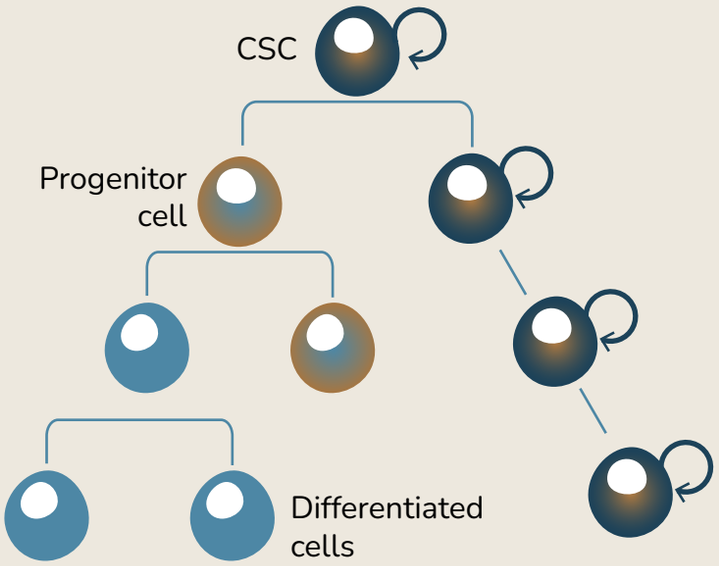
## Unified model

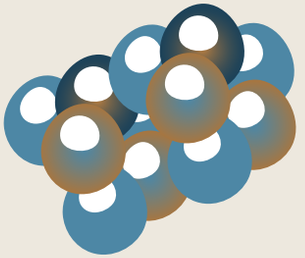



Tumor

High tumor heterogeneity
High plasticity & complexity
Dynamic system
Therapy failure

## Cancer stem cell model



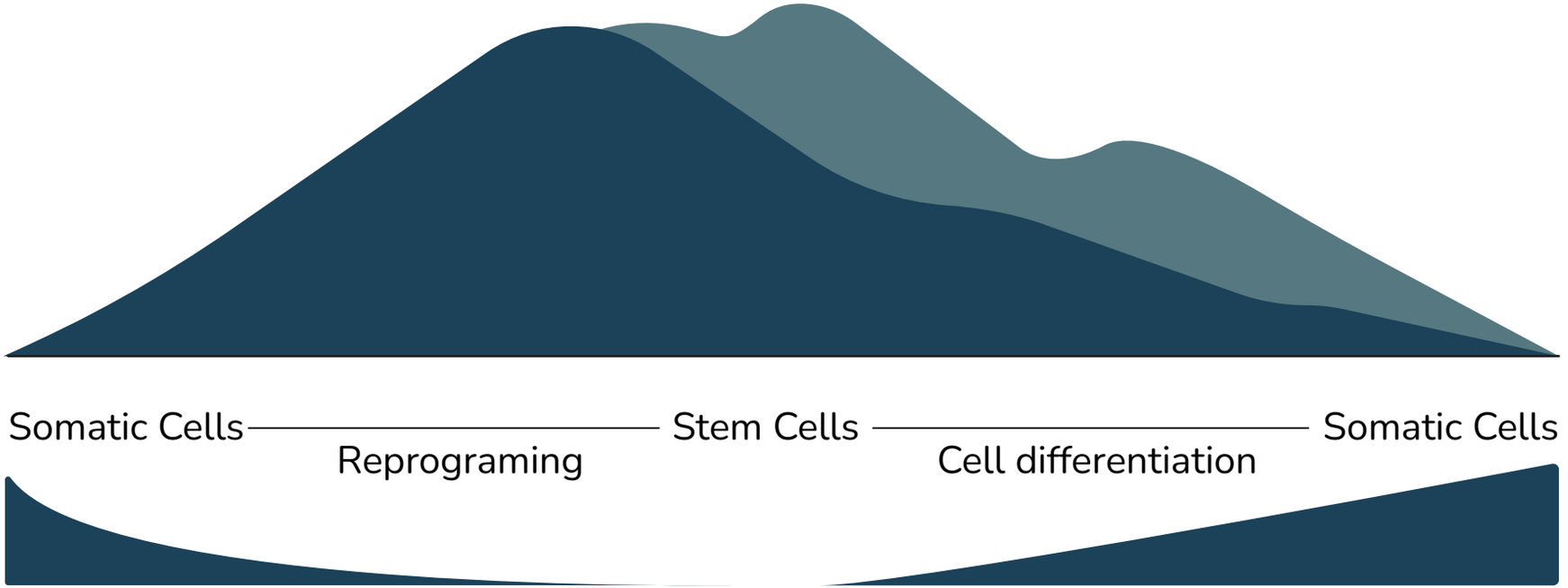
Tumor

Tumor propagation is driven by CSC

Cancer STEM Lab

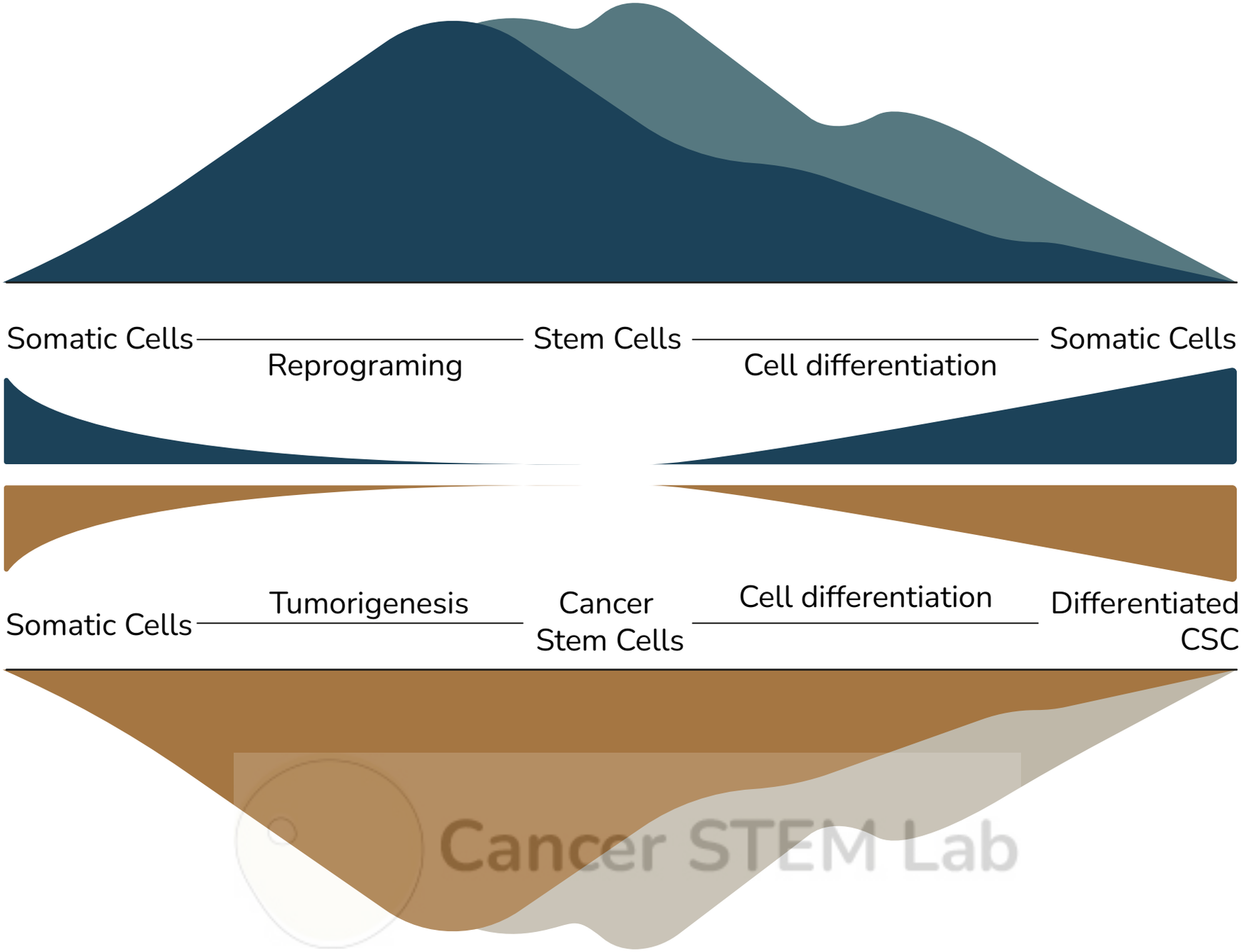
*Can we therapeutically target Cancer Stem Cells?*

# Stem Cells




Cancer STEM Lab

# Stem Cells in Cancer




Cancer STEM Lab

# Therapeutic targeting of cancer stem cells

The idea of anti-CSC therapy arose in the 1970s and 1980s from the observation that leukemic cells were blocked in an undifferentiated state, and it is exemplified by the use of **all-trans retinoic acid to induce terminal differentiation of CSCs**—currently the standard of care for the treatment of patients with acute promyelocytic leukemia.

The success of all-trans retinoic acid therapy inspired other therapies that were based on **inhibiting epigenetic regulators** to induce CSC differentiation in multiple hematological malignancies.

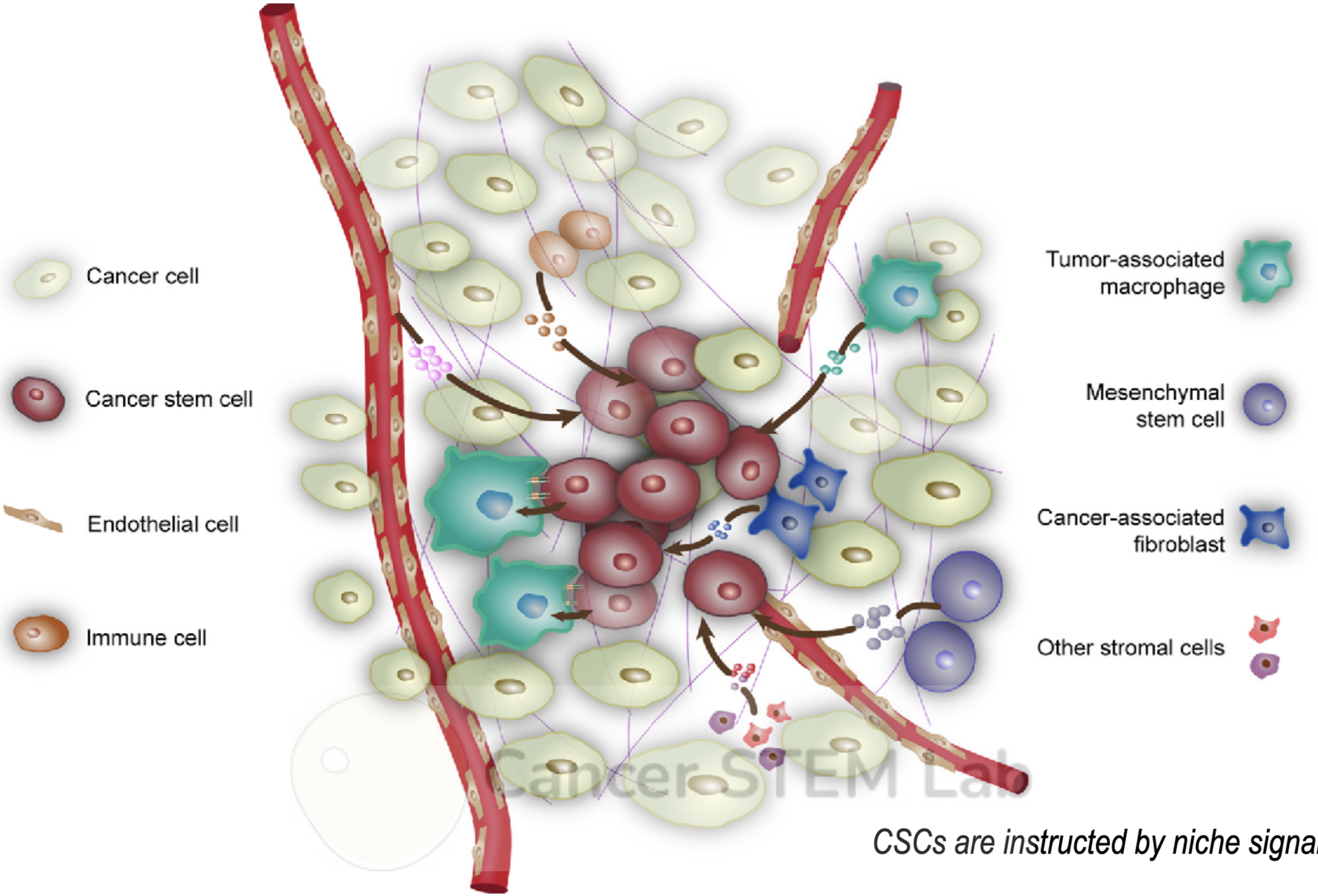
Cancer STEM Lab

# Therapeutic targeting of cancer stem cells

| Therapy | Potential drawbacks and limitations | Target | Progress to the clinic |
|---|---|---|---|
| Inhibition of key CSC signaling pathways | • Side effects on healthy stem cells that depend on the equivalent signals<br>• Acquisition of resistance mechanisms<br>• Regeneration of the CSC pool by plasticity of non-CSCs upon treatment cessation | WNT pathway | • Inhibitors of upstream WNT-signaling components (PORCN, FZD, anti-RSPO3) in clinical phases for colorectal, pancreas, and other tumor types<br>• PRI-724, an inhibitor of β-catenin/CBP interaction in phase 1 trials for several malignancies (for reviews, see refs. 136,144) |
| | | NOTCH pathway | • Several inhibitors of γ-secretase, NOTCH receptor or NOTCH ligands in distinct clinical phases for multiple cancer types (for reviews, see refs. 144,145) |
| CSC ablation using antibody–drug conjugates (ADCs) | • Toxicity associated with ADCs<br>• Lack of CSC-specific markers<br>• Depletion of normal stem cells that share surface markers with CSC<br>• Regeneration of CSCs by plasticity of non-CSCs upon treatment cessation<br>• Intratumor heterogeneity in CSC surface-marker expression | Several CSC surface markers, including CD33, LGR5, CD133, and DLL3 | • ADC directed against $CD33^{+}$ leukemic stem cells in AML was given FDA approval but it was later withdrawn due to toxicity[146].<br>• Many ADCs are being tested in clinical trials[147], but it is unclear whether any target CSCs<br>• Strategies based on bona fide CSC markers (for example, LGR5) remain at preclinical phases |
| Epigenetic therapy | • Toxicity owing to misregulation of gene expression in healthy stem cells<br>• Knowledge of the epigenetic regulation of CSCs in solid tumors is sparse<br>• Regeneration of the CSC pool by plasticity of non-CSCs upon treatment cessation | Multiple epigenetic regulators, including LSD1, HDACs, DOT1L, BET proteins, and IDH1/2 | • Differentiation therapy by all-*trans* retinoic acid is standard of care in APML<br>• HDAC inhibitors approved by FDA for several malignancies. Large number of other epigenetic regulator inhibitors in phase I-III trials for hematological and solid malignancies (for reviews, see refs. 115,148), some of which potentially target CSCs. |
| Targeting of quiescent CSCs | • Still limited knowledge of specific features of quiescent CSCs | Blockade of specific dependencies, such as metabolic requirements (e.g., anti-CD36 (ref. 91), inhibitors of OxPhos[82,83,111]) | • Preclinical research |

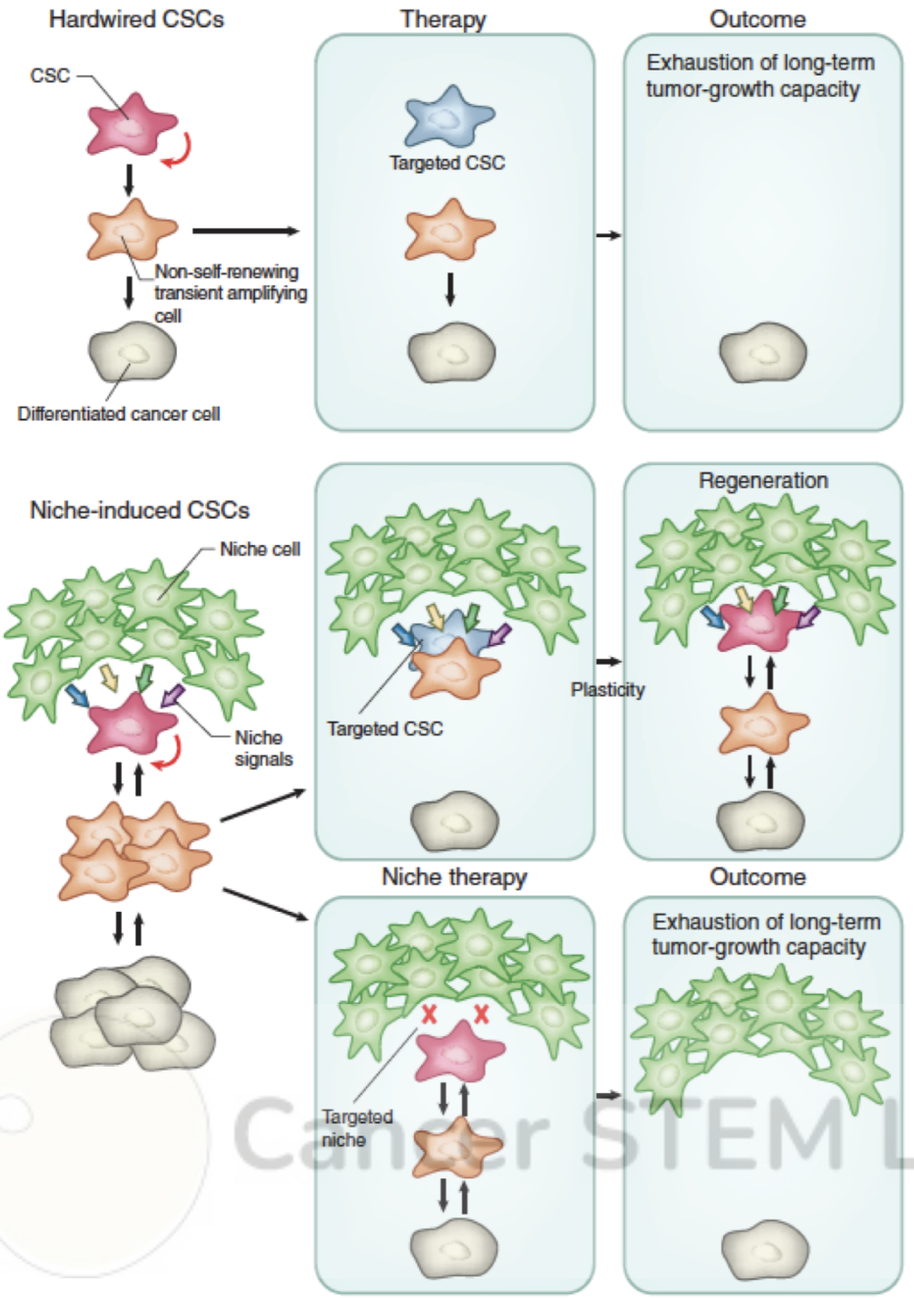
Cancer STEM Lab

# Emerging concepts in stem cell and cancer stem cell biology: NICHE dependency




*CSCs are instructed by niche signals*

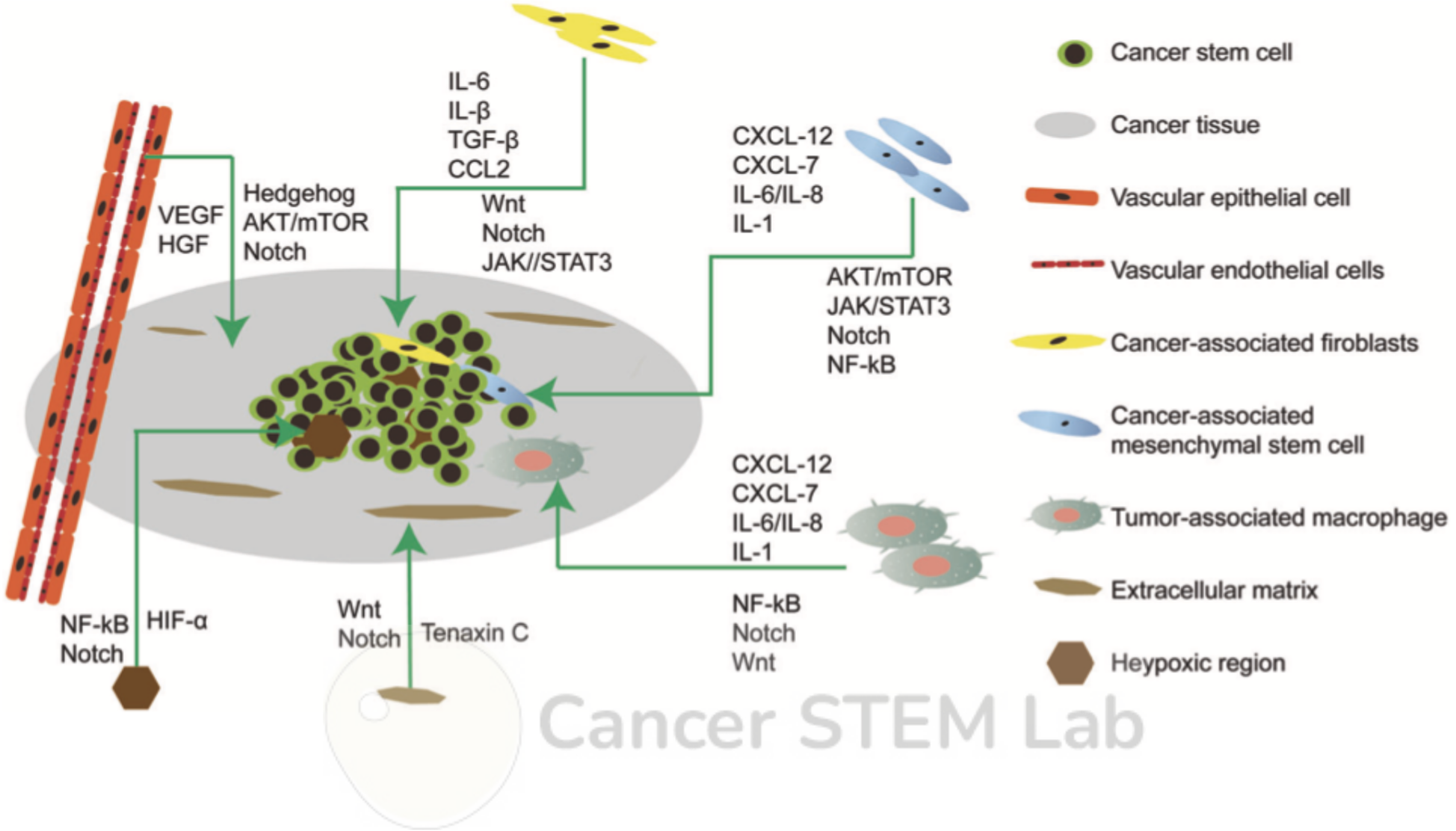
# What about targeting the cancer stem cell niche?



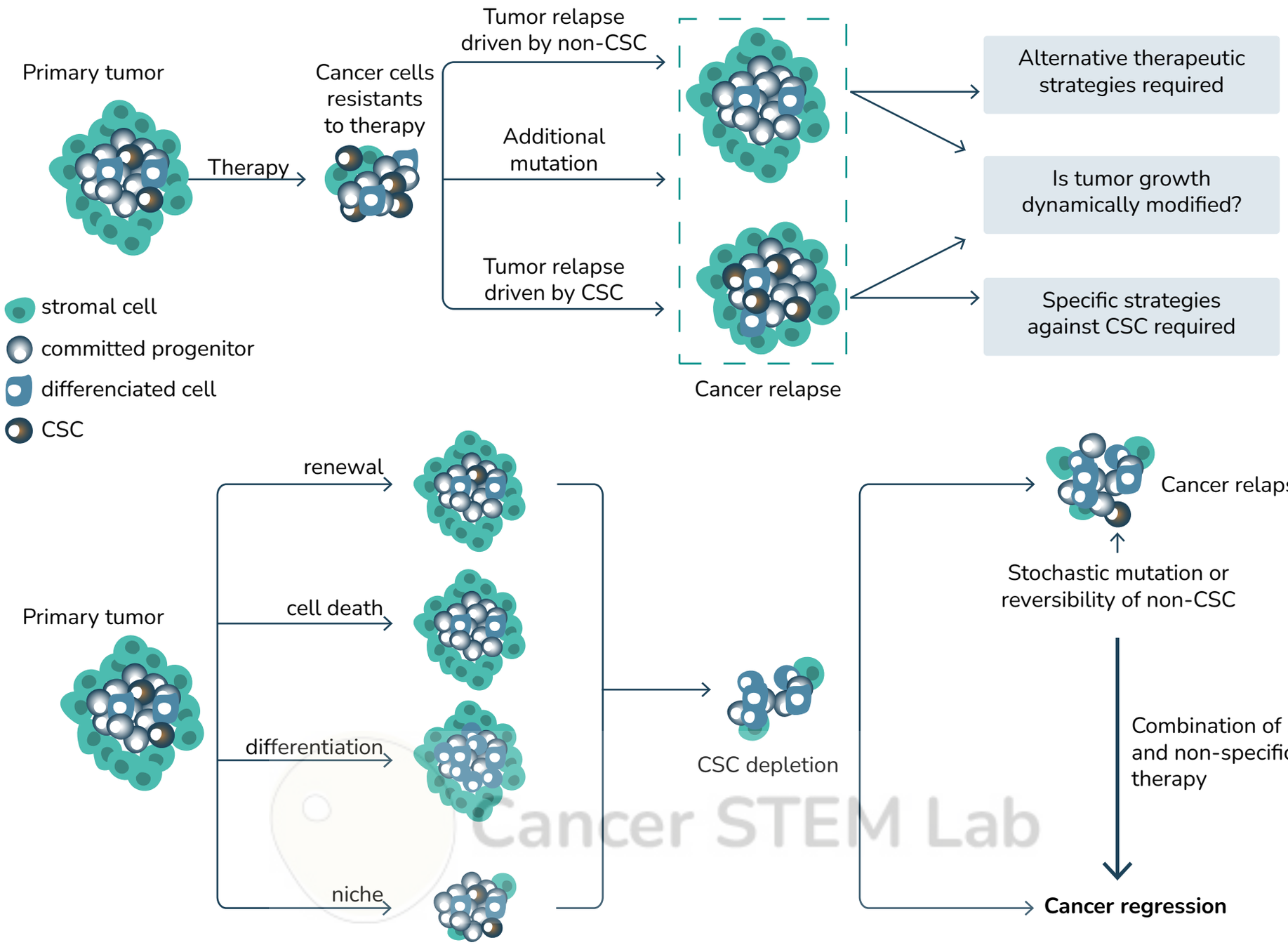

Cancer STEM Lab

# What about targeting the cancer stem cell niche?

## Pathways involved in CSC emergence, development, maintenance and survival



# Implications of cancer stem cells on tumor therapy and tumor relapse



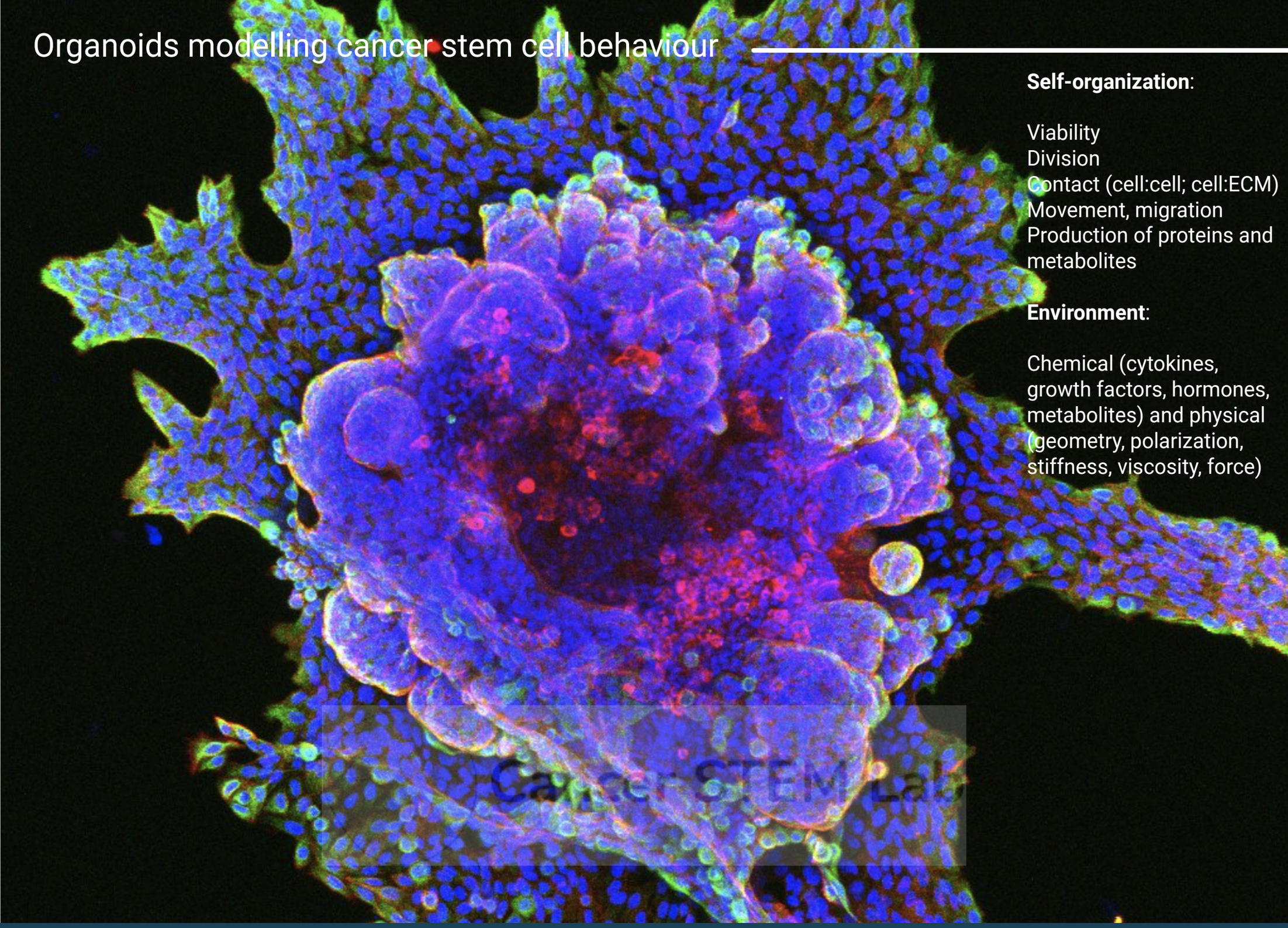

Cancer STEM Lab

*Organoids as a model to study cancer stem cell behaviour*



Cancer STEM Lab

# Organoids modelling cancer stem cell behaviour



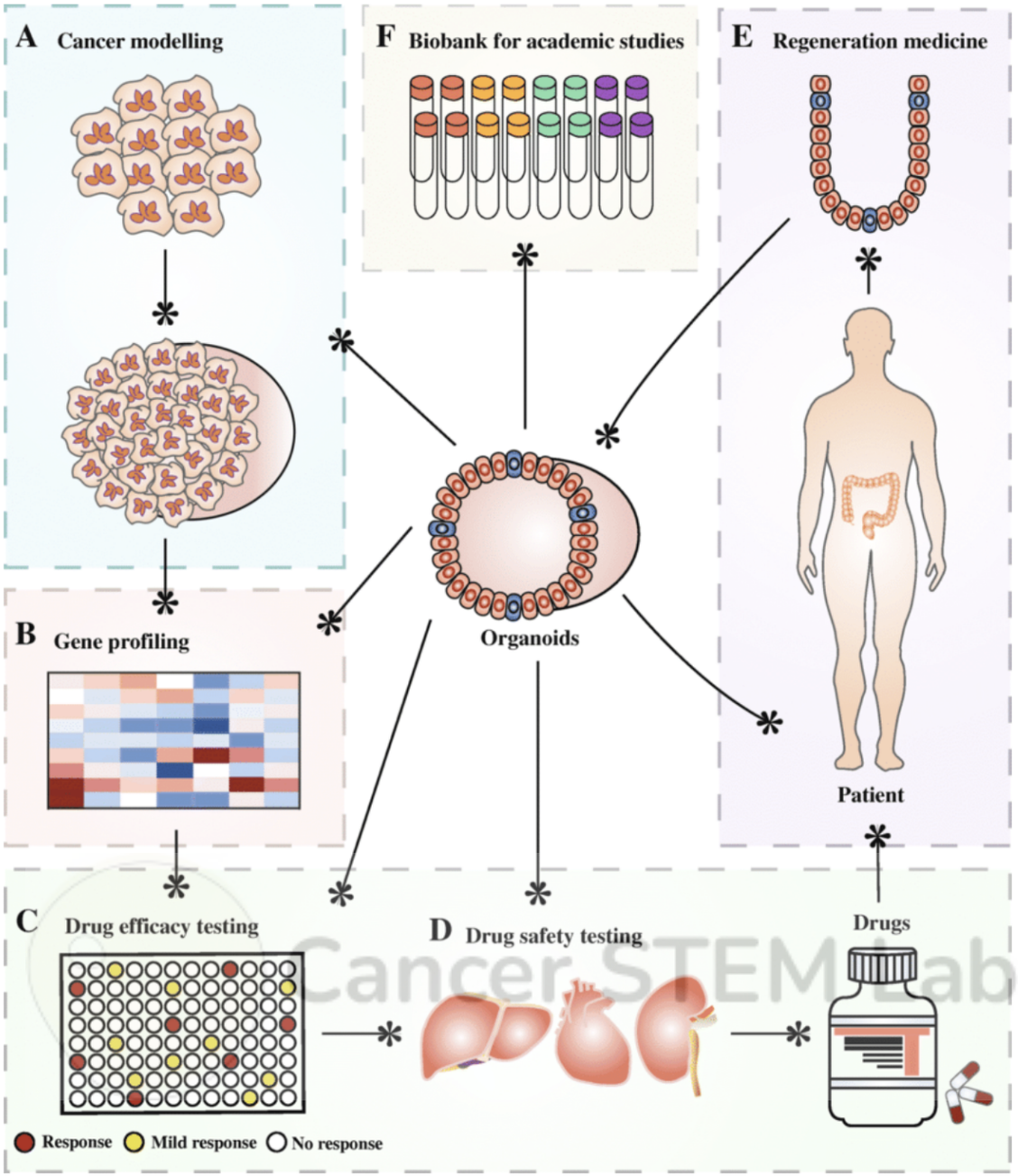

**Self-organization**:

Viability
Division
Contact (cell:cell; cell:ECM)
Movement, migration
Production of proteins and metabolites

**Environment**:

Chemical (cytokines, growth factors, hormones, metabolites) and physical (geometry, polarization, stiffness, viscosity, force)

# Taking advantage of niche dependency: Organoids that model cancer stem cell behaviour



# Taking advantage of niche dependency: Organoids that model cancer stem cell behaviour

These studies build upon the available knowledge of stem cell niche requirements:

Y-27632:ROCK inhibitor,

SB431542:TGF-β R Kinase Inhibitor IV,

ITS: Insulin Transferrin-Sodium,

NEAA: Non Essential Amino Acid Culture Supplement,

EGF: Epidermal Growth Factor,

RSPO1: R-spondin-1,

Wnt3A: Wingless-Type MMTV Integration Site Family Member 3A,

T3: Triiodothyronine,

FBS: Fetal Bovine Serum,

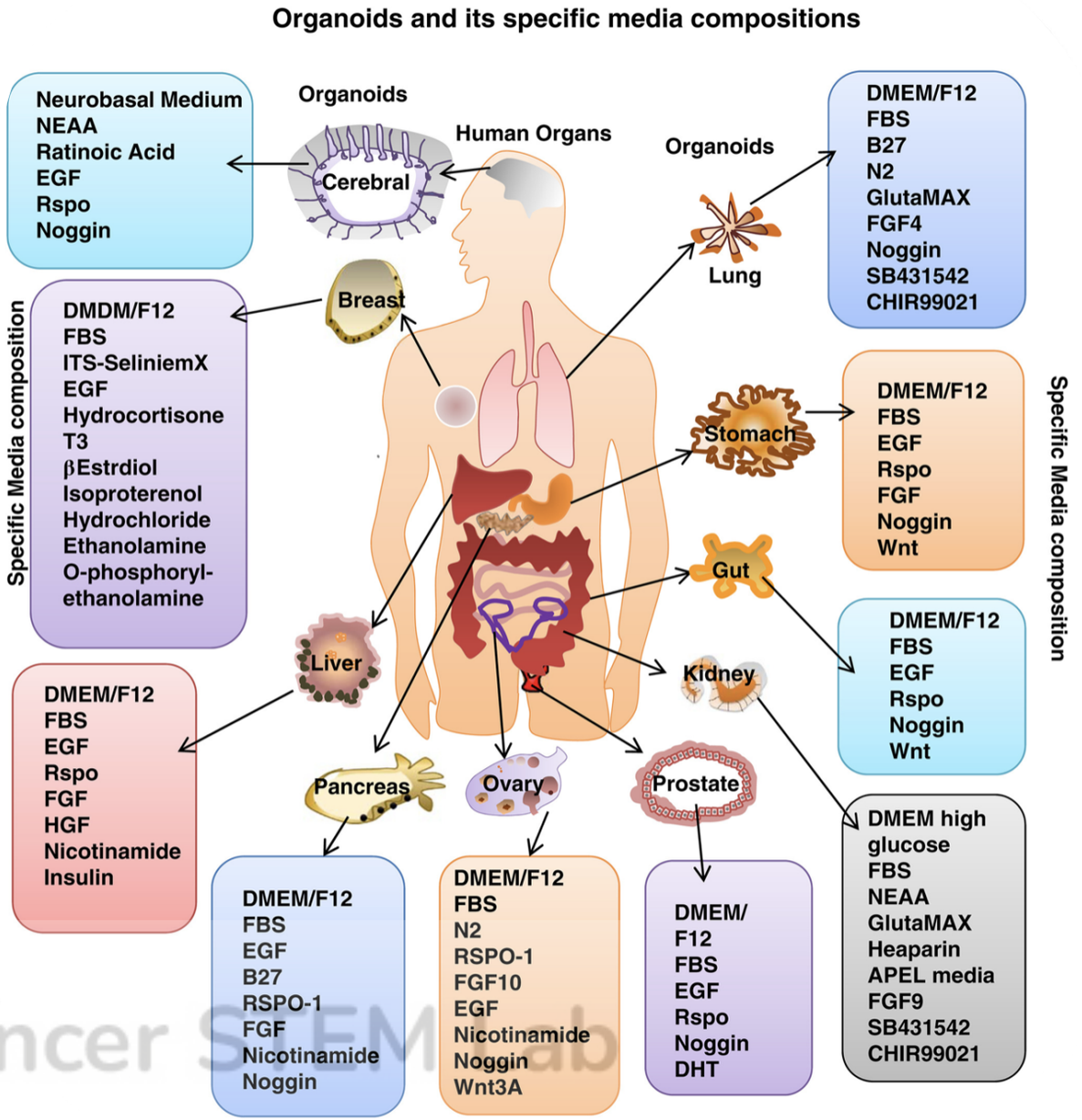
FGF: Fibroblast Growth Factor,

HGF: Hepatocyte Growth factor,

DMEM/F12: Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12,

DHT: Dihydrotestosterone,

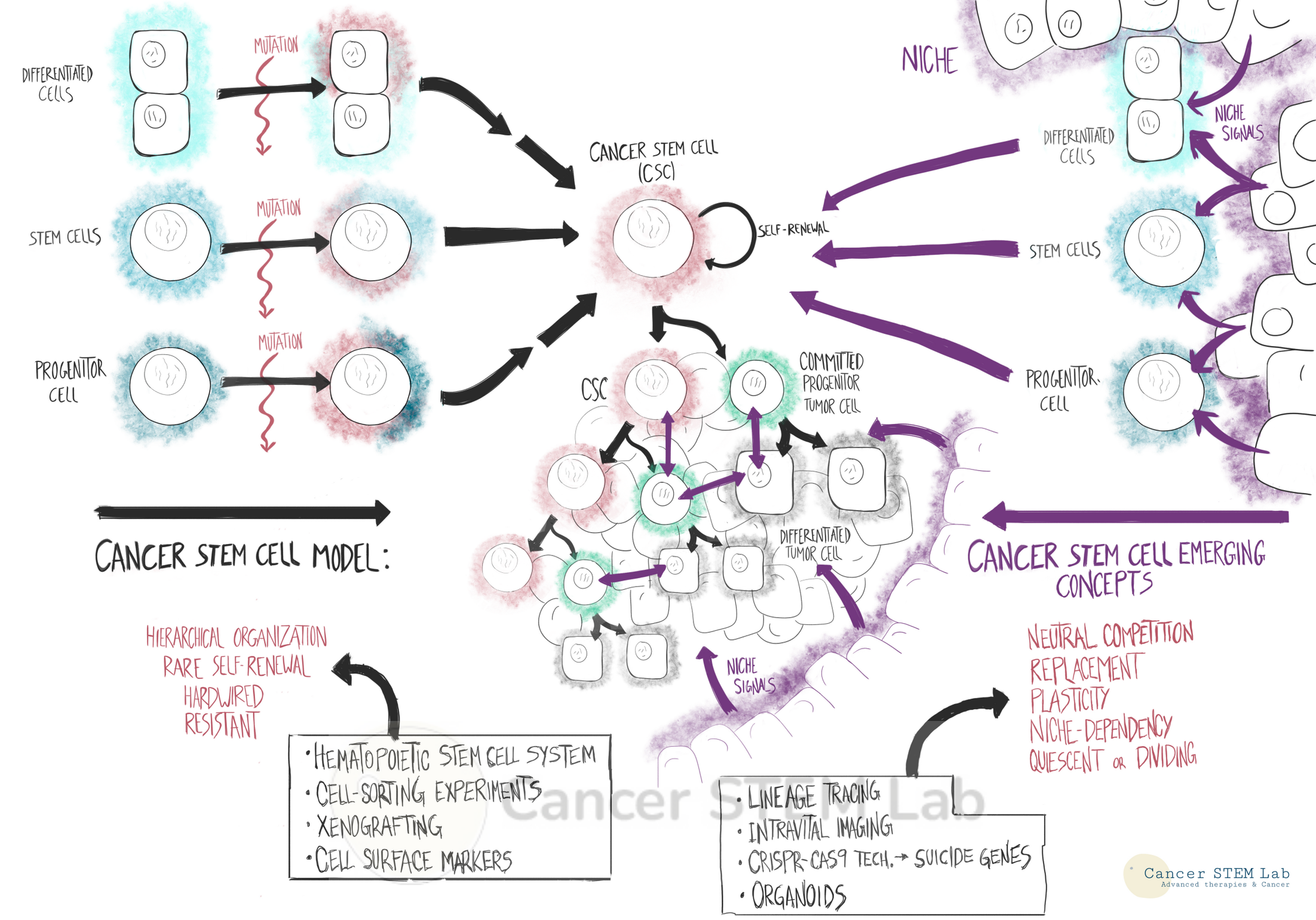
CHIR99021: glycogen synthase kinase 3 inhibitor




Cancer STEM Lab

# *Summary*

Cancer STEM Lab




Cancer STEM Lab

Cancer STEM Lab
Advanced therapies & Cancer