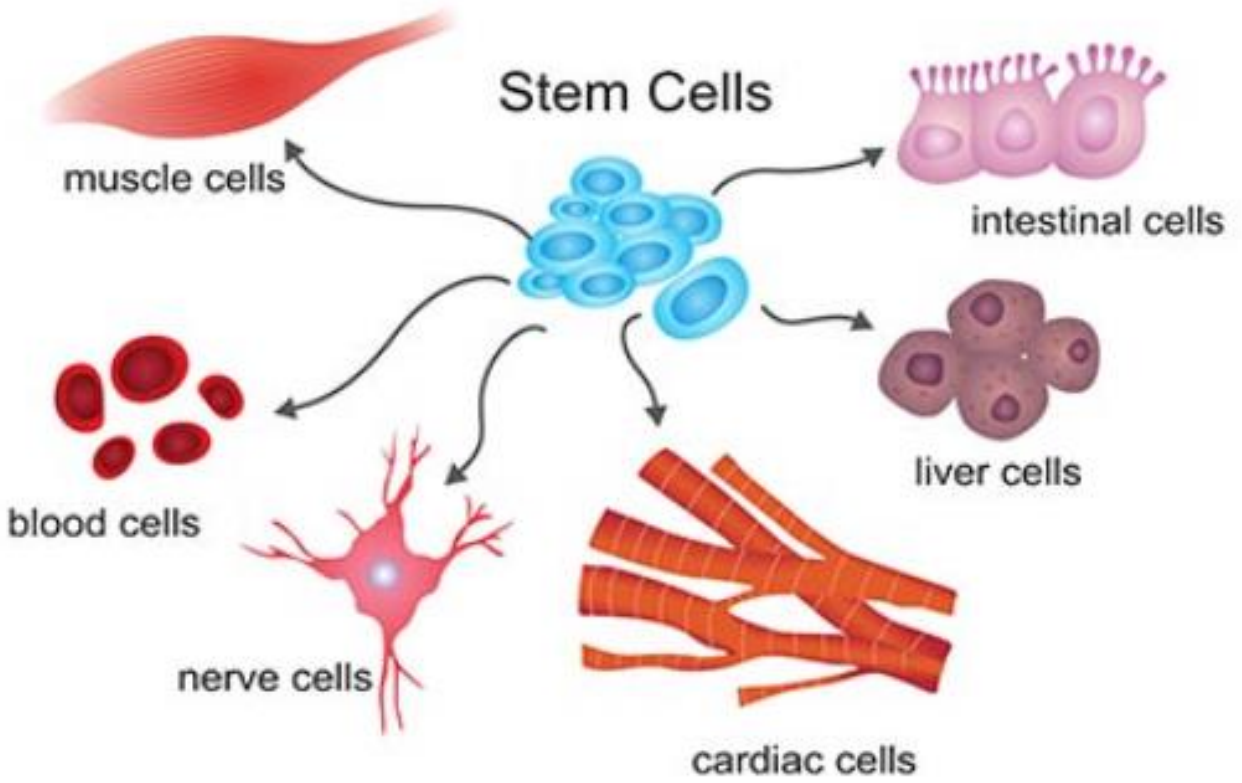
LABORATOIRE HOSPITALIER UNIVERSITAIRE DE BRUXELLES
LHUB-ULB
UNIVERSITAIR LABORATORIUM BRUSSEL

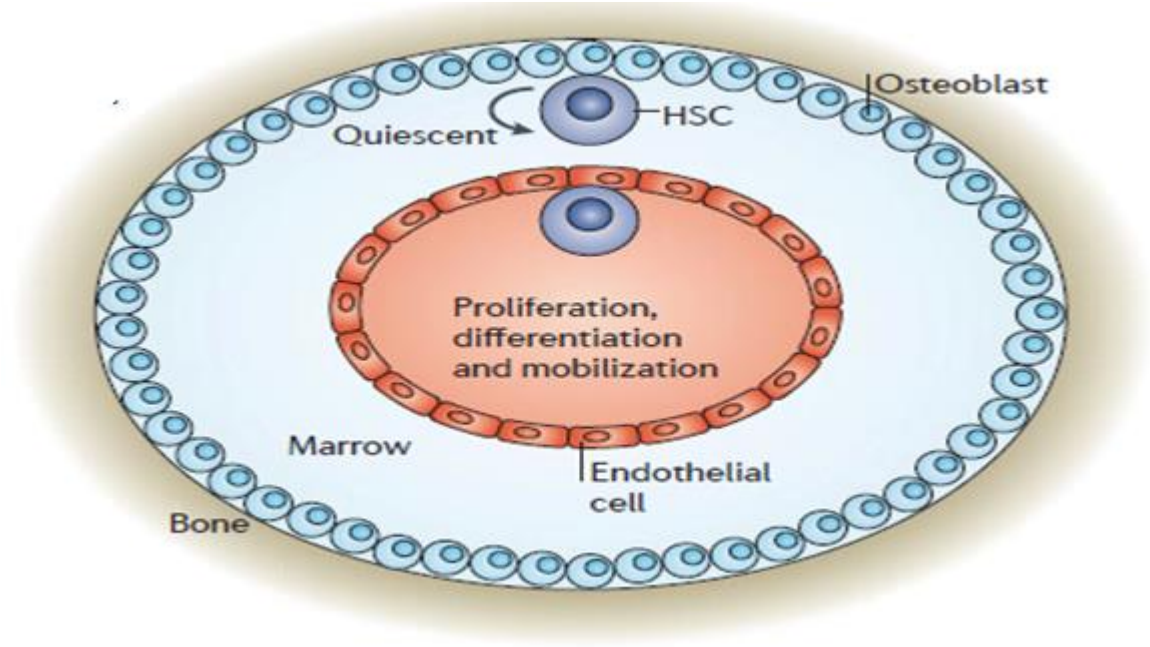
Transdifferentiation in onco-hematology

Mecanisms, actors & an illustrative case

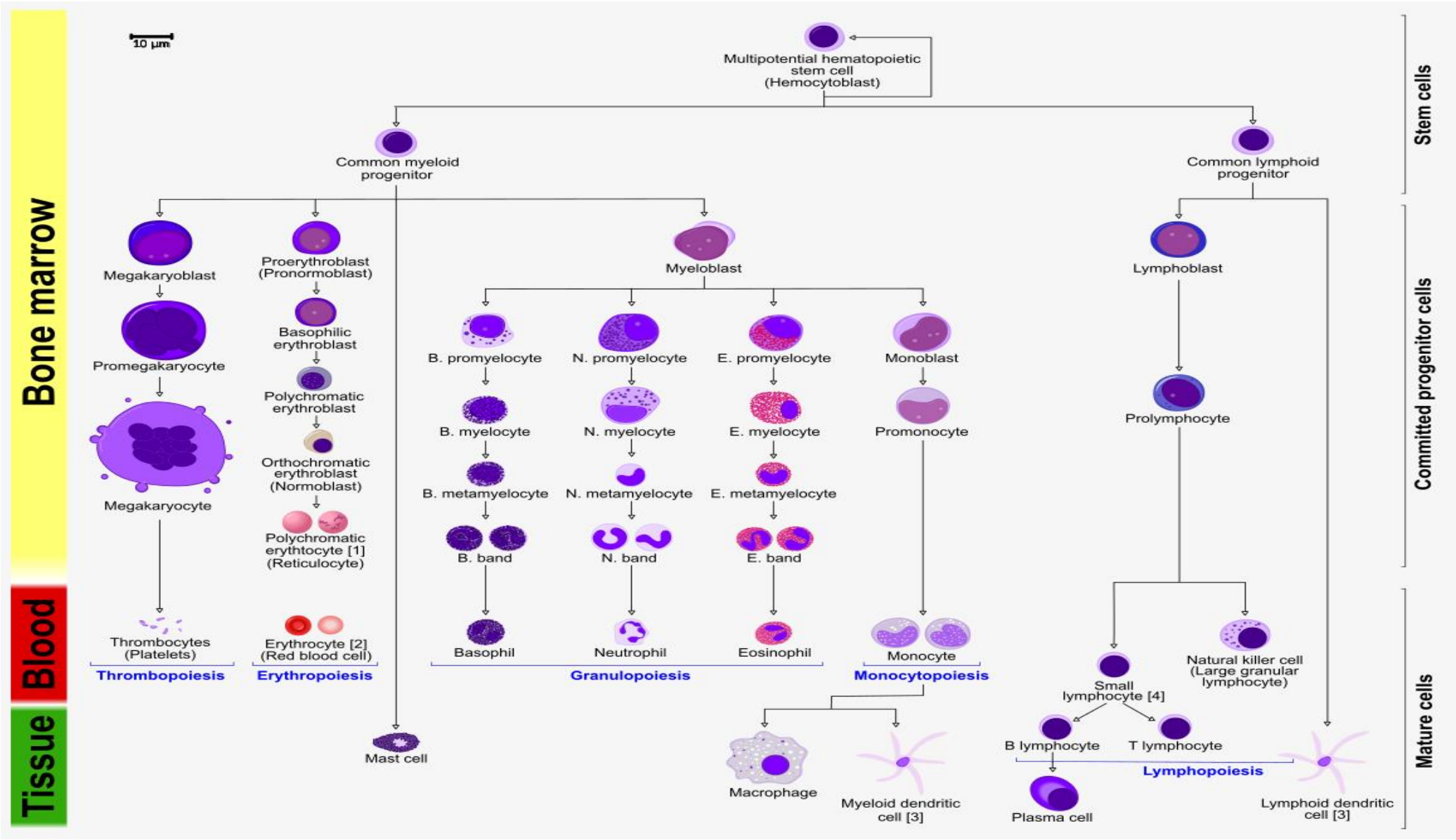
Cell Differentiation



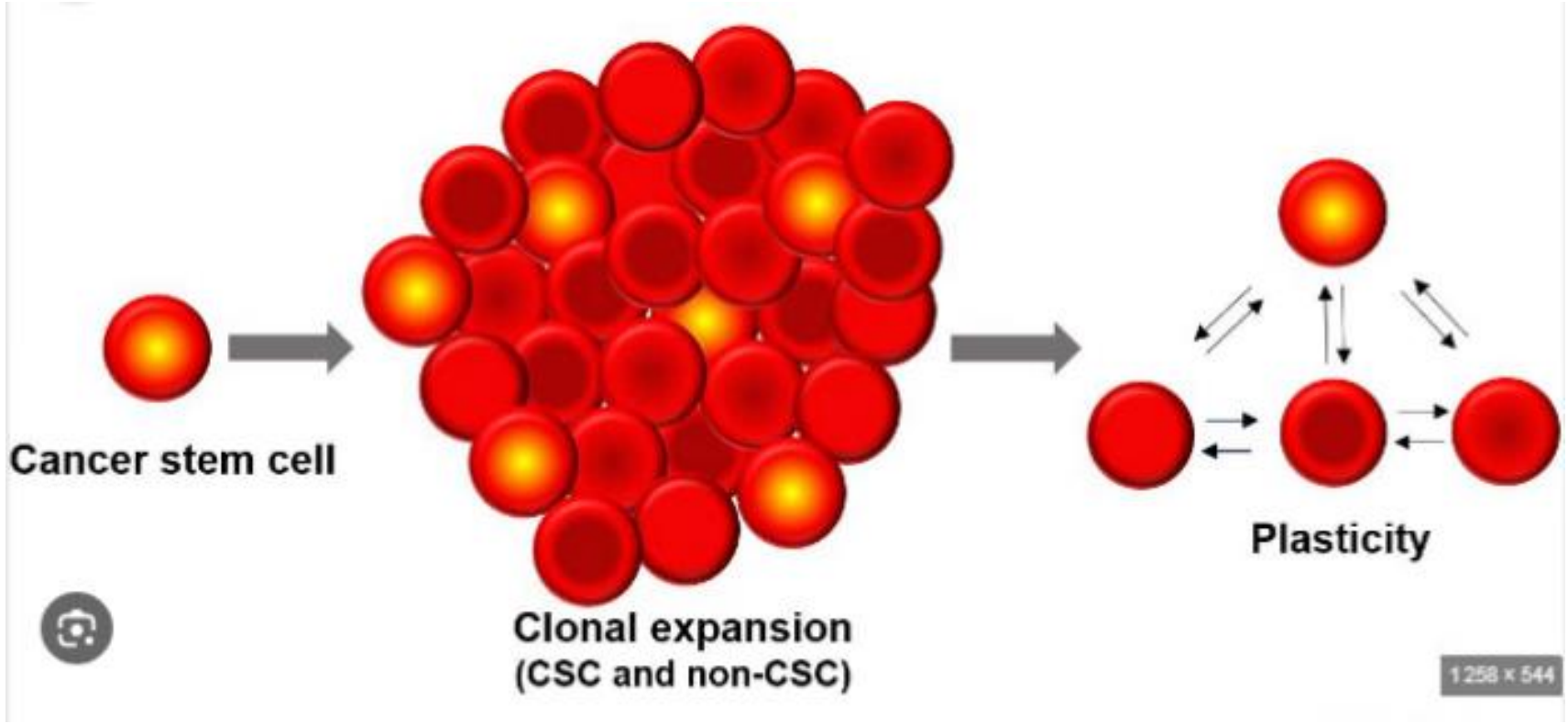
Hematopoïetic Stem Cells

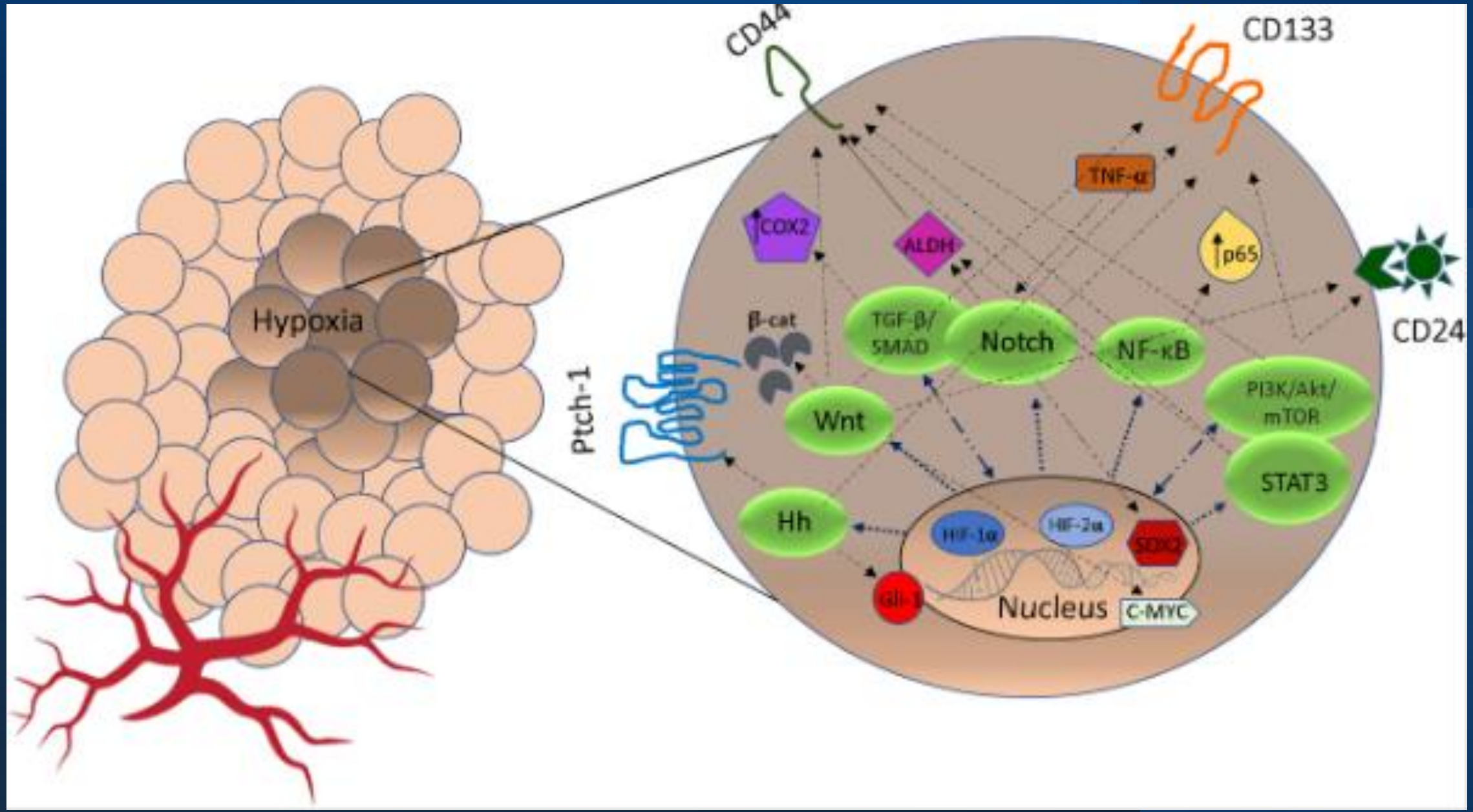


Differentiation of the hematopoïetic tissue



Cell Differentiation & Phenotypic plasticity




CD44
CD133
TNF-α
COX2
ALDH
p65
CD24
β-cat
TGF-β/
SMAD
Notch
NF-κB
Hypoxia
Ptch-1
Wnt
PI3K/Akt/
mTOR
STAT3
Hh
HIF-1α
HIF-2α
SOX2
Gli-1
Nucleus
C-MYC

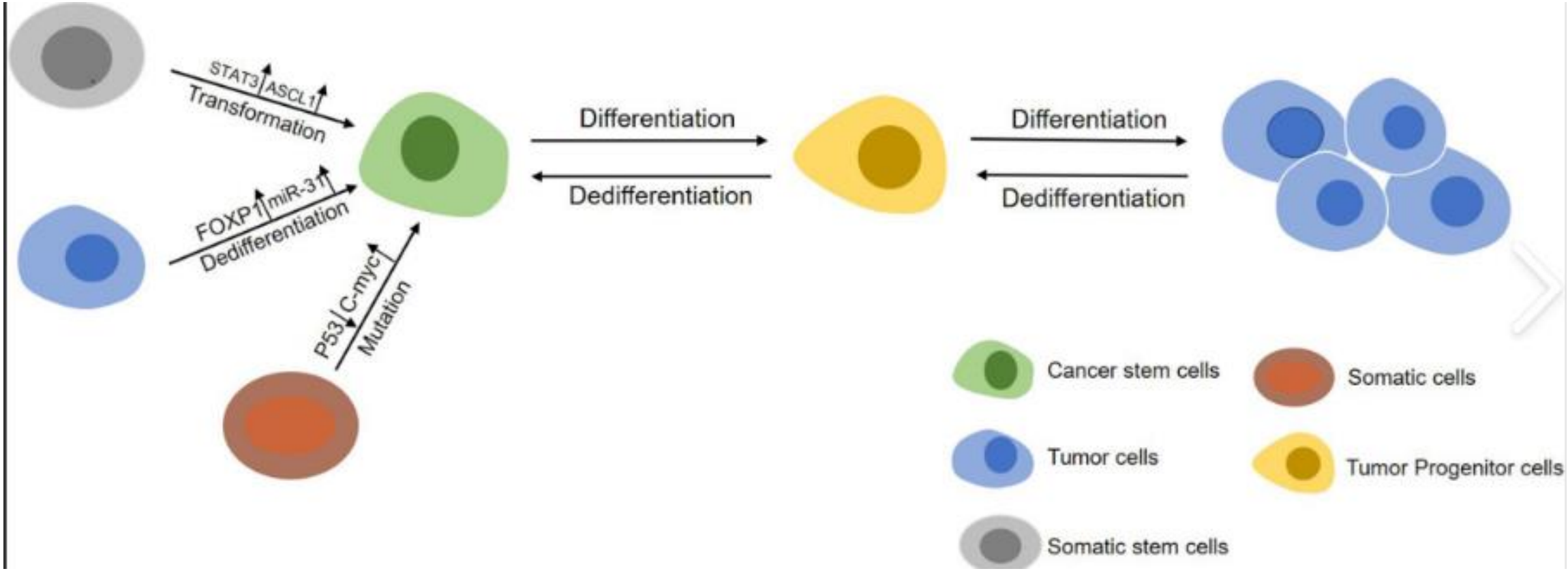
Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration

Chris Jopling, Stephanie Boue & Juan Carlos Izpisua Belmonte

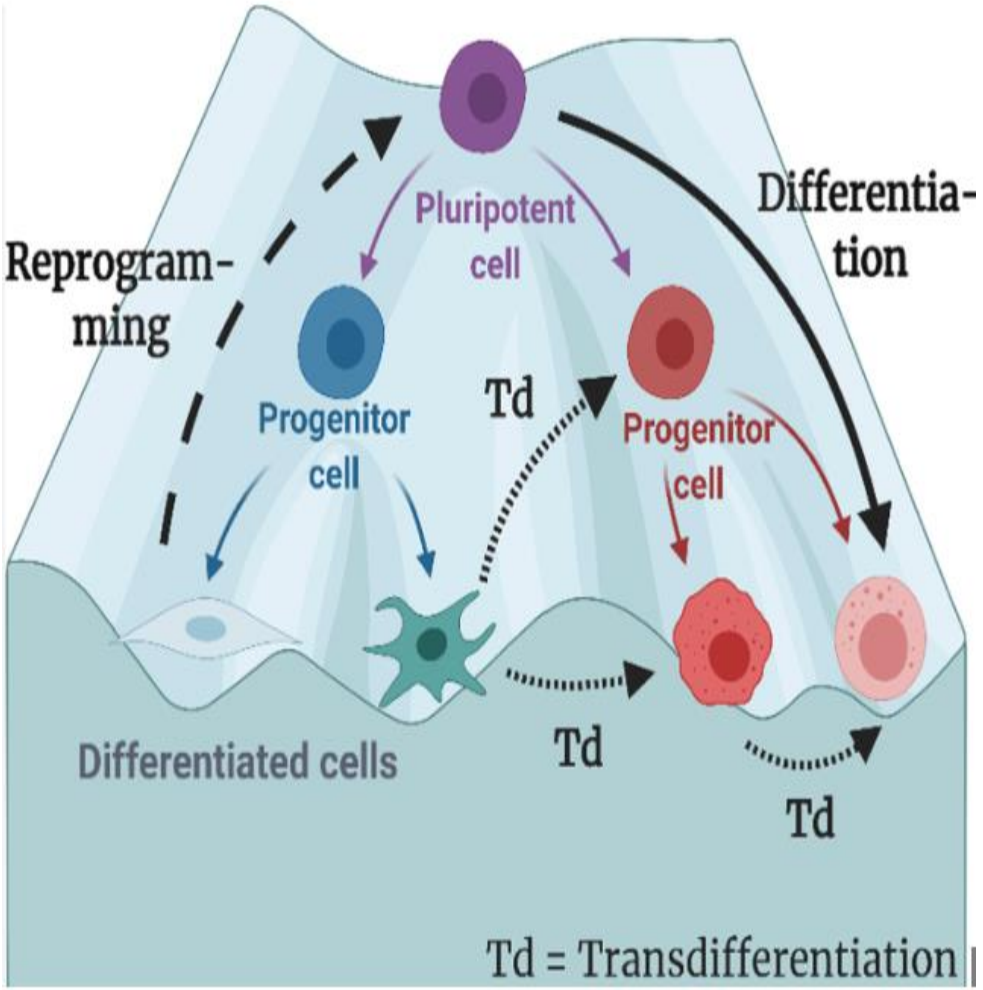
Nature Reviews Molecular Cell Biology **12**, 79–89 (2011) | Cite this article

Dedifferentiation

- During dedifferentiation, a terminally differentiated cell reverts back to a less-differentiated stage from within its own lineage, which allows it to proliferate.



Transdifferentiation



- Transdifferentiation: the conversion of one cell type (differentiated phenotype) to a different one.

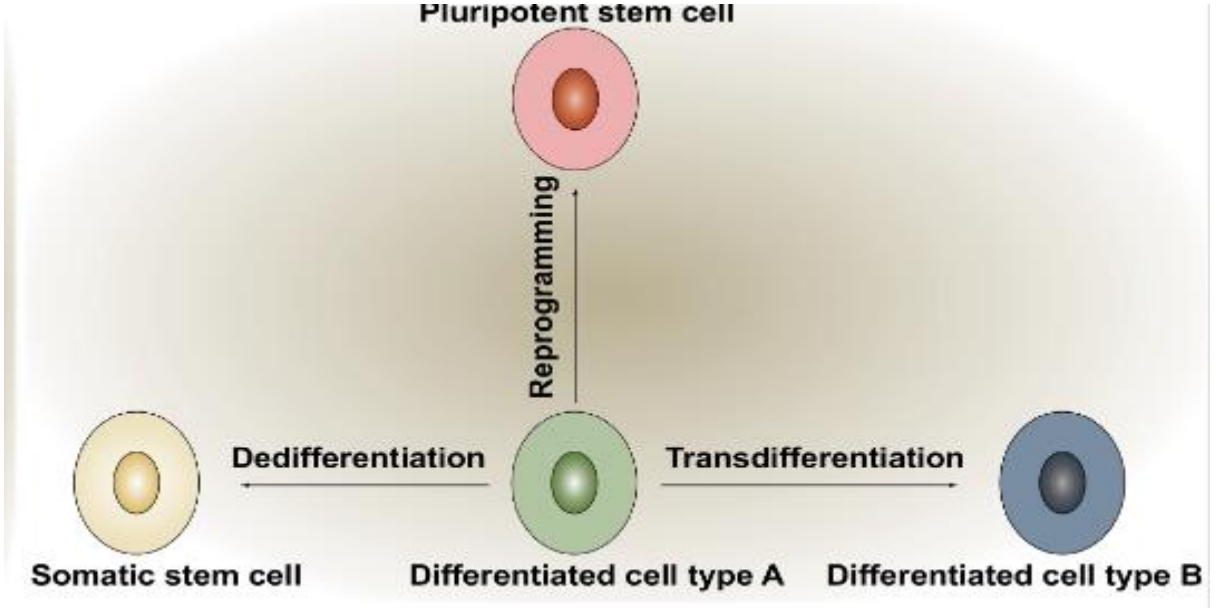
- Two alternative ways:

- by returning to an undifferentiated state to then re-differentiate into a different subtype

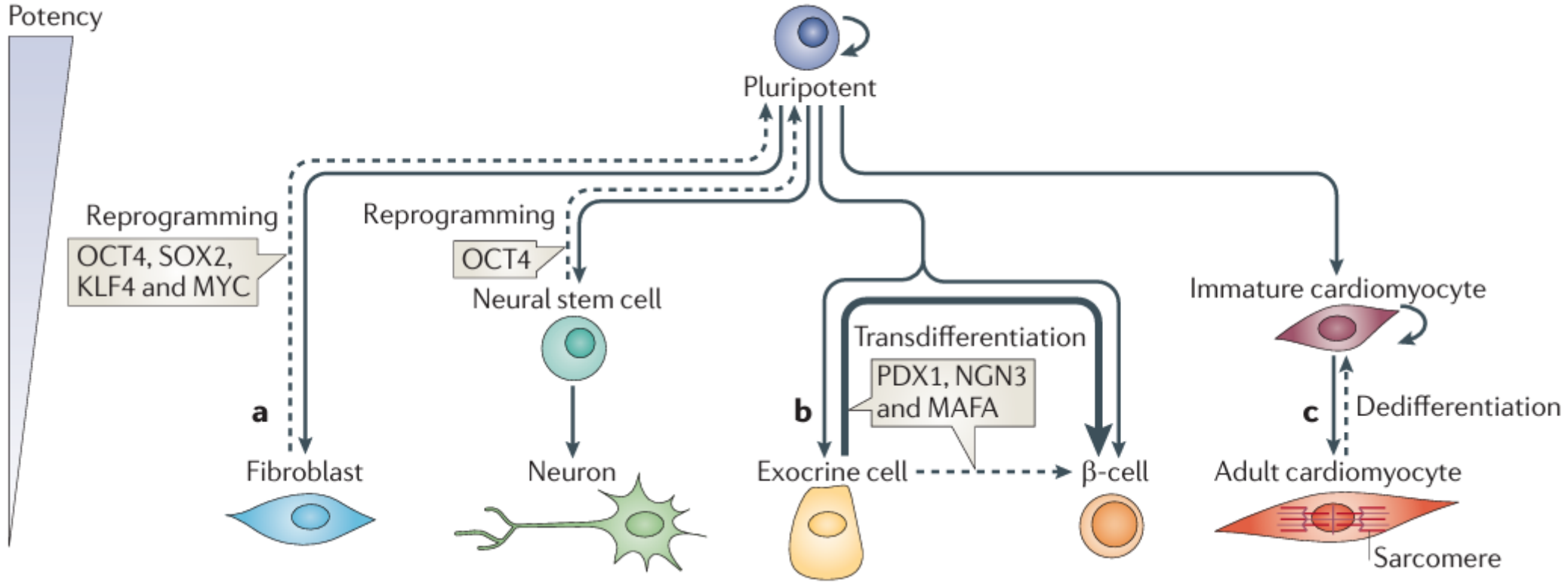
- or by direct transdifferentiation: cells completely change their developmental program →acquiring tissue-specific traits that were not predestined to their normal cells of origin.

Reprogramming

- Reprogramming aims to induce differentiated cells into reverting to pluripotency.
- From here, they can differentiate into almost any cell type.



Overview of reprogramming, transdifferentiation and dedifferentiation



An Illustrative onco-hematology case

- Emerging hallmark of cancer
- Molecular mechanisms
- Crossroads between tumor cells and their microenvironment

- Focusing on a particular example of tumor plasticity in hematological human cancer

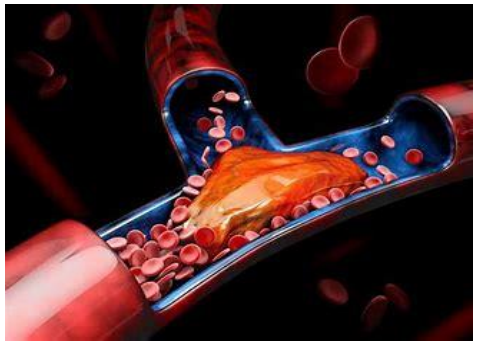
ALCL associated to a secondary clonally-related malignant histiocytosis in a young adult

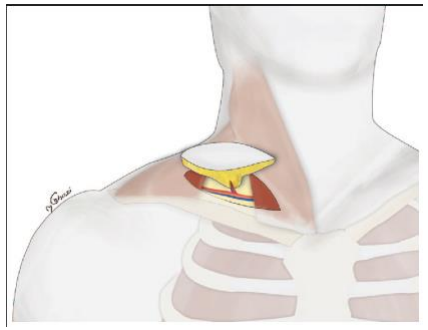
Clinical presentation

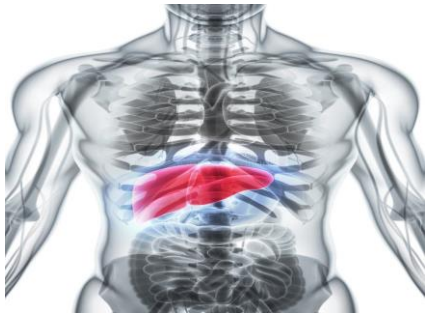
- **Chief complaint:**

- 26 yo man
- No PMH
- B symptoms

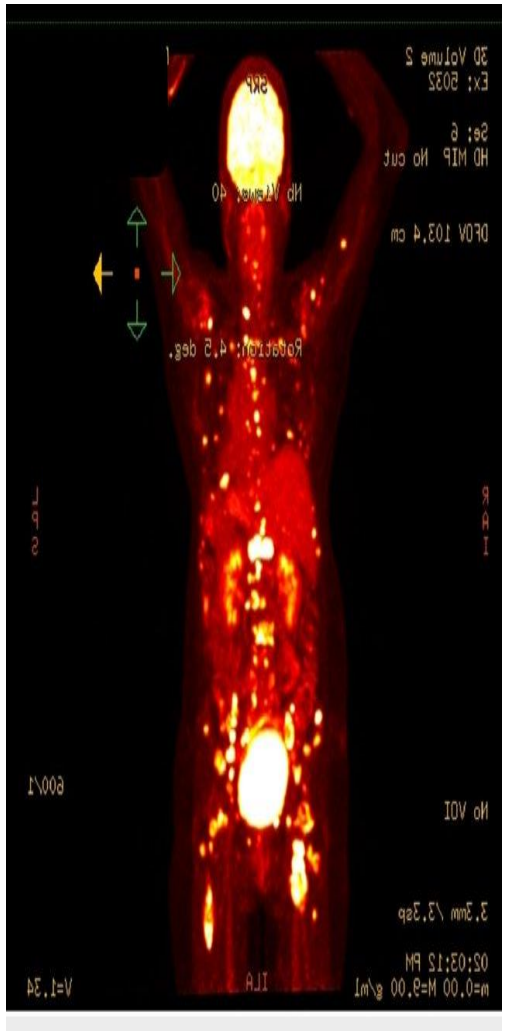
- Physical Exam:



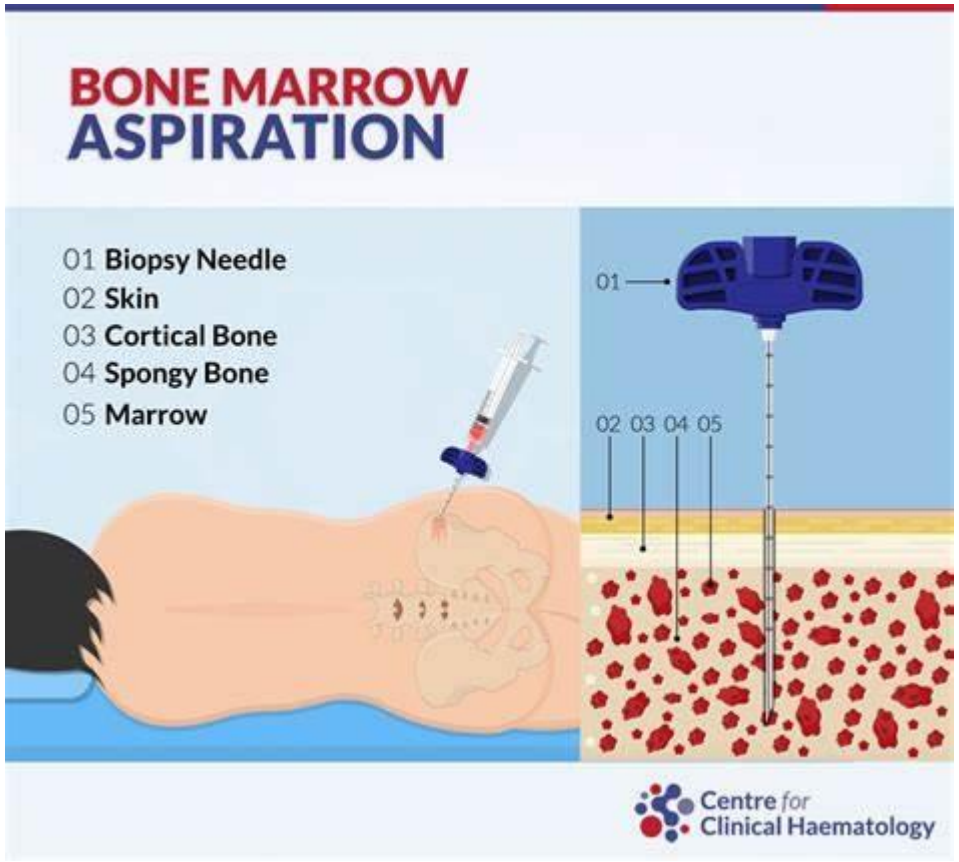


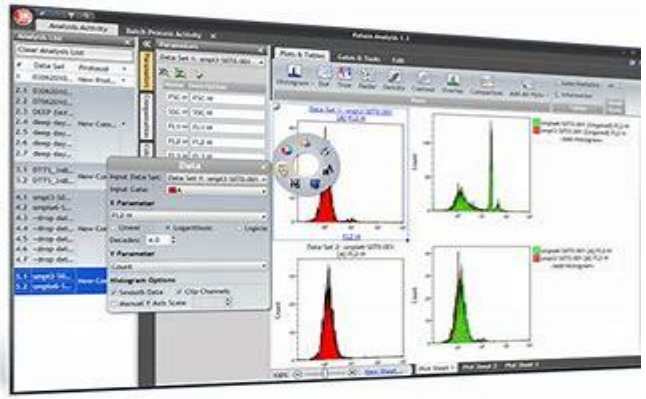


- Laboratory and radiology results



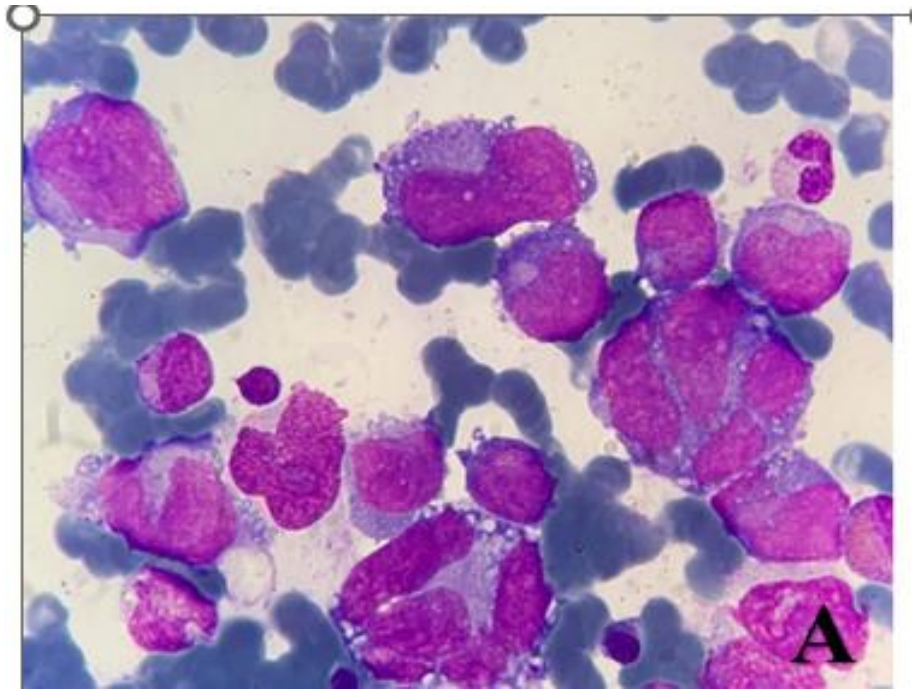
- Hemoglobin: 7,1 g/dL
- Platelets: 21 000/mm³
- Neutrophils: 2870/mm³
- Ferritin: 1800 µg/L
- LDH: 10,497 UI /L
- ASAT: 95 U/L
- PET CT-scan showed metabolic evidence in favor of high-grade lymphomatous disease stage IV - lymph node, spleen, liver, and bone marrow involvement


BONE MARROW
ASPIRATION
01 Biopsy Needle
02 Skin
03 Cortical Bone
04 Spongy Bone
05 Marrow
01
02 03 04 05
Centre for
Clinical Haematology

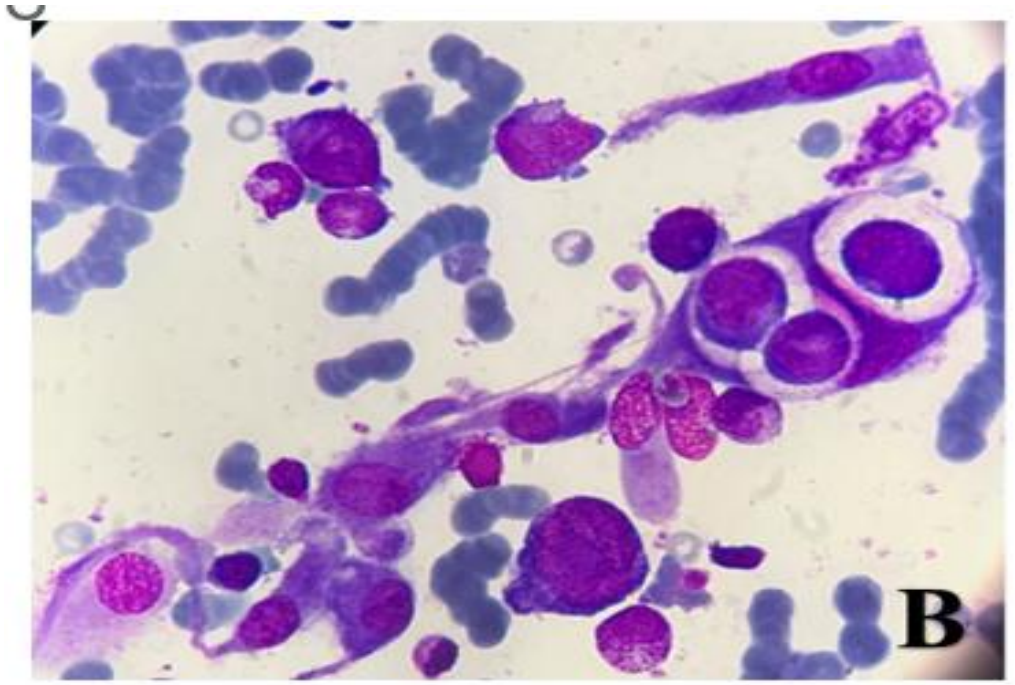


BMA cyto-morphology

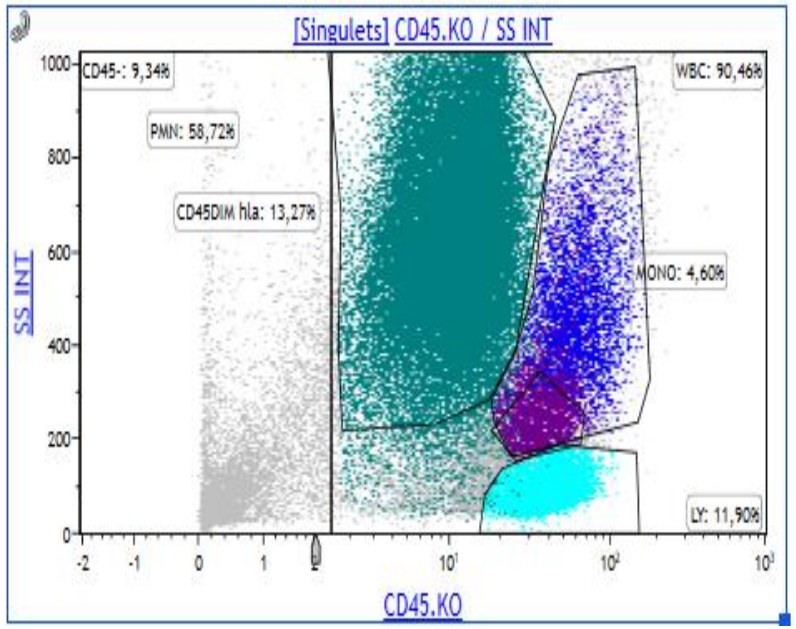
- Large pleiomorphic lymphoid cells, displaying partially “hallmark” cell morphology

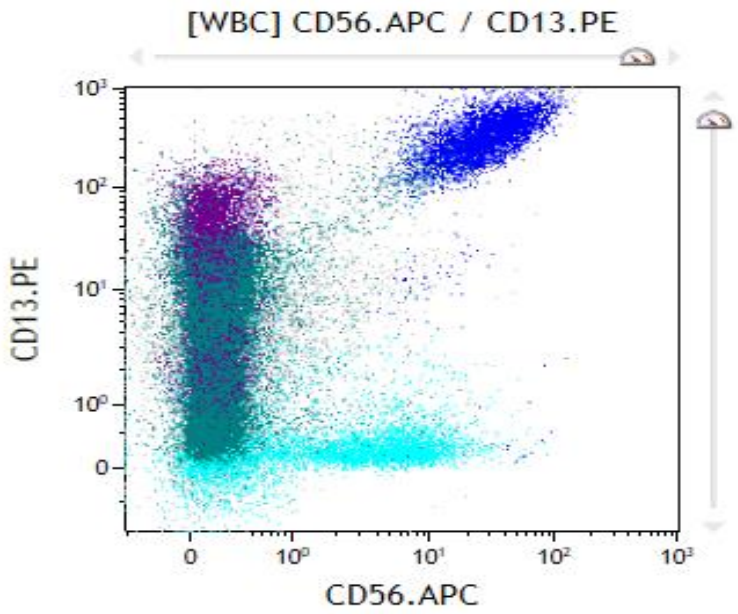


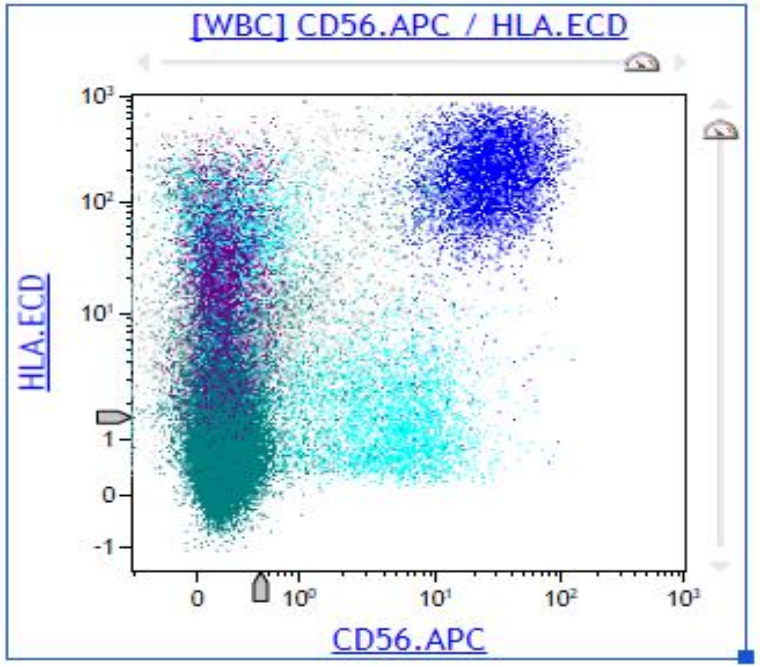
- Numerous histiocytes showing cytological atypias including gigantism, multinucleation, spindle shape, and emperipolesis images

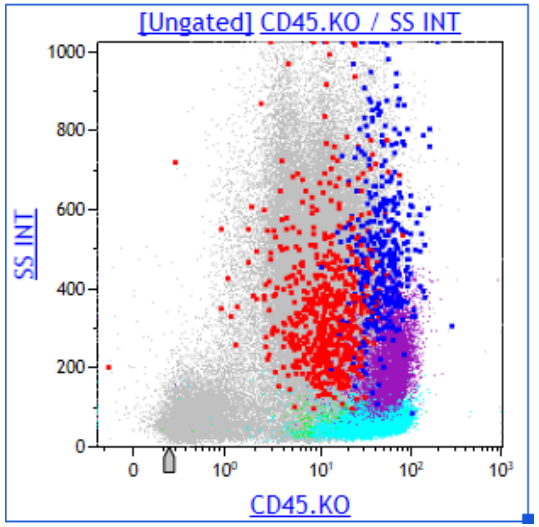


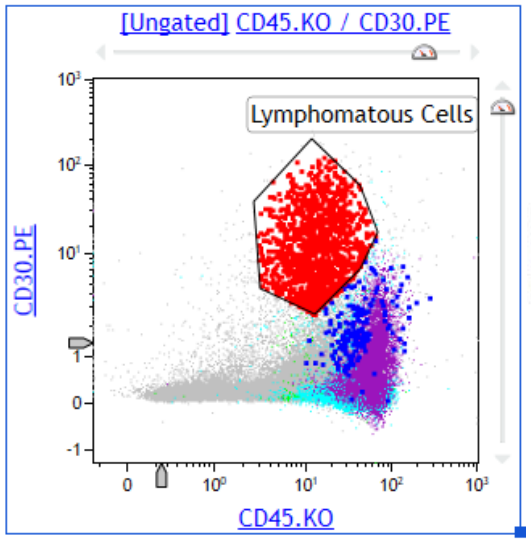
BMA Flow cytometry

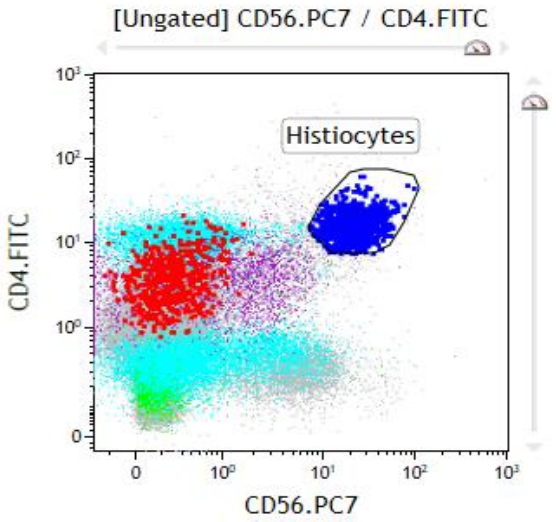


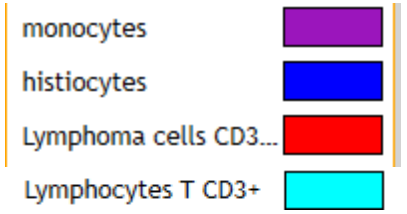


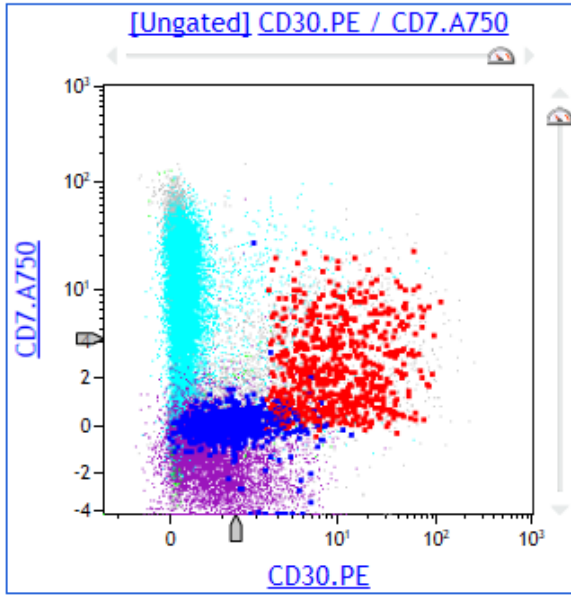


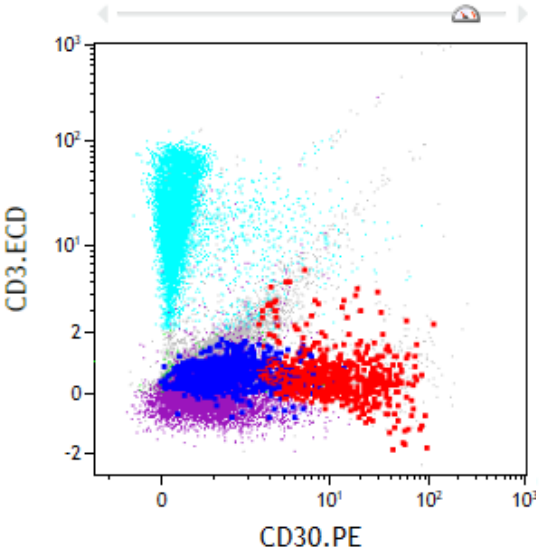

[Ungated] CD45.KO / SS INT
SS INT
CD45.KO

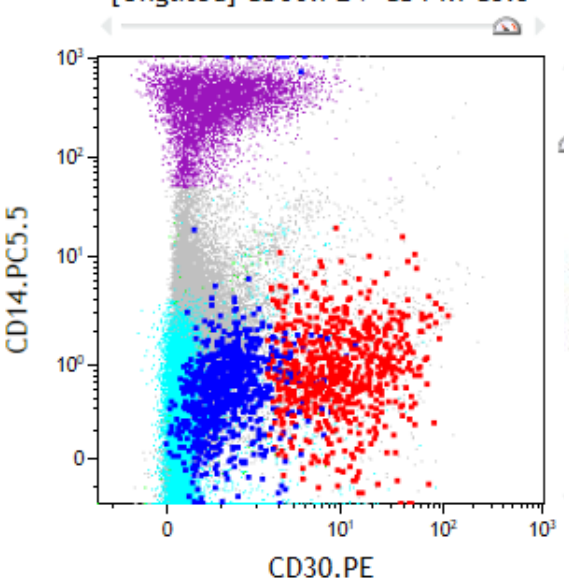

[Ungated] CD45.KO / CD30.PE
Lymphomatous Cells
CD30.PE
CD45.KO

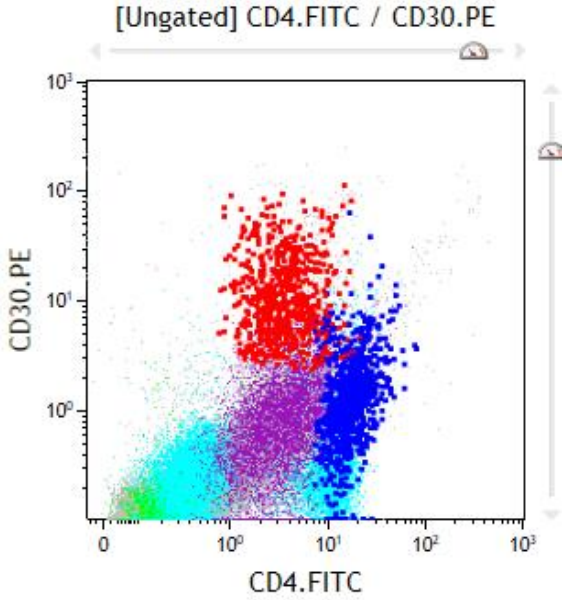

[Ungated] CD56.PC7 / CD4.FITC
Histiocytes
CD4.FITC
CD56.PC7


monocytes
histiocytes
Lymphoma cells CD3...
Lymphocytes T CD3+


[Ungated] CD30.PE / CD7.A750
CD7.A750
CD30.PE


[Ungated] CD30.PE / CD3.ECD
CD3.ECD
CD30.PE


[Ungated] CD30.PE / CD14.PC5.5
CD14.PC5.5
CD30.PE


[Ungated] CD4.FITC / CD30.PE
CD30.PE
CD4.FITC

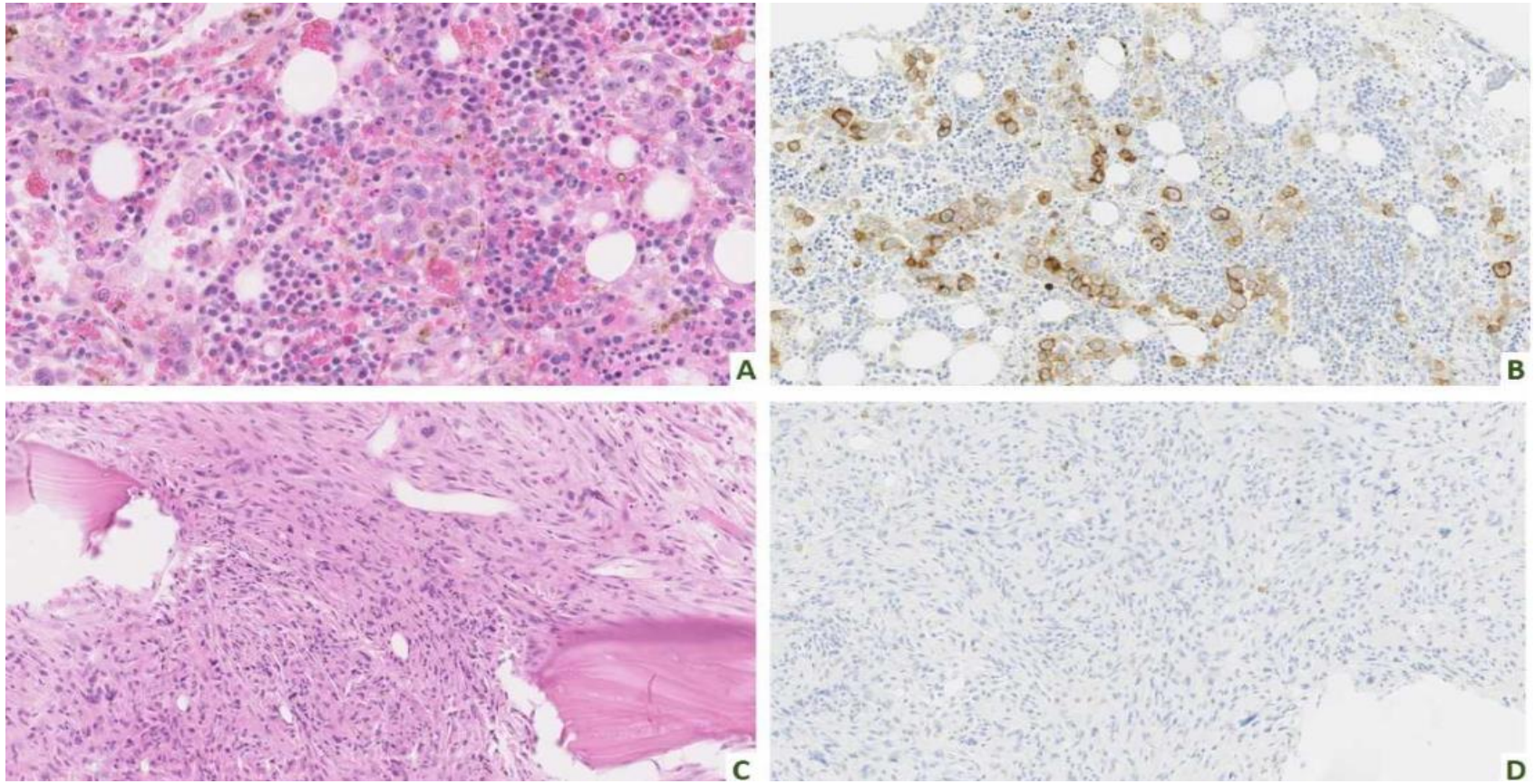
- Lymphomatous cells expressed by flow cytometric analyses the following immunophenotype:

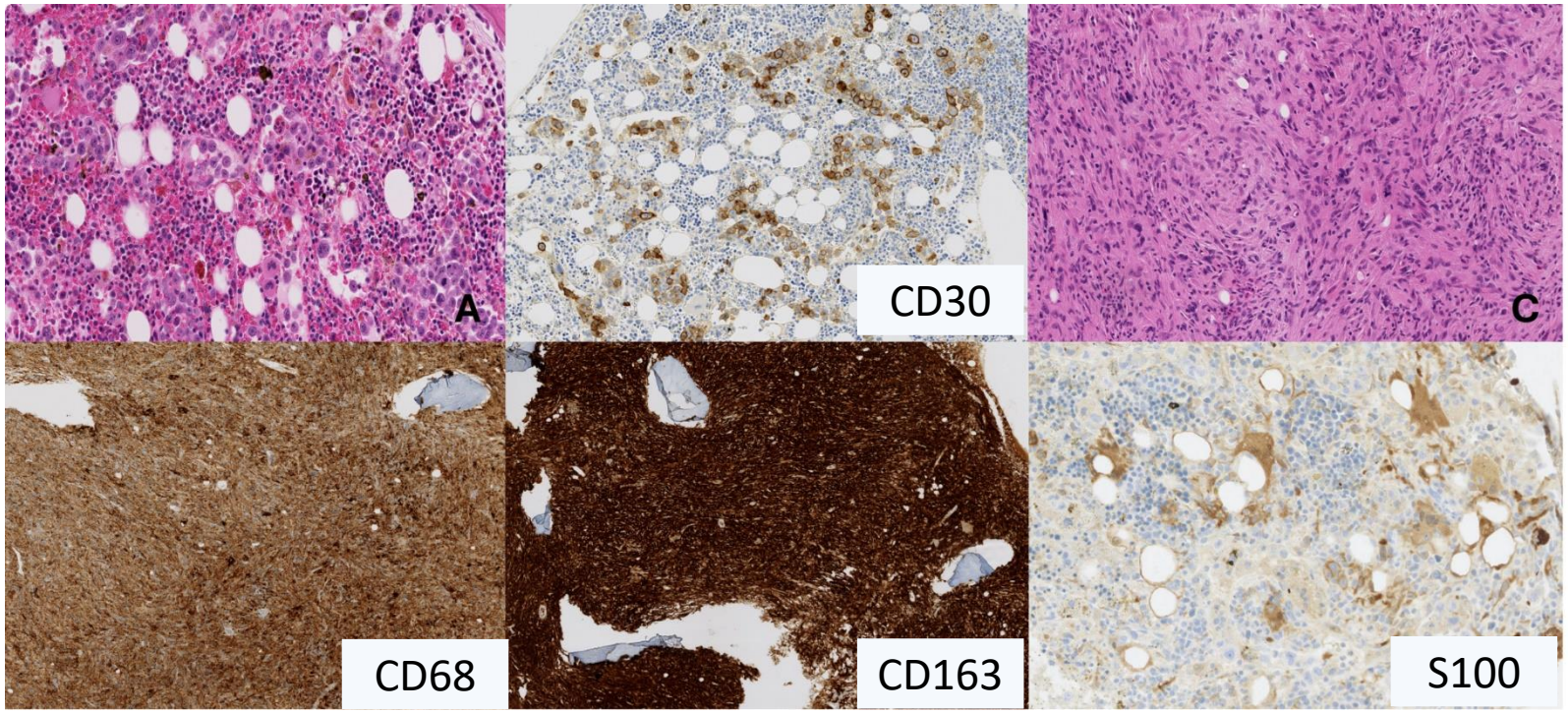
- CD2-
- **CD3-**
- **CD4+/-**
- CD5-
- **CD7+/-**
- CD8-
- CD13-
- CD14-
- **CD30+**
- CD33-
- CD56-
- HLADR-

- Histiocytes expressed an aberrant immunophenotype by flow cytometry:

- CD2-
- CD3-
- **CD4++**
- CD5-
- CD7-
- CD8-
- **CD13++**
- CD14-
- CD33-
- CD30-
- **CD56++**
- **HLADR++**

Immunohistochemistry: CD30





Anaplastic lymphoma kinase negative anaplastic large cell lymphoma [ALK-ALCL] associated with secondary malignant histiocytosis

- 1st intriguing question : Is the **histiocytic** population **reactive** to the lymphoma clone or **malignant** by itself ?

→ The **NGS** that was done after cell sorting of the BMA detected the oncogenic ***KRAS^{G12V}*** mutation exclusively in the histiocytic population → proving its clonality.

Molecular analysis

Cell sorting

Lymphomatous cells:

- clonal TCR Gamma gene rearrangement

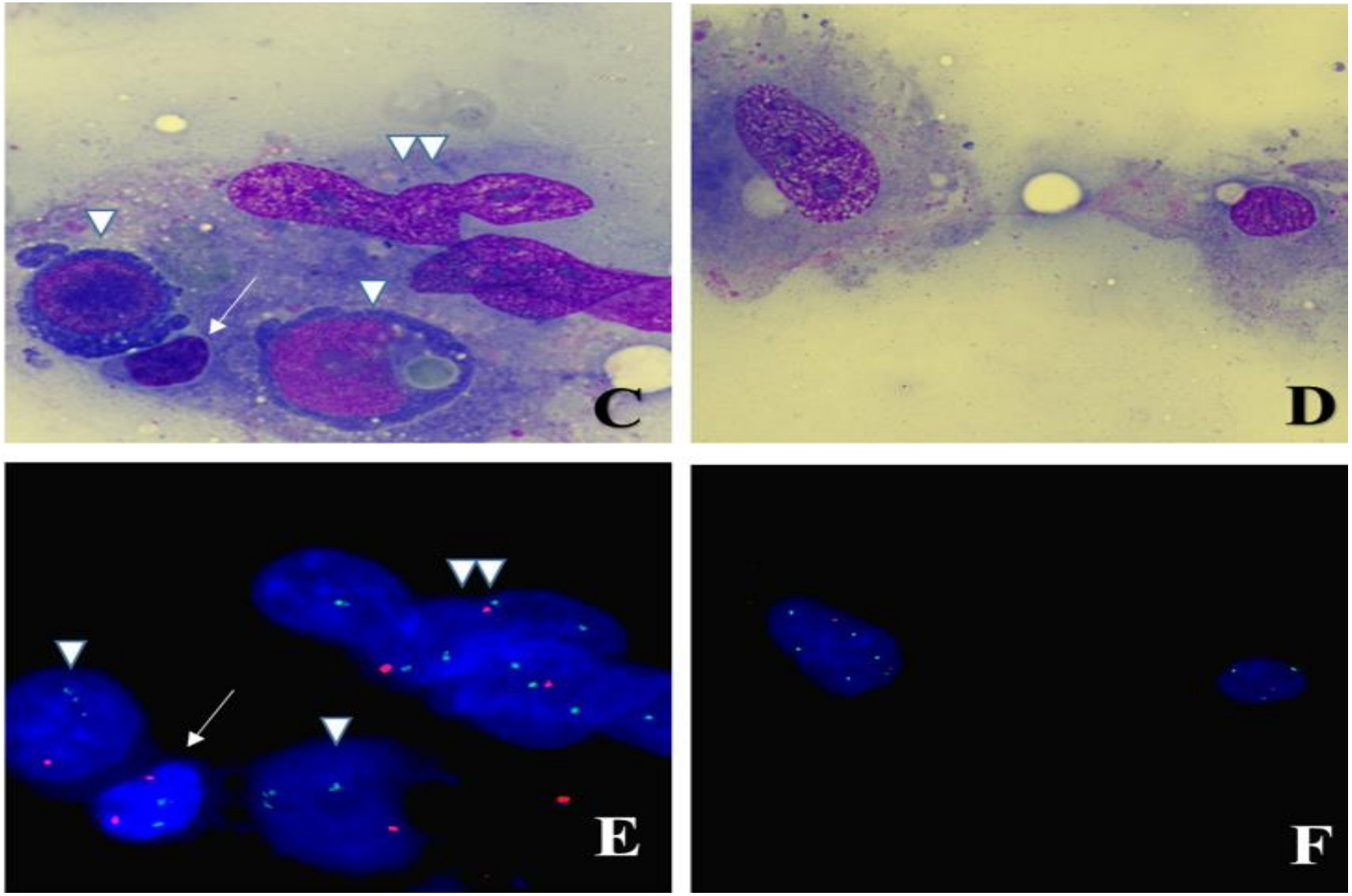
Histiocytes and monocytes:

- *KRAS* c.35G>T, p.[Gly12Val]
- *TP53* c.844C>T, p.[Arg282Trp] mutations

- 2nd intriguing question : are the two populations related?

→The sharing of identical chromosomal abnormalities between the 2 populations suggested a common precursor

Cytogenetics



Treatment

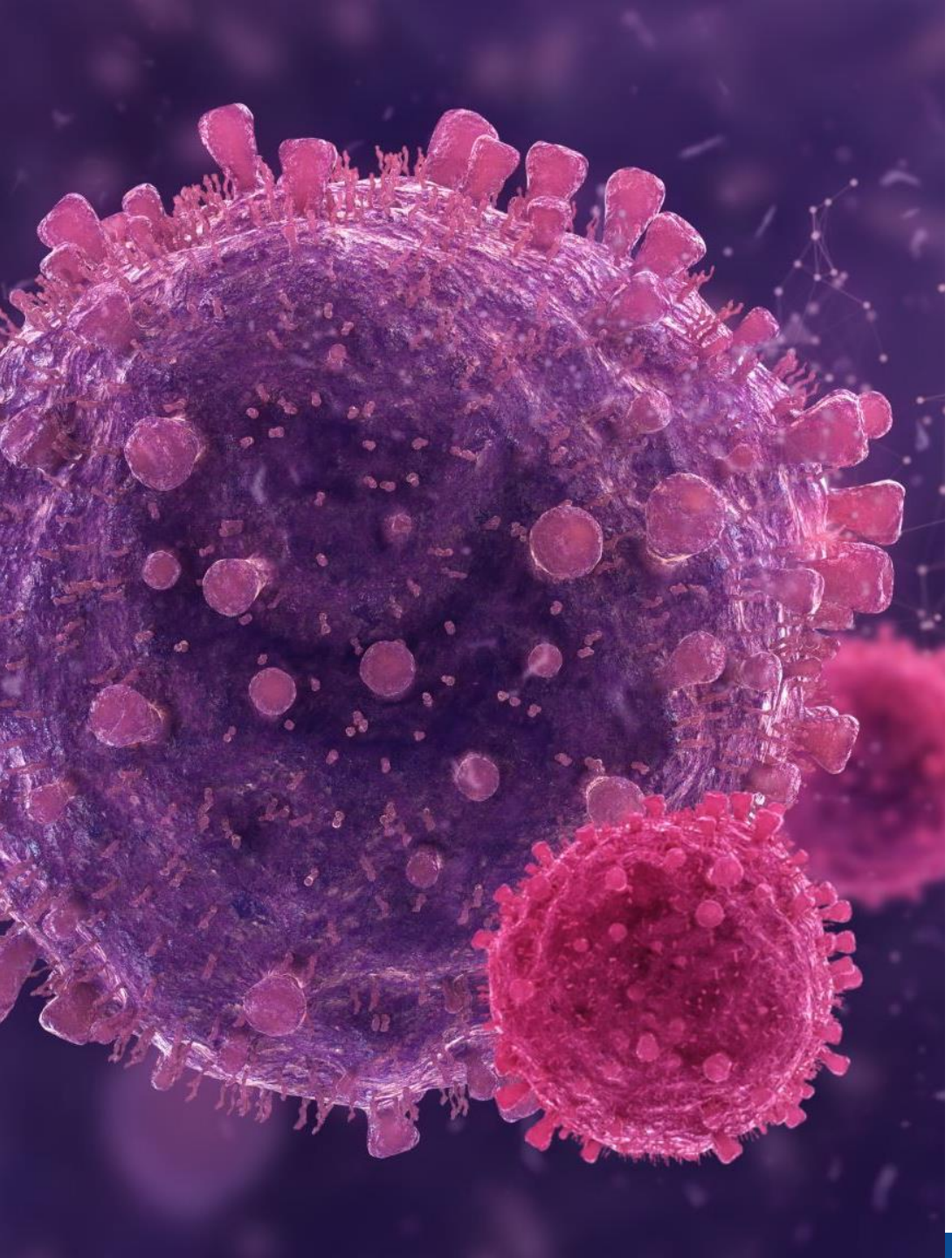
- First-line treatment protocol [Bv-CHP] included:
  - Brentuximab vedotin
  - Cyclophosphamide
  - Doxorubicin
  - Methylprednisolone
- Two cycles were administered separated by a 3 weeks interval
- The patient was not eligible for etoposide or MEK-inhibitor [for the clonal histiocytic component] because of his low platelets count and the high risk of hemorrhage.

Outcome

- Multiple complications occurred including:
- severe encephalopathy
- septic shock with febrile neutropenia
- severe thrombocytopenia
- hemorrhagic shock
- Pneumonia

- Despite a partial clinical and biological response after cycle 1, the patient was refractory at the end of cycle 2

- He died in the intensive care unit from a multiple-organ failure related to lymphohistiocytic hemophagocytosis



Discussion

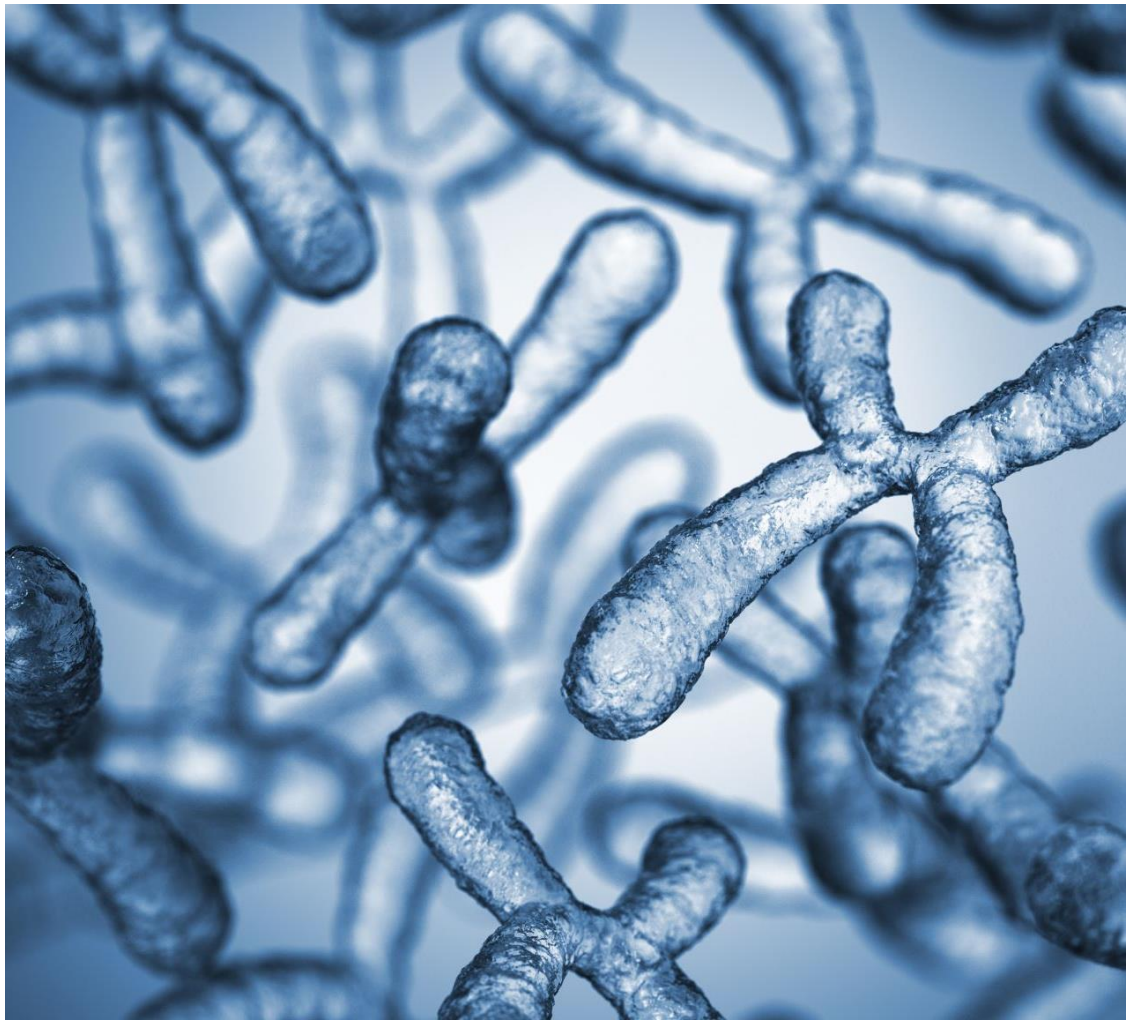
- To our knowledge, this is the first case of anaplastic T-cell lymphoma to be reported in association with secondary malignant histiocytosis.

- In this case, we succeeded in proving both:

- the clonal nature of the malignant histiocytosis
- & the sharing of identical chromosomal abnormalities between the 2 populations → suggesting a common precursor.

- However, proliferating cells are distinguished by the exclusivity of the rearrangement of TCR genes within the lymphoma cells, whereas mutations in the *KRAS* and *TP53* genes selectively affected some monocytes and histiocytic cells.

- Three hypotheses can be proposed regarding the presence of shared chromosomal abnormalities.



[1] Reprogramming of the lymphomatous population

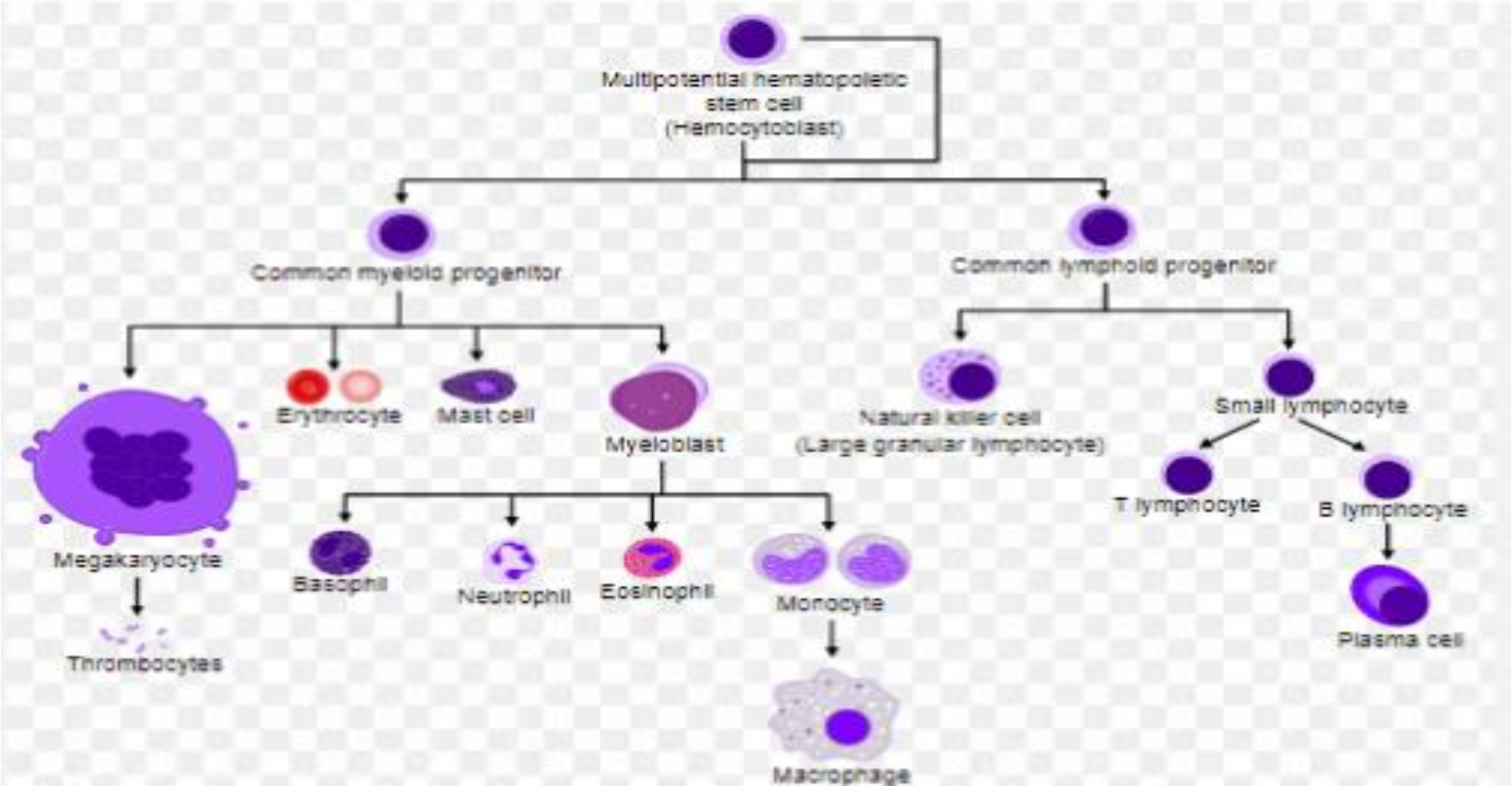
Hypothesis:

possible reprogramming of the lymphoma cells while retaining the cytogenetic abnormalities pre-existing to this process

followed by a subsequent histiocytic differentiation driven by the $KRAS^{G12V}$

We assume that the histiocyte population has undergone loss of its monoclonal TCR rearrangement during the reprogramming process.

Reminder of the differentiation of the hematopoïetic tissue



[2] Transdifferentiation of the lymphomatous population

Hypothesis:

possible transdifferentiation of the lymphoma cells while retaining the cytogenetic abnormalities pre-existing to this process

followed by a subsequent histiocytic differentiation driven by the $KRAS^{G12V}$

We assume that the histiocyte population has undergone loss of its monoclonal TCR rearrangement during the transdifferentiation process.

[3] Chromosomal abnormalities could have emerged early in tumorigenesis, preceding the differentiation into two distinct populations

The common progenitor for both populations likely acquired its chromosomal abnormalities at an early stage & was subsequently differentiated into:

either a T lymphomatous cell [by rearranging its TCR gene]

or into a histiocytic cell [driven by the acquirement of the oncogenic $KRAS^{G12V}$ mutation and the increased PU.1 expression].

This hypothesis may explain the absence of the TCR rearrangement in the histiocytic lineage.

Conclusion

- Histiocytosis associated with LPD: **mostly reactive**

- **Clonally related** are **rare** and frequently associated with mutations affecting genes of the **RAS/MAPK pathway**

- Theories:

- **chromosomal abnormalities were acquired early** in the differentiation process of the common progenitor
- **Reprogramming / Transdifferentiation / dedifferentiation and subsequent differentiation**

- **Mutation in the MAPK pathway → Targeted therapy**

Take home messages

Tumors are indeed **highly dynamic**, & they can evolve and undergo various changes in their characteristics during disease progression.

The classification of tumors into **subtypes**, characterized by **phenotypes** determined by specific **differentiation** pathways, **aids diagnosis** and **directs therapy** towards targeted approaches.

The picture becomes even more complex when the tumor responds to a therapy.

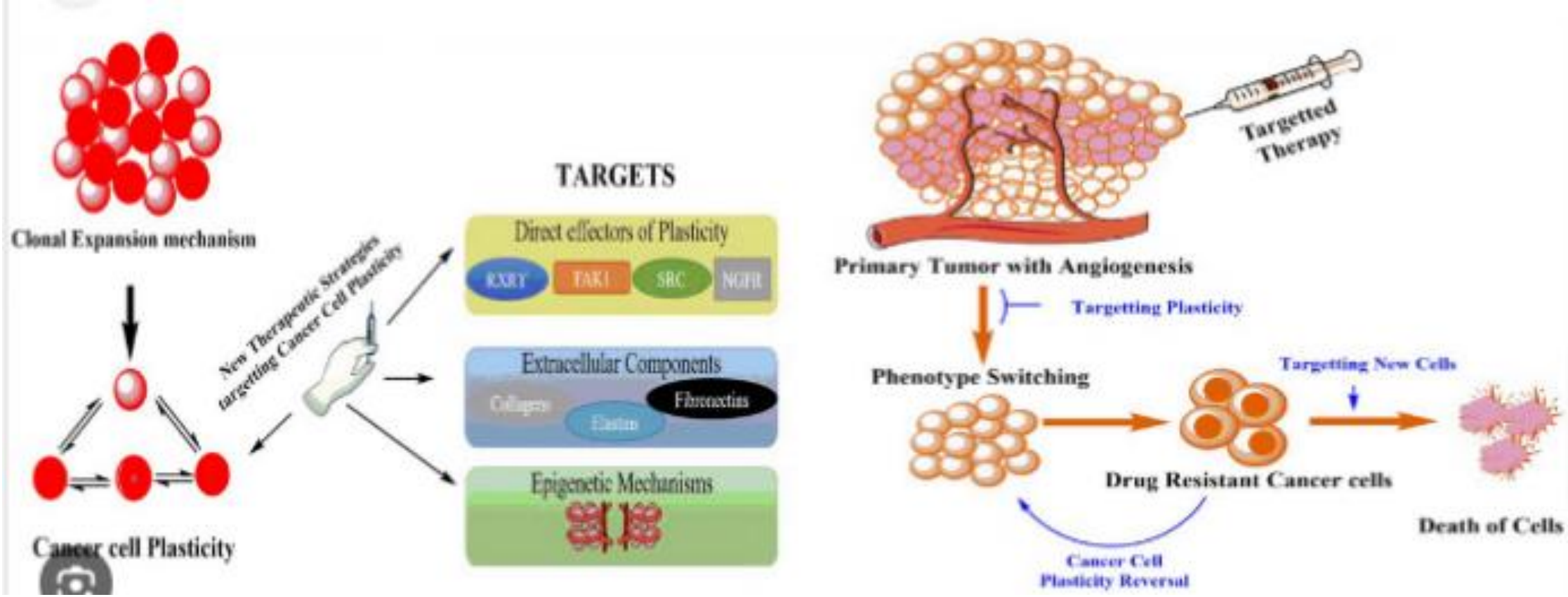
Take home messages

cancer cells → **transdifferentiate**, changing subtype, adapt to changing microenvironments.

These modifications affect the tumor's **growth rate**, invasiveness, **response to treatment**, and overall clinical behavior.

Studying tumor subtype transitions:

- understanding tumor evolution
- predicting disease outcomes
- & **developing personalized treatment strategies**.


Clonal Expansion mechanism
Cancer cell Plasticity
New Therapeutic Strategies targetting Cancer Cell Plasticity
TARGETS
Direct effectors of Plasticity
RXRY
TAK1
SRC
NGFR
Extracellular Components
Collagen
Fibronectins
Elastin
Epigenetic Mechanisms
Targetted Therapy
Primary Tumor with Angiogenesis
Targetting Plasticity
Phenotype Switching
Targetting New Cells
Drug Resistant Cancer cells
Death of Cells
Cancer Cell Plasticity Reversal

Thank you for your attention

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- Dr. Danaï POUTAKIDOU
- Dr. Anne-Laure TREPANT
- All the staff of the hematology, molecular biology and cytogenetics laboratory