Cancer Stem Cells:
Getting to the root of Cancer

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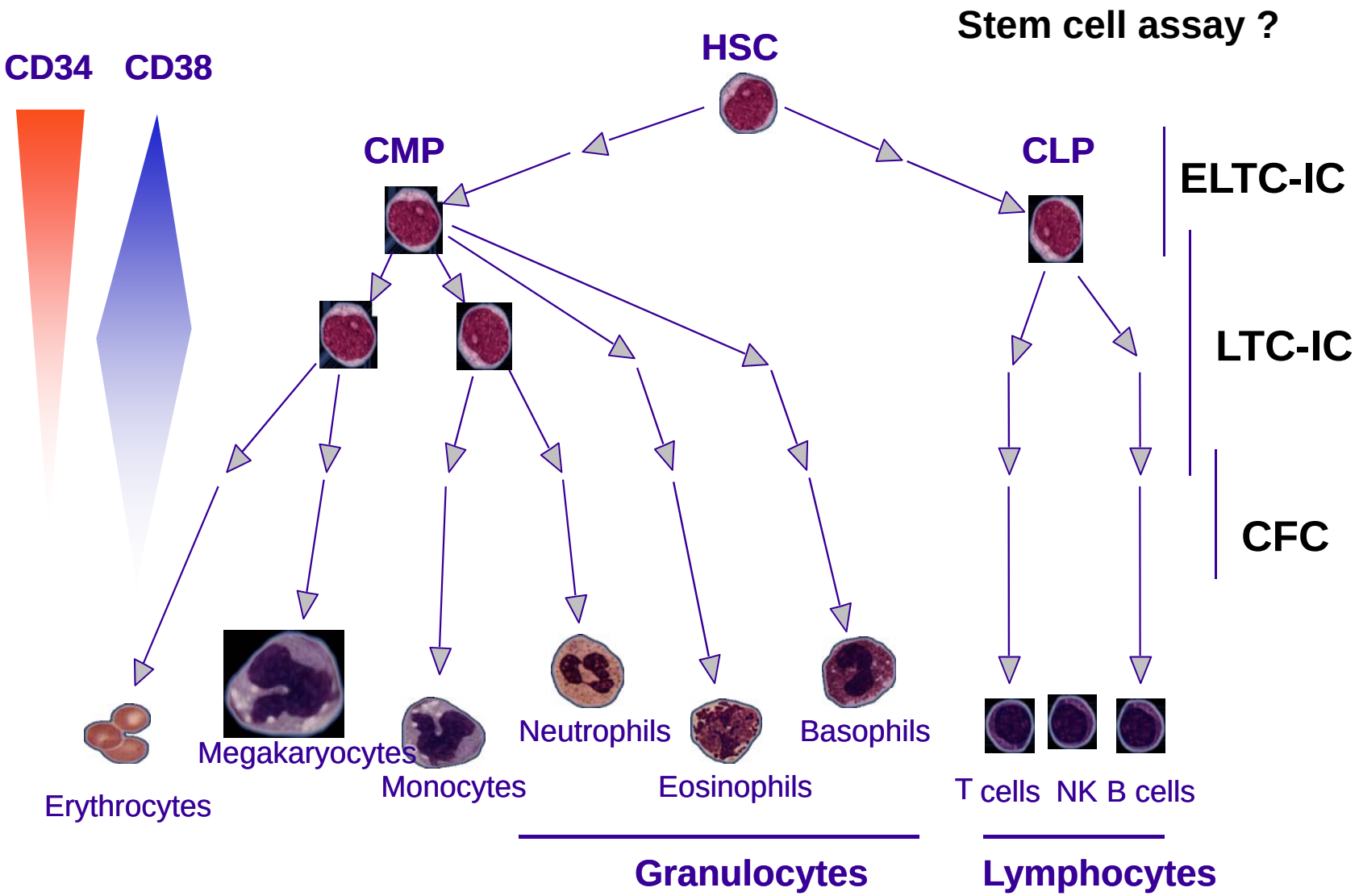
Overview

- Evidence for the existence of Cancer Stem Cells in Acute Myeloid Leukaemia (AML).
- Current implications of Leukemic Stem Cell (LSC) for therapy
- Considerations for targeting LSCs
- Future directions

The "Hematopoietic" (Blood) System



Gold Standard for Hematopoietic Stem Cells

Hematopoietic stem cells are assayed *in vivo* by their ability to repopulate the entire blood system

- ✓ Capable of extensive proliferation and multilineage differentiation
- ✓ Capable of self-renewal

Background of Acute Myeloid Leukemia (AML)

- Heterogeneous disease characterized by the overproduction of leukemic blasts.
- Leukemic blasts have a short life-time and thus needed to be replenish by a subset of self-renewing cells
- No good *in-vitro* model to study AML progenitor / stem cells

MAJOR ISSUES

- ✓ Which leukemic cells is capable of initiating and maintaining the disease ?
  - need functional quantitative assay

- ✓ Which normal cell(s) does LSC originate from?
  - need to purify LSC and functionally and phenotypically compared Normal and LSCs

NOD/SCID model for AML

Peripheral Blood
or BM of AML patients

375 cGy irradiation

Unpurified
mononuclear cells

or

Purified cells expressing
primitive surface phenotype

Ficoll Gradient

Tail vein injection
into NOD/SCID mouse

6-12 weeks ±
GF injections

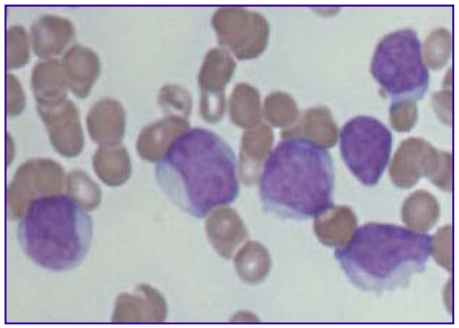
Southern Blotting
to quantify percentage of
human cells in mouse BM

FACS analysis to
phenotype Human cell
present in mouse BM

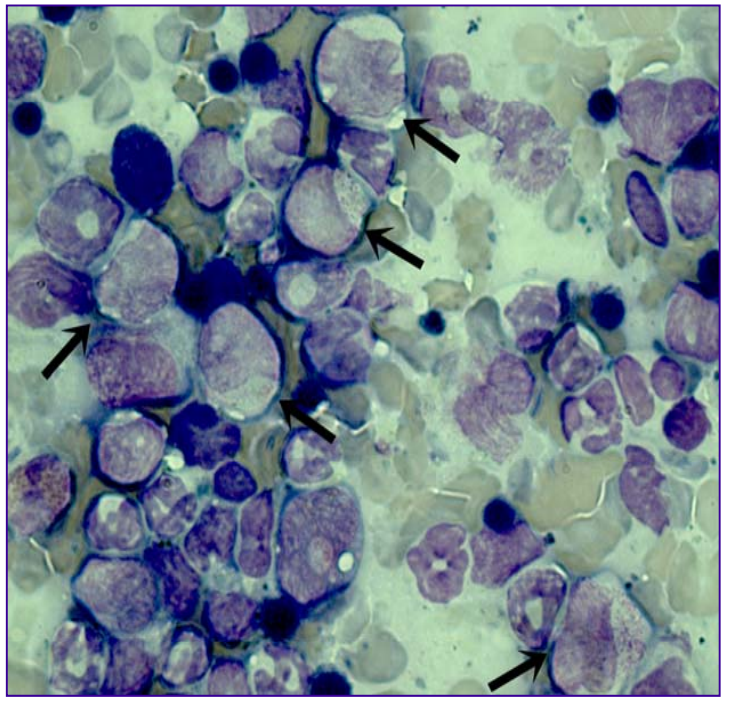
- Model that faithfully reproduces the AML disease in mice
- Enables characterisation of engrafted cells

Confirmation of AML: Morphology

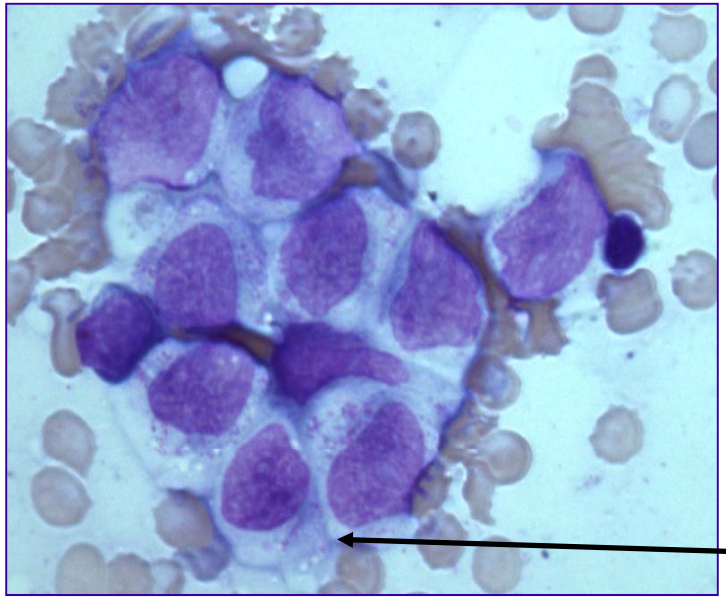
Original AML-M2



NOD/SCID Marrow
after 6 weeks



Sorted as
Human
$CD45^{+}$



"Auer Rod"

NOD/SCID model for leukemia

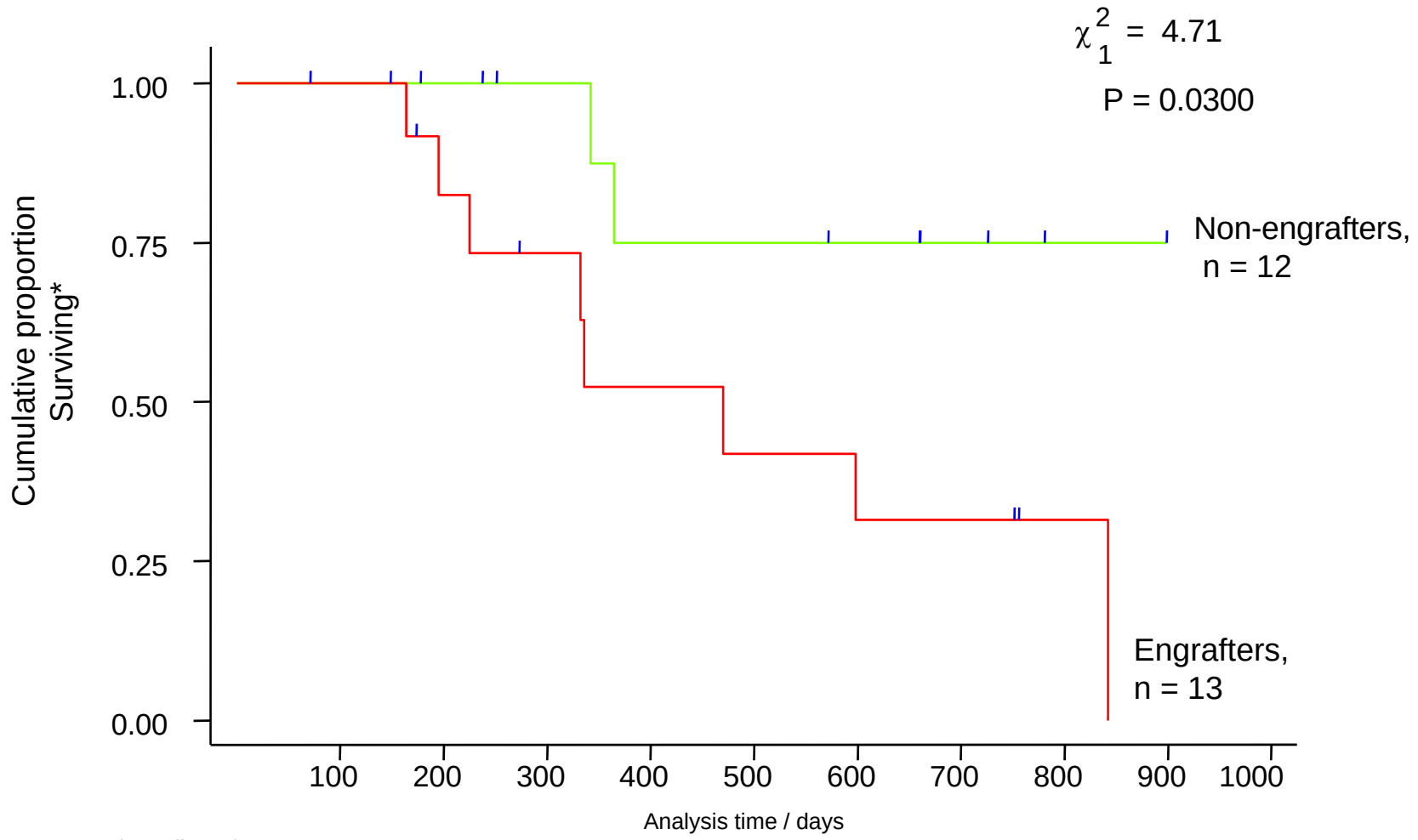
- AML shares similar morphology, phenotype and FISH abnormalities as original sample
- Similar profile of gene expression before and after engraftment

LIMITATION: Only 60-70% AMLs engraft in NOD/SCID

Engraftment in NOD/SCID assay correlates with karyotypically defined prognostic group

NOD/SCID / Cytogenetics	**Engrafter**	**Non-engrafter**
Good risk	0	11
Intermediate risk	13	14
Poor risk	8	0

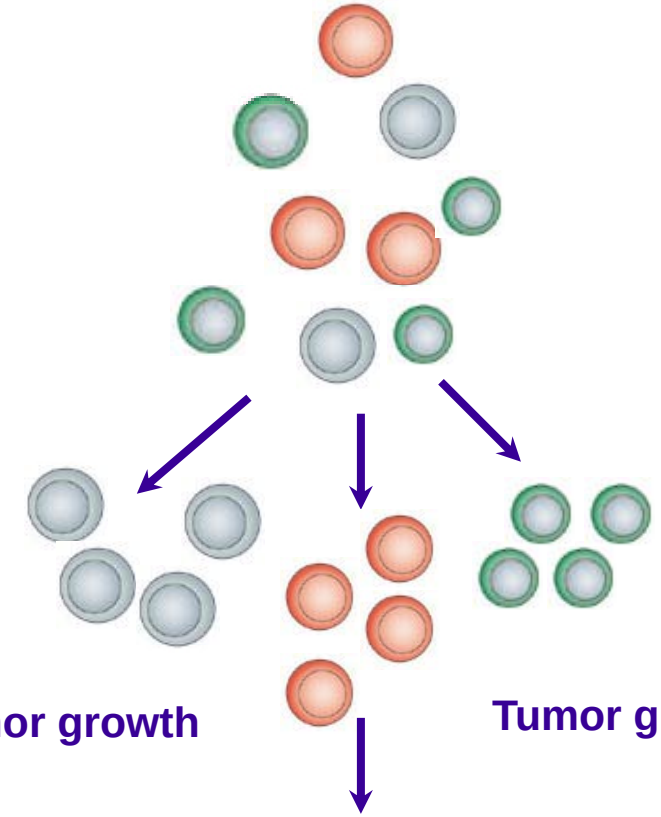
Overall survival of AML patients with intermediate risk cytogenetics (<60 years) with respect to engraftment observed in NOD/SCID mice



*Censored at allograft

Model of Tumor Cell Proliferation

Stochastic model

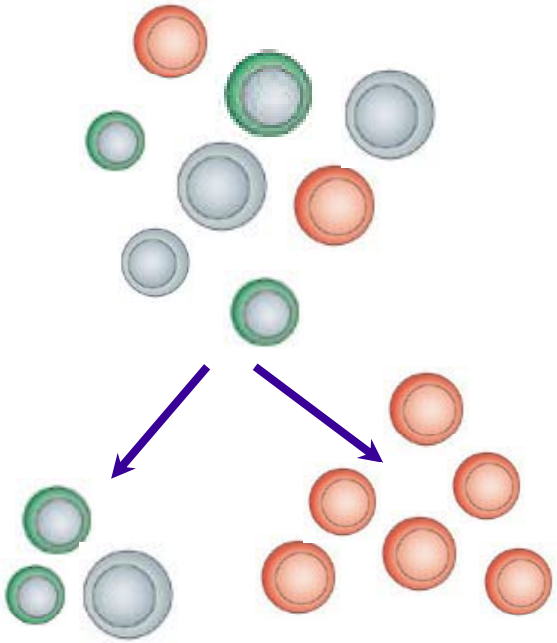


Tumor growth

Tumor growth

Tumor growth

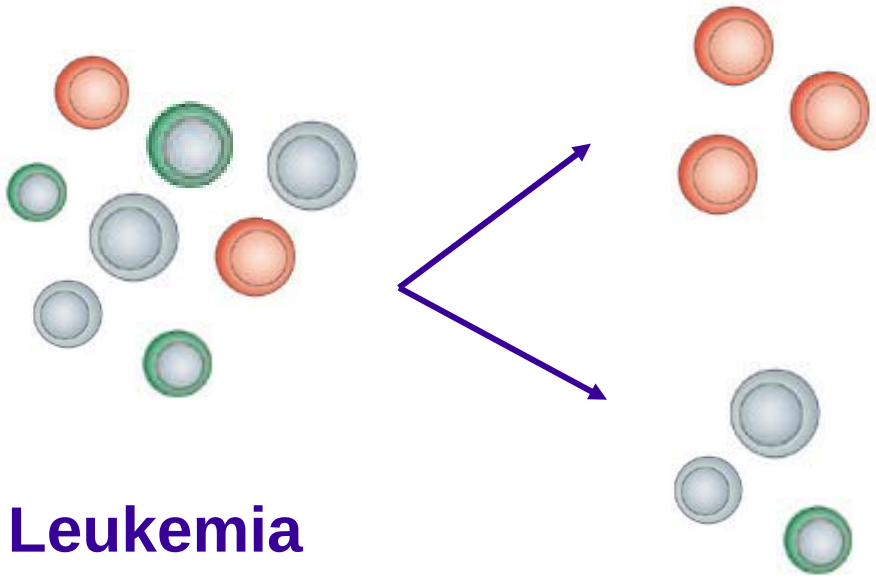
Cancer Stem Cell model



No Tumor

Tumor growth

Identification of Leukemic Stem Cells

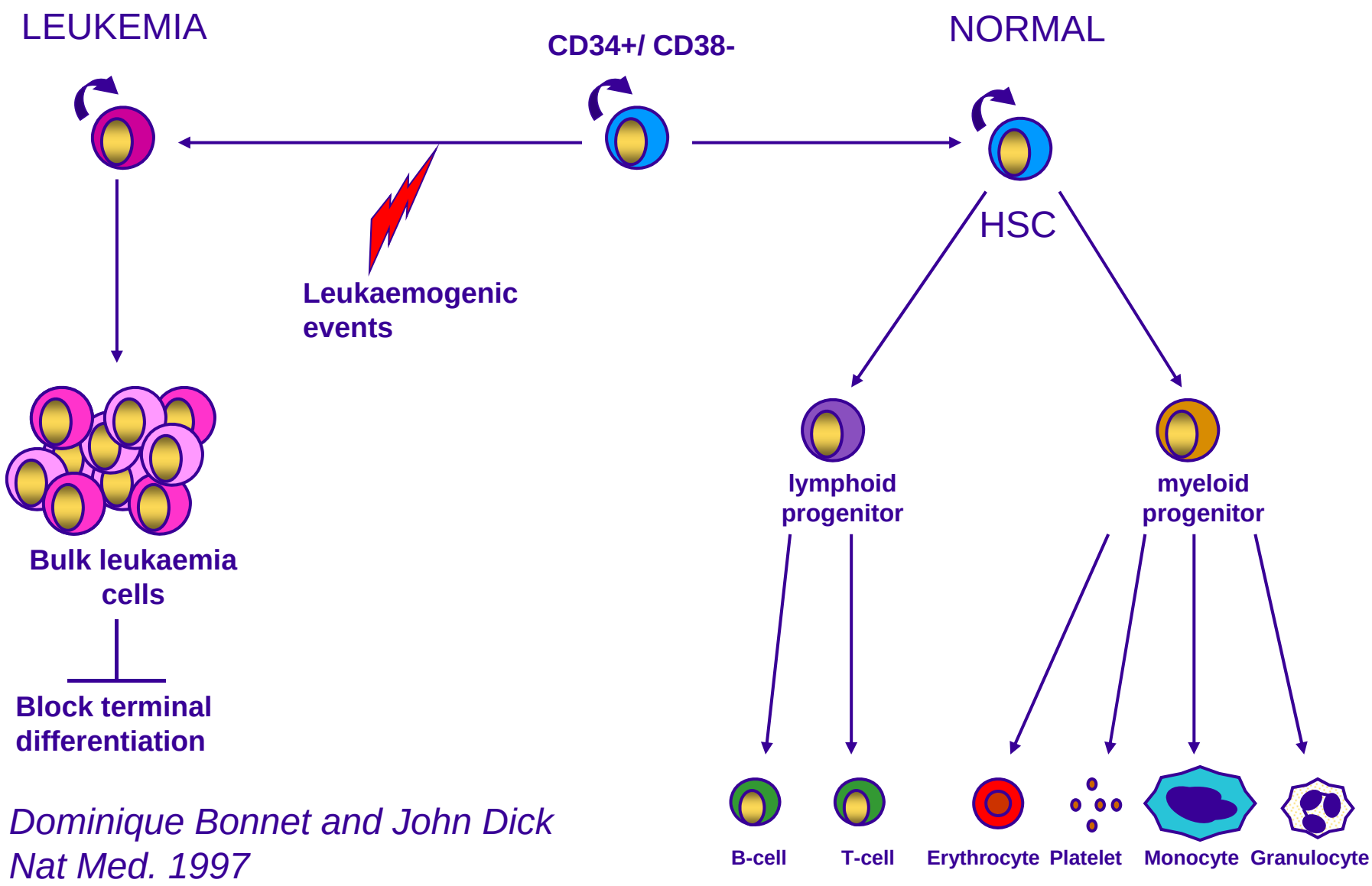


Leukemia

Leukemia formation

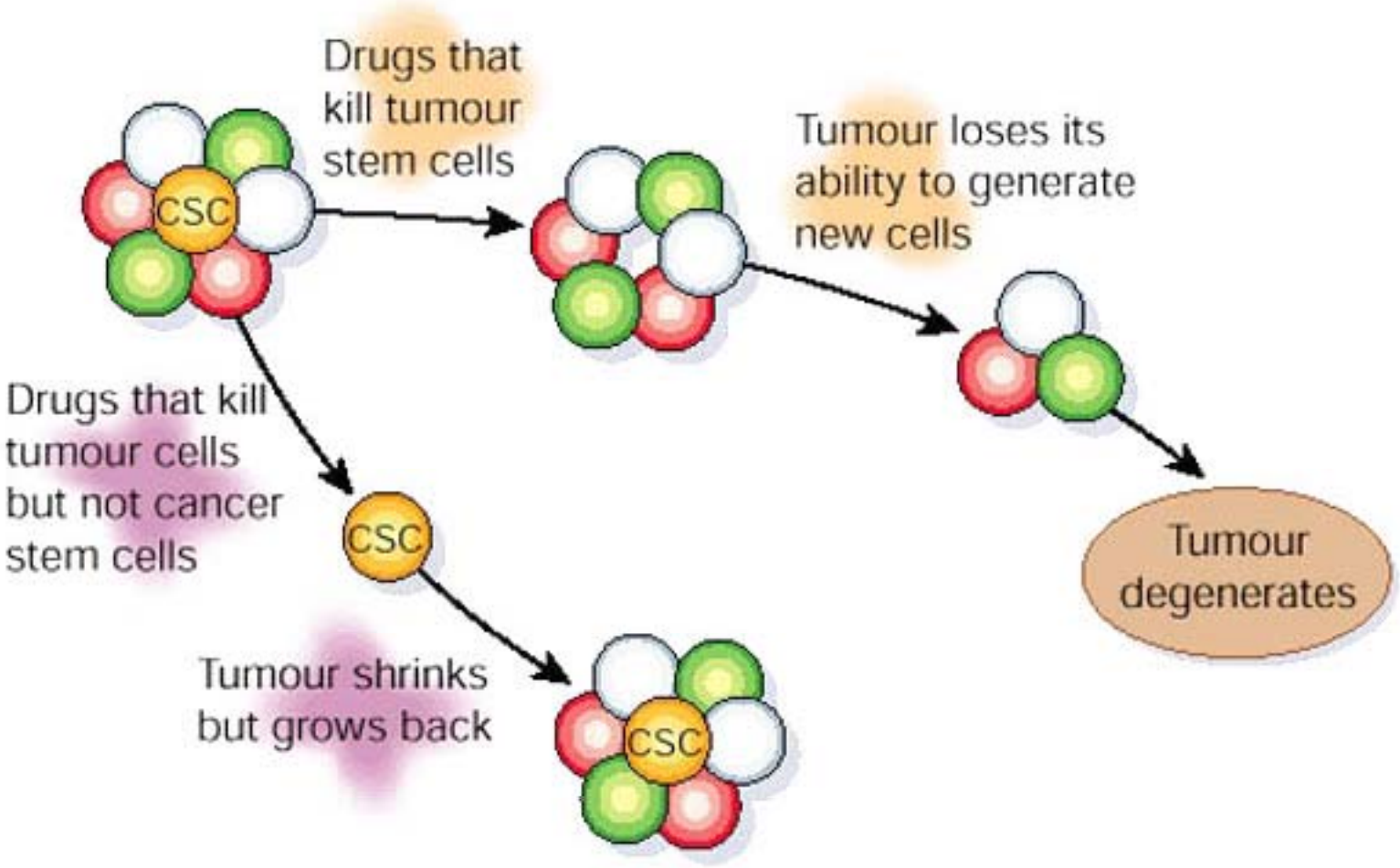
No Leukemia

Leukemia is arranged as a hierarchy similar to normal hematopoiesis



Dominique Bonnet and John Dick
Nat Med. 1997

What is the significance of AML-ICs ?



What is the significance of LSC ?

Despite years of research and therapeutic advancement, the majority of patients succumb to the disease.

Elimination of the LSC is crucial to obtain cure

Nevertheless, must of the new therapeutic drugs are being develop against the bulk blast population not against the leukemic stem cells

Considerations in targeting LSC

- Effectively target AML-ICs while selectively sparing normal HSC function.

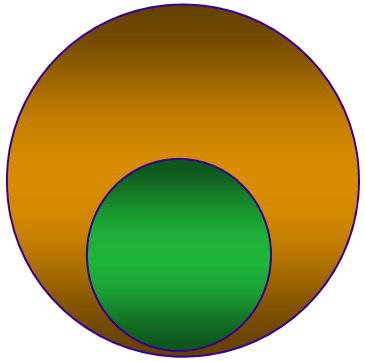
- Target potential biological differences between LSC and normal HSC:

  - Surface phenotype

  - Self-renewal mechanisms.

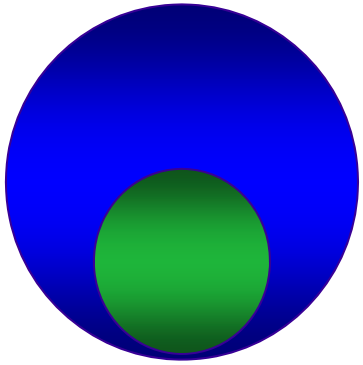
  - Interfered with the LSC microenvironment

Antigen expression on LSC in AML



- Normal HSC phenotype –

CD34+
CD38-
CD123-
HLADR-
CD33+
CLL-1- ?
CD90+
CD96- ?



- AML HSC phenotype –

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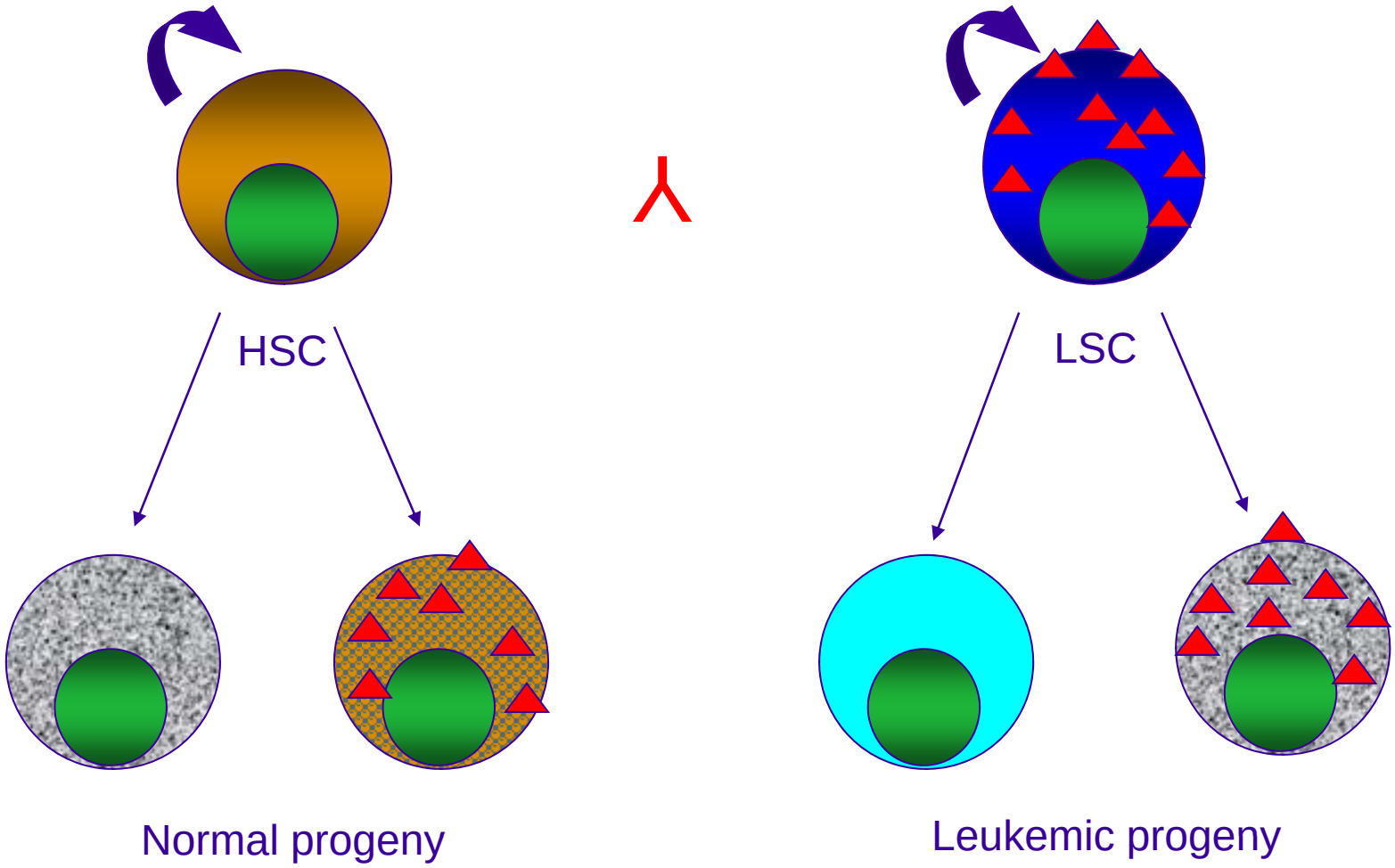
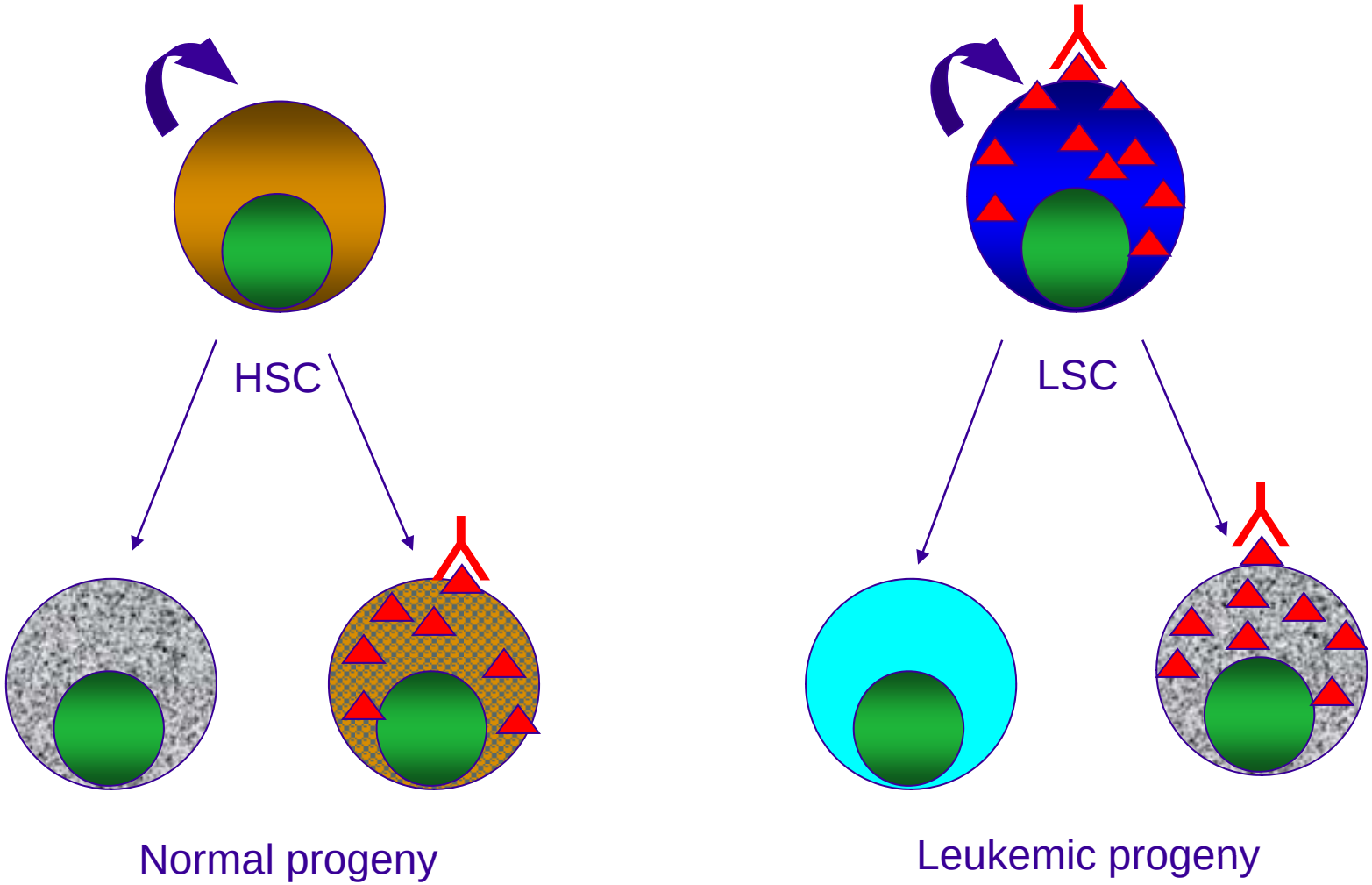
Immunotherapy of AML LSC



Immunotherapy of AML LSC



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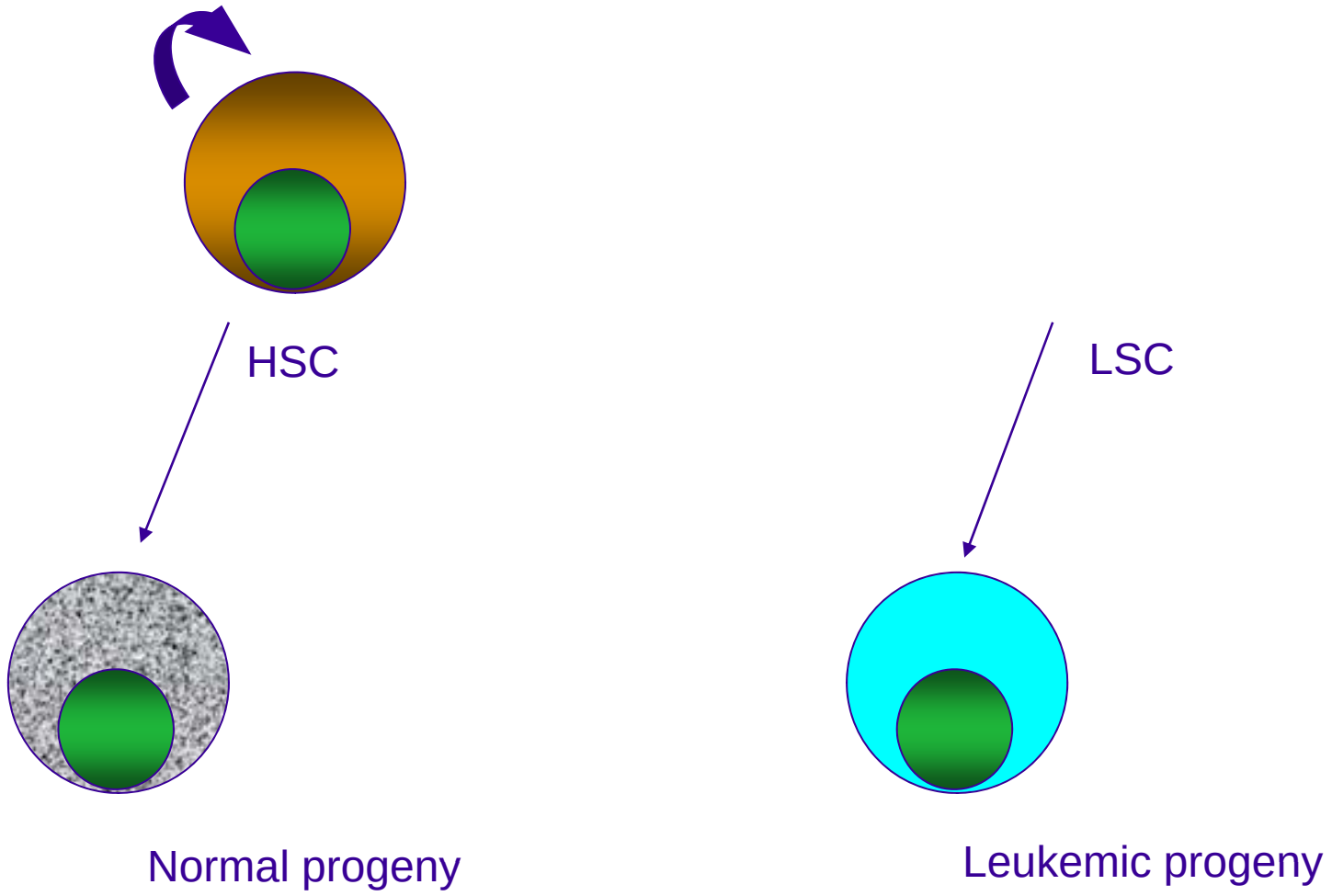

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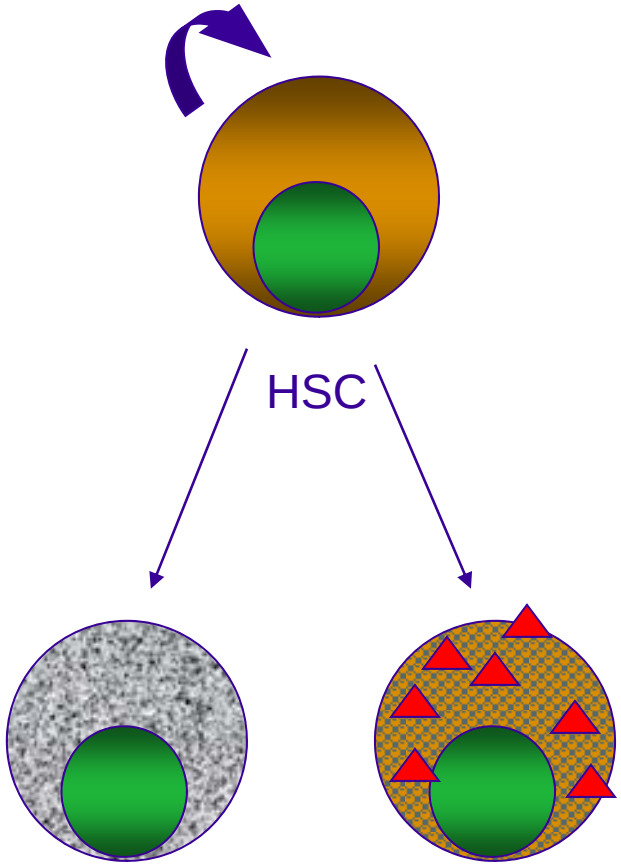


Eradication of disease

Mechanisms of self-renewal in AML-IC

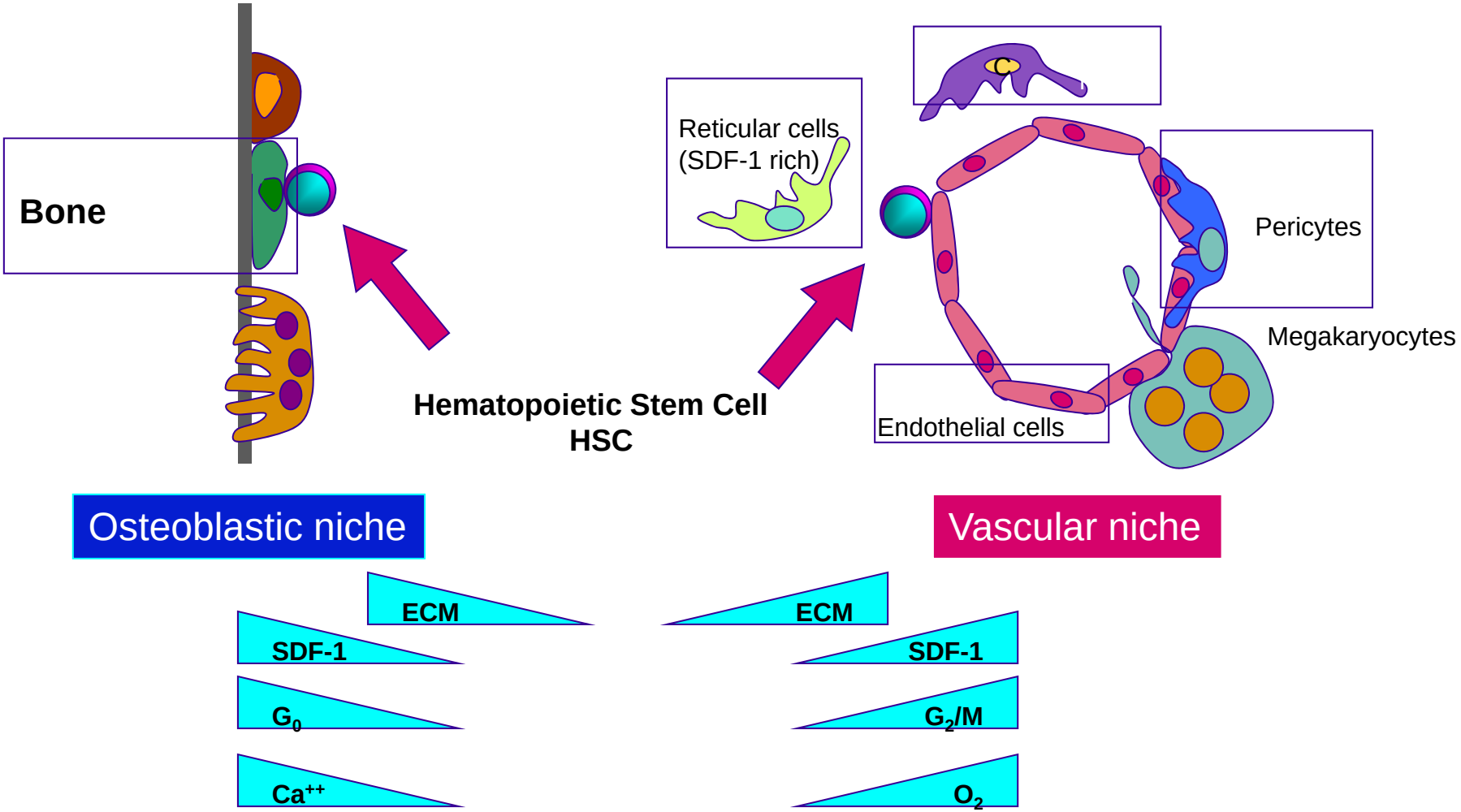
- Proposed mechanisms of self-renewal in AML-ICs.

- Wnt/β-Catenin
- Notch
- BMI-1
- Shh
- HOX genes

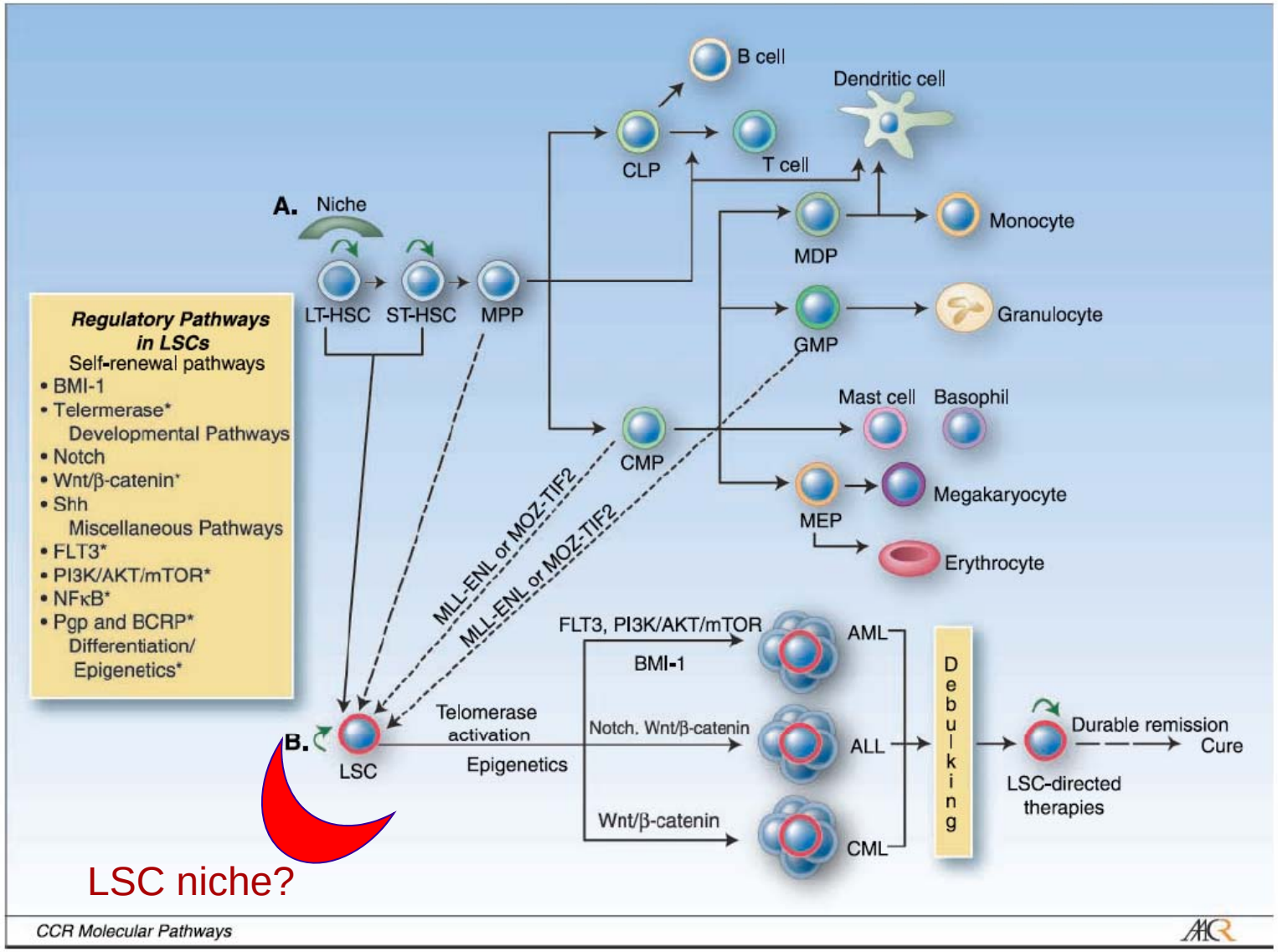
- ALL ALSO IMPLICATED IN NORMAL STEM CELL SELF-RENEWAL

Need to Identify self-renewal pathways preferentially utilized by LSC

The stem cell niche model



Mechanisms that regulate LSCs ?



Conclusions

- The leukemia stem cell (LSC) is the critical target in AML therapy.
- A further understanding of the biology of both the LSC and the normal HSC is required.
- Target pathways preferentially utilized by LSC.
- Growing body of evidence that differences in biology between LSC and HSC may be exploited for therapeutic benefit.

Impact of our work

Generalisation of the concept of “Cancer Stem Cell” to other tumours

- Brain tumours (Singh et al, 2003)
- Breast cancer (Al-Hajj et al, 2003)
- Colon Cancer (O’Brien et al. , Ricci-Vitiani et al. 2007)
- Prostate Cancer (Li C et al., Collins A et al. 2007)
- Head and Neck Cancer (Dalerba et al., 2007)

Our work on LSCs may have some impact on other cancers