# Hepatic stem cells and progenitors

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***First International Course of Translational Hepatology, Florence, 2011***

# Liver Stem Cells

- Multidisciplinary field of hepatology
  - Physiology of liver regeneration
  - Liver disease pathogenesis
  - Liver oncology
  - Cell and tissue banking and manipulation
  - Experimental treatments

- Interest in the field of liver stem cells is constantly increasing

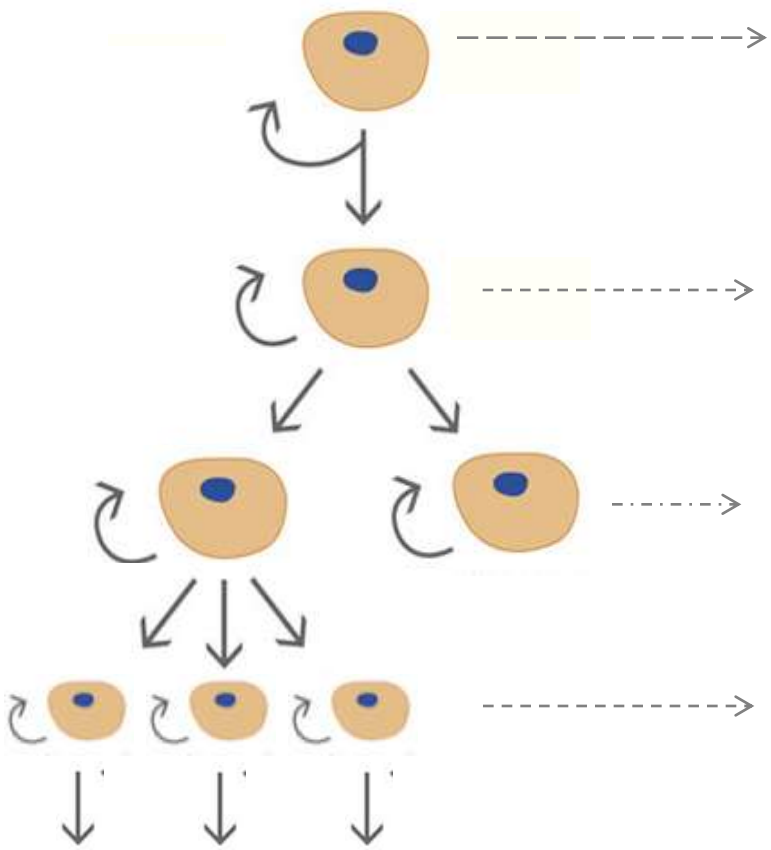
# Liver Stem Cells

- Stem cell therapies offer the opportunity to transform the approach to hepatic disease.
- Although current pharmacological therapy may target specific pathways or receptors, stem cell therapies can provide a living agent able to influence a range of biological processes.
- The potential to enhance hepatic regeneration after partial hepatectomy in advanced cirrhosis or provide a bridge to orthotopic liver transplantation has focused research.

# Potency and hierarchy of stem cells

EMBRYO

ADULT



Specialized cells

e.g.: RBC, WBC, PLT

1. Totipotent stem cells

can differentiate into embryonic and extraembryonic cell types.

Can construct a complete, viable, organism.

2. Pluripotent stem cells

Can differentiate into nearly all cells,

i.e. cells derived from any of the three germ layers

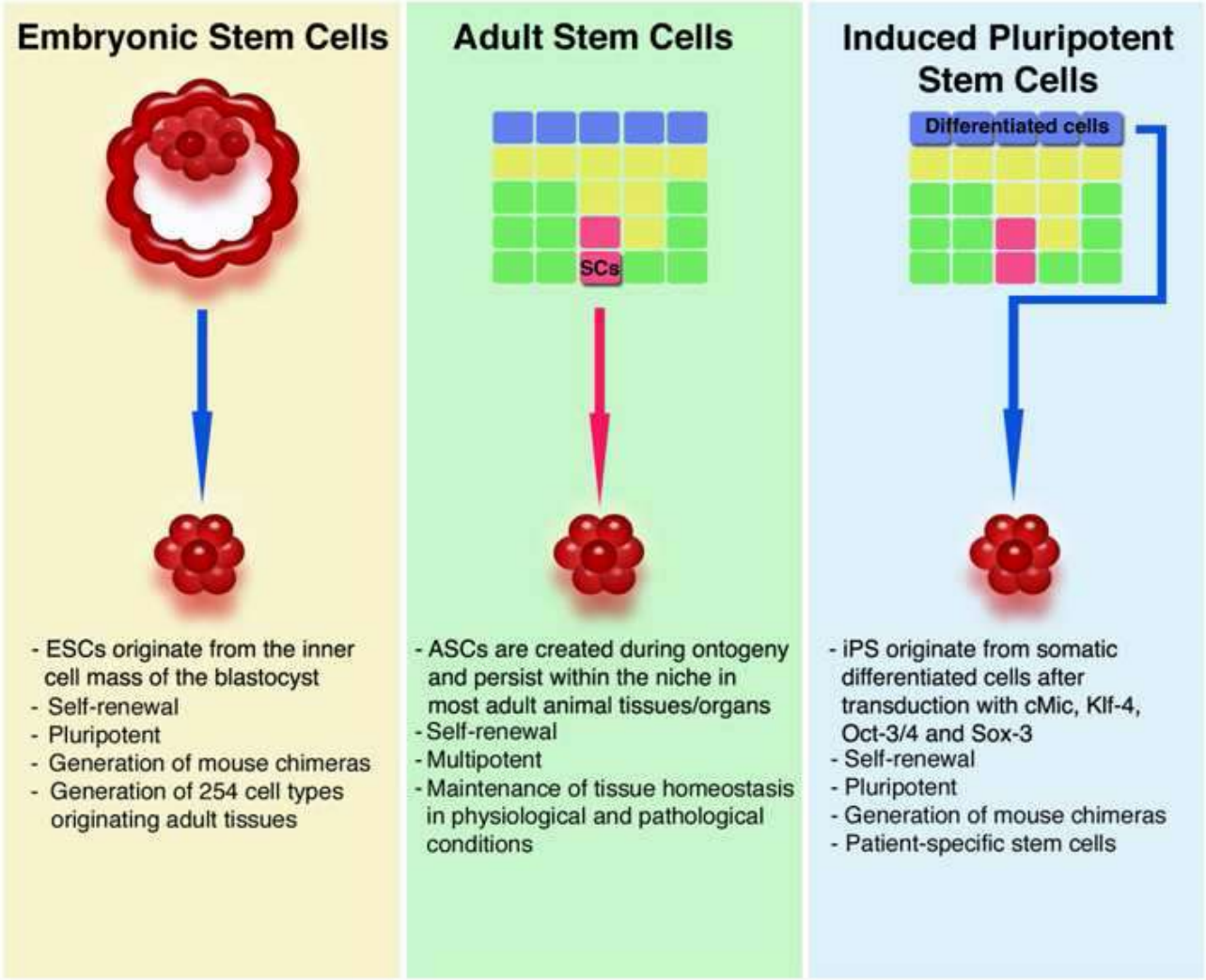
3. Multipotent stem cells

Can differentiate into a number of cells, but only those of a closely related family of cells. E.g.: HeSC

4. Oligopotent stem cells

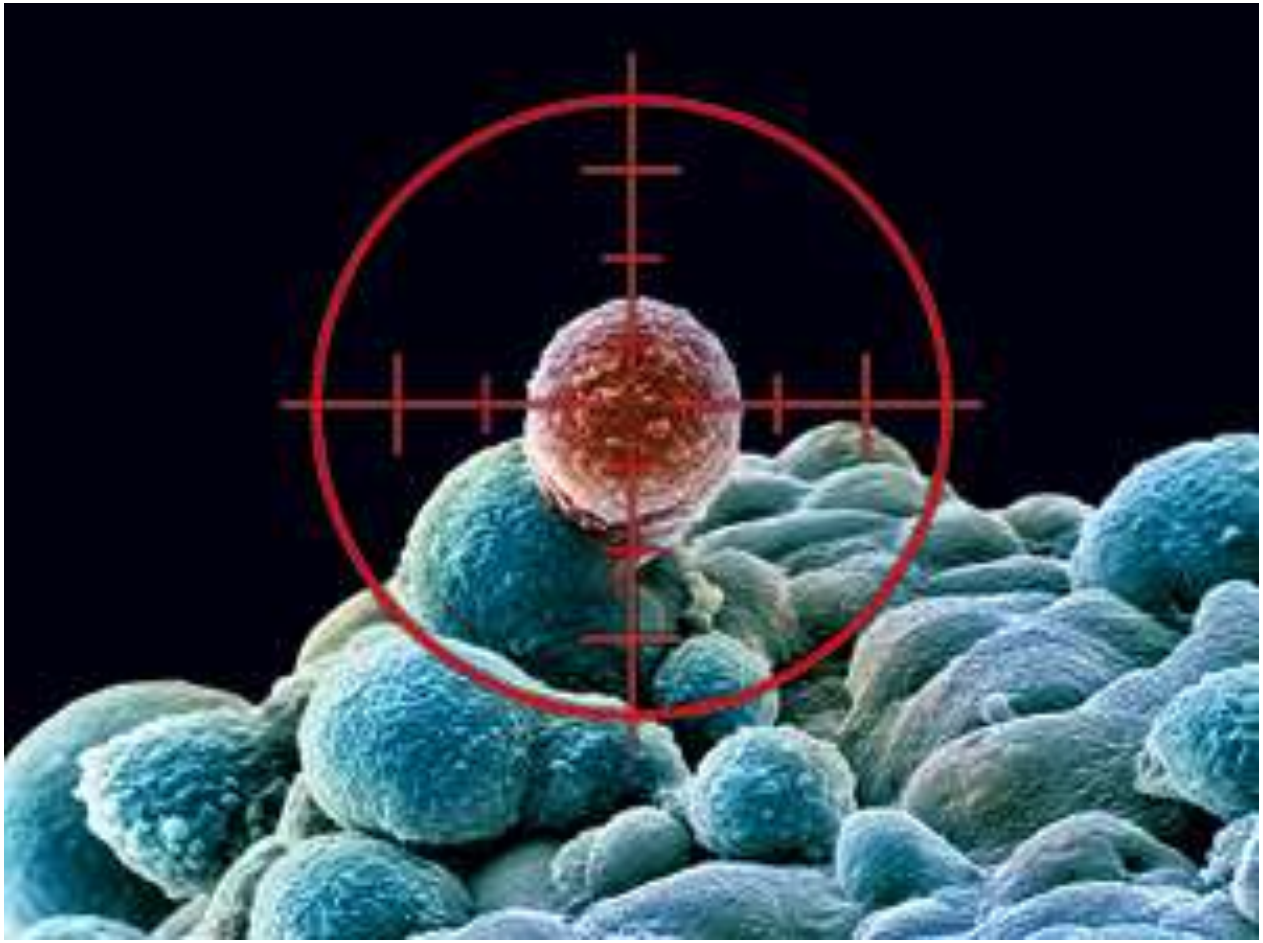
Can differentiate into only a few cells, E.g.: lymphoid or myeloid stem cells

# Stem cells with potential clinical use




*First International Course of Translational Hepatology, Florence, 2011*

# Cancer stem cells



# Outline of the presentation

- Overview of intrahepatic cell compartment
- Clinical trials on stem cell transplantation in the treatment of liver disease
- Liver stem cells and cancer

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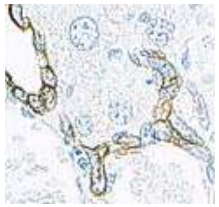
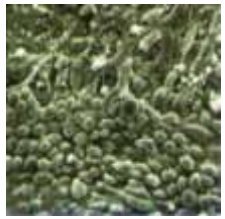
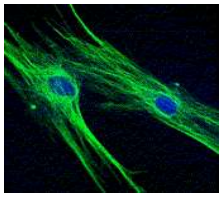
# The adult human liver is a complex tissue composed of different mature elements



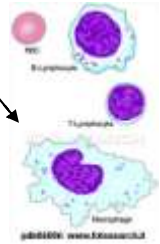
Endothelial cells
(sinusoidal endothelial liver cells…)



Epithelial cells
(hepatocytes, cholangiocytes)



Mesenchymal cells
(stellate cells, myofibroblasts…)



Hematopoietic cells
(intrahepatic WBCs)

- Accordingly, the intrahepatic stem cell population is likely an heterogeneous pool
- Possible immunophenotypic/functional overlap between stem cells of different origins

Cytometry Part A • 77A: 31–40, 2010

# Simultaneous Characterization of Progenitor Cell Compartments in Adult Human Liver

Laura Porretti,[1] * Alessandra Cattaneo,[1] Federico Colombo,[1] Raffaella Lopa,[1] Giorgio Rossi,[2] Vincenzo Mazzaferro,[3] Carlo Battiston,[3] Gianluca Svegliati-Baroni,[4] Francesco Bertolini,[5] Paolo Rebulla,[1] Daniele Prati[1,6]

**Table 1.** Commitment of intrahepatic stem cells towards different lineages

| | DONORS ($n$ = 10) | PATIENTS ($n$ = 20) | $P$ |
|---|---|---|---|
| | Median (range) | Median (range) | |
| Hematopoietic stem cells | | | |
| $CD34^+/CD45^+$ | 0.05 (0.01–0.2) | 0.2 (0.03–1) | 0.01 |
| $CD133\text{-}2^+/CD45^+$ | 0.08 (0.01–0.33) | 0.08 (0.01–1) | NS |
| Endothelial progenitors | | | |
| $KDR^+/CD146^+/CD45^+$ | 0.025 (0–0.22) | 0.11 (0–0.17) | NS |
| $KDR^+/CD146^+/CD45^-$ | 0.02 (0–0.13) | 0.13 (0.01–0.77) | 0.02 |
| Epithelial stem cells | | | |
| $CD29^+/CD49f^+/CD45^-$ | 3.5 (0.7–13.6) | 2.7 (0.1–18.4) | NS |
| Mesenchymal stem cells | | | |
| $CD73^+/CD105^+/CD45^-$ | 0.6 (0.4–2.5) | 0.6 (0.1–3.7) | NS |

Median percentages (range) of freshly isolated mononuclear liver cell fraction.
The groups were compared using the Mann-Whitney test.
$P$ values of $<0.05$ were considered significant.

Cytometry Part A • 77A: 31–40, 2010

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- Hematopoietic
- Endothelial (ECFCs)
- Endothelial (CFU-ECs)
- Epithelial
- Mesenchymal

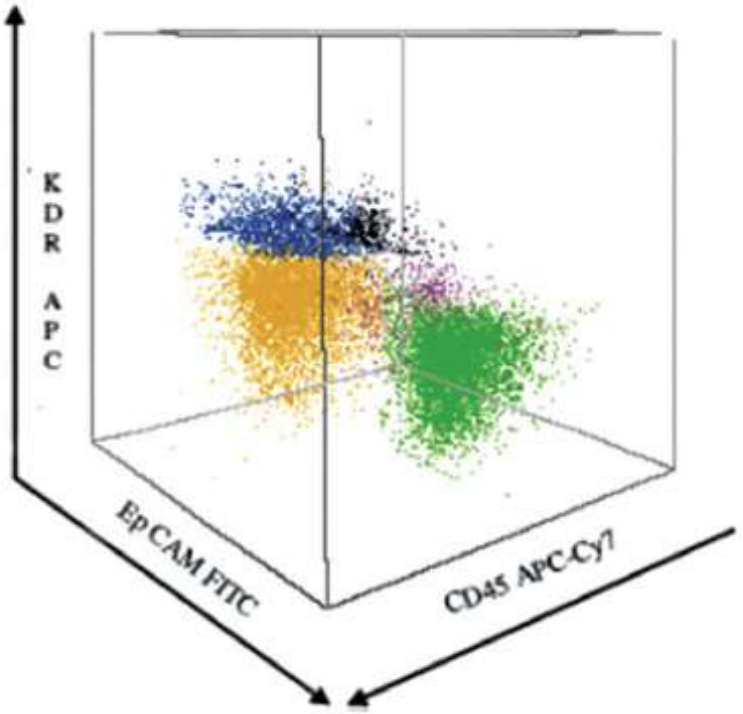
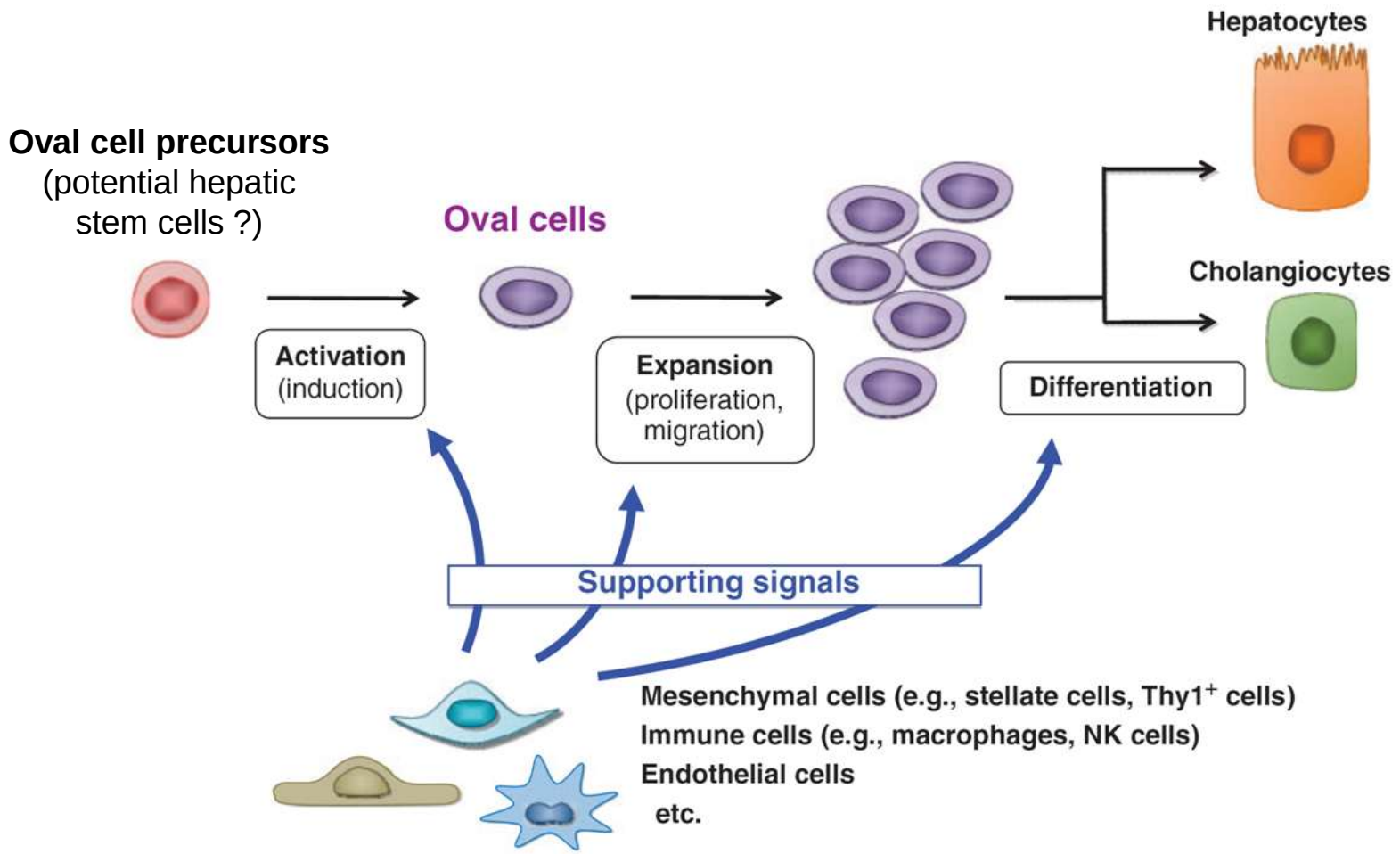


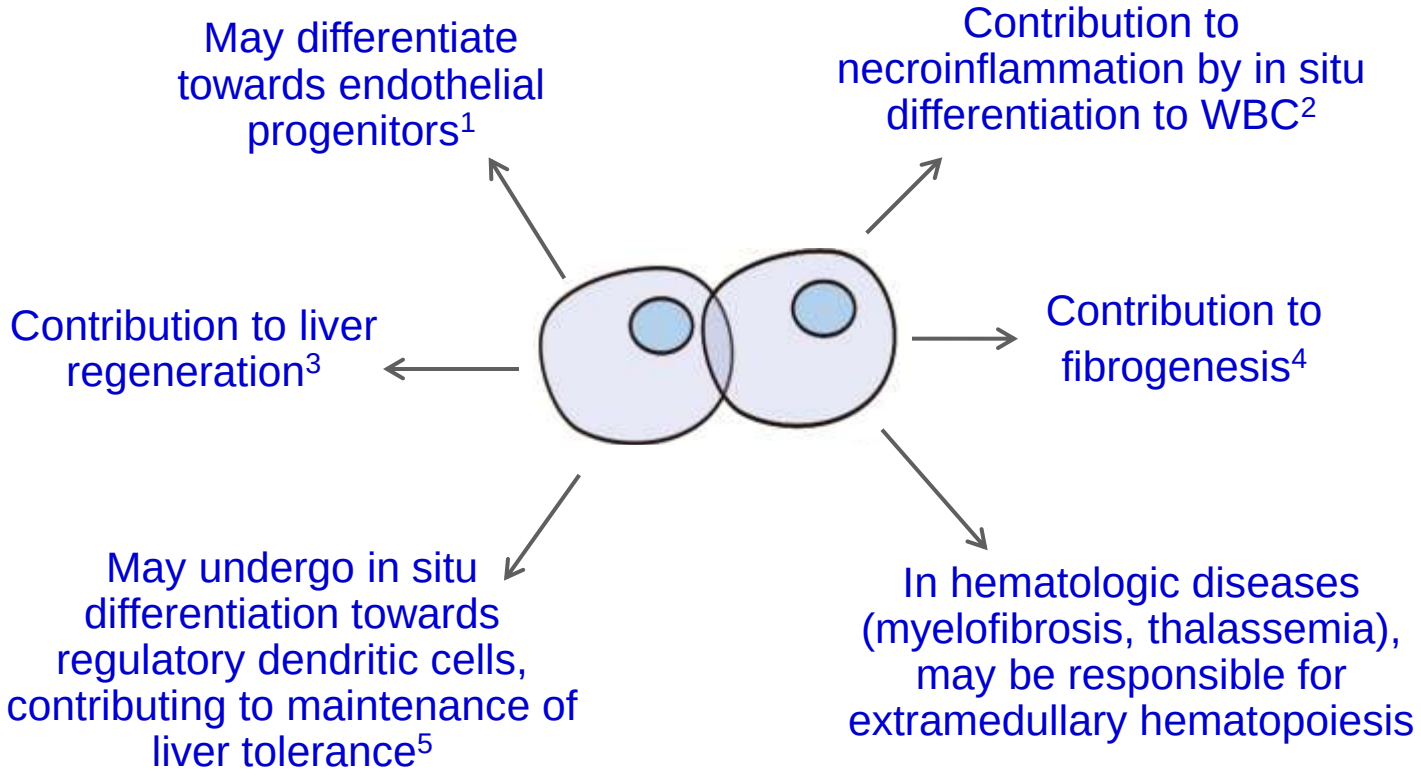

**Figure 4.** Three-dimensional plot of liver stem and progenitor cells analyzed in a single tube combination of EpCAM/KDR/7-AAD/CD34/Thy-1 (CD90)/CD45. FACSDiva files, adequately compensated as reported in the text, were exported as FCS 2.0 in Paint-a-gate software. The following gating strategy was then applied: L-HSCs (viable $CD45^+/CD34^+$ cells, yellow dots), ECFCs (viable $CD45^-/KDR^+$ cells, black dots), CFU-ECs (viable $CD45^+/KDR^+$ cells, blue dots), L-EpPCs (viable $CD45^-/EpCAM^+$ cells, green dots), L-MSCs (viable $CD45^-/Thy\text{-}1^+$ cells, violet dots). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

# Liver cell differentiation from epithelial precursors



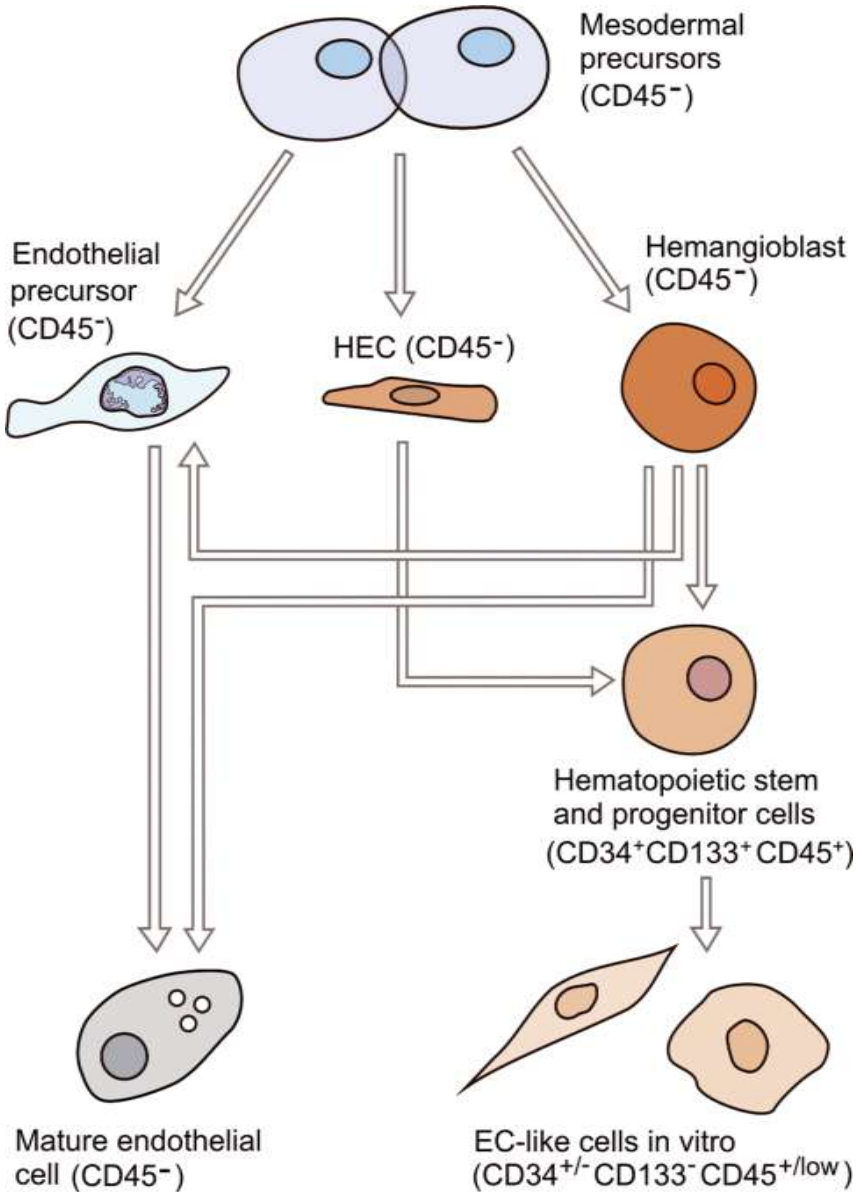

Tanaka M, J Biochem 2011

# Intrahepatic Hematopoietic SC and progenitors - Issues of potential clinical importance




[1]Harb R, Hepatology 2009 ; [2]Golden Mason L, Hepatology 2000; [3]rev. by Duncan A, Gastroenterology 2009; [4]Russo FP, Gastroenterology 2006); [5]Xia S, Blood 2009.

Endothelial and hematopoietic precursors share common mesodermal origin



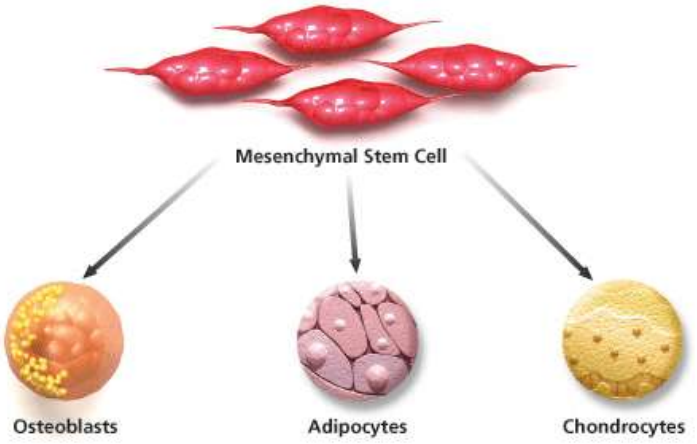

Timmermans F, J Cell Mol Med 2009

# Intrahepatic EPC – Why should be further studied?

- Novel potential antitumoral strategies
  - inhibition of EPC to retard tumor angiogenesis and vasculogenisis;
  - In situ delivery of antitumoral agents
- Experimental treatment of vascular liver disorders
- Crosstalk with stromal cells – experimental treatment of liver fibrosis

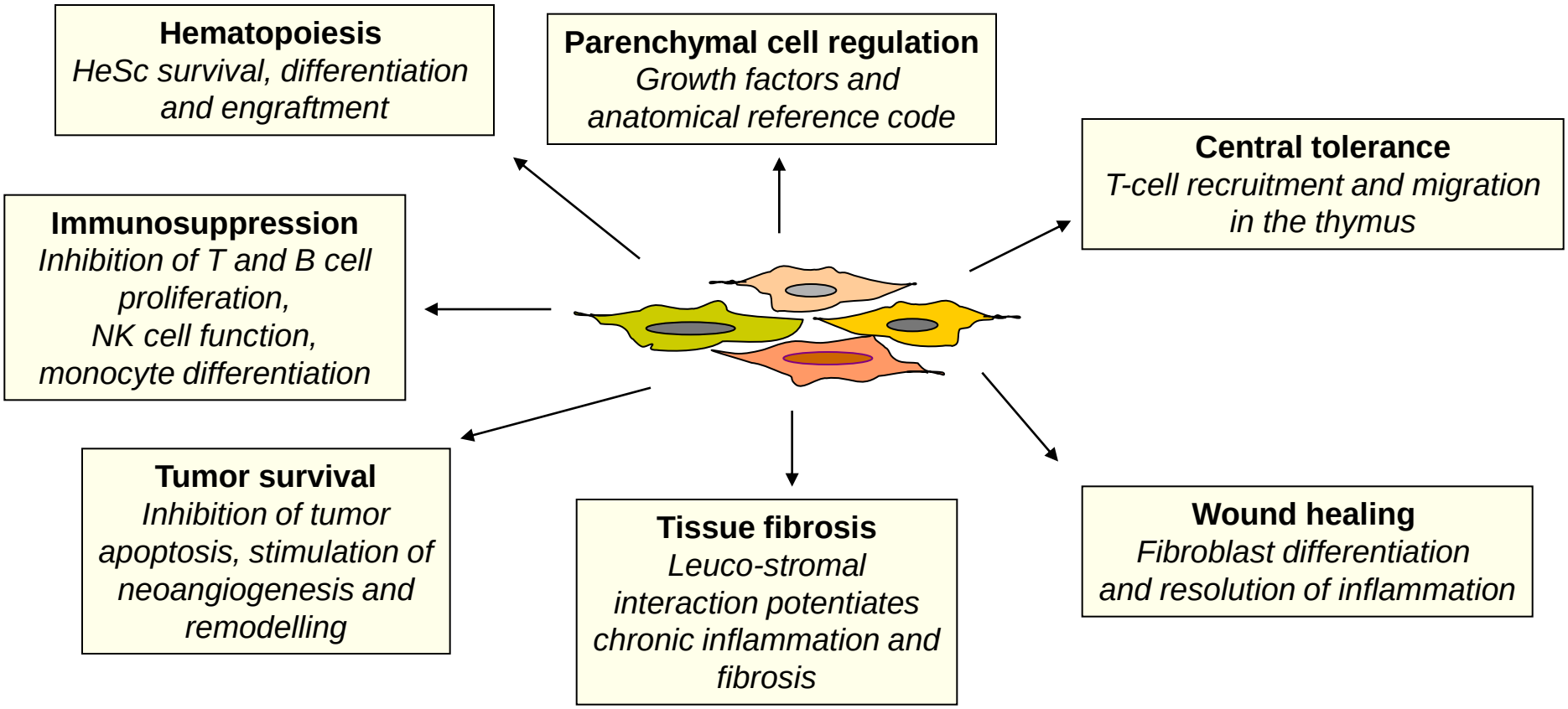
# Mesenchymal Stem Cells

- Originally identified in bone marrow, but virtually present in all organs
- Plastic adherent, multipotential spindle shaped cells expressing CD73, CD105 and negative for the hematopoietic markers CD14, CD34 and CD45[1]
- Self renewal, clonogenic properties, multilineage differentiation potential[1]




[1]As defined by the International Society for Cytotherapy

# Stromal/mesenchymal cells modulate diverse biological processes




Reviewed in Haniffa MA, Haematologica 2009

# Outline of the presentation

- Overview of intrahepatic cell compartment
- Clinical trials on stem cell transplantation in the treatment of liver disease
- Liver stem cells and cancer

# Approaches of Autologous Stem Cell Therapies

| Approach | Cell Source | Intended Objectives |
|---|---|---|
| 1. Undifferentiated Stem/ progenitor cells | Embryo, Adult stem/progenitors | Liver engraftment, cell differentiation and function |
| 2. Ex vivo differentiated hepatocytes | Embryo, Adult Stem/progenitors IPSCs | Liver engraftment, cell function |
| 3. Bio-Artificial liver support (BALS) systems | Ex vivo differentiated hepatocytes | Extracorporeal support by hepatocyte contact |
| 4. Promoting endogenous processes | Hematopoietic stem cells, BM mesenchymal cells | Matrix & vascular remodeling, immunomodulation, facilitation of resident liver cell proliferation |

# 4. Promoting endogenous processes

•**Cell source**: Hematopoietic stem cells, BM mesenchymal cells

•**Objective**: Matrix & vascular remodeling, immunomodulation, facilitation of resident liver cell proliferation

•**Evidences**: Improved physiological parameters in some phase 1 clinical trials[1]

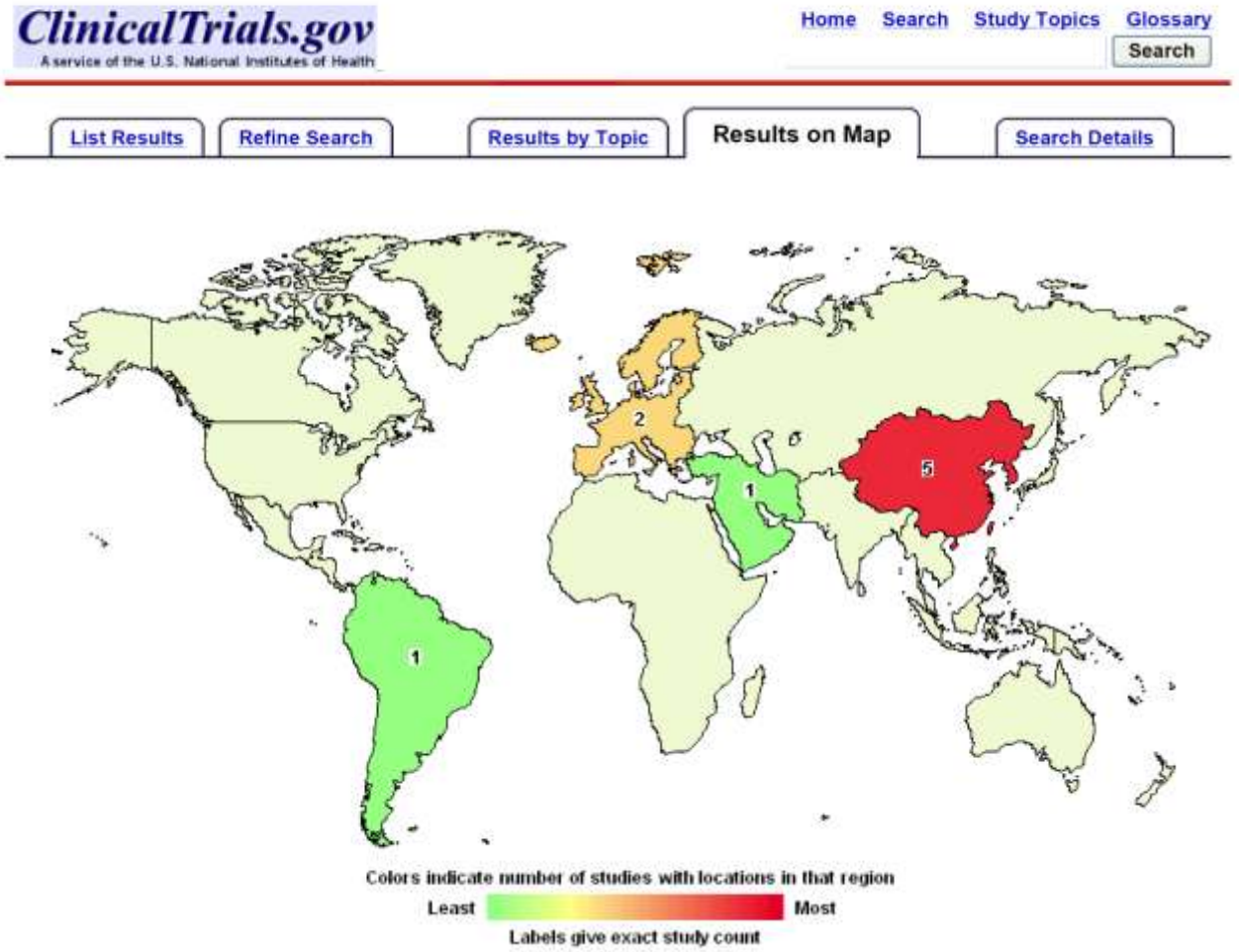
•**Comments**: Still limited clinical evidence - MSC problematic: potential increased hepatic fibrosis?

[1]Reviewed in Stutchfield BM, Liver Transplantation 2010

# Stem cell therapies and clinical trials

- Autologous stem cells derived from BM are the only stem cell types undergone clinical investigation
- 9 cohorts/phase 1 trials investigating BMSCs to enhance hepatic regeneration have been published
- A total of 47 patients with chronic liver failure or inadequate future liver volume prior to partial hepatectomy (17 with HCV)
- Outcome differently assessed (MELD, CP score, liver Volumetry, LFTs)
- The majority of trials (8/9) have observed modest clinical benefit

# Phase I/Phase II clinical trials on stem cell therapy in liver cirrhosis, currently recruiting patients




Access: March 10, 2011

# BM stem cell mobilization in chronic liver disease

- Research in rodent models suggests that BM stem cells may lodge in the cirrhotic liver after massive bone marrow mobilization and undergo hepatocyte like differentiation (*Liu F, Liver Transpl 2006; Piscaglia AC, Gastroenterology 2007*)

- In two studies, G-CSF has been administered to small cohorts with chronic liver disease (n=8 and n=13) *(Gaia S, J Hepatol 2006; Spahr L, Hepatology 2008)*

- Clinical improvement has been observed in some cases (n=4) and it could be attributed to the mobilized cells or perhaps to the direct effect of G-CSF itself on endogenous hepatic repair mechanisms *(Yannaki e, Exp Hematol 2005; Stutchfield BM, Liver Transplantation 2010)*

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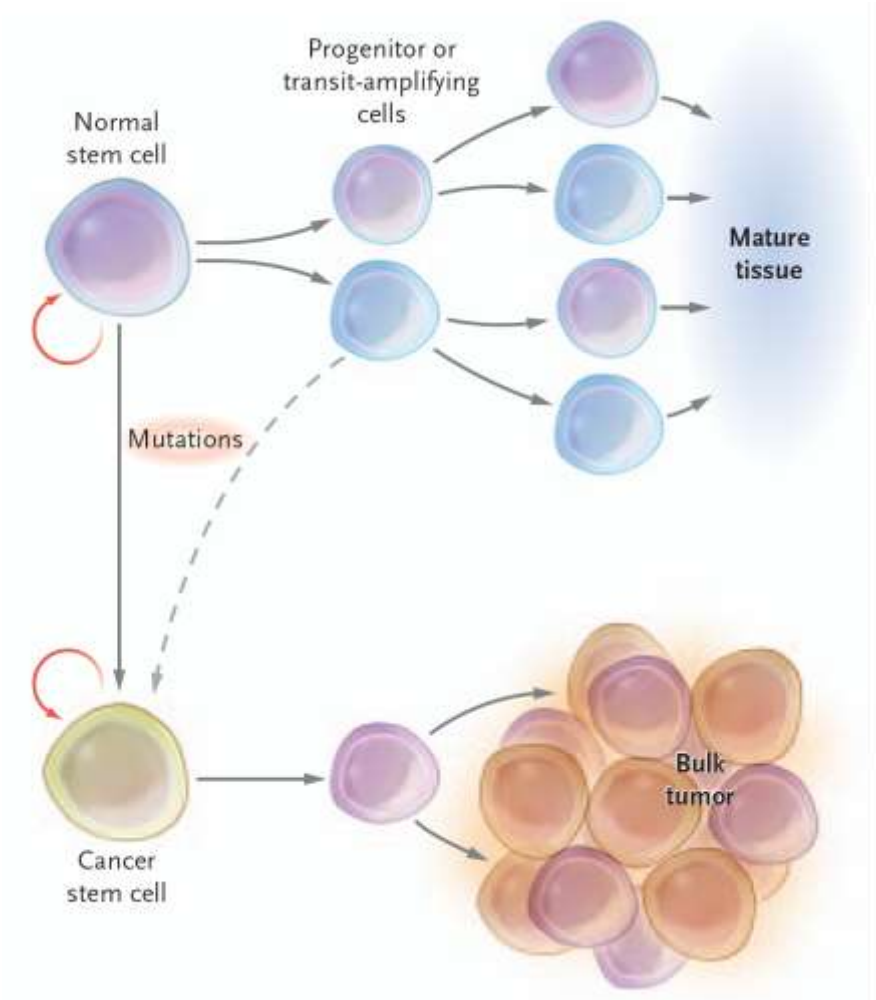
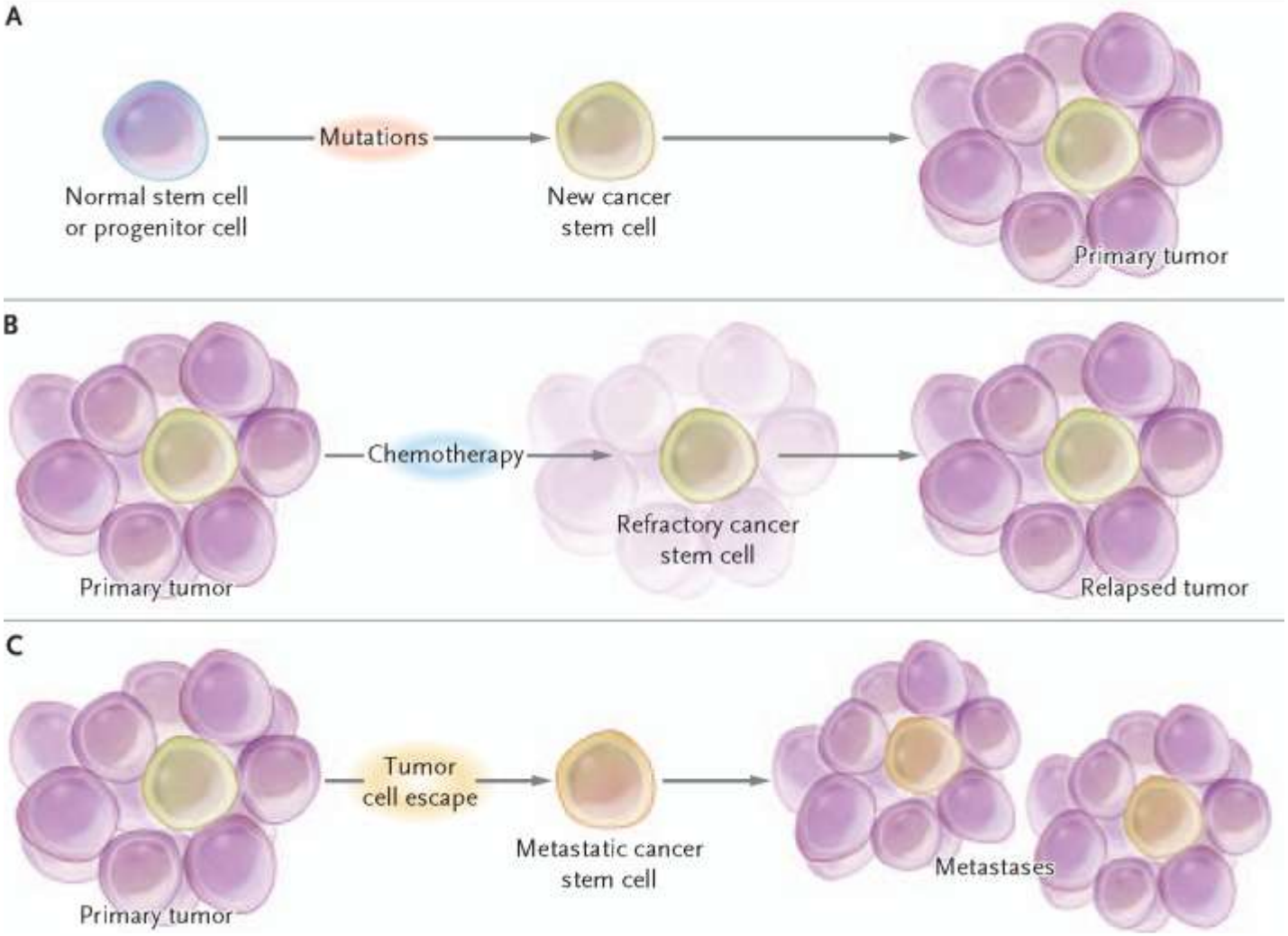
# “Pathological” stem cells: cancer stem cells (tumor initiating cells) can be found in chronic liver disease patients




Craig TJ, NEJM 2006

# Cancer stem cells may form new tumors and be responsible for tumor recurrence and metastasis

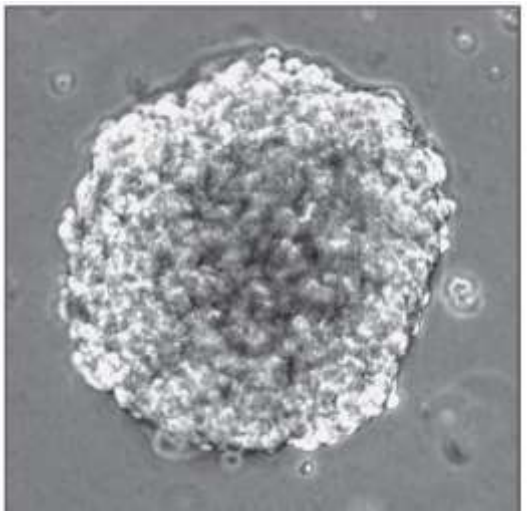



Craig TJ, NEJM 2006

# Cancer stem cells have growth properties similar to normal stem cells

- Self regeneration
- Generation of large progenies of daughter cells
- Can give rise to differentiated cells

Similarly to normal cells, they can be studied in cell cloning assays

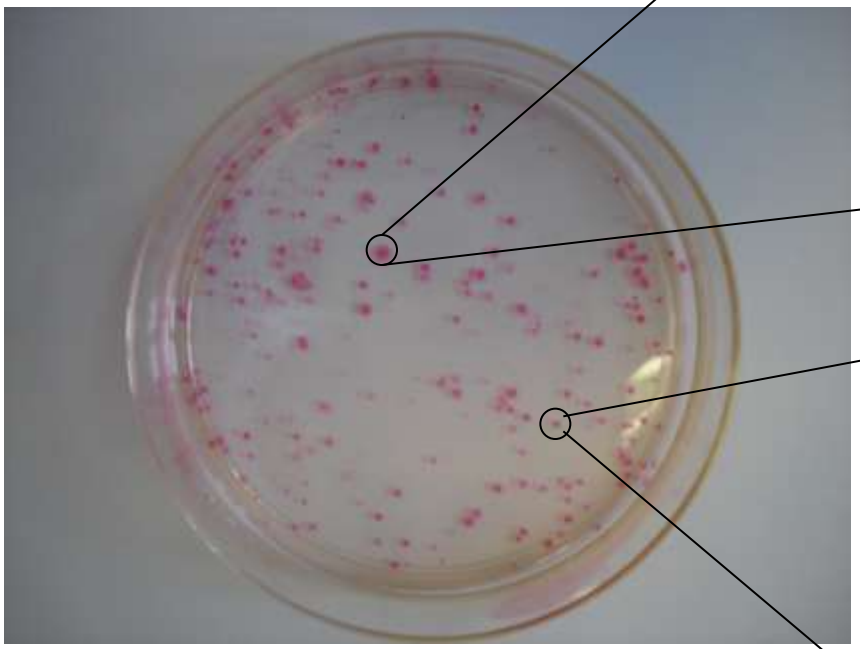


*Brain tumors:*
*primary human neurosphere*

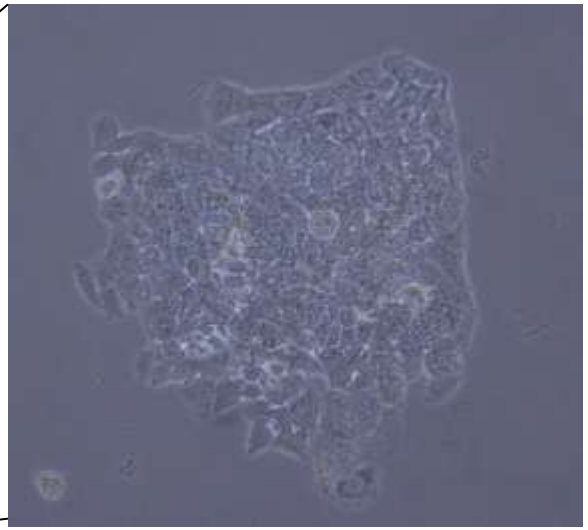
# Cancer stem cells in different human tumors

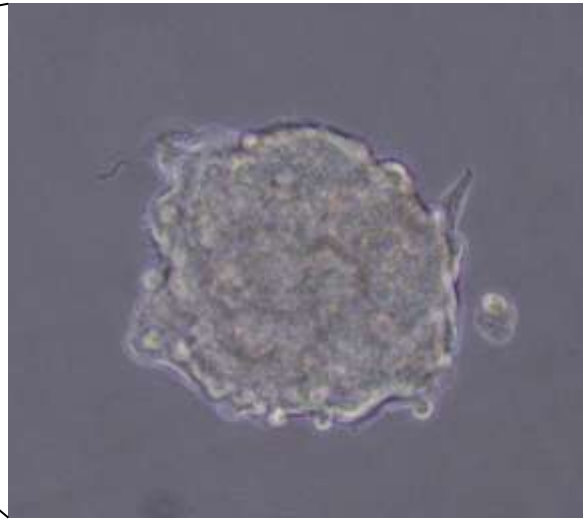
- Leukemia/Lymphoma
- Brain tumors
- Breast tumors
- Prostate tumors
- Colon cancer
- Hepatocellular carcinoma (HCC)
- …

Single cell clone generation an HCC specimen



*Eosine staining*





F. Colombo. et al (submitted), 2011

*Int. J. Cancer:* **120,** 1444–1450 (2007)


## CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity

**Shengyong Yin[1], Jinjun Li[2], Chen Hu[1], Xinhua Chen[1], Ming Yao[3], Mingxia Yan[3], Guoping Jiang[1], Chao Ge[2], Haiyang Xie[1], Dafang Wan[2], Shengli Yang[2,4], Shusen Zheng[1*] and Jianren Gu[2*]**

# Side Population Purified From Hepatocellular Carcinoma Cells Harbors Cancer Stem Cell–like Properties

Tetsuhiro Chiba,[1,2] Kaoru Kita,[1] Yun-Wen Zheng,[1] Osamu Yokosuka,[3] Hiromitsu Saisho,[3] Atsushi Iwama,[2] Hiromitsu Nakauchi,[4] and Hideki Taniguchi[1,5,6]

HEPATOLOGY, Vol. 44, No. 1, 2006

# Identification and Characterization of Tumorigenic Liver Cancer Stem/Progenitor Cells

STEPHANIE MA,[*,‡] KWOK–WAH CHAN,[*] LIANG HU,[‡] TERENCE KIN–WAH LEE,[§] JANA YIM–HUNG WO,[§] IRENE OI–LIN NG,[*] BO–JIAN ZHENG,[‖] and XIN–YUAN GUAN[‡]

GASTROENTEROLOGY 2007;132:2542–2556

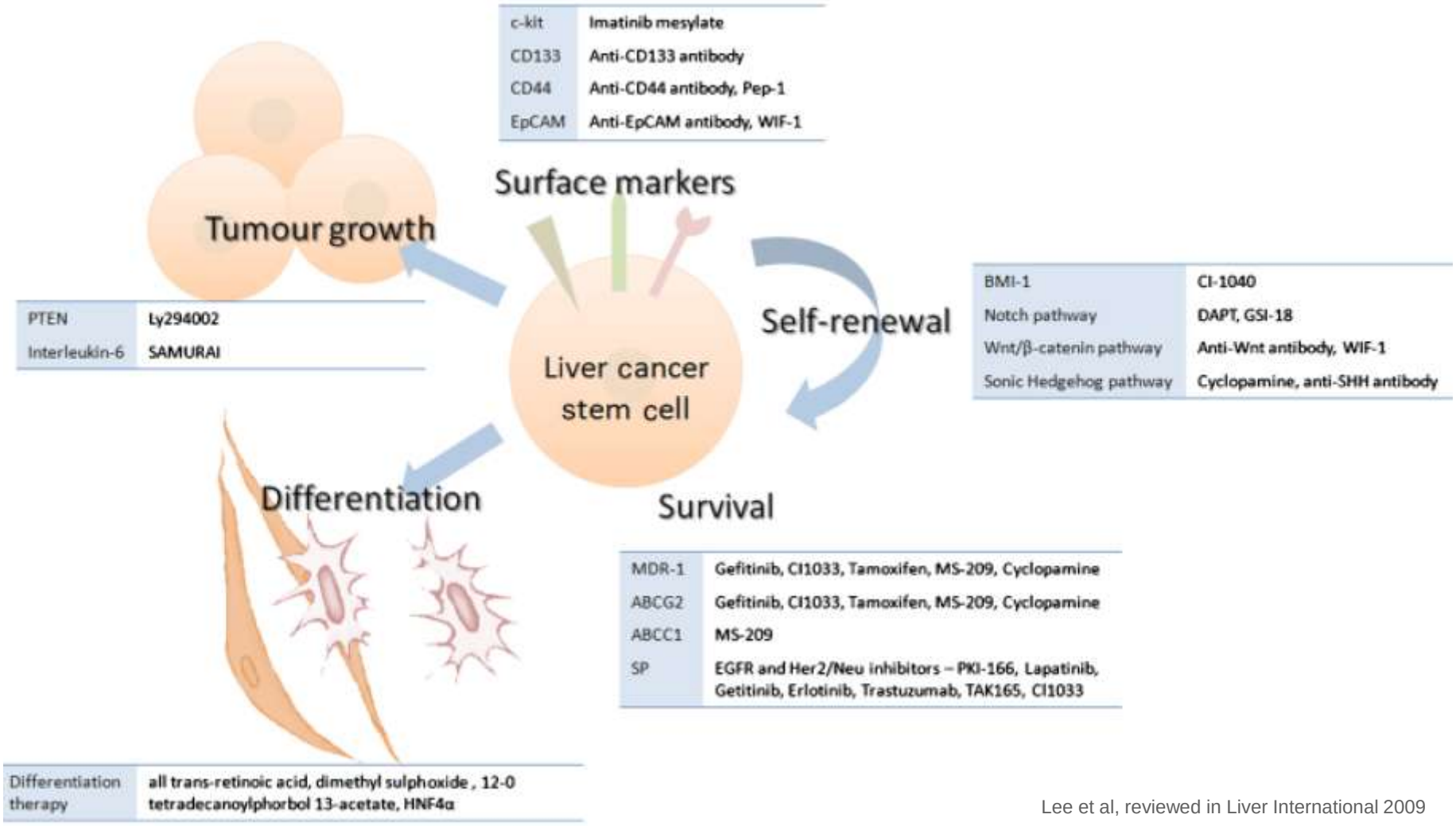
# Current putative liver cancer stem cell markers

| Markers | References |
|---|---|
| 1. CD133 | Ma *et al*. (76) |
| 2. CD44 | Yang *et al*. (84) |
| 3. CD90 | Yang *et al*. (80) |
| 4. OV6 | Yang *et al*. (89) |
| 5. SP | Chiba *et al*. (90) |
| 6. EpCAM | Yamashita *et al*. (88) |

EpCAM, epithelial cell adhesion molecule; SP, side population.

Lee et al, reviewed in Liver International 2009

# Potential inhibitors of liver cancer stem cells

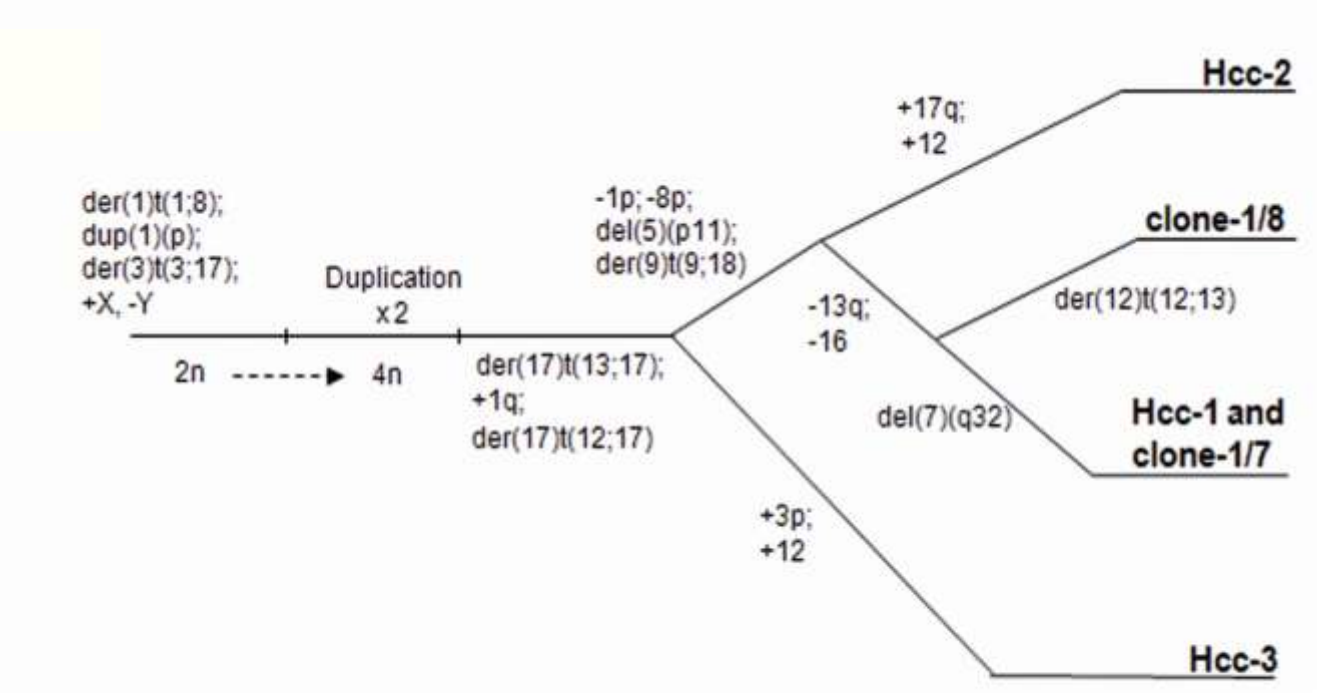



Lee et al, reviewed in Liver International 2009

# Challenges for therapies targeted against cancer stem cells

- Knowledge of identity and functions of “healthy” stem cells and progenitors
- Further definition of pathological pathways in cancer stem cells; is there a common Achille’s heel in different types of stem cells?
- Effect of conventional chemotherapy regimens on cancer stem cells: do they provide a competitive advantage to cancer stem cells? Definition of novel treatment strategies
- “Clonal evolution” of cancer stem cells?

# Clonal evolution of cancer stem cells from a single HCC mass




The same tumor can contain genetically distinct cell populations with independent tumour initiating capability, but significantly different phenotypical and growth characteristics.

Colombo F, submitted

# Summary

- After more than a decade of intensive investigation, stem cell based treatment for liver disease remains a hope.
- No clinical indication outside clinical trials
- Growing interests in physiopathological aspects of liver stem cells in the study of hepatic regeneration and carcinogenesis.