Hepatocyte Transplantation: The Next Frontier

Research

Clinical Care

Education

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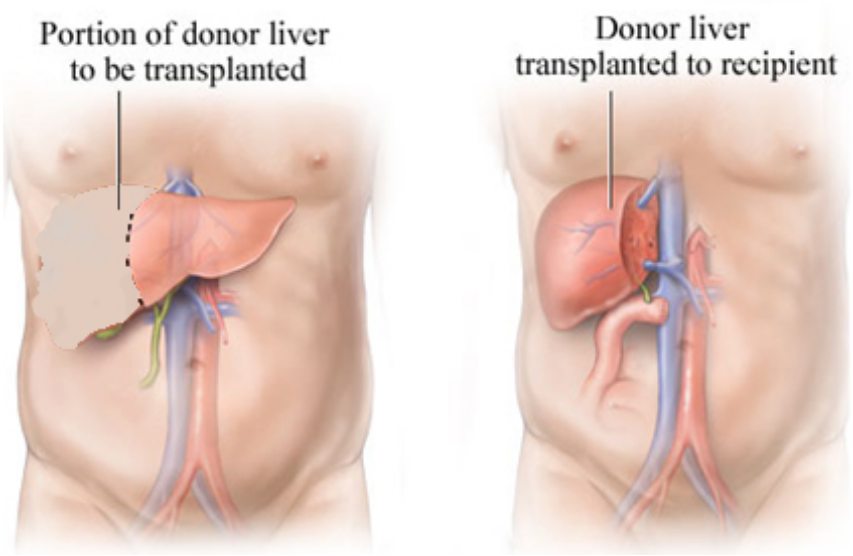
Singapore Hepatology Conference

Disclosure

No conflict of interest with respect to the content of this presentation

The liver is an organ of tremendous regenerative capability-



Basis of LDLT- getting parts of the liver to regenerate

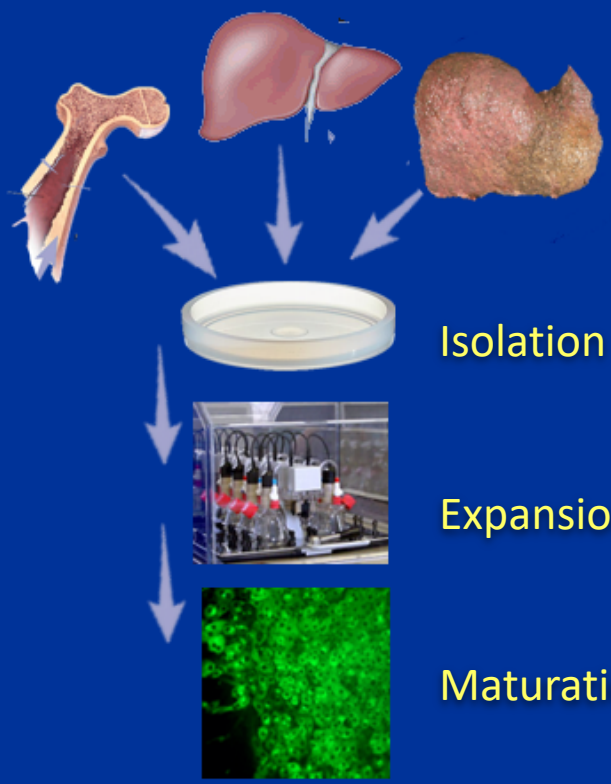
Unmet need:

1. Unsuitable for transplant
2. No access to transplant
3. Cannot wait for transplant

Therapeutic Potential of Hepatic Stem Cells

Gene therapy

Cellular therapeutics:
Hepatocyte Transplant



Isolation

Expansion

Maturation

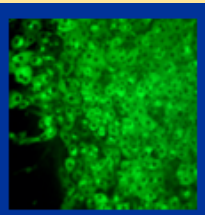
Toxicology studies
drug development

Bioartificial Liver
Assisted Device

Therapeutic Potential of Hepatic Stem Cells

The Liver Cell is only a hepatocyte when it is in the liver
To date, it has eluded all efforts to expand
and maintain function in vitro

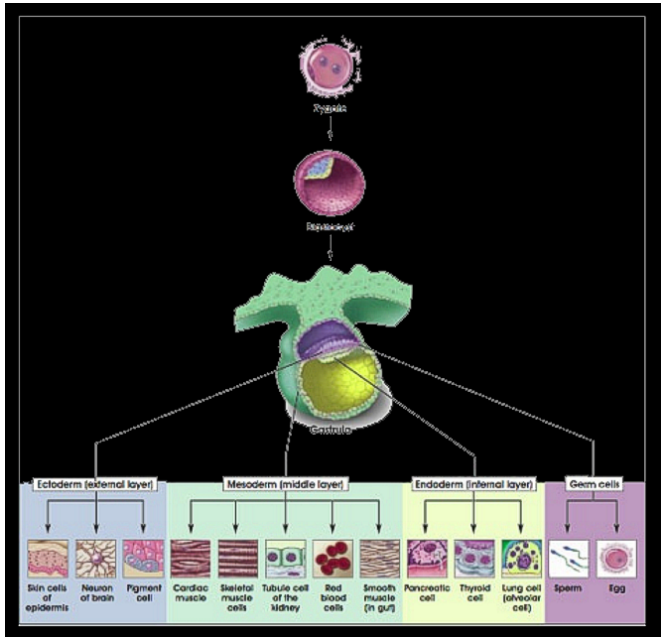
Toxicology studies
drug development

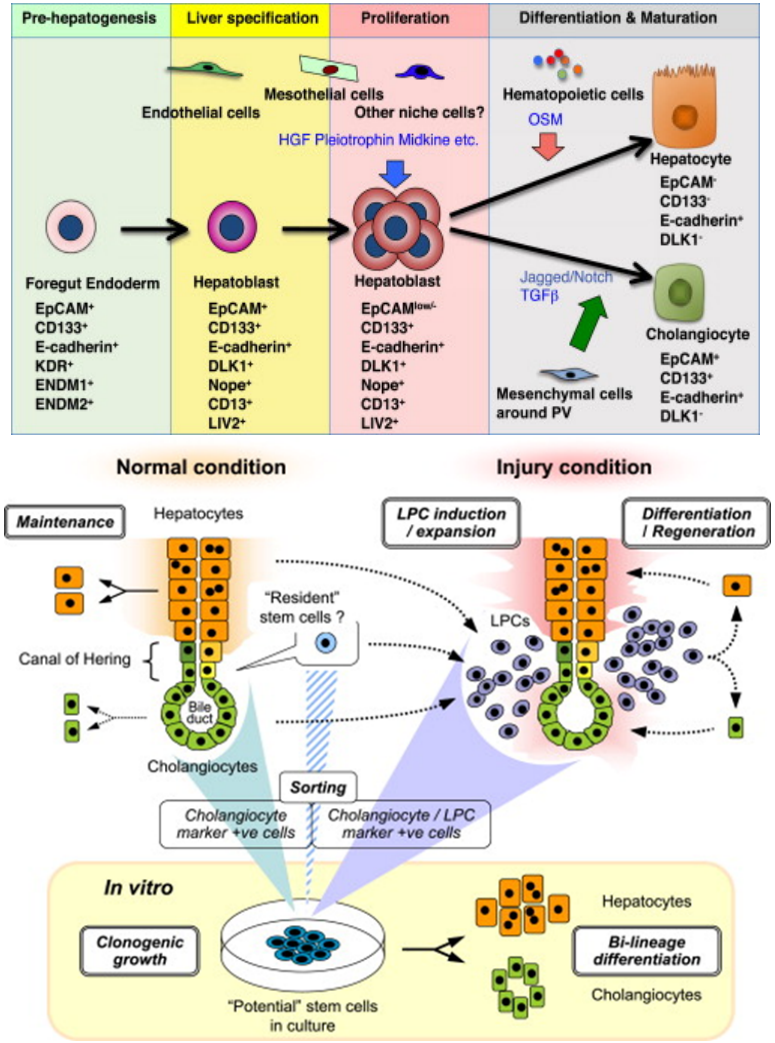


Maturation

Bioartificial Liver
Assisted Device

Stochastic cell fate concept





In response to liver injury :
Hepatocytes and cholangiocytes will expand and repair themselves

In more severe injury: Progenitor Cells will proliferate and differentiate into hepatocyte

Miyajima Cell Stem Cell, 2014

Scope

- The putative Liver Stem Cell
- Approach to therapy with liver stem cell
- Reviewing the evidence to date
- Challenges with stem cell therapy
- Where are we heading?

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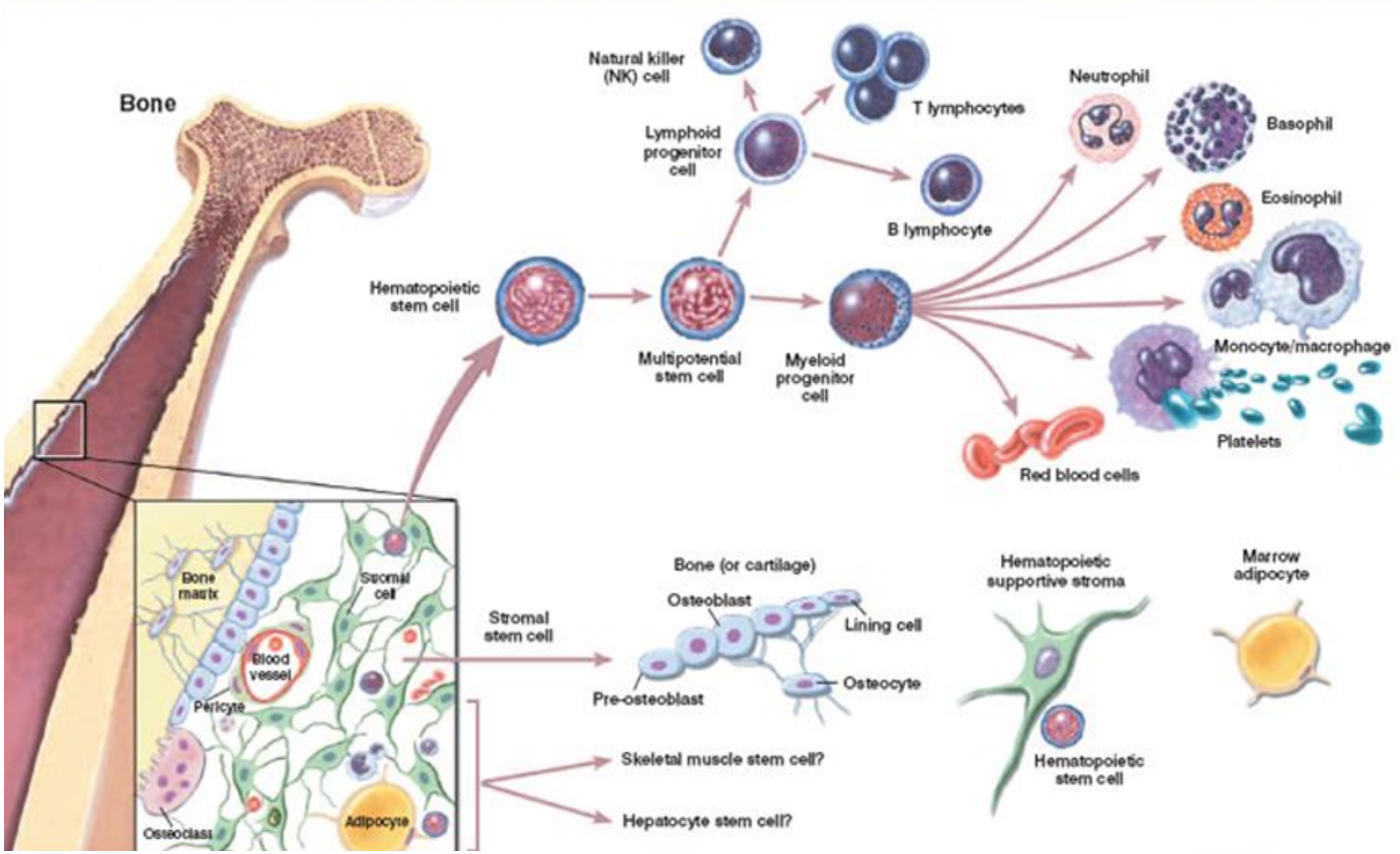
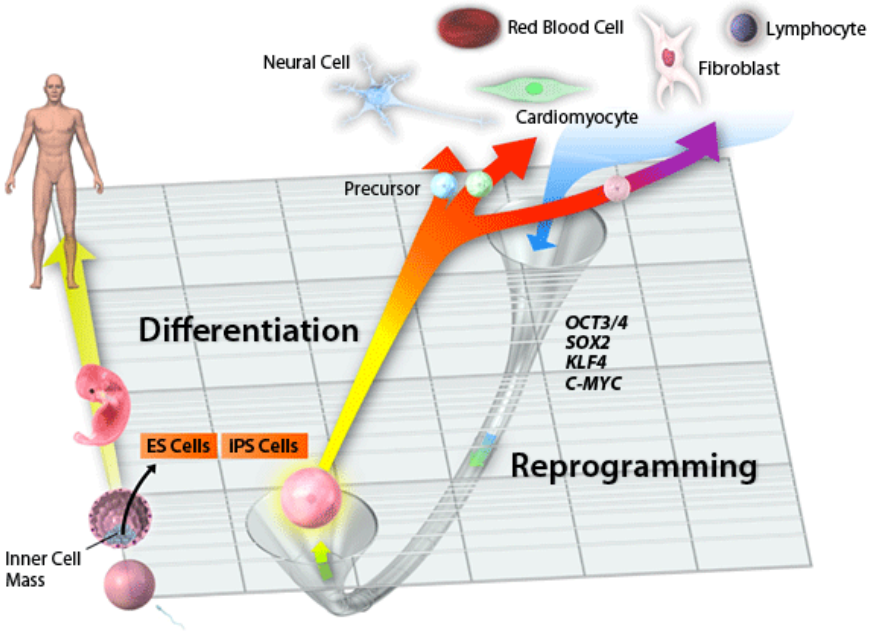
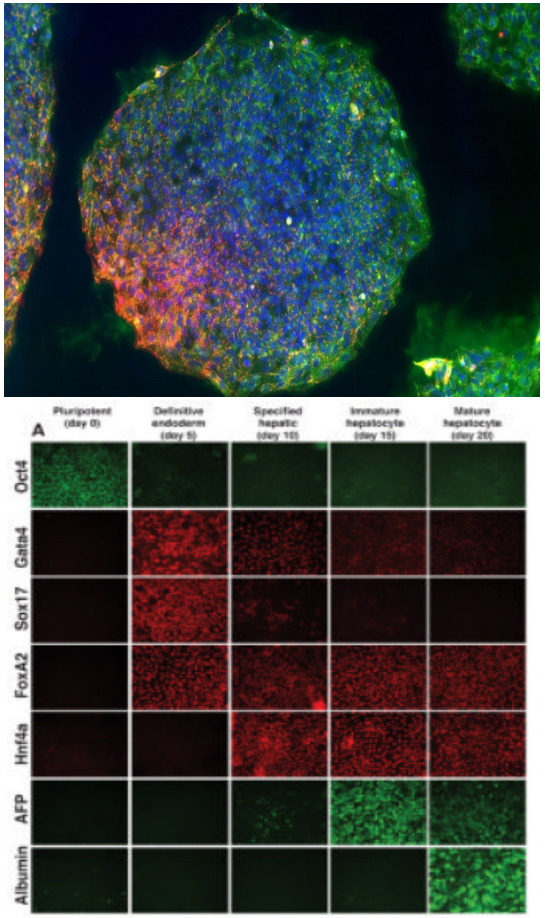
Bone Marrow hematopoietic stem cell does not become hepatocytes



Novel promising stem cell candidates

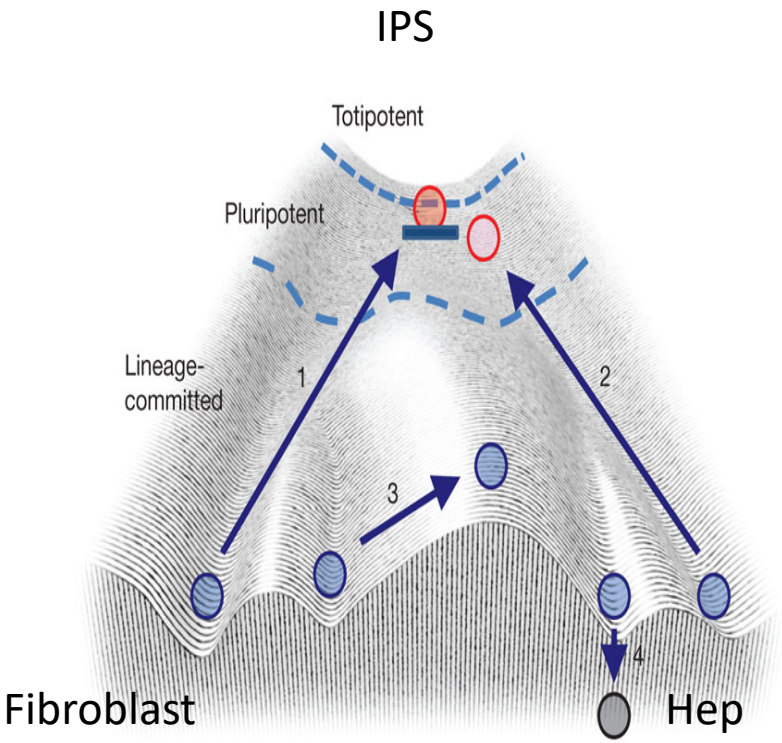
1. Induced Pluripotent Cell

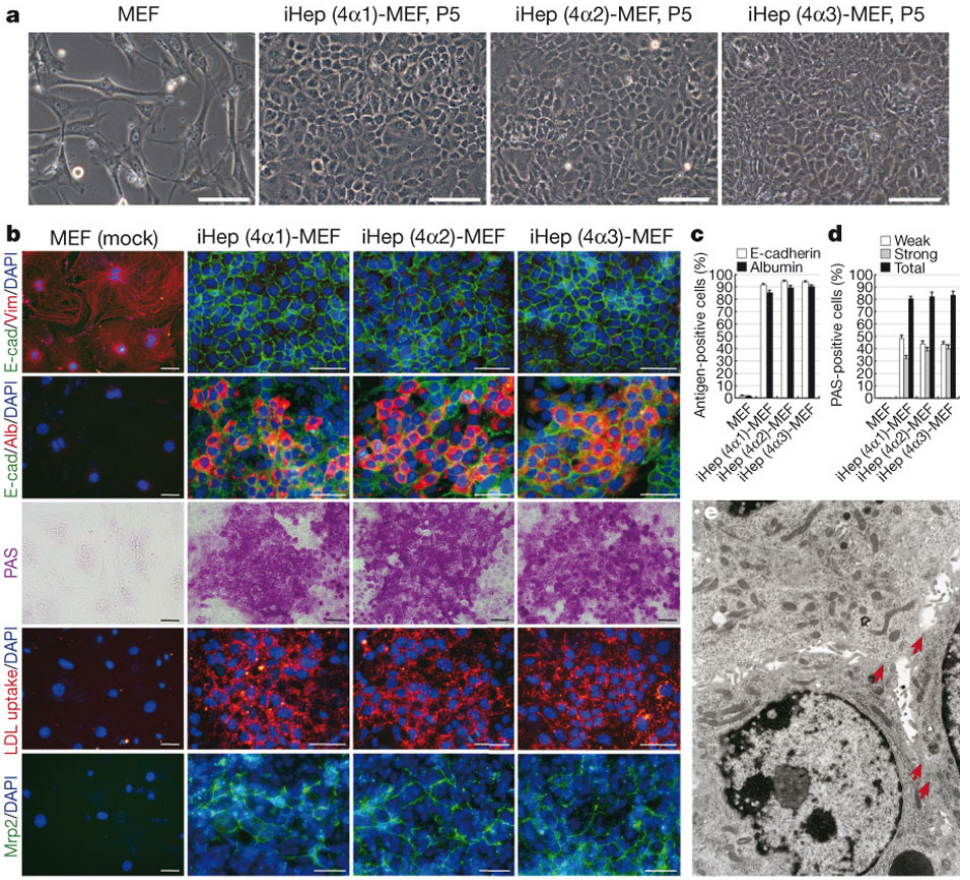




Takahashi K, et al., Cell *2007*
Si-Tayeb Hepatology 2010

Induced Hepatocytes

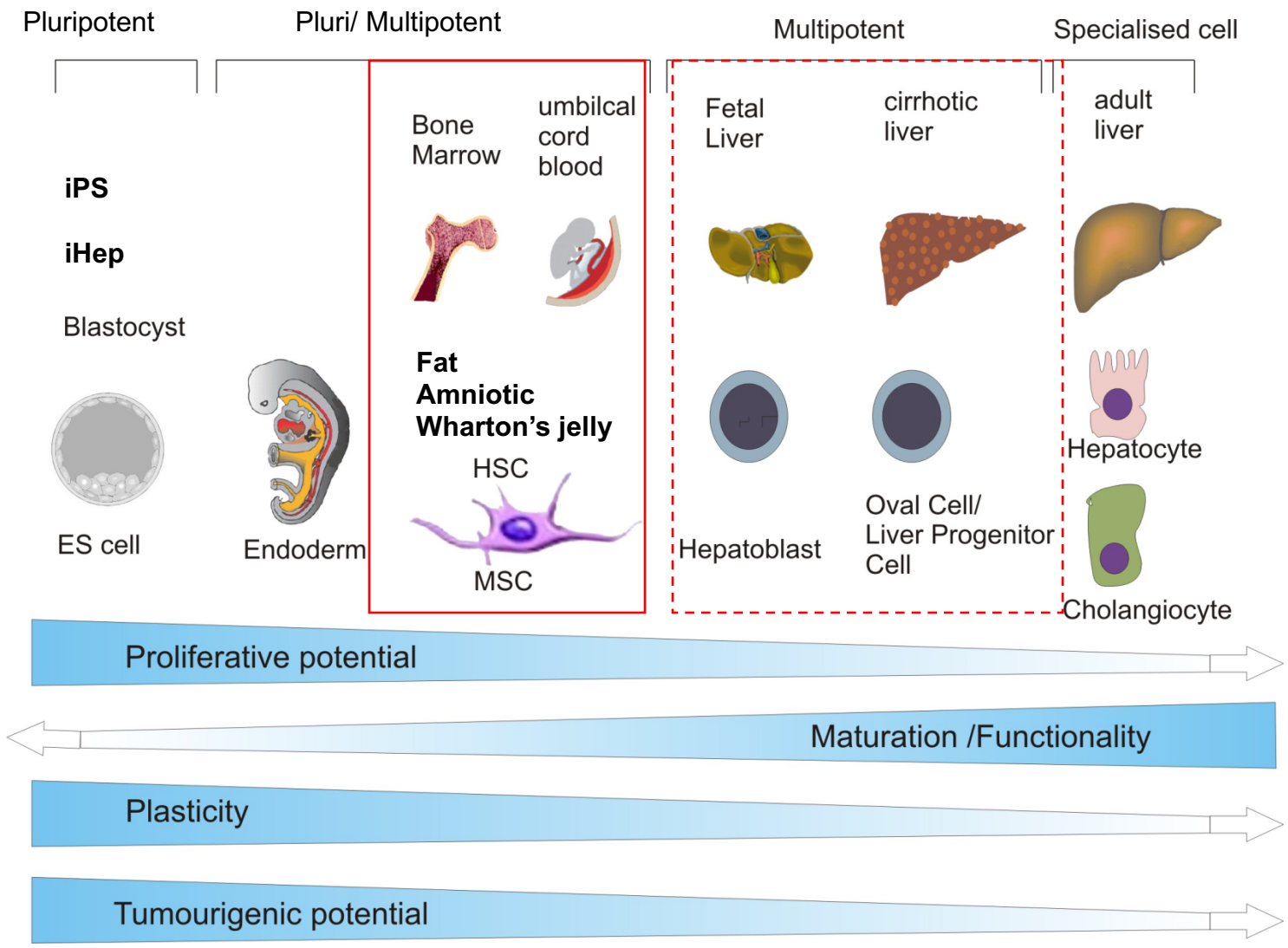




S Sekiya & A Suzuki *Nature* **000**, 1-4 (2011) doi:10.1038/nature10263
PY Huang *et al. Nature* **000**, 1-4 (2011) doi:10.1038/nature10116

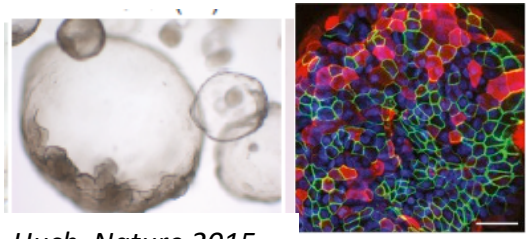
nature

Which is the ideal stem cell candidate?

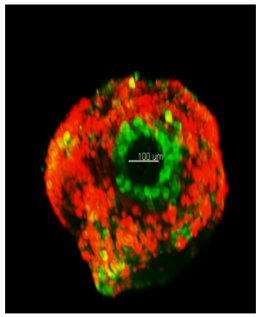


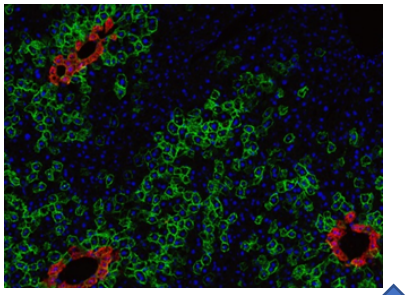
Liver stem cell controversy

"de novo stem cell sox-9+"
Crelox lineage tracing shows that ductular stem cells were actually hepatocytes dedifferentiating into proliferating cells



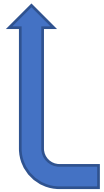
Huch Nature 2015

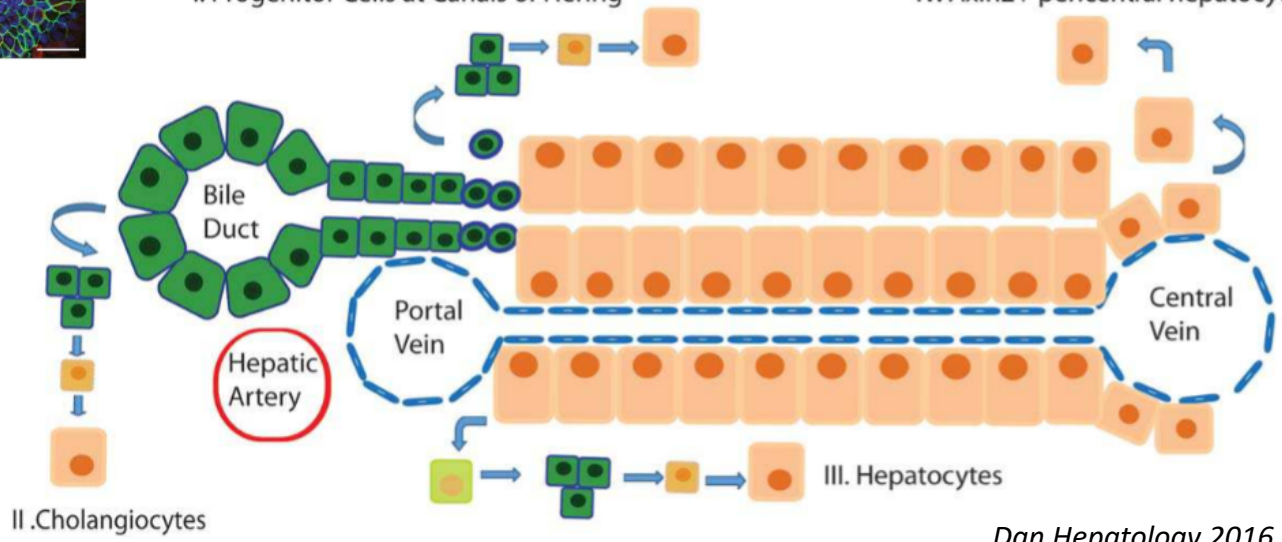




Hepatocyte homeostasis /renewal

Wang Nature 2015





Dan Hepatology 2016

Scope

- The putative Liver Stem Cell
- Approach to therapy with liver stem cell
- Reviewing the evidence to date
- Challenges with stem cell therapy
- Where are we heading?

Approach 1: Human Hepatocytes

- Hepatocytes from discarded liver grafts
  - Limited supply
  - Marginal livers
  - Number of hepatocytes are limited

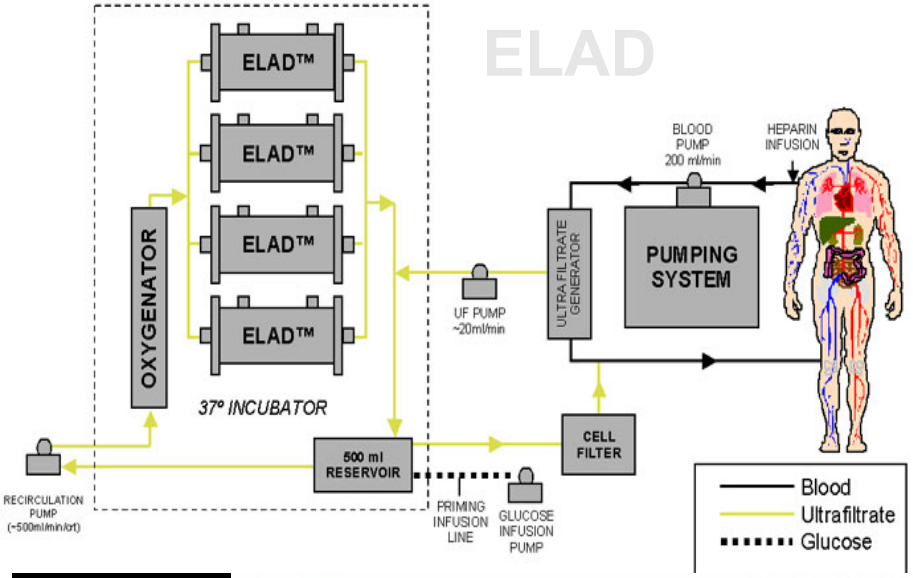
Metabolism Defect- Hepatocyte Transplant

Crigler–Najjar	30–50% Reduction in bilirubin	Fox 98
Familial hyperlipidemia	20% Reduction in LDL cholesterol in 3/5 patients	Grossman 95
Glycogen storage disease	Partial correction	Puppi 2009
Urea cycle defect	No transplant free benefit Decreased ammonia level	Strom 97
Hemophilia	Partial correction but still required FVII	Dhawan 04
Alpha-1 antitrypsin	No benefit	Strom 2009
Infantile Refsum's	Partial correction	Puppi 2009
Progressive familial intrahep cholestasis	No benefit	Puppi 2009

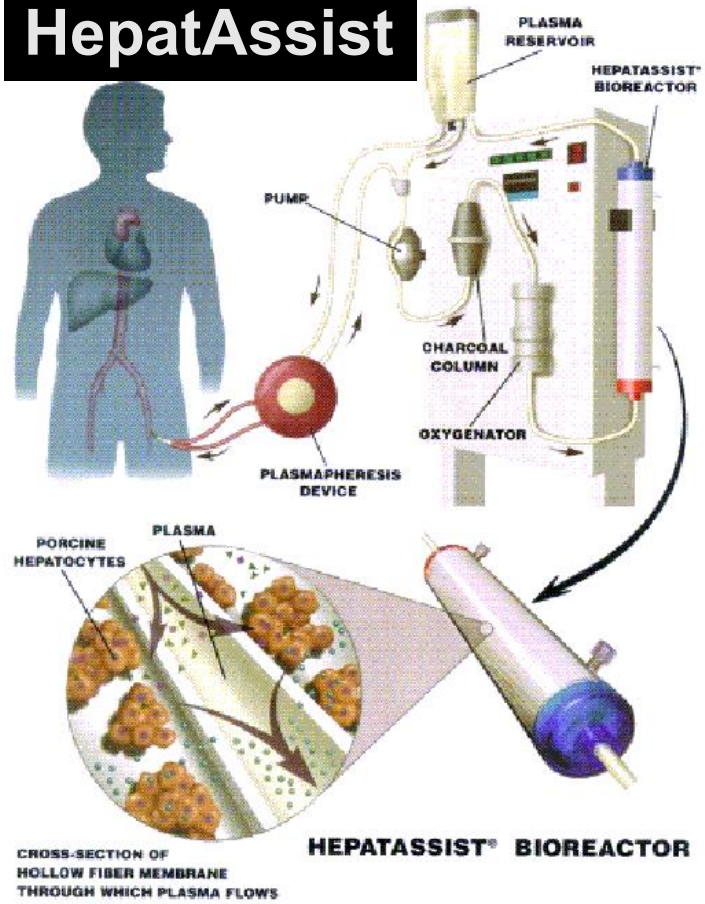
Approach 2: Immortal Cell lines

- Expand in vitro
- Hepatocyte function limited
- Safety concerns with transformation and genetic manipulation
- Use outside the human body to support liver functions

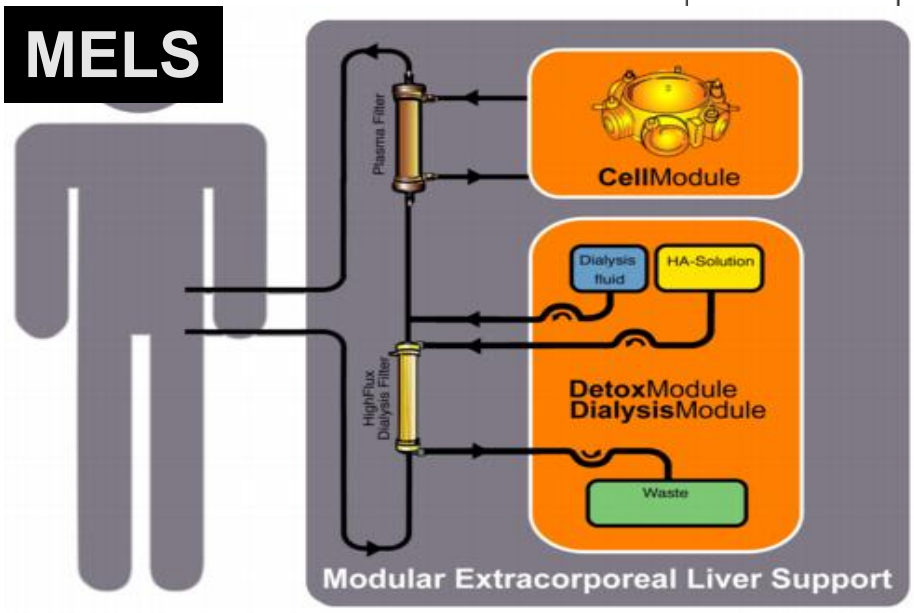
Using Stem Cells in bioreactors



HepatAssist



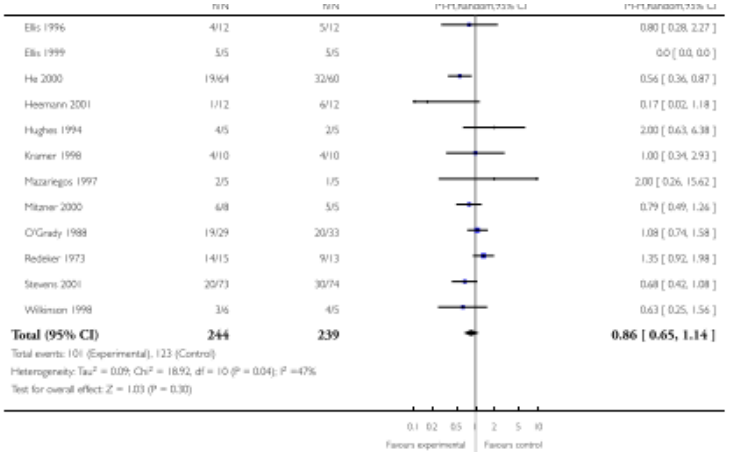
MELS



Various Bioartificial Liver Systems

Issues with Liver Assist Devices

- Lack of survival efficacy
  - 2 Systematic reviews
  - Kjaergard LL. JAMA 2003 pts)
  - Liu et al Cochrane review (2004)

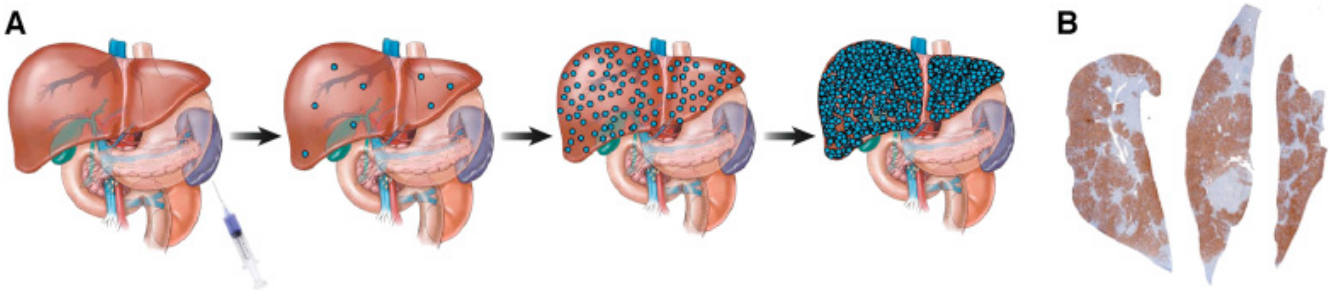


  - (12 RCT- 483
  - No survival benefit between liver support systems and standard treatment of care (RR, 0.86; 95% CI, 0.65-1.12).
  - However Meta-regression using stratified meta-analyses, showed mortality reduction by 33% in acute-on-chronic liver failure (RR, 0.67; 95% CI, 0.51-0.90)
  - *caveat: definition of acute on chronic liver failure

Proof of Principle?- Cirrhosis

Terai et al	NR cohort	autologous bone marrow cell infusion	Improvement in CPS	Stem Cells 2006
Mohamad nejad	Phase I	autologous bone marrow-hematopoietic stem cell	Safety	WJG 2007
Khan	N=4	autologous bone marrow stem cell CD34+	improve	Transplant proc 2008
Pai	N=9 Alcoholic cirrhosis	autologous bone marrow stem cell CD34+	improved	Am J Gastro 2008
Kharaziha	N=8 cohort	autologous mesenchymal stem cell	improved	*Eur J Gastro Hep 2009*
Salama	N=48 Retrosp	Autologous CD34+	improved	Cell Transplant 2010
Am esch	HCC open label	CD133+ cells to augment segmental embolization	Improved regen	Stem Cells 2005

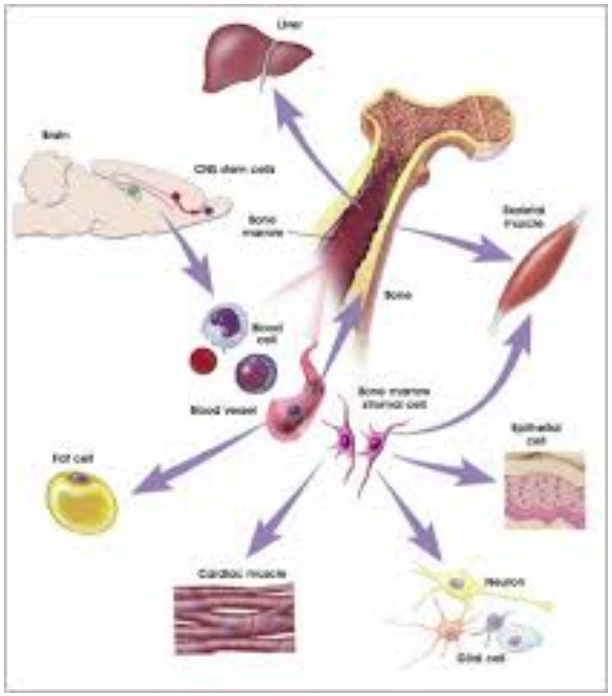
Approach 3: Farming human Hepatocytes

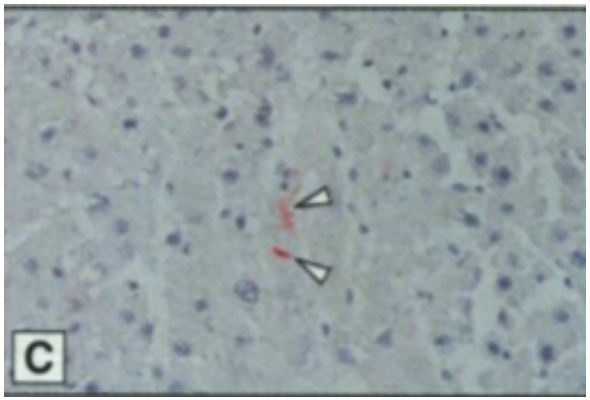


Using FAH -/- mice selection pressure, it is possible to expand human hepatocyte engraftment up to 90% of the mice liver.

Replicating this in humanized large animal models will all invivo farming of human hepatocytes

Approach 4: Other Stem Cell Source





- The bone marrow HSC does not contribute significantly to regeneration of hepatocytes in most of liver regeneration to injury
- Bone marrow mesenchymal stem cell and possibly endothelial stem cell may help support liver regeneration
- Peripheral Blood Stem Cell after gCSF
  (CD34, CD134)
- Umbilical Cord stem cell : HSC and MSC
- Adipose tissue MSC

NIH Stem Cells Primer 2000

Petersen., Science *1999*

How are bone marrow stem cells contributing to liver regeneration?

Attenuation of inflammation	Fibrosis Reversal	Improvement in liver function	Survival Benefit

Dan JGH 2009

?? Non-parenchymal fraction

?? Growth factors

?? Exosomes

?? Anti-inflammatory antifibrotic effect

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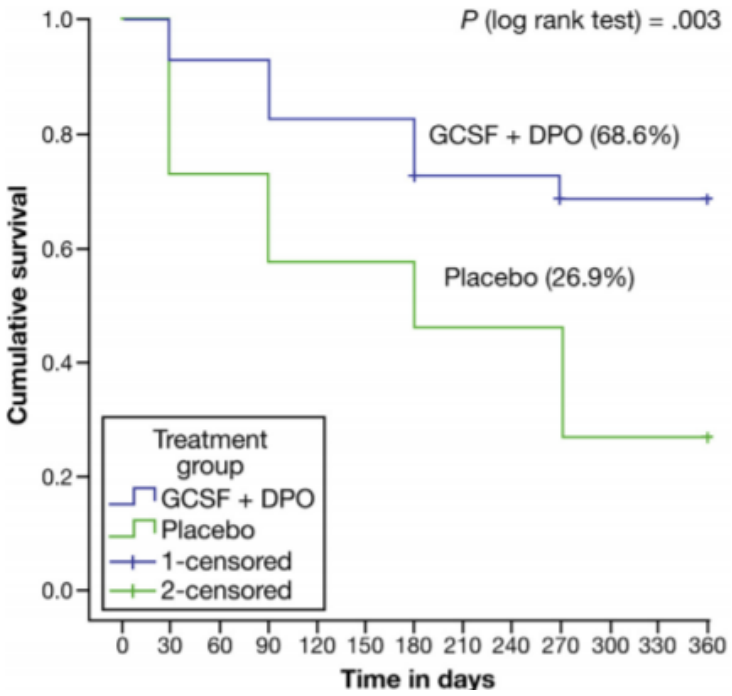
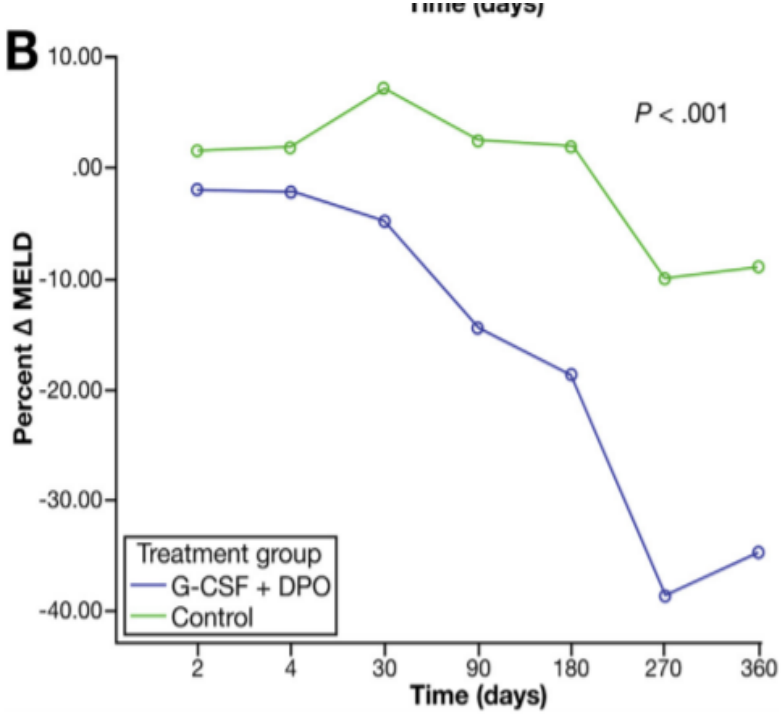
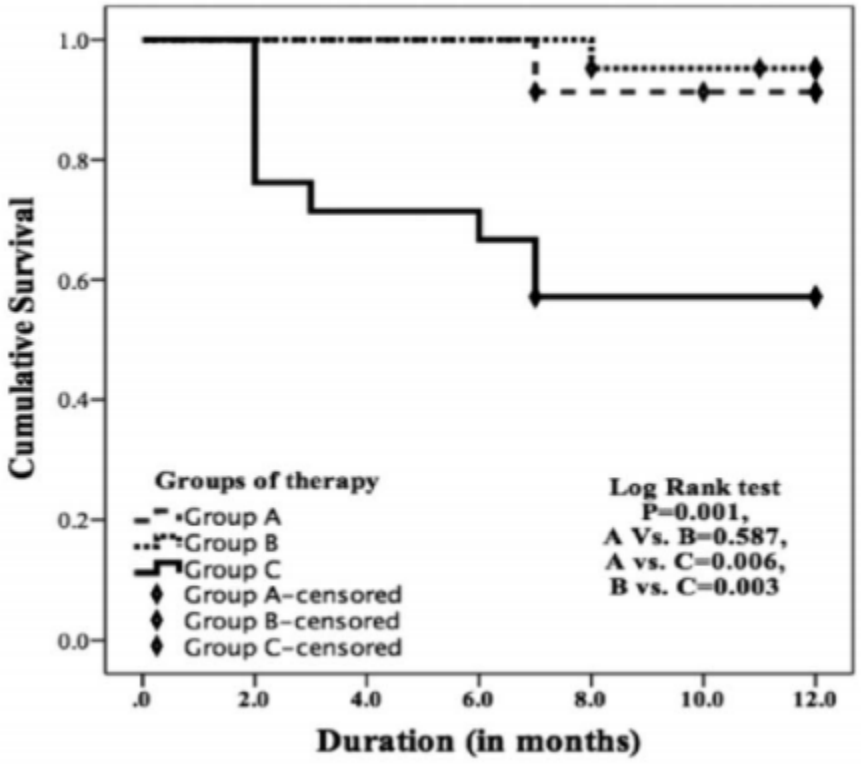
Combination of Granulocyte Colony-Stimulating Factor and Erythropoietin Improves Outcomes of Patients With Decompensated Cirrhosis

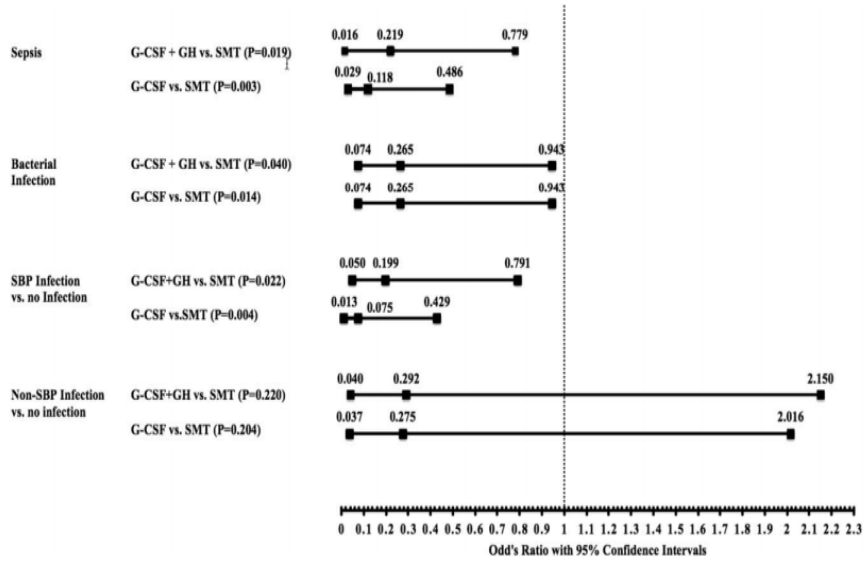
N=55 : subcutaneous G-CSF (5 mg/kg/d) for 5 days and then every third day (12 total doses)
+ subcutaneous darbopoietin a(40 mcg/wk) for 4 weeks follow up 12 months





Outcomes After Multiple Courses of Granulocyte Colony-Stimulating Factor and Growth Hormone in Decompensated Cirrhosis: A Randomized Trial





Multiple courses of G-CSF improved 12-month TFS, mobilized hematopoietic stem cells, improved disease severity scores, nutrition, fibrosis, QOL scores, ascites control, reduced infections, and the need for LT in patients with DC.

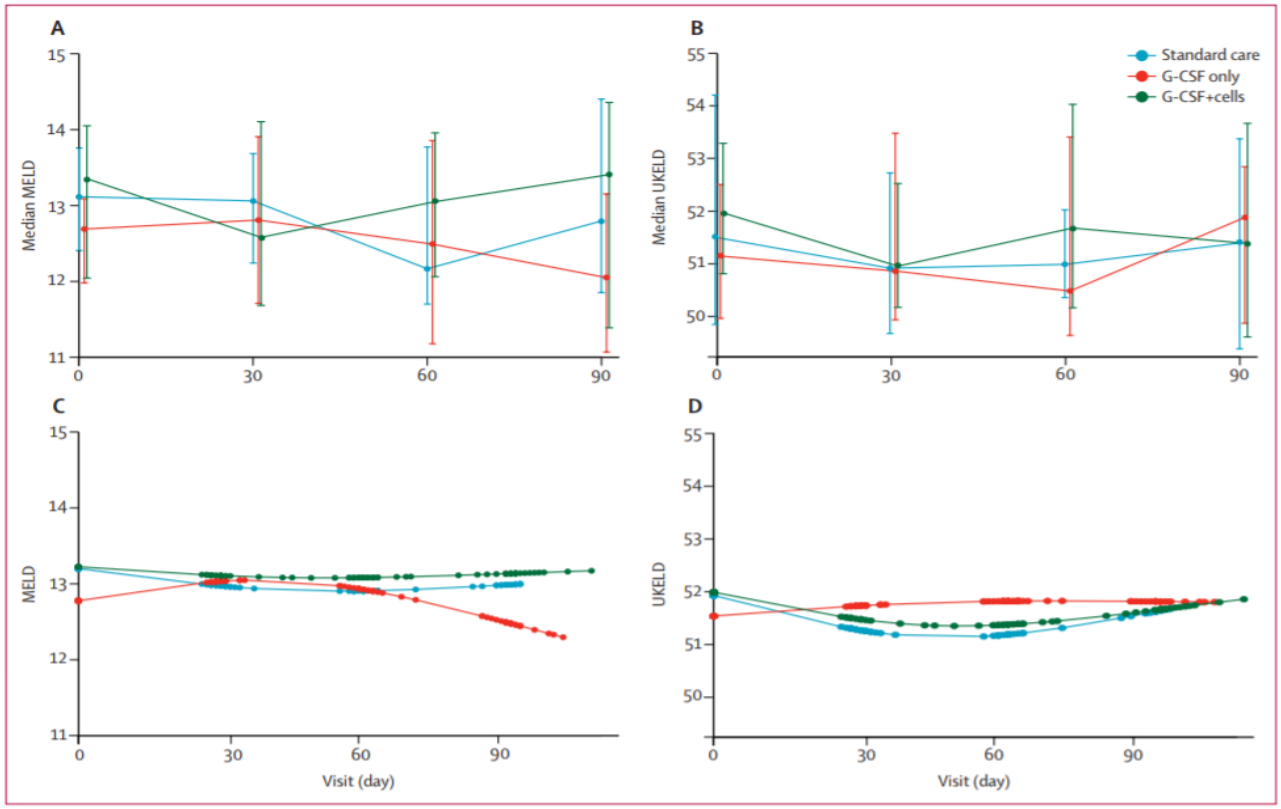
Verma Hepatology 2017

RCT in clinical cell transplant – 17 trials to date

Mohamadnejad Liver Int 2013	RCT n=27 Decomp cirrhosis Intravenous	Auto BM-MSC	No difference in MELD and liver volume	
Salama Stem Cell Res Therapy 2012	RCT. N=40 HCV decom Intravenous	Auto BM-MSC	Improvement in CPS	
El-Ansary Stem Cell Reviews	Ph II n=25 HCV Deomp Intravenous	BM-MSC vs MSC diff hepatocytes vs controls	Improvement in MELD 3/12 and 6/12	
Amer EJGH 2011	RCT n=40 HCV Intrasplenic vs intrahepatic	Auto BM-MSC hepatic lineage	Improvement in CPS and MELD	
Mohamadnejad Stem Cells Transl Medicine 2016	RCT n=27 Decomp cirrhosis Intraportal	PB: CD133+ vs BM MNC vs Control	Transient improvement in CD133+. No significant survival	
Spahr PLOS One 2013	RCT n=58 Alcoholic cirrhosis MELD 19	PB-MNC	No difference in MELD score at 3/12 or HPC proliferation	
Salama World J Gastro 2010	RCT. N=90 Decompensated Intraportal	PB: CD34+, CD133+ vs controls	54% improvemenrt vs 0% improvement at 3/12	

Granulocyte colony-stimulating factor and autologous CD133-positive stem-cell therapy in liver cirrhosis (REALISTIC): an open-label, randomised, controlled phase 2 trial

Philip Noel Newsome, Richard Fox, Andrew L King, Darren Barton, Nwe-Ni Than, Joanna Moore, Christopher Corbett, Sarah Townsend, James Thomas, Kathy Guo, Diana Hull, Heather A Beard, Jacqui Thompson, Anne Atkinson, Carol Bienek, Neil McGowan, Neil Guha, John Campbell, Dan Hollyman, Deborah Stocken, Christina Yap, Stuart John Forbes



Lancet Gastroenterol Hepatol 2017

G-CSF with or without haemopoietic stem-cell infusion did not improve liver dysfunction or fibrosis and might be associated with increased frequency of adverse events compared with standard care

RCT in clinical cell transplant – 17 trials to date

Peng Hepatology 2011	Matched n=158 Hep B cirrhosis Hepatic Artery	Auto BM-MSC	Improvement in MELD score	
Suk Hepatology 2016	RCT n=34 Alcoholic cirrhosis Hepatic Artery	Auto BM-MSC	Improvement in CTP and fibrpsis	
Xu JGH 2014	RCT n=39 Hep B Hepatic Artery	Auto BM-MSC	Improvement in MELD	
Fang JGH 2018	RCT n=83 Hep B cirrhosis Intravenous	UC -MSC	Improvement in MELD and survival	
Lin Hepatology 2017	RCT n=110 AoCLF Intravenous	Auto BM-MSC	Improvement in MELD up to 2 weeks	
Tang WJG WJG 2003	RCT n=153 AoCLF Intravenous	UC blood +/- Plasma exchange	Improvement in function	
Li Stem Cells Review 2016	Consecutive 44 HBV AoCLF Hepatic Artery	UC -MSC	Improvement in MELD 4/52 and function at 24/62 and survival	

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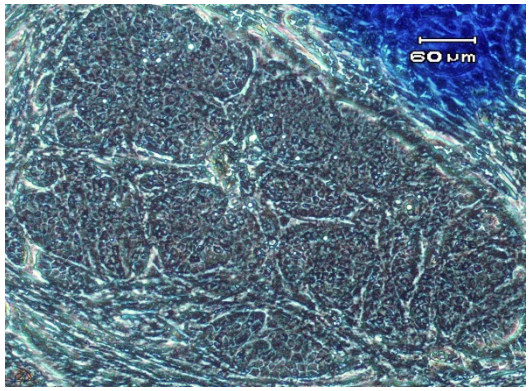
The Challenge in Translation to cell transplantation

1. Do we have a good cell candidate with meaningful hepatocyte production
    1. Functional hepatic differentiation
    2. Sufficient numbers
    3. Clinically safe – free from genetic manipulaton

The best technology today can only produce fetal hepatocyte
Limitless supply not demonstrated yet
Genetic and epigenetic alterations in IPS and iHEP cells

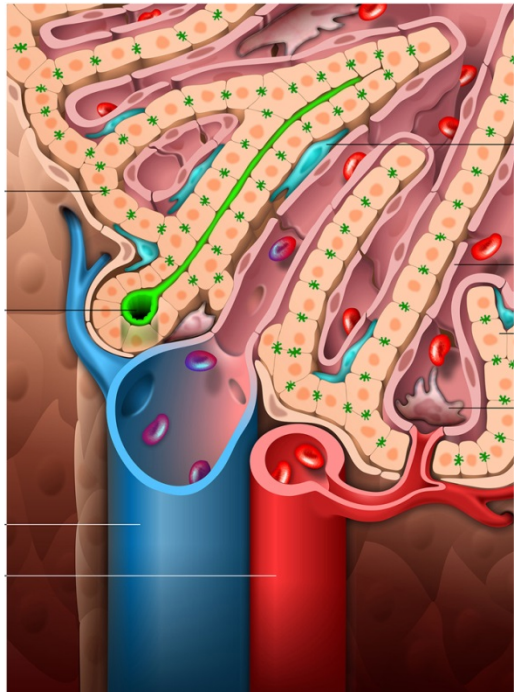
Can we make enough cells?

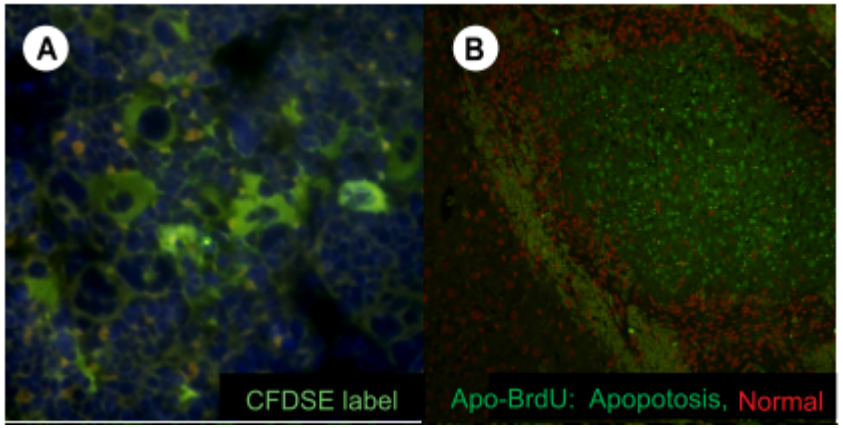
- A liver has 240 billion cells
- 60% hepatocytes and 40% non parenchymal
- Replacing 5% of hepatocyte => 6 billion cells



Delivery of Cells?

- Intraarterial
- Intraportal
- Intravenous
- Intrahepatic





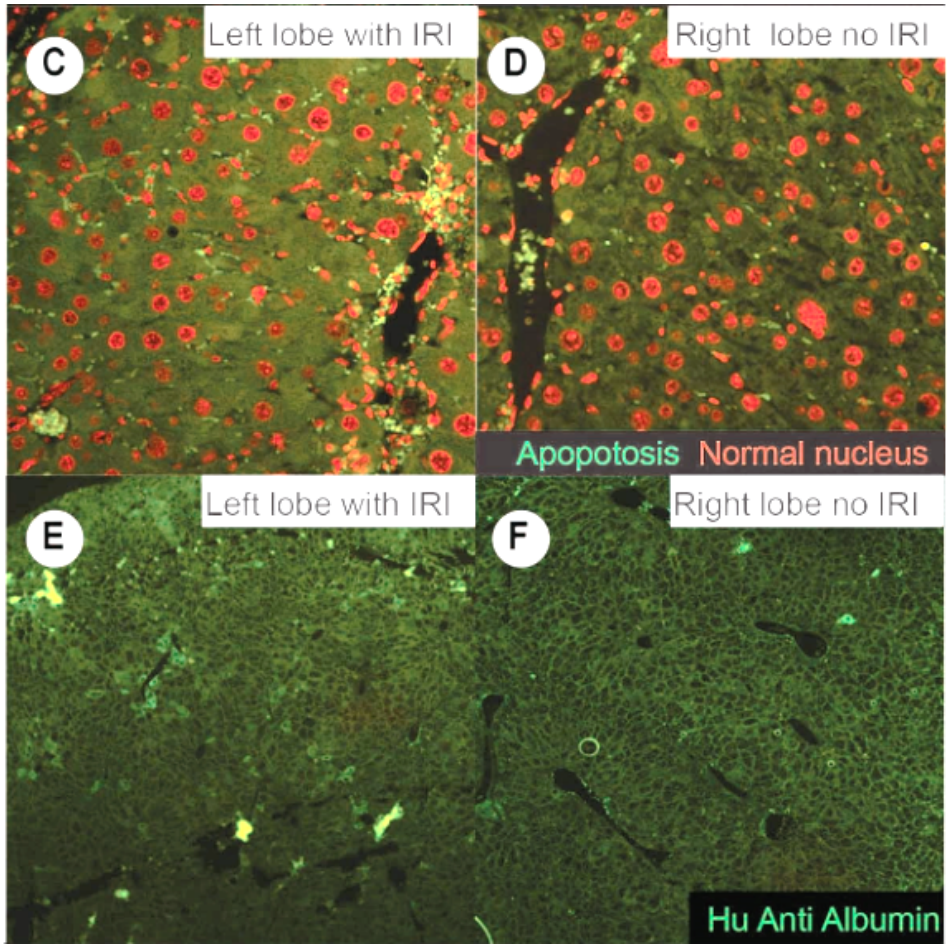
Engraftment efficiency is still low

Excessive cells will result in thrombosis, ischemia and worsening of liver function

Arterial thromobosis has been documented as adverse event with disastrous outcomes

We need to **open the door** and **create space** for stem cells to enter the hepatic sheets -
Opening the sinusoidal endothelium to improve engraftment

1. SEL injury
2. Conditioning
3. Radiation.



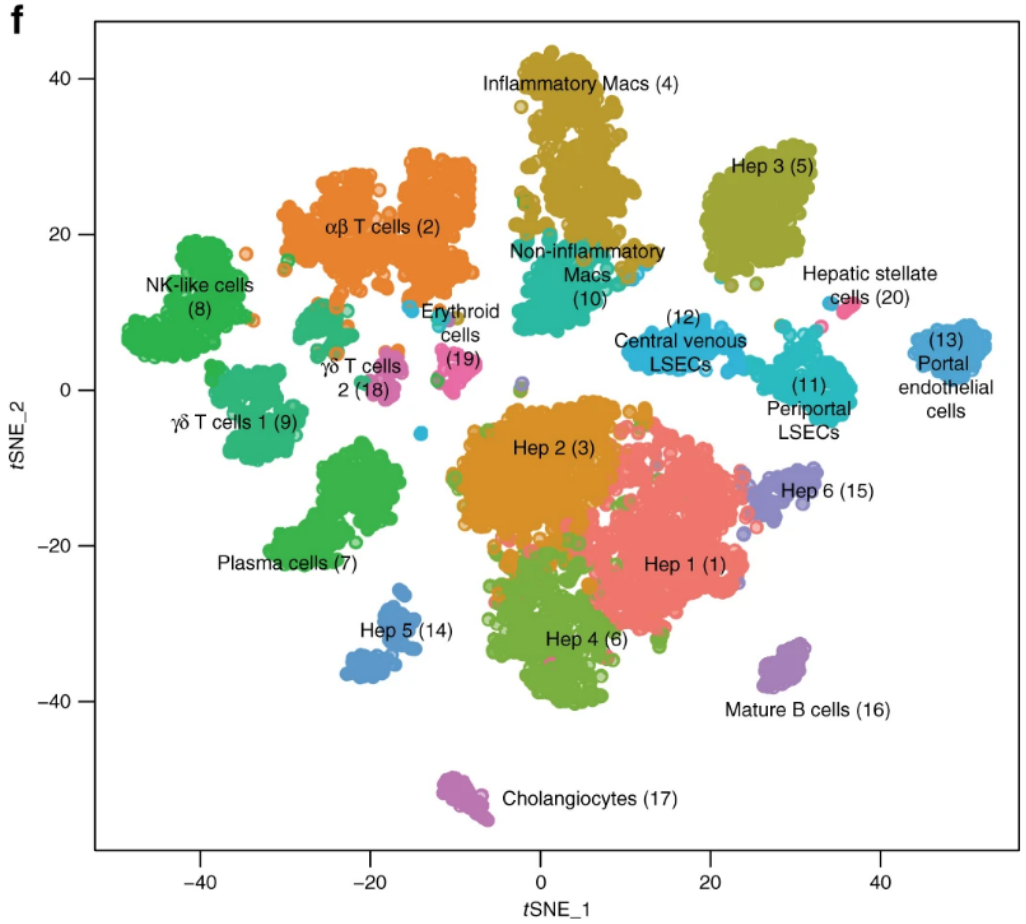
Antony,Mark Stem Cells 2017

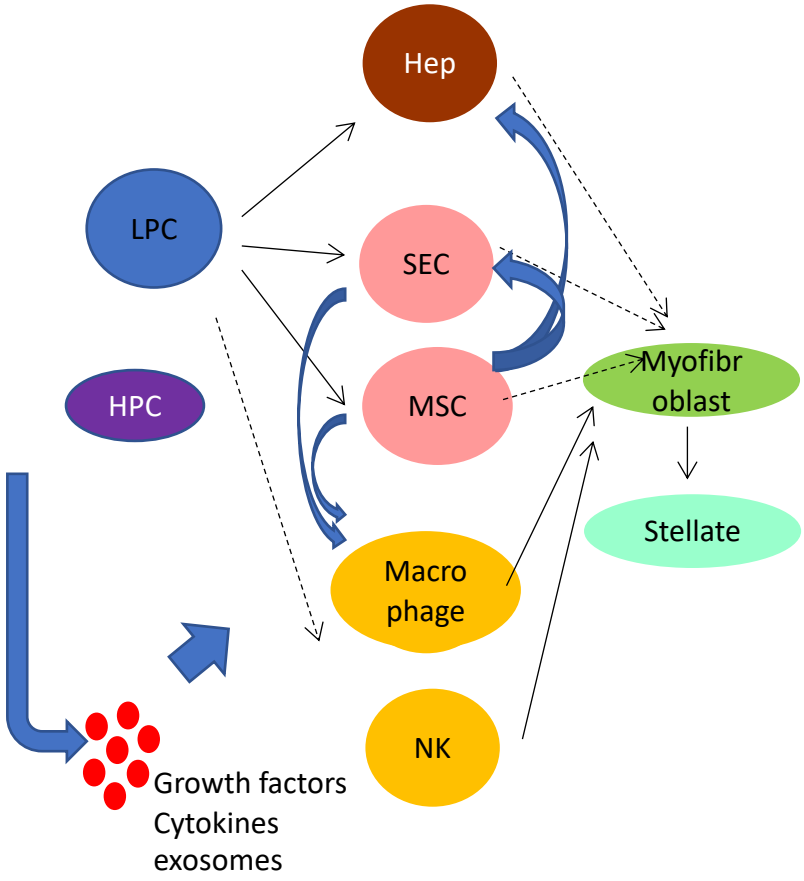
Adverse Events

- SAE were more frequent the in G-CSF and stem-cell infusion group (43% vs 11% in the G-CSF vs12% in controls.
- Worsening of MELD - Ascites, sepsis, encephalopathy
- Theoretical risks of HCC

- THE Liver Stem Cell
- Does it work?
- Why is it not working?
- Where are we heading?

Changing concept of Liver

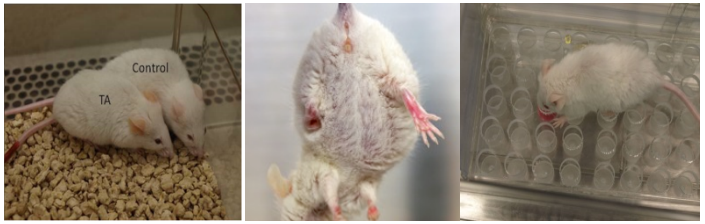


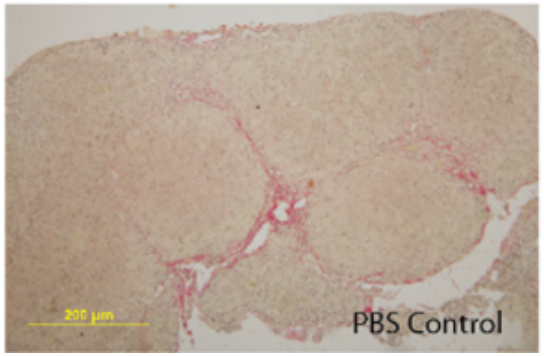


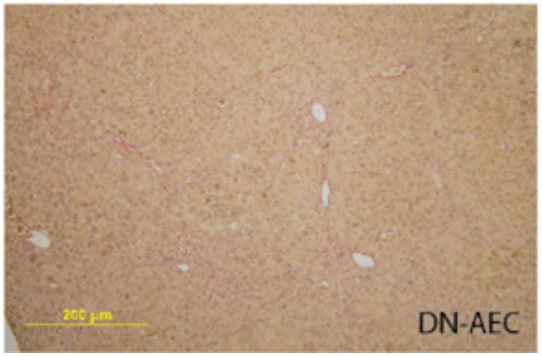
20 discrete cell populations of hepatocytes, endothelial cells, cholangiocytes, hepatic stellate cells, B cells, conventional and non-conventional T cells, NK-like cells, and distinct intrahepatic monocyte/macrophage populations

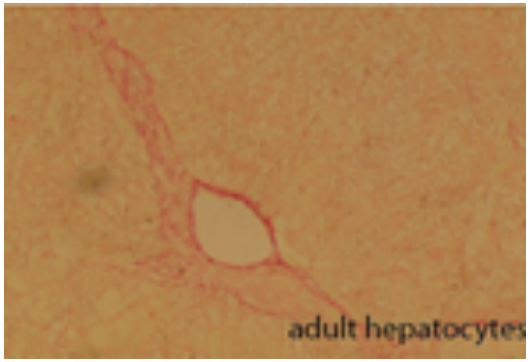
McParland Nat Comms 2018

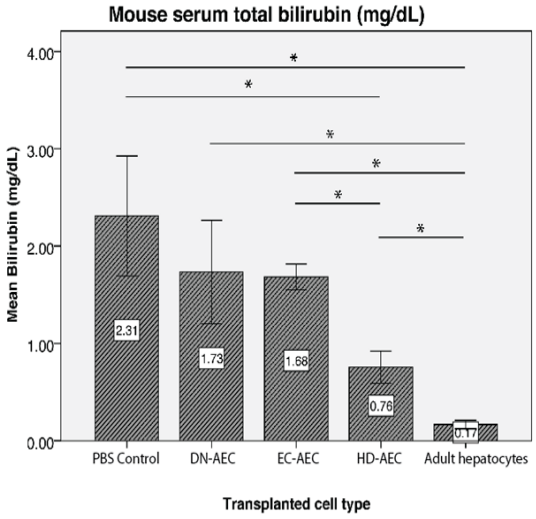
Anti Fibrosis effect

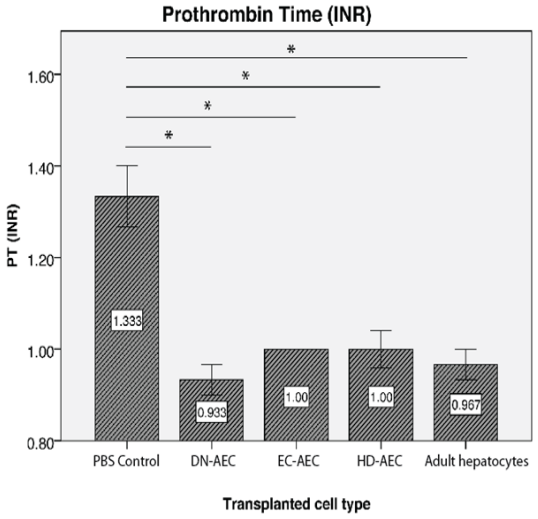


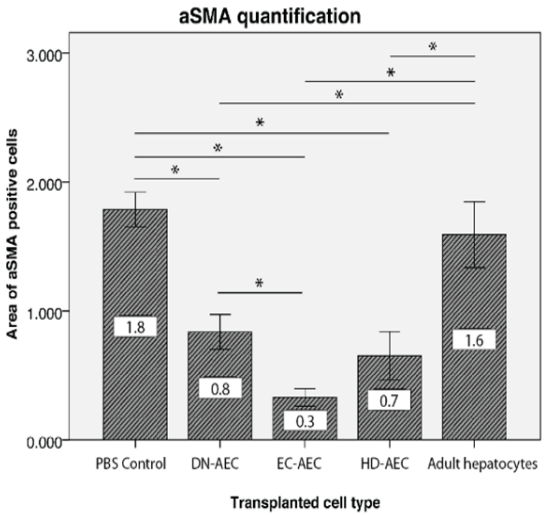








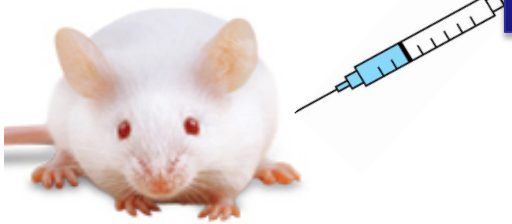




Hepatocytes provide liver function but do not contribute to fibrosis reversal
Non-parenchymal effect reverses fibrosis and improve function

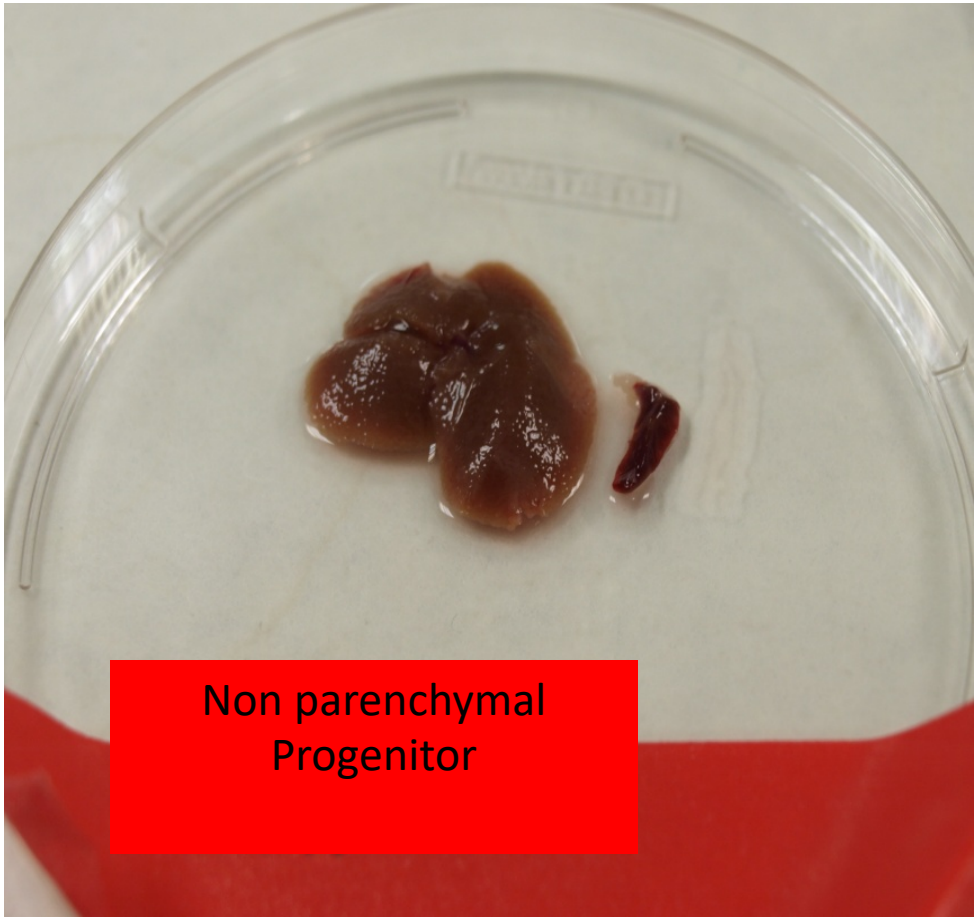
Unpublished Data

In-Vivo



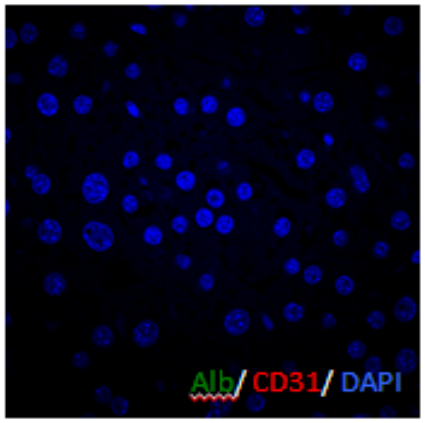
Intra-splenic transplantation

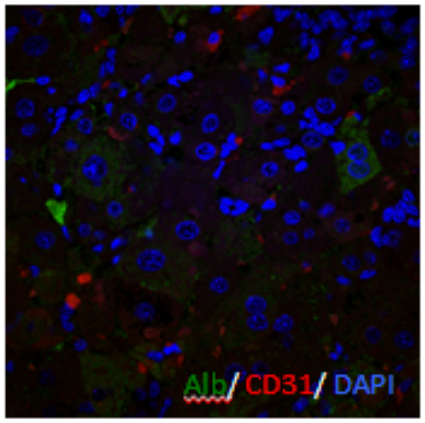
NSG mice with chronic liver failure

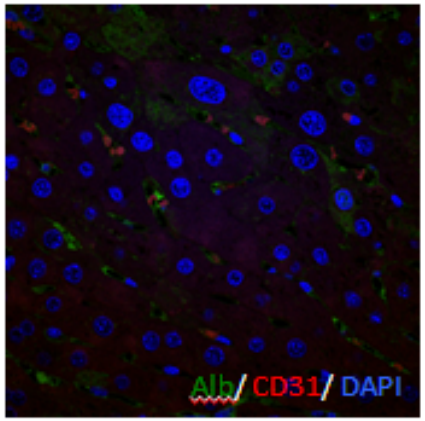


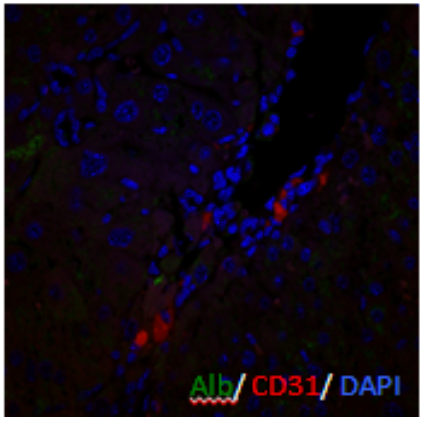
- Endothelial Progenitors?

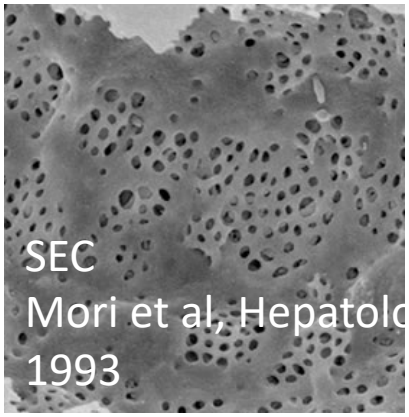
- PBS Fully Dx Partially dx De novo

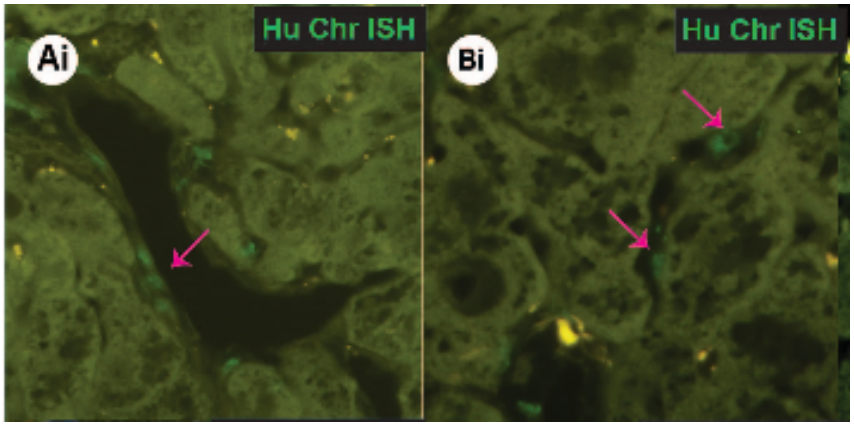


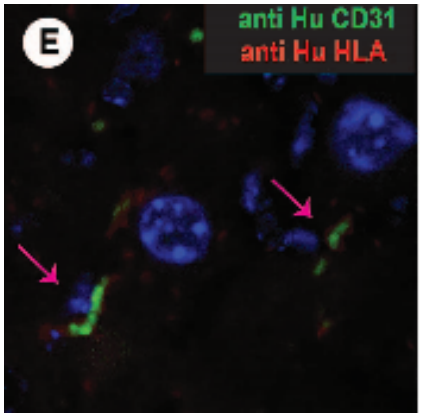








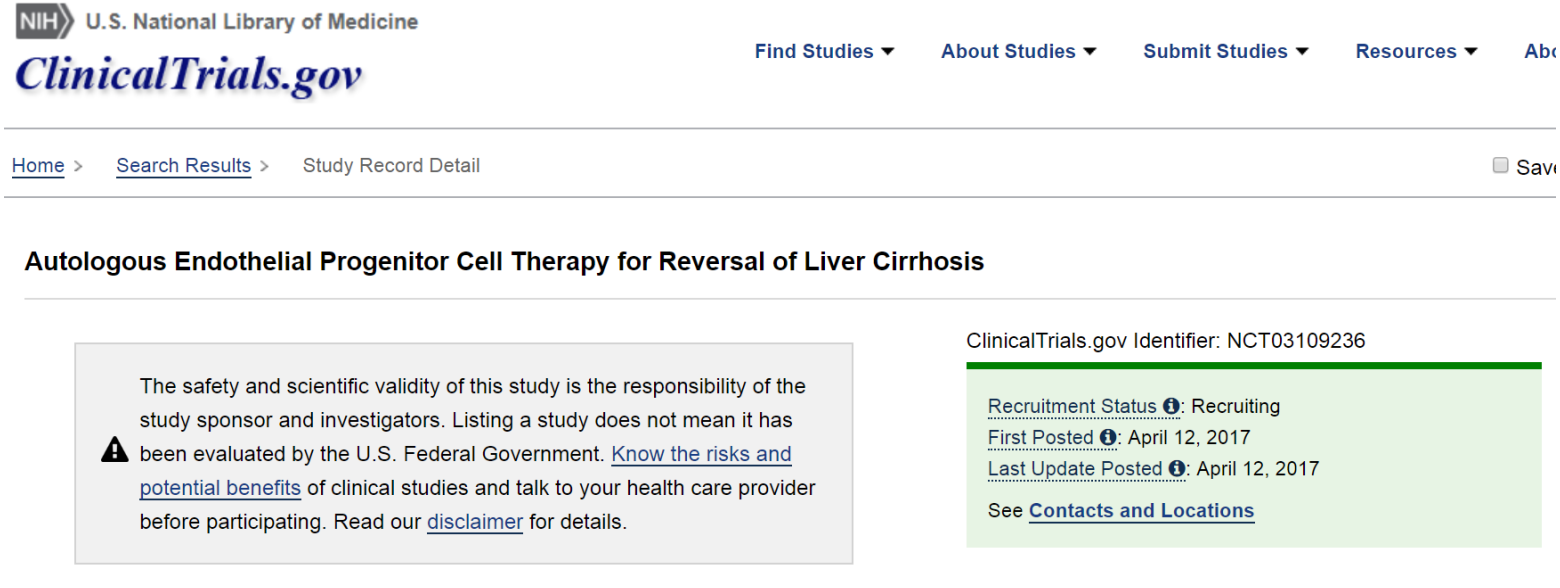




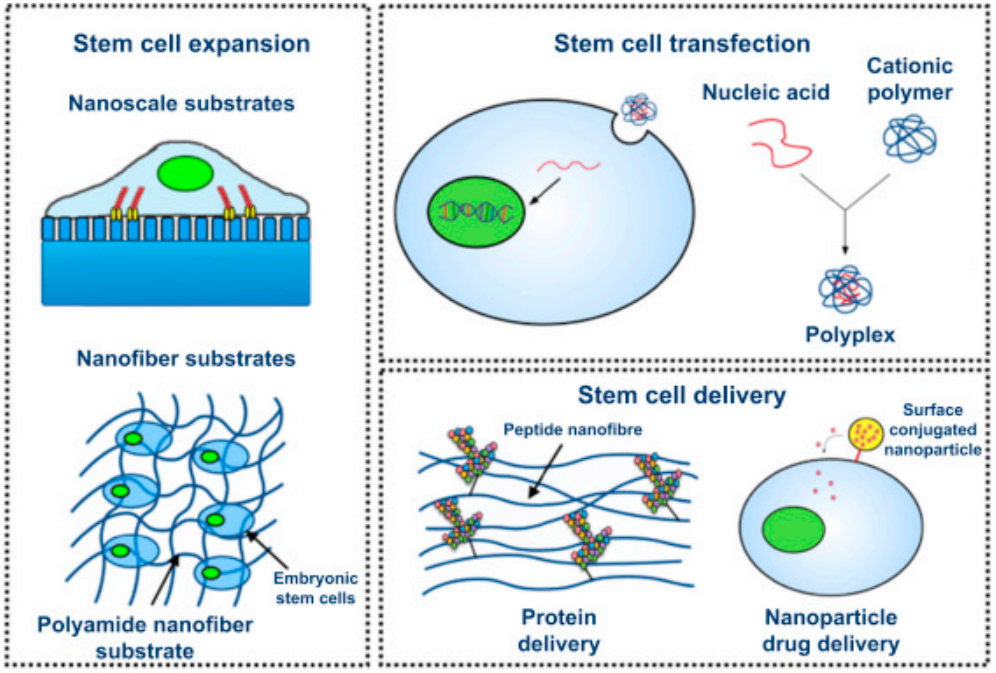
Antony,Mark Stem Cells 2017

BM CD133+ Endothelial Progenitor Cell transplant Study

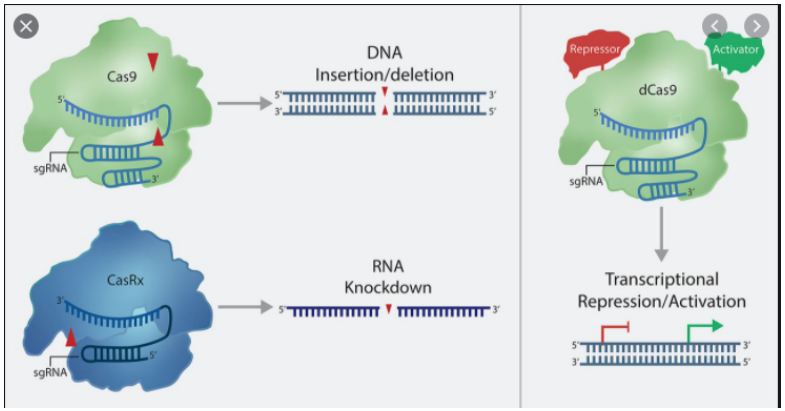
- Autologous transplant
- direct transplant into liver via portal vein
- Repair of Sinusoidal endothelium
- Stabilise or reverse fibrosis
- Improve liver function



New Frontiers in Stem Cells



Nanotechnology to expand and optimise liver stem cells



CRISPR, siRNA, exosomes to influence gene regulation and cell processes of inflammation , fibrosis and repair

Stem Cells as biological army of drones

Summary

- Stem cells provide an exciting potential therapy for patient with liver cirrhosis especially for those who do not qualify for transplant but the success remains elusive

- The bottle neck remains in having anoptimal source of clinically useful cells that can be delivered successfully into the liver.

- Ideal strategy may need to combine efforts to reverse cirrhotic microenviroment and to trigger a pro-regenerative response in the liver to improve outocmes.

- Promising advances in nanotechnology, gene editing and understanding of cell-cell interactions in controlling inflammation and fibrosis allow us to insert stem cell drones to tweak cellular processes for optimal clinical outcomes.


Thank you for your attention
Freiburg Germany 2003