MICROGRAVITY MAINTAINS STEMNESS AND ENHANCES GLYCOLYTIC METABOLISM IN HUMAN HEPATIC AND BILIARY TREE STEM/PROGENITOR CELLS.

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65th International Congress of Aviation and Space Medicine

Rome, 10-14 September 2017

The global burden of chronic liver diseases

Total deaths worldwide from cirrhosis and liver cancer rose by 50 million per year over 2 decades,

according to the first-ever for World Health Organization (WHO) study of liver disease mortality

The main causes of chronic liver diseases

Viral: HCV, HBV, HDV

Alcol:

NAFLD: non alcoholic fatty liver diseases (Metabolic Syndrome) **!**

HBV prevalence in european population !

Figura 3: Prev

Very effective treatment !
(entecavir, tenofovir…)

Fonte: TECHNICAL REPORT Hepatitis B and C in the EU neighbourhood: prevalence, burden of disease and screening policies September 2010

HCV prevalence in european population !

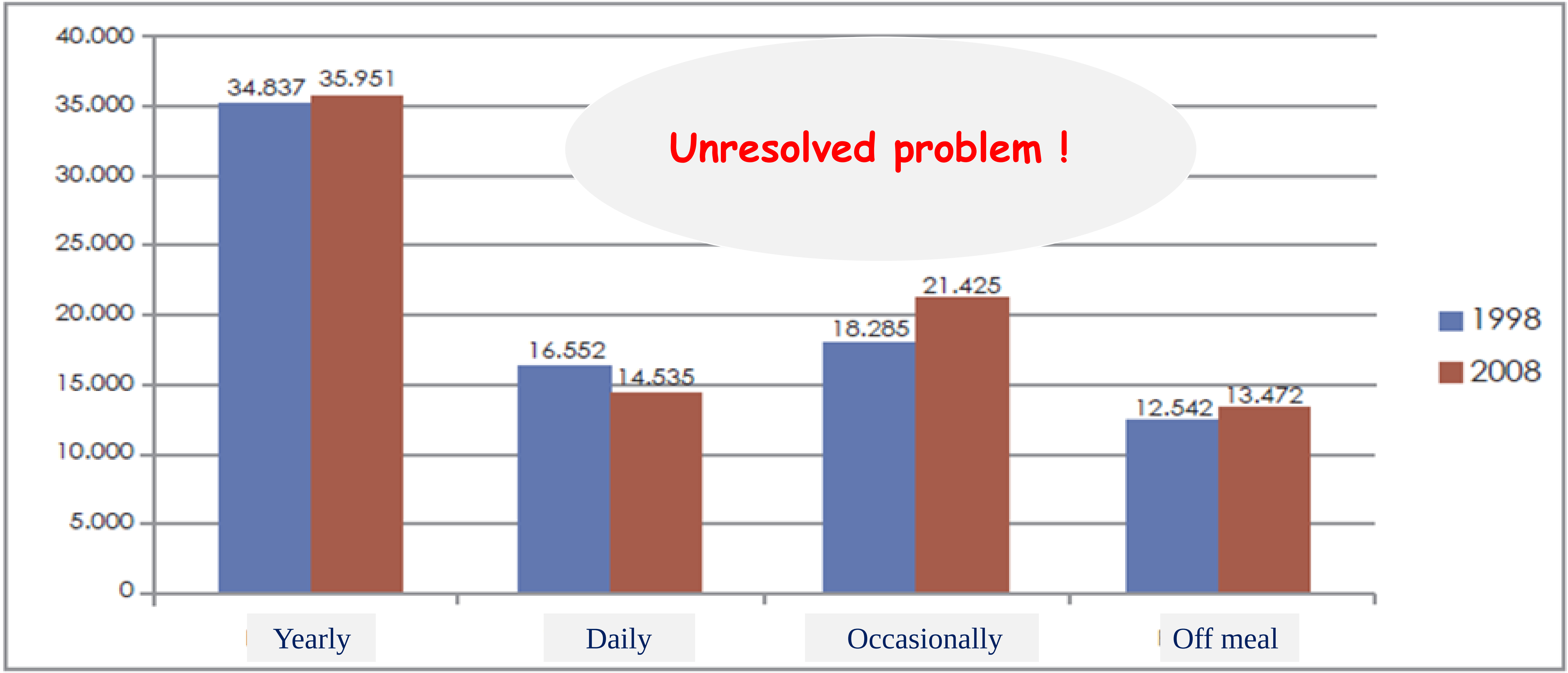
Figura 2.b

Very effective treatment !
(DAA)

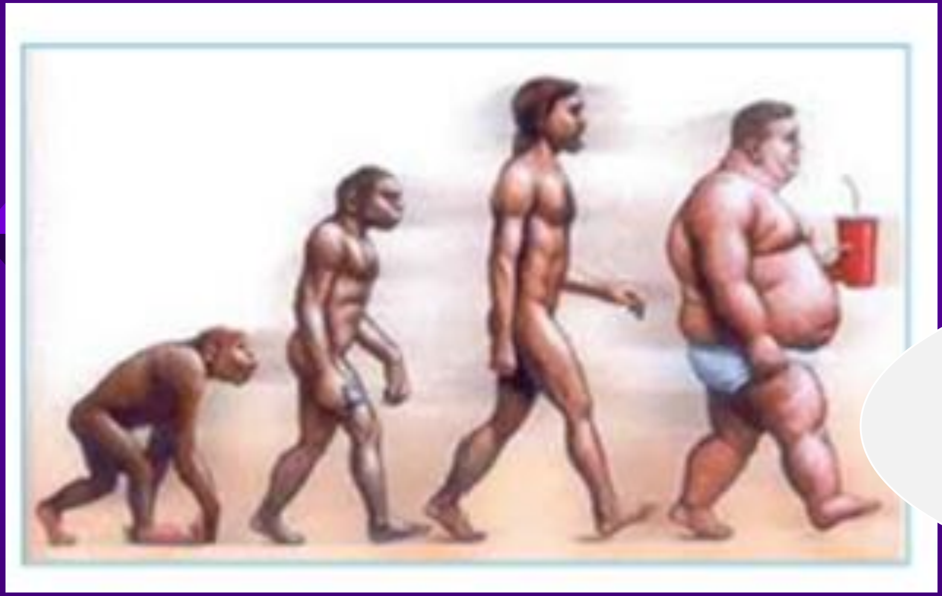
TECHNICAL REPORT Hepatitis B and C in the EU neighbourhood: prevalence, burden of disease and screening policies September 2010

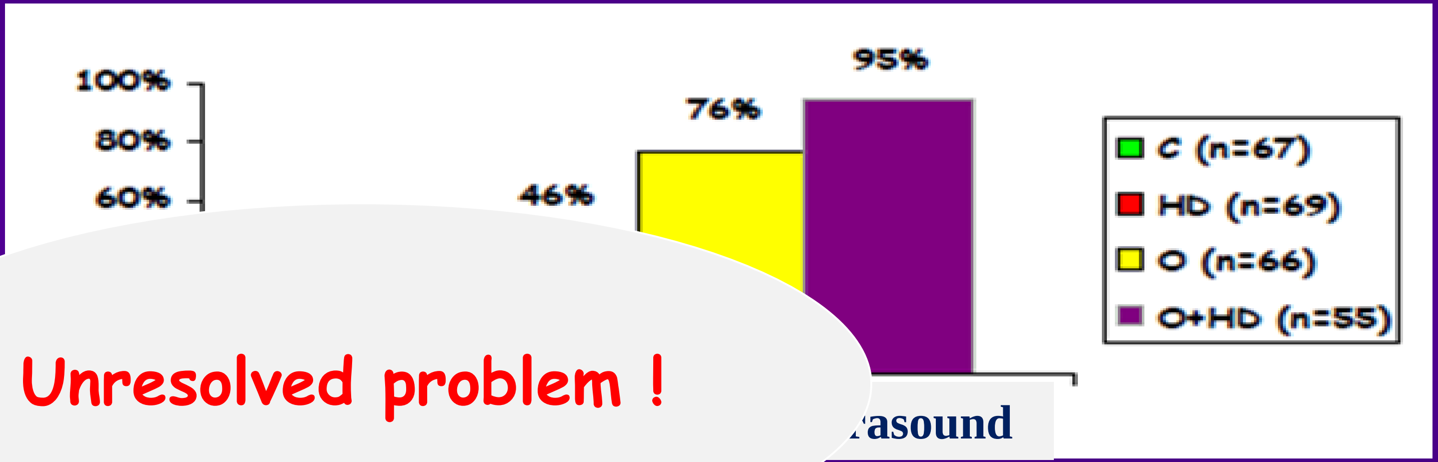
Alcol and liver diseases

N. of pts. consuming doses of alcol responsible of progressive liver diseases.



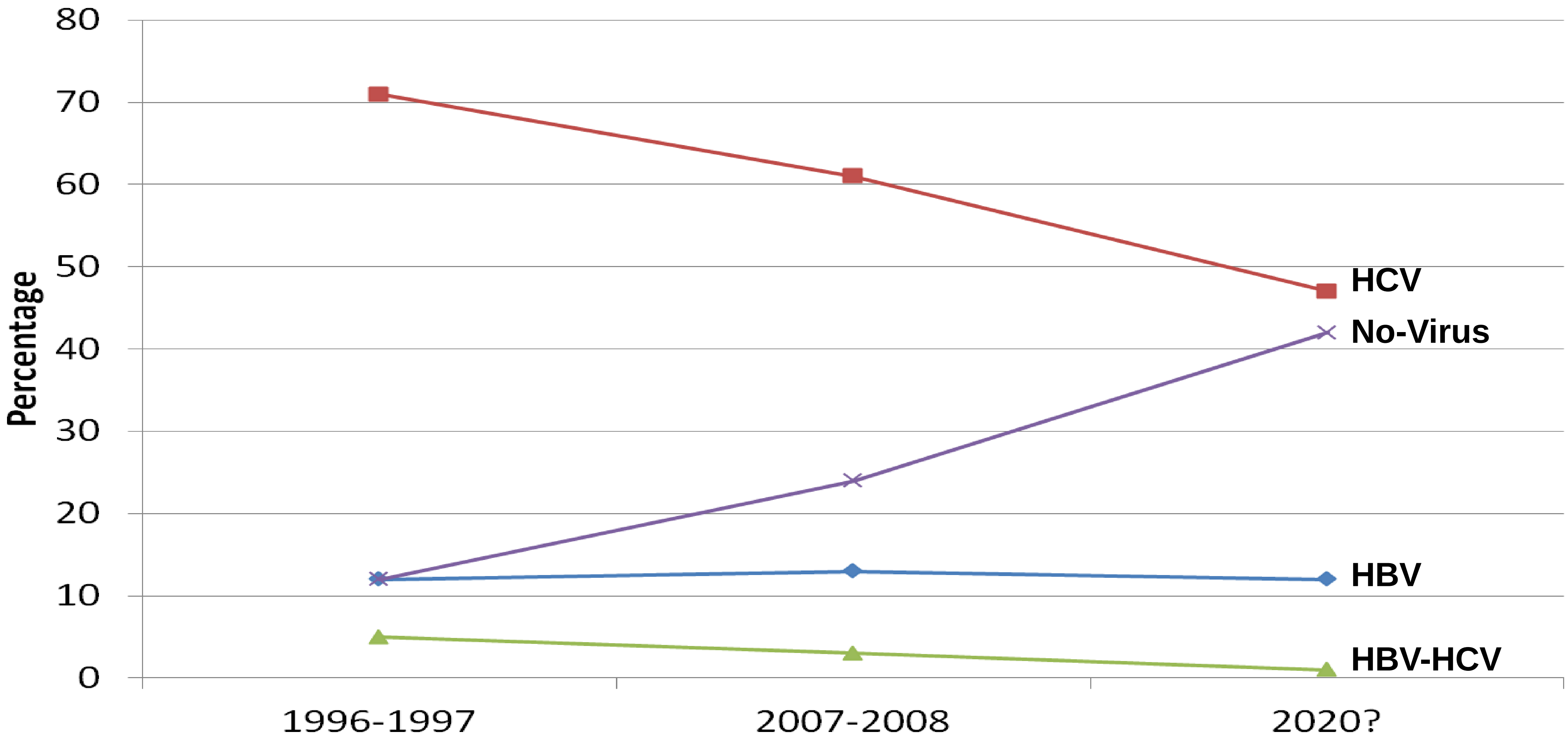
Fatty Liver
The epidemy of obesity in western countries





Unresolved problem !

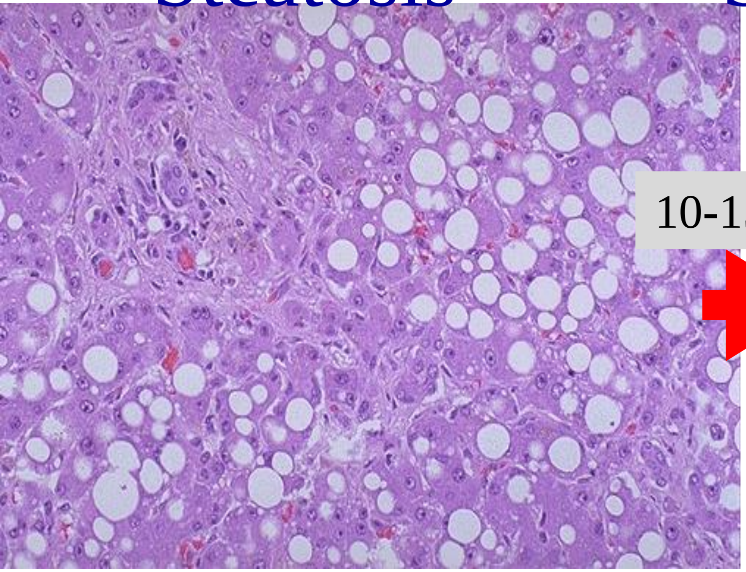
Etiology of HCC in Italy: observed and expected temporal trends (23 centres: 1733 HCC)



Stroffolini et al. 2009

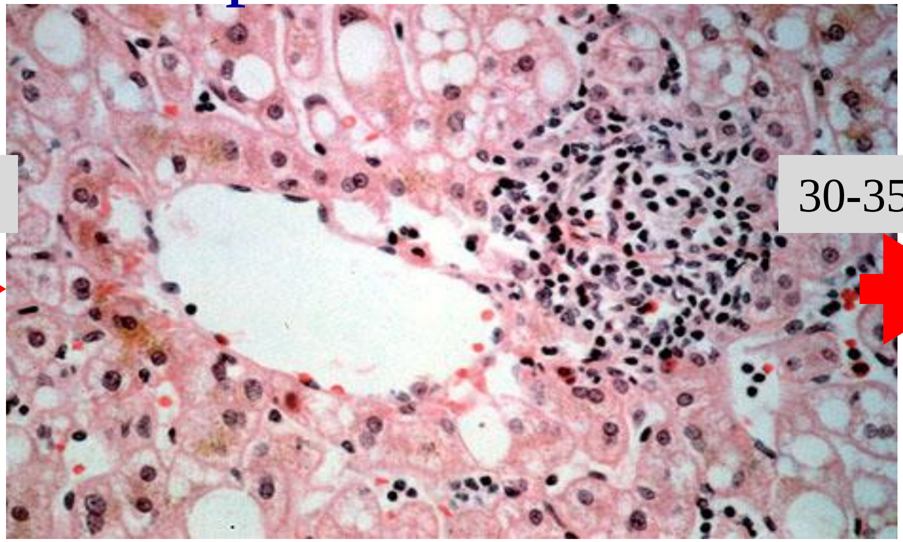
NAFLD: natural history

Steatosis



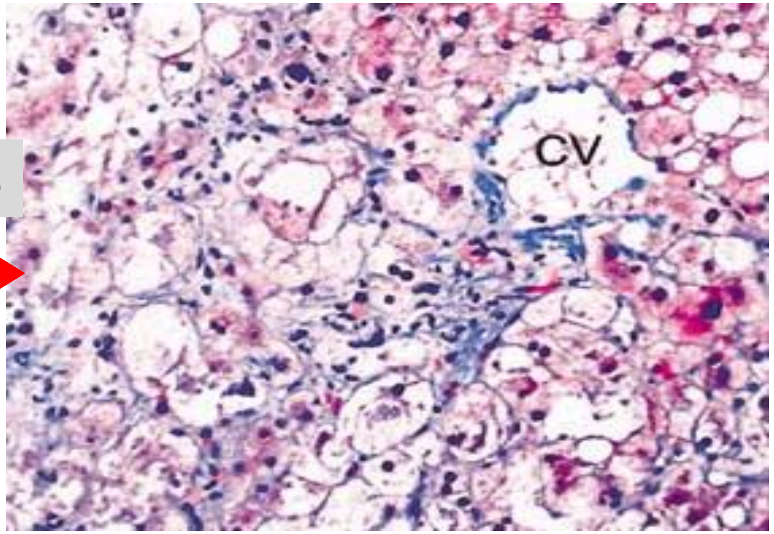
10-15%

SteatoHepatitis



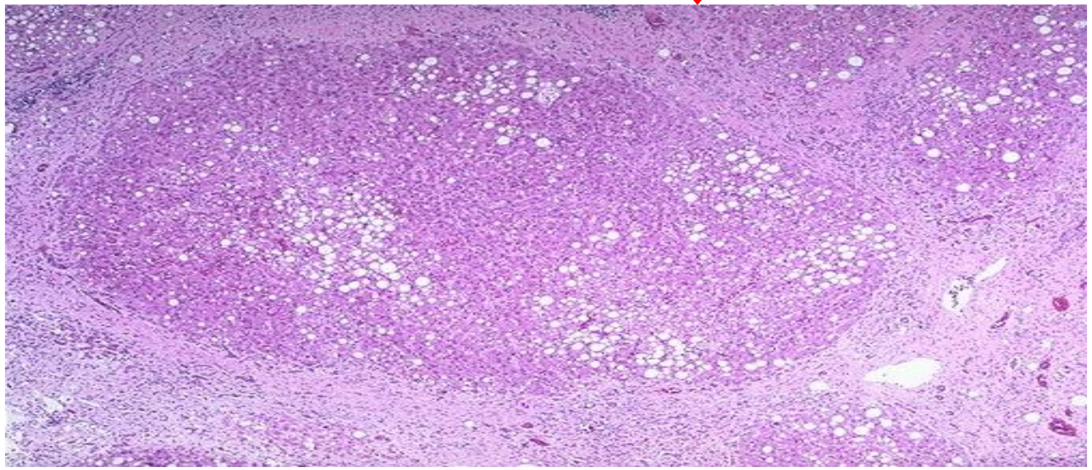
30-35%

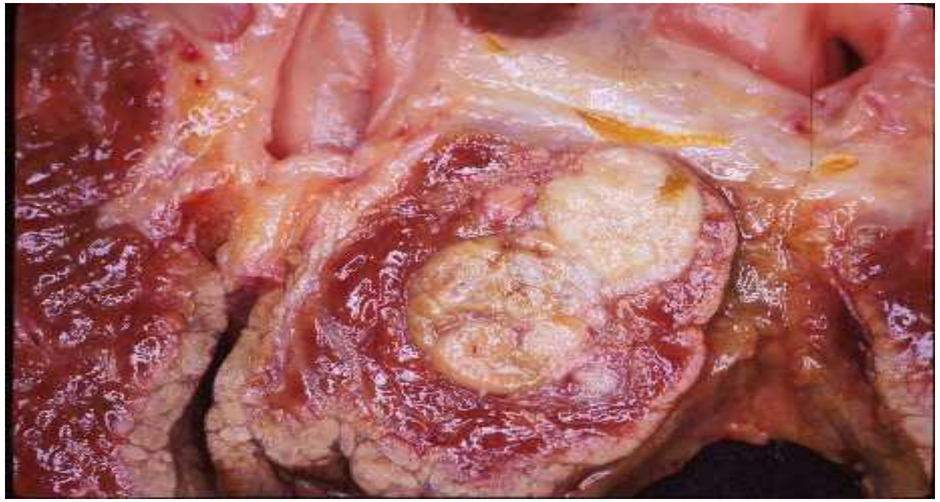
SteatoHepatitis+fibrosis



10-15%

Cirrhosis





Cirrhosis+hepatocellular carcinoma (HCC)

LIVER CIRRHOSIS: CLINICAL FEATURES

COMPENSATED CIRRHOSIS

↓

DECOMPENSATED CIRRHOSIS:

-Ascitis and (PBS, HRS..)
-Bleeding (varices…..)
-Hepatic Hencefalophaty
-Portal Trombosis
-HCC

The treatment of chronic liver diseases

Liver Transplantation

the only option for untreatable liver diseases !

Liver Transplantation (OLT)

However ……………..

--The number of donated livers is limited !

--OLT ca[…]e very ad[…] contrai[…]

--Post-sur[…]ant problen[…]

--High co[…] and first-year medical follow-up !

OLT in Italy, year 2015

N. 1.090

Pts in list = 1288 !

Fig. 1. Primary diseases leading to liver transplantation in Europe (01/1988–12/2011) [40]. *Others: Budd-Chiari: 792, Bening liver tumours or polycystic diseases: 1228, Parasitic diseases: 80, Other liver diseases: 1304.

Alternative to OLT
CELL THERAPY and LIVER DISEASES

The cell therapy for treatment of liver diseases is the object of extensive investigations but,

the ideal cell sources still represent an unresolved issue !

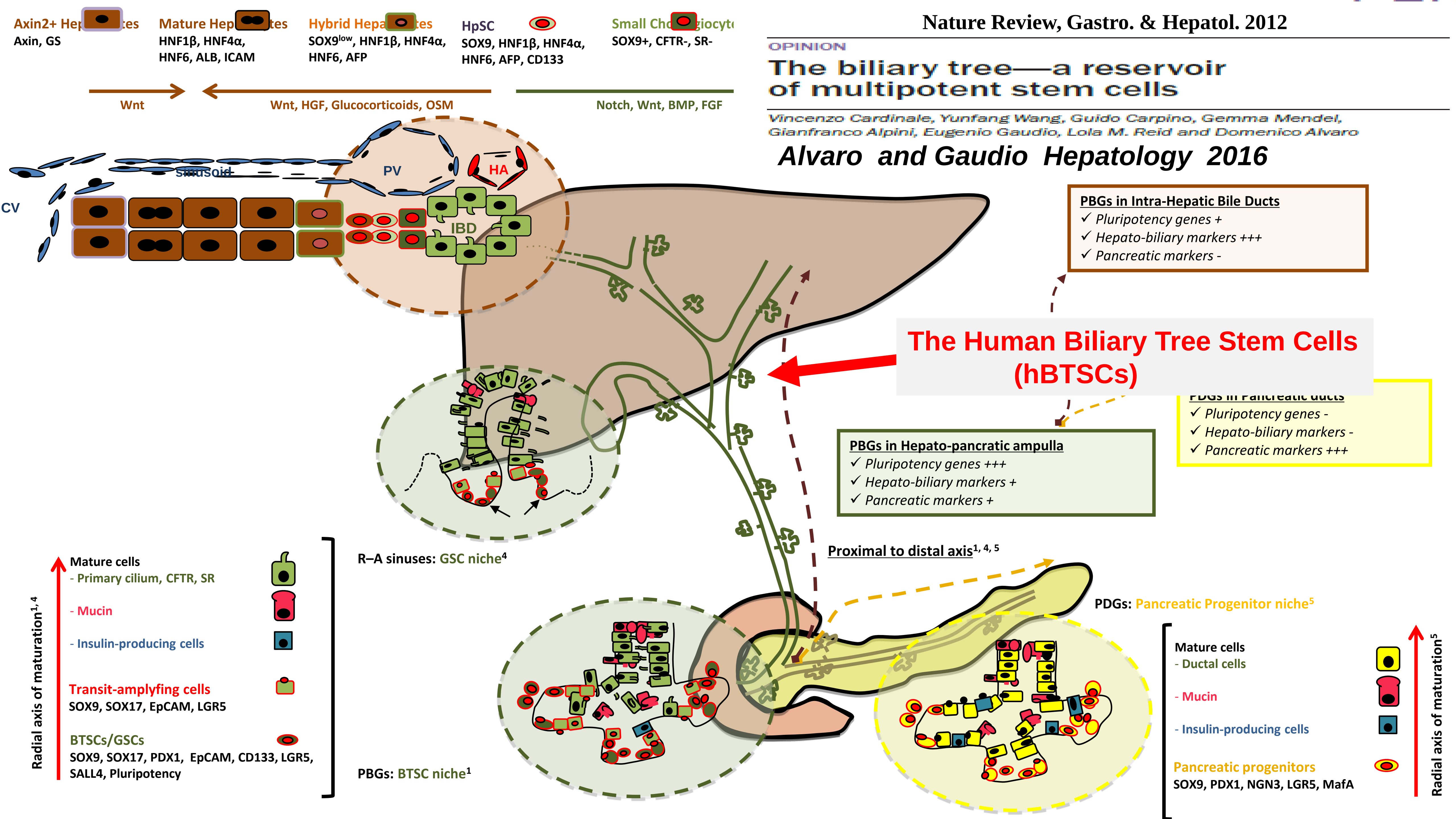
CELL THERAPY and LIVER DISEASES

Cell Sources:

Adult Hepatocytes

Stem/progenitor-cells:

1. Fetal stem cells
2. Adult hepatic stem cells
3. Mesenchymal stem cells
4. Amniotic fluid-derived stem cells
5. Induced pluripotent stem cells (iPS)



Axin2+ Hep tes
Axin, GS
Mature Hep tes
HNF1β, HNF4α, HNF6, ALB, ICAM
Hybrid Hepa tes
SOX9low, HNF1β, HNF4α, HNF6, AFP
HpSC
SOX9, HNF1β, HNF4α, HNF6, AFP, CD133
Small Cho giocyte
SOX9+, CFTR-, SR-
Wnt
Wnt, HGF, Glucocorticoids, OSM
Notch, Wnt, BMP, FGF
Nature Review, Gastro. & Hepatol. 2012
OPINION
The biliary tree—a reservoir of multipotent stem cells
Vincenzo Cardinale, Yunfang Wang, Guido Carpino, Gemma Mendel, Gianfranco Alpini, Eugenio Gaudio, Lola M. Reid and Domenico Alvaro
Alvaro and Gaudio Hepatology 2016
CV
Sinusoid
PV
HA
IBD
PBGs in Intra-Hepatic Bile Ducts
✓ Pluripotency genes +
✓ Hepato-biliary markers +++
✓ Pancreatic markers -
The Human Biliary Tree Stem Cells (hBTSCs)
✓ Pluripotency genes -
✓ Hepato-biliary markers -
✓ Pancreatic markers +++
PBGs in Hepato-pancratic ampulla
✓ Pluripotency genes +++
✓ Hepato-biliary markers +
✓ Pancreatic markers +
Proximal to distal axis1, 4, 5
R–A sinuses: GSC niche4
PBGs: BTSC niche1
PDGs: Pancreatic Progenitor niche5
Radial axis of maturation1, 4
Mature cells
- Primary cilium, CFTR, SR
- Mucin
- Insulin-producing cells
Transit-amplyfing cells
SOX9, SOX17, EpCAM, LGR5
BTSCs/GSCs
SOX9, SOX17, PDX1, EpCAM, CD133, LGR5, SALL4, Pluripotency
Mature cells
- Ductal cells
- Mucin
- Insulin-producing cells
Pancreatic progenitors
SOX9, PDX1, NGN3, LGR5, MafA
Radial axis of maturation5

Multipotent Stem/Progenitor Cells in Human Biliary Tree Give Rise to Hepatocytes, Cholangiocytes, and Pancreatic Islets

The study was also supported by Consorzio Interuniversitario Trapianti d'Organo, Rome, Italy
HEPATOLOGY 2011;54:2159-2172

Vincenzo Cardinale,[1,2*] Yunfang Wang,[1*] Guido Carpino,[3] Cai-Bin Cui,[4] Manuela Gatto,[2] Massimo Rossi,[5] Pasquale Bartolomeo Berloco,[5] Alfredo Cantafora,[2] Eliane Wauthier,[1] Mark E. Furth,[6] Luca Inverardi,[7] Juan Dominguez-Bendala,[7] Camillo Ricordi,[7] David Gerber,[4] Eugenio Gaudio,[3*,†] Domenico Alvaro,[2*,†] and Lola Reid[1,6*,†]

human biliary tree stem cells (hBTSCs)

1. Wide availability and easy supplying (specially from extrahepatic sources)

2. *in vitro*: sufficient amount of cells for transplantation

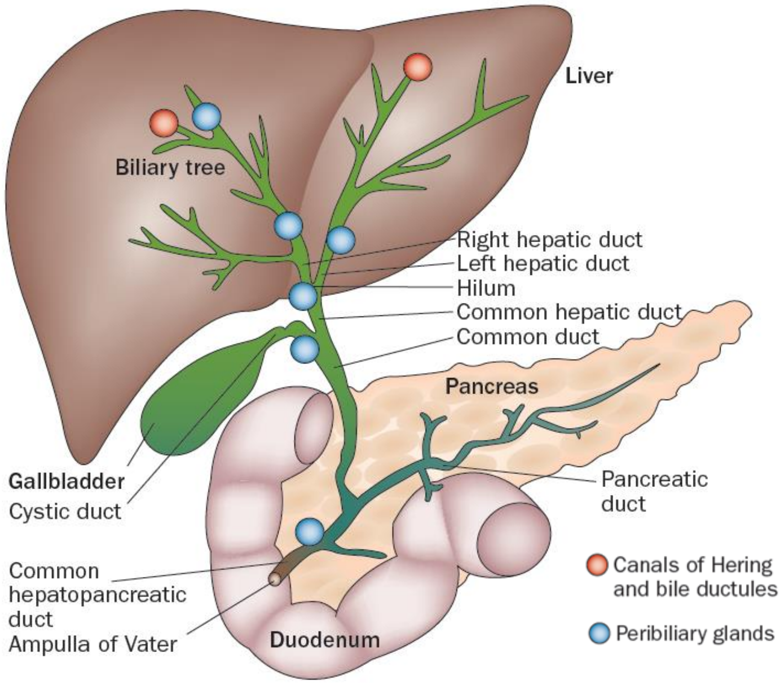
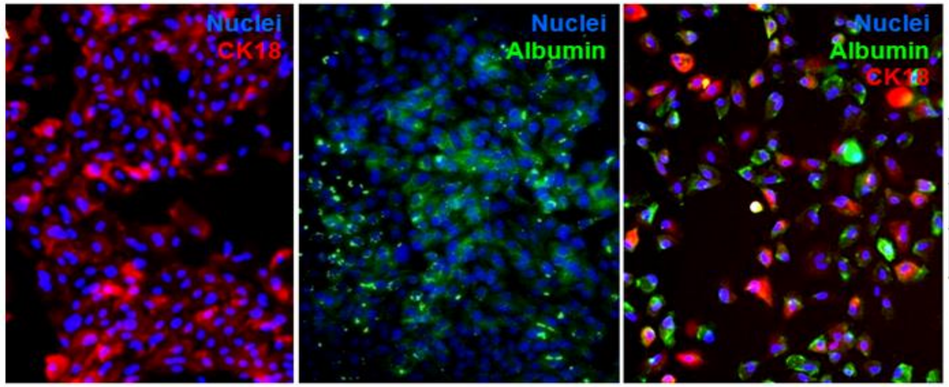


Image from: Cardinale V. et al. Nat. Rev. Gastroenterol. Hepatol. (2012)

Adult hBTSCs: Multipotency in vitro

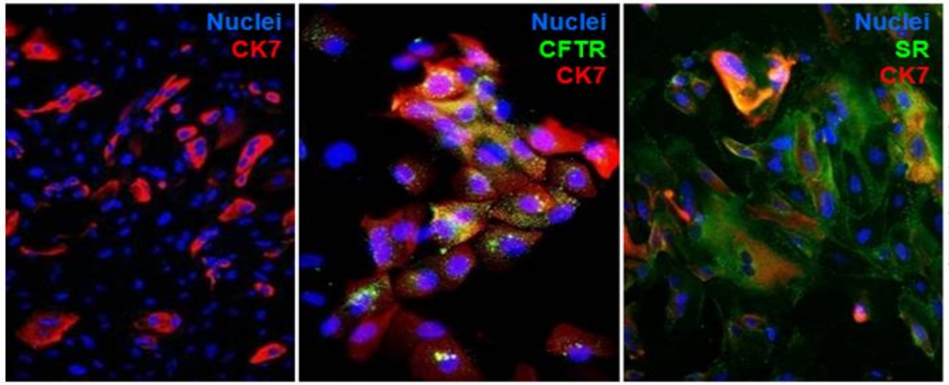
Differentiation towards

Hepatocytes



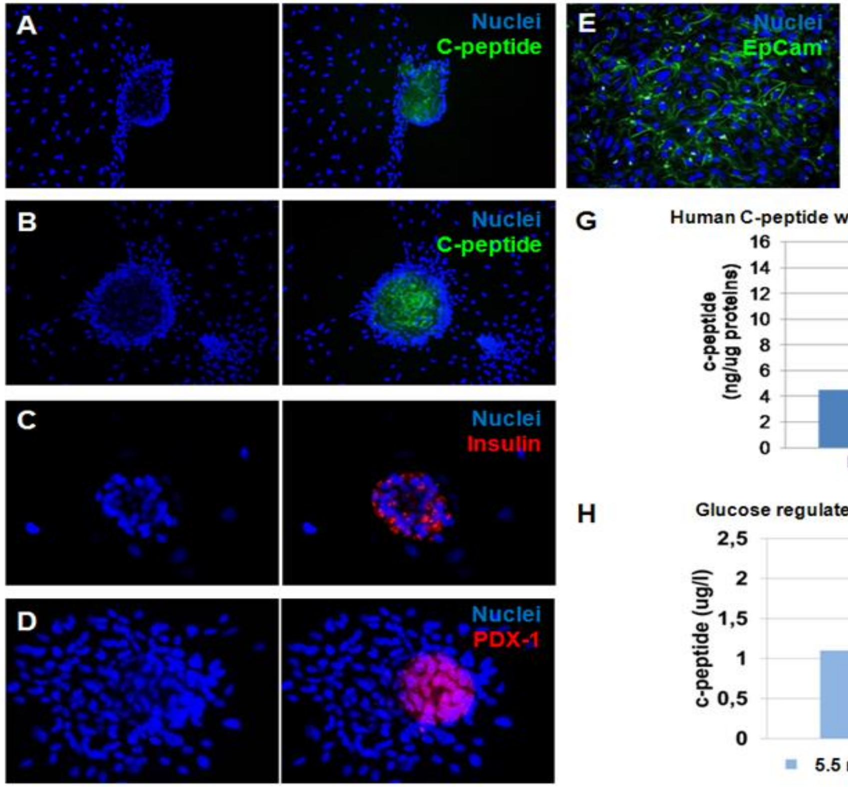
% of ALB+/CK18+ cells	
KM	0
HDM-L	36.66 ± 10.41*

Cholangiocytes



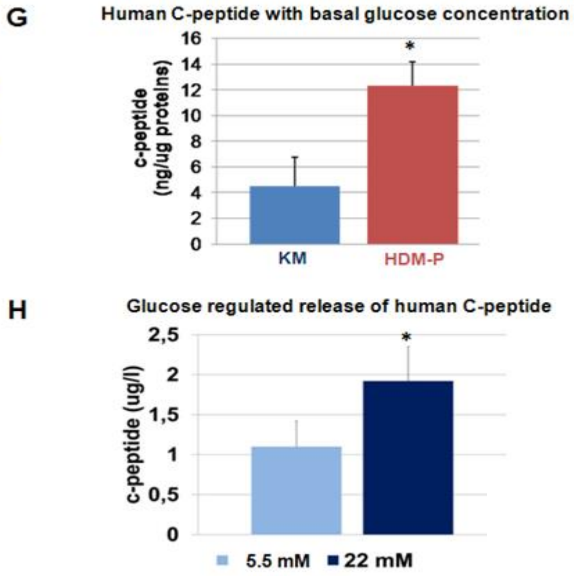
% of SR+/CK7+ cells	
KM	3.16 ± 2.64
HDM-C	49.17 ± 11.14*

Pancreatic isltes



F

N° of Islet-like structures (c-pep+)	
KM	1 ± 0.7
HDM-P	3.8 ± 1.3*



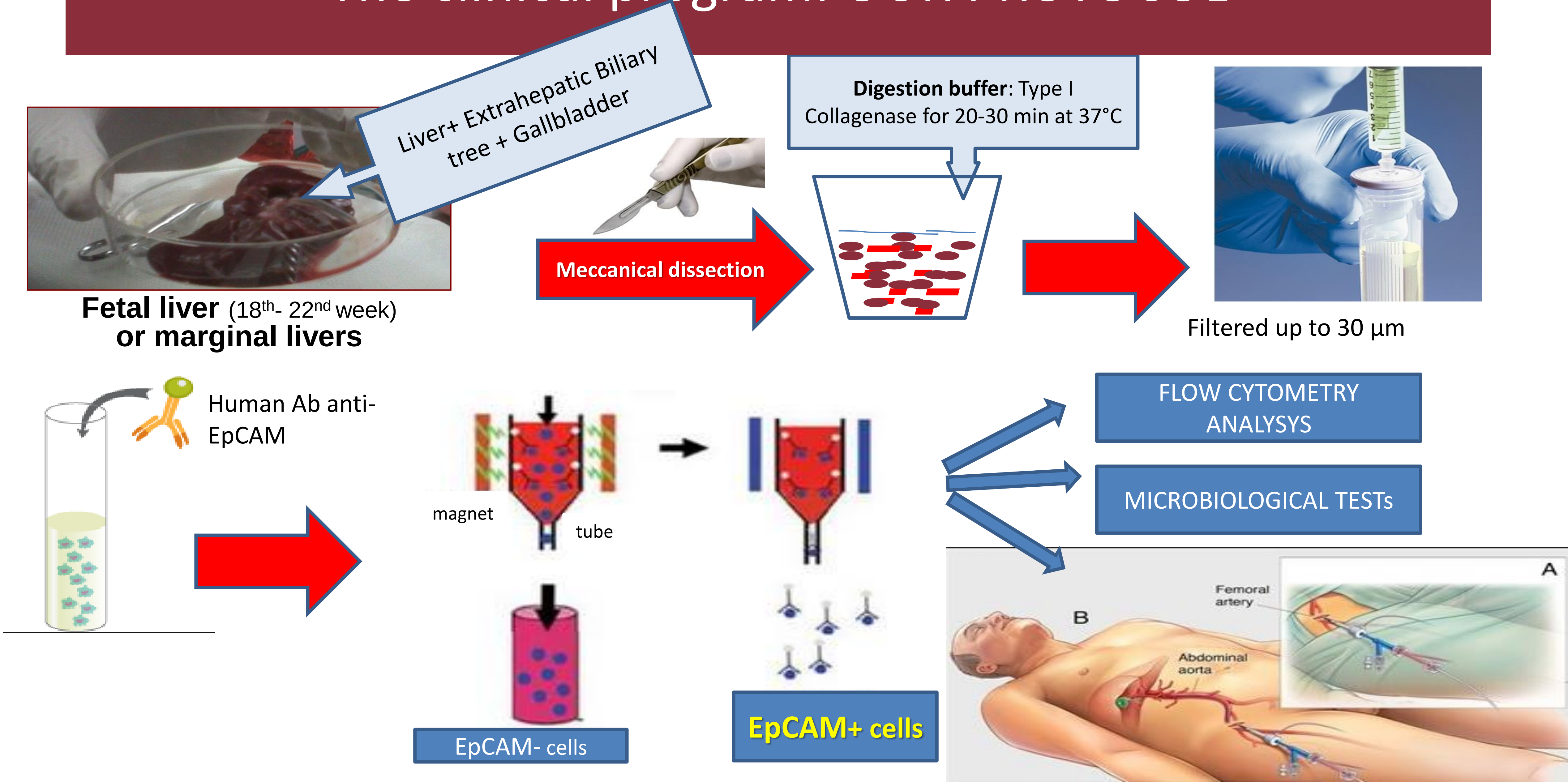
Phenotype and functions were evaluated under **<u>conditioned media</u>**

for hepatocytes, cholangiocytes or pancreatic isltes.

Transfer into differentiation conditions resulted in distinct mature fates.

Cardinale V, Wang Y, …, Gaudio E, Alvaro D, Reid L. Hepatology. 2011

The clinical program: OUR PROTOCOL



Phase I/II study on advanced cirrhosis transplanted with fetal hBTSCs. 2° Paz.

Pz. A.P., 71 yrs, Cirrhosis-HCV, Child-Pugh 12, Meld 21

60 millions freshly isolated fetal BTScs, EpCAM+ (50% Lgr5+) injected via hepatic artery

Time:	0’	24 months
Child-Pugh score	12	10
Meld score	21	17
Bilirubin	3.04	2.08 mg/dl
Albumin	3.2	3.44 g/dl
INR	2.0	1.72
Creatinine	1.3	1.1

However …..

Only 7-10 % of cells infused as suspension engraft the liver!

How to improve the benefits of hBTSC transplantation ?
Ongoing projects:

1. Enhance the engraftment efficiency by infusing tridimensional cell clusters !

2. Cryopreservation of hBTSCs and HA-hBTSCs, the generation of a cell bank;

3. Identify additional subpopulations of hBTSCs possessing the properties of the ideal stem cell and suitable for clinical programs?

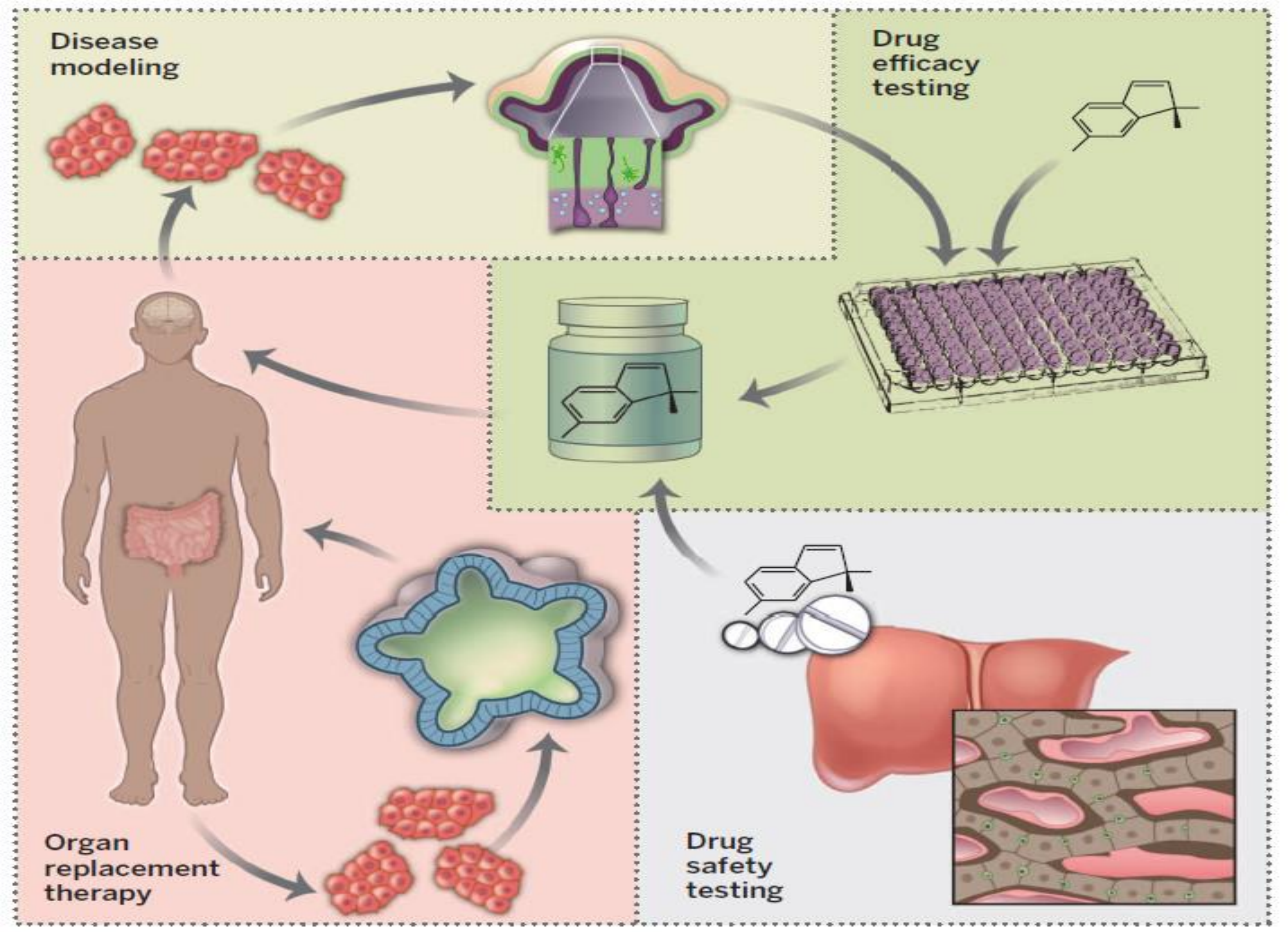
Science 2014

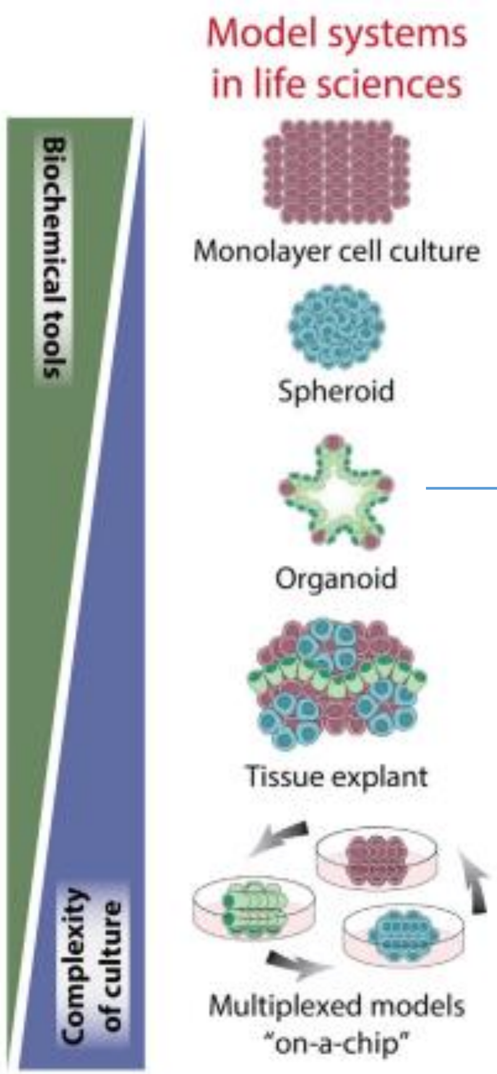
REVIEW

ORGANOID G

Organ
develo
organ

Madeline A. L





- spatial organization
- cell-cell interactions
- cell-matrix interactions
- physiological functions

Bioengeneering strategies to obtain 3D cultures

- *In vitro* specific conditions
- Bioprinting
- Biomimetic scaffolds
- Microfluid devices
- **Microgravity** based

Thyroid Organoid Formation in Simulated Microgravity: Influence of Keratinocyte Growth Factor

To cite this article:
A. Martin,1A. Zhou,1R.E. Gordon,2S.C. Henderson,3A.E. Schwartz,4E.W. Friedman,4T.F. Davies1. Thyroid. January 2009, 10(6): 481-487. doi:10.1089/thy.2000.10.481.

Published in Volume: 10 Issue 6: January 30, 2009

Microgravity exerts different effects on the gravity-evolved organisms

- Systemic effects on the human health
- Research focused mainly on musculoskeletal apparatus, cerebellum and cardiovascular system

Alteration of carbohydrate and lipidic metabolism and reduction of the number of macrophages.
(Racine et al. 1992)

Changes in the content and activity of CYP450 in rats during spatial flights.
(Rabot et al. 2000)

Microgravity Reduces the Differentiation and Regenerative Potential of Embryonic Stem Cells

Elizabeth A. Blaber,[1,2] Hayley Finkelstein,[1] Natalya Dvorochkin,[1] Kevin Y. Sato,[3] Rukhsana Yousuf,[1] Brendan P. Burns,[2,4] Ruth K. Globus,[1] and Eduardo A.C. Almeida[1]

STEM CELLS AND DEVELOPMENT
Volume 24, Number 22, 2015
DOI: 10.1089/scd.2015.0218

Stem Cell Health and Tissue Regeneration in Microgravity

Elizabeth Blaber, Kevin Sato, and Eduardo A.C. Almeida*

Stem Cells and Development
Vol. 23, Supp. 1 2014 • DOI: 10.1089/scd.2014.0408

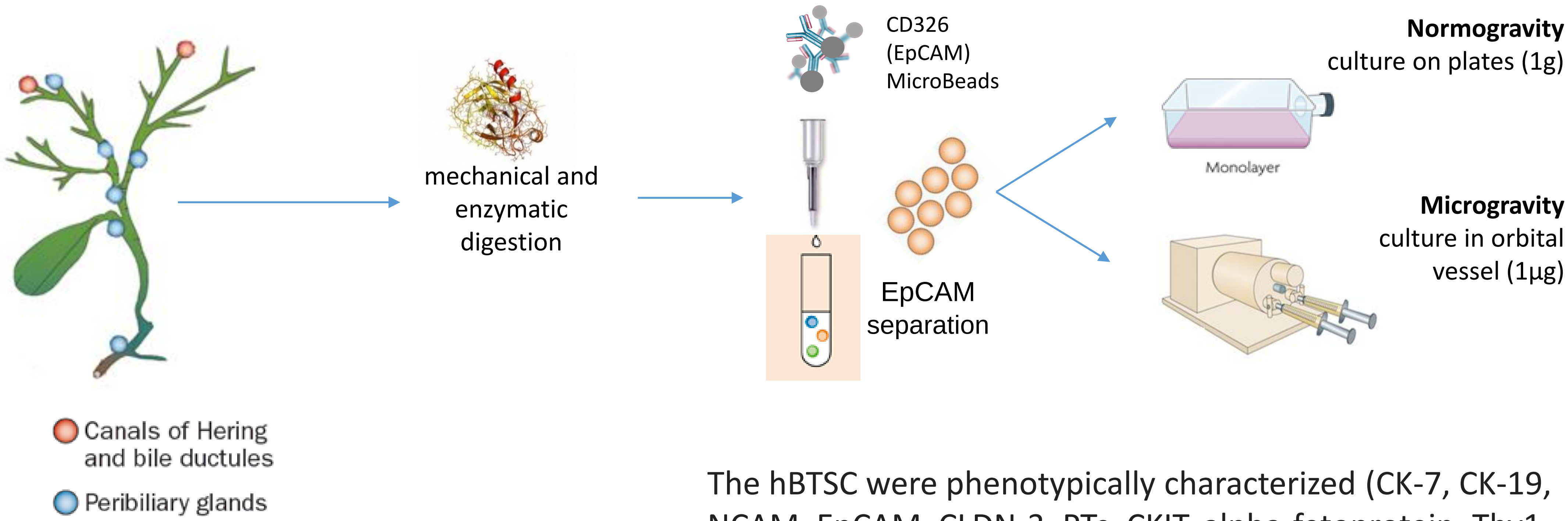
AIMs

1. To evaluate whether microgravity may help the development of tridimensional cultures of human biliary tree stem cells (**hBTSCs**), to be used for the regenerative medicine of liver diseases and for development of liver devices;

2. To evaluate the effects of microgravity on biological properties and functions of isolated **hBTSCs;**

Isolation of human Biliary Tree Stem Cells

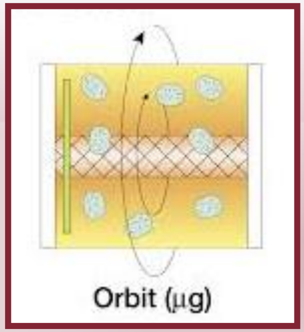
normogravity and microgravity cultures

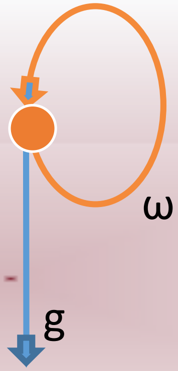


The hBTSC were phenotypically characterized (CK-7, CK-19, NCAM, EpCAM, CLDN-3, PTc, CKIT, alpha-fetoprotein, Thy1, albumin), plated and cultured in basal and differentiation medium in both normogravity and microgravity.

Rotary Cell Culture System (RCCS)

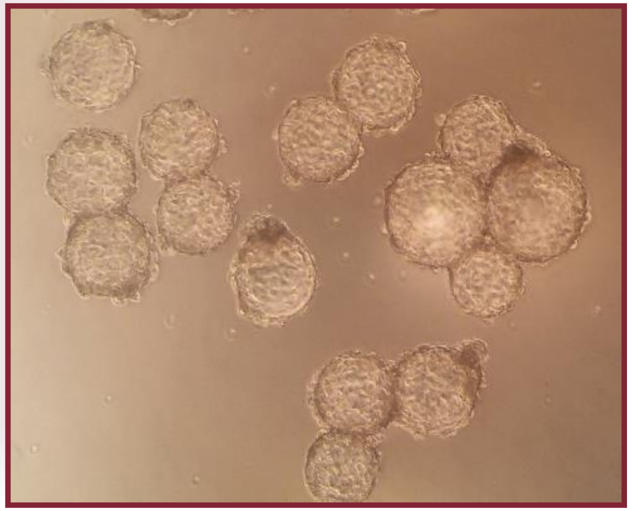
- Cell growth with or without solid support (scaffold, microcarrier beads);
- Versatility - more than 50 cell types grown successfully;
- Spontaneous formation of 3D tissue;
- Propagation of mono- and co-cultures;





10-30 rpm:
10.000 fold less than g

Cytodex 3 microcarrier

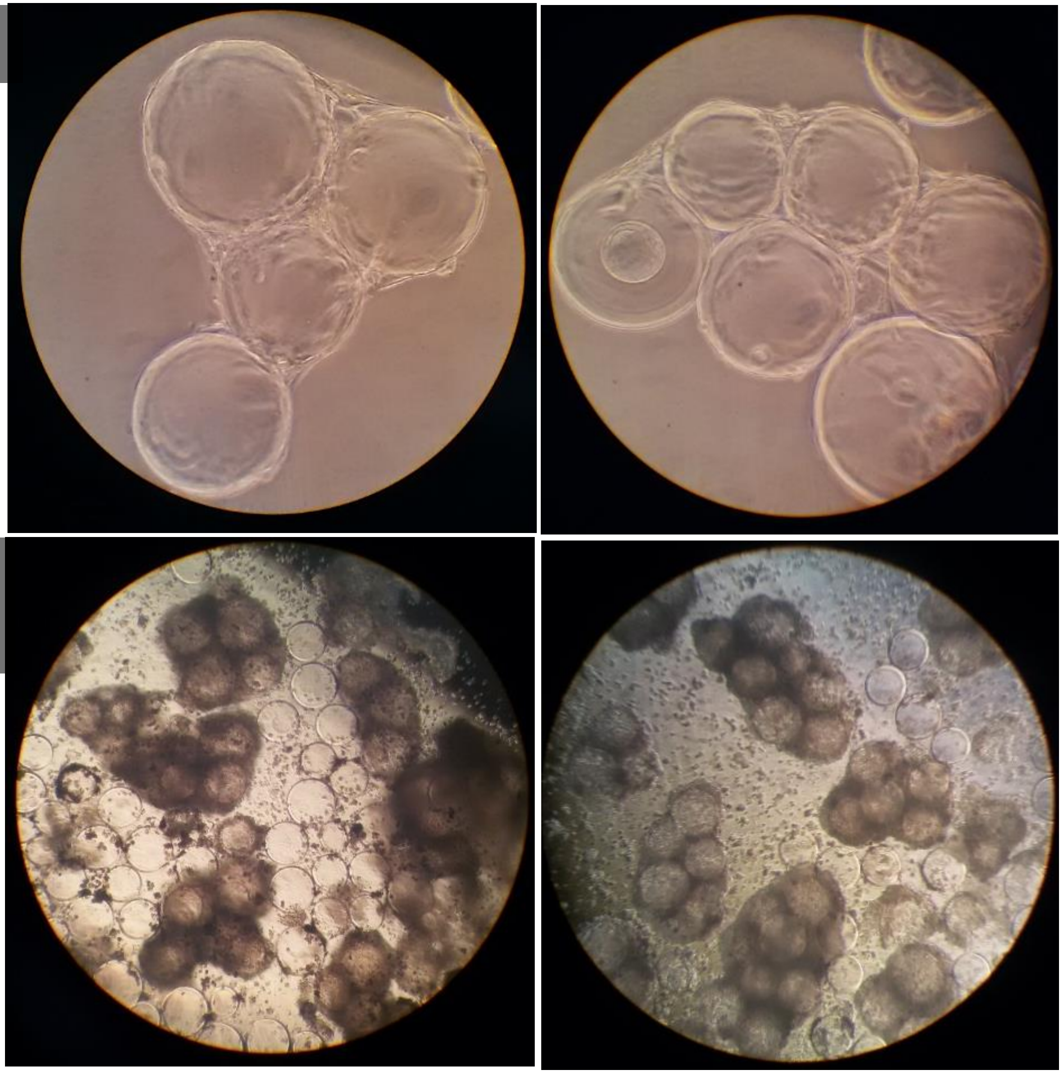


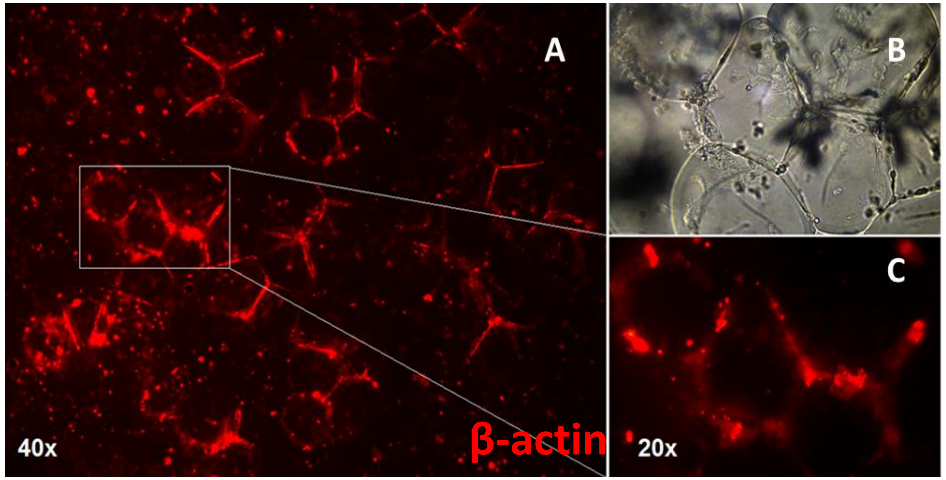
Simulated microgravity favors development of tridimensional cultures

14 days

hBTSCs

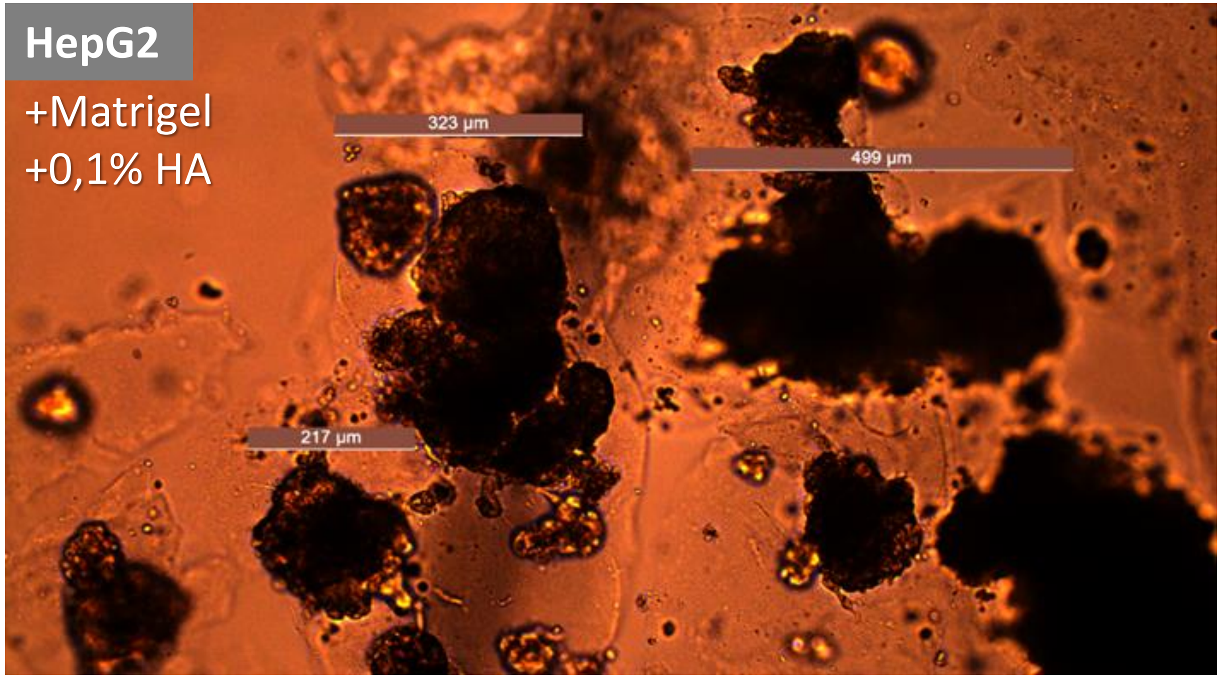
HepG2 cells

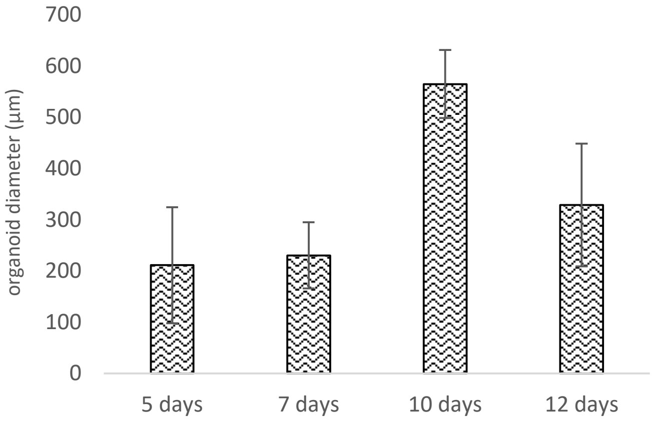




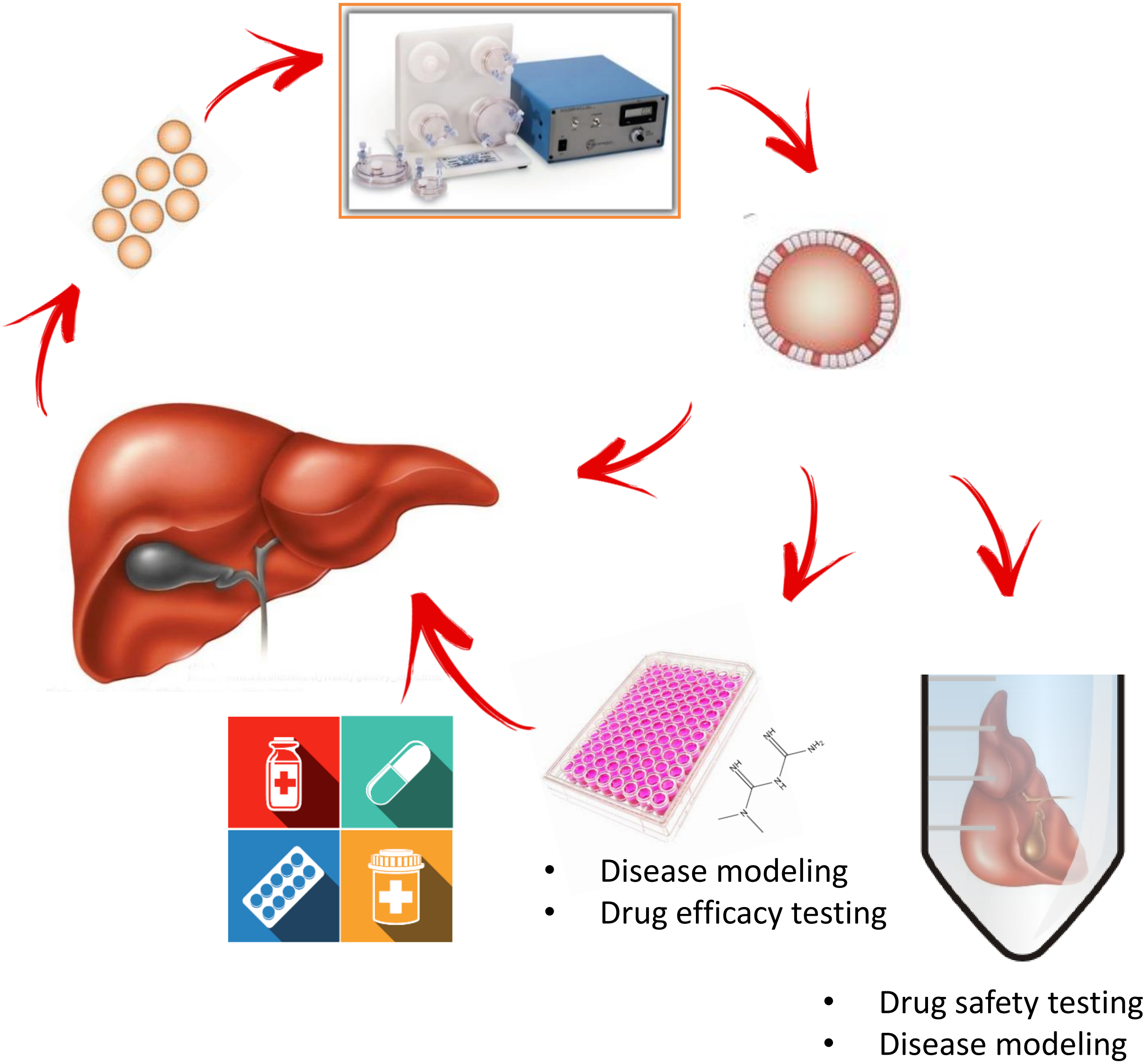
Cluster Ø:
350 µm - 787,5 µm ca

Tridimensional cultures in microgravity conditions and organoid-based technologies for research and medicine applications





Cells were able to readhere on plates to form colonies

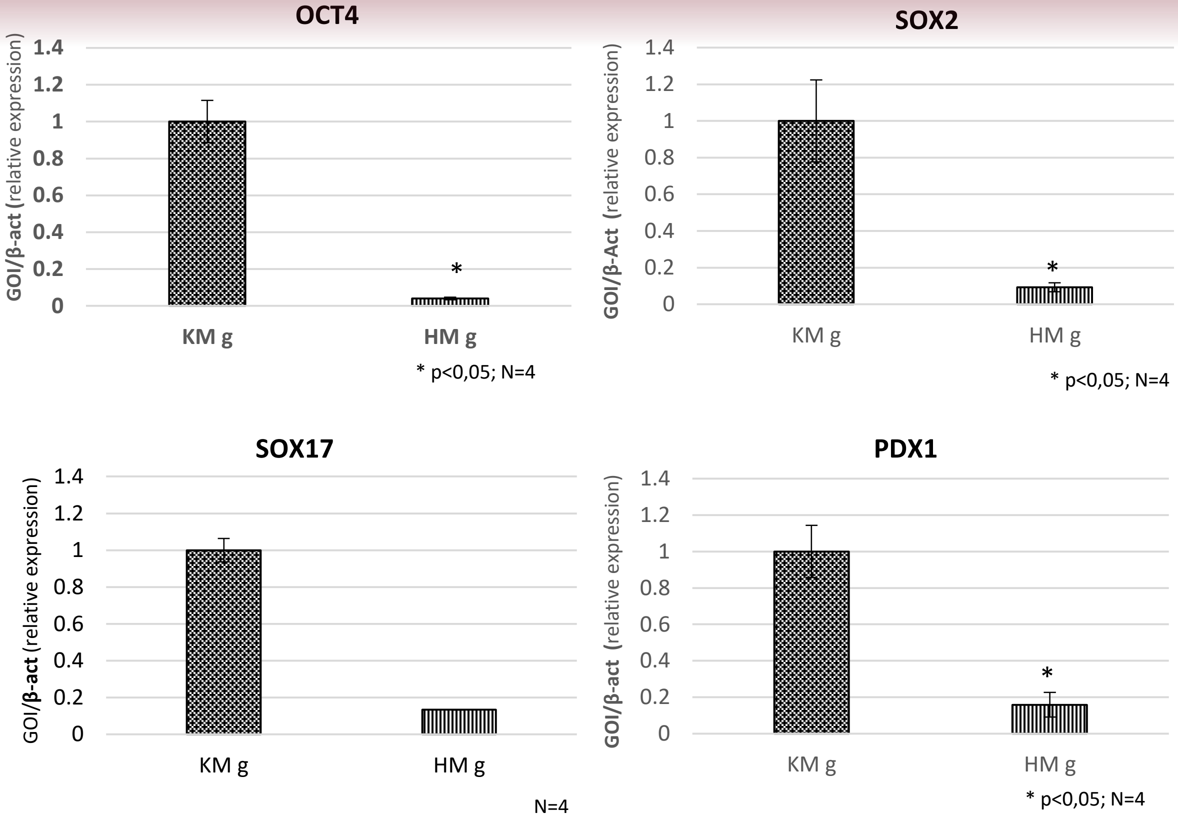


Expression of Stem Cell Markers in human Biliary Tree Stem Cells cultured in hepatocyte differentiation medium, **normogravity**

KM = Kubota's medium (basal medium)

HM = Hepatocyte Differentiation Medium

Gravity



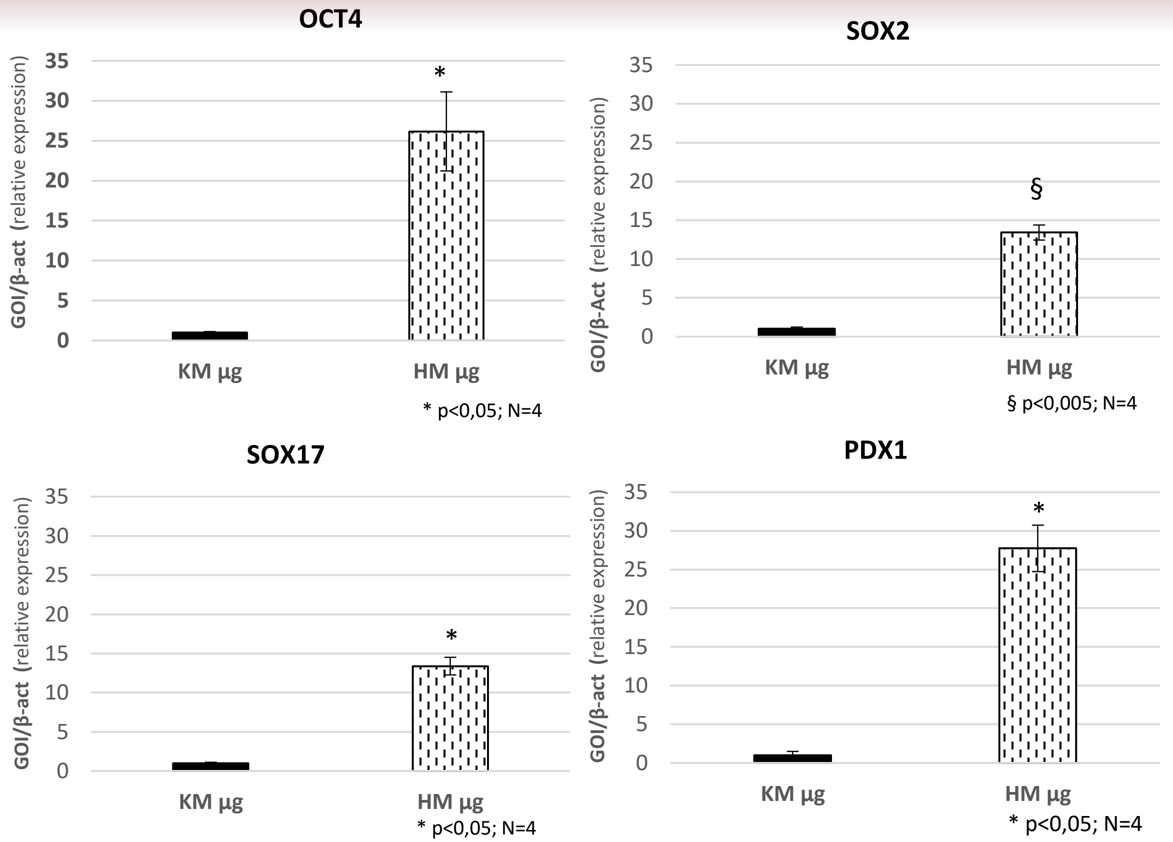
Normogravity downregulated Stem Cell Markers in hBTSCs cultured in hepatocyte differentiation medium.

Expression of Stem Cell Markers in human Biliary Tree Stem Cells cultured in hepatocyte differentiation medium, microgravity

KM = Kubota's medium (basal medium)

HM = Hepatocyte Differentiation Medium

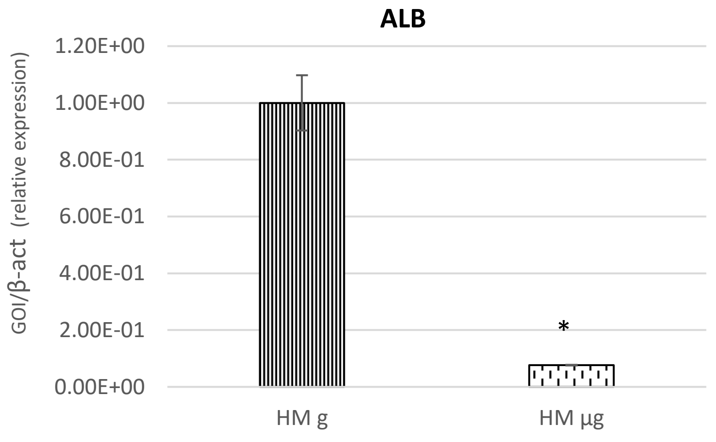
Microgravity



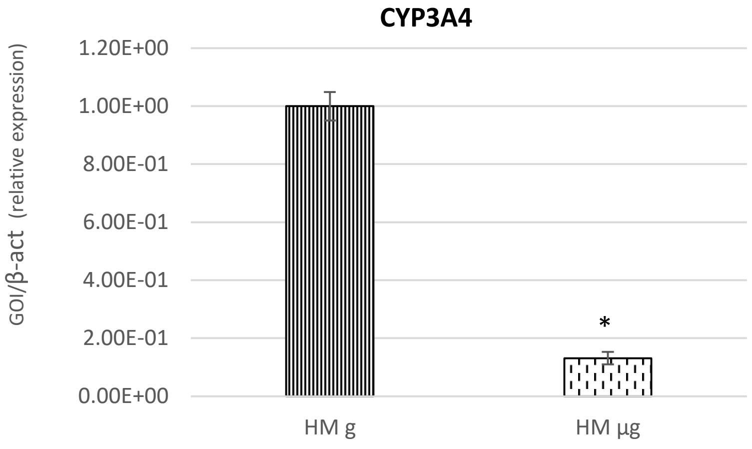
The main hBTSCs stem cell markers were surprinsingly upregulated when cultured in hepatocyte differentiation medium in microgravity conditions

Expression of typical genes of mature hepatocytes in human Biliary Tree Stem Cells cultured in hepatocyte differentiation media

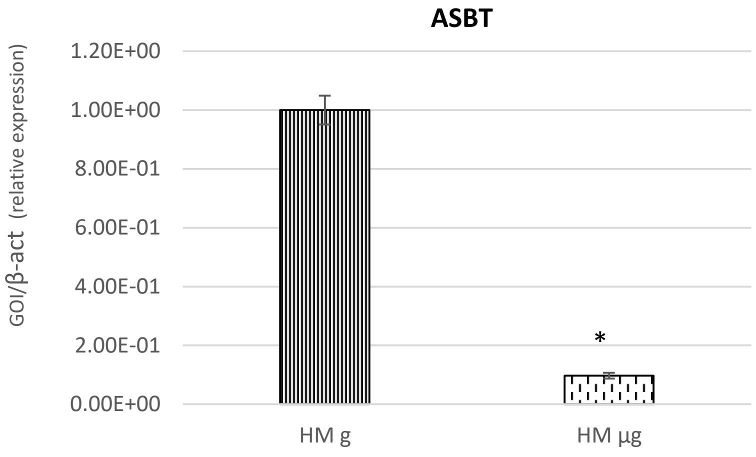
Normogravity vs Microgravity (14 days in culture)



* p < 0,05; N=4



* p < 0,05; N=4

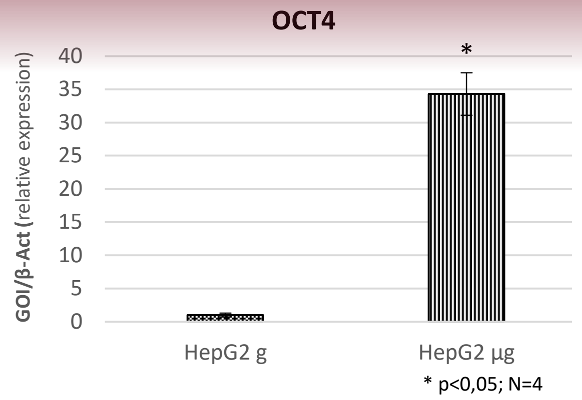


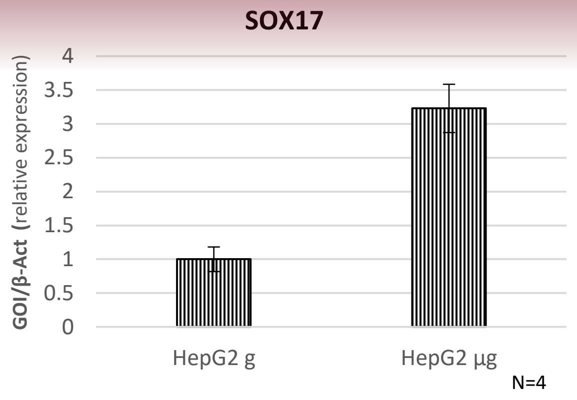
* p < 0,05; N=4

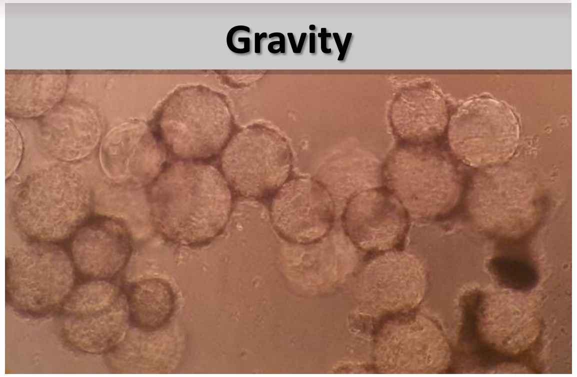
The expression of “mature hepatocytes” genes was significantly downregulated in microgravity conditions!

Expression of stemness genes and genes of "mature hepatocytes" in HepG2 cells

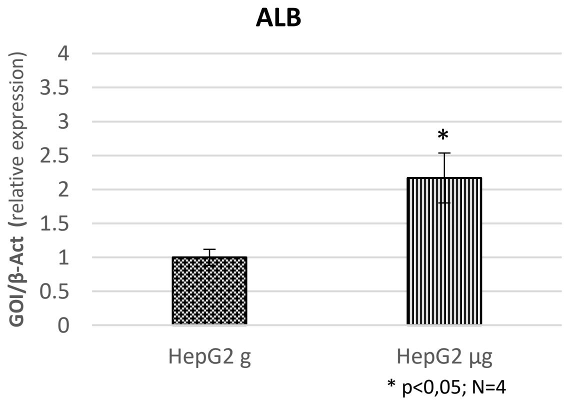
Normogravity vs Microgravity (14 days in culture)

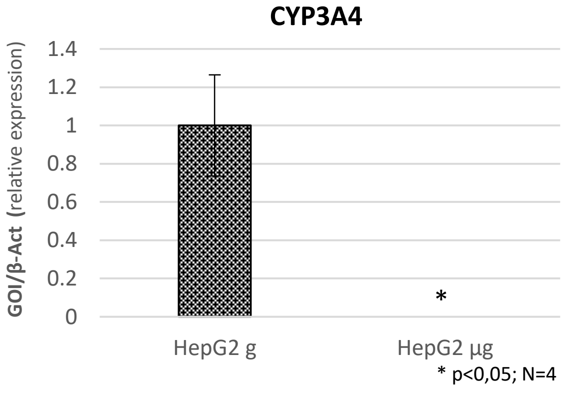


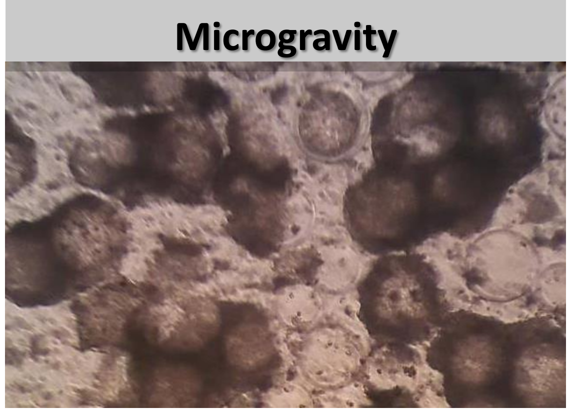




Important stemness marker were upregulated in HepG2 cultures in microgravity compared to normogravity





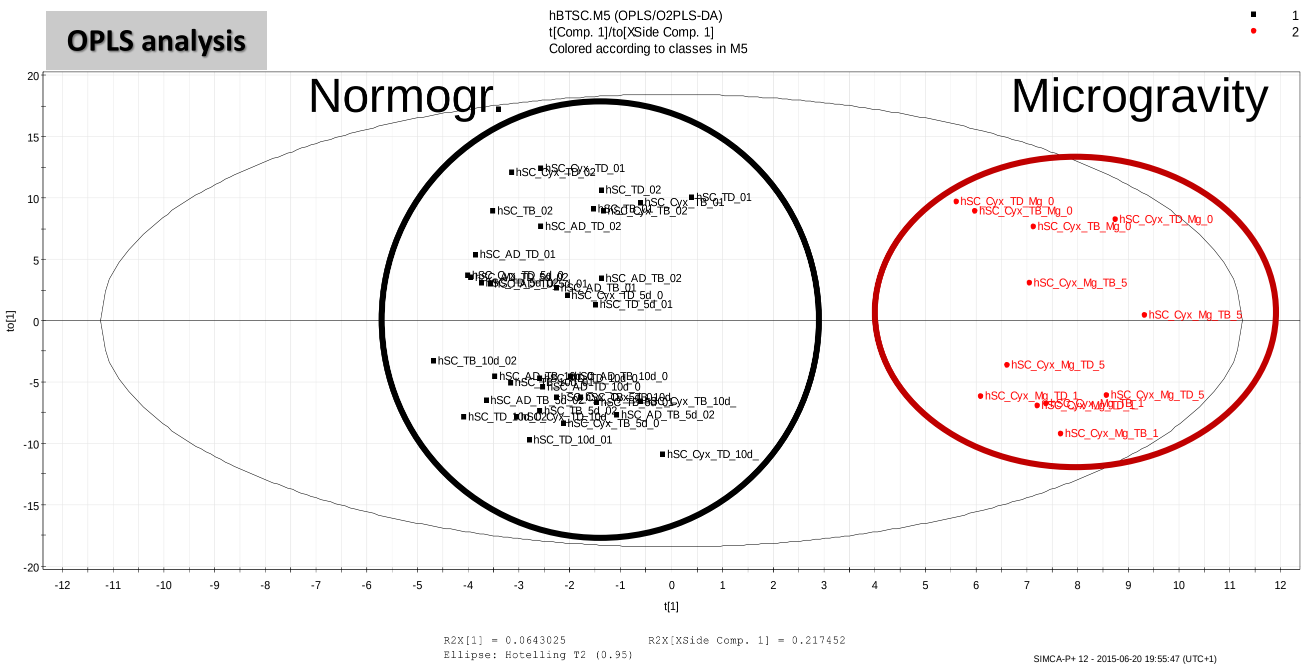


Alb was upregulated - but not Cyp3A4 - in HepG2 cultures in microgravity compared to normogravity

hBSTC: exometabolome analyses

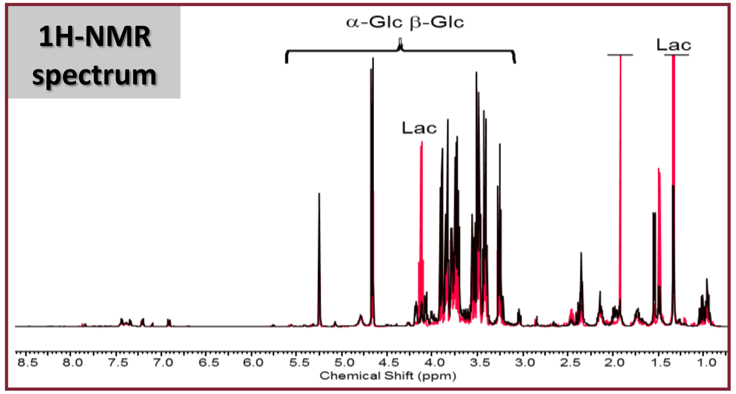
by **Nuclear Magnetic Resonance**

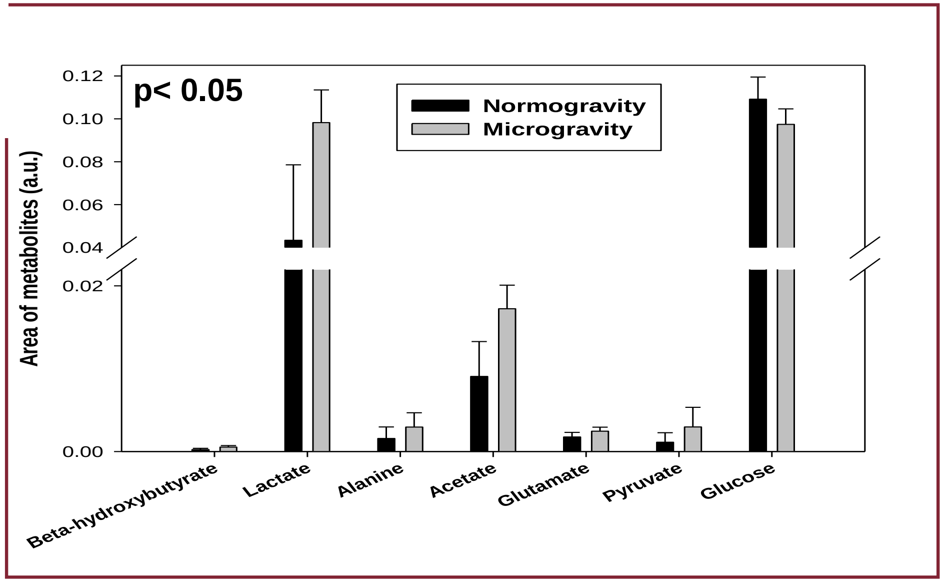
OPLS analysis



- Microgravity:
  no significant differences between the metabolisms of hBSTCs in basal vs differentiation medium;

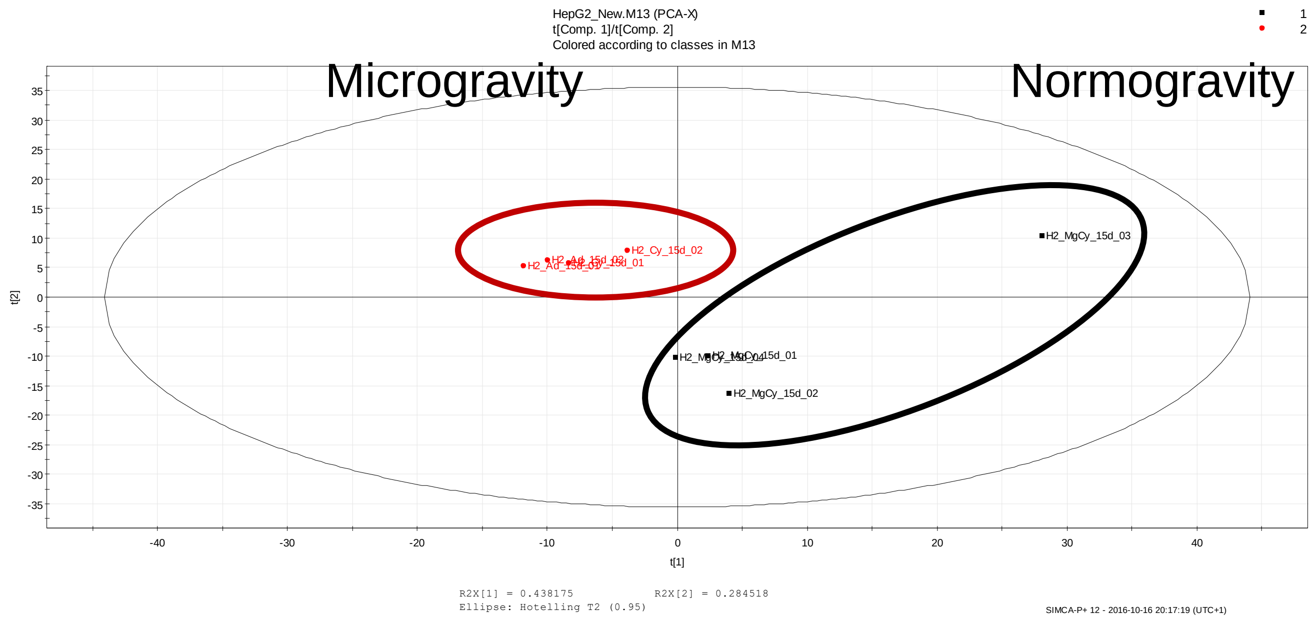
- when compared with normogravity, hBTSCs in microgravity consumed more glucose and produced more lactate, acetate, glutammate;





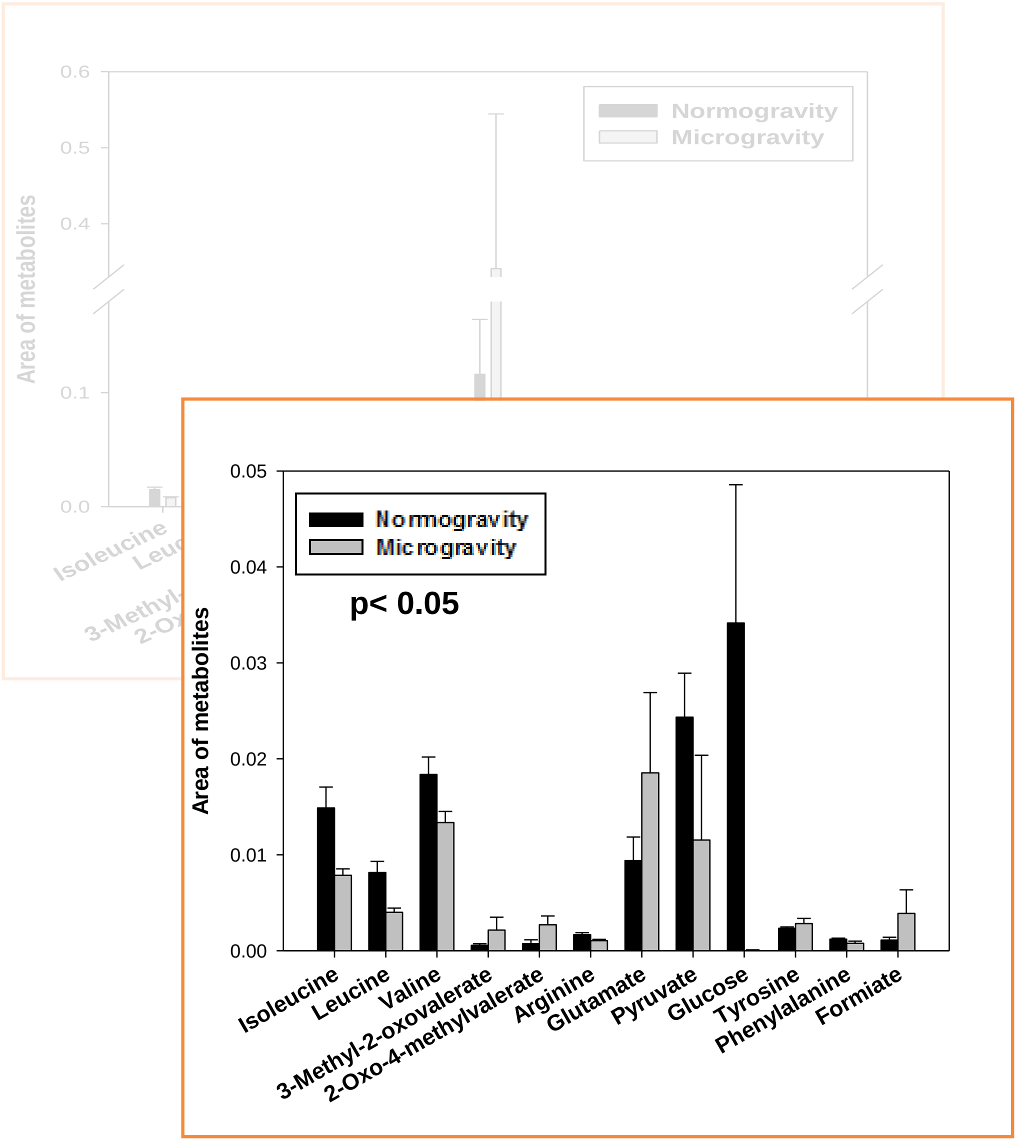
HepG2: exometabolome analyses

by **Nuclear Magnetic Resonance**

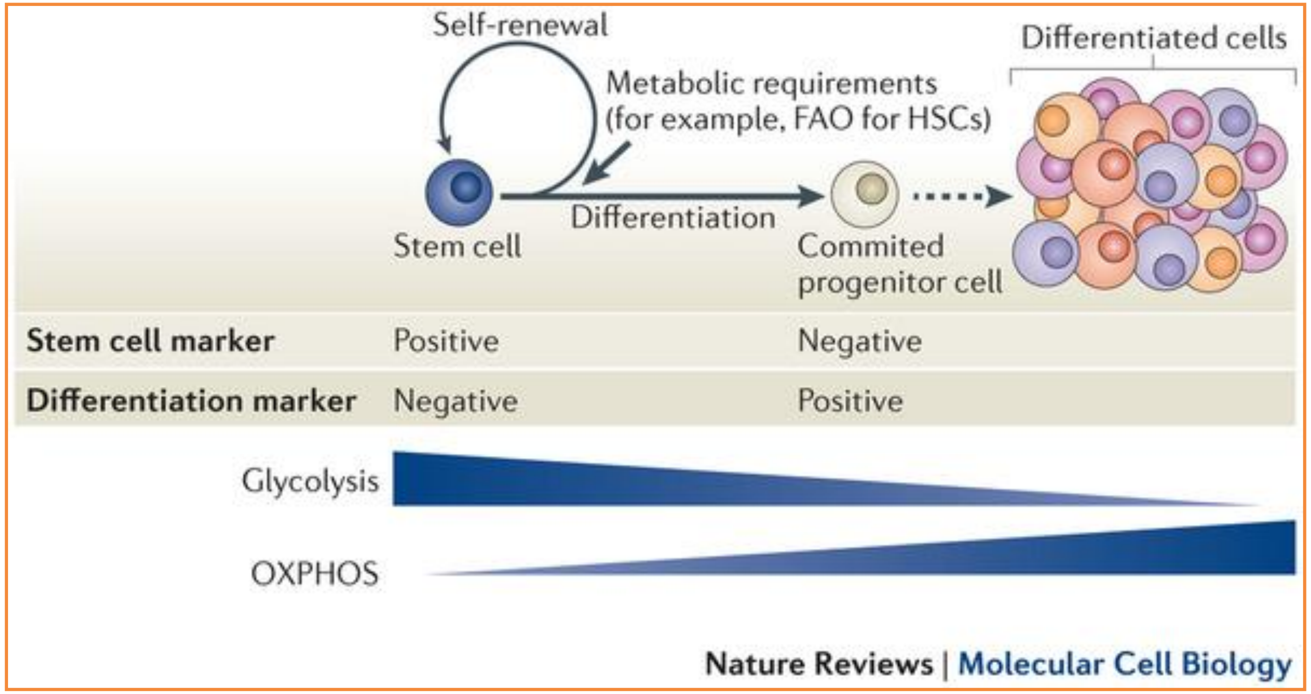


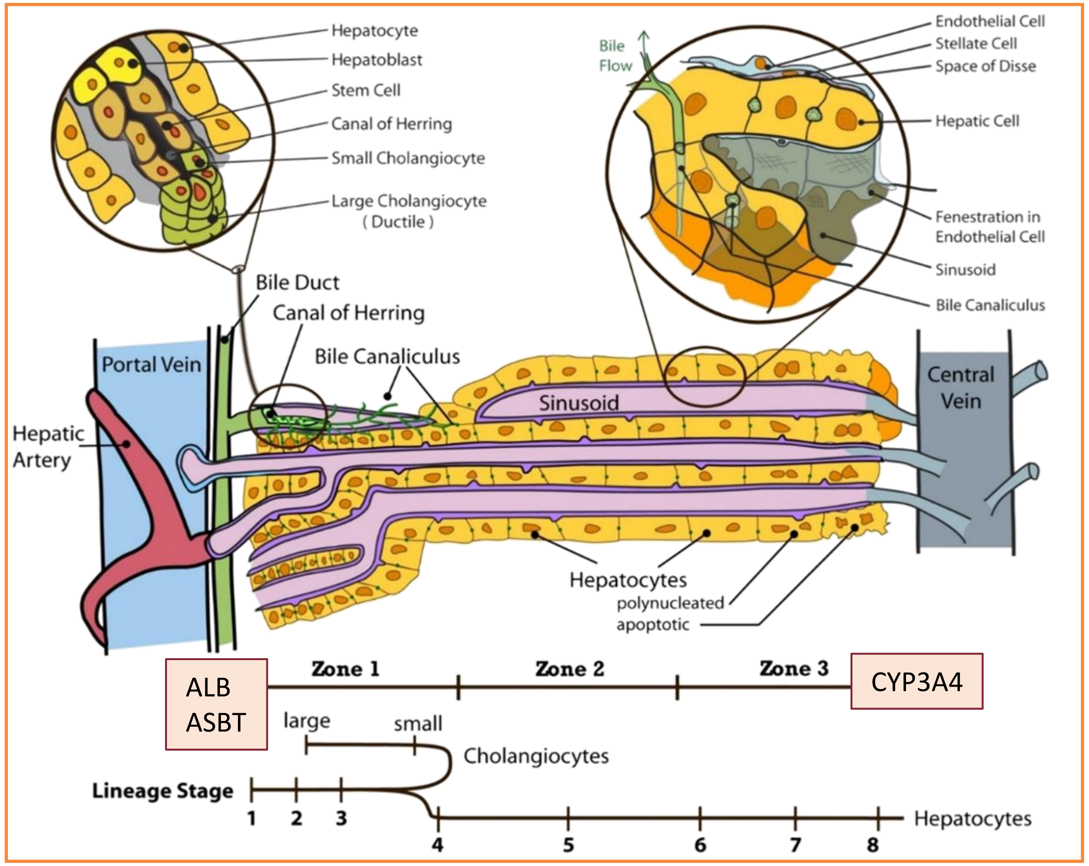
The metabolism of HepG2 that have been cultured in microgravity were significantly different from the metabolism of HepG2 in normogravity (p<0.05)

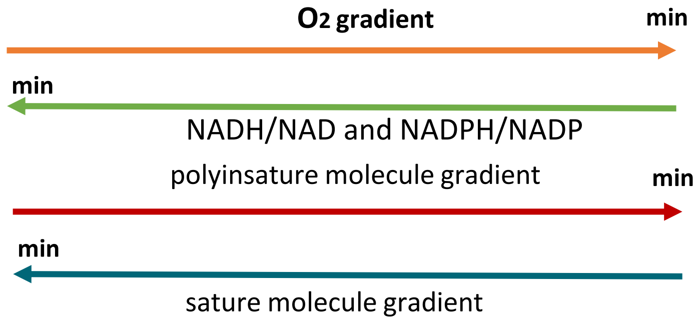
HepG2 in microgravity consumed more glucose and released a more amount of fermentation derivatives and glutammate when compared to the cells grown in normogravity.



Hepatic acinus zonation







Differentiation is associated to:

- a development of the mytochondrial network amd cristae
- a metabolic shift to prevalent oxidative phosphorilation (OXPHOS)

Ito et al. (2014)

Yanes et al., Nat Chem Biol 2010

RESULTS:
Summary

1. Microgravity favors the organization of hBTSCs in tridimensional clusters.

2. Microgravity favors the maintenance of stemness features and counteracts the differentiation of hBTSCs toward mature hepatocytes;

3. The effects of microgravity on hBTSCs are associated with a metabolic shift to glycolysis and to the detriment of OXPHOS.

Perspectives

Regenerative medicine:
--Microgravity could help the generation and maintainance of tridimensional cultures of pluripotent stem cells to be used for regenerative medicine;

Implications:
--Identifyng molecular and biologic mechanisms associated with the maintenance of stemness in microgravity could help the identification of putative terapeutical target to modulate stem cell differentiation.

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Thank you for attention

study
effect
differentiation
Microgravity
hBTSC
RMN
gene expression
organoids
liver
biliary tree
in vitro
stemness
model

The progression of liver diseases

Normal → Cirrhosis