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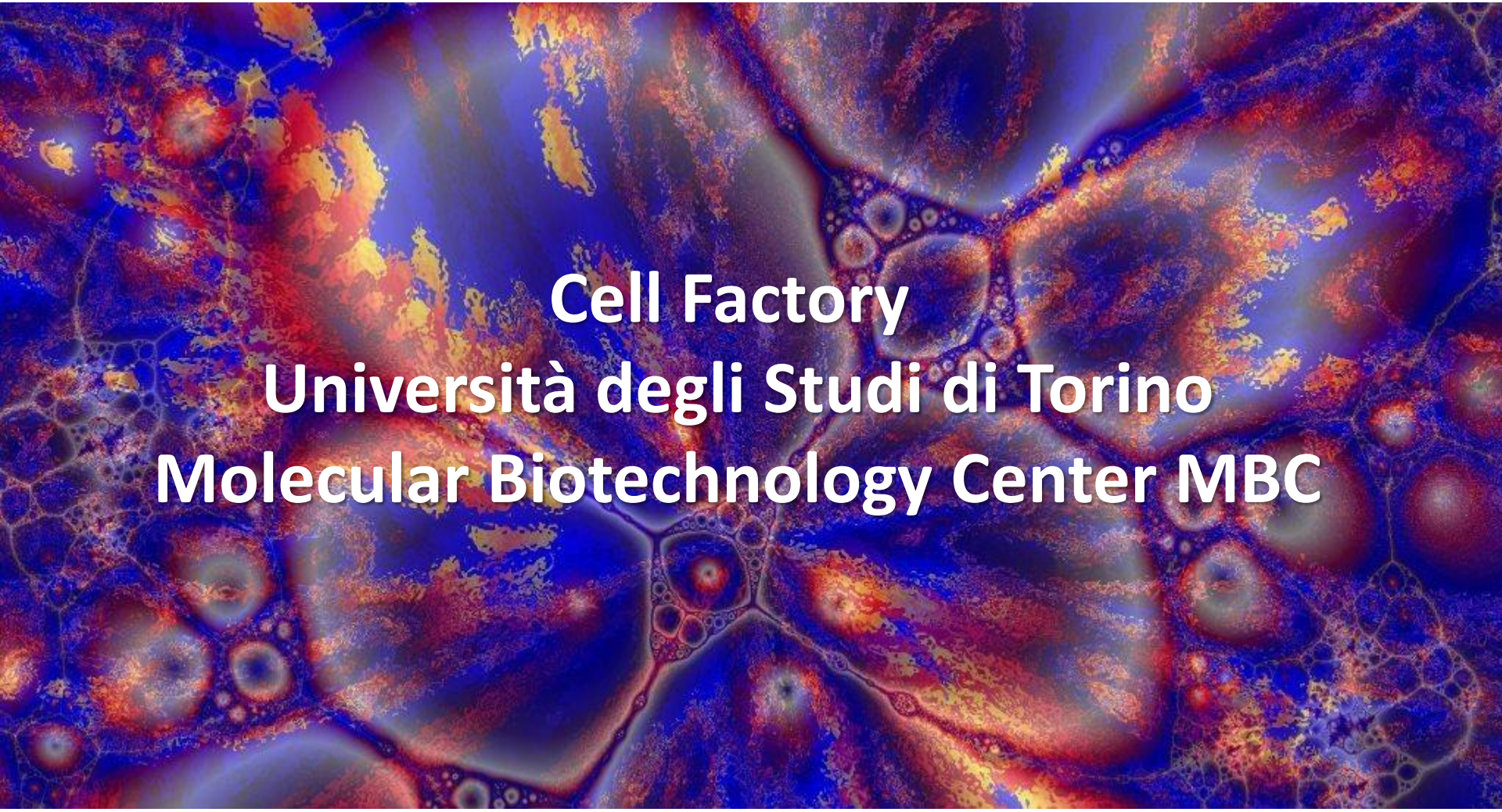
M B C
Molecular Biotechnology Center

UNIVERSITÀ DEGLI STUDI DI TORINO



Cell Factory
Università degli Studi di Torino
Molecular Biotechnology Center MBC

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From the discovery…

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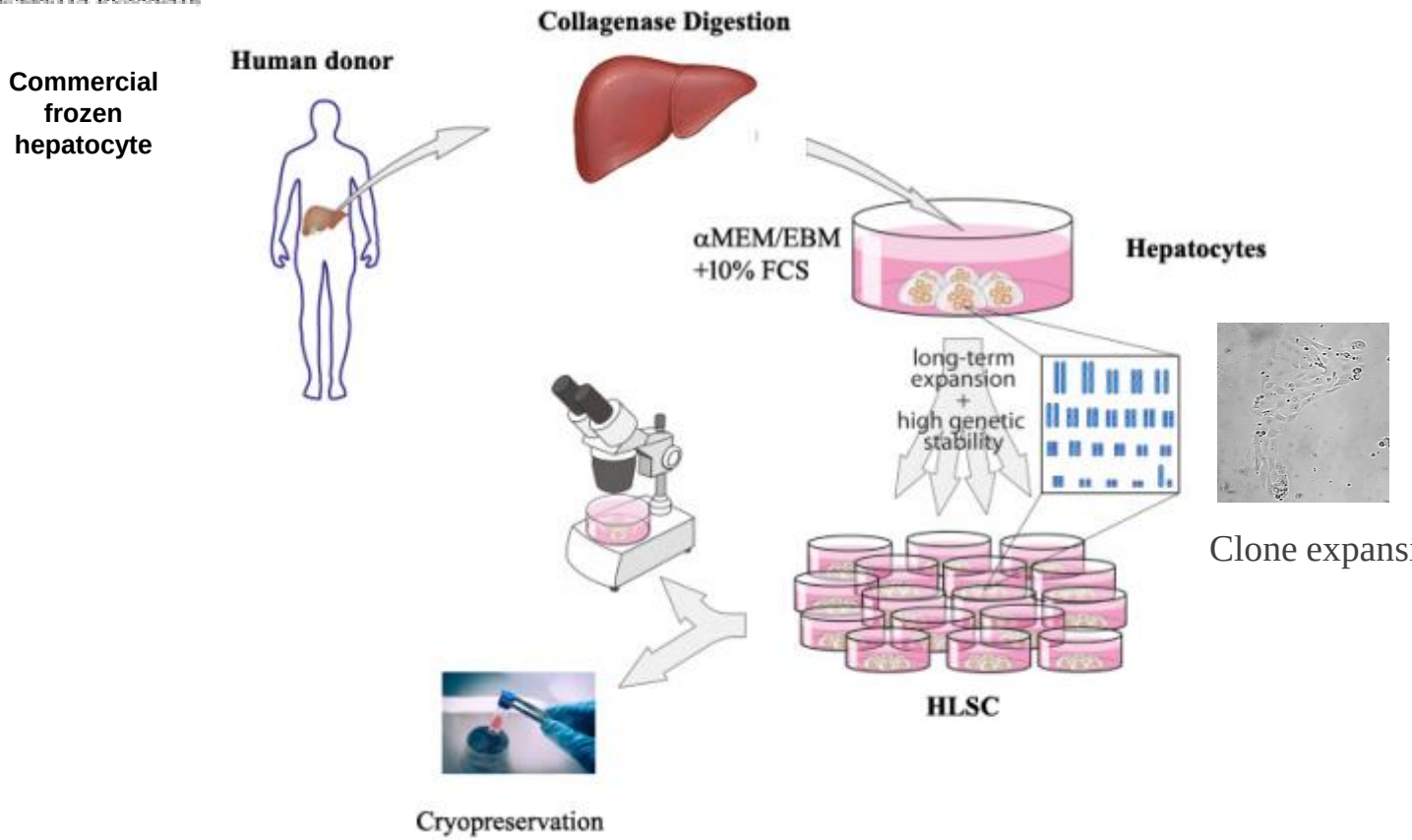
STEM CELLS
TISSUE-SPECIFIC STEM CELLS

Isolation and Characterization of a Stem Cell Population from Adult Human Liver

MARIA BEATRIZ HERRERA,[a,b] STEFANIA BRUNO,[a,b] STEFANO BUTTIGLIERI,[a,b] CIRO TETTA,[c] STEFANO GATTI,[d] MARIA CHIARA DEREGIBUS,[a,b] BENEDETTA BUSSOLATI,[a]



Clone expansion

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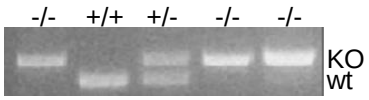
HLSC therapy in immunocompromised Crigler-Najjar mouse

HLSC injection prolongs NSG/Ugt1$^{-/-}$ mice survival by expressing UGT1A1

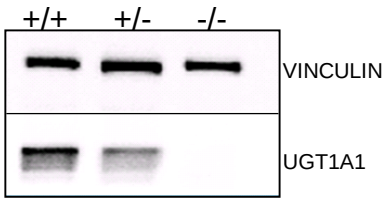
2 days old pups

5 days old pups

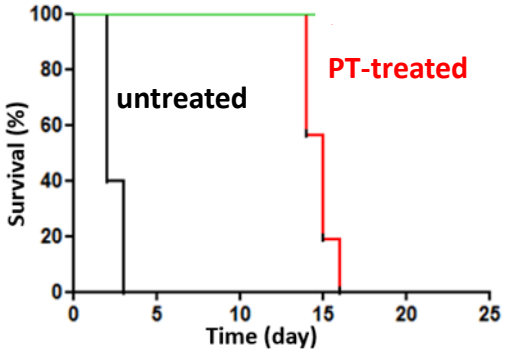
PCR



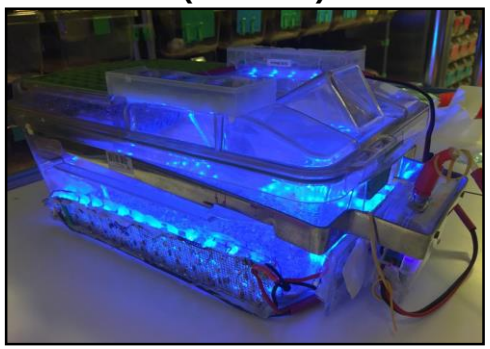
Western Blot



NSG/Ugt1$^{-/-}$ mice survival



Sterile cages with blue light (λ 450nm)



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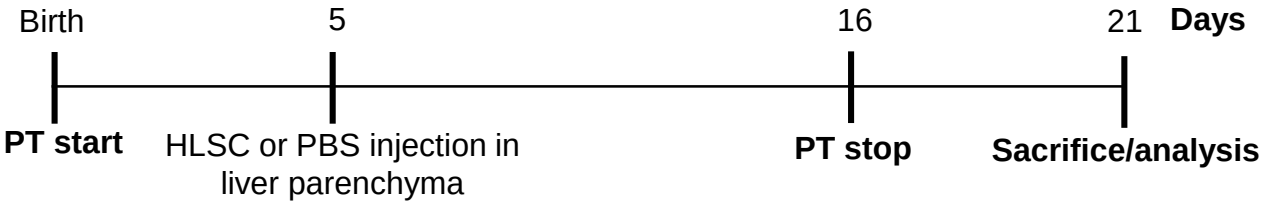
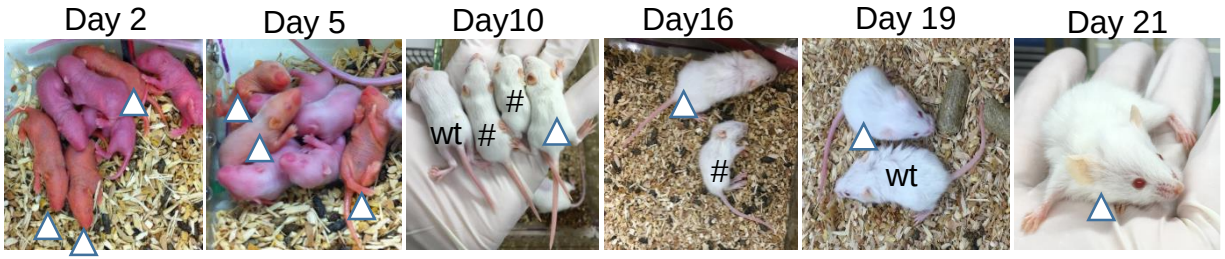
HLSC injection prolongs NSG/Ugt1$^{-/-}$ mice survival by expressing UGT1A1

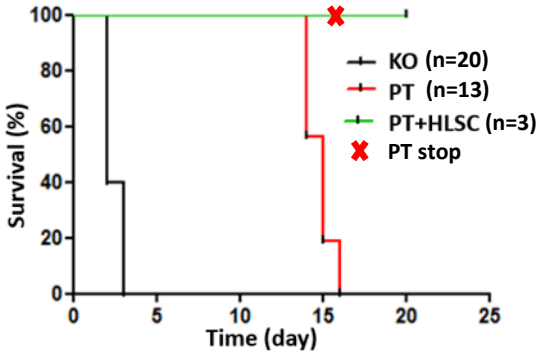




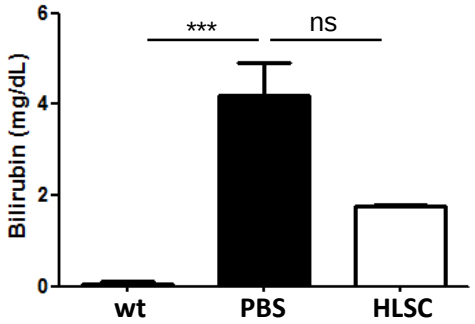
[redacted]

[redacted]

NSG/Ugt1$^{-/-}$ mice survival



Total bilirubin level



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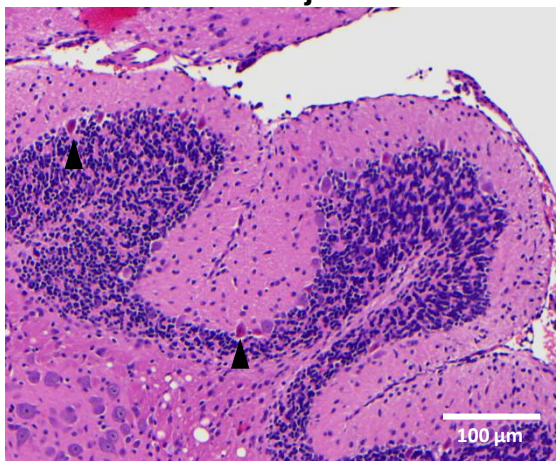
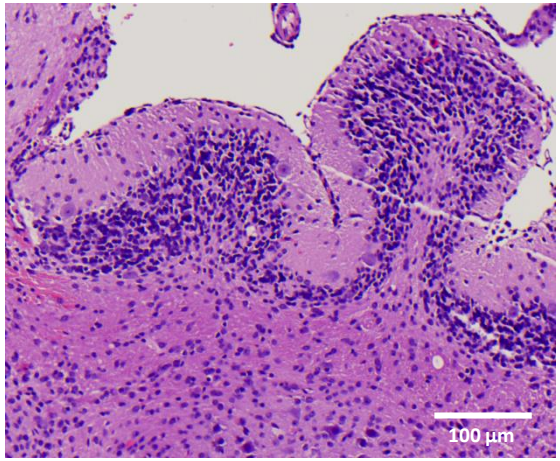
HLSC injection reduces brain injury in NSG/Ugt1$^{-/-}$ mice

Analysis of mouse cerebellum

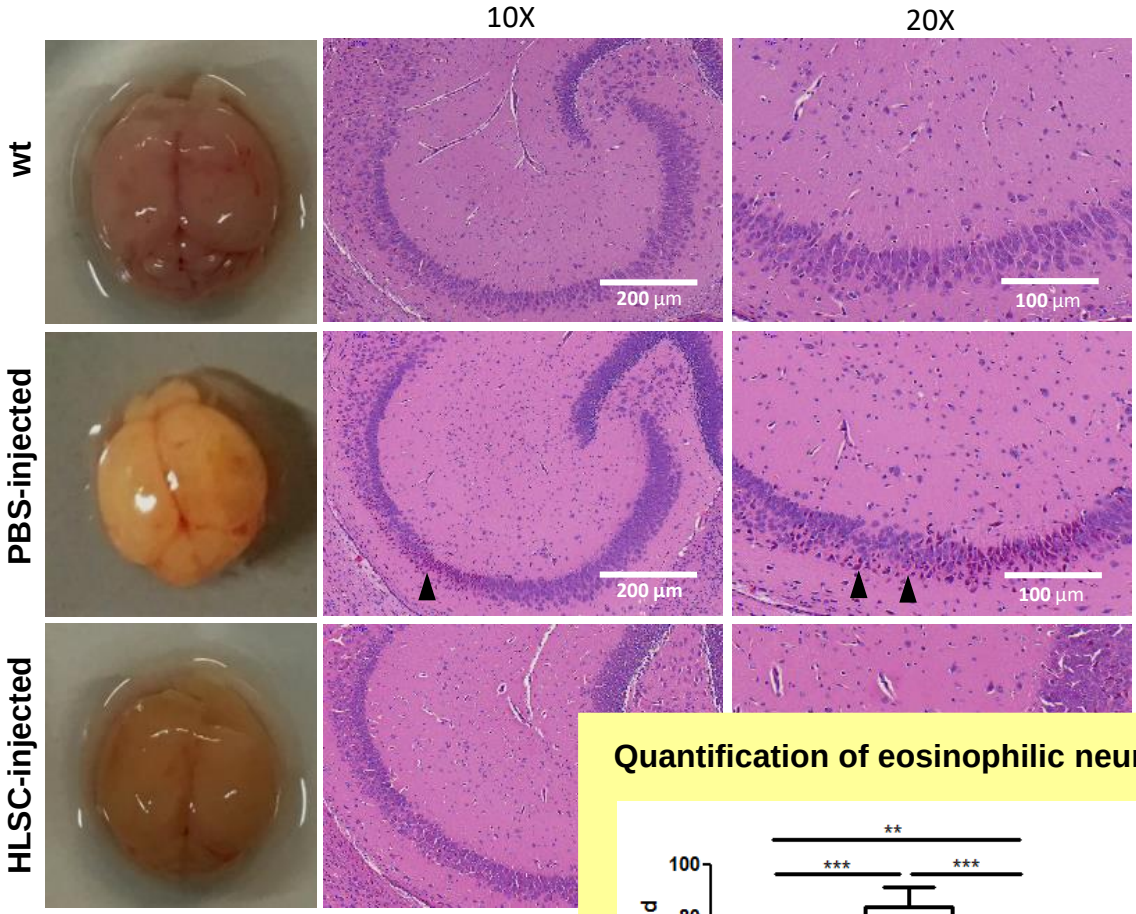
PBS-injected



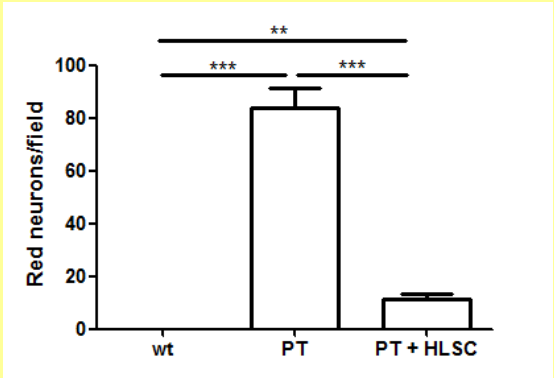
HLSC-injected



Analysis of mouse hippocampus



Quantification of eosinophilic neurons



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Conclusion

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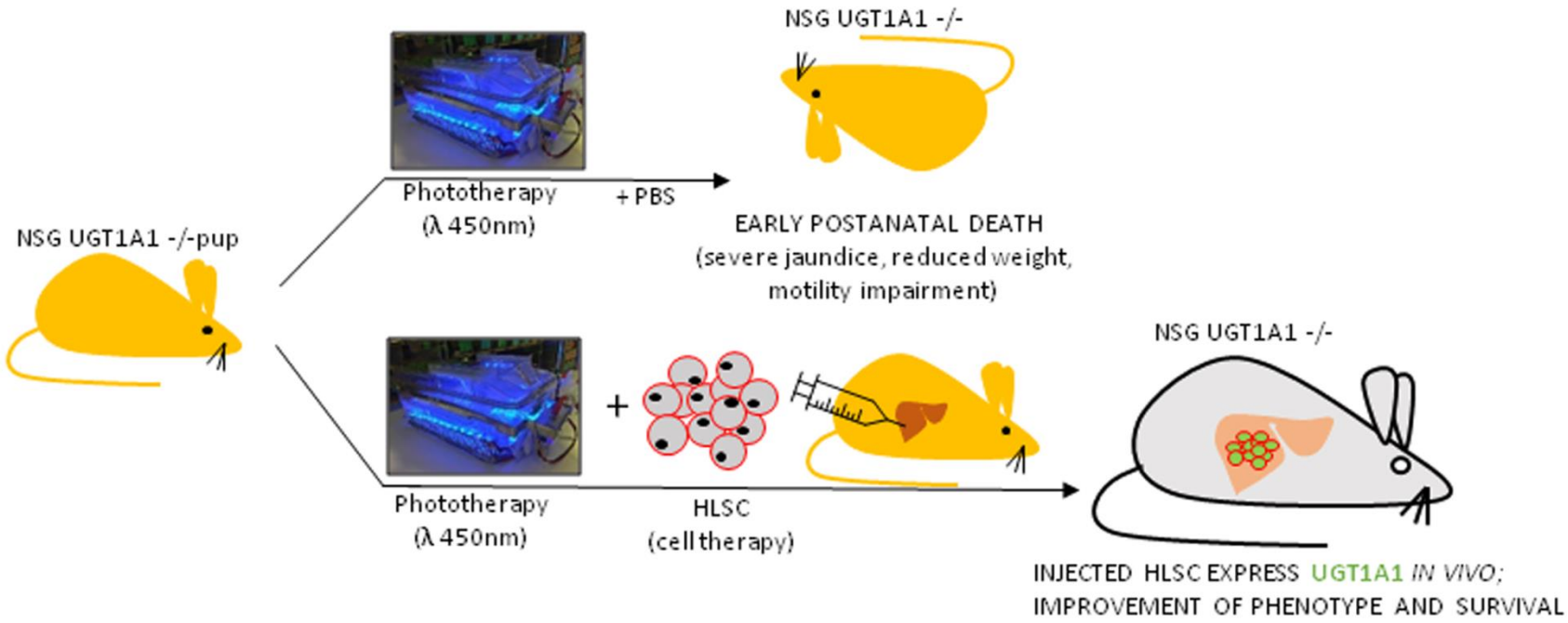
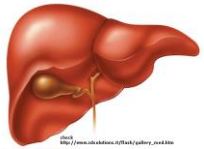
Our results show that a single HLSC injection ameliorates the phenotype and survival of NSG/Ugt1$^{-/-}$ mice by differentiating into UGT1A1-expressing hepatocyte-like cells with UGT1A1 enzyme activity



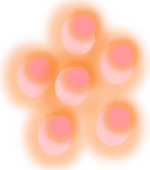
HLSC could develop into a therapeutic opportunity for pediatric patients with CNSI as well as other metabolic disorders of the liver

Famulari, S., …….Fagoonee, S*. and Altruda, F*. manuscript in revision (* equally contribution)

…to the clinic: UCD Process
Urea cycle disorders in newborn



Enzymatic dissociation



hepatocytes

α-MEM/EBM
+ 10%FCS
+ hEGF + hFGF

areta international

MCB

3 T175

12 T175

1° expansion 1:4

60 T175

2° expansion 1:5

DP

Harvest
Freezing

areta international

TO DATE CLINICAL APPLICATION OF HLSC

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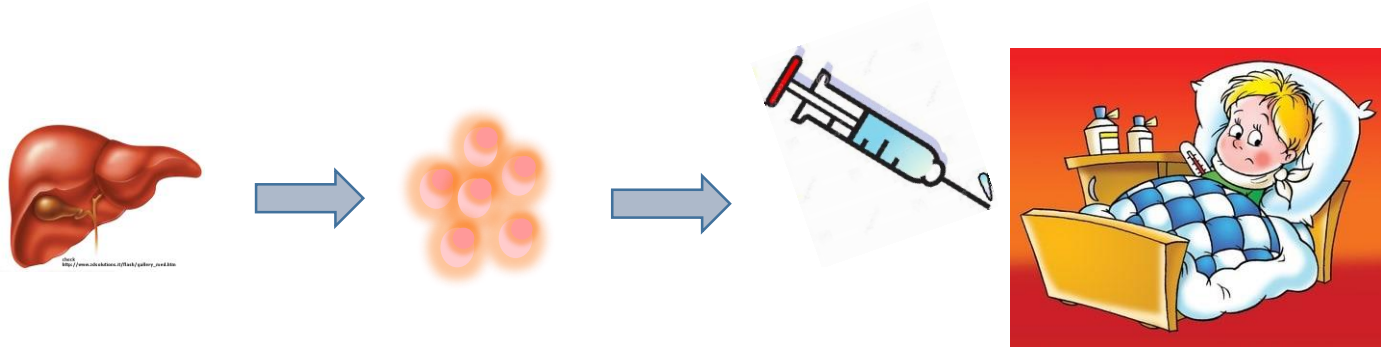


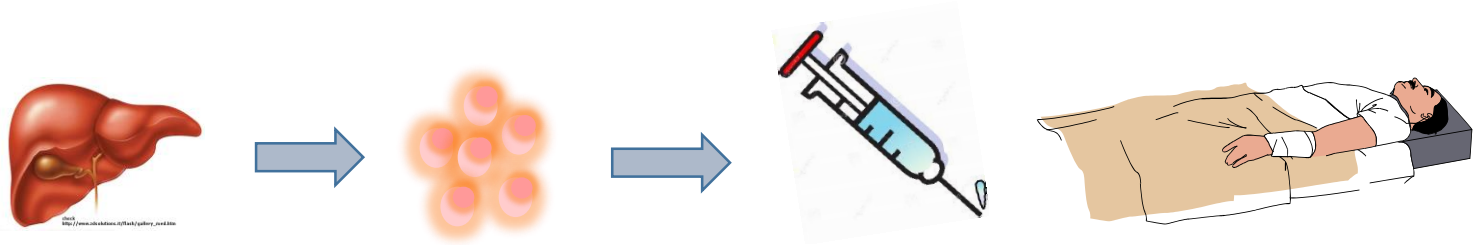
Phase I clinical study *(CONCLUDED)*: "Human Liver Stem Cells (HLSCs) in patients suffering from inborn liver metabolic diseases causing life-threatening neonatal-onset of hyperammonemic encephalopathy". (Clinical Study Protocol N°: HLSC01-11; EudraCT 2012-002120-33)

areta international

PI: Dott. Marco Spada

SPONSOR

TO DATE CLINICAL APPLICATION OF HLSC



Phase I clinical study: “Human Liver Stem Cells (HLSCs) in adult patients affected by Acute Liver Failure (ALF) and ineligible for liver transplantation”. (Clinical Study Protocol N°: HLSC01-14; EudraCT 2014-003889-24)

PI: Prof. Renato Romagnoli

SPONSOR

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Layout CLF

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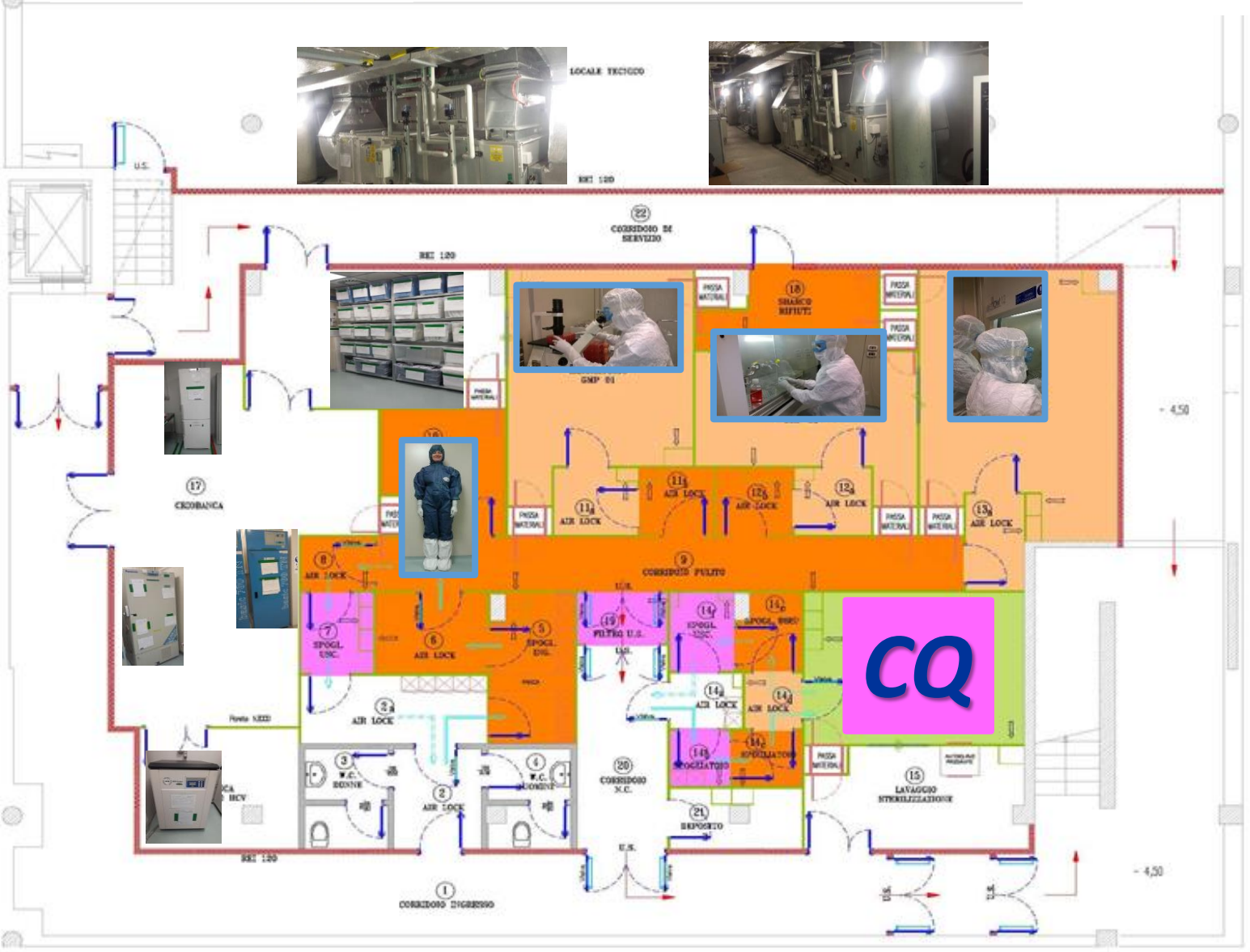
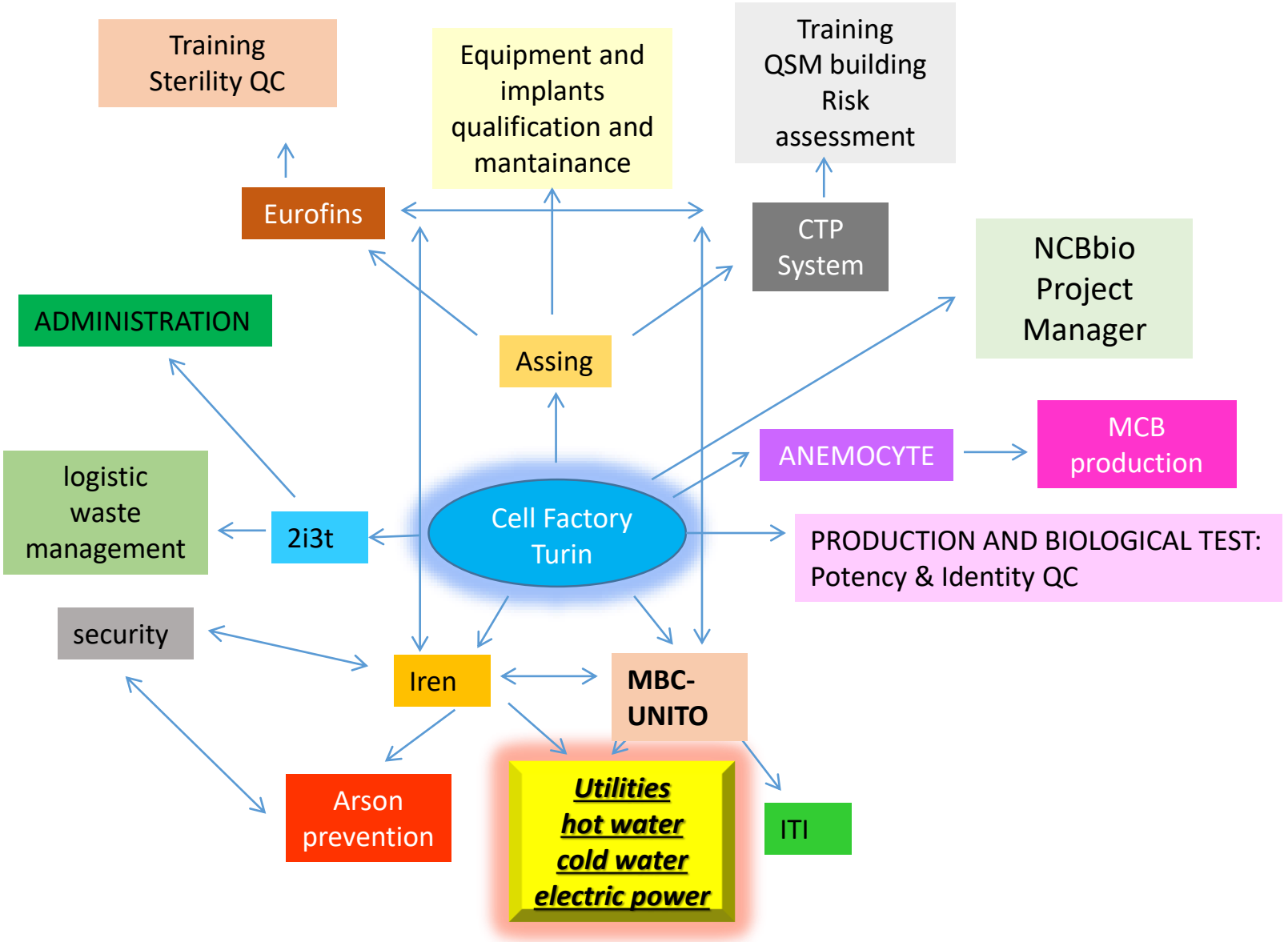


CLF network


Training
Sterility QC
Equipment and implants qualification and mantainance
Training
QSM building
Risk assessment
Eurofins
CTP System
NCBbio Project Manager
ADMINISTRATION
Assing
logistic waste management
2i3t
Cell Factory Turin
ANEMOCYTE
MCB production
PRODUCTION AND BIOLOGICAL TEST: Potency & Identity QC
security
Iren
MBC-UNITO
Arson prevention
Utilities
hot water
cold water
electric power
ITI

Organization chart

RAPPRESENTANTE LEGALE CELL FACTORY
Prof. Gianmaria Federico Ajani
(Magnifico Rettore Unito)

SITE MANAGER CELL FACTORY
Prof.ssa Fiorella Altruda
(Direttore Molecular Biotechnology Center MBC-Unito)

VICE SITE MANAGER CELL FACTORY
Prof. Lorenzo Silengo
(Presidente Molecular Biotechnology Center MBC-Unito)

DIREZIONE AMMINISTRATIVA CELL FACTORY
Prof. Silvio Aime
(Rappresentante Legale 2i3T)

RESPONSABILE FINANZIARIO CELL FACTORY
Dott. Giuseppe Serrao
(Responsabile Operativo 2i3T)

QUALIFIED PERSON (QP) CELL FACTORY
Dott.ssa Valentina Fonsato

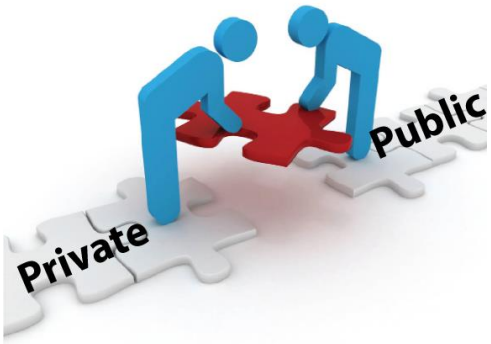
QUALITY ASSURANCE (QA) CELL FACTORY
Dott.ssa Michela De Lena

DIRETTORE DI PRODUZIONE (PRD) CELL FACTORY
Dott. Federico Figliolini

DIRETTORE CONTROLLO DI QUALITÀ (QC) CELL FACTORY
Dott.ssa Chiara Pasquino

OPERATORI QUALIFICATI SETTORE PRODUZIONE (OpQ PR) CELL FACTORY
Dott.ssa Claudia Cavallari
Dott.ssa Rossella Selvaggio
Dott.ssa Veronica Dimuccio

OPERATORI QUALIFICATI SETTORE CONTROLLO DI QUALITÀ (OpQ CQ) CELL FACTORY
Dott.ssa Federica Guido
Dott.ssa Marta Tapparo



CNSI: approches to treatment

- phototherapy

- Cell therapy (primary hepatocytes, induced pluripotent stem cells)
- Gene therapy (AAV-based)

} Bridging therapy

- **Liver transplantation**

Our approach: Human liver stem cell (HLSC)-based therapy

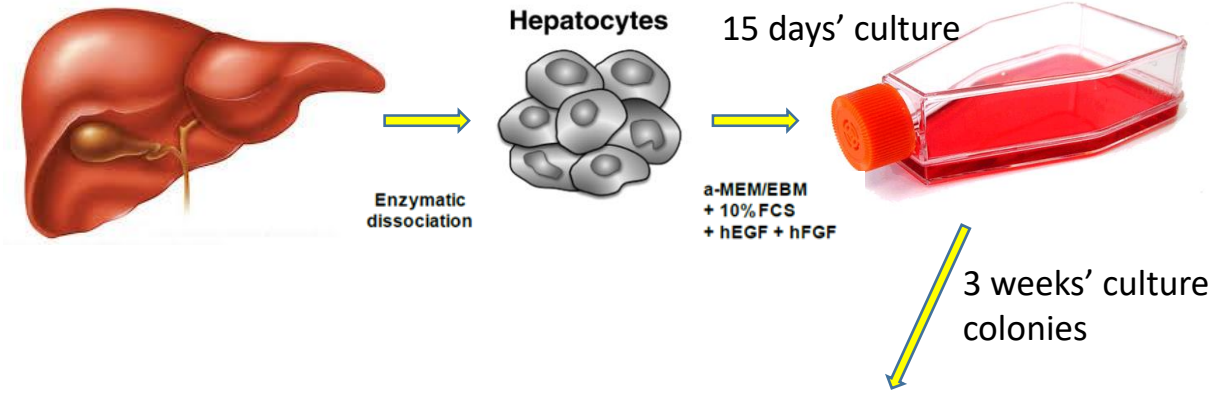
Why stem cells?

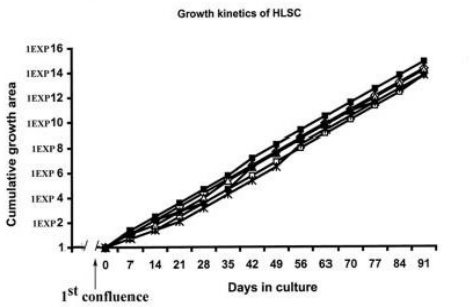
- Stem cells can be cryopreserved and thawed with no loss in proliferation or differentiation capacity w.r.t. differentiated cells
- obtain a permanent expression of the wild type gene in mutant livers with the final goal of achieving organ repopulation and long-term recovery of liver functions.
- Adult stem cells→ less risk of tumorigenicity

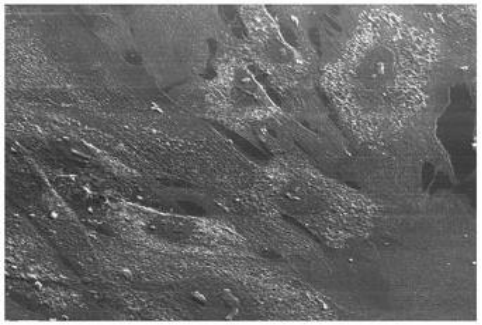
Crigler-Najjar Syndrome Type 1 (CNSI)

- Rare monogenic disease affecting 1/1000 000 persons
- Caused by deficiency in the only enzyme responsible for bilirubin conjugation in the liver, uridine-diphosphate (UDP)-glucuronosyltransferase (UGT) 1A1
- Normal liver, but patients die of bilirubin-induced neurotoxicity

Our approach: Human liver stem cell (HLSC)-based therapy



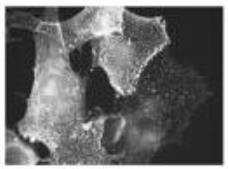




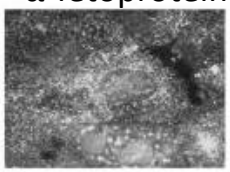
Human liver stem cells (HLSC)

Hepatocyte markers+

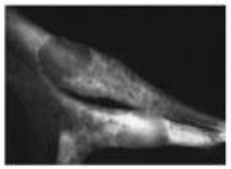
Albumin



α-fetoprotein

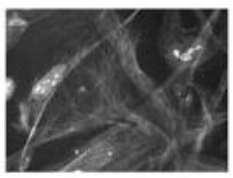


CK18

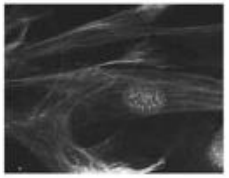


MSC markers+

Vimentin



Nestin



Herrera MB et al., Stem cells 2006

Generation of a new immunodeficient Crigler-Najjar mouse

Ugt1a1+/- C57Bl/6

X

NOD/LtSz-scid
(NOD-scid IL2Rgamma(null)
NSG mice)

8 generation backcrosses
NSG/Ugt1+/- mice

NSG/Ugt1+/- mice X NSG/Ugt1+/- mice

NSG/Ugt1-/- mice