13TH PARIS HEPATOLOGY CONFERENCE (PHC) 2020
JANUARY 13, 2020-JANUARY 14, 2020

PATHOPHYSIOLOGY OF NAFLD AND NASH

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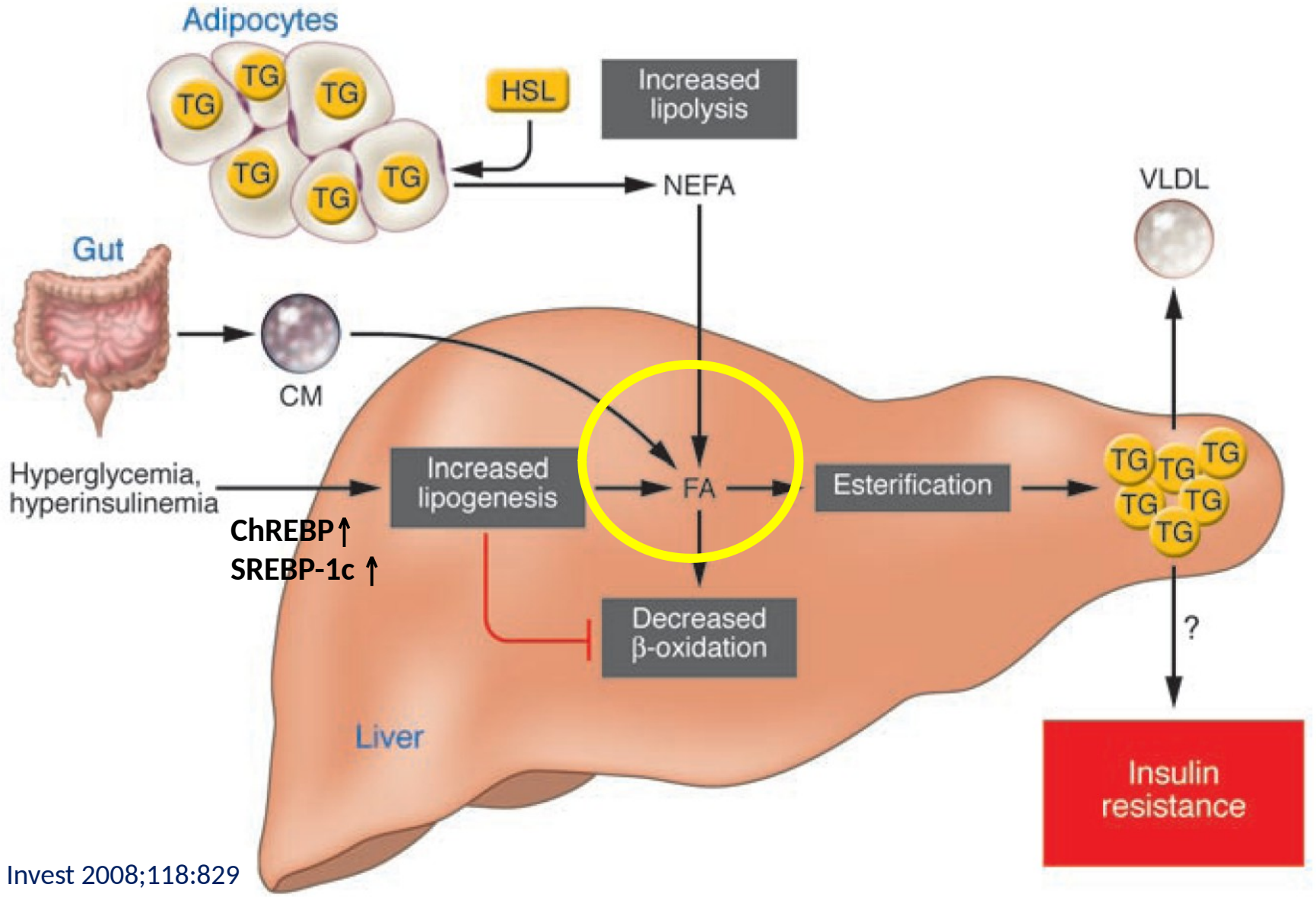
Disclosures (2020)

1. Inventor and patent holder ELF test (Siemens)
2. Speakers bureau: Echosens (Paris, France)
3. SAB/Consultancy: Promethera (Belgium); NeuroVive (Sweden); Chemomab (Israel); Median Technology (France/USA); Boheringer-Ingelheim (Germany); Takeda (USA)
4. Co-Founder and Director, Engitix Ltd (UCL Spin-out) (UK)*
5. Co-Founder and Director, 3P-Sense Ltd (UCL Spin-out) (UK)**
7. Chair EASL Consortium for Regenerative Hepatology (2019-2022)

* Regenerative medicine and tissue engineering

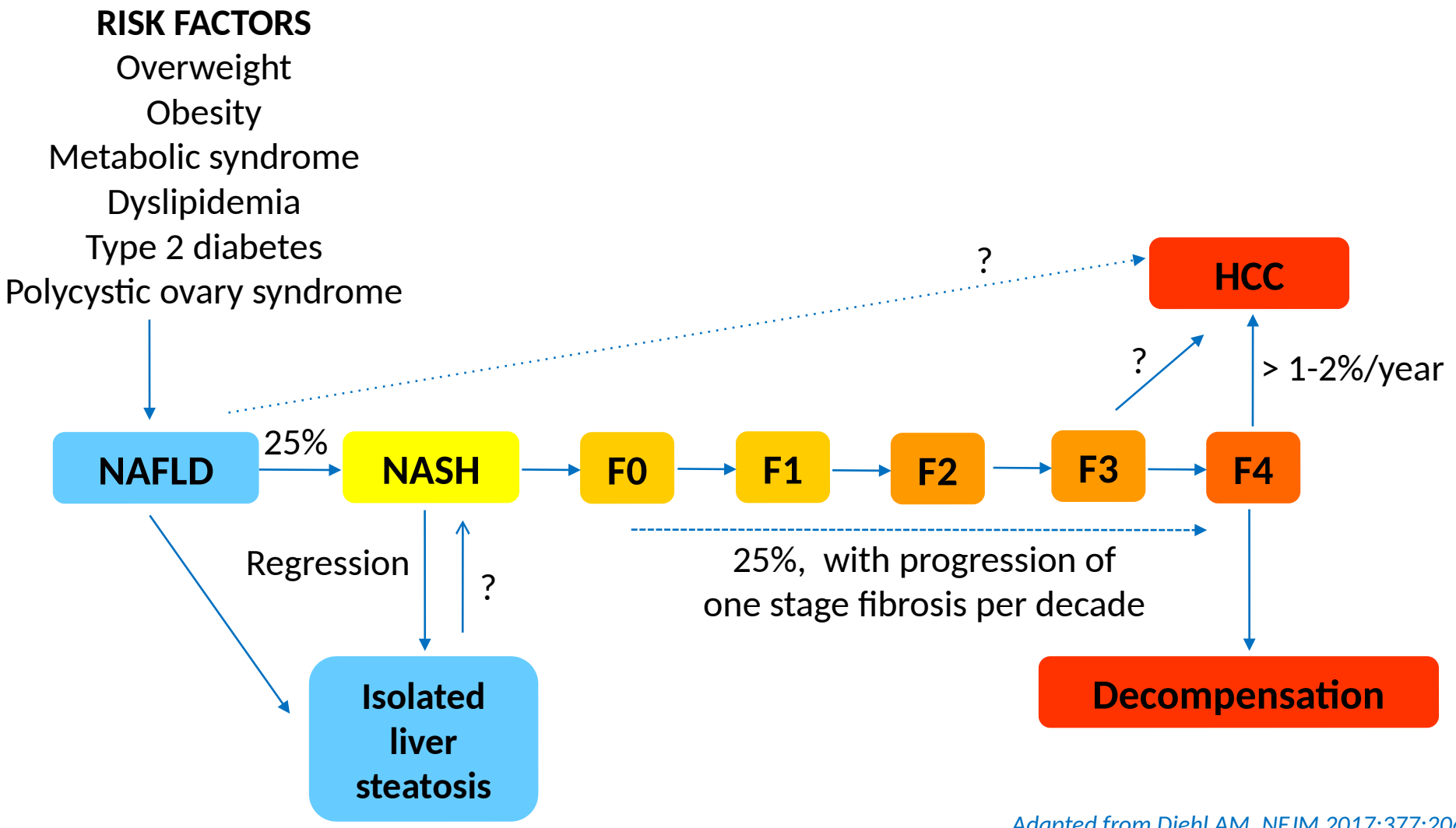
** Nanotechnology diagnostics

Metabolic Defects Leading to Steatosis



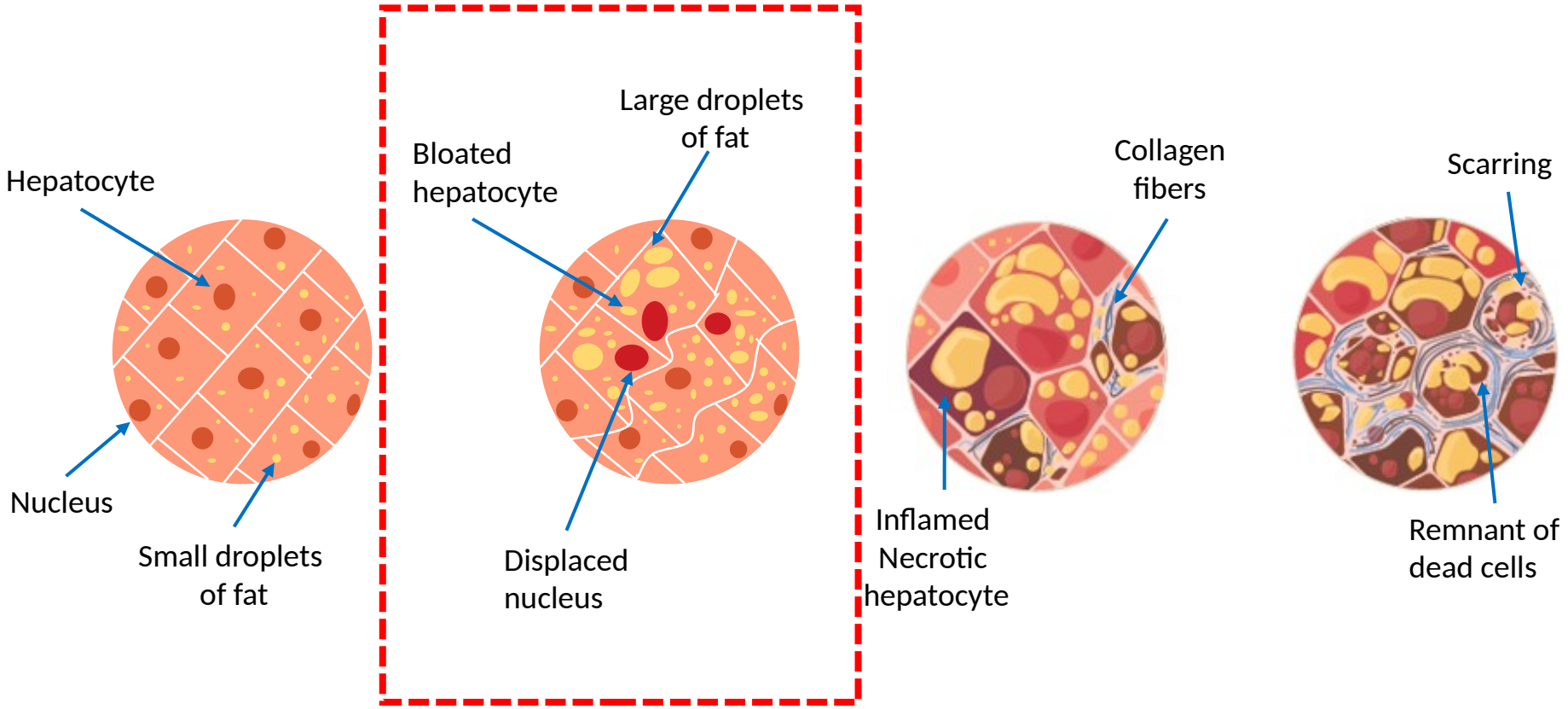
Postic and Girard, J Clin Invest 2008;118:829

NAFLD: Natural History



Adapted from Diehl AM. NEJM 2017;377:2063-72

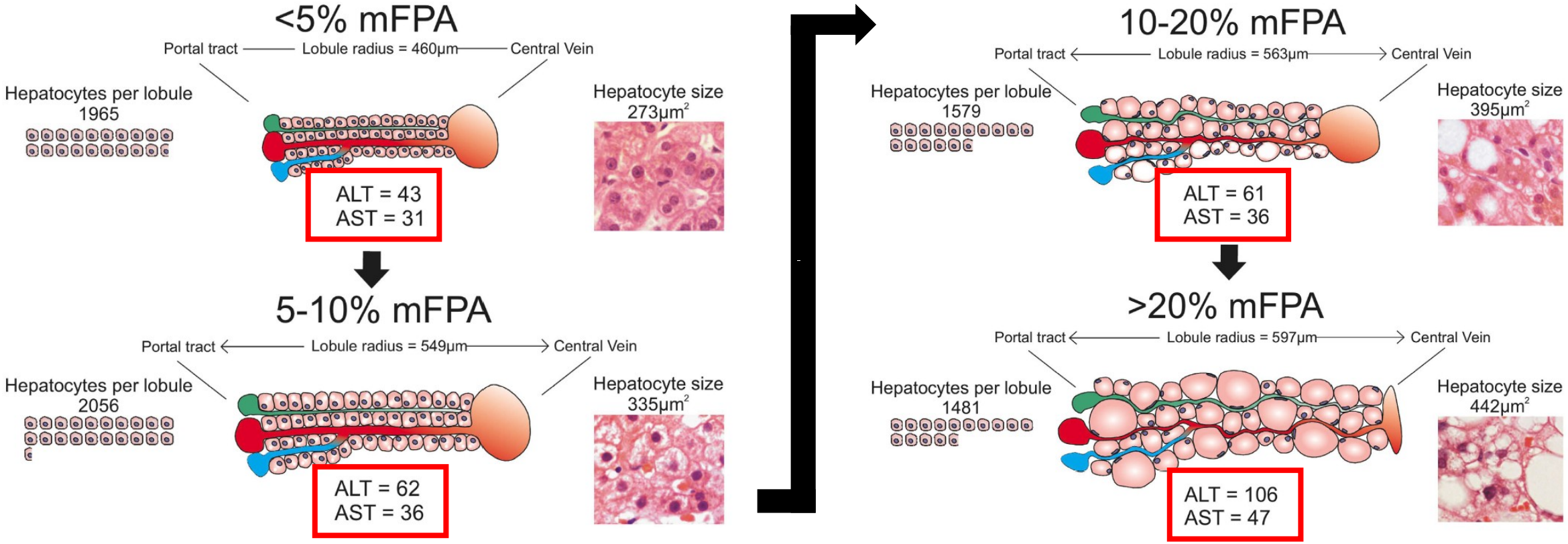
NAFLD: Natural History



ADAPTATION

PATHOLOGY

Adaptation of the Liver Lobule with Increasing Fat Accumulation



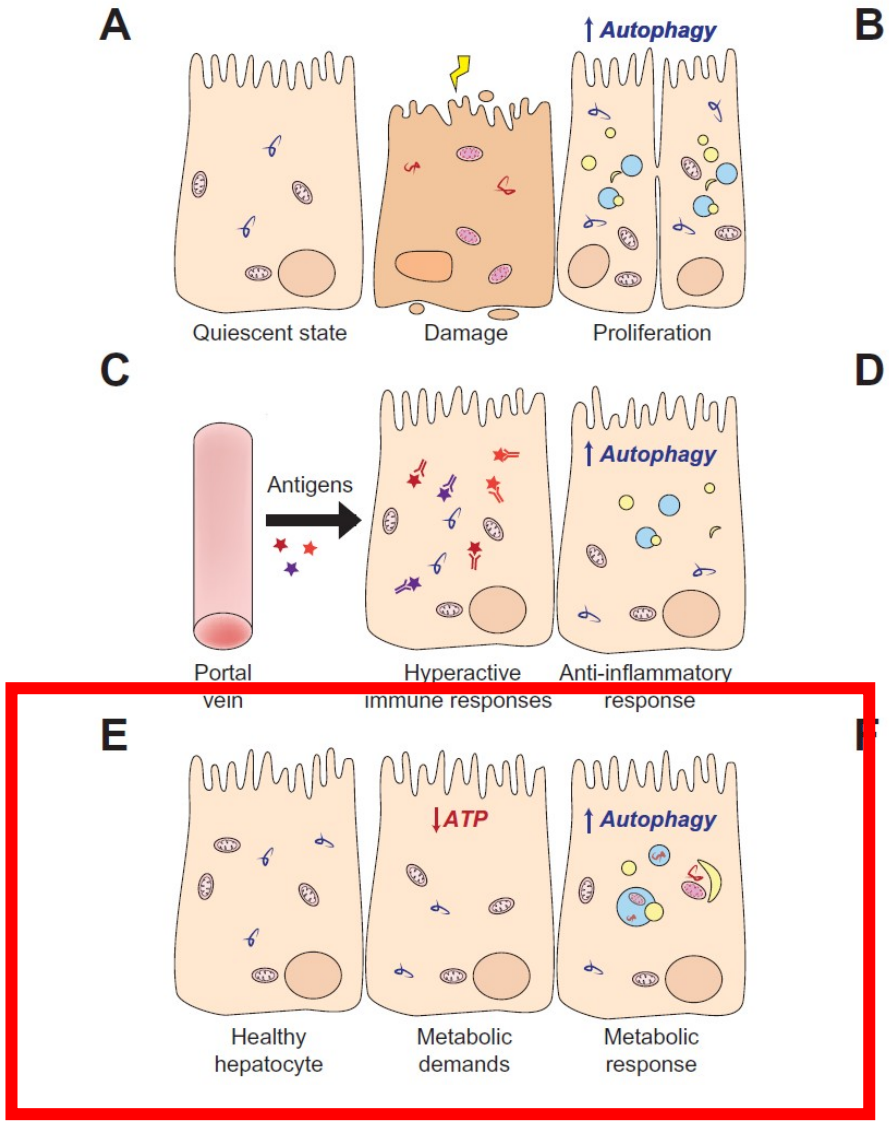
Micro-environmental predisposing condition to oxidative stress?
Role of Autophagy?

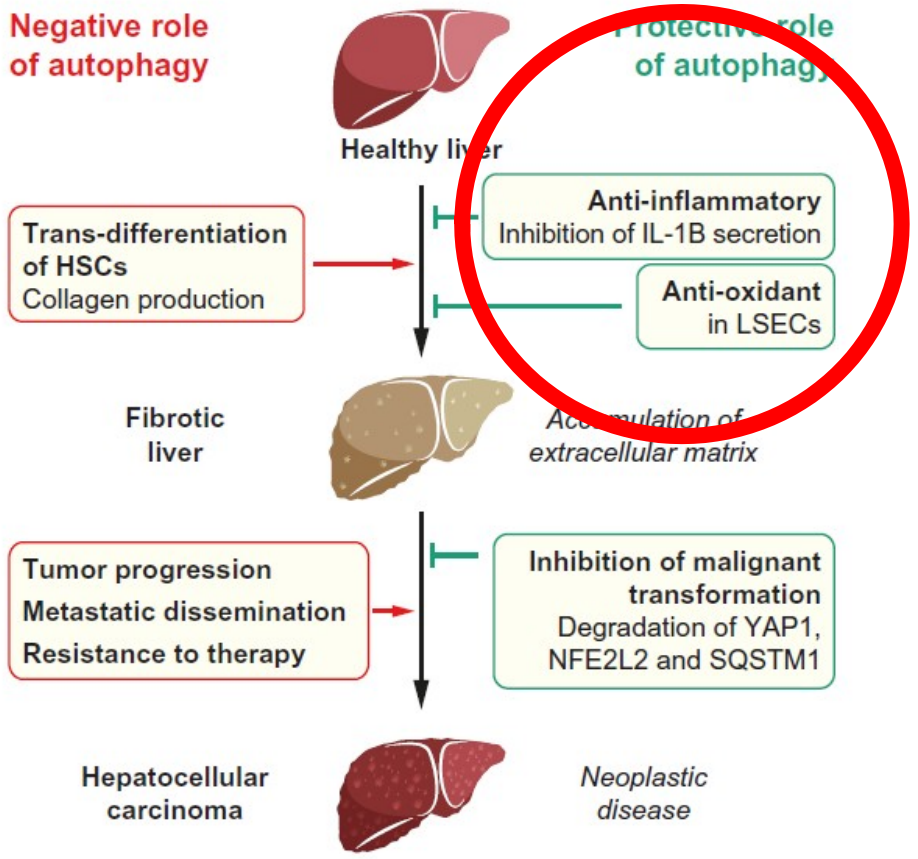
Hall A et al., Scientific Reports 2017

Journal of Hepatology **2020** vol. 72 | 183–196

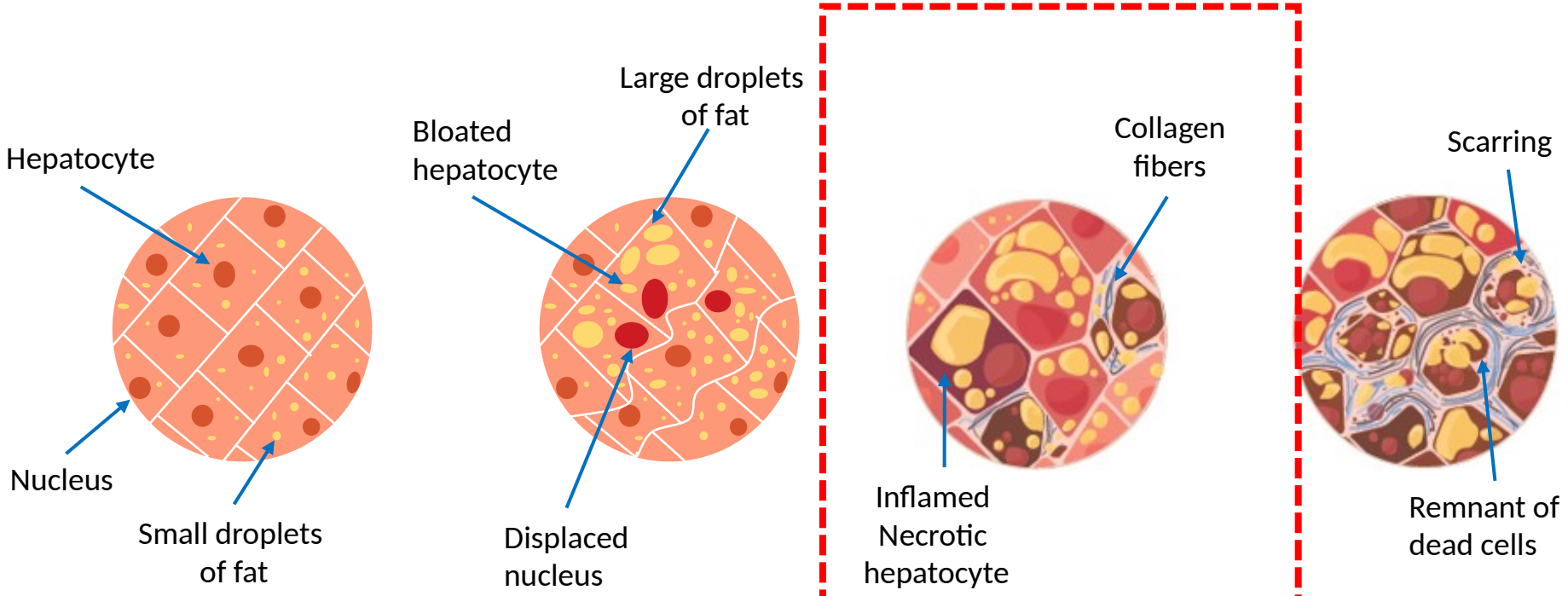
Autophagy in hepatic adaptation to stress

Younis Hazari[1,2,3], José Manuel Bravo-San Pedro[4], Claudio Hetz[1,2,3,5,*], Lorenzo Galluzzi[6,7,8,9,†], Guido Kroemer[4,9,10,11,12,13,*,†]

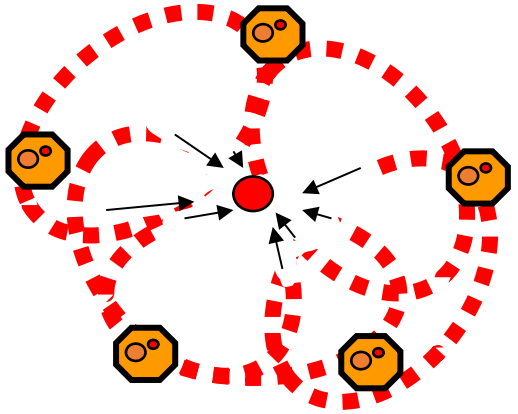
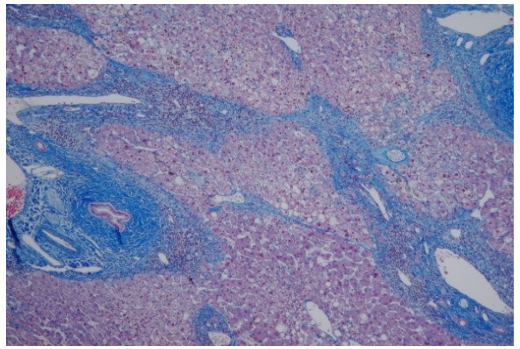
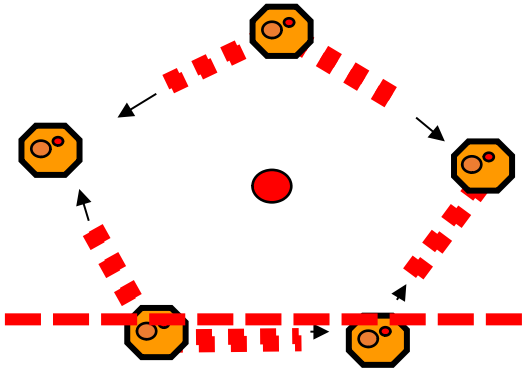
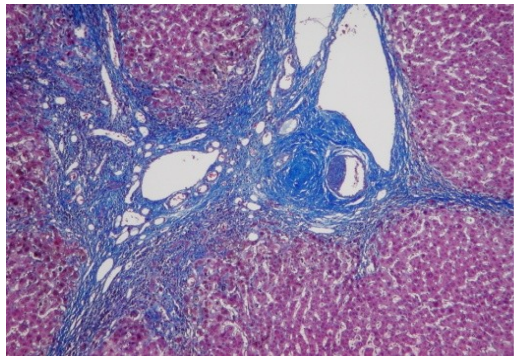
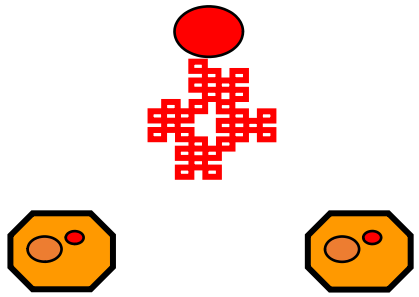
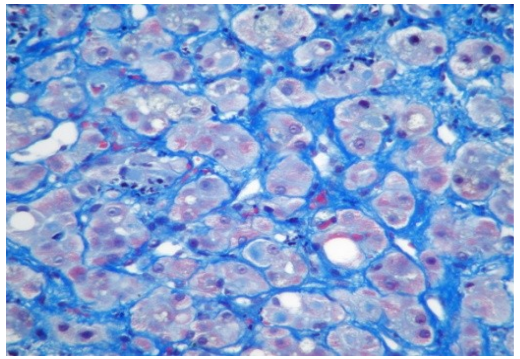




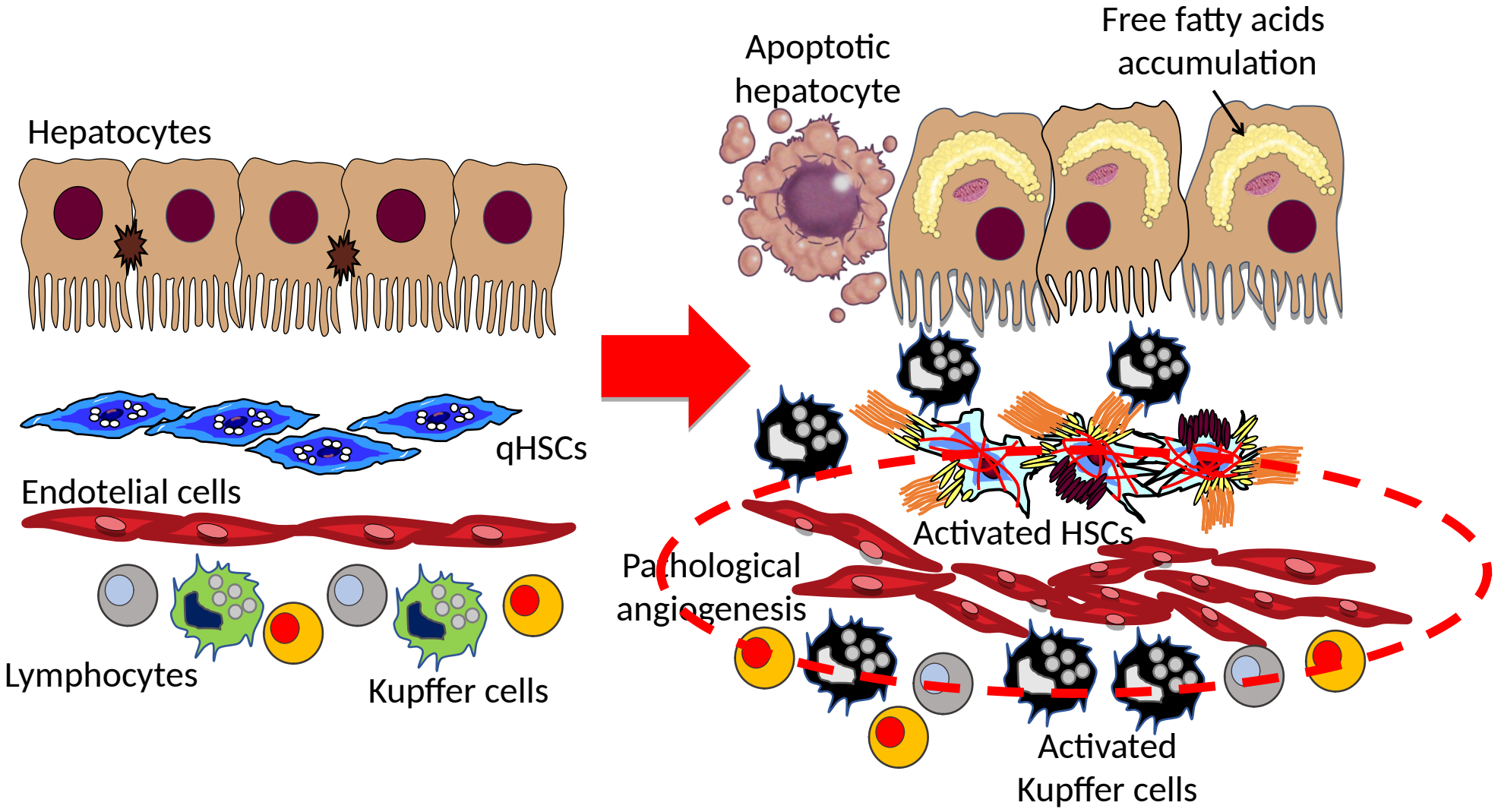
NAFLD: Natural History



ADAPTATION → PATHOLOGY

Type of Fibrosis	Pattern	Histology	Prevalent Mechanisms
Post-necrotic: Viral Hepatitis, Autoimmune Hepatitis			Chronic Wound Healing
Biliary: Primary Biliary Cirrhosis, Primary Sclerosing Cholangitis, Secondary Biliary Cirrhosis			Epithelial-Mesenchymal Disruption, Reactive Cholangiocytes, Bile salt toxicity. Deranged gut-liver immunity?
Pericellular: Alcoholic Steatohepatitis, Non Alcoholic Steatohepatitis (Haemochromatosis/ Wilson Disease)			Oxydative Stress, Reactive Aldehydes, Lipotoxicity

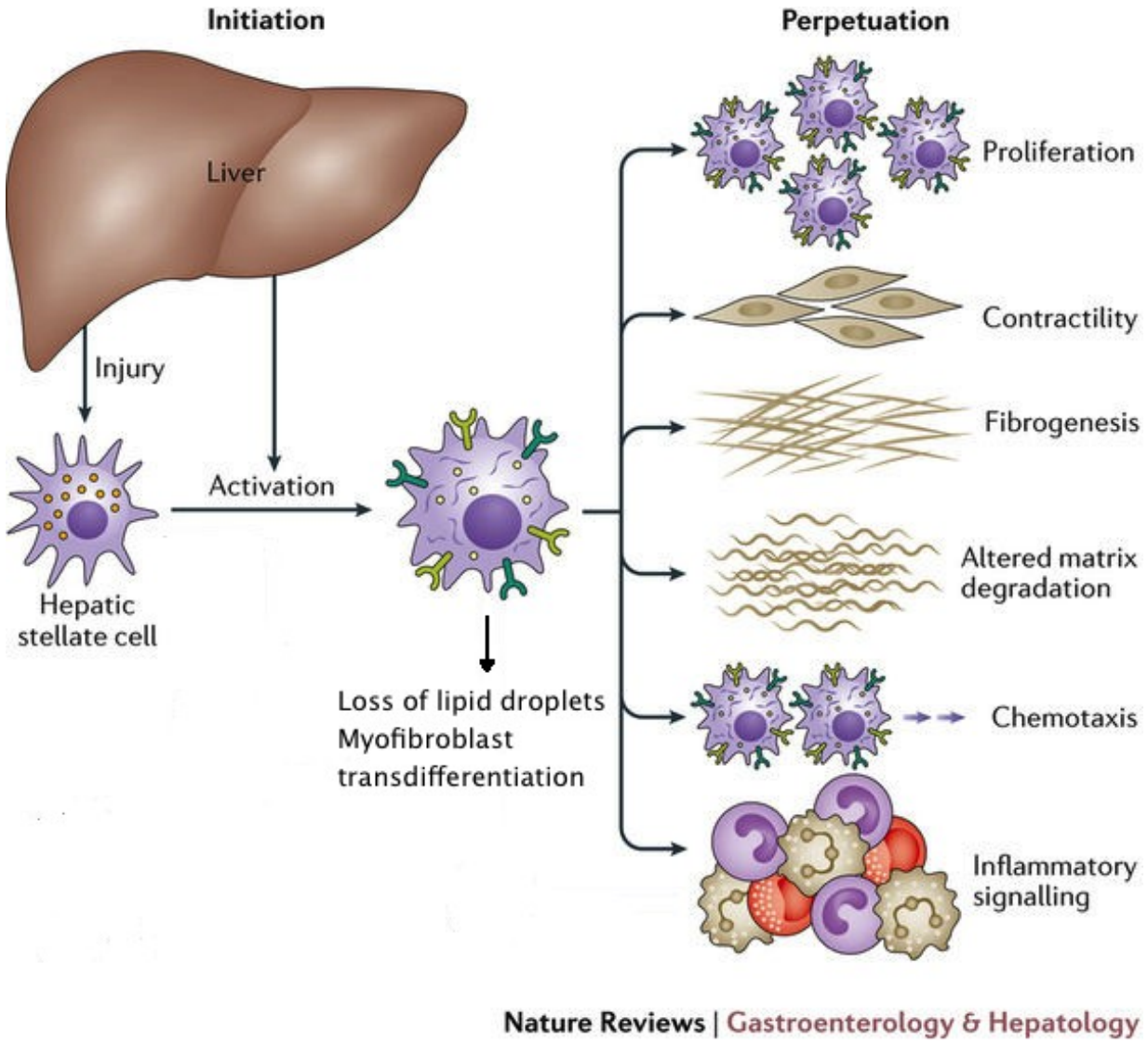
Pathogenesis of NASH



Normal Liver

NAFLD/NASH

Pathways of Stellate Cell Activation in Liver Injury



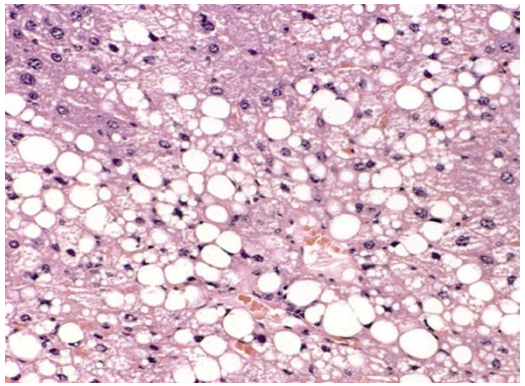
Increased tissue tension and stiffness

Changes in ECM quantity and relative composition

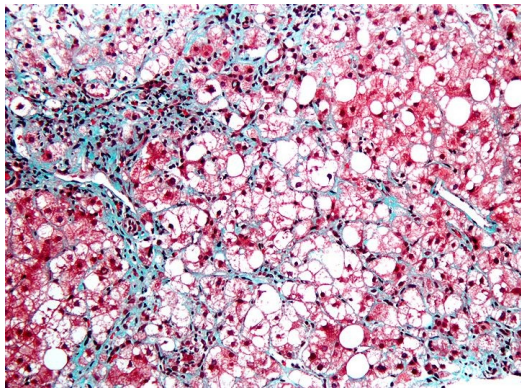
ECM as a bio-reservoir of pro-inflammatory and pro-angiogenic factors

Fibrotic Evolution of NASH

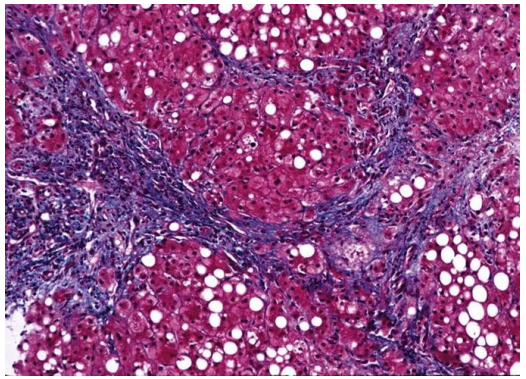
Steatosis (Fatty Liver)

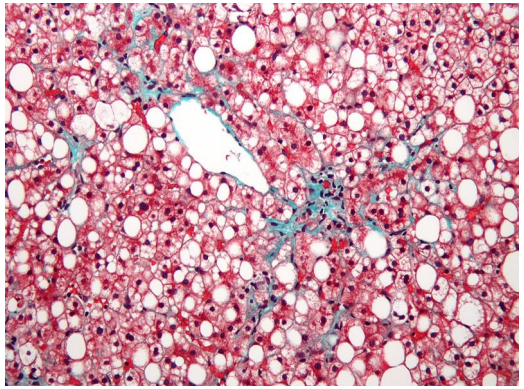


Steatohepatitis with bridging fibrosis



Cirrhosis



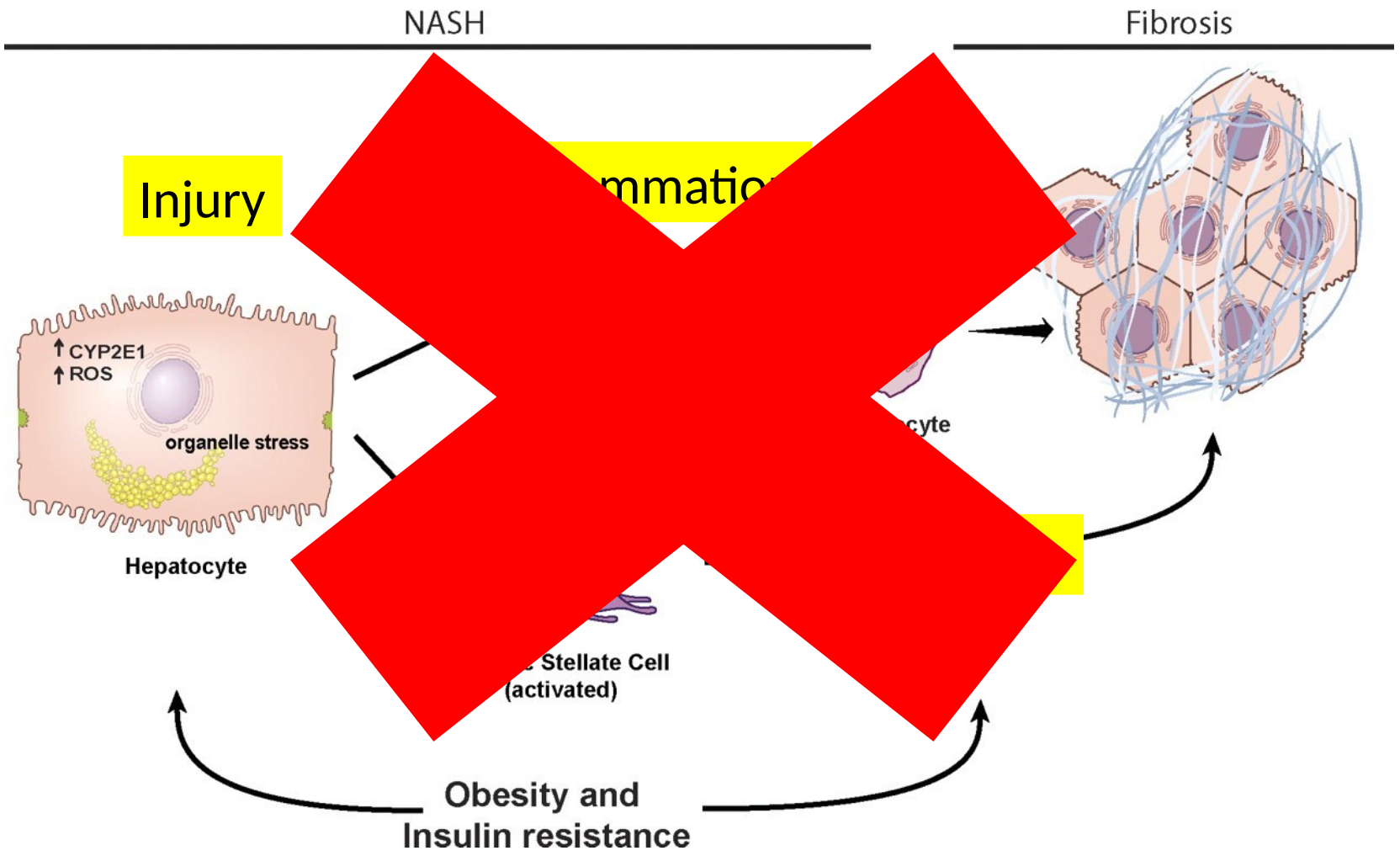


Steatosis with Pericellular Fibrosis

Steatofibrosis?

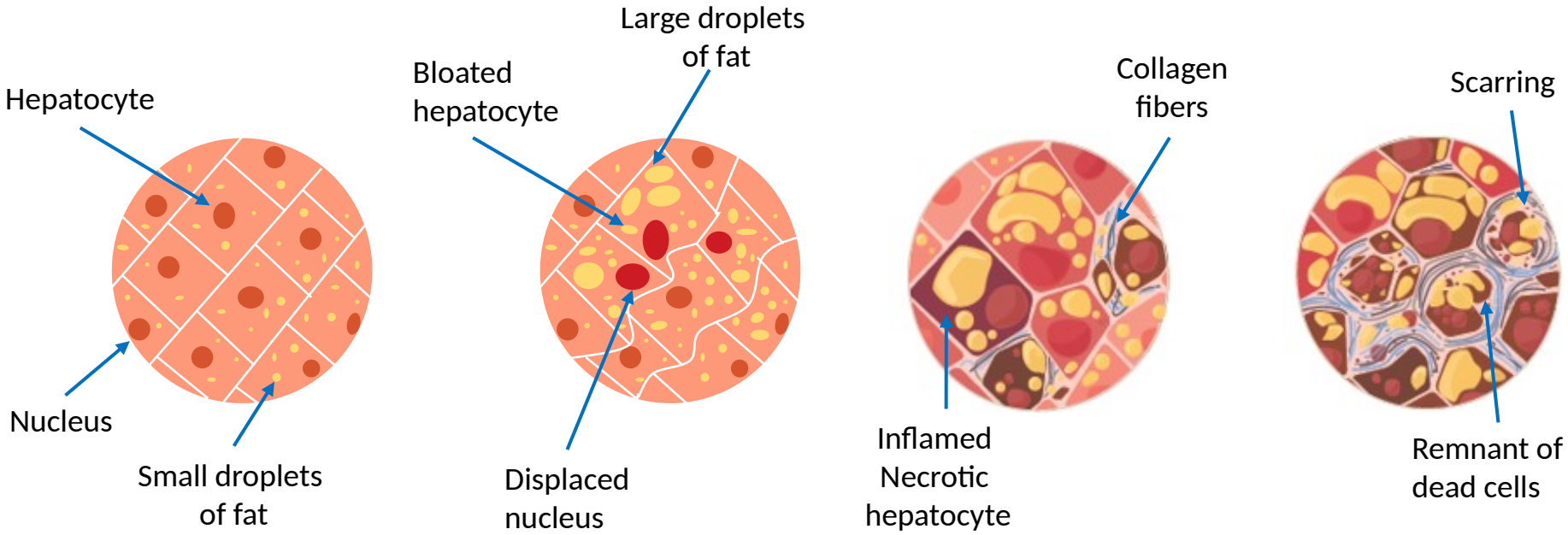
Fibrogenesis in NAFLD may be partially independent of cell necrosis and inflammation

Fibrosis in NASH: A Chronic Wound Healing Response?



Modified from Chiang et al., AJP-GI 2011

NASH Fibrosis: Stage-dependent Mechanisms



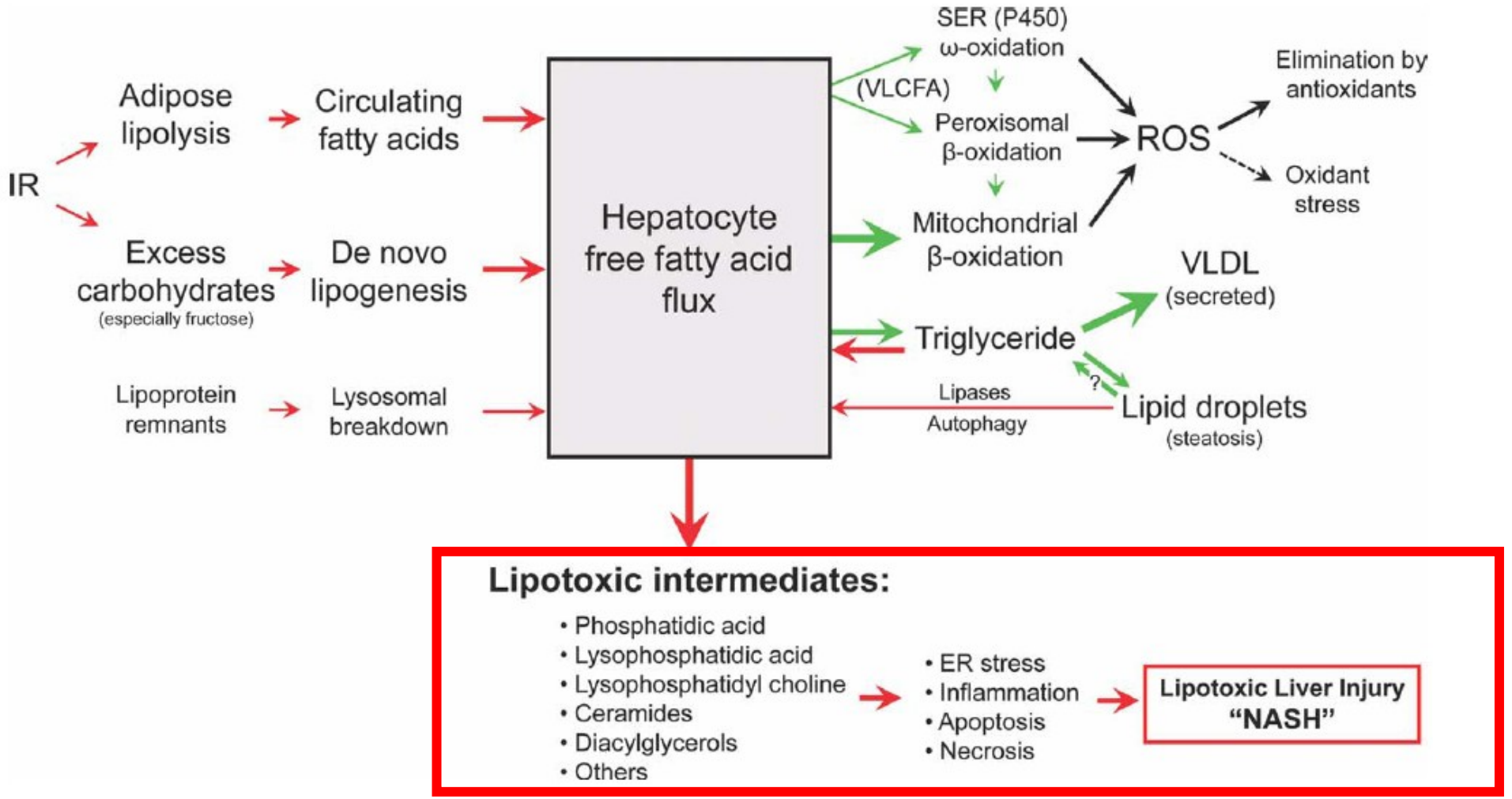
No evident necrosis

Defective Autophagy
LIPOTOXICITY
Oxidative Stress
Genetic factors

Evident necrosis

Chronic Wound Healing
Increase intestinal permeability
Complex inflammatory networks
Genetic factors

Pathways of Lipotoxic Liver Injury

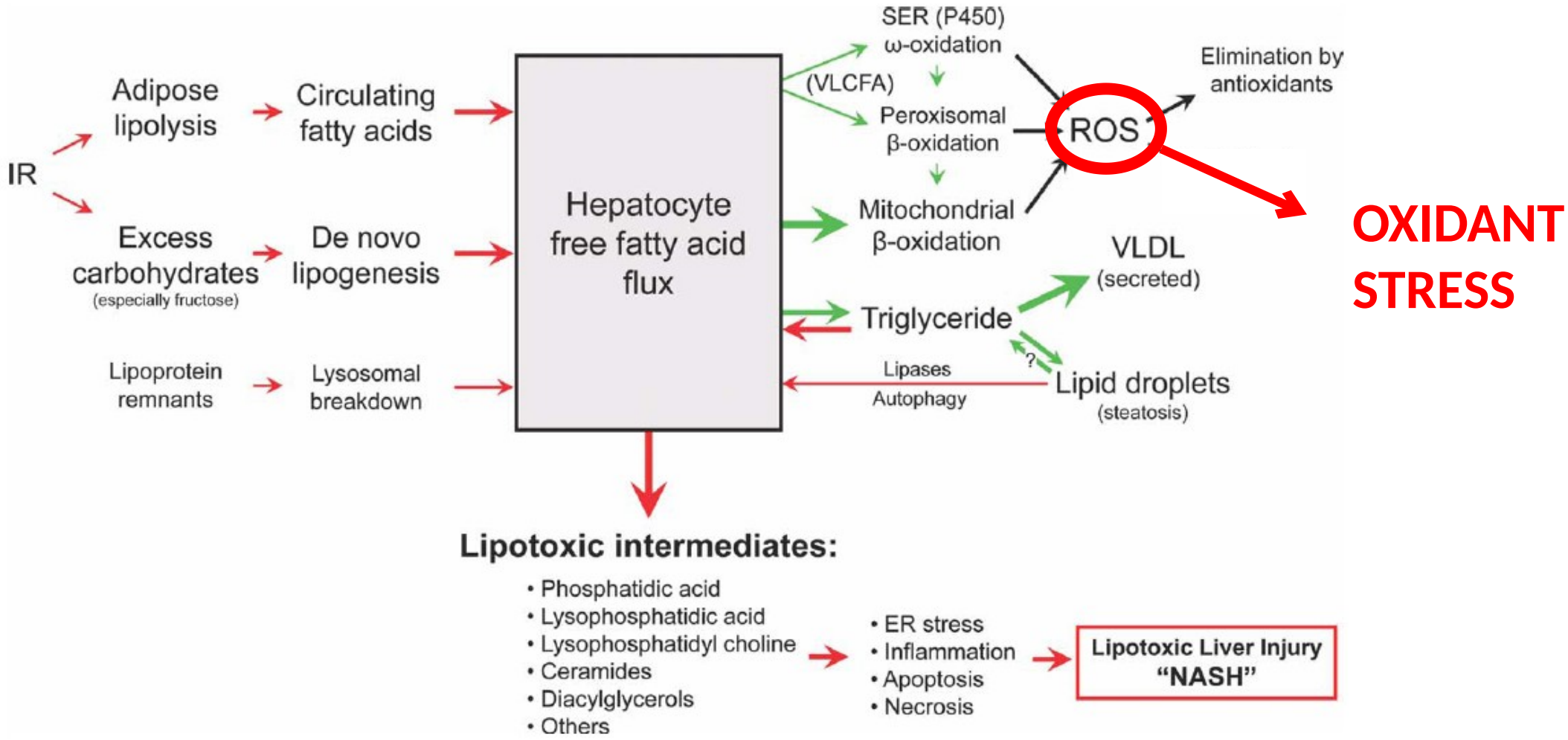


Neuschwander-Tetri Hepatology 2010;52:774C

Effectors and Targets of Lipotoxicity

EFFECTORS	TARGETS
Free fatty acids	Mitochondria
Free cholesterol	Lipid droplets
Ceramides	Autophagy
Sphingosines	Cholangiocytes
Phospholipids	
Leukotrienes	

Pathways of Lipotoxic Liver Injury



Neuschwander-Tetri Hepatology 2010;52:774C

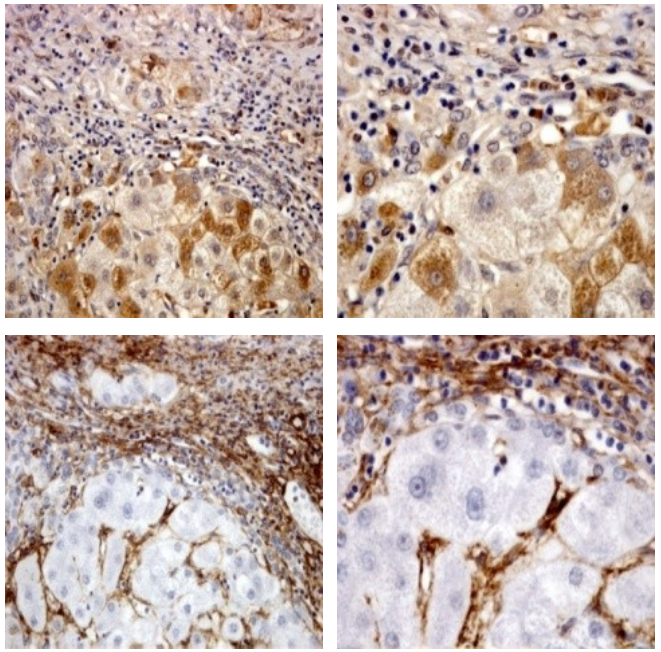
Oxidative Stress a Common Denominator in Chronic Liver Diseases

Increased intracellular ROS levels potentially any liver cell but mainly hepatocytes and activated HSC

200x 400x

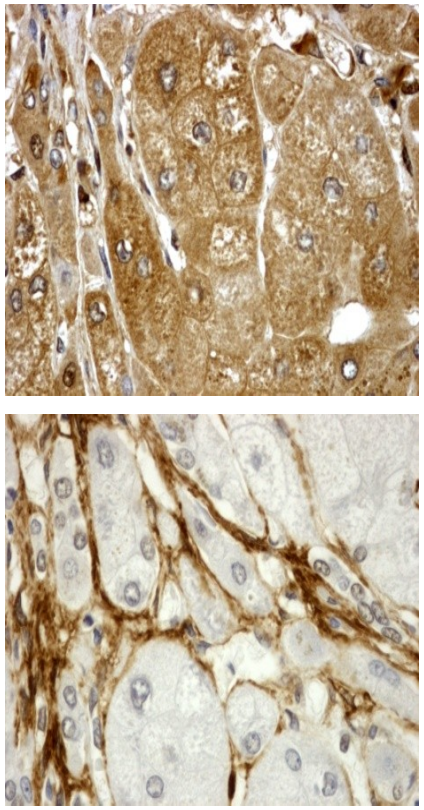
HO1

α-SMA



HO1

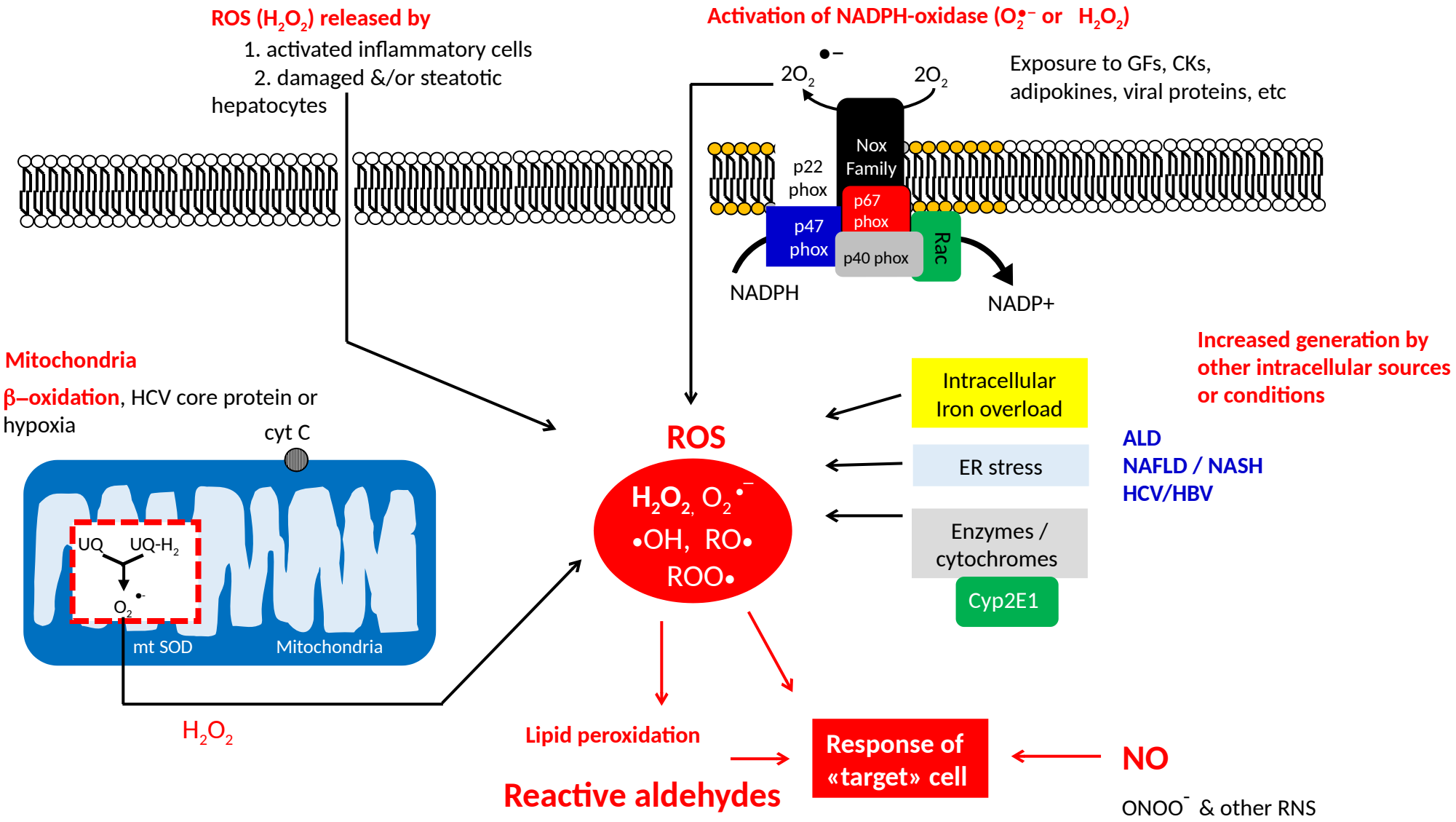
α-SMA



HO1: heme-oxygenase 1, a redox sensitive gene up-regulated in cells undergoing or exposed to oxidative stress

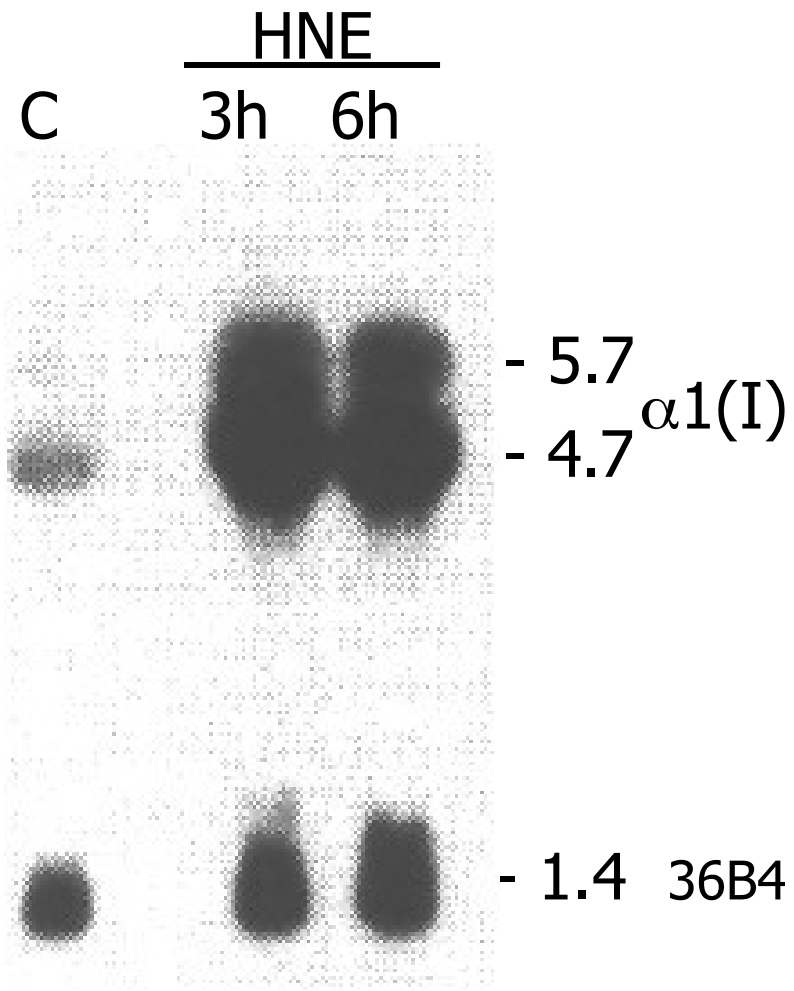
Novo et al. J Pathol 2012, 226:588-97

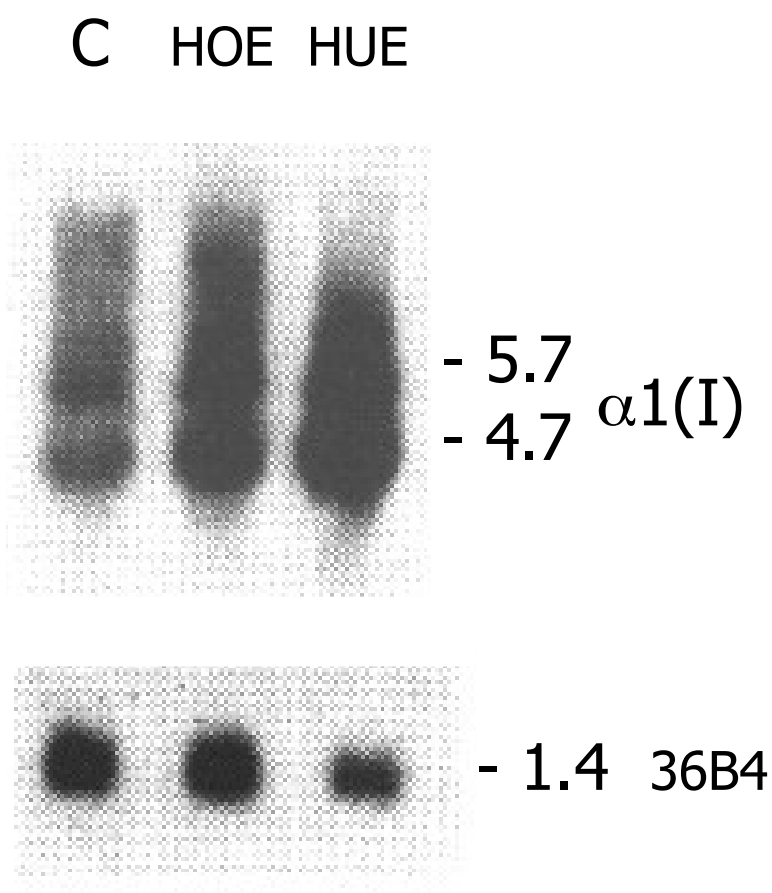
Sources of Intracellular ROS and Related Intermediates in Chronic Liver Injury



Courtesy of Professor Maurizio Parola, Turin, Italy

Reactive Aldehydes induce a direct pro-fibrogenic effect in human HSC

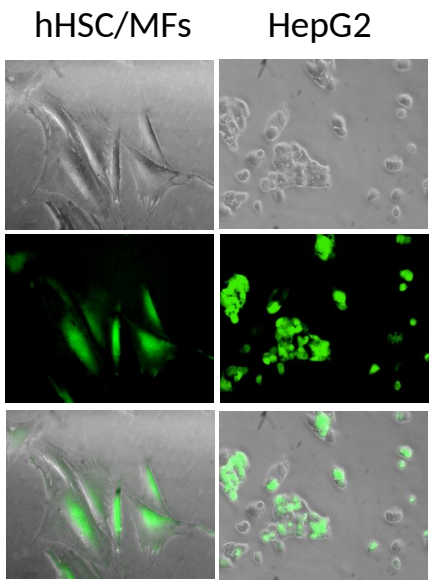




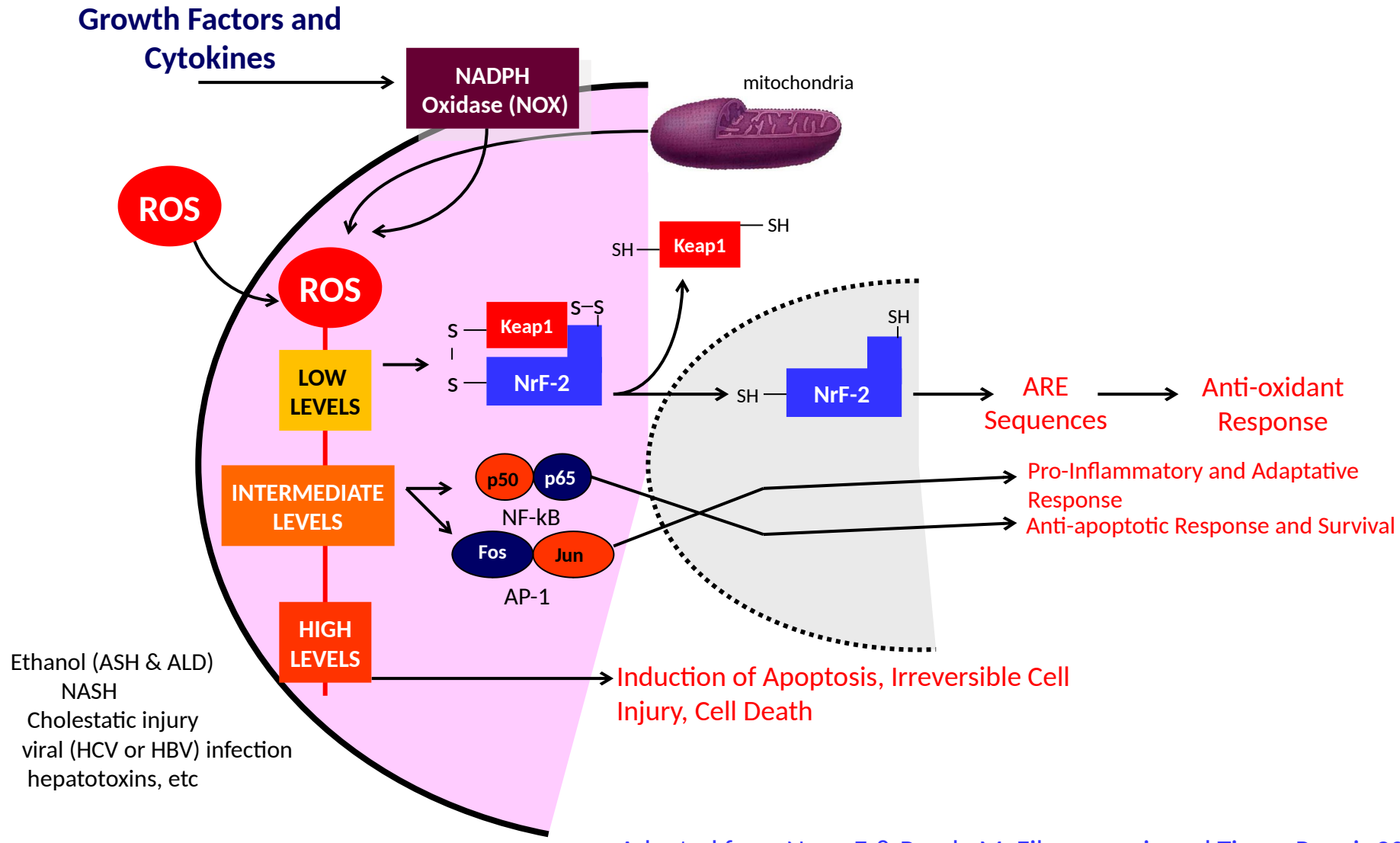
Parola M. et al., Biochem Biophys Res Comm 1996; 222:261-264

Parola M. et al., J Clin Invest 1998; 102:1942-1950

Cellular Responses Following Increased Intracellular ROS



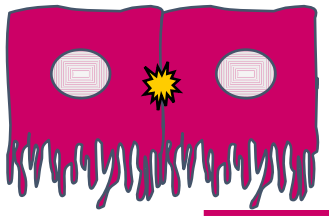
DCFH-DA technique



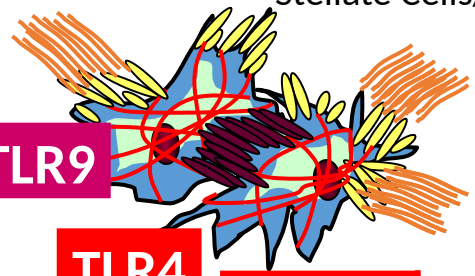
Adapted from Novo E & Parola M, Fibrogenesis and Tissue Repair 2008

Intestinal Permeability and the Activation of Gut-Liver Innate Immunity

Damaged /Apoptotic Hepatocytes



Activated Hepatic Stellate Cells/MFs



TLR9

TLR4

Damage-Associated Molecular Patterns "**DAMPs**" (i.e. apoptotic hepatocyte DNA)

Increased Collagen I synthesis

Increased Pro-fibrogenic (TGF-β) and Pro-inflammatory (IL-6, MCP-1) action

Chronic Liver Disease

Changes in Microbiota
Bacterial Overgrowth

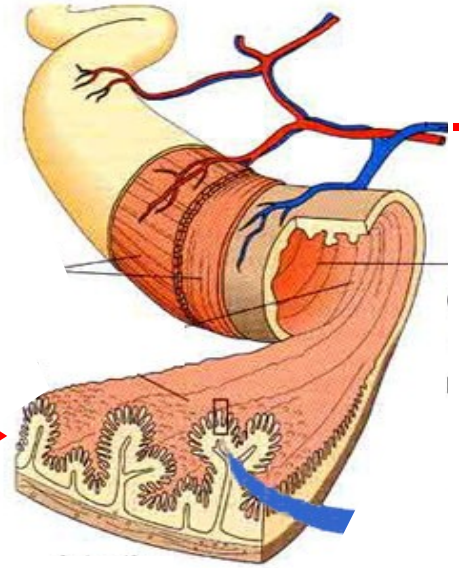
Decreased Bile Acids

Mucosal Injury (ETOH)

Mesenteric Vein Congestion

Immunosuppression

Obesity/Overweight
High Fat Diet

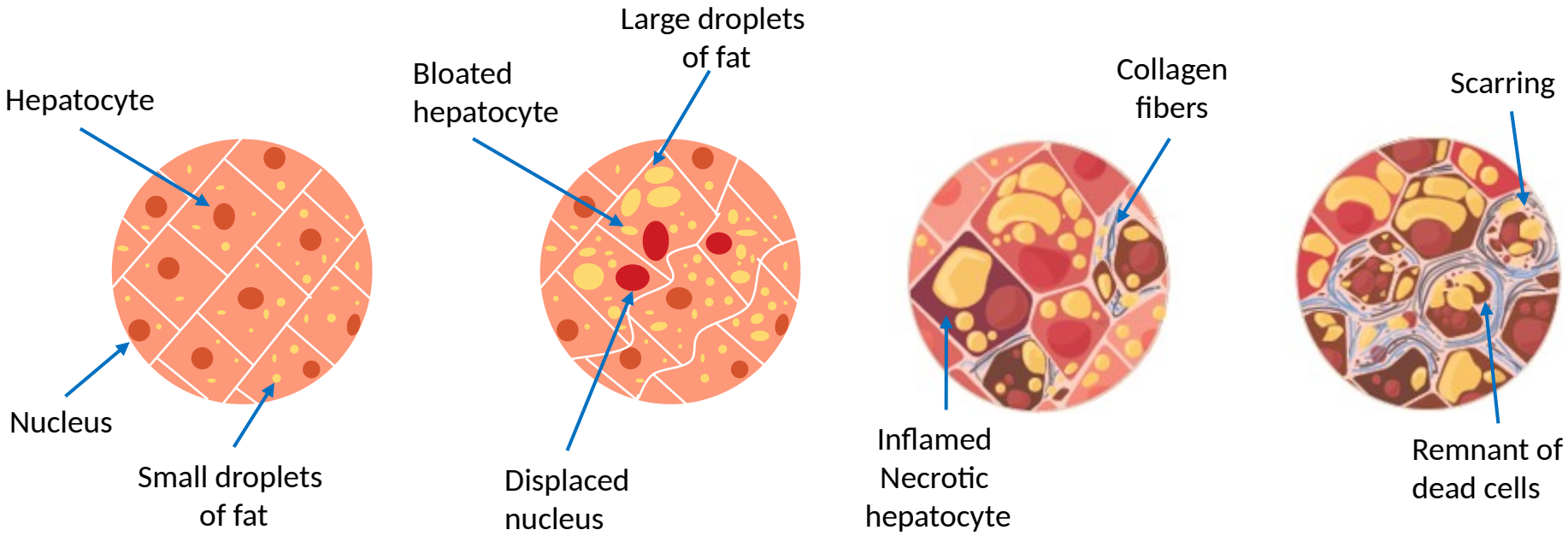


Increased *Pathogen-Associated Molecular Patterns* "**PAMPs**" (i.e. LPS) in the portal circulation (<u>proportional to disease stage</u>)

Brun P., et al., Am J Physiol Gastrointest Liver Physiol 2005; 289:G571-G578
Brun P., et al., Am J Physiol Gastrointest Liver Physiol 2007; 292:G518-G525
Seki E and Brenner DA. Hepatology 2008; 48:322-335
Seki E, et al., Nat Med. 2007; 13:1324-1332
Watanabe A, et al. Hepatology 2007; 46:1509–1518

Pinzani M and Macias Barragan J. Expert Rev Gastroenterology and Hepatology 2010

NASH Fibrosis: Stage-dependent Mechanisms



No evident necrosis

Defective Autophagy
LIPOTOXICITY
Oxidative Stress
Genetic factors

Evident necrosis

Chronic Wound Healing
Increase intestinal permeability
Complex inflammatory networks
Genetic factors

PNPLA3 and NAFLD

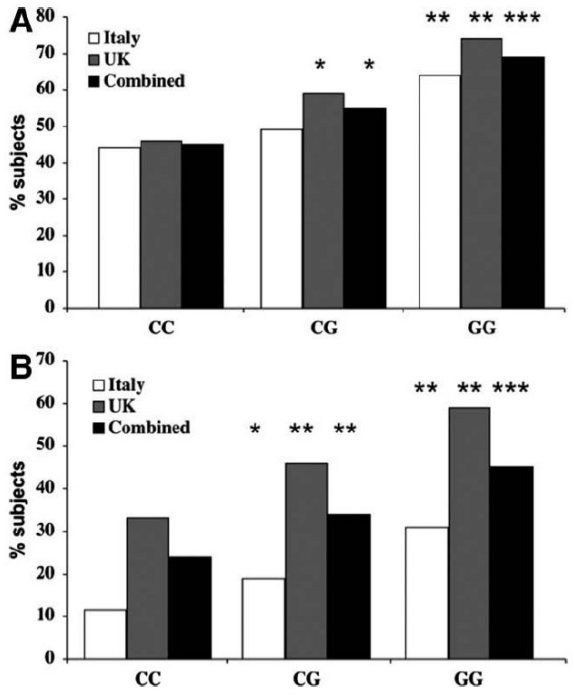
Severity of liver disease

Presence of NASH

OR 1.5 (1.12-2.04)

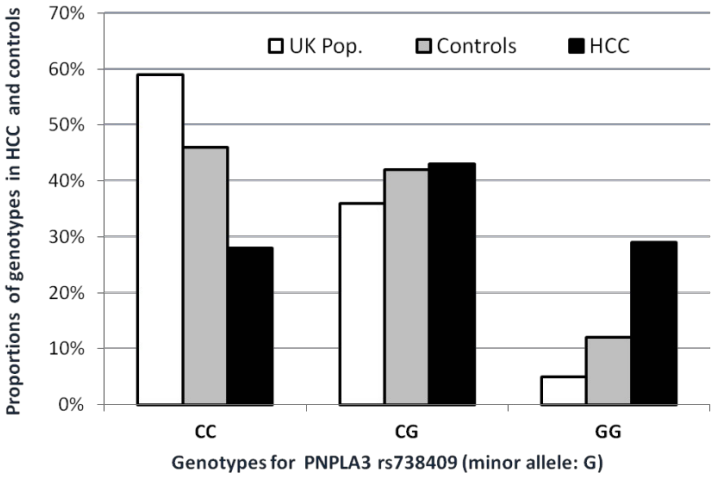
Fibrosis >F1

OR 1.5 (1.09-2.12)



Valenti et al. Hepatology 2010

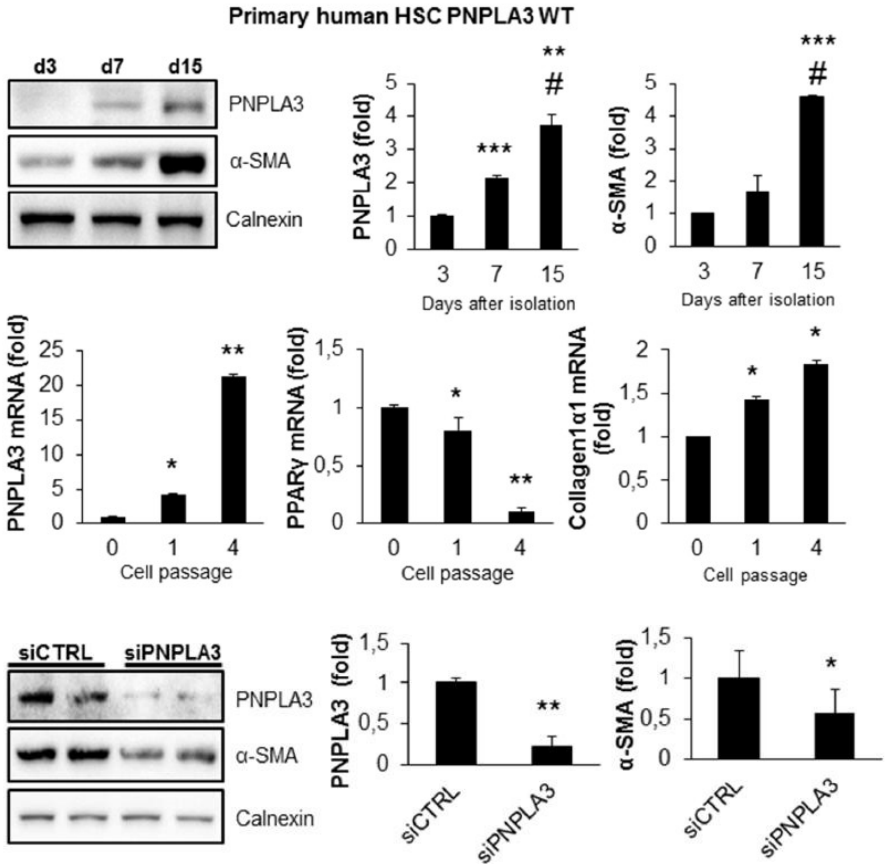
HCC



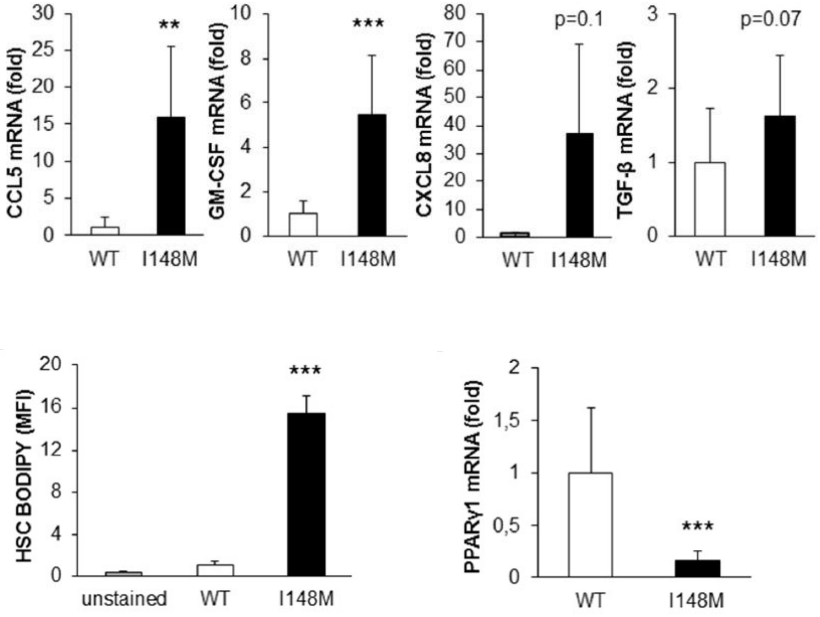
Variables	OR (95% CI)	P-value
PNPLA3 rs738409	2.26 (1.23-4.14)	**0.0082**
Age	1.24 (1.17-1.32)	**<0.0001**
Gender (Male)	11.11 (4.17-33.33)	**<0.0001**
BMI	0.94 (0.87-1.02)	0.148
Diabetes	2.33 (0.93-5.81)	0.070
Cirrhosis	9.37 (3.82-23.00)	**<0.0001**

Liu et al. J Hep 2014

PNPLA3 is required for HSC activation and its genetic variant I148M potentiates the pro-fibrogenic phenotype of human HSC



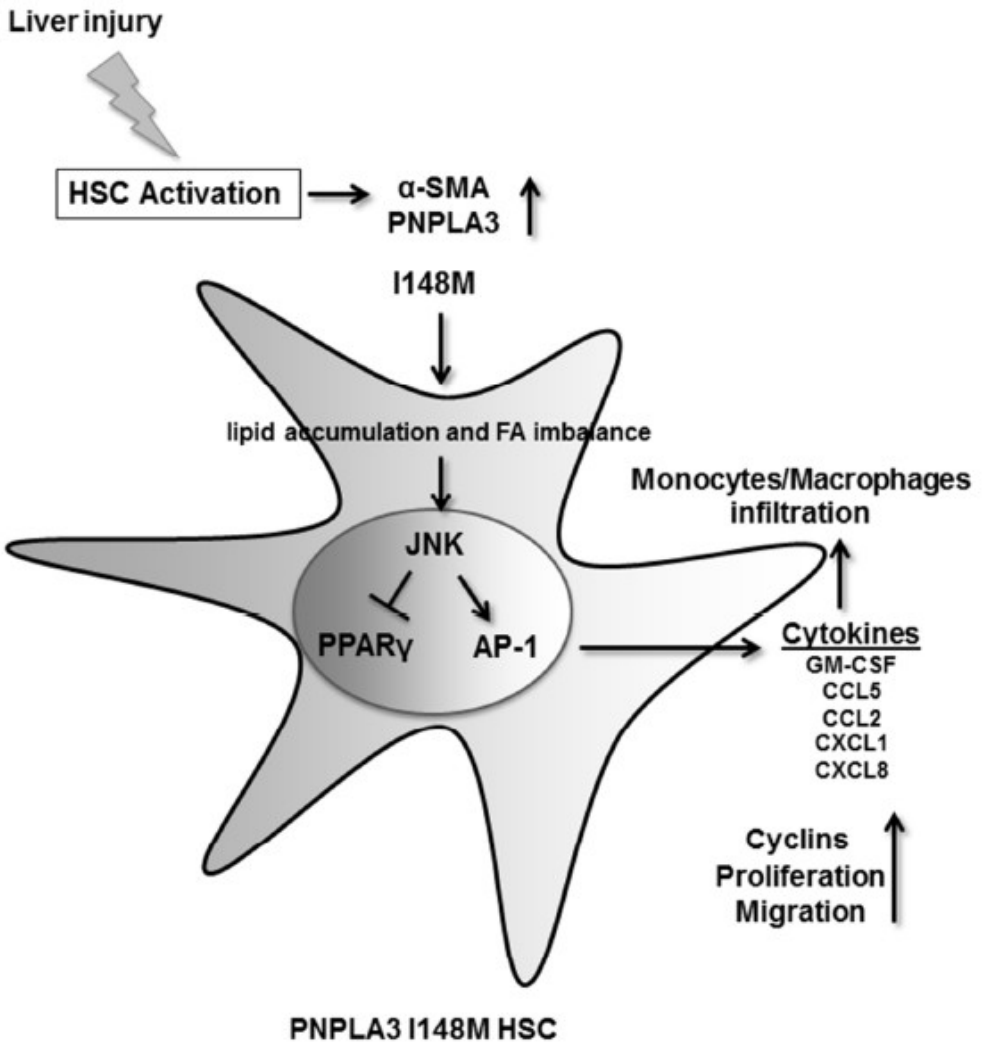
PNPLA3 expression increases with HSC activation
Silencing PNPLA3 reduces HSC activation



PNPLA3 I148M confers a pro-inflammatory and pro-fibrotic profile

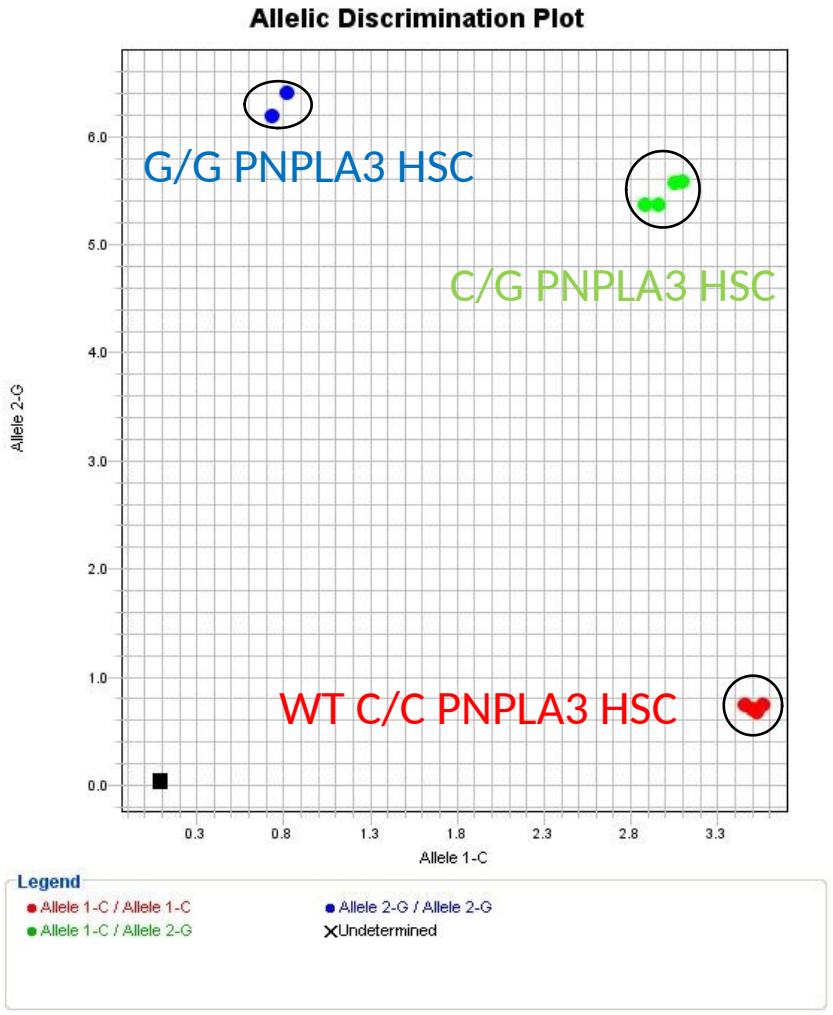
Bruschi F. et al. Hepatology 2017

Mutated PNPLA3 confers a pro-fibrogenic phenotype to human HSC



Bruschi F. et al. Hepatology 2017

Human HSC: Primary Genotyping for PNPLA3 I148M PNPLA3 SNP variant

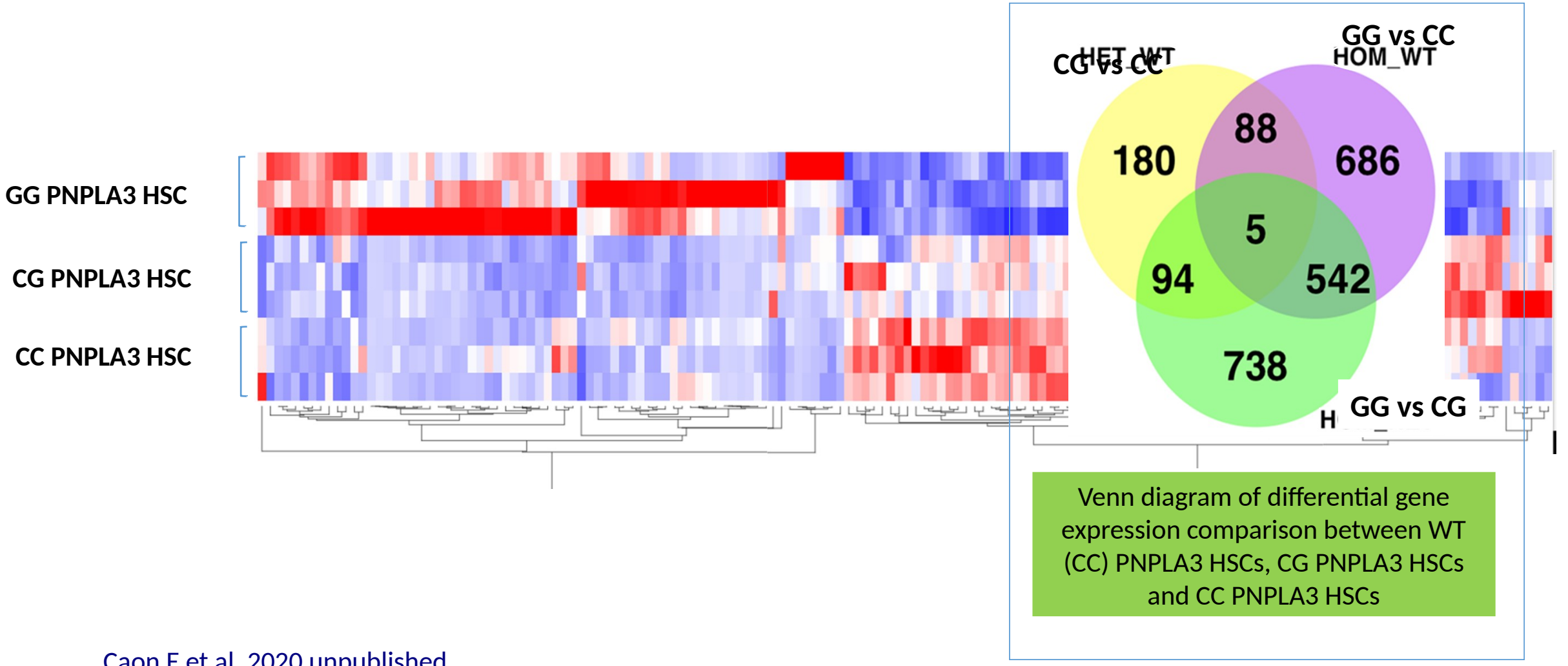


Primary hHSC were isolated (n = 23 donors), cultured in 2D followed by genotyping for PNPLA3(I148M) and RNAseq data analysed with **Ingenuity pathway analysis (IPA).**

- WT PNPLA3 HSC: homozygous for Allele C (C/C)
- C/G PNPLA3 HSC: heterozygous for Allele C and G (C/G)
- G/G PNPLA3 HSC: homozygous for Allele G (G/G)

Caon E et al. 2020 unpublished

PNPLA3 (148M) SNP promotes the activation of human HSC through a dysregulated oxidative stress response

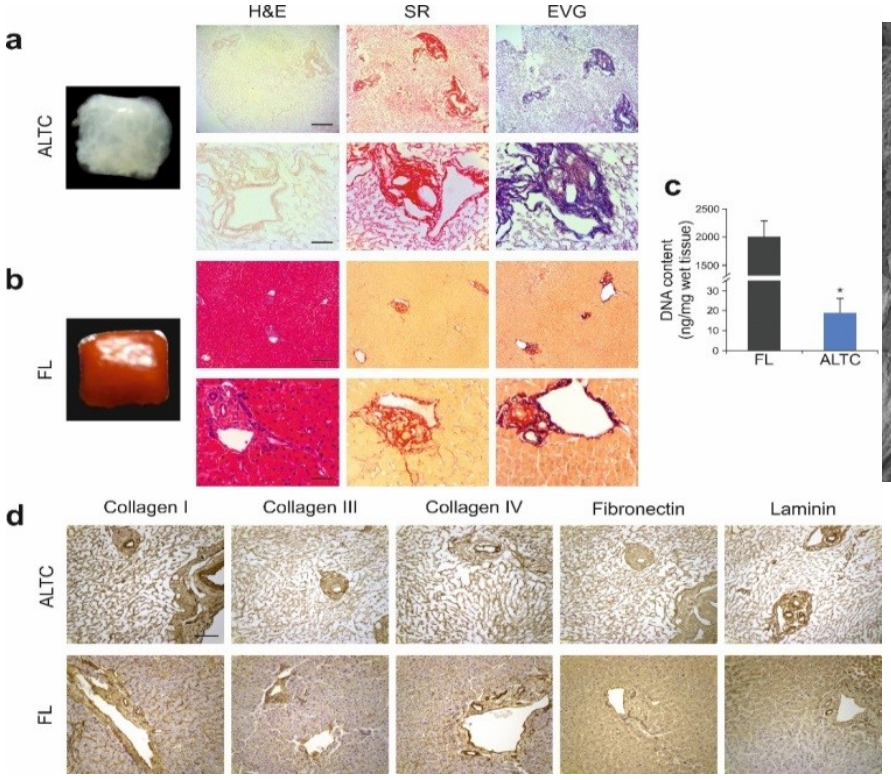


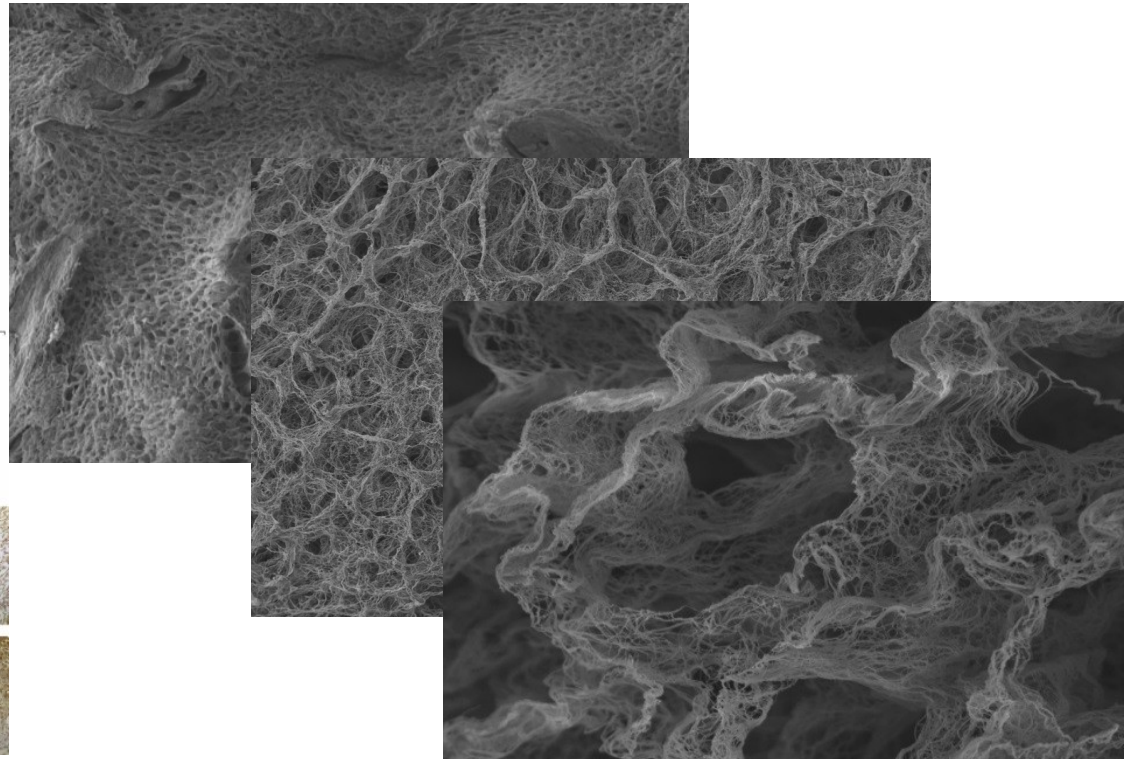
Venn diagram of differential gene expression comparison between WT (CC) PNPLA3 HSCs, CG PNPLA3 HSCs and CC PNPLA3 HSCs

Caon E et al. 2020 unpublished

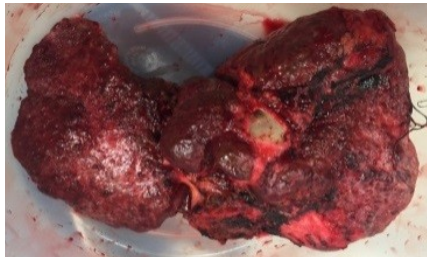
3D Human Healthy and Fibrotic Liver ECM

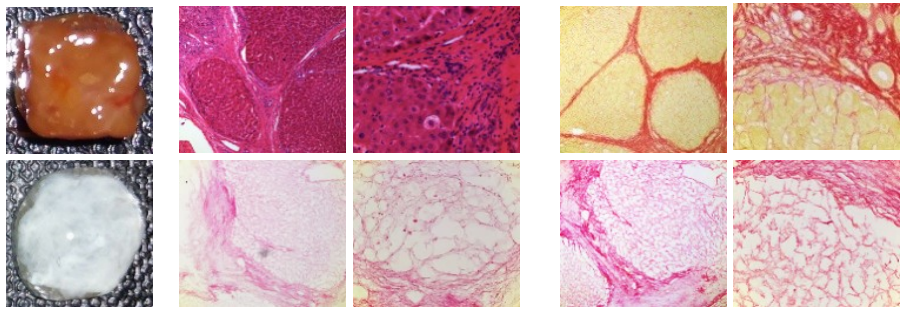
Donor healthy human liver

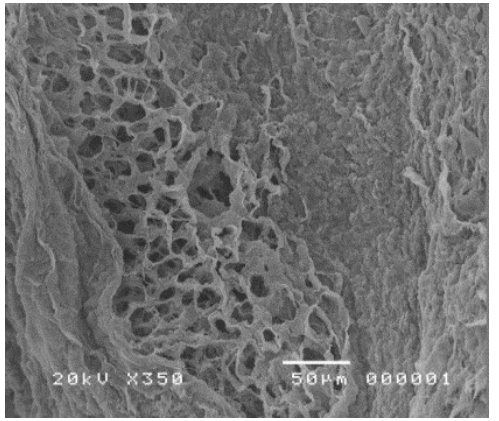


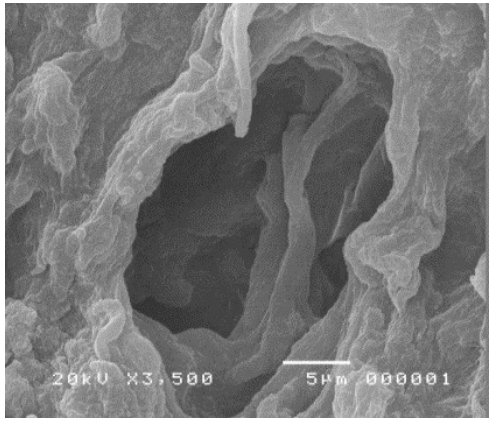


Explanted cirrhotic liver



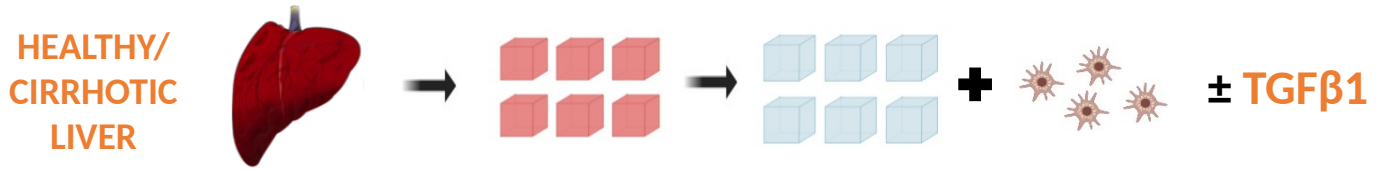


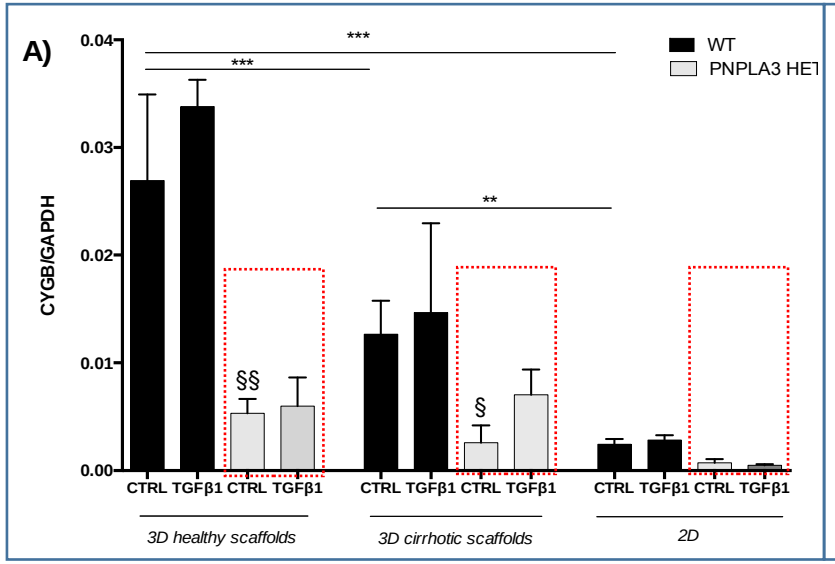


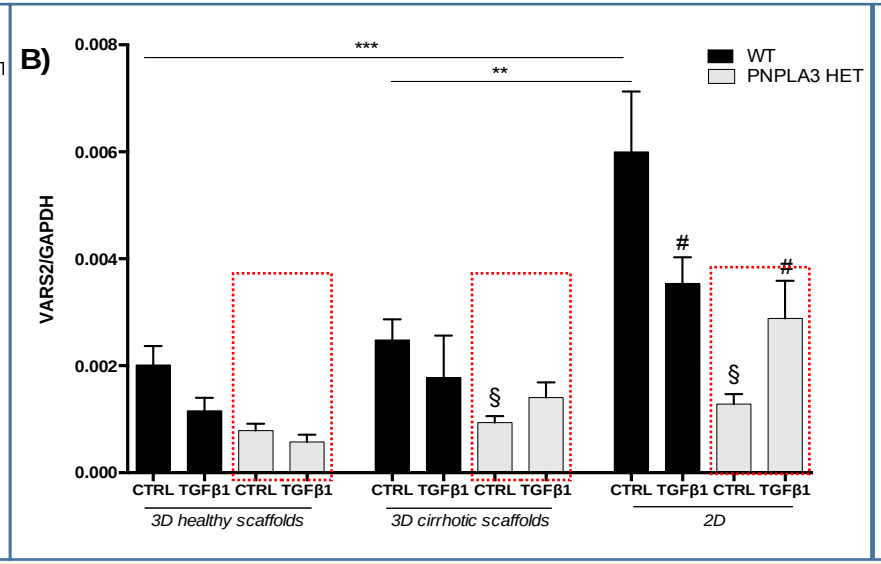


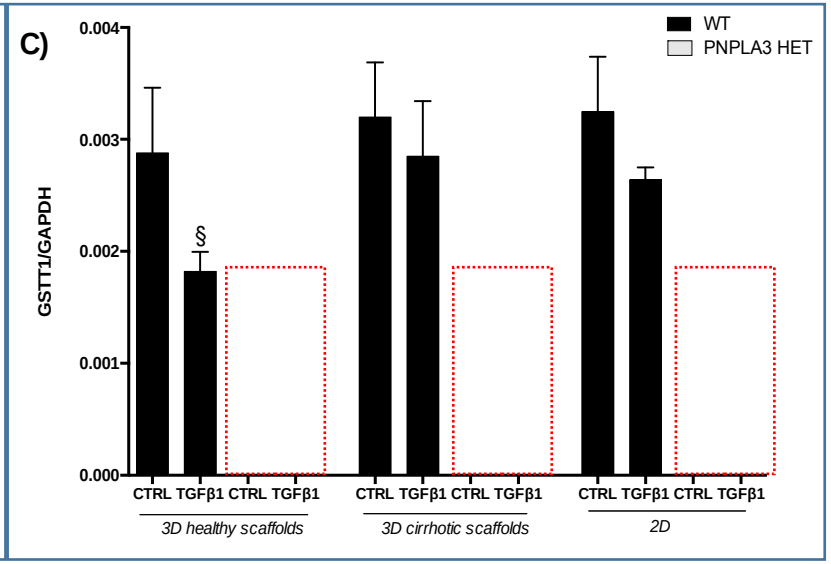
Mazza G et al, Nature Scientific Report 2015; Mazza G et al, Nature Scientific Report 2017

PNPLA3 (148M) SNP promotes the activation of human HSC through a dysregulated oxidative stress response









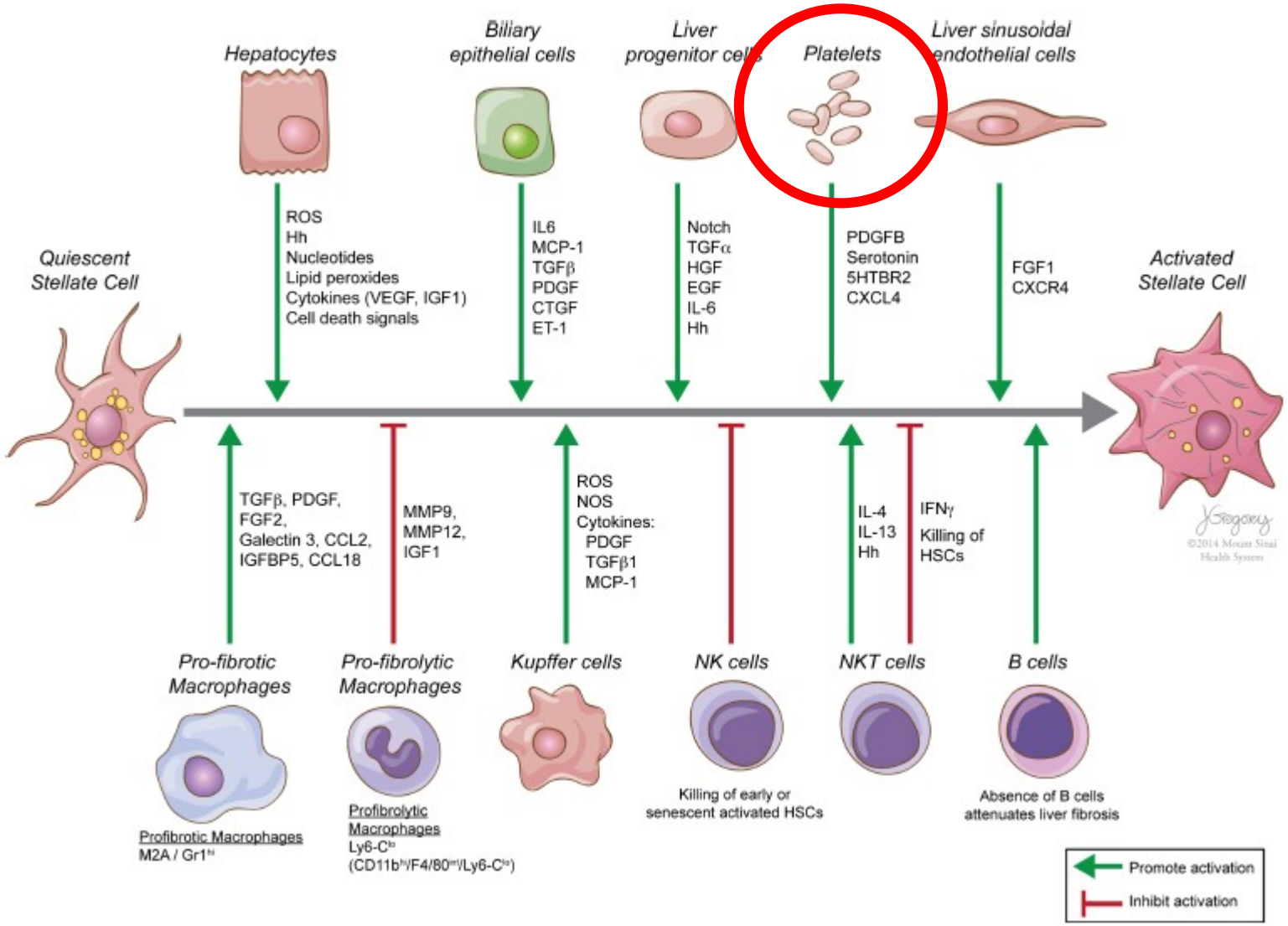
Cytoglobin B: HSC quiescence marker

VARS2: a mitochondrial enzyme involved in fatty acid metabolism

GSTT1, a Glutathione-S-Transferase

Caon E et al. 2020 unpublished

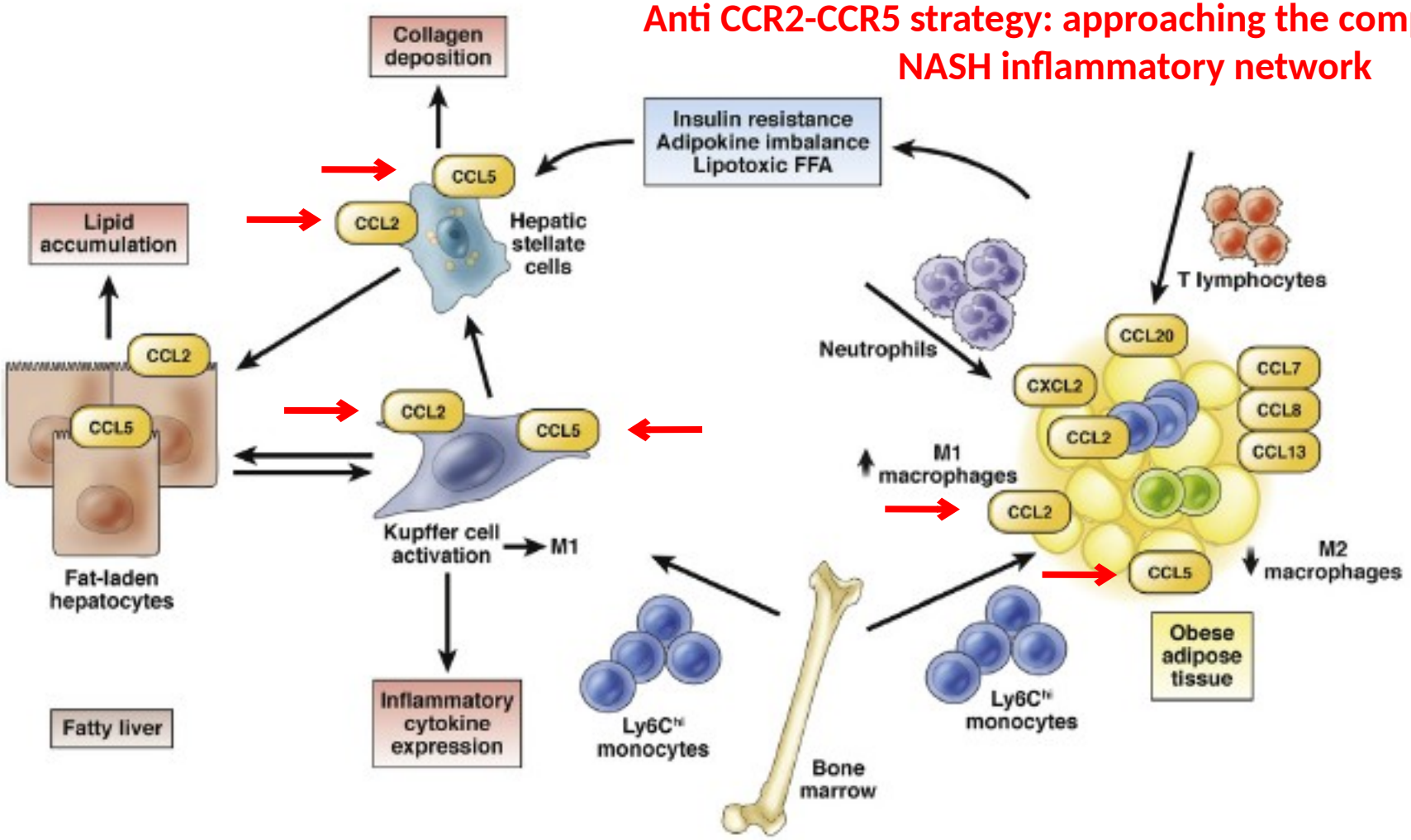
Cellular Cross-Talk in Liver Fibrogenesis



Lee et al., Gut 2015

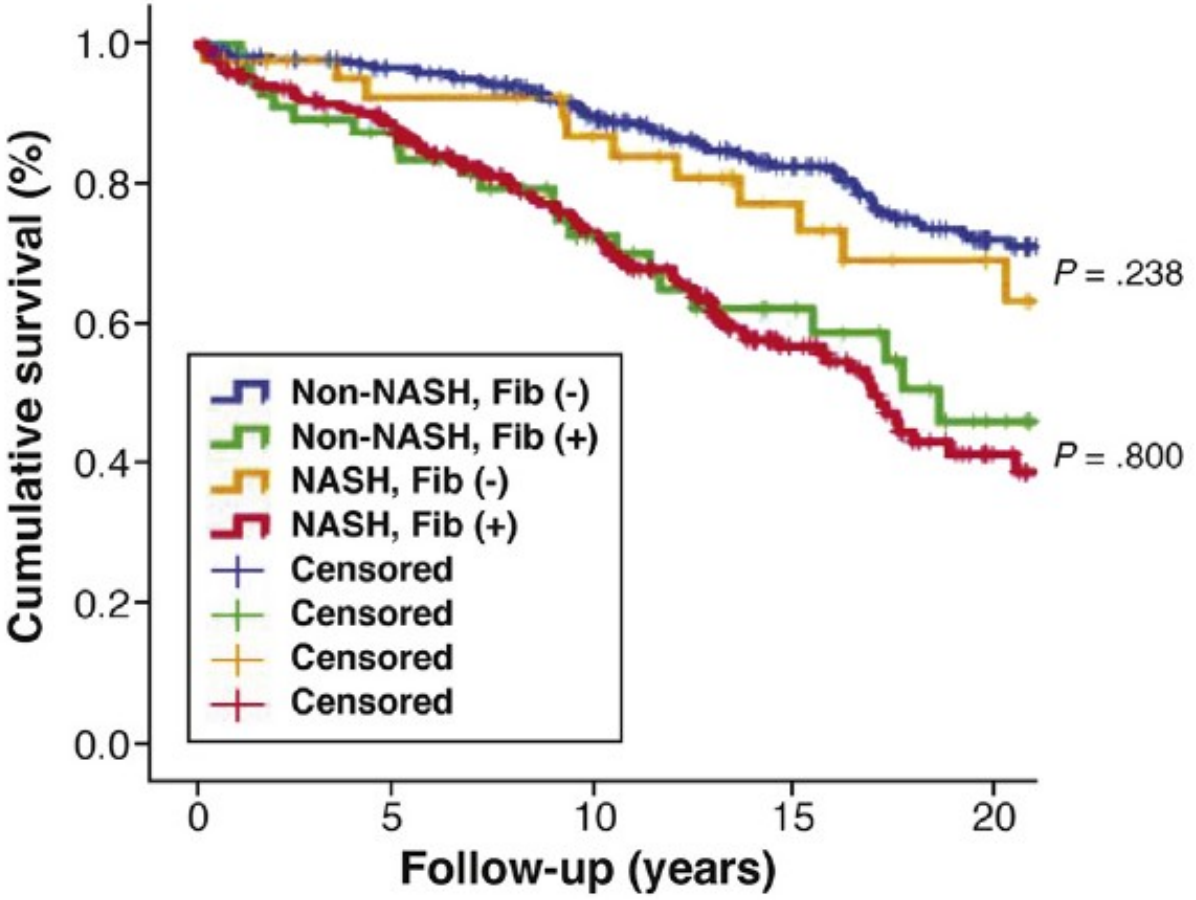
Chemokines and NASH Progression

Anti CCR2-CCR5 strategy: approaching the complexity of the NASH inflammatory network



Marra & Tacke, Gastroenterology 2014

Fibrosis and NAFLD Outcome

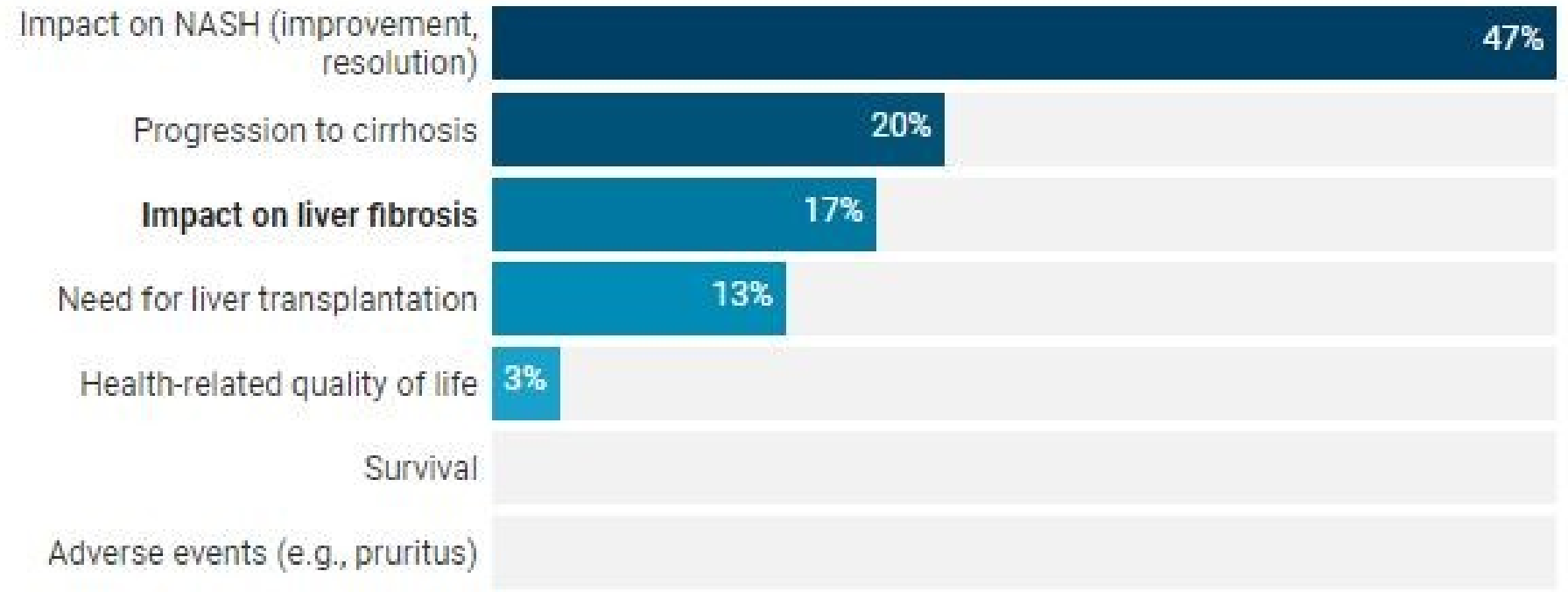


279	241	197	137	72	Non-NASH, Fib (-)
56	46	30	19	7	Non-NASH, Fib (+)
43	35	31	20	12	NASH, Fib (-)
241	197	124	58	18	NASH, Fib (+)

Angulo et al., Gastroenterology 2015

Fibrosis in NASH: Still a Confused End-point!!!!

Despite liver fibrosis being the only end point currently a strong predictor of negative outcomes in NASH patients – it's not the most important factor to payers…



Which of the following clinical measures would you consider most important when assessing the cost-effectiveness of a novel drug for the treatment of NASH. Percentage of MCO PD/MDs (n=30). Survey data collected December 2017.

NASH pipeline crowds at mid-stage, but has few advanced candidates

PHASE I (14 drugs)

- butanoic acid
- CER209
- evogliptin
- DUR928
- MK-4074
- OPRX-106
- PF06865571
- PF06882961
- PXS-5382A
- RG-125
- RYI-018
- seladelpar
- SGM-1019
- TVB-2640

PHASE II (29 drugs)

medications

- ARX618
- BI 1467335
- DS102
- EDP-305
- emricasan
- gemcabene
- GR-MD-02
- GRI-0621
- GS-0976
- GS-9674
- IMM-124E
- IONIS-DGAT2Rx
- IVA-337
- lipaglyn
- LJN452
- LMB763
- MGL-3196
- MN-001
- MSDC-0602K
- NC101
- NGM282
- NS-0200
- ozempic
- PF-05221304
- PF-06835919
- remogliflozin etabonate
- SHP626
- TVB-2640
- VK2809

PHASE III (5 drugs)

- cenicriviroc
- elafibranor
- Ocaliva (obeticholic acid)
- Selonsertib
- aramchol


UCL ILDH
Giuseppe MAZZA
Krista ROMBOUTS
Walid Al-Akkad
Luca Frenguelli
Lisa Longato
Kessarin Thanapirom
Maria Giovanna Vilia
Elisabetta Caon
Martina Marrali
Eric Felli
Bailin Chen
Yutaka Yasui
Kevin Moore
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Fotis Sampaziotis
Imperial College London
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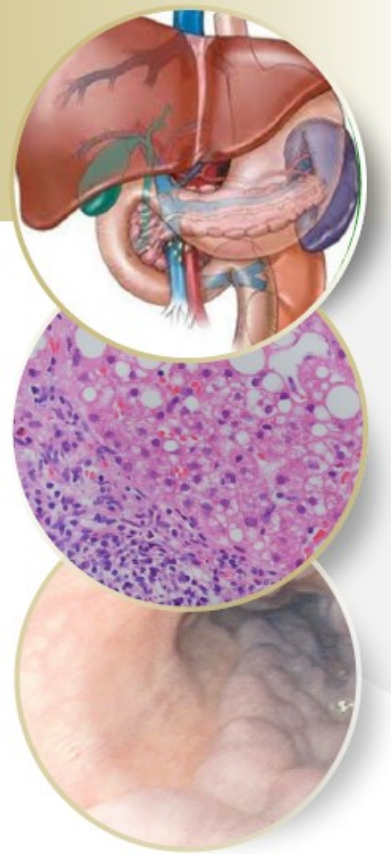
Benjamin Robinson
Universita' di Pavia, Italy
Antonio Di Sabatino
Paolo Giuffrida
ENGITIX THERAPEUTICS
UCL Institute for Child Health, Div. Of Surgery
Paolo De Coppi
Panagiotis Maghsoudlou
Luca Urbani
NIHR | University College London Hospitals Biomedical Research Centre
cnBio innovations
A member of cn innovations
HARVARD APPARATUS
NORDIC BIOSCIENCE
TAPB
Tissue Access for Patient Benefit
UCLB
UCLPartners
Academic Health Science Partnership
royal free charity
Royal Free London NHS

ROYAL FREE SHEILA SHERLOCK

HEPATOLOGY

POSTGRADUATE COURSE



SAVE THE DATE

Tuesday 5th » *Thursday* 7th

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A comprehensive guide to liver disease in the clinic, acute take & ICU featuring:

- *Interactive grand rounds*
- *Real-life case discussions*
- *Hot topics in hepatology*
- *Q&As with the experts*

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