Animal model for liver disease

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Contains

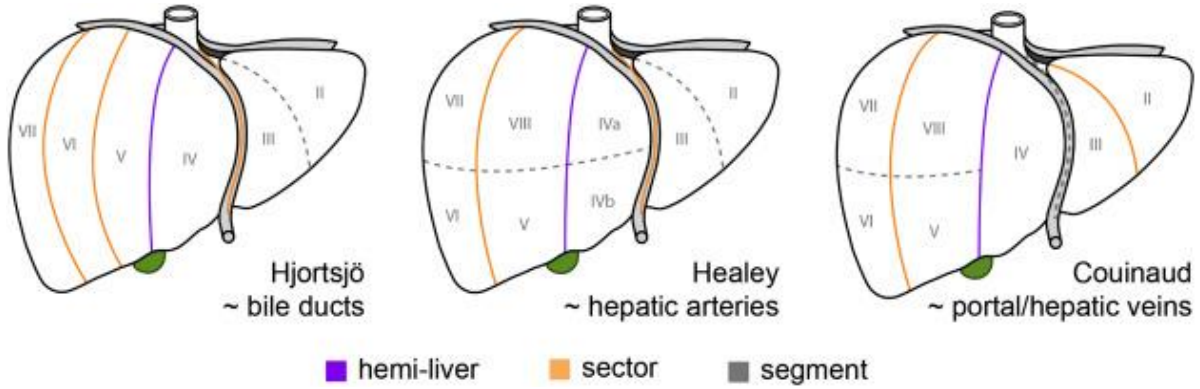
Introduction

Liver disease

Animal model

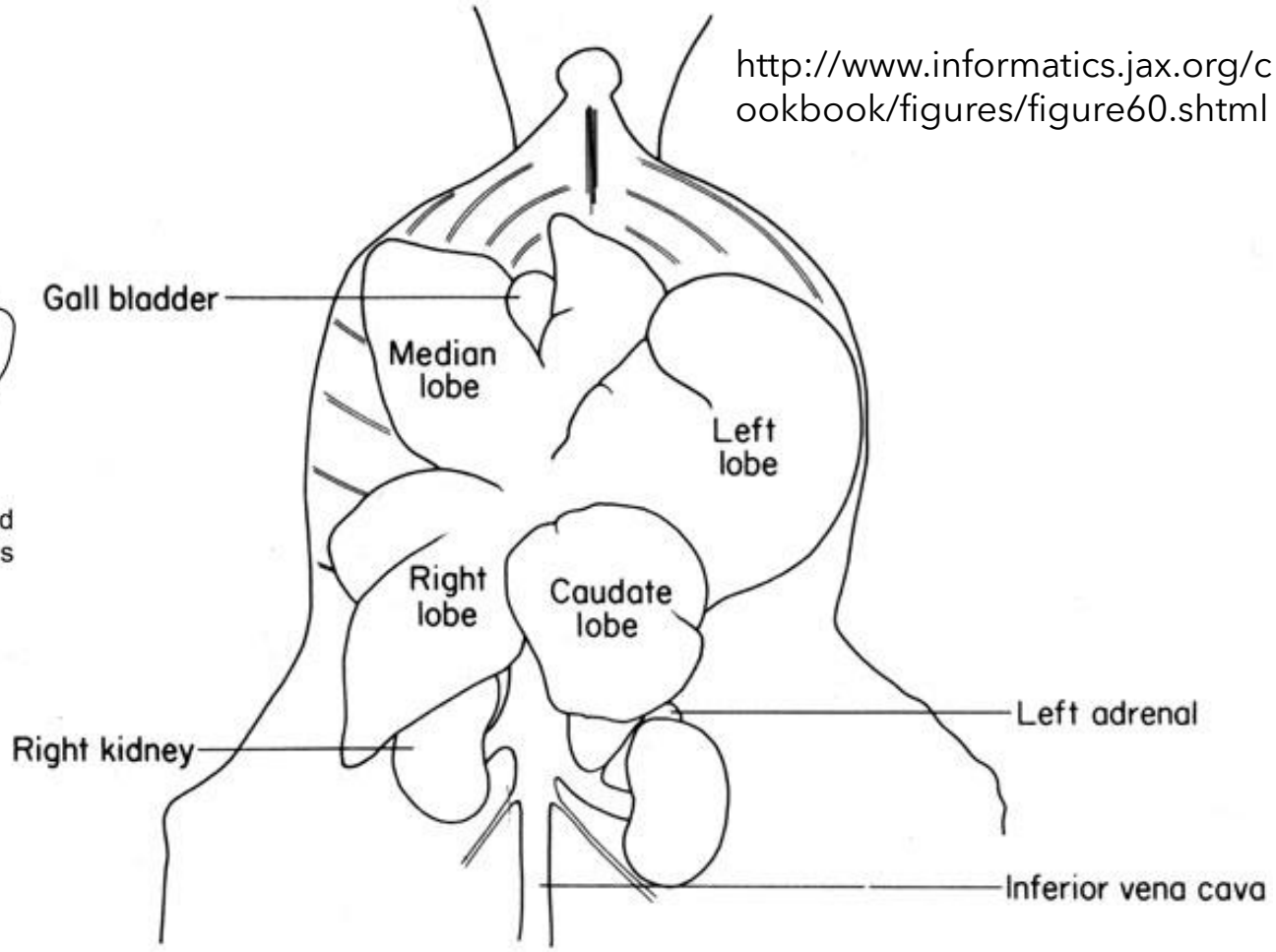
I. Introduction

- Liver gross anatomy



https://doi.org/10.1016/j.bbadis.2018.05.019

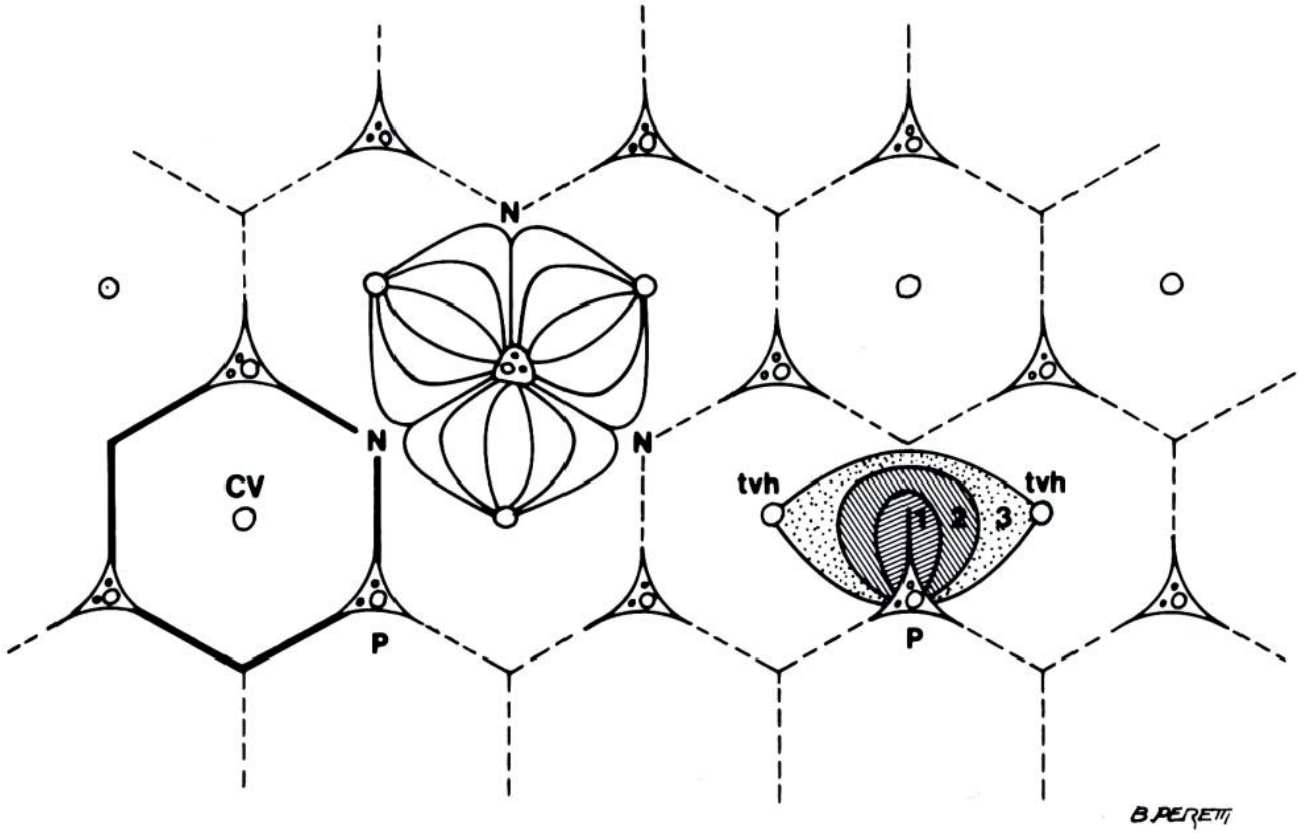
http://www.informatics.jax.org/cookbook/figures/figure60.shtml



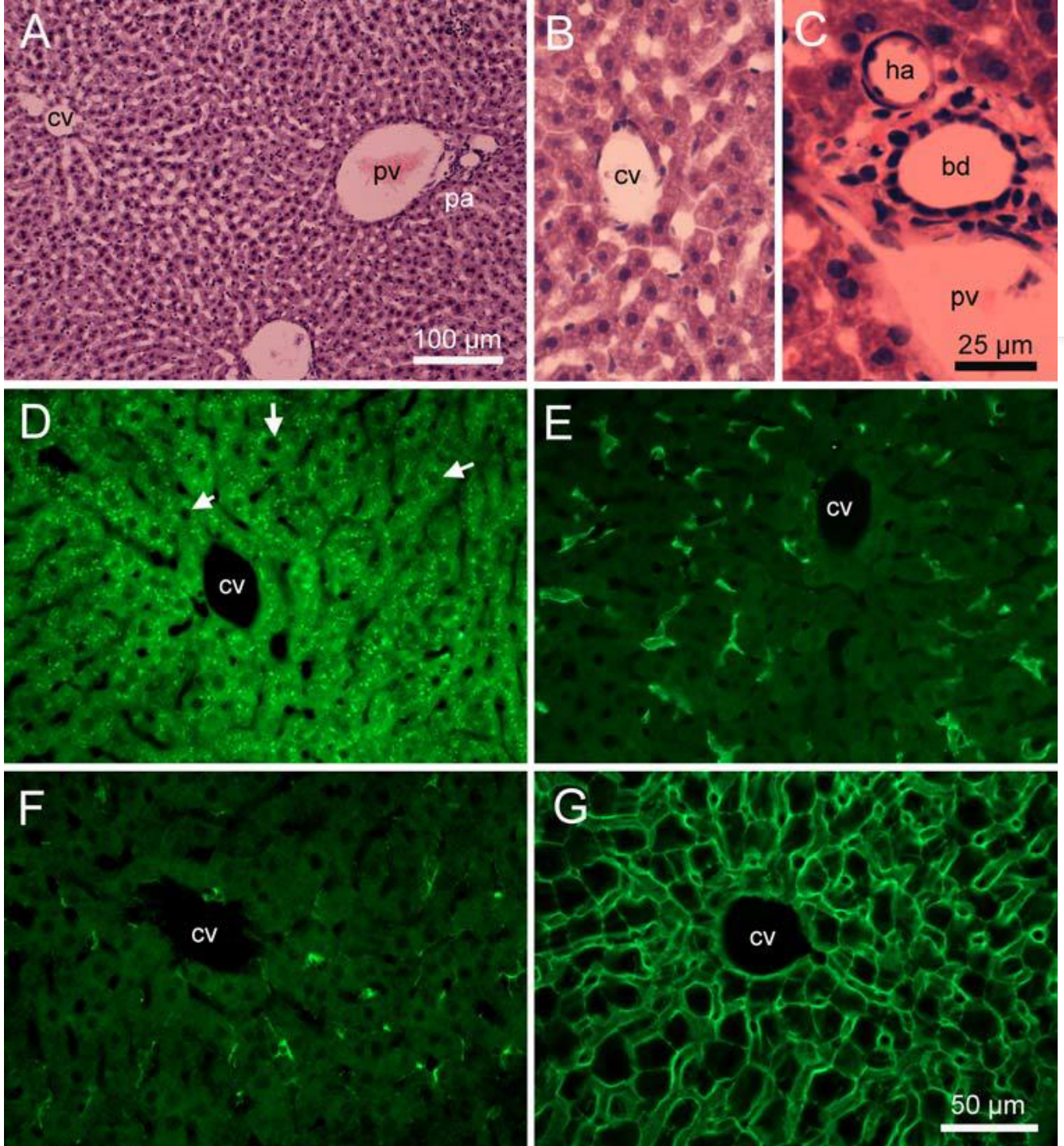
I. Introduction

The microscopic architecture: lobule/unit/acinar

- Vascular
- Cell
- Matrix



Dieter Sasse 1992 Liver architecture



A B C: H&E

D: IHC Albumin

E: IHC F4-80

F: IHC GFAP

G: Tomato lectin

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I. Introduction

- Liver function: The liver plays a major role in metabolism
  - Storage: blood, glycogen, vitamin, ion…
  - Protein synthesis: Albumin, blood clot factor, hormone…
  - Modification, and excretion of exogenous and endogenous substances
  - The synthesis of cholesterol, bile salts, and phospholipids
  - The transformation of carbohydrates

II. Liver disease

Inherited hyperbilirubinemia
Gilbert syndrome
Crigler-Najjar syndrome, types I and II
Dubin-Johnson syndrome
Rotor syndrome

Viral hepatitis
Hepatitis A
Hepatitis B
Hepatitis C
Hepatitis D
Hepatitis E
Others (mononucleosis, herpes, adenovirus hepatitis)
Cryptogenic hepatitis

Harrison's Principles of Internal Medicine, 18th ed

II. Liver disease

Immune and autoimmune liver diseases
Primary biliary cirrhosis
Autoimmune hepatitis
Sclerosing cholangitis
Overlap syndromes
Graft-versus-host disease
Allograft rejection

Genetic liver diseases
Alpha-1-antitrypsin deficiency
Hemochromatosis
Wilson disease
Benign recurrent intrahepatic cholestasis
Progressive familial intrahepatic cholestasis
Others (galactosemia, tyrosinemia, cystic fibrosis, Neiman-Pick disease, Gaucher disease)

Harrison's Principles of Internal Medicine, 18th ed

II. Liver disease

Vascular injury
Venoocclusive disease
Budd-Chiari syndrome
Ischemic hepatitis
Passive congestion
Portal vein thrombosis
Nodular regenerative hyperplasia

Acute fatty liver of pregnancy
Liver involvement in systemic diseases
Sarcoidosis
Amyloidosis
Glycogen storage diseases
Celiac disease
Tuberculosis
Mycobacterium avium-intracellulare

Harrison's Principles of Internal Medicine, 18th ed

II. Liver disease

Alcoholic liver disease
Acute fatty liver
Acute alcoholic hepatitis
Laennec cirrhosis
Nonalcoholic fatty liver
Steatosis
Steatohepatitis

Cholestatic syndromes
Benign postoperative cholestasis
Jaundice of sepsis
Total parenteral nutrition (TPN)–induced jaundice
Cholestasis of pregnancy
Cholangitis and cholecystitis
Extrahepatic biliary obstruction (stone, stricture, cancer)
Biliary atresia
Caroli disease
Cryptosporidiosis

Harrison's Principles of Internal Medicine, 18th ed

II. Liver disease

Drug-induced liver disease
Hepatocellular patterns (isoniazid, acetaminophen)
Cholestatic patterns (methyltestosterone)
Mixed patterns (sulfonamides, phenytoin)
Microvesicular and macrovesicular steatosis (methotrexate)

Mass lesions
Hepatocellular carcinoma
Cholangiocarcinoma
Adenoma
Focal nodular hyperplasia
Metastatic tumors
Abscess
Cysts
Hemangioma

Harrison's Principles of Internal Medicine, 18th ed

II. Liver disease

Acute or Chronic

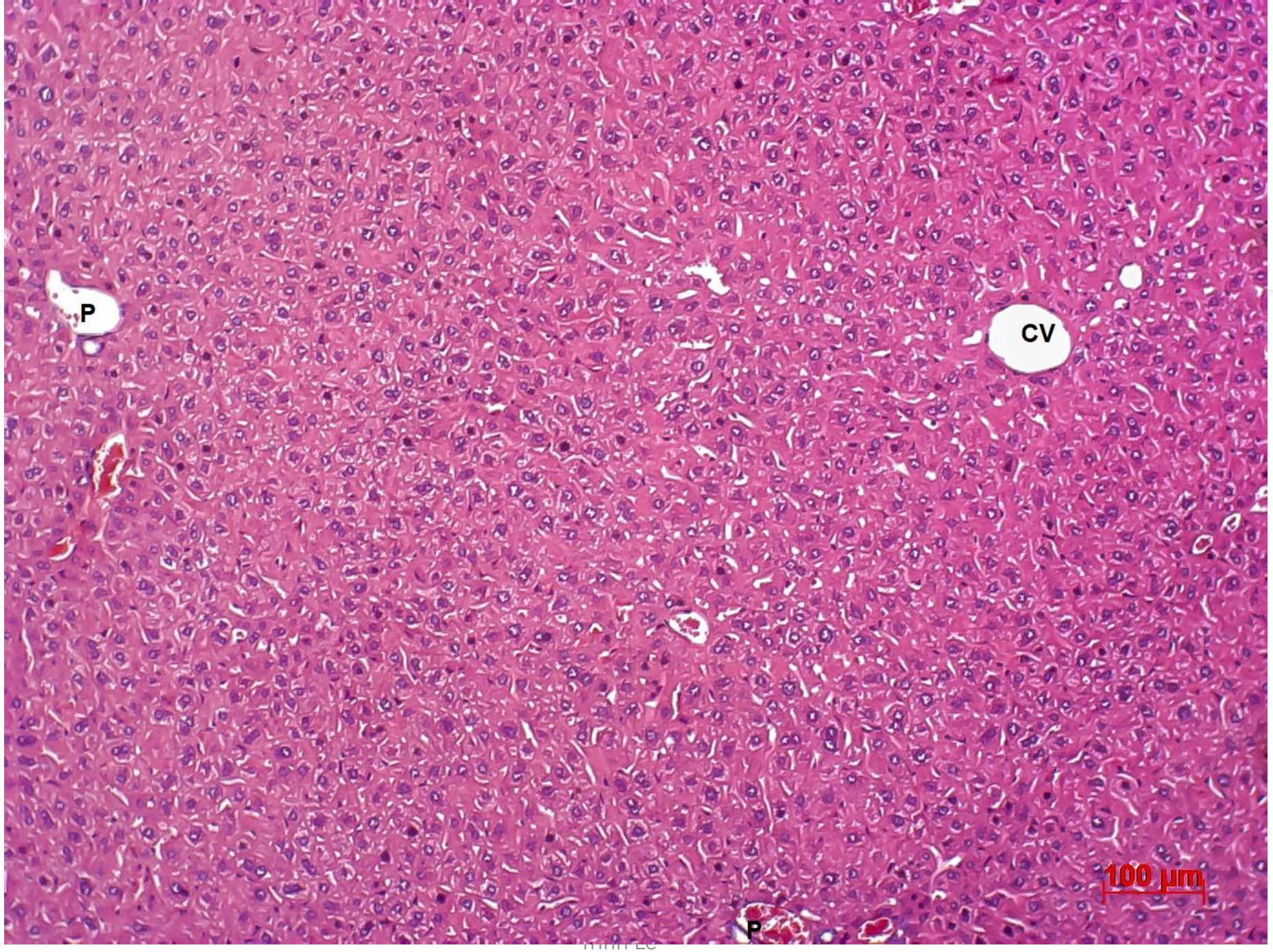
Focal or Diffuse

Mild or Severe

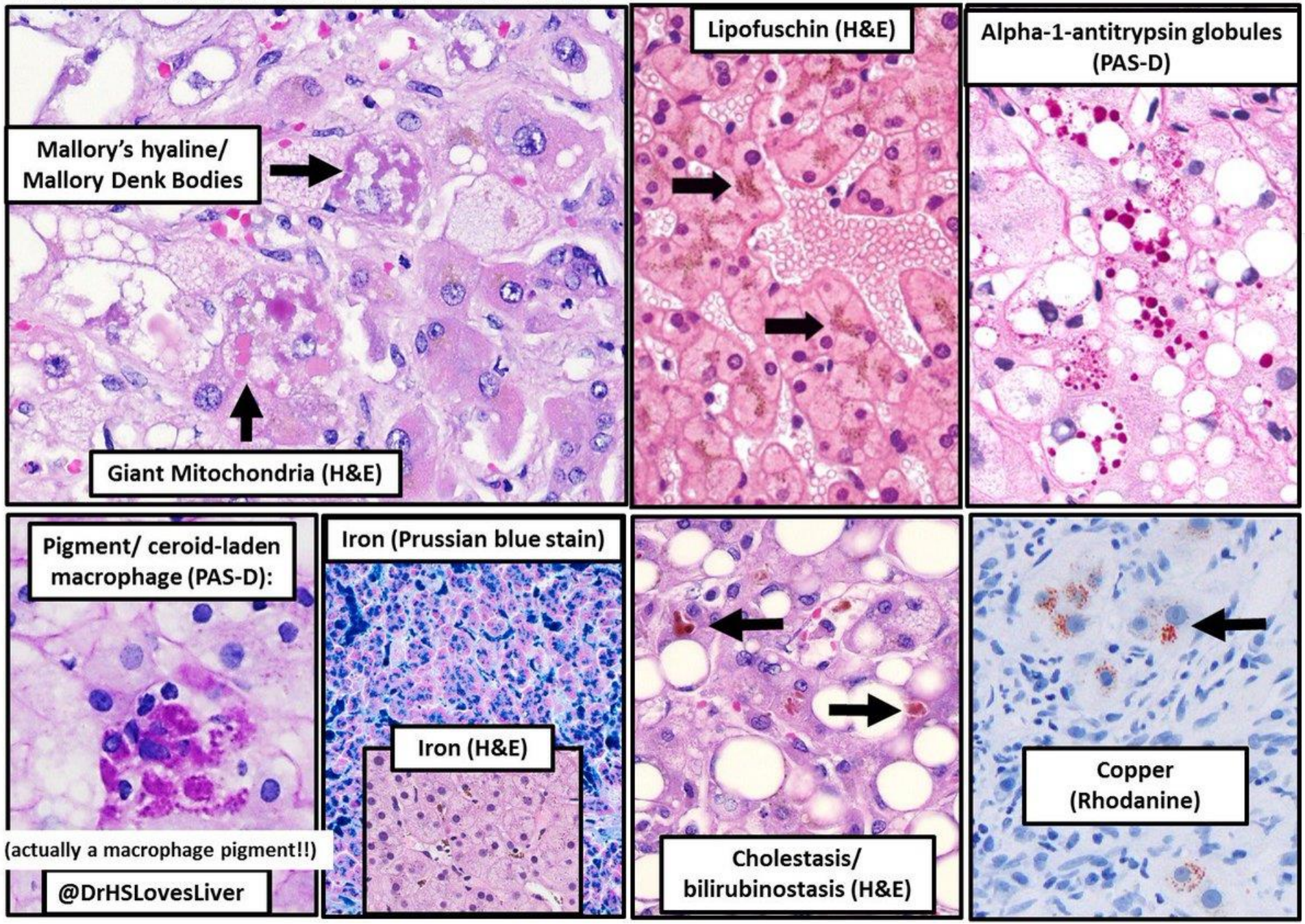
Reversible or Irreversible

II. Liver disease: Lesions/Paterns

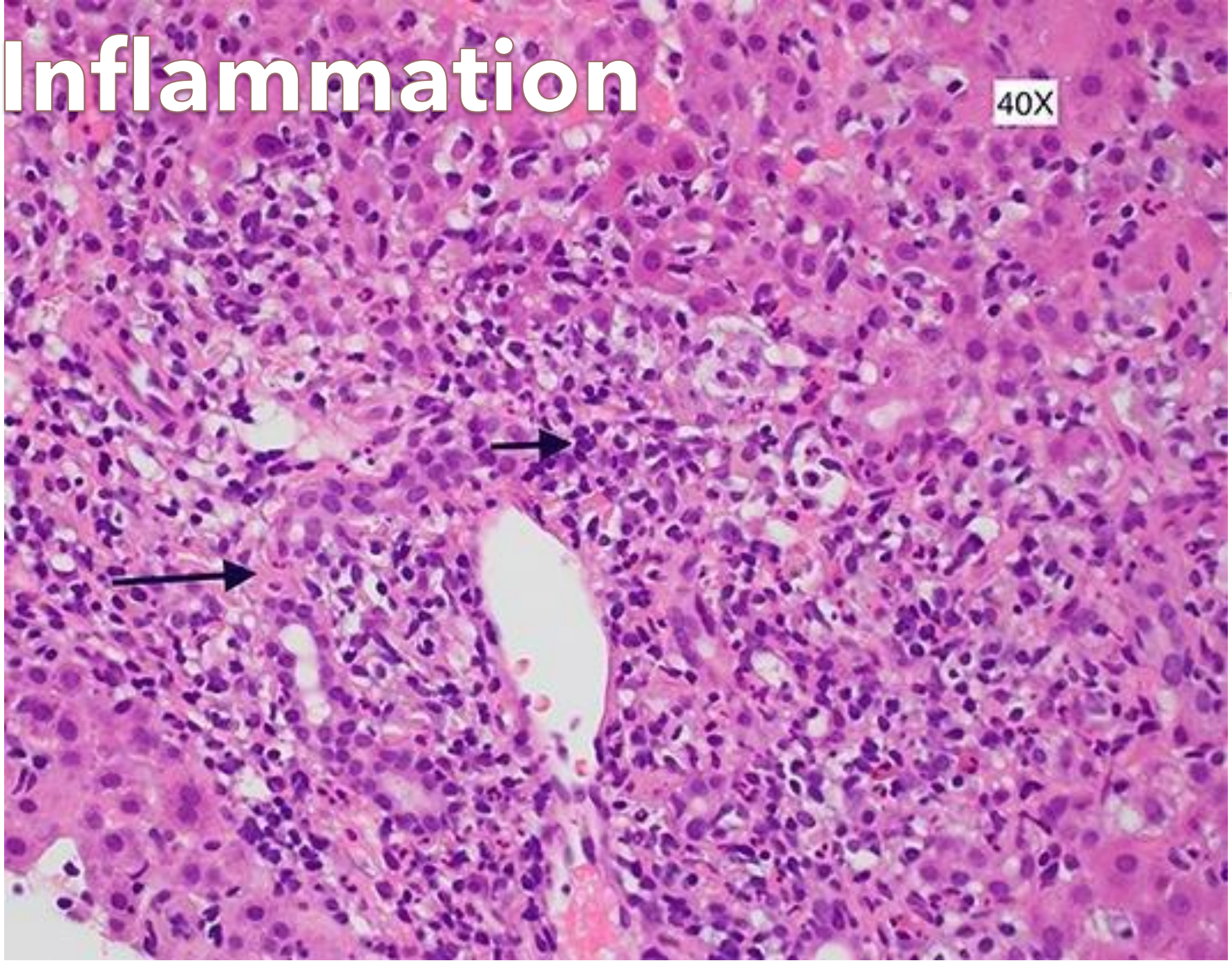
- **Cellular**
  - Cell changes: Pale/ Acidophil/ Ballooning-hydropic swelling/ Clear cell/Mallory bodies/ Hyaline/ Megamitochondria/ Mitochondriosis/ Sanded nuclei/ Cytomegalovirus (CMV) inclusions/ Herpes simplex viral inclusions/ Nuclear vacuolation/ Atrophy of hepatocytes/ Hypoxic vacuoles.
  - Necrosis: Acidophil body, Apoptosis, coaglutive, lytic, confluent, briding, central-central, portal central, surgical, Piecemeal, Lymphocytic piecemeal, Biliary, Fibrous, ductular piecemeal, Liver cell rosettes, focal, spotty, Emperipolesis.
  - Storage phenomena: Fatty/lipidosis, iron, copper, glycogen, α1-Antitrypsin, Fibrinogen, *Lipofuscin, The pigment in Dubin–Johnson syndrome, Ceroid pigment, Erythropoietic protoporphyria.*
- **Biliary and cholestatic**: visible bilirubin deposition, Portal and periportal changes.
- **Extracellular matrix = Fibrosis**: *Portal fibrosis, Concentric periductal fibrosis, Centrolobular* (*acinar zone 3*) *fibrosis, pericellular or perisinusoidal fibrosis, Perivenular fibrosis, Septal fibrosis, Primary/Secondary collapse, Hepatoportal sclerosis, Biliary fibrosis, Cirrhosis.*
- **Vascular change**: Hepatic artery, Afferent and efferent veins, Sinusoids and the space of Disse, Kupffer cell reaction and granuloma.
- **Histology**
  - Inflammation: *Lobular disarray, Acute hepatitis, Portal hepatitis, Periportal hepatitis, Central* (*centrolobular*) *hepatitis, Chronic lobular hepatitis.*
  - Regeneration: *Parenchymal giant cells* (*multinucleated hepatocytes*), *Liver cell dysplasia* (*dysplastic liver cells*), *Twin-cell plates, Nodular regeneration.*
- **Carcinoma: cancer neoplasia cell**


P
CV
100 µm
P

PRETTY HEPATOCYTE PIGMENTS



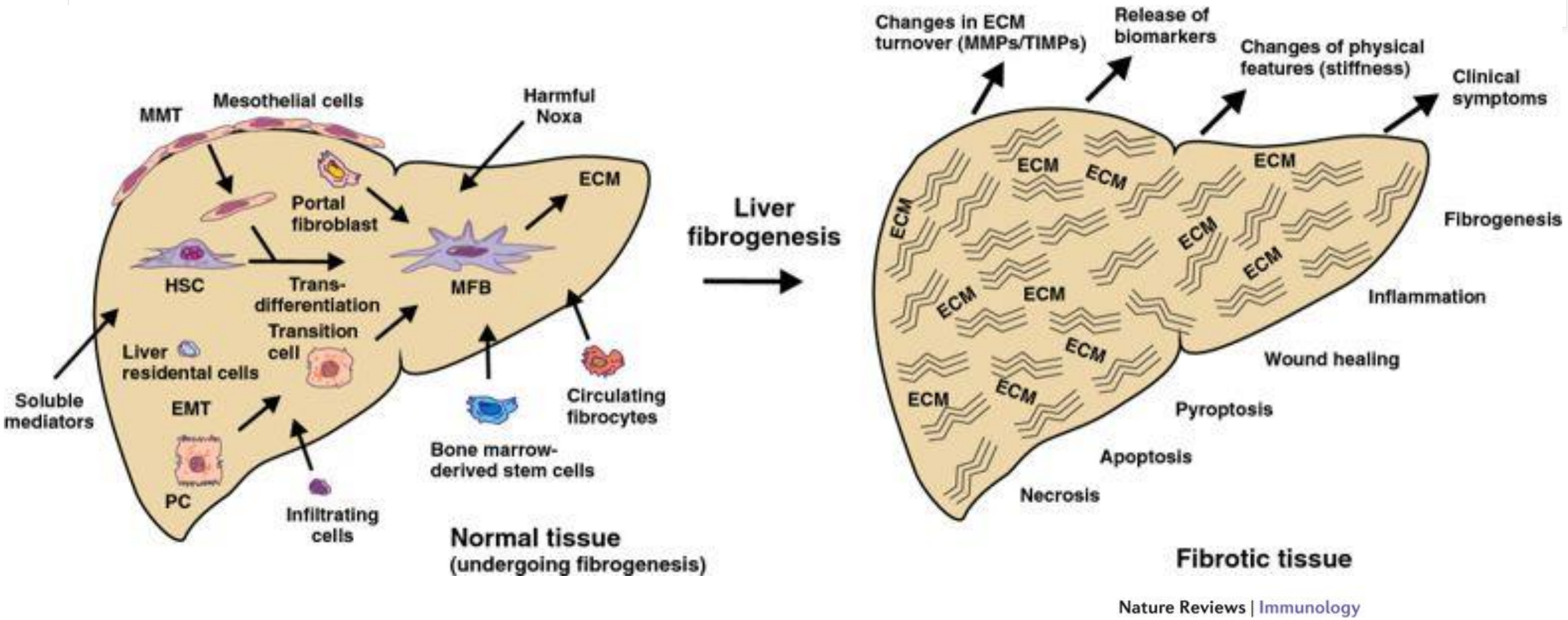
Inflammation



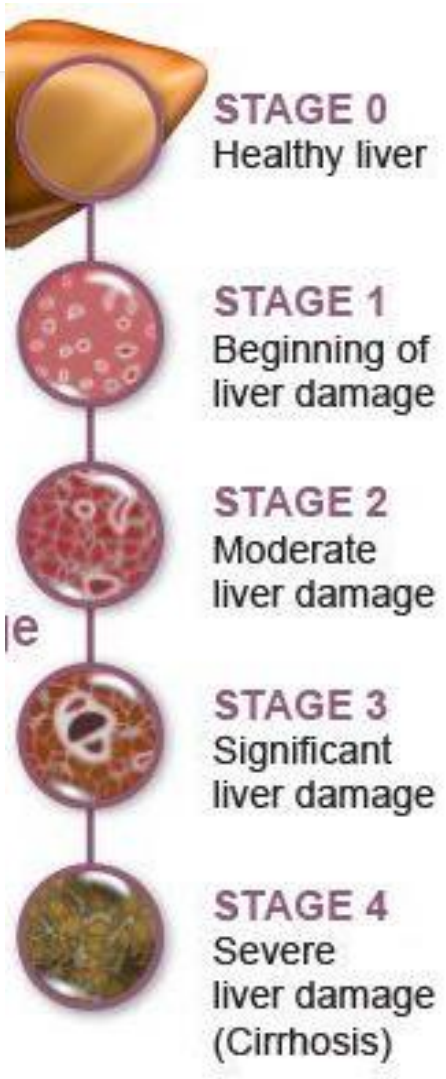
II. Liver disease: general manifestations

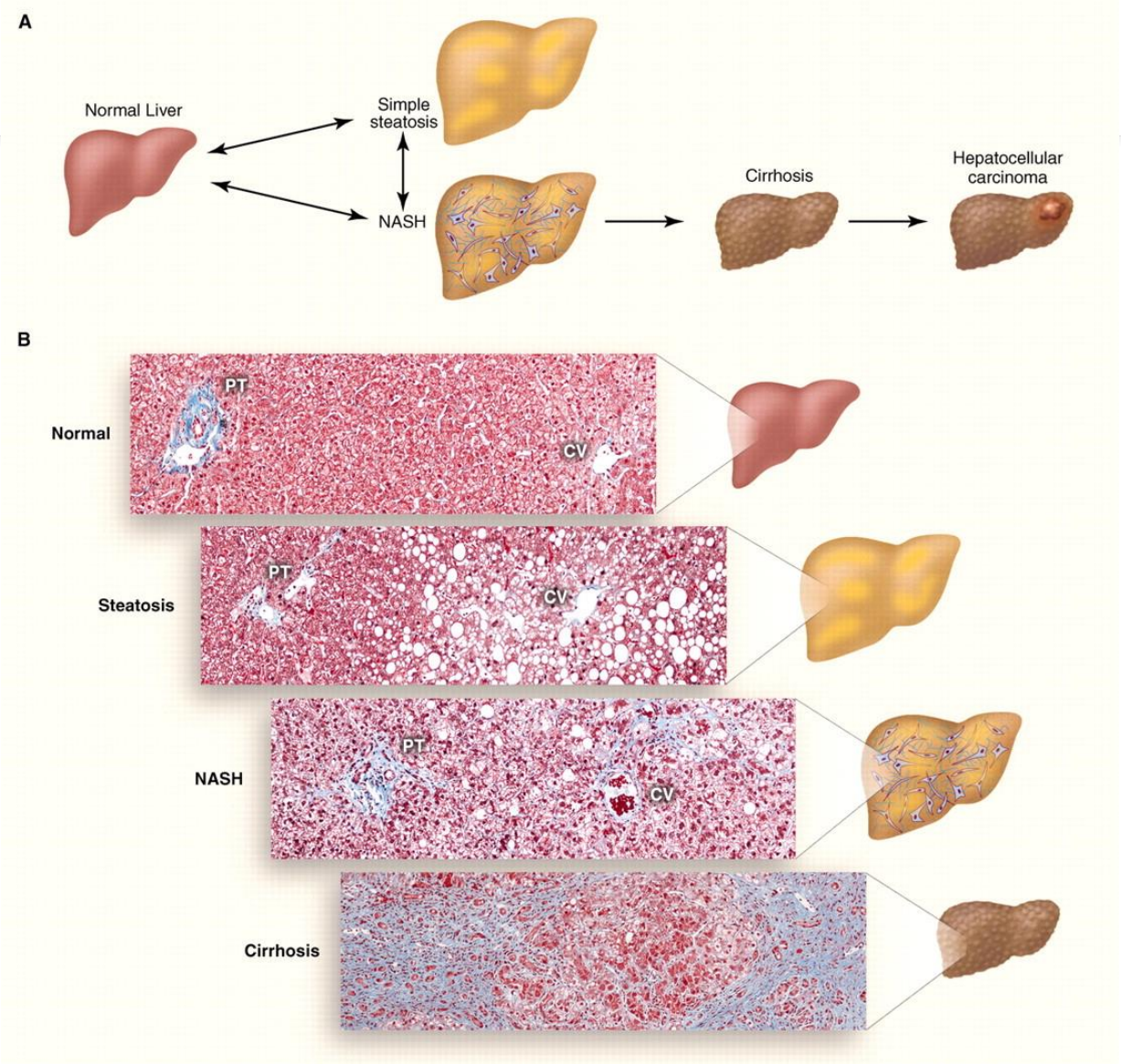
- **Hepatocellular Failure: jaundice, muscle wasting, ascites, excessive bleeding, deficiencies.**
- **Portal Hypertension: Gastroesophageal Varices**
- **Portal Systemic Encephalopathy: Hepatic Encephalopathy, Cerebral Edema**
- **Complications of Advanced Liver Disease: Ascites, Spontaneous Bacterial Peritonitis, Hepatorenal Syndrome**

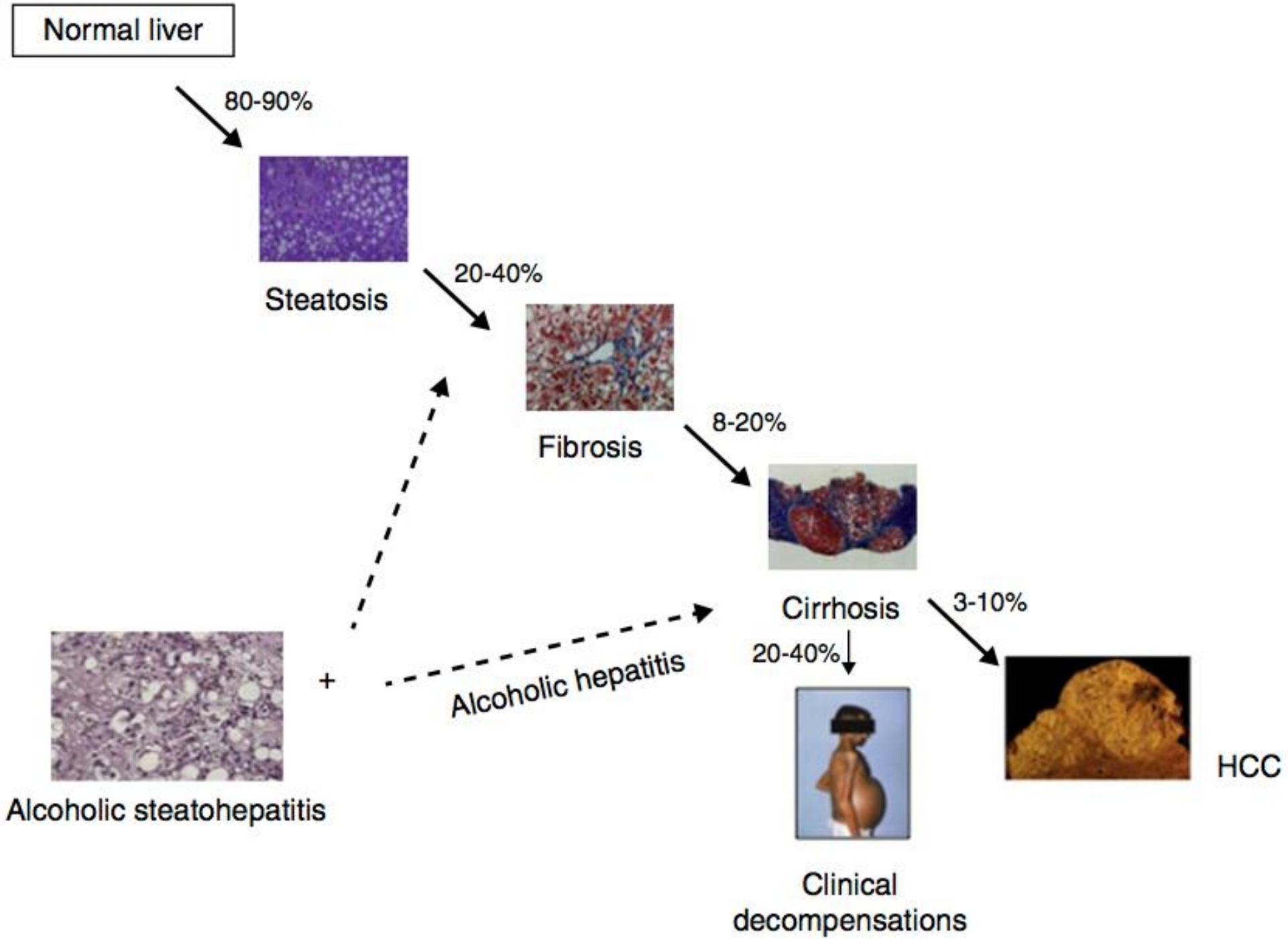
II. Liver disease: progession

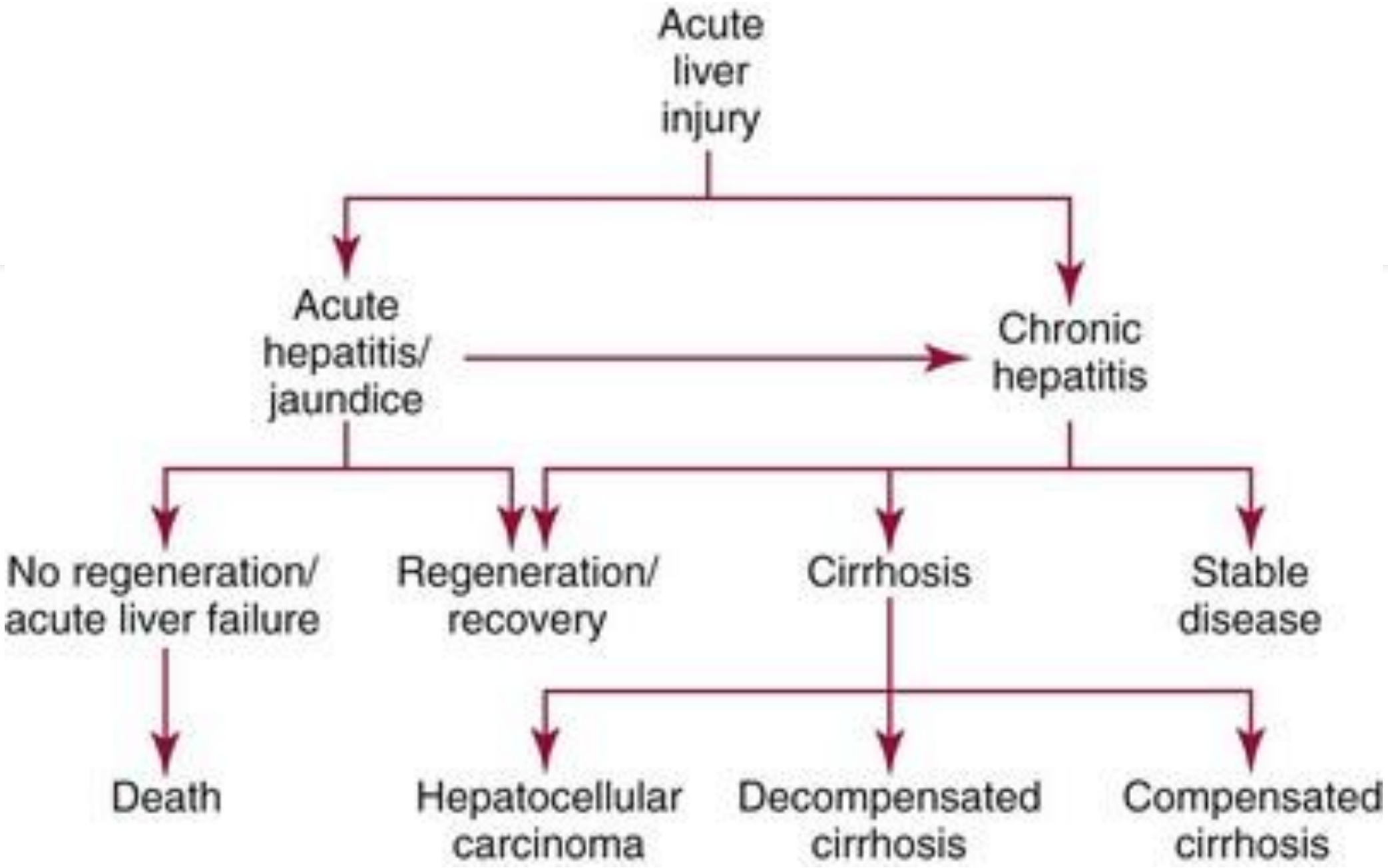


II. Liver disease: progession






Normal liver
80-90%
Steatosis
20-40%
Fibrosis
8-20%
Cirrhosis
3-10%
HCC
20-40%
Clinical decompensations
Alcoholic hepatitis
+
Alcoholic steatohepatitis

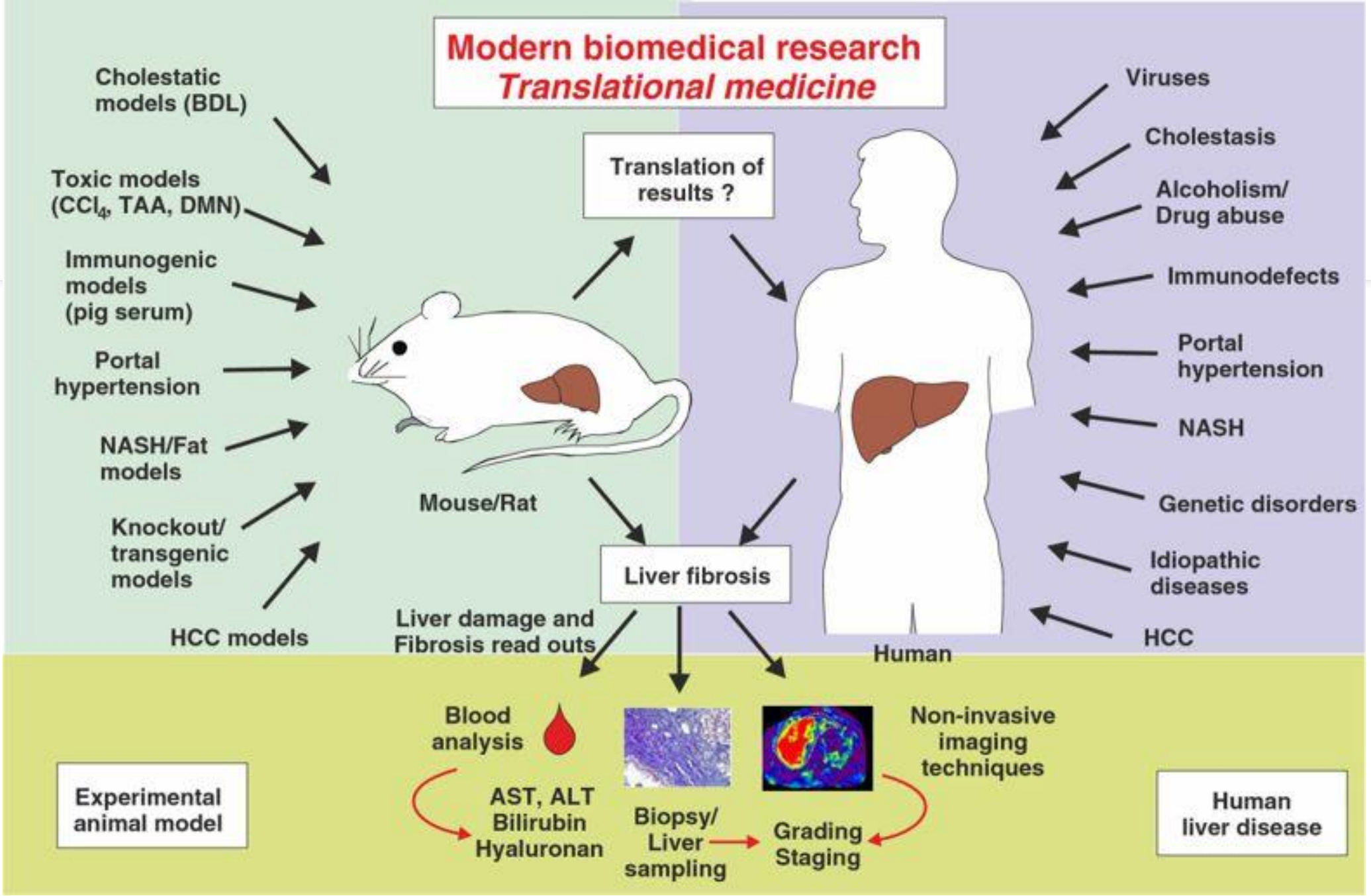

Acute liver injury
Acute hepatitis/ jaundice
Chronic hepatitis
No regeneration/ acute liver failure
Regeneration/ recovery
Cirrhosis
Stable disease
Death
Hepatocellular carcinoma
Decompensated cirrhosis
Compensated cirrhosis

III. Animal model of liver disease

- Aims: pathogenesis mechanisms, therapeutic targets, novel therapies, drug toxicity…
- Main objective: mice/rodent

III. Animal model of liver disease

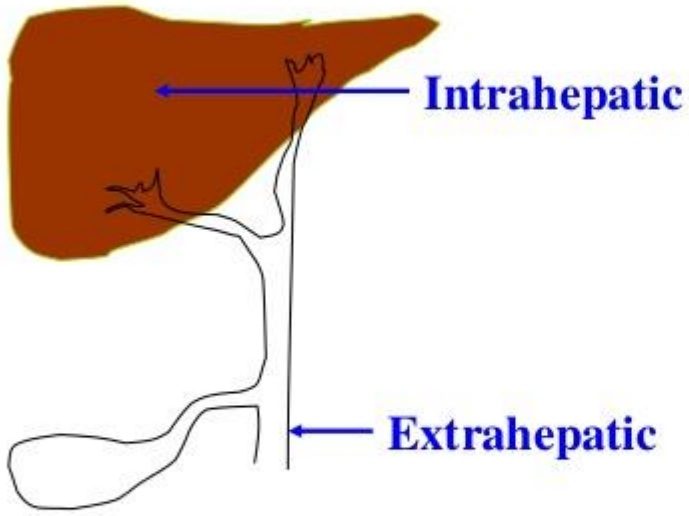
- Surgical:
- Chemical:
- Virus:
- Autoimmune:
- Diet:
- Gene:


Modern biomedical research
Translational medicine
Cholestatic models (BDL)
Toxic models (CCl_4, TAA, DMN)
Immunogenic models (pig serum)
Portal hypertension
NASH/Fat models
Knockout/ transgenic models
HCC models
Translation of results ?
Mouse/Rat
Liver fibrosis
Liver damage and Fibrosis read outs
Viruses
Cholestasis
Alcoholism/ Drug abuse
Immunodefects
Portal hypertension
NASH
Genetic disorders
Idiopathic diseases
HCC
Human
Blood analysis
AST, ALT
Bilirubin
Hyaluronan
Biopsy/ Liver sampling
Grading Staging
Non-invasive imaging techniques
Experimental animal model
Human liver disease

III. Animal model of liver disease

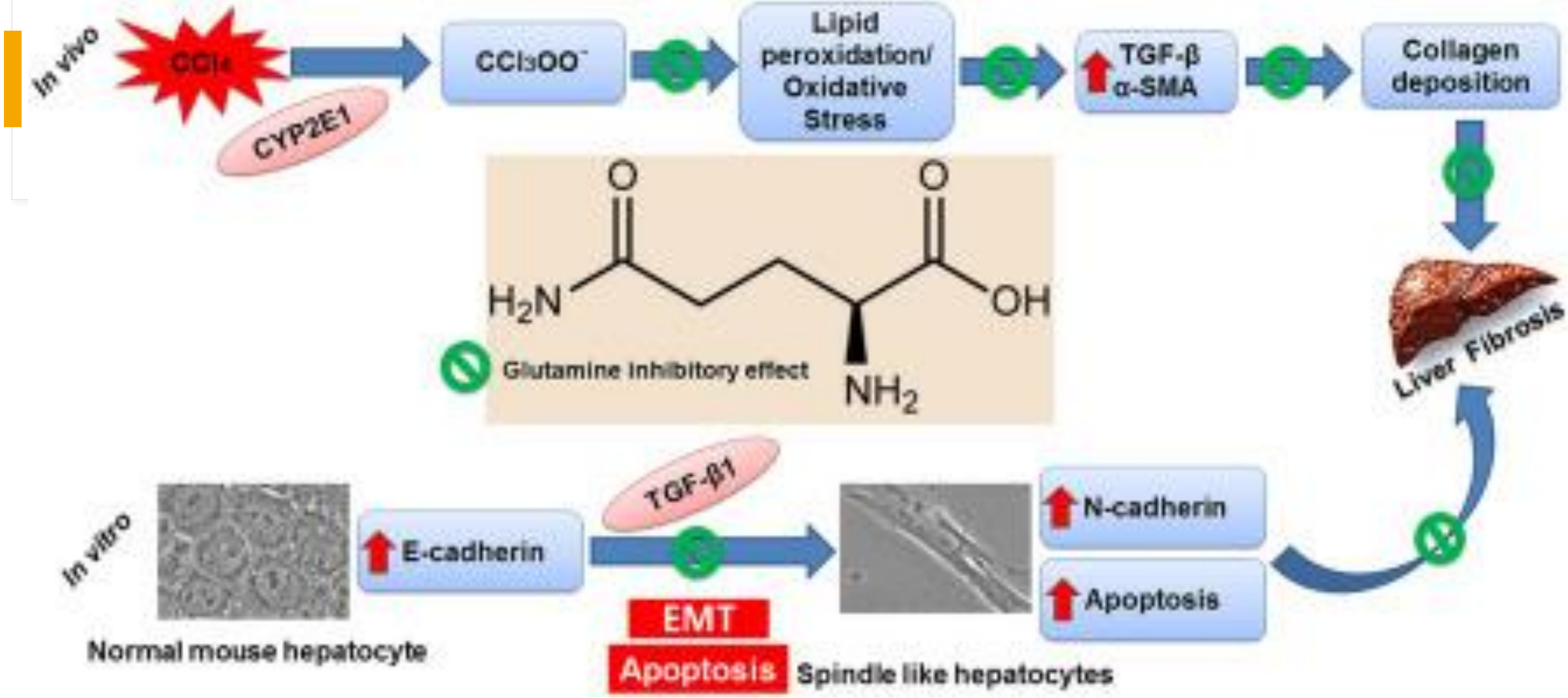
Cholestasis (Greek-bile stoppage)

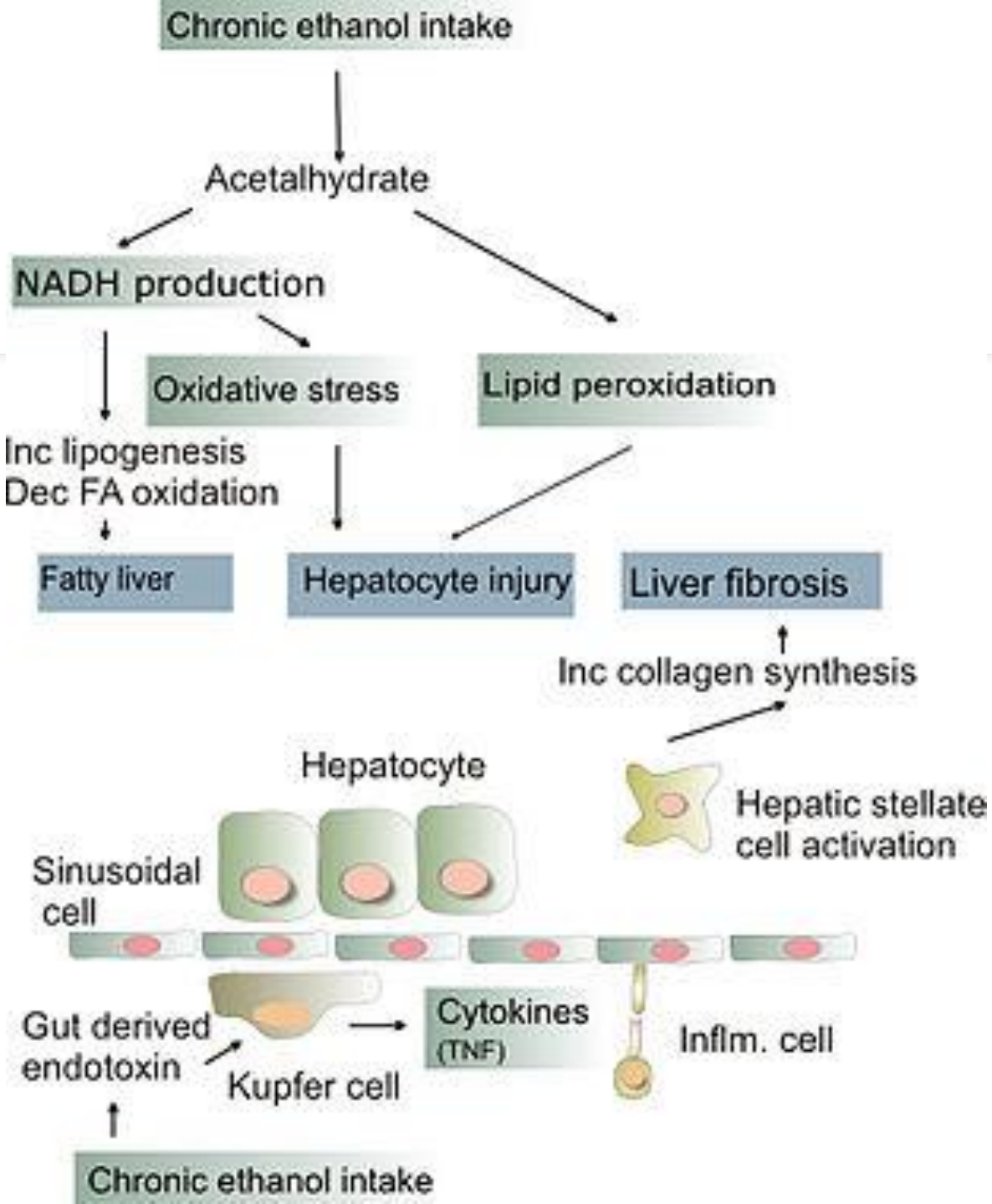
Reduction or absence of bile flow into duodenum

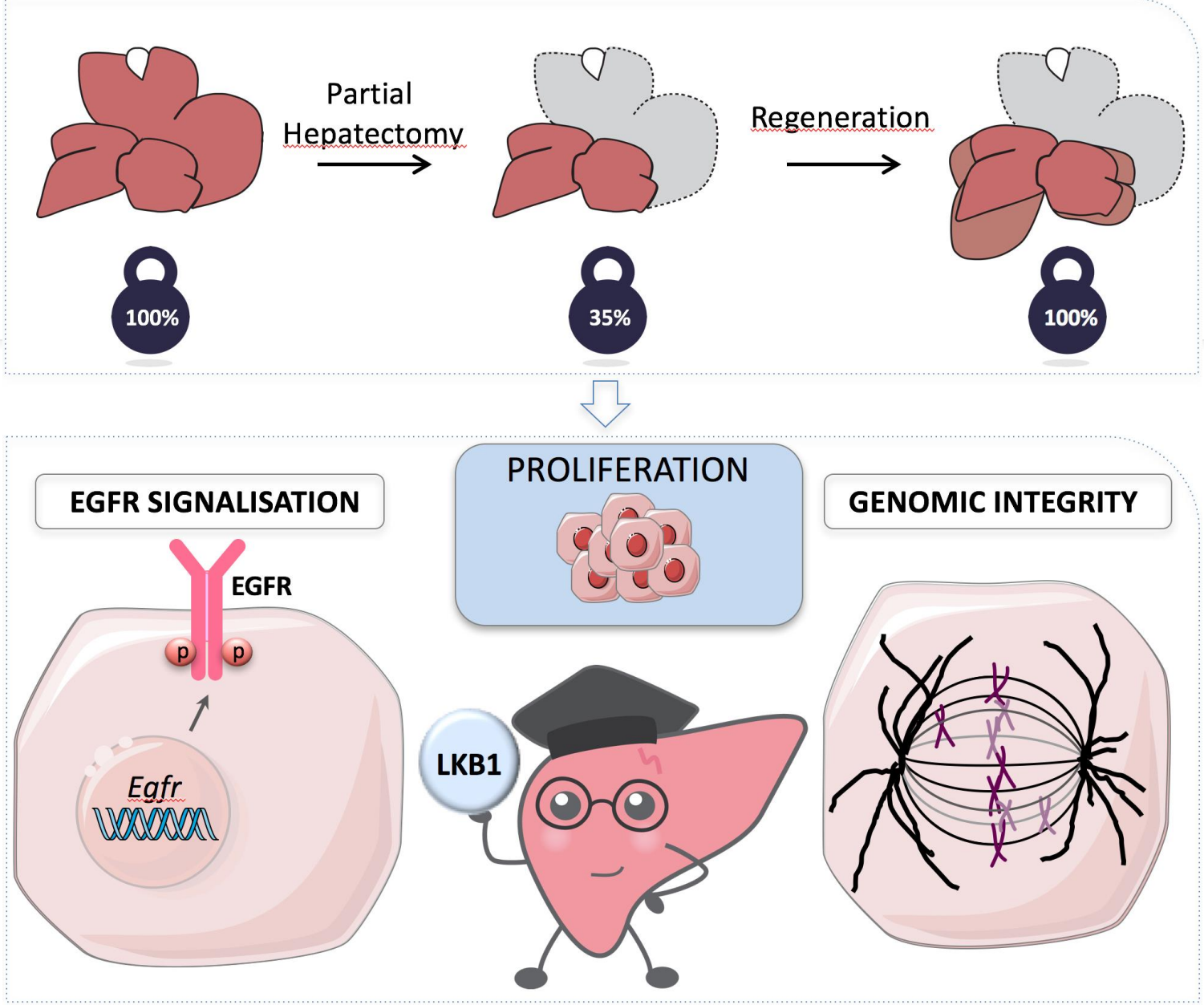


- Impairment of bile secretion at the level of bile ductules (ductular cholestasis)
- Functional defect in bile formation at hepatocyte level (hepatocellular chlestasis)

Chronic if > 6mo duration
Etiology: differs across ages
Alkaline phosphatase >1.5ULN, GGT> 3ULN*

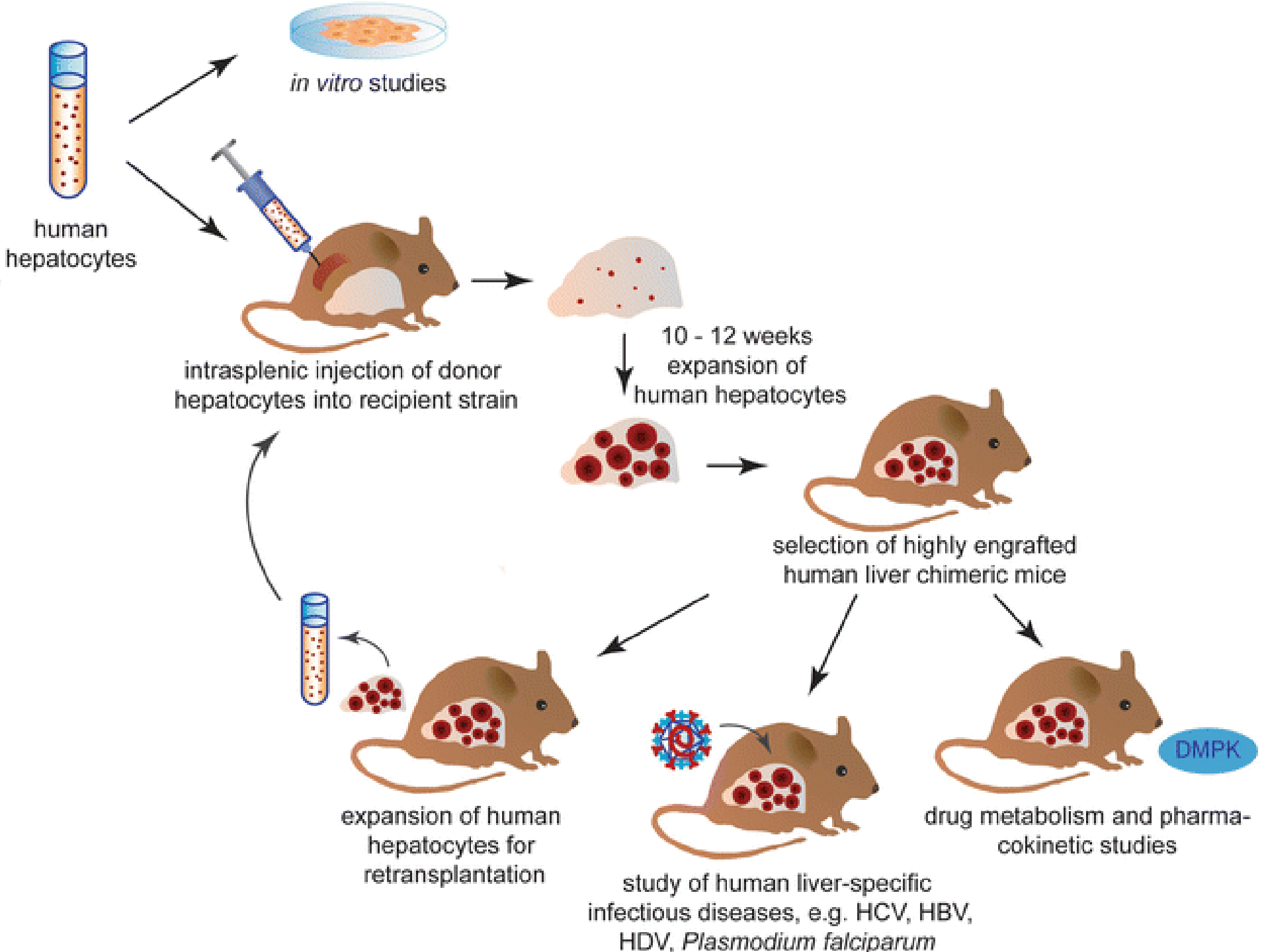

In vivo
CCl_4
CYP2E1
CCl_3OO^-
Lipid peroxidation/ Oxidative Stress
TGF-β α-SMA
Collagen deposition
Liver Fibrosis
H_2N
O
O
OH
NH_2
Glutamine inhibitory effect
In vitro
Normal mouse hepatocyte
E-cadherin
TGF-β1
EMT
Apoptosis
Spindle like hepatocytes
N-cadherin
Apoptosis

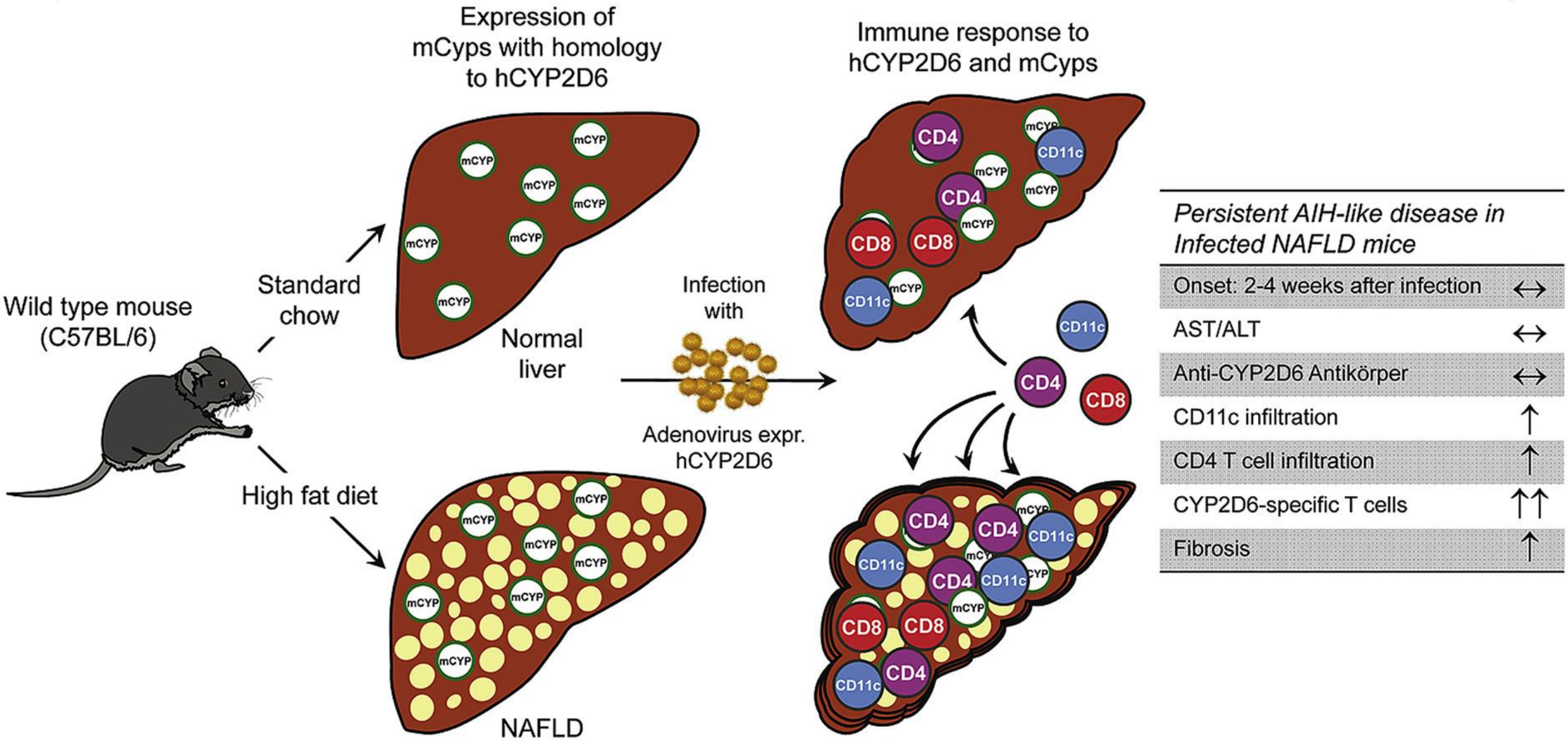

Chronic ethanol intake
Acetalhydrate
NADH production
Oxidative stress
Lipid peroxidation
Inc lipogenesis
Dec FA oxidation
Fatty liver
Hepatocyte injury
Liver fibrosis
Inc collagen synthesis
Hepatocyte
Hepatic stellate
cell activation
Sinusoidal
cell
Gut derived
endotoxin
Kupfer cell
Cytokines
(TNF)
Inflm. cell
Chronic ethanol intake

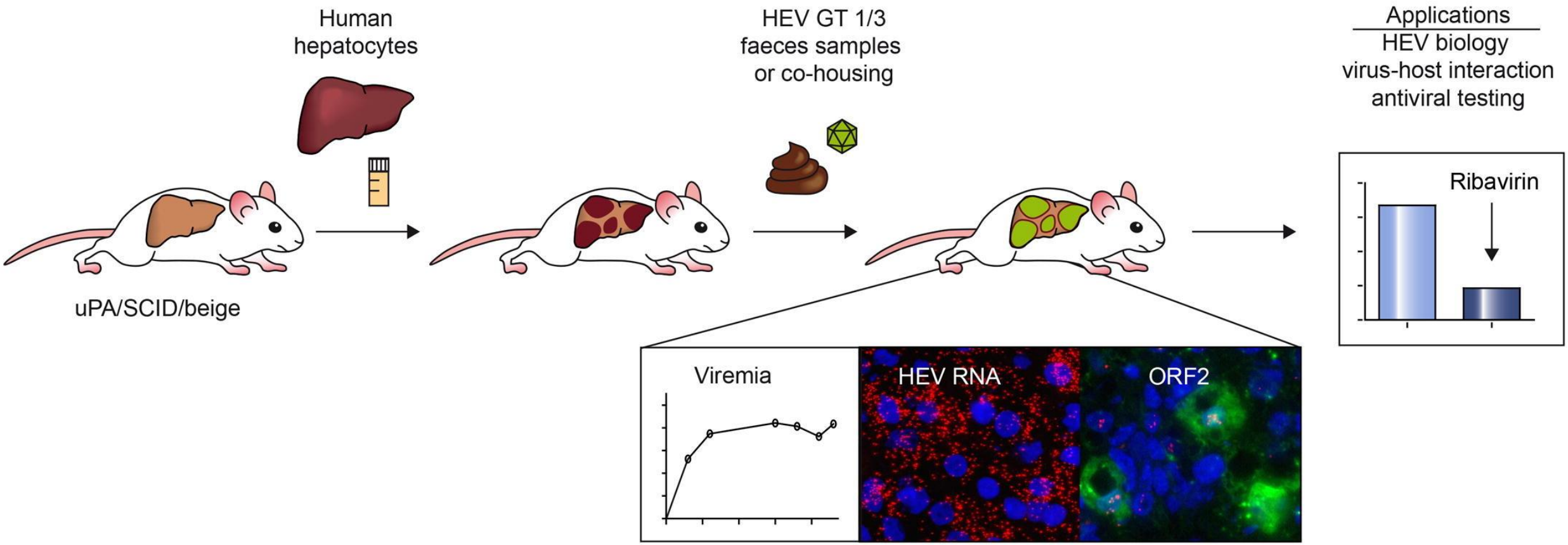


LKB1 : a Gatekeeper of Controlled Hepatocyte Proliferation

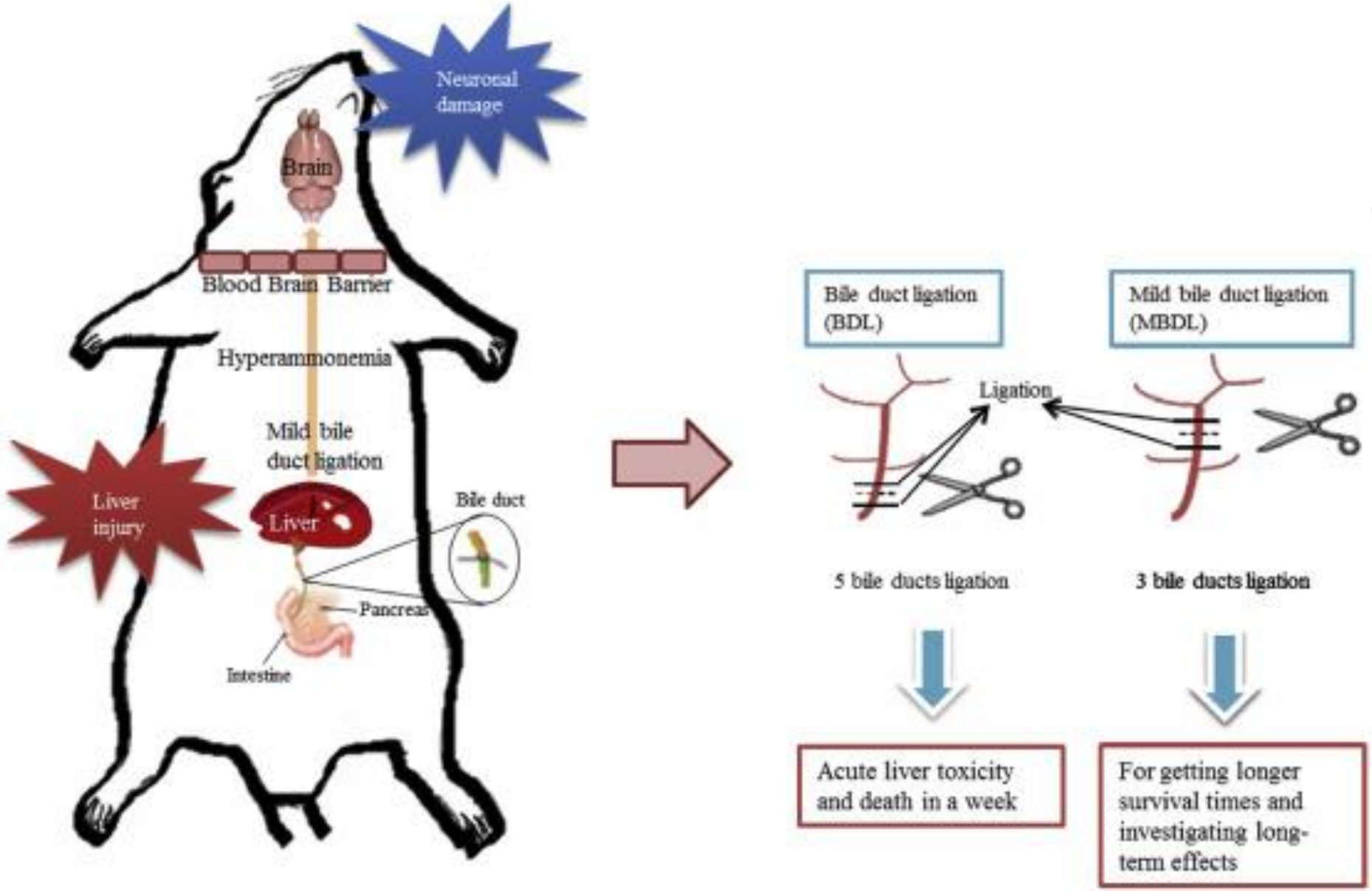
The authors performed liver partial hepatectomy in wild-type and hepatocyte-specific LKB1 knockout mice. Without LKB1, hepatocyte proliferation and genomic integrity are altered during compensatory cell renew consecutive to hepatectomy. These results reveal the importance to monitor that LKB1 kinase activity is not impaired in the liver of patient undergoing surgical resection.


in vitro studies
human hepatocytes
intrasplenic injection of donor hepatocytes into recipient strain
10 - 12 weeks expansion of human hepatocytes
selection of highly engrafted human liver chimeric mice
DMPK
drug metabolism and pharma-cokinetic studies
study of human liver-specific infectious diseases, e.g. HCV, HBV, HDV, Plasmodium falciparum
expansion of human hepatocytes for retransplantation


Expression of
mCyps with homology
to hCYP2D6
Immune response to
hCYP2D6 and mCyps
Wild type mouse
(C57BL/6)
Standard
chow
Normal
liver
Infection
with
Adenovirus expr.
hCYP2D6
High fat diet
NAFLD
mCYP
CD4
CD8
CD11c
Persistent AIH-like disease in Infected NAFLD mice
Onset: 2-4 weeks after infection ↔
AST/ALT ↔
Anti-CYP2D6 Antikörper ↔
CD11c infiltration ↑
CD4 T cell infiltration ↑
CYP2D6-specific T cells ↑↑
Fibrosis ↑


Human
hepatocytes
HEV GT 1/3
faeces samples
or co-housing
Applications
HEV biology
virus-host interaction
antiviral testing
uPA/SCID/beige
Ribavirin
Viremia
HEV RNA
ORF2

Mild Bile Duct Ligation (MBDL) Model



III. Animal model of liver disease

- Results:???

III. Animal model of liver disease

Liver model	System	Disease	Culture	Advantages	Weaknesses
Spheroids[182]	*In vitro*	NAFLD/NASH	PHH, IC, NPC	High-throughput drug screening, co-cultures with NPC possible without any specialised system	Cell choice can modify the output, suboptimal cell–cell interaction, access to fresh human tissue, healthy tissue not really extracted from healthy individuals, lack of complete hepatocyte polarity
Organoids[108]	*In vitro*	NAFLD/NASH, HCC/CCA	PSC	A genetically stable 3D model, long-term culture, unlimited source of cells, high-throughput drug screening and personalised medicine (gene therapy)	PSCs express foetal markers, limited cell maturation/cell function, failure to recapitulate multiple cell types of the liver
Liver-on-a-chip[109]	*In vitro*	NAFLD/NASH, ACLD	PHH, IC, NPC	Dynamic fluid flow, commercially available, single and multi-chamber design, sustained functionality for at least 4 weeks, modelling zonal liver phenotypes possible	Low throughput drug screening, access to fresh human tissue, healthy tissue not really extracted from healthy individuals
Precision-cut liver slices[111]	*Ex vivo*	ALD/ASH, NAFLD/NASH, HCC	Whole tissue	Reproducible, low cost, hepatic environment and ECM present, low amount of tissue	Access to fresh tissue, healthy tissue not really extracted from healthy individuals, functions as metabolic capacity only maintained 3 days
Decellularized liver scaffold[113]	*Ex vivo*	Under development	PHH, IC, NPC	Provides a neutral environment that mimics (patho)physiological conditions, low antigenicity, simple and safe protocol	Divergence in decellularisation and recellularisation protocols, reendothelisation is a concern, long-term engraftment

ACLD, advanced chronic liver disease; ALD, alcohol-related liver disease; ASH, alcohol-related steatohepatitis; CCA, cholangiocarcinoma; ECM, extracellular matrix; HCC, hepatocellular carcinoma; IC, immortalised cell lines; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NPC, non-parenchymal cells; PHH, primary human hepatocytes; PSCs, pluripotent stem cells.

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Thank you