Hepatitis tóxica (DILI): una hoja de ruta para investigar en Europa

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ROADMAP TO DILI RESEARCH IN EUROPE

STRATEGIC LINE	Low risk	Medium risk	High risk
	SHORT-TERM	INTERMEDIATE-TERM	LONG-TERM
I. Creation of an integrative database and optimised case report form for prospective identification of DILI cases, biological samples and imaging data	**1.1** Creation of a **prospective** and **integrative** database of DILI patients: **ID-DILI** **1.2** Prospective identification and characterization of **atypical DILI phenotypes**	**1.3** Challenges in the establishment of **causality assessment** **1.4** Development of an **adaptive** and optimized case report form to capture **real-world** information in DILI patients	
II. Preclinical models in DILI to improve the assessment and prediction of hepatotoxicity to guide future drug safety testing	**2.1 Advanced *in vitro*** approaches to move forward personalized medicine in DILI: **patient-derived** pluripotent stem cells and complex cell culture configurations	**2.2 Humanized mouse** models: a game-changer in DILI research **2.3** Mode of liver injury and impact of **metabolic liver disease** in DILI	**2.4 Zebrafish** model: simplicity to get effectiveness **2.6** Perspectives of **extracellular vesicles** (EVs) in DILI **2.5 Computational** Approaches for **Integrating** Multi-Scale Data in DILI research
III. Clinical research and Healthcare system Implementation	**3.1** DILI as a **rare disease** and the possibility of **family studies**	**3.2** DILI **risk stratification** and Prognostic Biomarkers including **pharmacogenetic** studies **3.3 Artificial intelligence**-based integrative research	
IV. Networking and regulatory aspects	**4.1 EASL DHILI** Consortium **4.2** Criteria to appoint centers as **Networks of Excellence**	**4.3** Proof of concept, mechanistic, targeted-oriented **Clinical Trials** in DILI	

Lucena MI.., Andrade RJ et al. Pharmacol Res. 2024

I. Creation of an integrative database and optimised case report form for prospective identification of DILI cases, biological samples and imaging data

Creation of a prospective and integrative database of DILI patients

- Prospective registries are the most valuable source of data for idiosyncratic DILI research.
- Comparability between registries is to some extent limited due to differences in data collection, case definition, or causality assessment.
- Thus, the creation of a prospective and integrative database has great interest.

Map the existing databases → Create a list of metadata → **Integrate the information in this new database**

I. Creation of an integrative database and optimised case report form for prospective identification of DILI cases, biological samples and imaging data

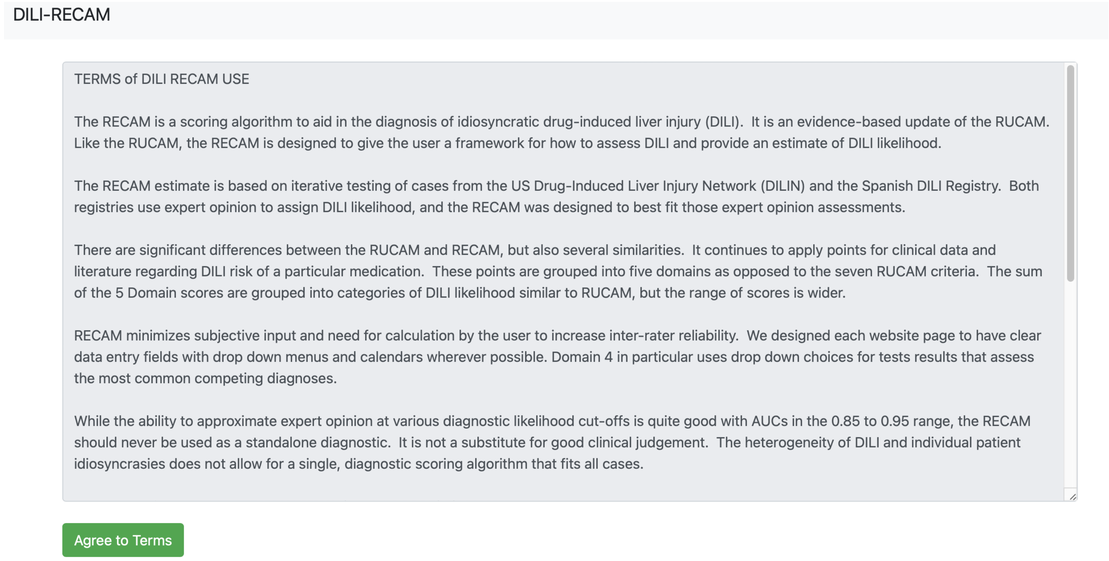
Challenges in the establishment of causality assessment

CIOMS/RUCAM

Criteria		Score
1. TIME TO ONSET OF THE REACTION		
	Highly suggestive	*+ 3*
	Suggestive	+ 2
	Compatible	+ 1
	Inconclusive	*0*
If incompatible, then case "unrelated"		
If information not available, then case "insufficiently documented"		
2. COURSE OF THE REACTION		
	Highly suggestive	+ 3
	Suggestive	+ 2
	Compatible	+ 1
	Against the role of the drug	- 2
	Inconclusive or not available	0
3. RISK FACTOR(S) FOR DRUG REACTION	Presence	+ 1 to + 2[a]
	Absence	0
4. CONCOMITANT DRUG(S)[c]	Time to onset incompatible	0
	Time to onset compatible but unknown reaction	- 1
	Time to onset compatible and known reaction	- 2
	Role proved in this case	- 3
	None or information not available	0
5. NON DRUG-RELATED CAUSES[c]	Ruled out	+ 2
	Possible or Not investigated[b]	+ 1 to - 2
	Probable	- 3
6. PREVIOUS INFORMATION ON THE DRUG		
	. Reaction unknown	0
	. Reaction published but unlabelled	+ 1
	. Reaction labelled in the product's characteristics	+ 2
7. RESPONSE TO READMINISTRATION	Positive	+ 3
	Compatible	+ 1
	Negative	- 2
	Not available or Not interpretable	0
or PLASMA CONCENTRATION of the drug known as toxic		+ 3
or VALIDATED LABORATORY TEST with high specificity, sensitivity and predictive values	Positive	+ 3
	Negative	- 3
	Not interpretable or not available	0

Danan G & Benichou C. J Clin Epidemiol. 1993

RECAM

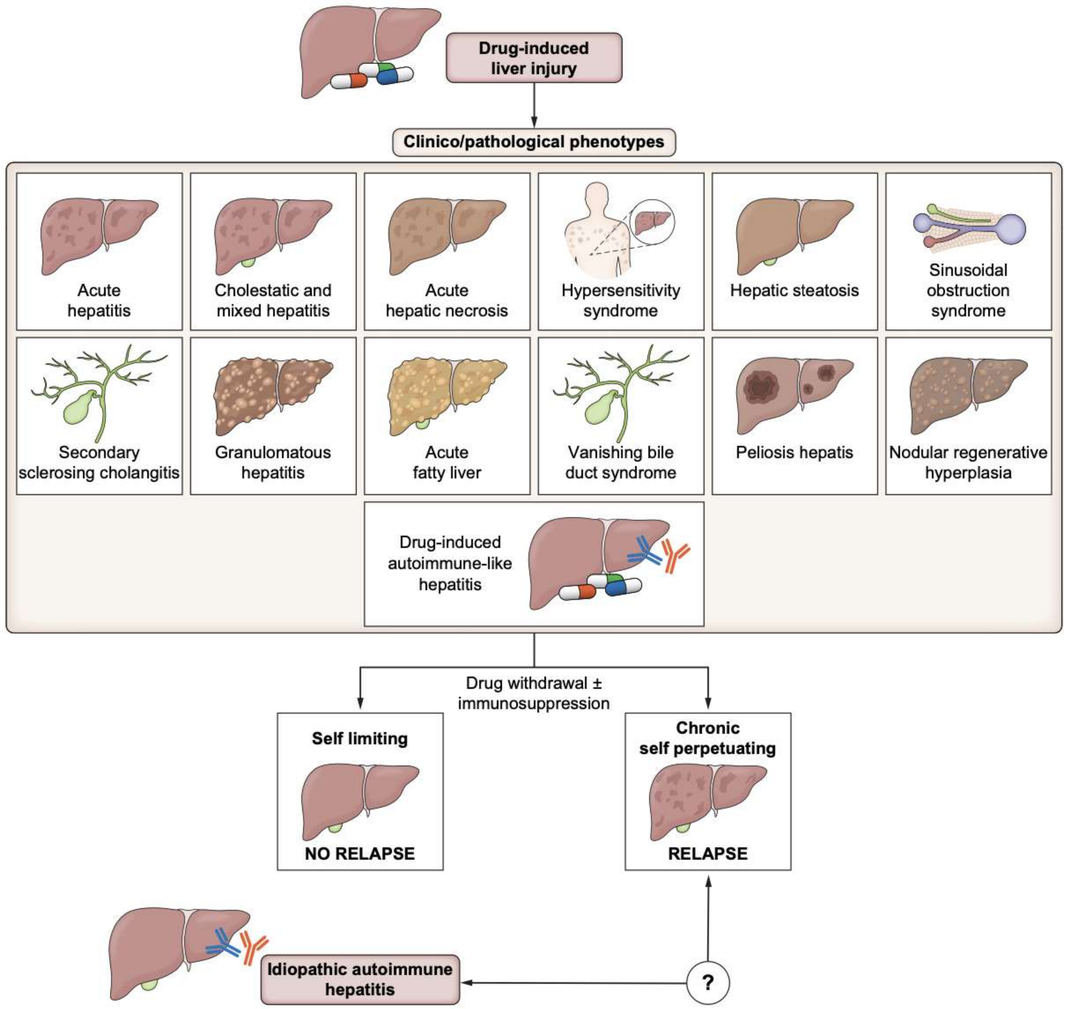


Hayashi P, Lucena Mi, et al. Hepatology. 2022

- **Should the CIOMS/RUCAM be replaced? Is still the expert opinion model the chosen causality assessment method in the future in clinical drug development?**

I. Creation of an integrative database and optimised case report form for prospective identification of DILI cases, biological samples and imaging data

Prospective identification and characterization of atypical DILI phenotypes

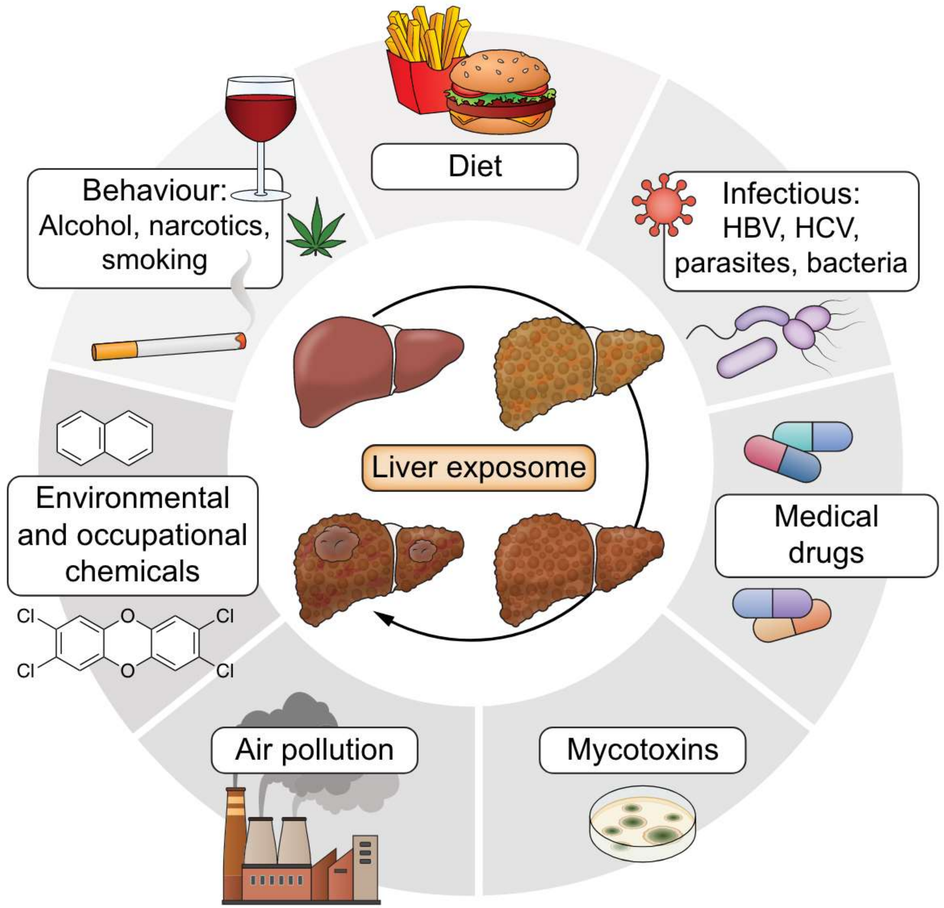


Andrade RJ, Aithal GP, de Boer YS, Liberal R, et al. J Hepatol. 2023

- DILI due to immune checkpoints inhibitors.
- Drug-induced autoimmune hepatitis (DI-ALH).
- DILI in pediatrics.
- DILI due to herbal and dietary supplements.
- **Collection of biological samples collection will aid in the study of the pathophysiology of these phenotypes.**

I. Creation of an integrative database and optimised case report form for prospective identification of DILI cases, biological samples and imaging data

Development of an adaptive and optimized case report form to capture real-world information in DILI patients

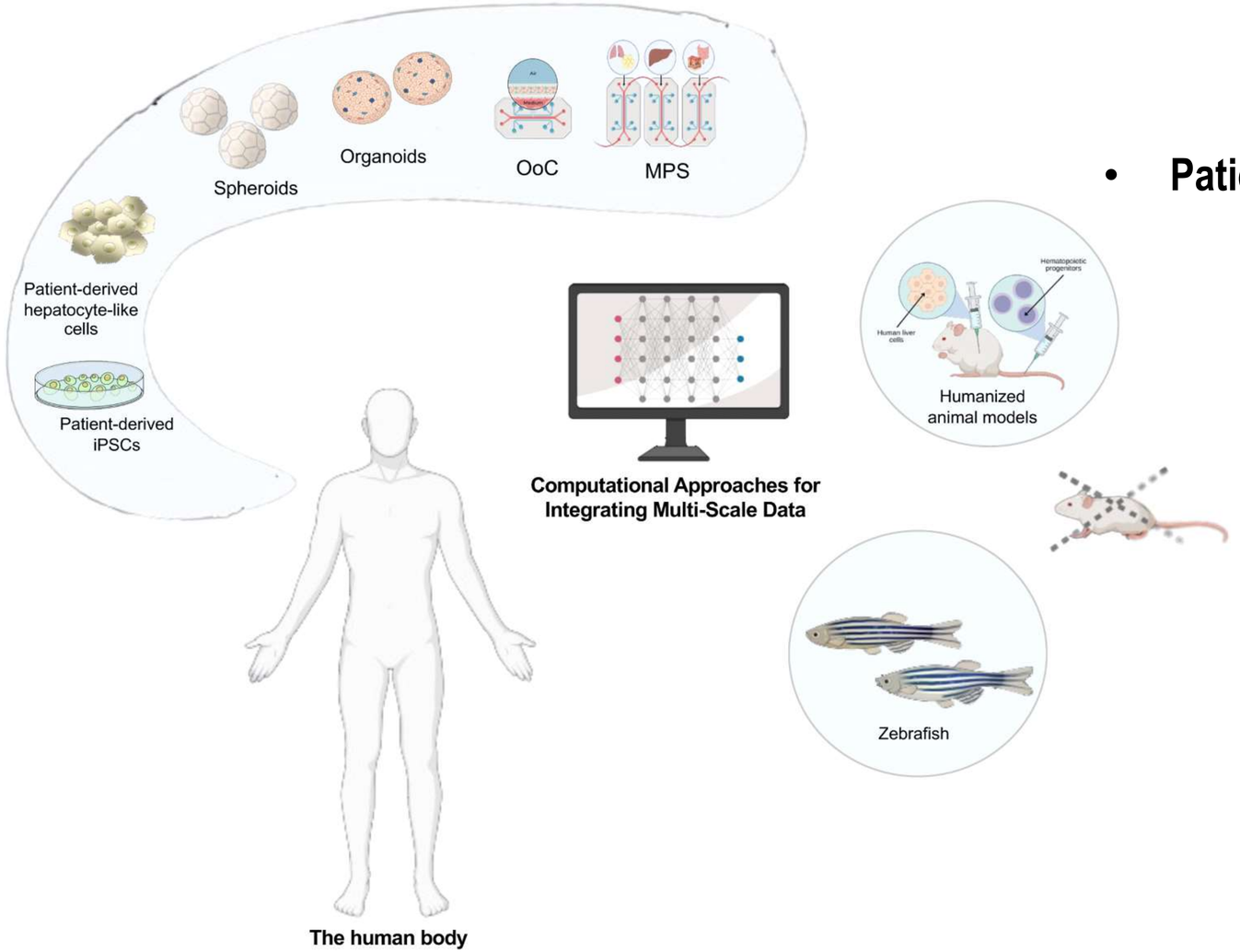


Barouki R, et al. J Hepatol. 2023

- The **exposome** provides novel, additional insights into innovative study designs to test novel mechanistic hypotheses in DILI.
- Newly designed case report forms should include **patient liver histology data for prospective registries**.
- Use of tools such as digitized liver biopsies and the development of text-processing machine learning models to extract structured information from narrative descriptions will aid in the analysis.

II. Preclinical models in DILI to improve the assessment and prediction of hepatotoxicity to guide future drug safety testing

Mechanisms of idiosyncratic DILI are still unknown



1. Multidisciplinary approach:

- **Patient-derived preclinical models + non-invasive imaging + integrative multi-omics analyses**

…complemented with

- **Emerging 3D–4D multicellular *in vitro* platforms + humanized animal models**

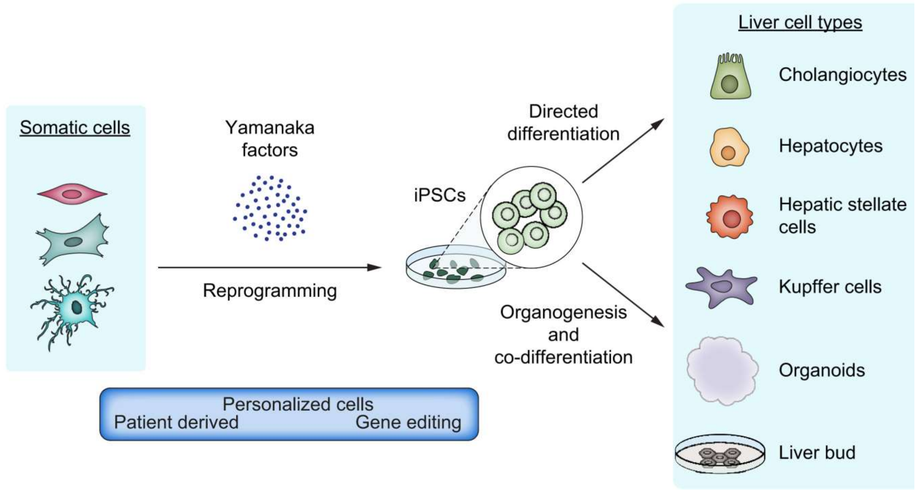
2. New preclinical tools
- **Hepatic extracellular vesicles (EVs)**

Lucena MI.., Andrade RJ et al. Pharmacol Res. 2024

II. Preclinical models in DILI to improve the assessment and prediction of hepatotoxicity to guide future drug safety testing

➢ Advanced *in vitro* approaches to move forward personalized medicine in DILI

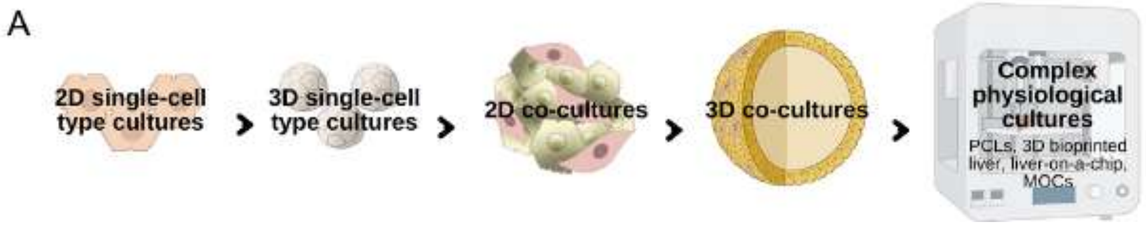
○ Patient-derived pluripotent stem cells

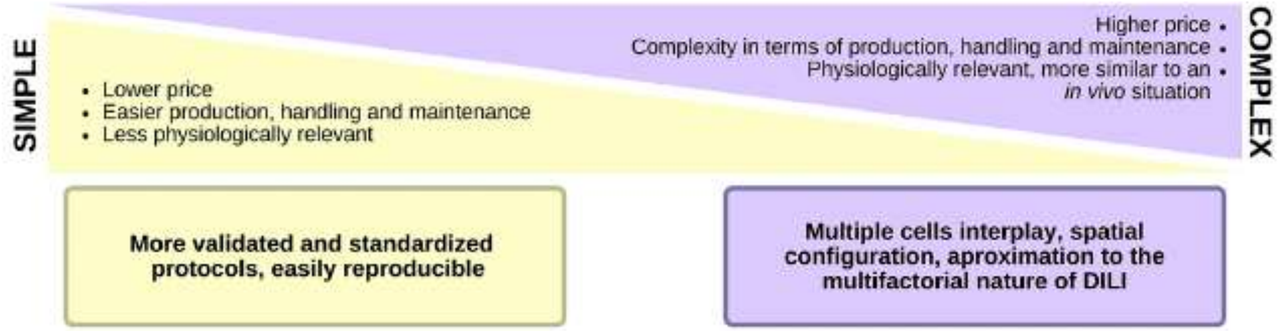


- Extensive proliferation
- Genetic stability
- Patient genetic background----personalized medicine
- Susceptible to genetic modifications
- Capacity to differentiate into different liver cell types
- Multicellular system with the same genetic background

Fernández-Checa JC et al. J Hep. 2021

○ Complex cell culture configurations

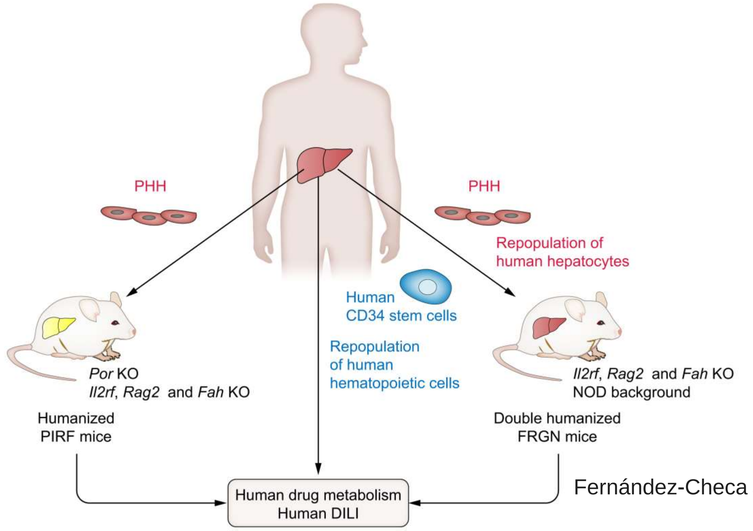




Segovia-Zafra A. et al. Acta Pharm Sin B. 2021

II. Preclinical models in DILI to improve the assessment and prediction of hepatotoxicity to guide future drug safety testing

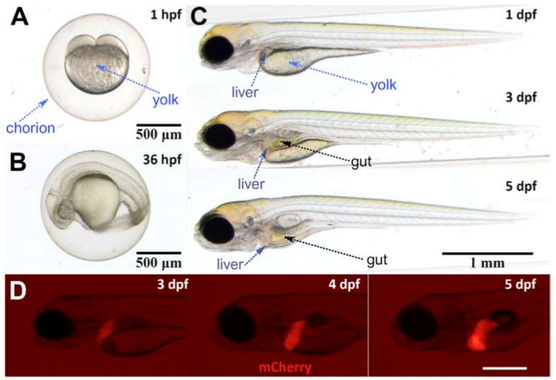
➢ Humanized mouse models: a game-changer in DILI research



Fernández-Checa JC et al. J Hep. 2021

- Mouse lines with humanized liver
- Immunosuppressive environment
- Possibility to generate double chimeras with humanized adult hepatocytes and hematopoietic cells
- Genetic modifications to study specific pathways.
- Example: FRGN mice model: triple (Il2rg-/-/Rag2-/-/Fah-/-) knockout line in the NOD background.

➢ Zebrafish model: simplicity to get effectiveness



Cakan-Akdogan G,et al. Explor Dig Dis. 2023.

Specific metabolic drug reactions reported in zebrafish compared with humans

Compound	Drug metabolism in zebrafish Reaction observed in zebrafish	Similar to human	Human P450 isotype	Ref.
Ibuprofen	Hydroxylation	Yes	CYP2C8/9	[33]
Paracetamol	Hydroxylation	Yes	CYP3A4	[37]
Testosterone	Hydroxylation	Yes	CYP3A4	[37]
Cisapride	Sulfate conjugation	No	CYP3A4	[38]
Verapamil	*N*-Dealkylation and hydroxylation	Yes	CYP3A4, CYP2C8/9, CYP1A2	[38]
Chlorpromazine	Hydroxylation, oxidation, *N*-demethylation, glucuronidation and sulfation	Yes	CYP1A2, CYP2D6	[38]
Phenacetin	De-ethylation	Yes	CYP1A2	[38]
Dextromethorphan	Demethylation	Yes	CYP2D6	[38]
Bupropion	Hydroxylation	Yes	CYP2B6	[38]

Vliegenthart, A.D.B. et al. Br J Clin Pharmacol, 2014.

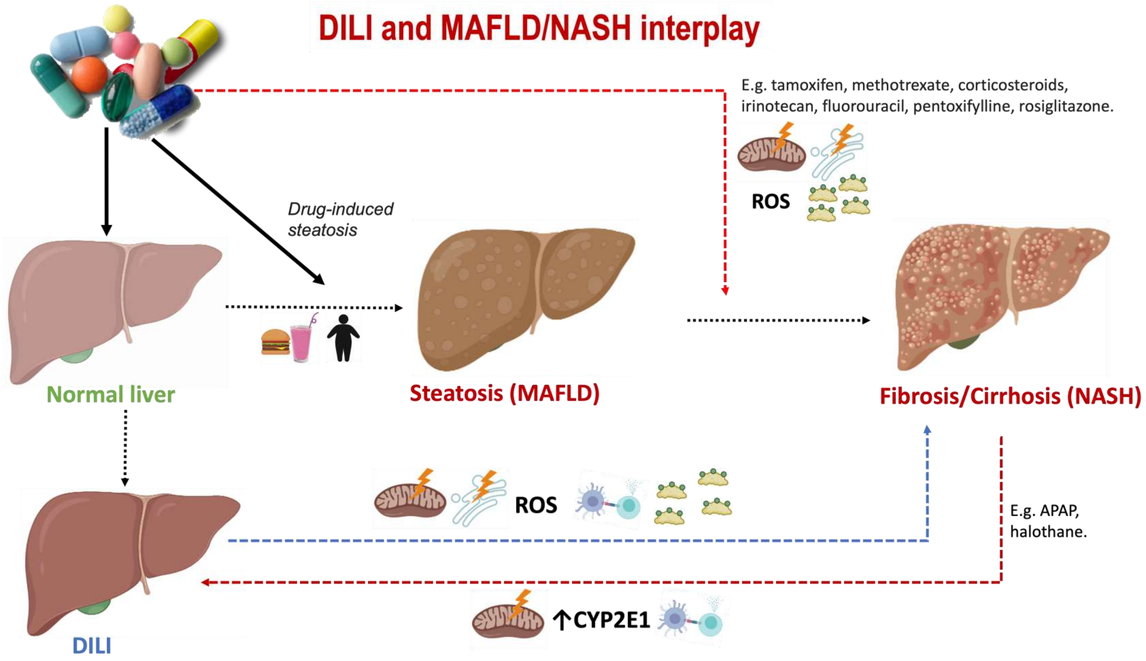
II. Preclinical models in DILI to improve the assessment and prediction of hepatotoxicity to guide future drug safety testing

➢ Mode of liver injury and impact of metabolic liver disease on DILI

Table 2 | **Case definitions and phenotypes of DILI**

Case definition	Drugs associated with phenotypes
Hepatocellular pattern of DILI	
ALT (or AST) alone is increased ≥5-fold above ULN or a ratio of ≥5	Acetaminophen, diclofenac, disulfiram, efavirenz, fenofibrate, isoniazid, lamotrigine, minocycline, nevirapine, nitrofurantoin, pyrazinamide, rifampicin and sulfonamide
Cholestatic pattern of DILI	
ALP alone is increased ≥2-fold above ULN or ratio ≤2	Amoxicillin–clavulanate, androgens, cephalosporins, chlorpromazine, erythromycin, flucloxacillin, oral contraceptives, penicillins, sulfonamide and terbinafine
Mixed pattern of DILI	
Ratio of >2 to <5	Carbamazepine, lamotrigine, phenytoin and sulfonamides

Andrade, R. J., et al. Nat Rev Dis Primers, 2019.

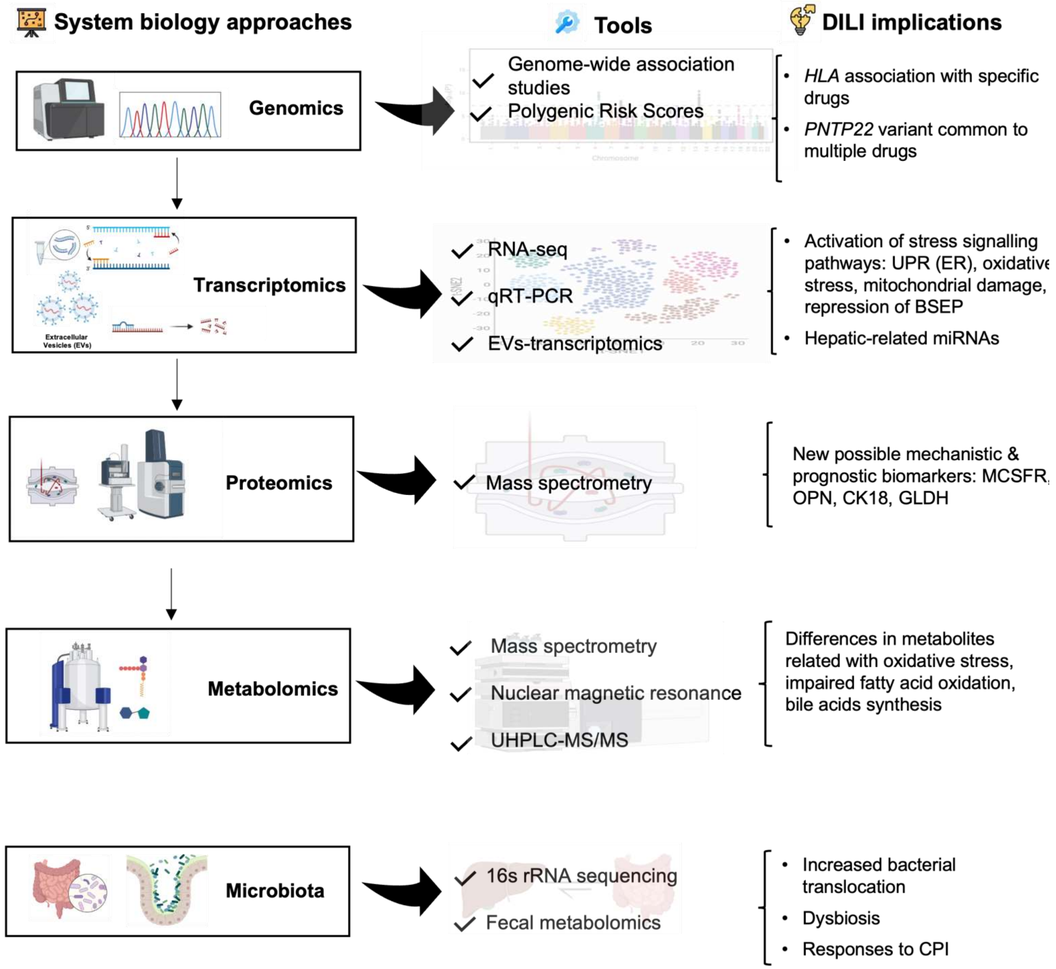


Source: own preparation

Liver damage in response to drugs reflects a hepatocellular/cholestatic/mixed injury, which exhibits different biochemical and morphological characteristics underlying distinct modes of cell death

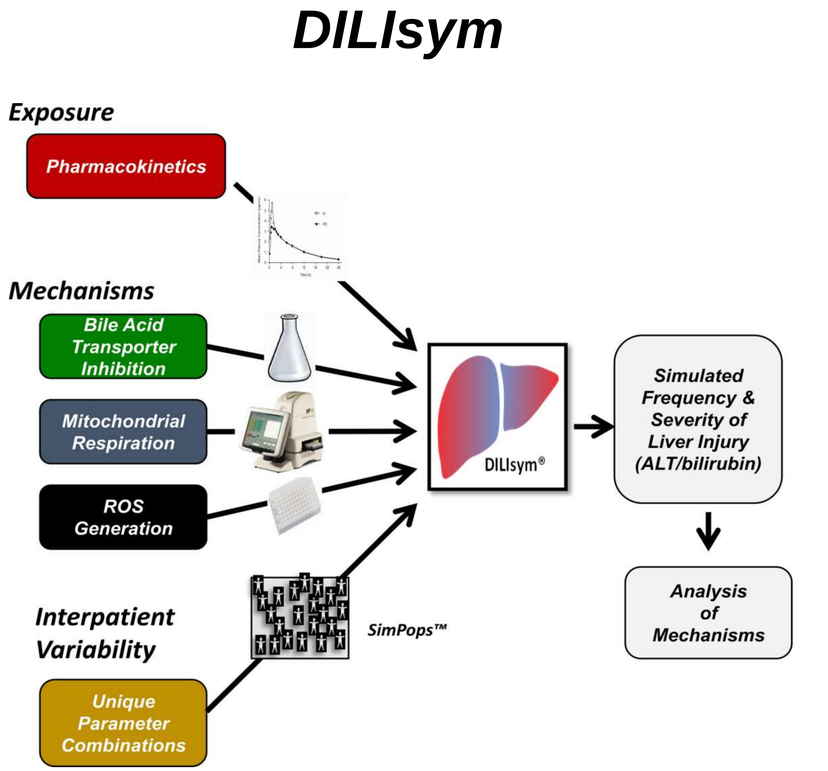
II. Preclinical models in DILI to improve the assessment and prediction of hepatotoxicity to guide future drug safety testing

➢ Computational approaches for integrating multi-scale data in DILI research



Source: own preparation

Data integration, AI, *in silico* models

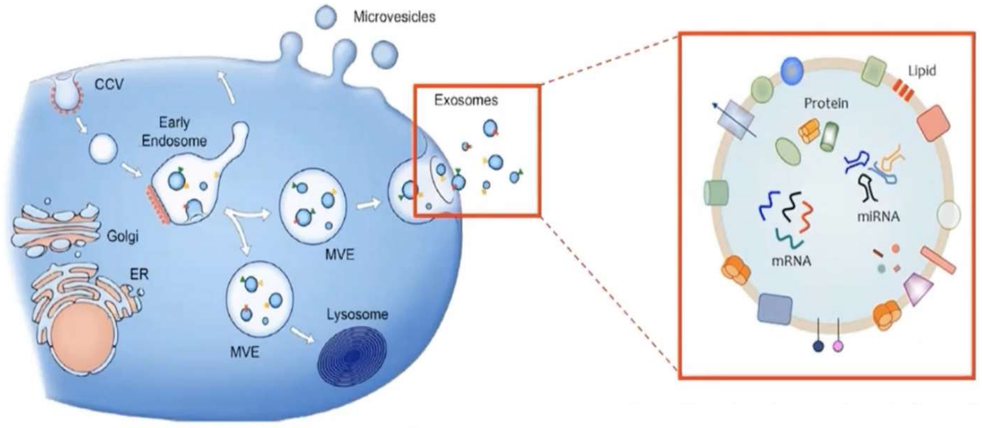


Watkins, P.B. Clin Transl Sci, 2019.

II. Preclinical models in DILI to improve the assessment and prediction of hepatotoxicity to guide future drug safety testing

➢ Perspectives of extracellular vesicles (EVs) in DILI

Liver injury signals come in the form of hepatic cell-derived extracellular vesicles (EVs)



Raposo, G. *et al.* J Cell Biol. 2013 Feb;200(4):373-83.

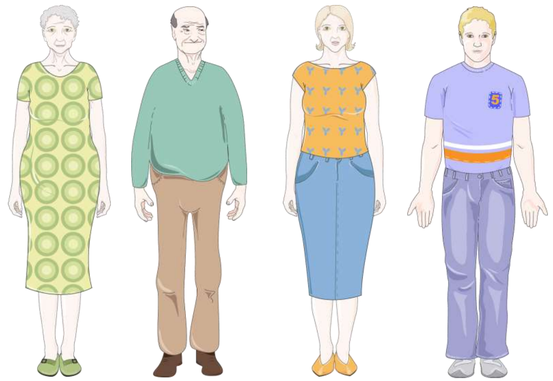
- Hepatotoxic drugs can alter EVs cargo (mRNA, miRNA, proteins...).
- EVs possess the capability to activate naïve T cells.
- EVs in plasma may serve as putative biomarkers for DILI.

DILI as a rare disease and possibility of family studies

- In terms of frequency, DILI is **neither a rare disease nor common disease.**

Causative drug	Frequency in those exposed to the drug	Reference
Amoxicillin-clavulanate	1 in 2,350	Björnsson et al., 2013
Amoxicillin-clavulanate	1 in 641	Suzuki et al., 2023
Azathioprine	1 in 133	Björnsson et al., 2013
Diclofenac	1 in 9,148	Björnsson et al., 2013
Flucoxacillin	1 in 7,065	Wing et al, 2017
Isoniazid	1 in 19	Jiang et al, 2021
Isoniazid	1 in 71	Björnsson et al., 2013
Infliximab	1 in 148	Björnsson et al., 2013
Nitrofurantoin	1 in 1,369	Björnsson et al., 2013

Incidence of selected forms of DILI reported in the literature.



- Family studies as an alternative to case-control studies involving unrelated individuals might be a useful means of obtaining new insights into genetic risk factors**.**
- **Genome sequencing studies on families with more than one affected member could be helpful in confirming data collected from case-control studies.**

III. Clinical research and healthcare system implementation

DILI risk stratification and prognostic biomarkers including pharmacogenetic studies

Category	Biomarker	AUC	95% CI
Traditional	ALT	0.990	0.984 – 0.996
Traditional	AST	0.975	0.963 – 0.987
Traditional	ALP	0.902	0.873 –0.930
Traditional	TBIL	0.857	0.821 – 0.892
Candidate	K18	0.947	0.928 – 0.966
Candidate	FABP1	0.916	0.890 – 0.941
Candidate	ccK18	0.911	0.887 – 0.935
Candidate	GLDH	0.907	0.870 – 0.945
Candidate	MCSFR**	0.854	0.822 – 0.887
Candidate	miR-122	0.831	0.779 – 0.883
Candidate	AFP	0.826	0.793 – 0.859
Candidate	GSTα	0.827	0.792 – 0.862
Candidate	SDH	0.819	0.763– 0.876
Candidate	OPN	0.758	0.718– 0.799
Candidate	CDH5	0.658	0.614 – 0.701
Candidate	PON1	0.612	0.542 – 0.682
Candidate	ARG1	0.564	0.519 – 0.609
Candidate	LECT2	0.519	0.450 – 0.588

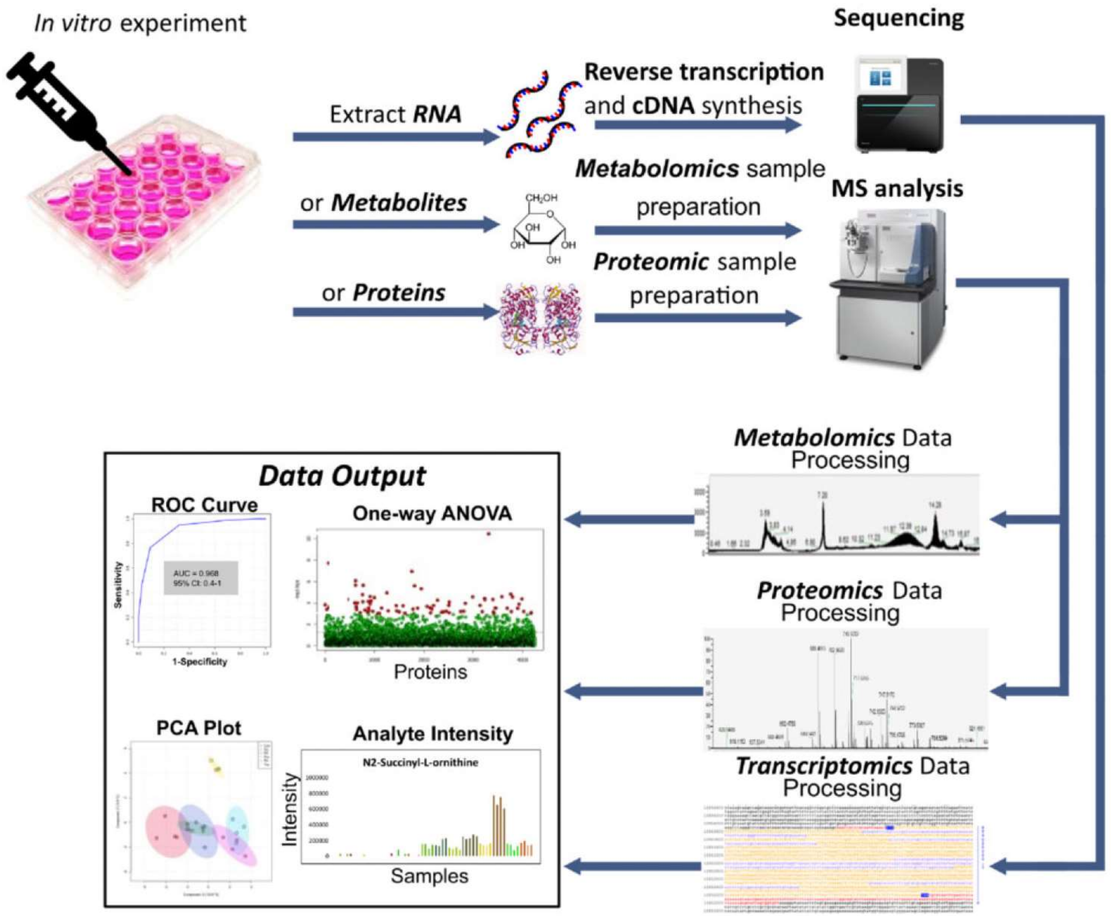
Church R, et al. Hepatology. 2019

- **Larger genetic studies on DILI generally, and on specific drug causes of DILI, are needed.**
- Biomarkers are urgently required for DILI detection during drug development, monitoring during clinical trials, early diagnosis in clinical practice and stratification of individuals whose disease will progress to acute liver failure or chronic liver disease.
- **TransBioLine, a large study on biomarker identification and qualification for DILI, is currently in progress.**

TransBioLine
Translational Safety Biomarker Pipeline

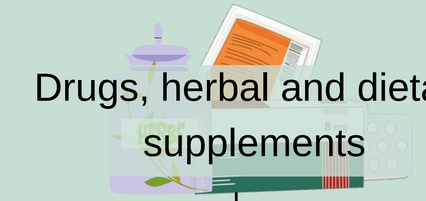
III. Clinical research and healthcare system implementation

Artificial intelligence-based integrative research



- AI modelling is founded on the design of algorithms that need to be *fed* with data.
- **The rapid advances in the field of omics have contributed to foster the development of analytical big data methodologies aimed to unveil new mechanistic pathways of the disease.**

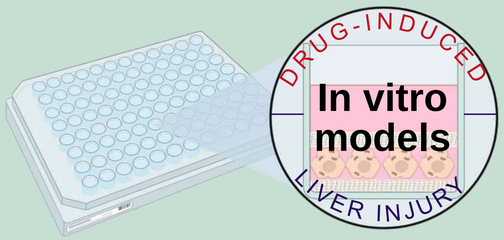
Kralj T, et al. Toxicol Sci. 2021



Drugs, herbal and dietary supplements

↑ALT ↑ALP ↑ASP ↑TB

Preclinical prediction



Lack of consensus about the compounds to test and validate in vitro models

SYSTEMATIC REVIEW ON THE CONTROL COMPOUNDS TO TEST *IN VITRO* IDIOSYNCRATIC DRUG-INDUCED LIVER INJURY (DILI) MODELS

PROTOCOL DESIGN AND DATABASE SEARCH

OSF REGISTRIES

Clarivate Web of Science™ Scopus® Embase® PubMed

51 publications included in the systematic review

RELIABILITY EVALUATION

Modified ToxRTool

A tool to assess the reliability of toxicological data

All the included studies were reliable

DATA EXTRACTION, DATA ANALYSIS AND CLINICAL DATA FROM:

PANISH DILI REGISTRY

DILIN Drug-Induced Liver Injury Network

LATINDILI

PRO-EURO DILI NETWORK
Prospective European Drug-induced Liver Injury Network

& adviced by an expert committee

CONCLUSIONS

There is great heterogeneity and lack of agreement regarding all experimental conditions in idiosyncratic DILI preclinical assays. This review pools the available data and proposes a unified list of DILI control compounds to validate *in vitro* models

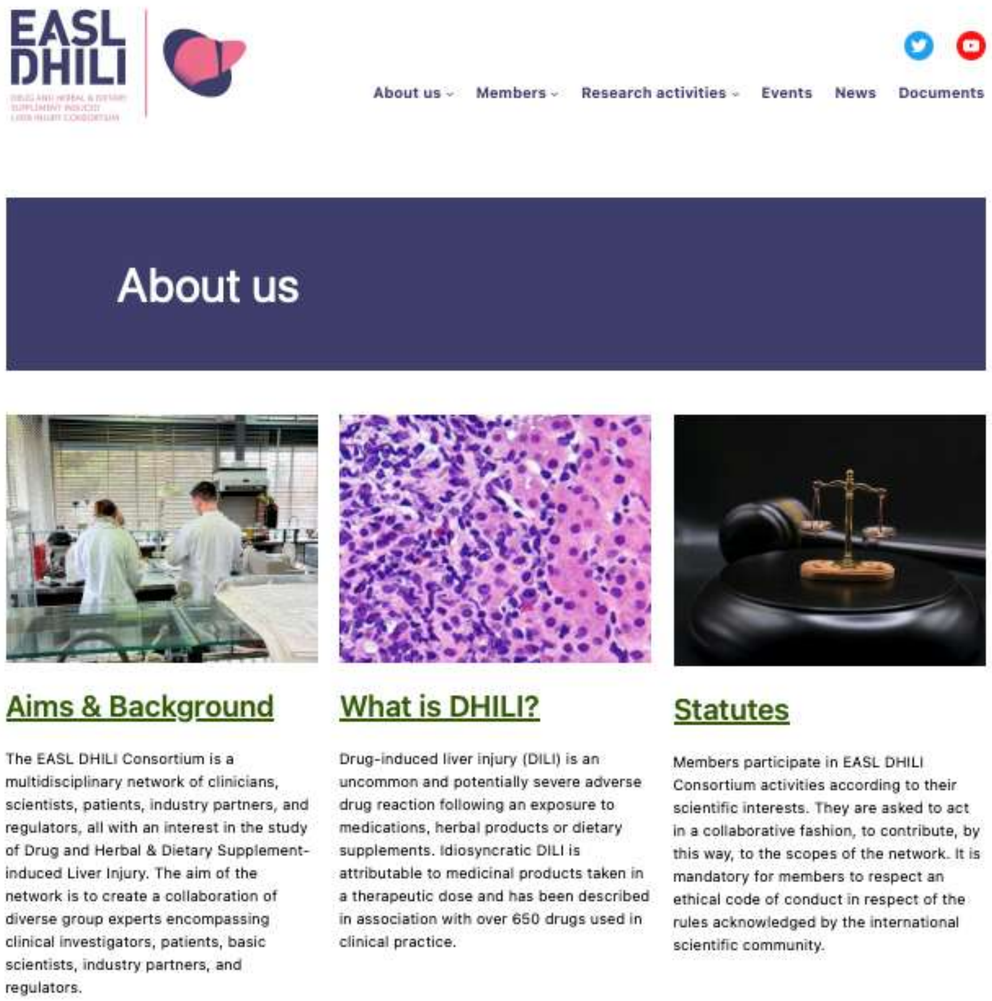
DILI+				
DICLOFENAC	TROGLITAZONE	AMIODARONE	KETOCONAZOLE	TAMOXIFEN
CHLORPROMAZINE	ISONIAZID	VALPROIC ACID	IMIPRAMINE	DANAZOL

DILI-				
DIPHENHYDRAMINE	ISOPROTERENOL	CAFFEINE	PRIMIDONE	STREPTOMYCIN
OXYBUTYNIN	LIDOCAINE	LOPERAMIDE	PYRIDOSTIGMINE	TOLTERODINE

Segovia-Zafra A,…, Andrade RJ, et al. J Hepatol. 2024 May 2:S0168-8278(24)00325-8. doi: 10.1016/j.jhep.2024.04.026.

IV. Networking and regulatory aspects

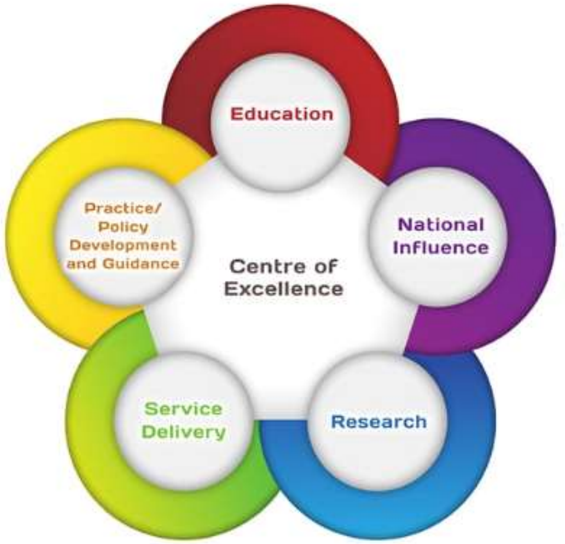
➢ EASL DHILI Consortium



- Proposing best practice on issues encompassing DILI.
- Harmonization of terminology for DILI in general and for liver injury attributed to HDS.
- Properly characterizing DI-AILH cases that occur during clinical development.
- Developing strategies to involve patients.
- Identifying gaps in clinical service relevant to DILI subjects and improve public awareness.

IV. Networking and regulatory aspects

- **Criteria to appoint centers as networks of excellence**



Clinical Centres of Excellence in DHILI

Centers should provide hepatobiliary services, including:
Pathway for Acute Liver/ Biliary Injury
Pathway for Jaundice and regular clinical pathology conferences.

It should also have access to the investigations necessary to assess suspected DHILI, research interests, projects, and publications in the field of DHILI, and teaching and training programs in hepatobiliary medicine.

Finally, these centres would contribute to substantial influence on professional societies, regulatory agencies, and policy makers.

IV. Networking and regulatory aspects

➢ Proof of concept, mechanistic, targeted-oriented Clinical Trials in DILI

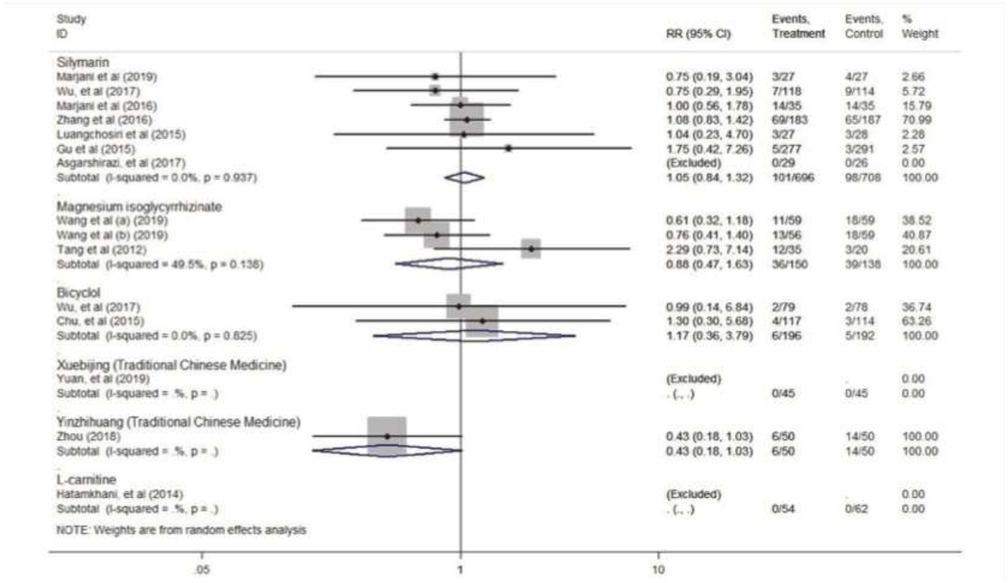
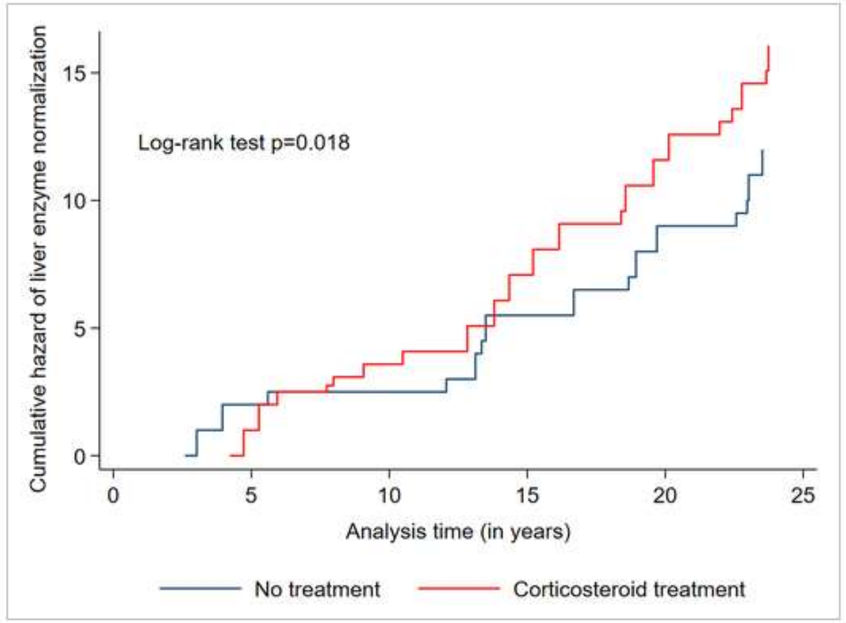


Fig. 6. Pooled effects of adverse events of pharmacological/herbal agents tested in randomised clinical trials in drug-induced liver injury prevention/management.

H Niu et al. Pharmacological Research, 2021



H Niu et al. Aliment Pharmacol Ther, 2023

- There is no available treatment with demonstrated efficacy other than withdrawing the suspected offending chemical agent and providing supportive care.
- Robust rationale for further investigating the use of corticosteroids in DILI.
- Urgent need to establish relevant endpoints to assess the efficacy of novel interventions.

TAKE HOME MESSAGES

- Operational framework for the advancement of DILI research
- A paradigm shift towards a more holistic approach that integrates basic, applied, translational and clinical research into the disease:

1. Creation of a database encompassing optimised case report form for prospectively identified DILI cases with well-characterised controls with competing diagnoses, biological samples, and imaging data.
2. Establishing of preclinical models to improve the assessment and prediction of hepatotoxicity in humans to guide future drug safety testing.
3. Emphasis on implementation science.
4. Enhanced collaboration between drug developers, clinicians and regulatory scientists.

Gracias por la atención

Hepatitis tóxica (DILI): una hoja de ruta para investigar en Europa

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