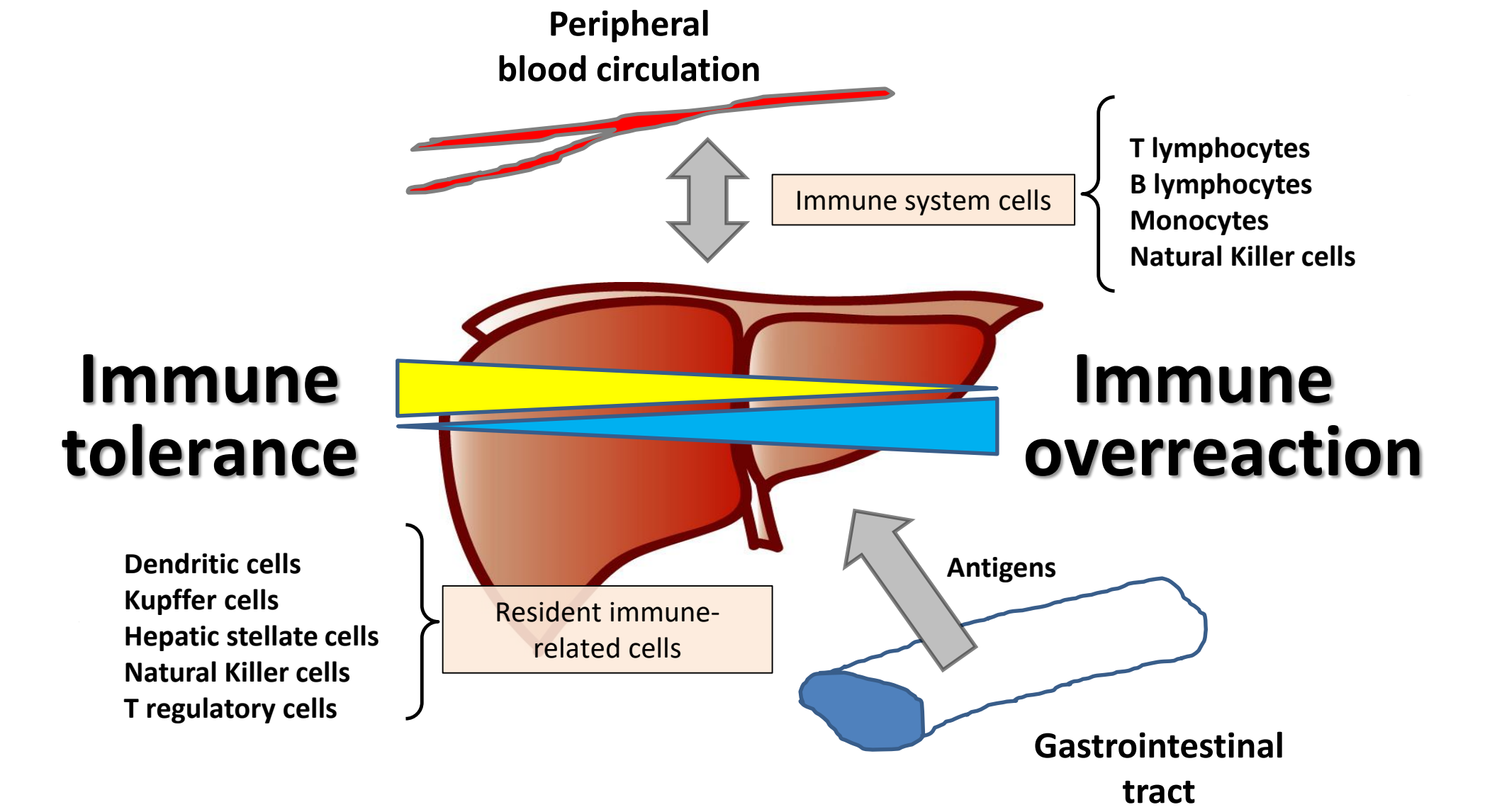
Primary biliary cholangitis

Pietro Invernizzi

Division of Gastroenterology and
Center for Autoimmune Liver Diseases
University of Milano-Bicocca
Monza, Italy

2 Mar 2022

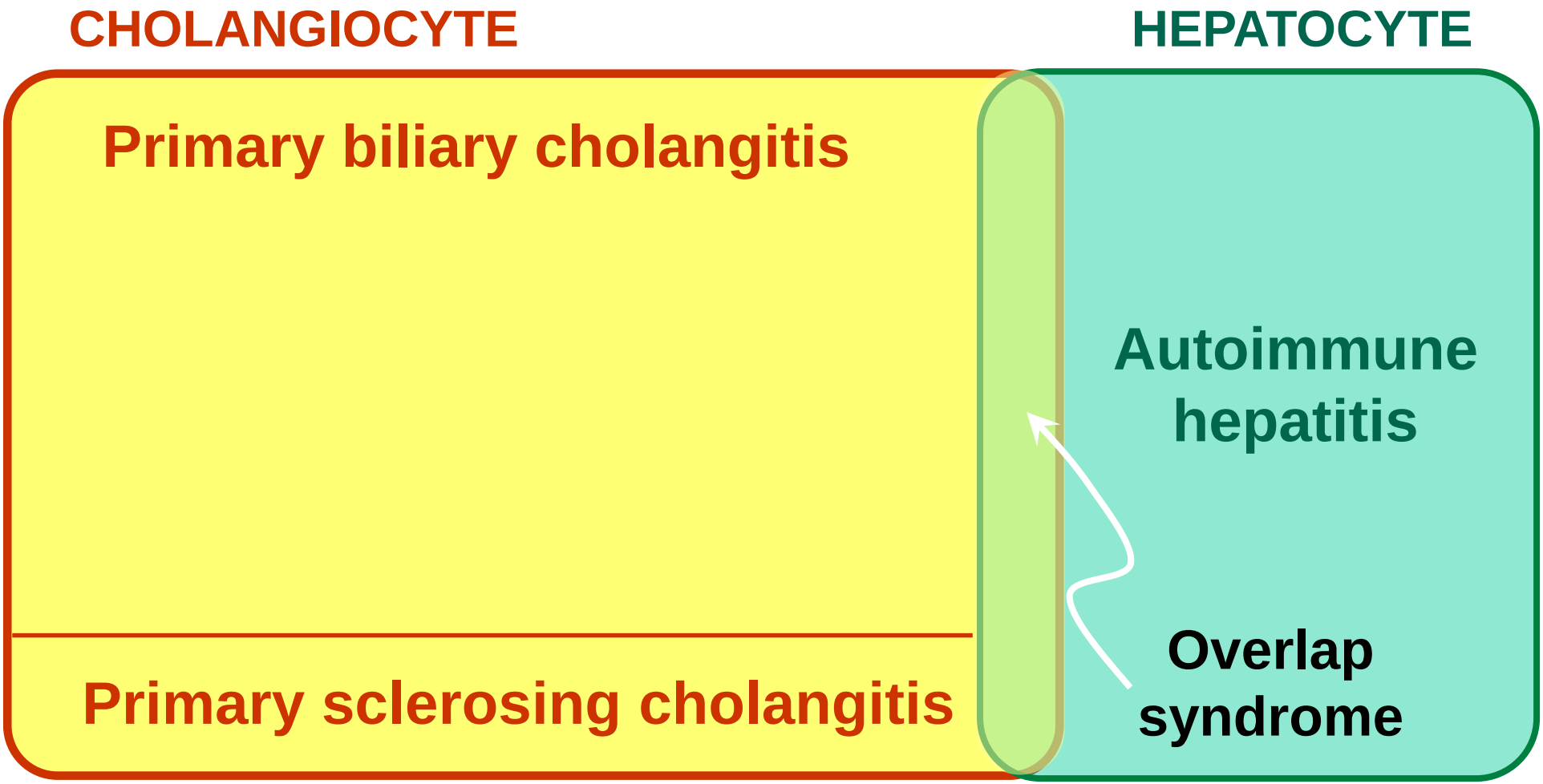
Liver: a peculiar immunologic organ



Invernizzi P J Autoimmun 2013

Autoimmune liver diseases

Primary target of immune-mediated injury



Invernizzi et al. Semin Liver Dis 2007

Our origins

Mauro Podda

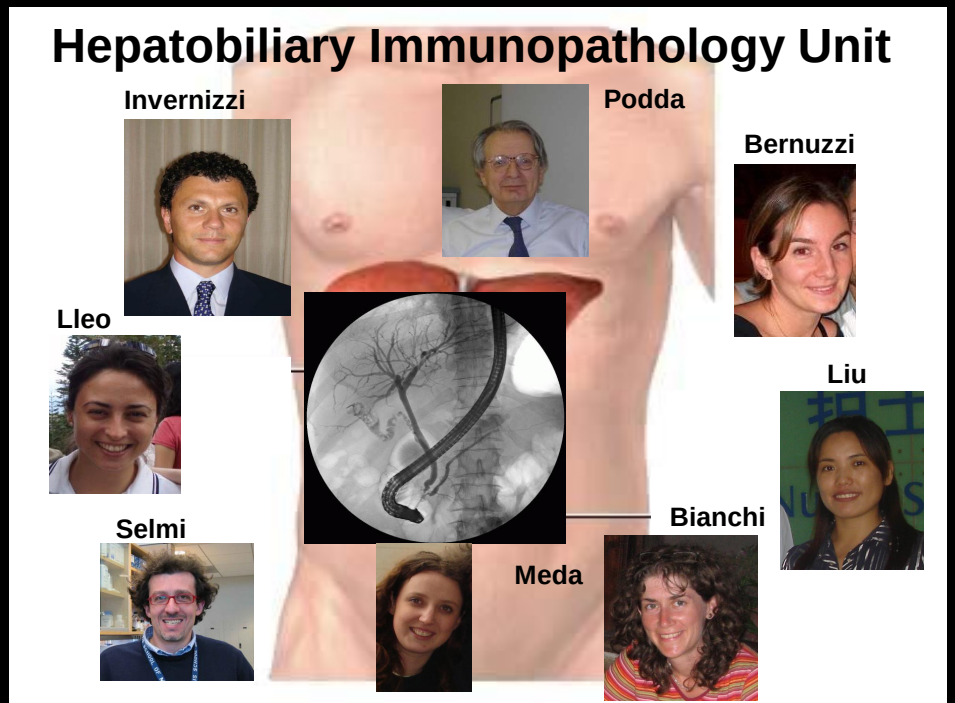
Massimo Zuin
Pier Maria Battezzati
Andrea Crosignani
Chicca Camisasca
Emanuela Bertolini
Paola Zermiani

Research interests

CHOLELITIASIS

BILE ACIDS METABOLISM

CHRONIC CHOLESTASIS



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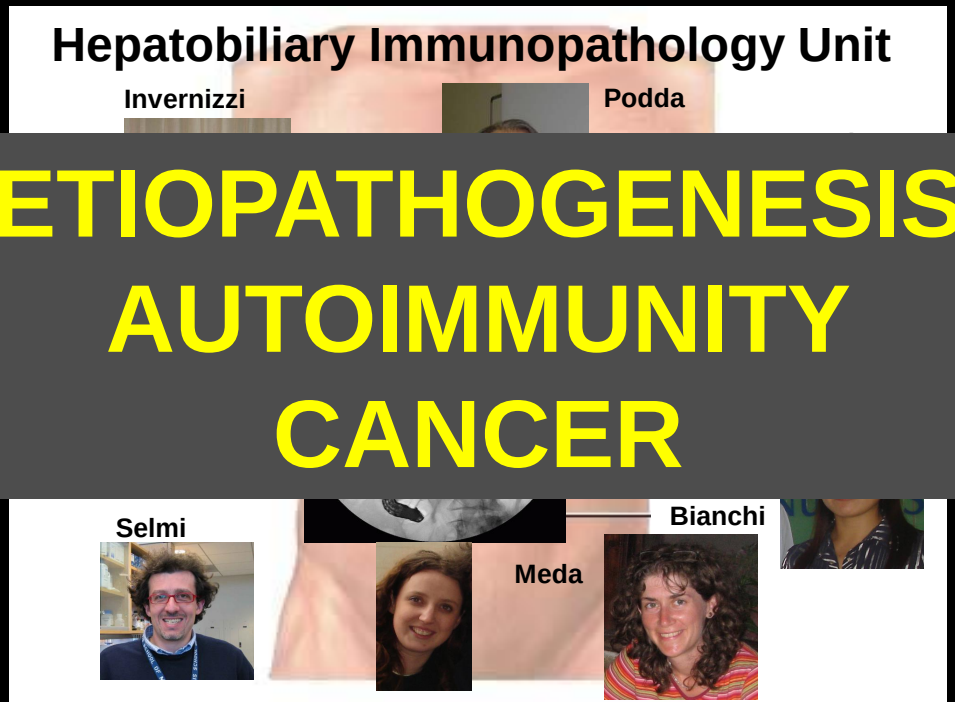


ETIOPATHOGENESIS
AUTOIMMUNITY
CANCER

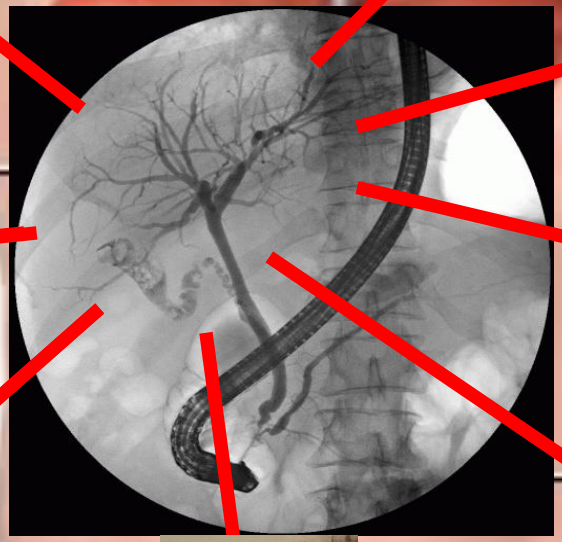
Hepatobiliary Immunopathology Unit

Invernizzi

Podda

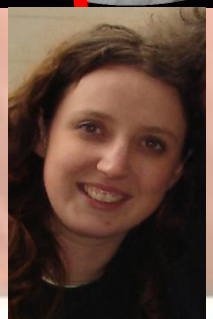
Bernuzzi

Lleo



Liu

Selmi



Meda

Bianchi

Center for Autoimmune Liver Diseases

ERN-RARE LIVER
European Reference Network

Pietro Invernizzi
University of Milan-Bicocca
Italy

Autoimmune hepatistis

Primary biliary cholangitis

Primary sclerosing cholangitis

(Cholangiocarcinoma)

2000 patients

BASIC

Genetics/Epigenetics
Immunology
Neuroendocrine
Carcinogenesis
New drugs

TRANSLATIONAL / CLINICAL

Biomarkers
Clinical trials
Epidemiology

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Italy

Autoimmune hepatistis

Primary biliary cholangitis

Primary sclerosing cholangitis

(Cholangiocarcinoma)

2000 patients

WET LAB

Blood
Cells
Animals
Chemicals

DRY LAB

Big data
Statistics
Calculators

Center for Autoimmune Liver Diseases

ERN-RARE LIVER
European Reference Network

Pietro Invernizzi
University of Milan-Bicocca
Italy

Autoimmune hepatistis

Primary biliary cholangitis

Primary sclerosing cholangitis

(Ch… …a)

2000 patients

CLINICAL LAB

Sponsered trials
Cross-over
?

WET

Blood
Cells
Animals
Chemicals

… LAB

…g data
Statistics
Calculators

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | MONOTHEMATIC CONFERENCE

PRIMARY BILIARY CIRRHOSIS

MAY 23 – 24 / 2014. **Milan,** Italy

www.easl.eu

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | MONOTHEMATIC CONFERENCE

PRIMARY BILIARY CIRRHOSIS

MAY 23 – 24 / 2014. **Milan,** Italy

New name

www.easl.eu

September 2015 - Position paper

“Changing Nomenclature for PBC: From ‘cirrhosis’ to ‘cholangitis’”

- **Hepatology**. 2015 Sep 15.
- **Gut**. 2015 Sep 14.
- **Gastroenterology**. 2015 Sep 15.
- **J Hepatol**. 2015 Sep 10.
- **Clin Gastroenterol Hepatol**. 2015 Sep 16.
- **Am J Gastroenterol**. 2015 Sep 29.
- **Dig Liver Dis**. 2015 Sep 23.
- **Clin Res Hepatol Gastroenterol**. 2015 Oct;39(5):e57-9.

PRIMARY BILIARY CHOLANGITIS

Outline

?

Genetics /environment

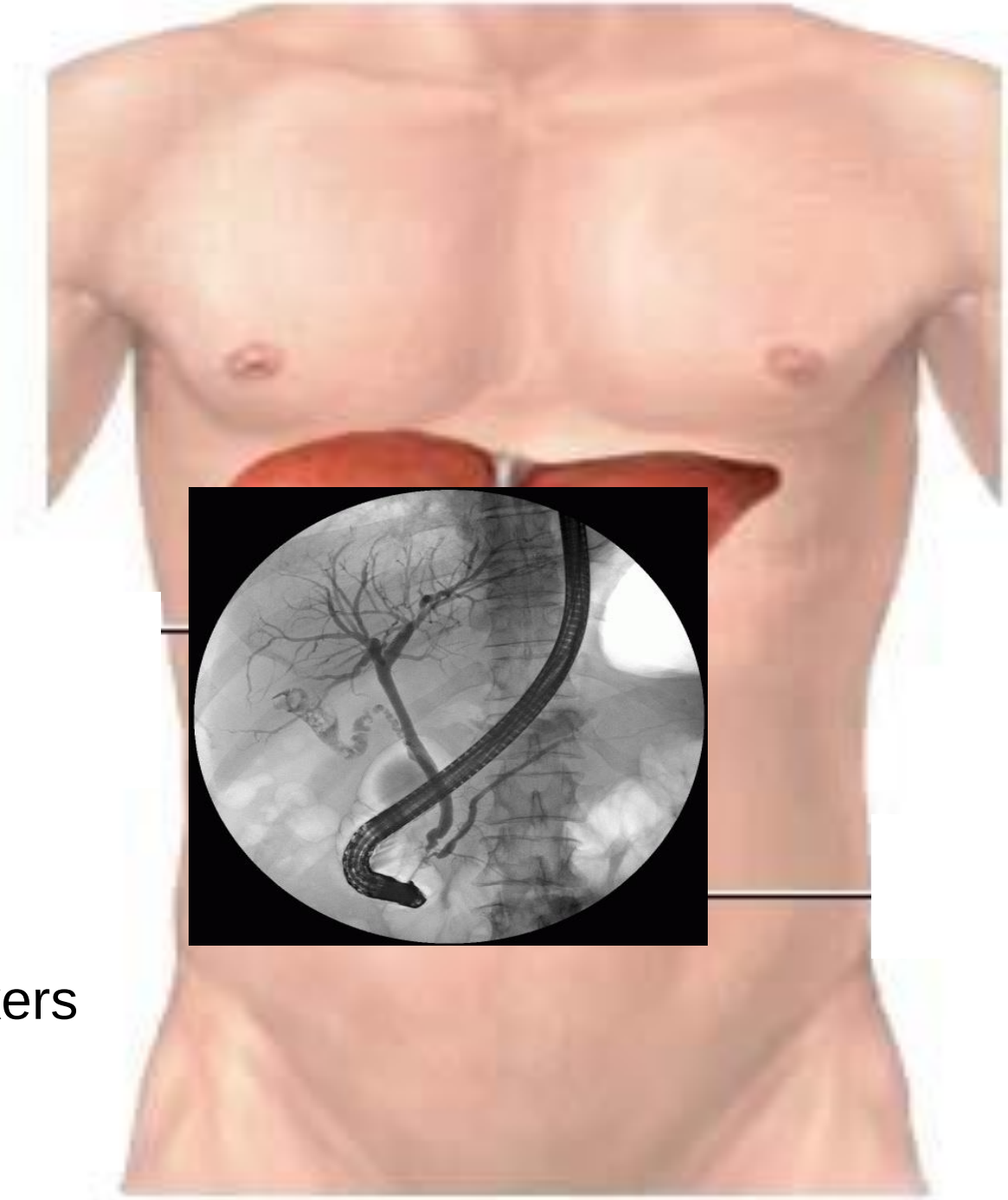
Networks

Female/male

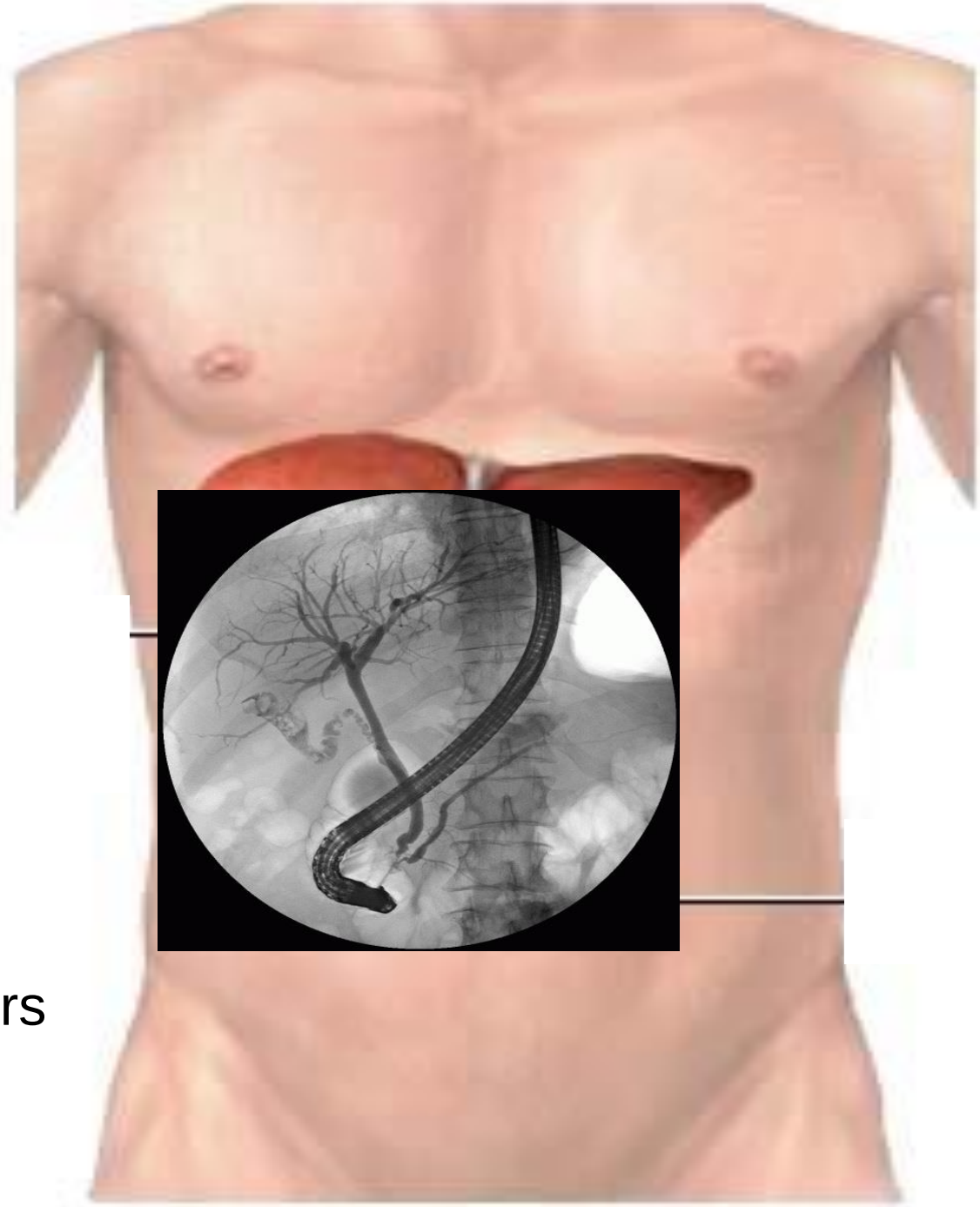
Target organ

Novel biomarkers

Novel drugs



Outline



?

Genetics /environment

Networks

Female/male

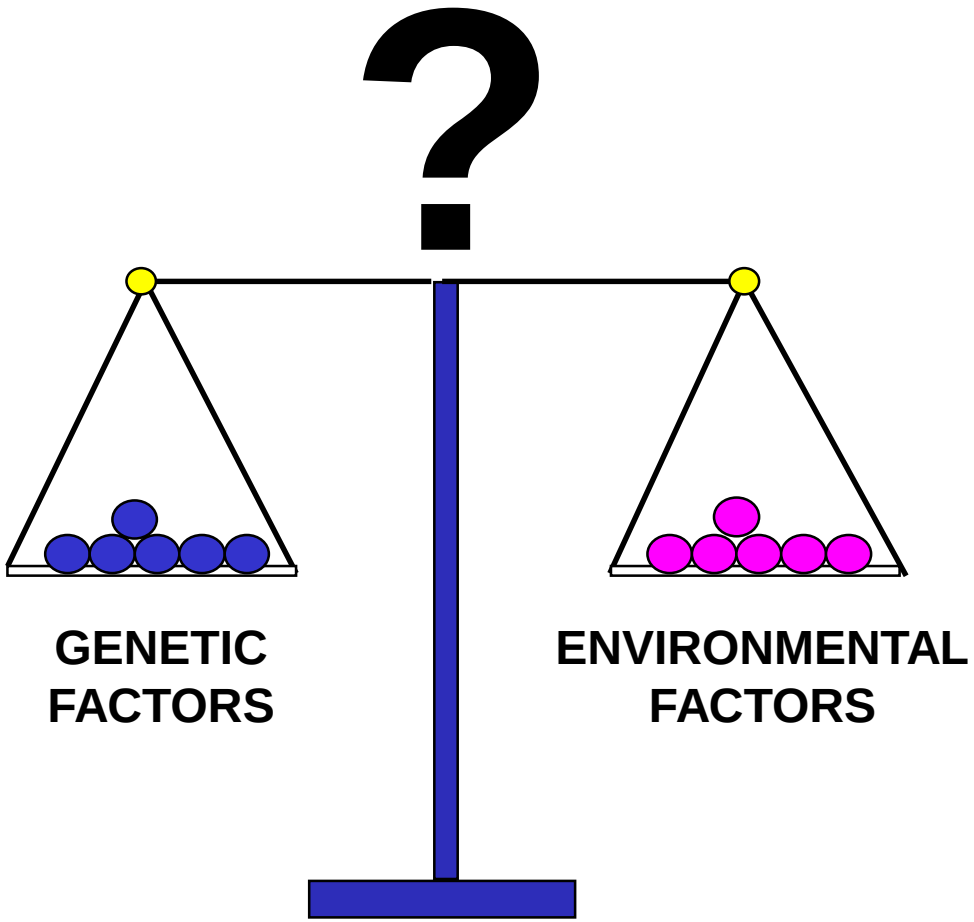
Target organ

Novel biomarkers

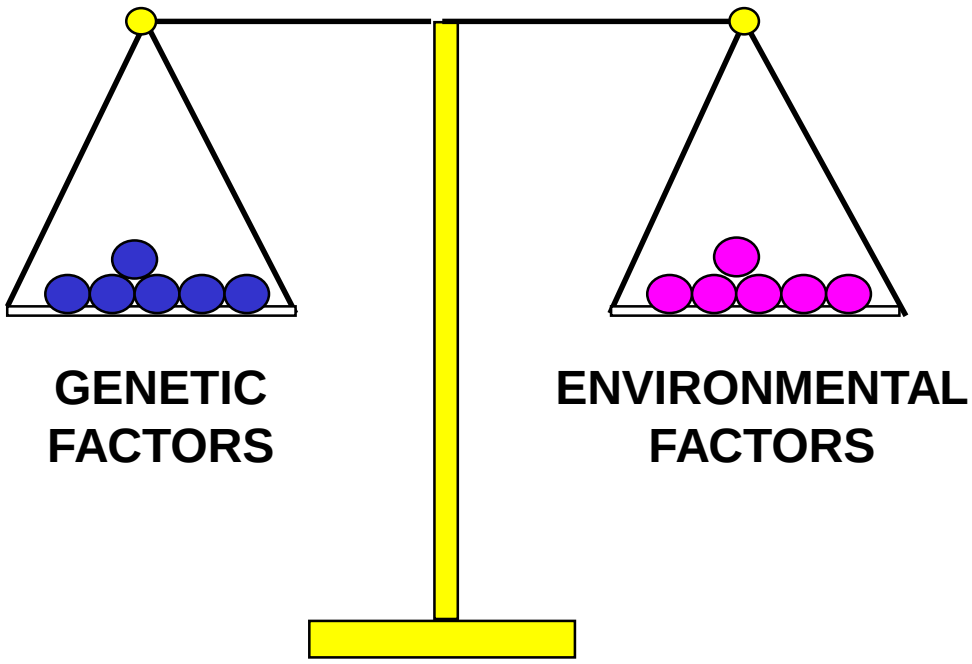
Novel drugs

Etiopathogenesis of PBC

Autoimmunity

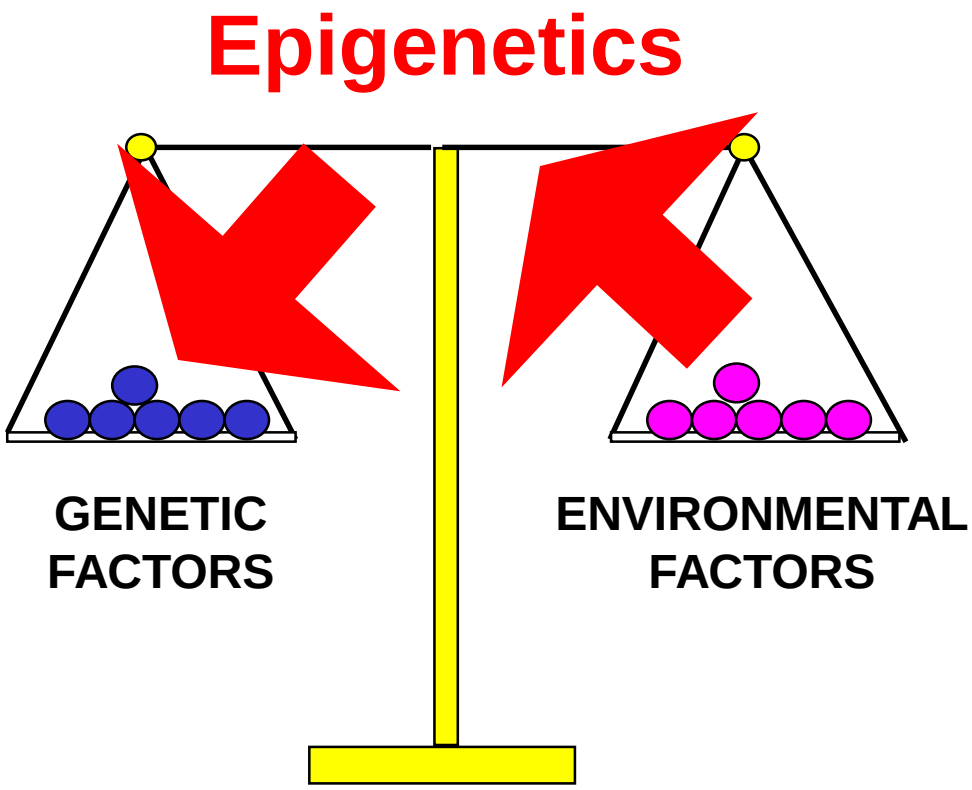


Environmental factors



- **Geo-epidemiology of PBC prevalence**
- **Local clustering**
- **Risk factors**
- **Experimental evidence for a role of xenobiotics, infectious agents**

Environmental factors



- Geo-epidemiology of PBC prevalence
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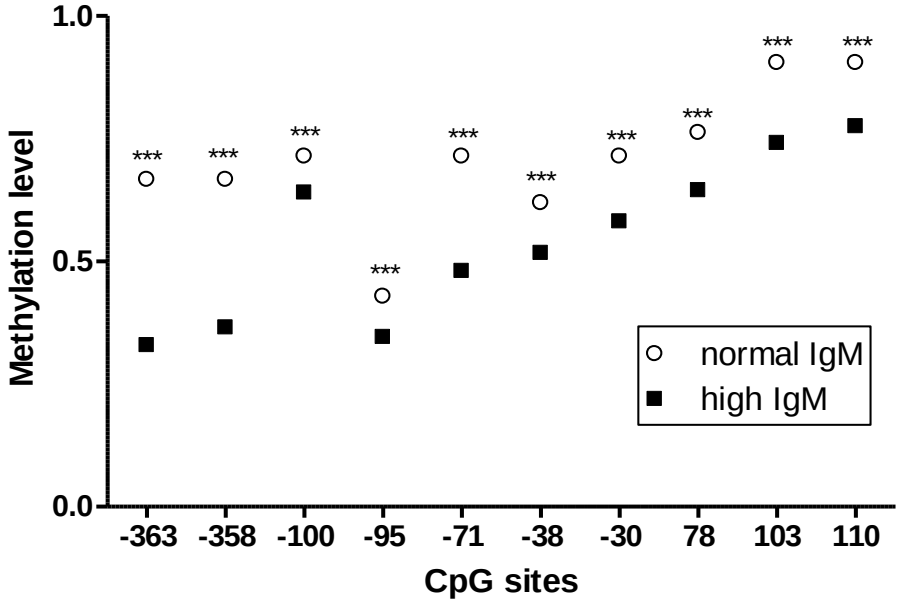
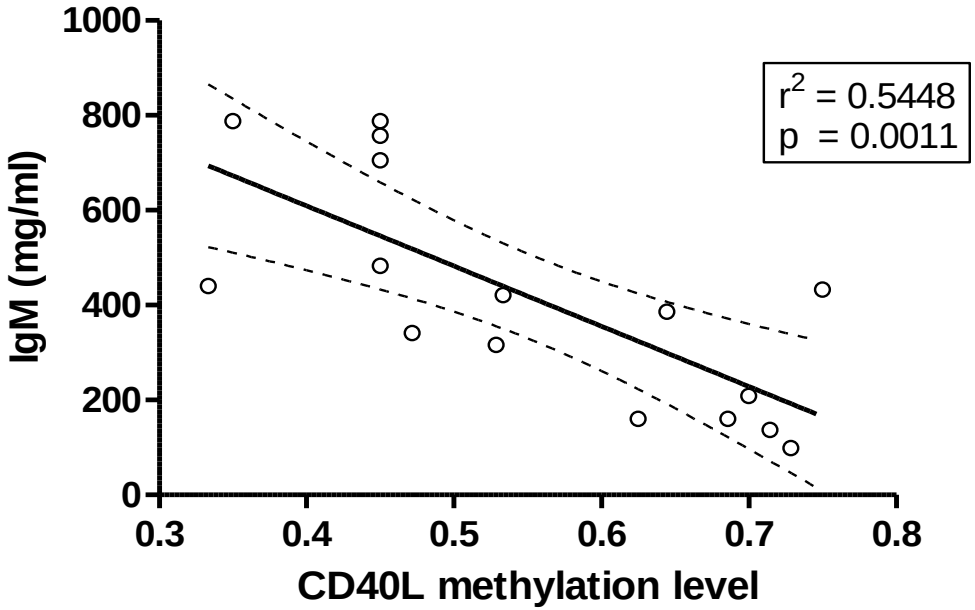
- Risk factors
- Experimental evidence for a role of xenobiotics, infectious agents

Epigenetics… alterations that result in changes in gene expression that do not involve changes in the DNA sequence

CD40L gene promoter methylation in PBC

PBC patients with high IgM levels show lower levels of CD40L promoter methylation





Lleo et al. Hepatology 2012

DNA methylation of X chromosome in PBC

PBMC from PBC (n=30) and controls (n=30)

CD8, CD4, CD14

Genomic DNA

RNA

Study of the methylation status of differentially expressed X-linked genes

Expression of selected genes

MeDIP-chip assay
PBC (n=10) healthy controls (n=10)

Bisulfite sequencing (validation)
PBC (n=20) healthy controls (n=20)

Lleo et al. Clinical Epigenetics 2015

DNA methylation of X chromosome in PBC

Demethylated:

CD4-**CXCR3**

CD8-**CXCR3**

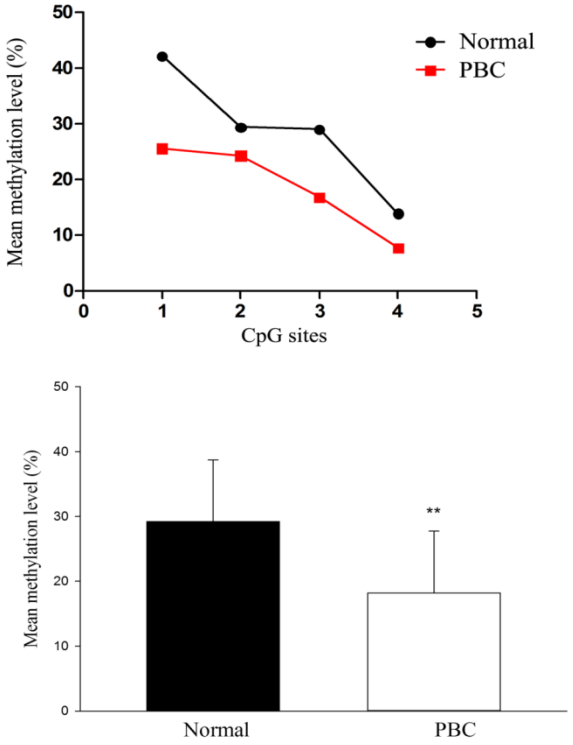
CD14-**CXCR3**

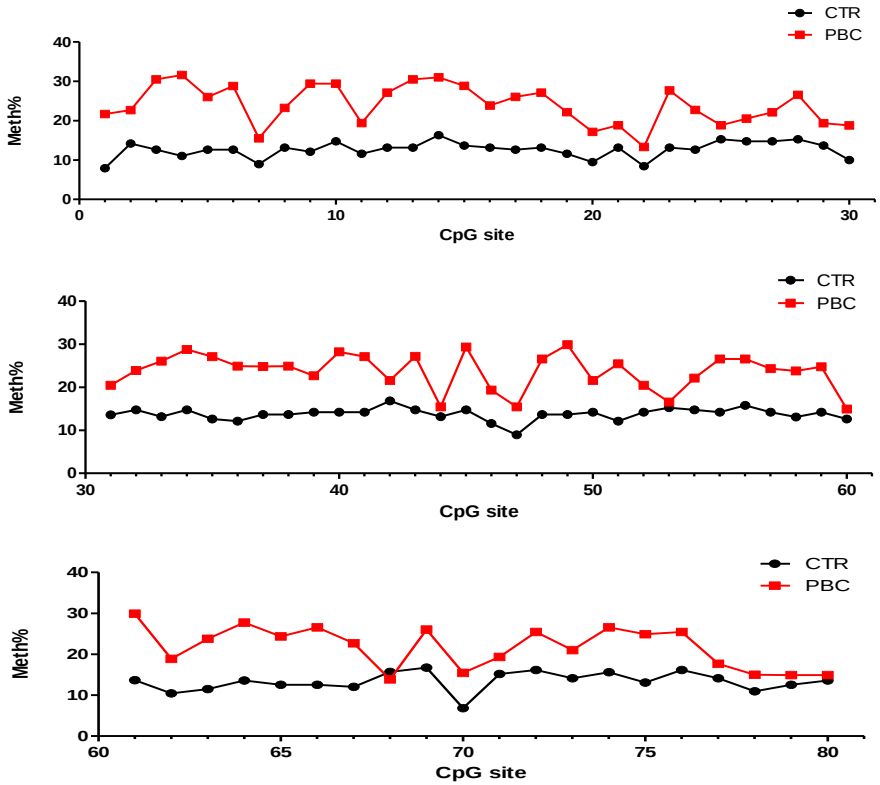
Hypermethylated:

CD4-**UBE2A**

CD8-**FUNDC2**

CD14-**IL1RAPL2**

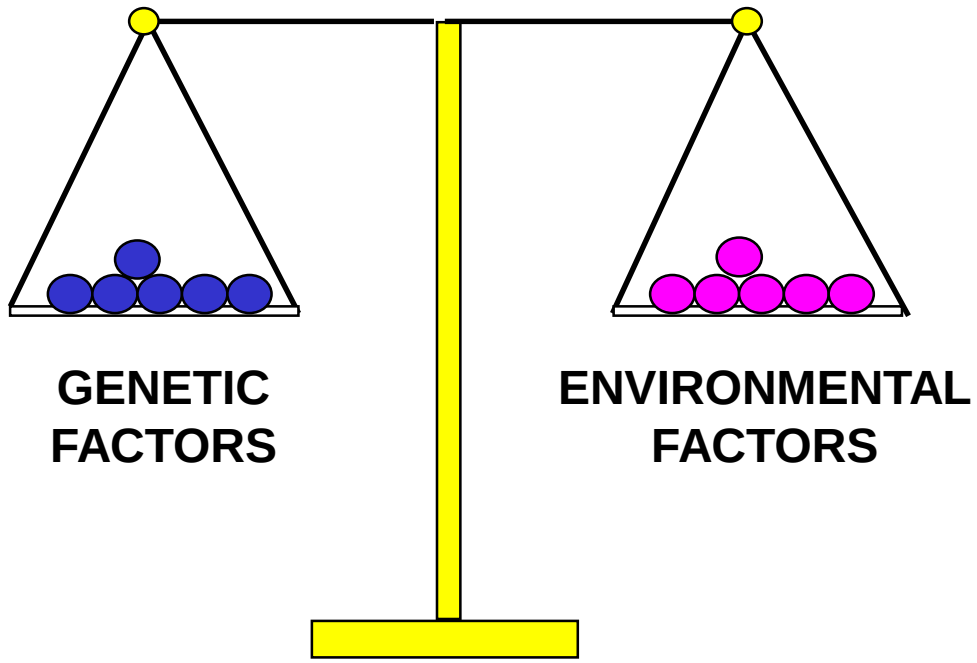




Lleo et al. Clinical Epigenetics 2015

Genetic factors

- **Familial clustering (high risk for PBC development within a family)**
- **High concordance rate of PBC in monozygotic twins**
- **Sex chromosomes defects**
- **Polymorphisms associated with susceptibility and progression**



Genetic factors in PBC

PBC concordance rate in twins

MZ Twins

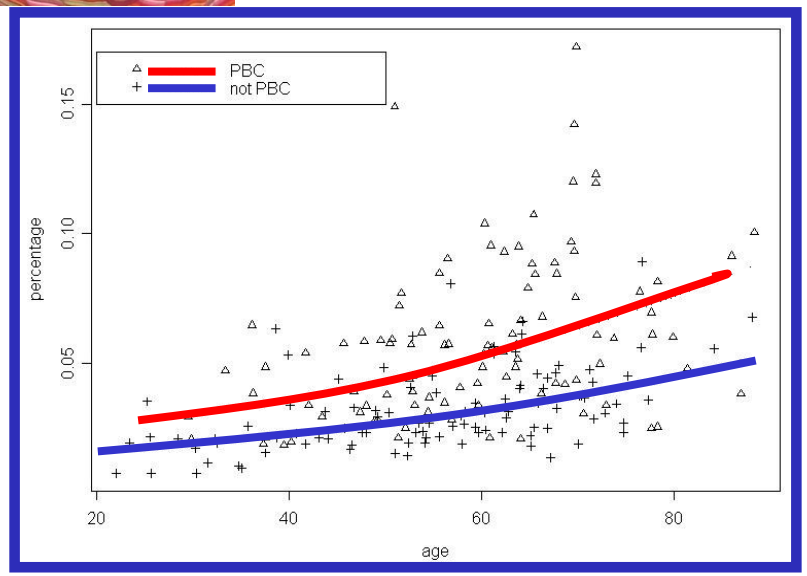
5 out of 8 pairs concordant for PBC (**CR 0.63**)

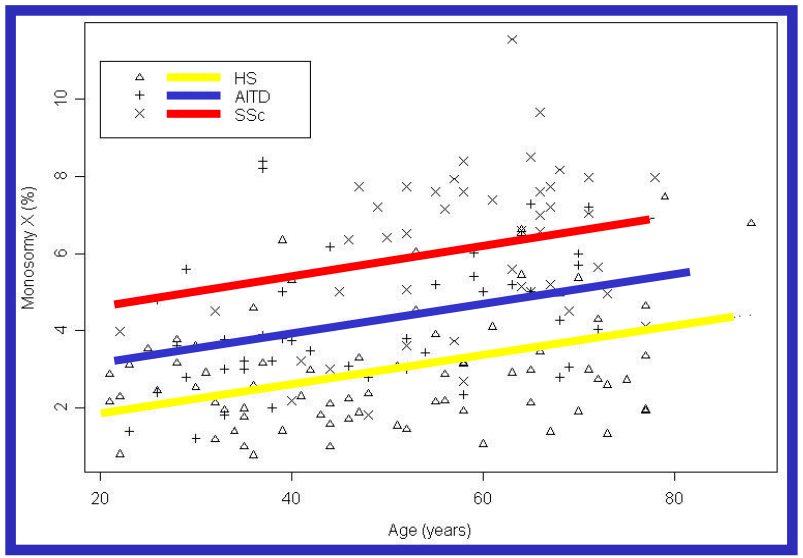
DZ Twins

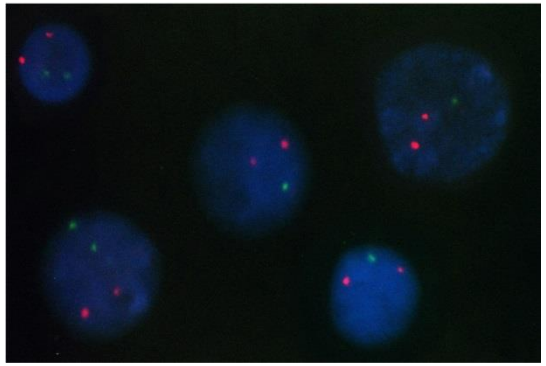
No pair [out of 9] concordant for PBC (**CR 0**)

Selmi et al. Gastroenterology 2004

X chromosome monosomy
In autoimmune diseases





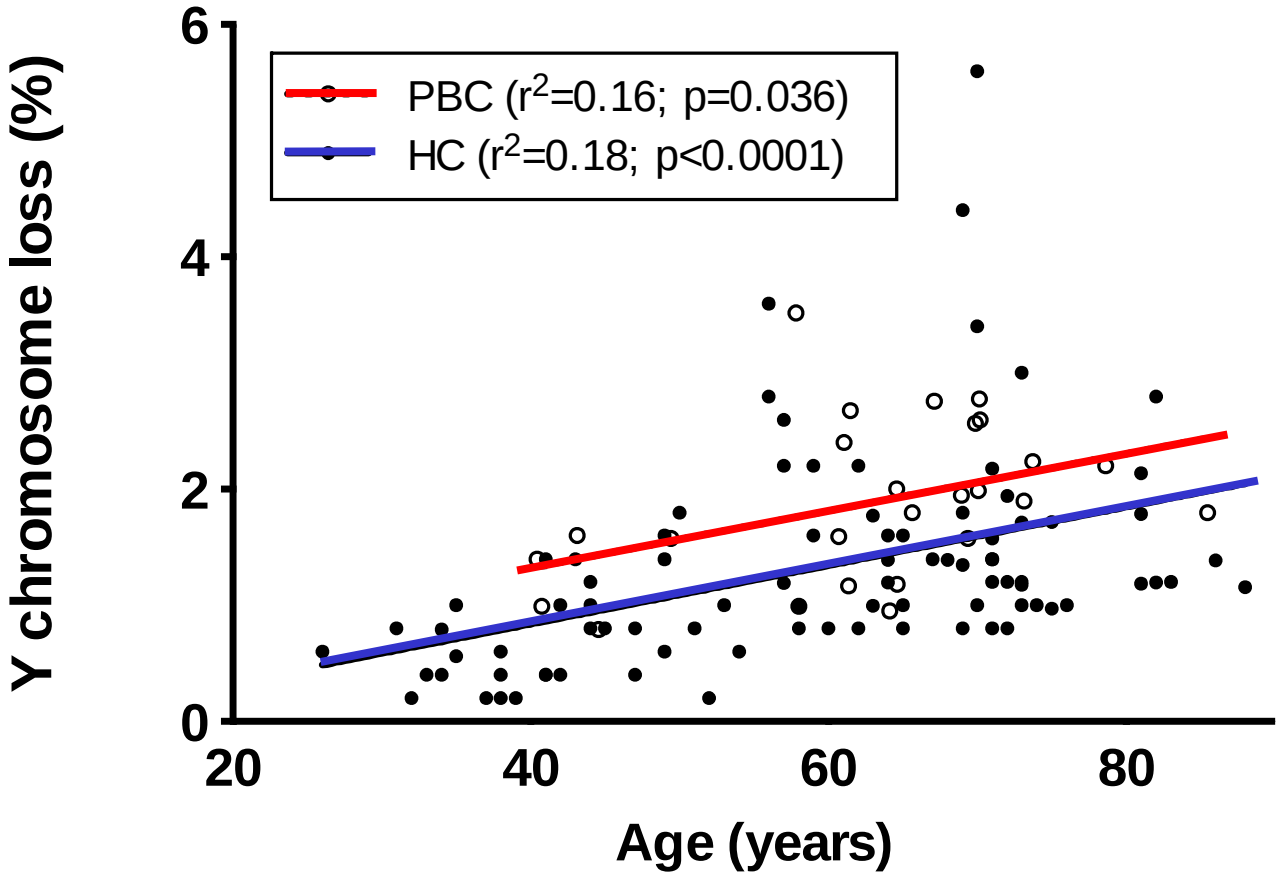


Monosomy X *(%)*

PBC (n=100)	**5.2 ± 1.7**
Scleroderma (n=44)	**6.2 ± 0.26**
AI thyroiditis (n=44)	**4.3 ± 0.26**
Chronic Hep C (n=50)	**3.2 ± 1.5**
Healthy (n=73)	**2.9 ± 0.20**

Invernizzi et al. Lancet 2004
Invernizzi et al. J Immunol 2005

Y chromosome loss in male PBC



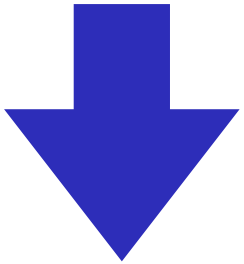
Lleo et al. J Autoimmun 2012

Sex chromosomes defects in PBC

Enhanced X monosomy (female)

Preferential X chromosome loss (female)

Increased Y chromosome loss (male)



Haploinsufficiency that unmasks PBC susceptibility genes

Invernizzi et al. Lancet 2004
Invernizzi et al. J Immunol 2005
Miozzo et al. Hepatology 2007
Svyryd et al. Autoimmun Rev 2012
Lleo et al. J Autoimmunity 2013

Genetic factors

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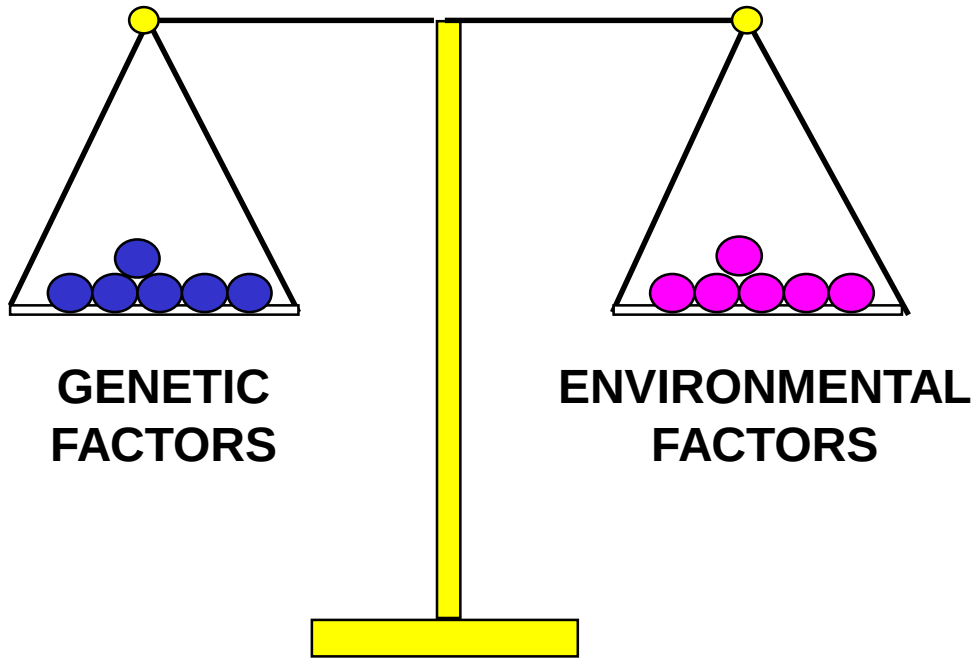
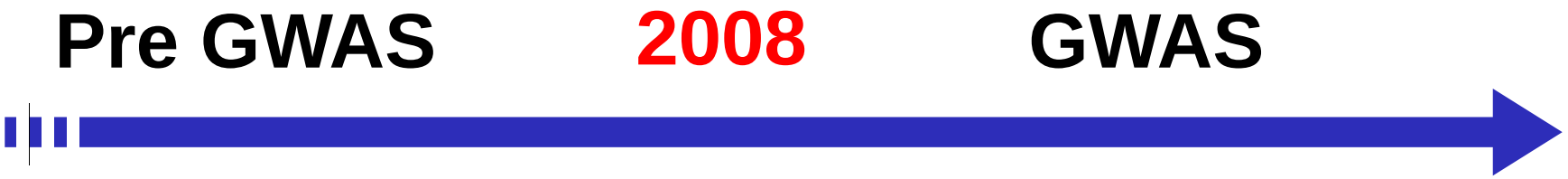
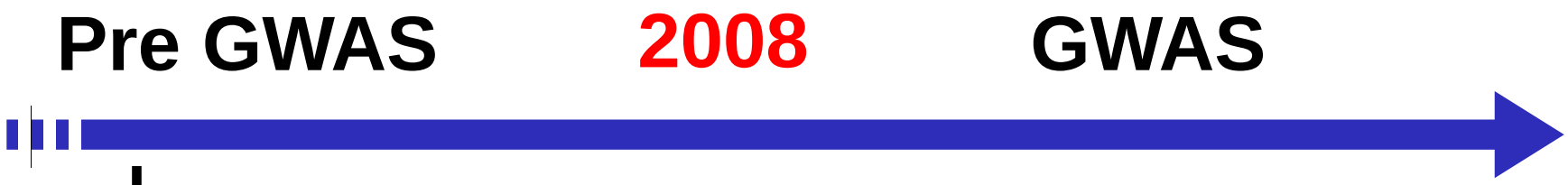


HLA story in PBC



HLA story in PBC



2003

Monocentric study

Italy

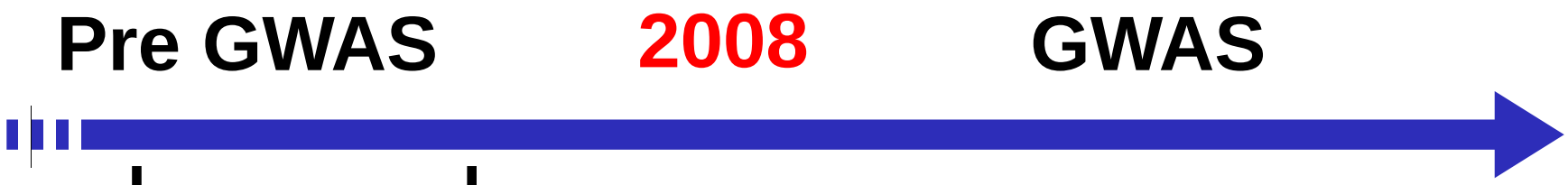
PBC = 186
HC = 558

HLA polymorphisms in Italian PBC
Monocentric Study

HLA	PBC (n=186) (%)	Controls (n=558) (%)	*Pc*	Odds Ratio	(95% C.I.)
DRB1*08	6.7	5.3	*N.S.*	-	-
DRB1*11	10.7	27.6	0.000	0.3	0.2-0.5

Invernizzi et al. J Hepatol 2003

HLA story in PBC



2003
Monocentric study

Italy

PBC = 186
HC = 558

2008
Multicentric Study

Italy

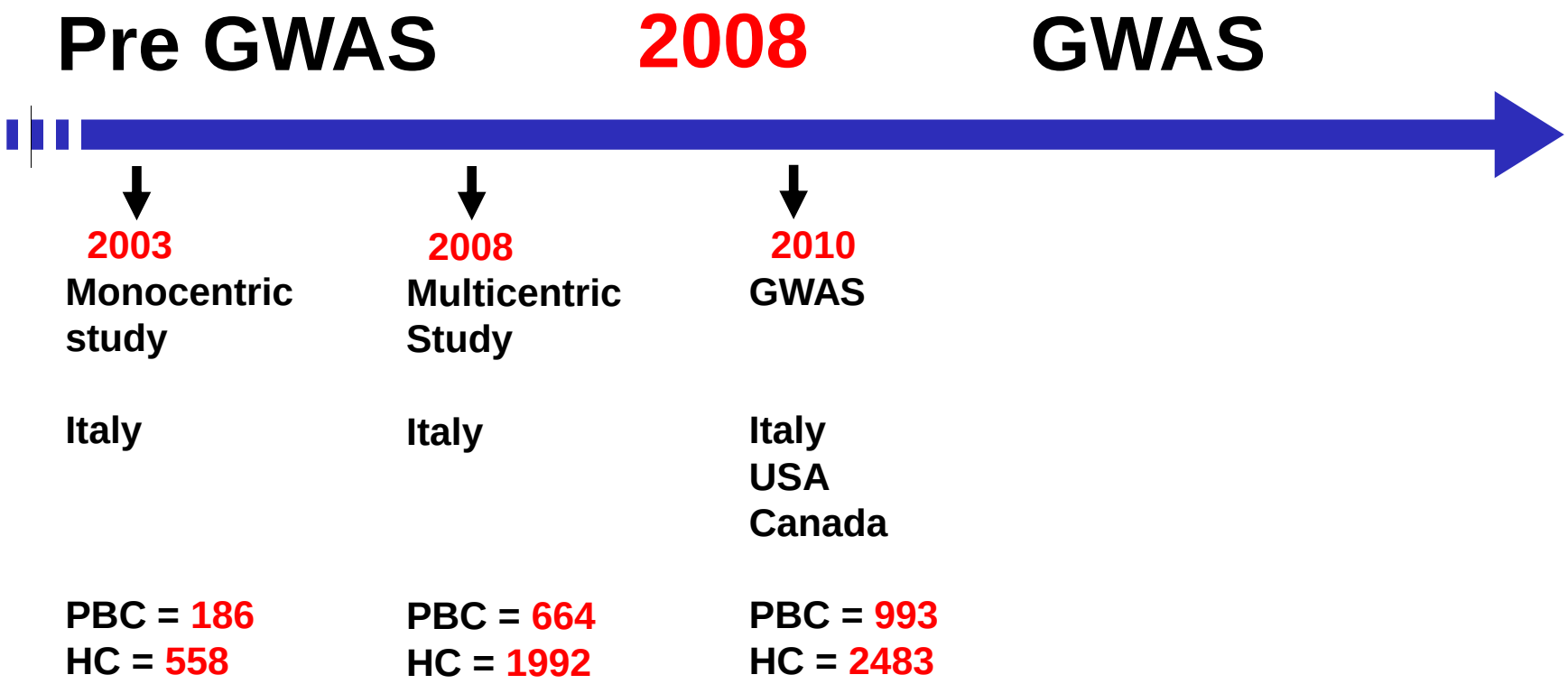
PBC = 664
HC = 1992

HLA polymorphisms in Italian PBC
Multicenter Study

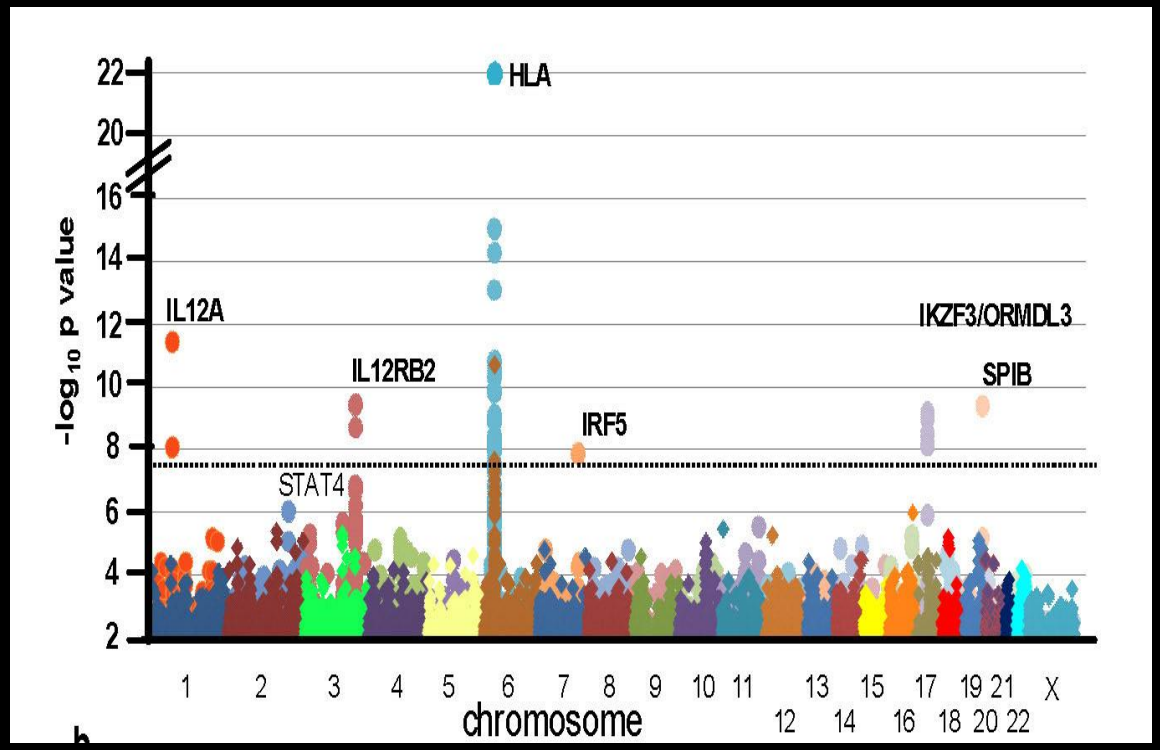
HLA	PBC (n=664) (%)	Controls (n=1992) (%)	*Pc*	Odds Ratio	(95% C.I.)
DRB1*08	7.2	2.3	0.000	3.3	2.4-4.5
DRB1*11	13.6	30.0	0.000	0.4	0.3-0.4
DRB1*13	8.6	11.2	0.000	0.7	0.3-0.9

Invernizzi et al. Hepatology 2008

HLA story in PBC



Genome-wide association study in PBC



Risk variants:

HLA

IL12A

IL12RB2

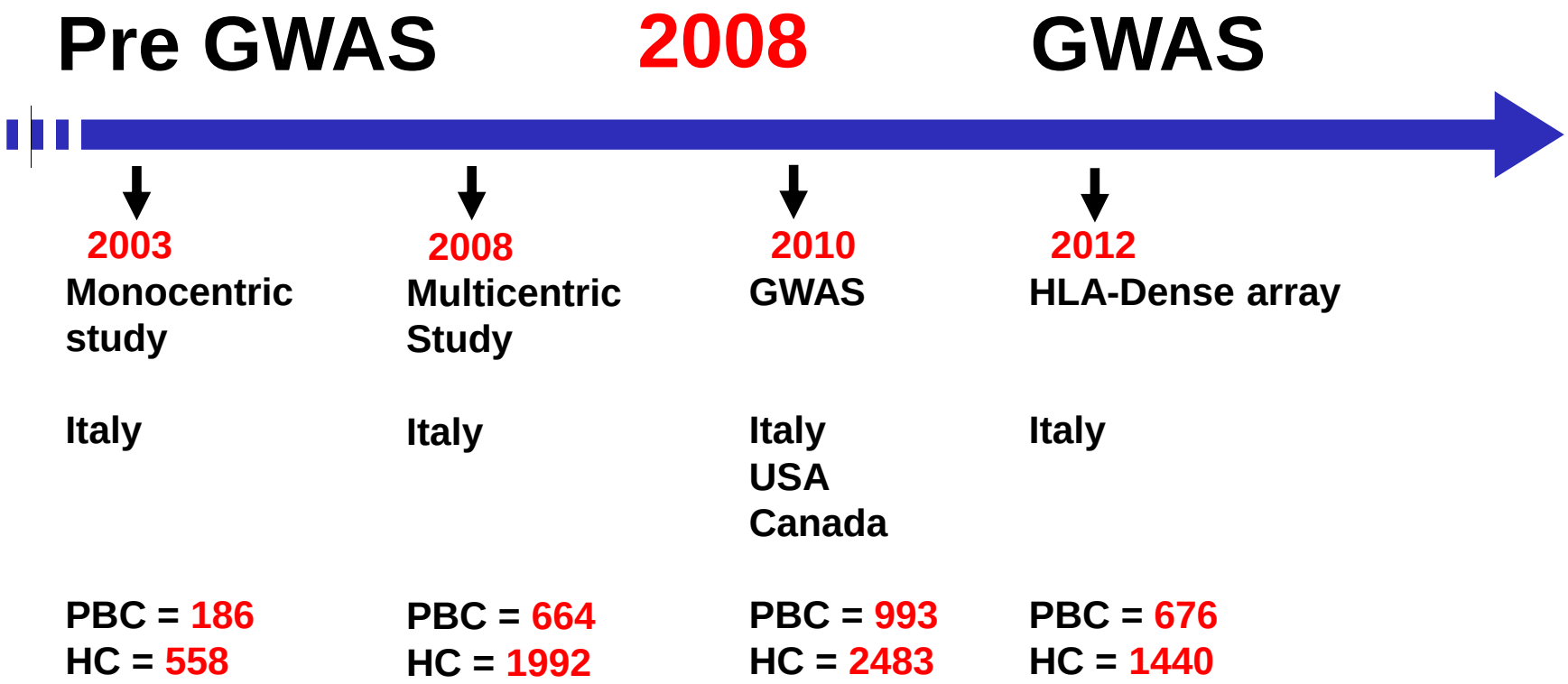
IRF5

IKZF3/ORMDL 3

SPIB

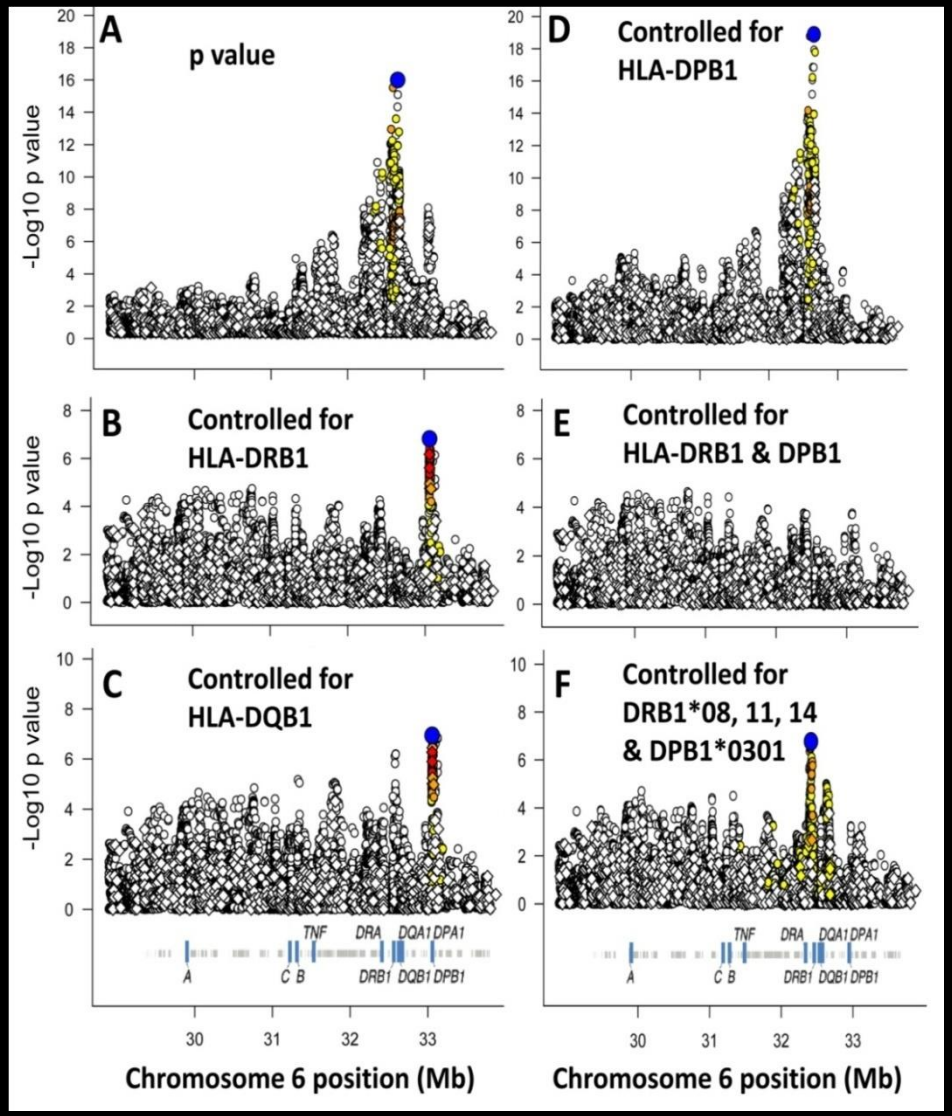
Liu & Invernizzi Nature Genetics 2010

HLA story in PBC



HLA associations in PBC

Dense array approach



676 Italian PBC

vs.

1440 controls

Results:

DRB1 (*08, *11)

DPB1

Invernizzi et al. Genes Immun 2012

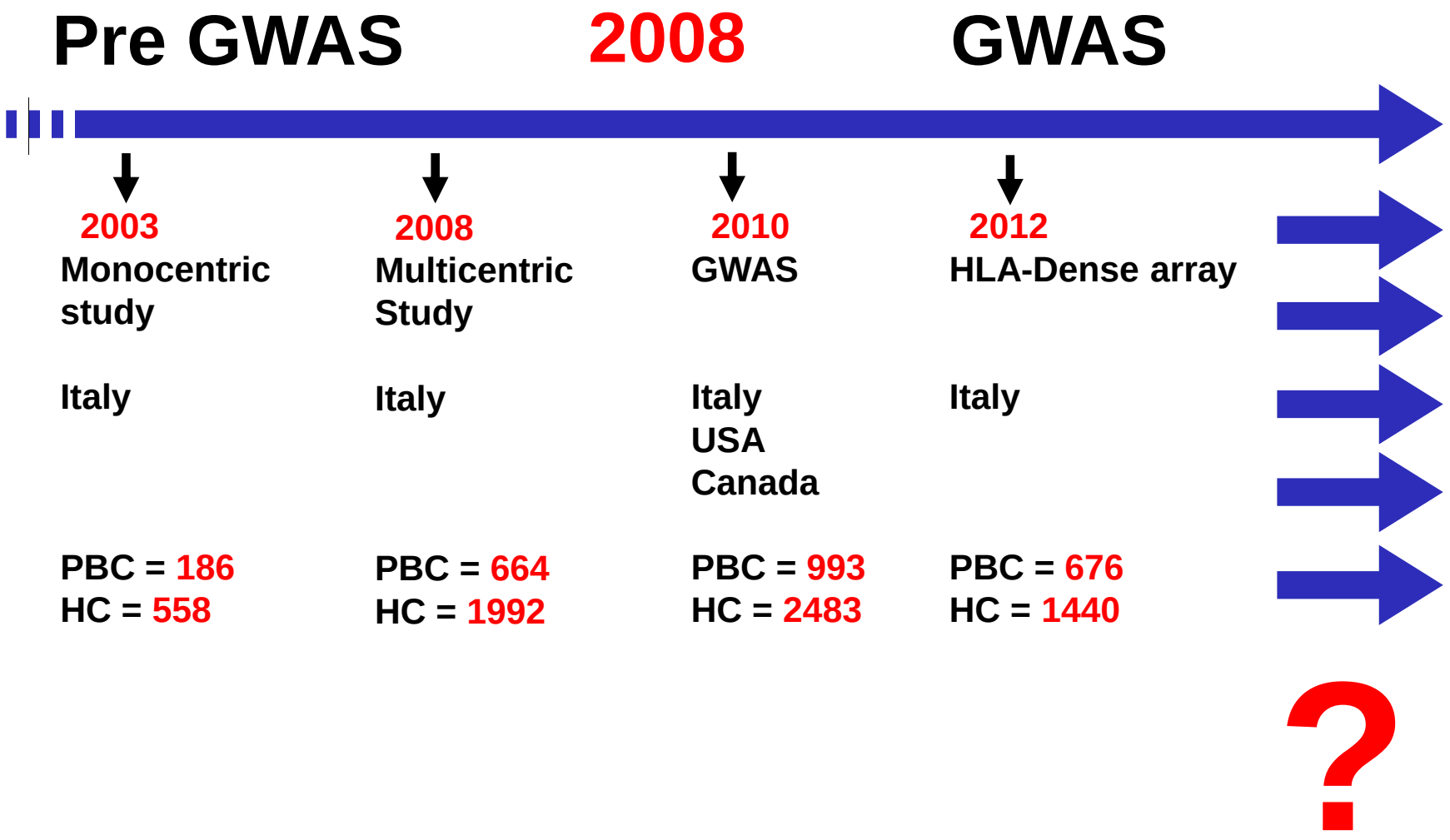
HLA story in PBC

Pre GWAS **2008** **GWAS**

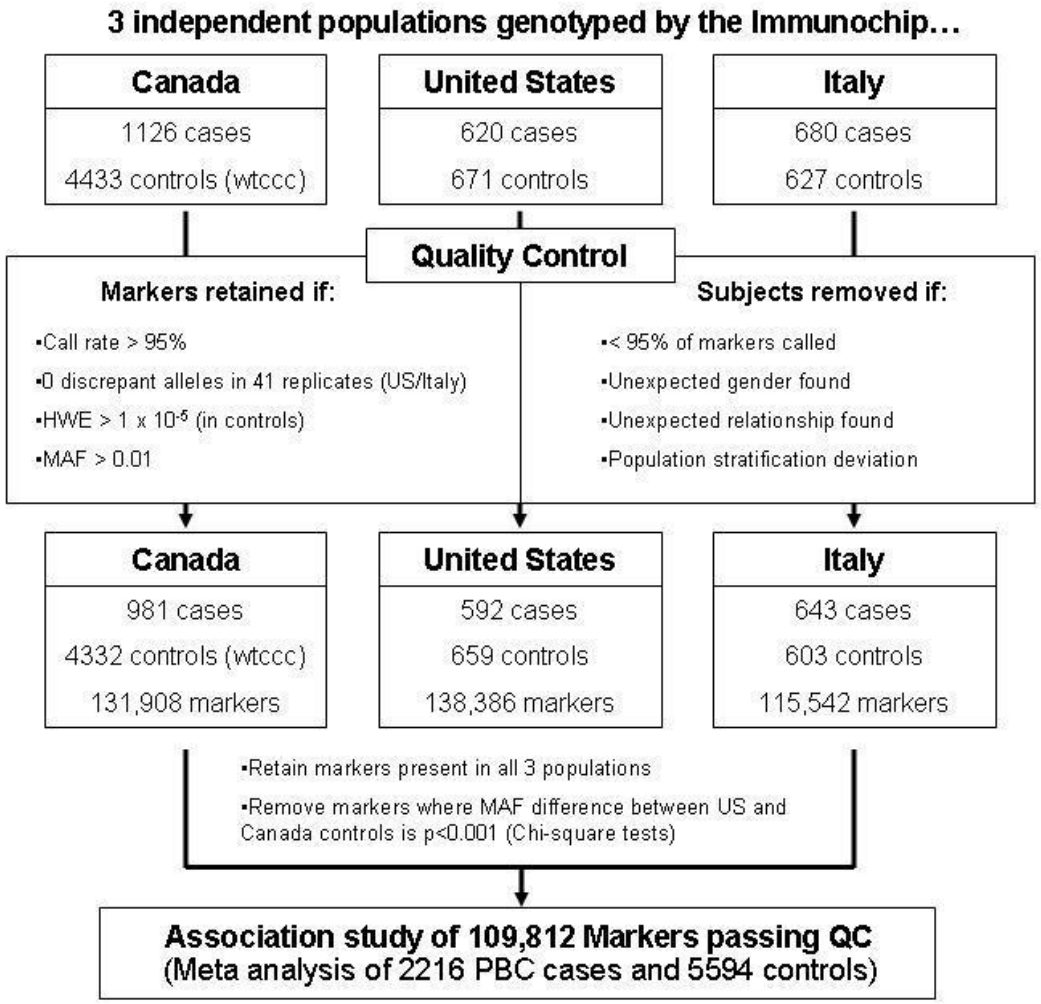
2003	2008	2010	2012
Monocentric study	Multicentric Study	GWAS	HLA-Dense array
Italy	Italy	Italy USA Canada	Italy
PBC = 186 HC = 558	PBC = 664 HC = 1992	PBC = 993 HC = 2483	PBC = 676 HC = 2483

Increasing study size

HLA / genetic story in PBC



Immunochip analyses in PBC



Novel risk locus:

TNFSF11 gene at 13q14

(RANKL)

PBC = 2426
Controls = 5731

Juran, Hirschfield, Invernizzi Hum Mol Genet 2012

International GWAS meta-analysis in PBC

Discovery and validation cohorts

Country	Discovery Cases	Discovery Controls	Validation Cases	Validation Controls
Canada	480	3,701	903	834
Italy	449	940	300	618
United Kingdom	1,816	5,161	1,792	2,515
United States of America	-	-	721	294
Total	**2,745**	**9,802**	**3,716**	**4,261**

Six novel risk loci:

2q12.1, 2q36.3, 4p16.3, 5q21.1
5q33.3, 6q23.3

PBC = 6461
Controls = 14.063

Cordell et al. Nat Commun 2015

PBC “gene” list 2017

Increasing study size

Gene Loci	Pre-GWAS	2009 Canada USA	2010 Italy-Canada USA	2011 UK	2012 Japan
HLA	Yes	Yes	Yes	Yes	Yes
IL12A	-	Yes	Yes	Yes	-
IL12RB2	-	Yes	Yes	Yes	-
IRF5/TNPO3	-	Yes	Yes	Yes	-
ORMDL3/IK ZF3	-	Yes	-	Yes	Yes
MMEL1	-	Yes	-	Yes	-
SPIB	-	Yes	Yes	Yes	-
DENND1B	-	-	Yes	Yes	-
CTLA-4		Yes	-	-	-
STAT4	-	Yes	-	Yes	-
CD80	-	-	-	Yes	Yes
NFKB1	-	-	-	Yes	-
IL7R	-	-	-	Yes	Yes
CXCR5	-	-	-	Yes	-
TNFRSF1A	-	-	-	Yes	-
TNFSF1	-	-	-	-	Yes
POU2AF1	-	-	-	-	Yes

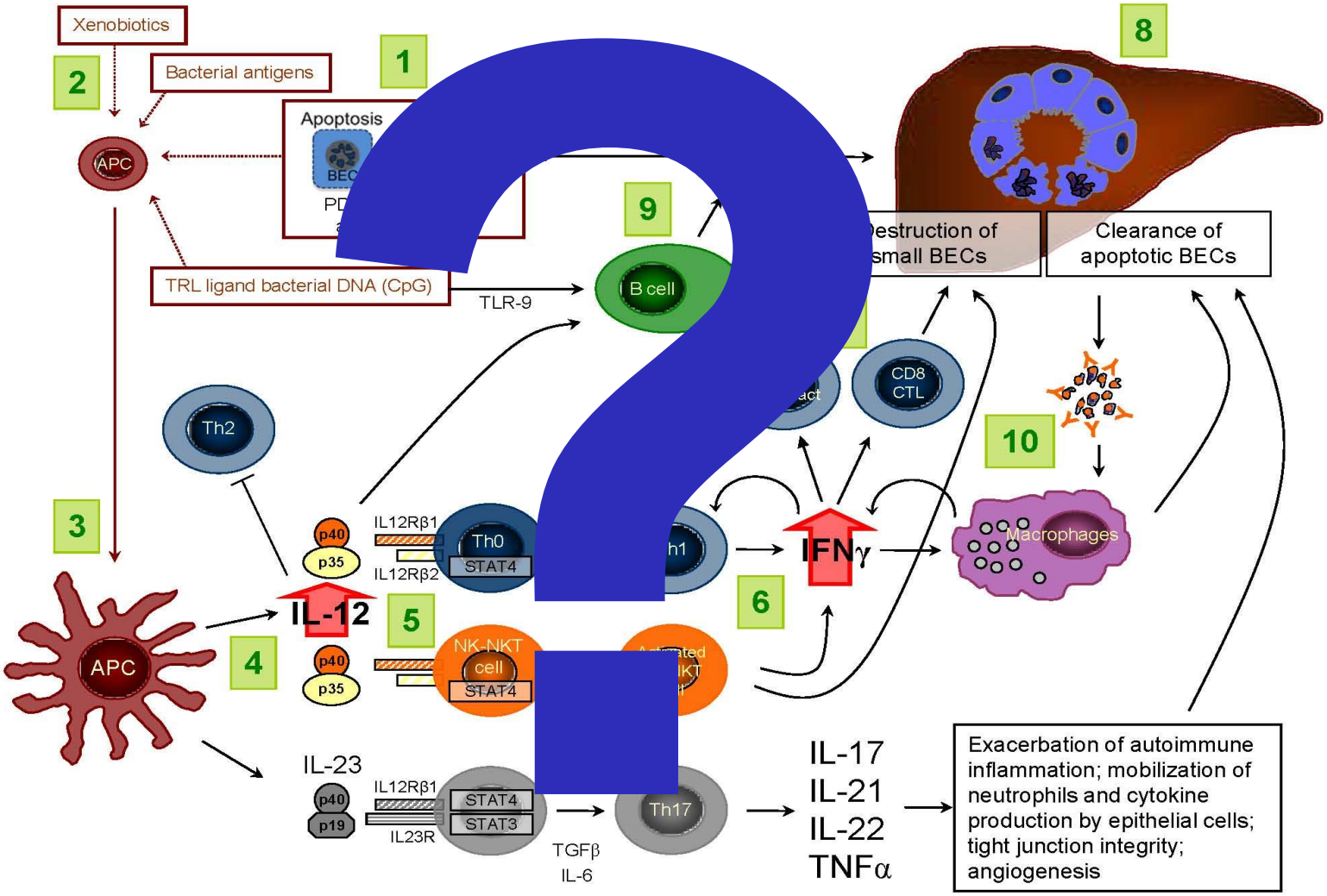
GENETICS

Big hopes for big data

Technology is allowing researchers to generate vast amounts of information about tumours. The next step is to use this genomic data to transform patient care.

And now?

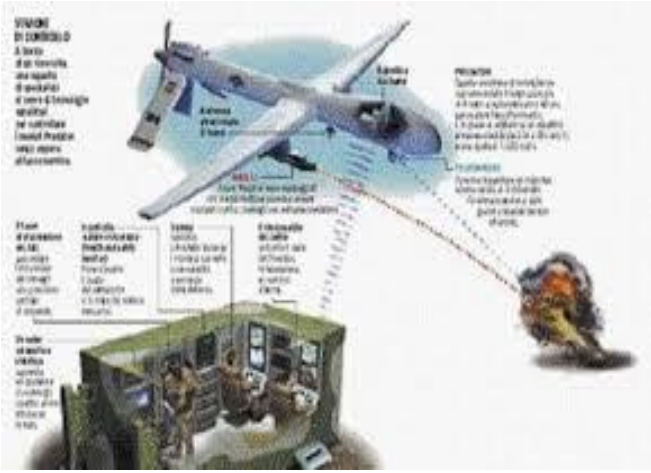
Pathogenesis of PBC



Lleo & Invernizzi. J Hepatol 2012

Precision drugs (weapons)

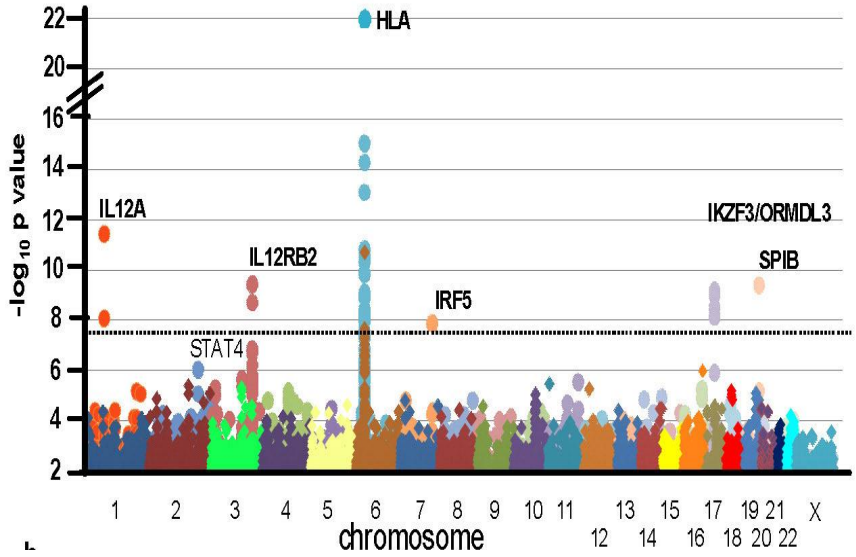
PRECISION DRUGS



- **FXR**
- **PPAR**
- **ASBT**
- **Anti-IL 12**
- **Anti-CXCL10**
- **Anti-CD20**
- **Anti-CD40L**
- **Anti-NOX 1 & 4**

Anti-IL12 for PBC

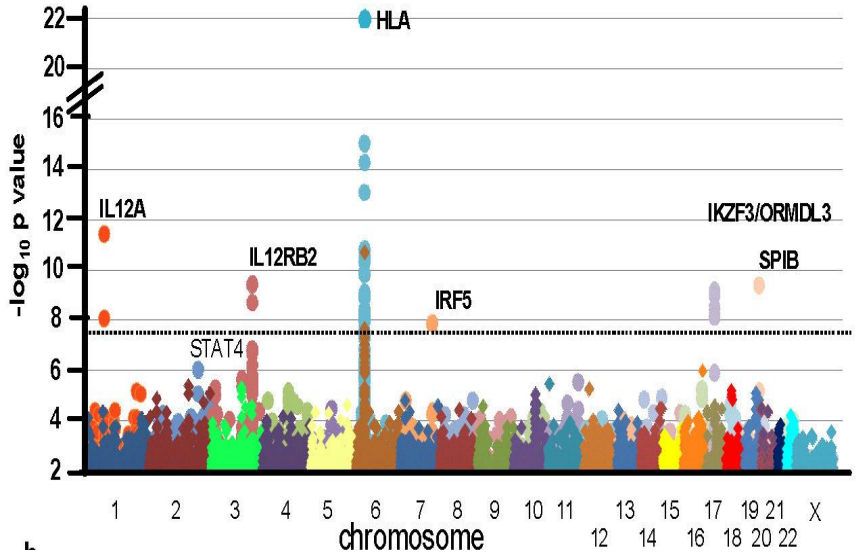
IL12
Genetic defect



Liu, Invernizzi, et al. Nature Genetics 2010

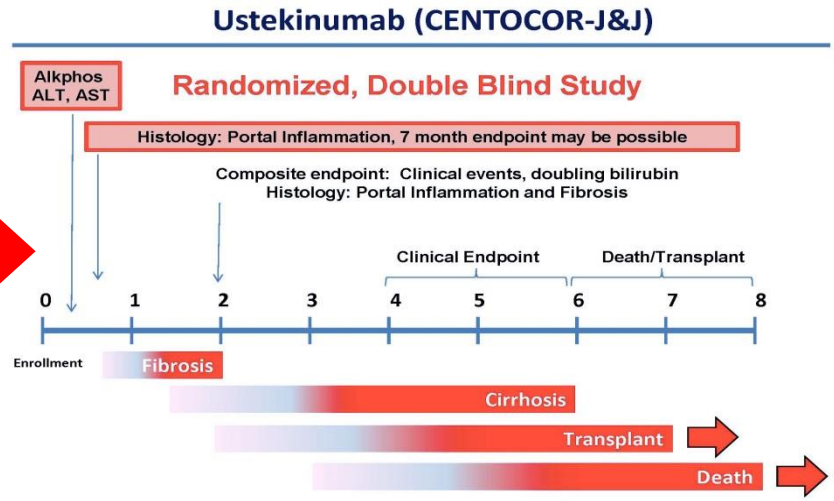
Anti-IL12 for PBC

IL12
Genetic defect



Liu, Invernizzi, et al. Nature Genetics 2010

Anti-IL12
Clinical trial



Hirschfield, et al. Hepatology 2016

?

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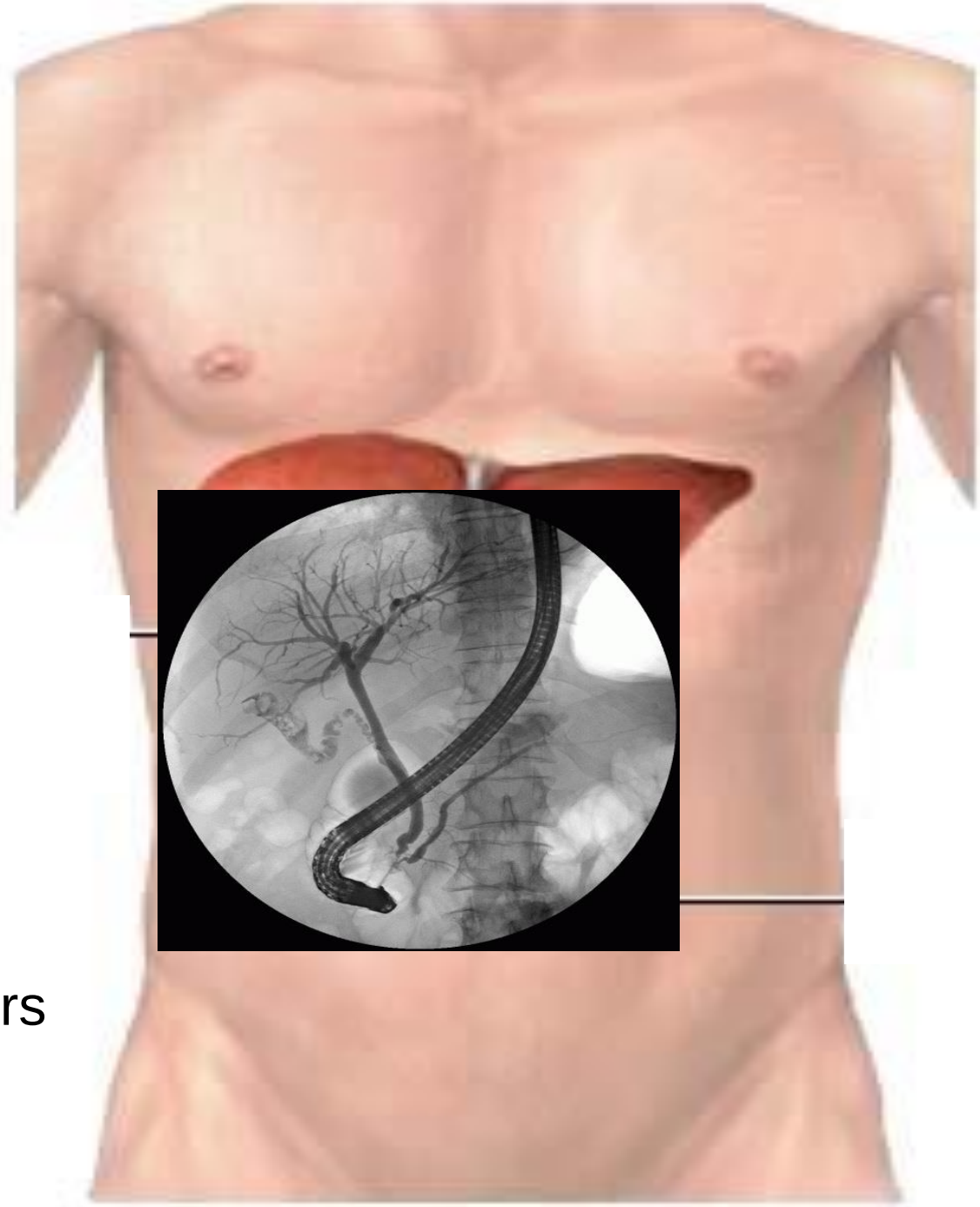
Female/male

Target organ

Novel biomarkers

Novel drugs

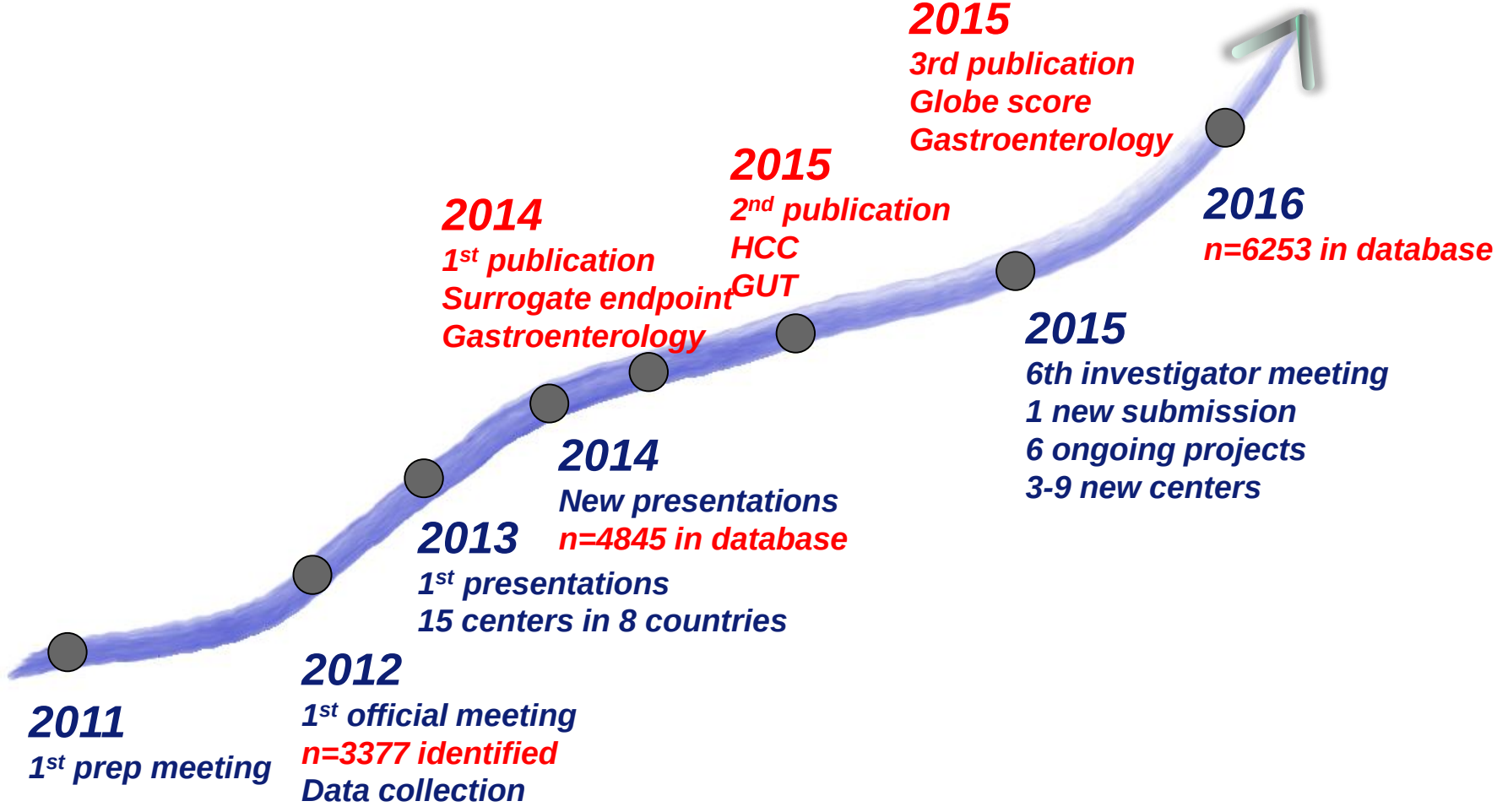


Networking story in PBC

PI's of the Global PBC Study Group (2012)

- Henk van Buuren, Rotterdam representing South of the Netherlands
- Gideon M. Hirschfield, Birmingham, UK
- Harry L.A. Janssen, Toronto, Canada
- Pietro Invernizzi, Milan, Italy
- Andrew L. Mason, Edmonton, Canada
- Cyriel Y. Ponsioen, Amsterdam representing North of the Netherlands
- Annarosa Floreani, Padova, Italy
- Christophe Corpechot, Paris, France
- Marlyn J. Mayo, Texas, USA
- Pier M. Battezzati, Milan, Italy
- Albert Parés, Barcelona, Spain
- Frederik Nevens, Leuven, Belgium
- Andrew K. Burroughs* & Douglas, London, UK
- Kris V. Kowdley, Seattle, USA
- Keith Lindor & Nicholas LaRusso, Rochester& Arizona, USA

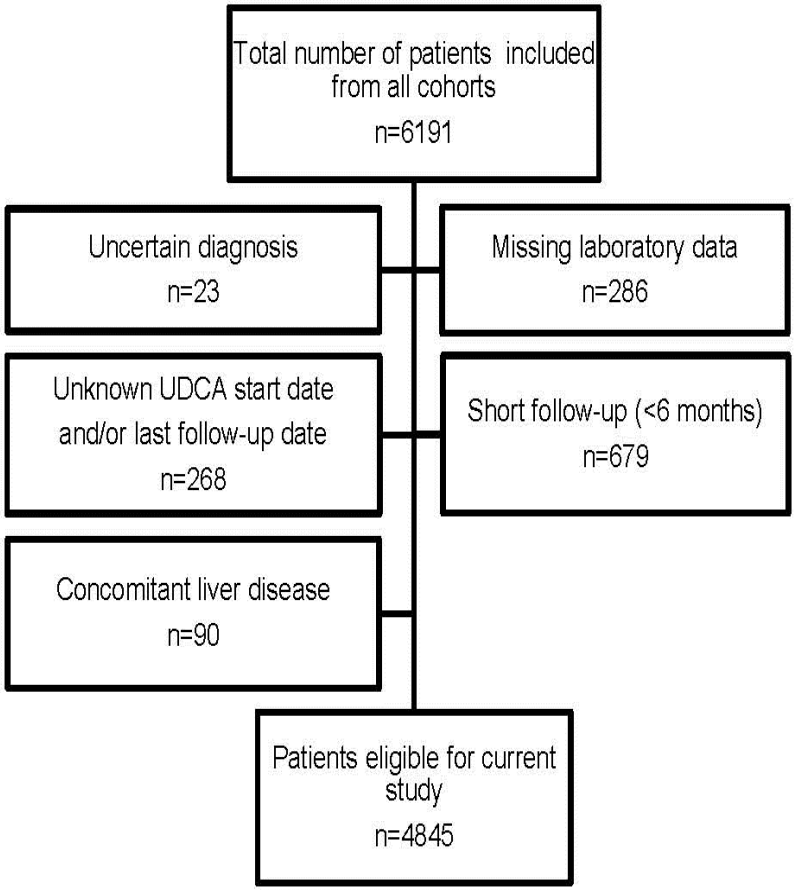
Continuous growth

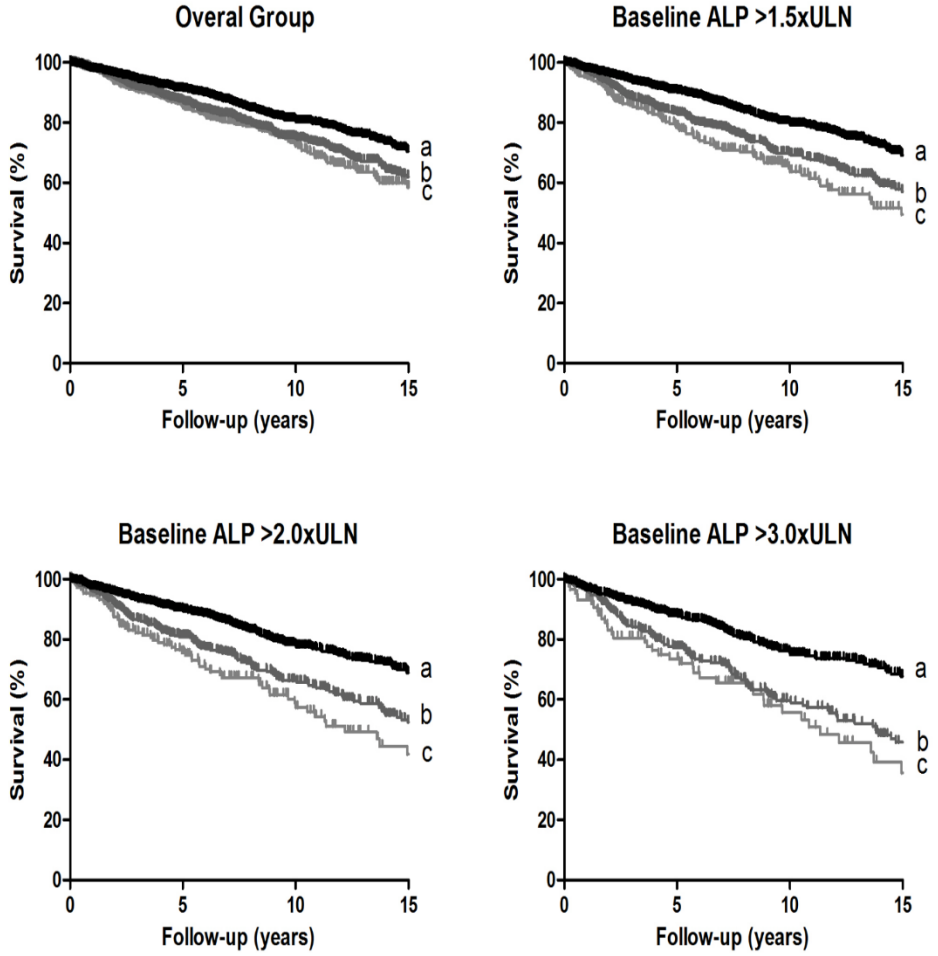


Gastroenterology 2014;147:1338–1349

CLINICAL—BILIARY

Levels of Alkaline Phosphatase and Bilirubin Are Surrogate End Points of Outcomes of Patients With Primary Biliary Cirrhosis: An International Follow-up Study





Lammers et al. Gastroenterology 2014

Global PBC projects

• Surrogate markers	*Gastroenterology 2014*
• HCC	*Gut 2015*
• Globe Score	*submitted, oral AASLD, poster EASL*
• Age and gender effects	*manuscript in preparation, poster EASL*
• Poise endpoint	*with the UK-PBC group* *manuscript in preparation, poster EASL*
• Dynamic prediction model	*analysis ongoing*
• Decompensation	*data collection running, poster EASL*
• Young Age	*data collection running*
• Liver transplantation	*data collection running*

• …..**PBC and pregnancy, MELD & optimal timing for transplantation, PBC North-South gradient,**

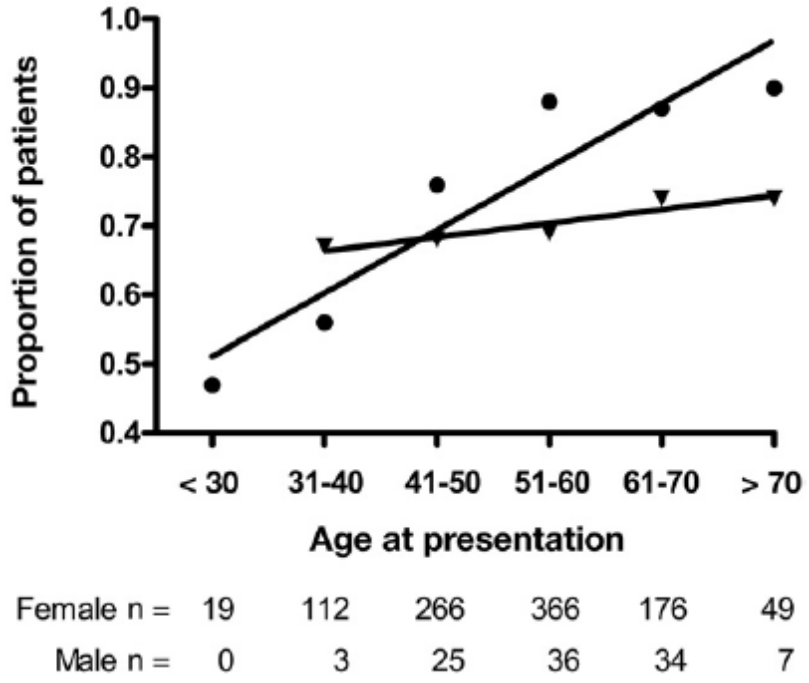
PBC-specific autoantibodies

Networking story in PBC

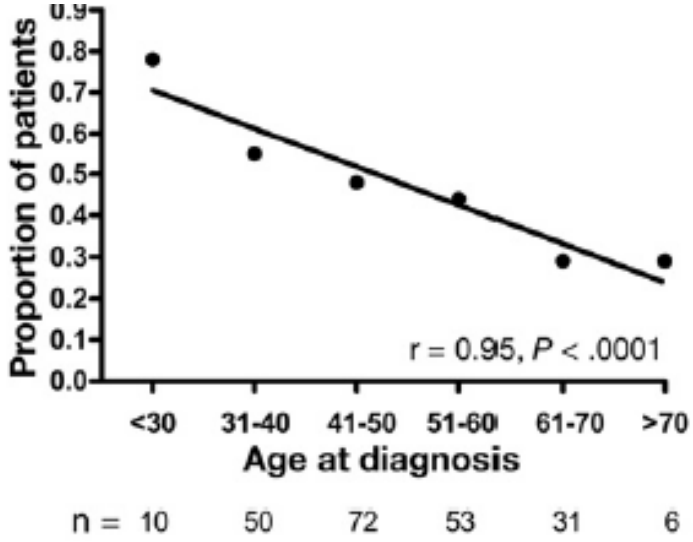
Predictors of response to UDCA Sex & Age

n=2353

Gender differences in the age-related likelihood of achieving UDCA response criteria



Proportion of patients who did not meet the criteria for response to UDCA after a minimum of 2 years treatment because of the ALT/AST criterion (2 ULN) related to their age at diagnosis

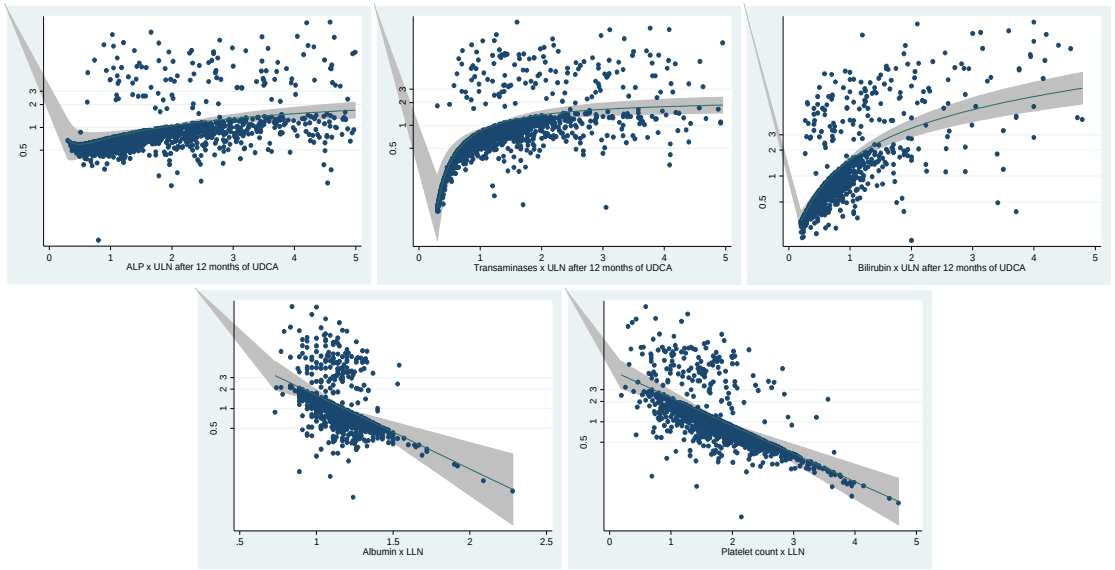


Carbone M et al. Gastroenterology 2013

Predictors of LT-free survival

n=3165

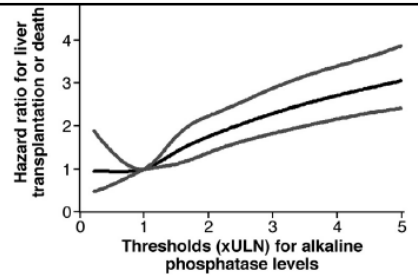
Fitted lines derived from the best fitting multivariable fractional polynomial model
UK-PBC cohort

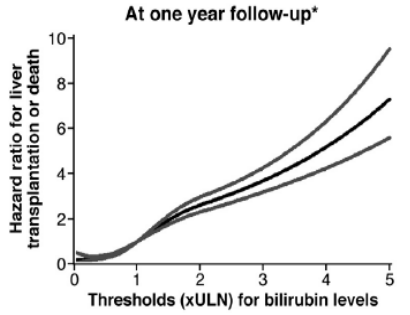


Carbone M, et al. Hepatology 2016

n=4119

Cubic spline function GLOBE cohort





Lammers et al. Gastroenterology 2015

Networking story in PBC

THE GLOBAL PBC STUDY GROUP

UK-PBC

Italian PBC Study Group

ERN-RARE LIVER
European Reference Network

Swiss PBC

Austro-German PBC

Outline

?

Genetics /environment

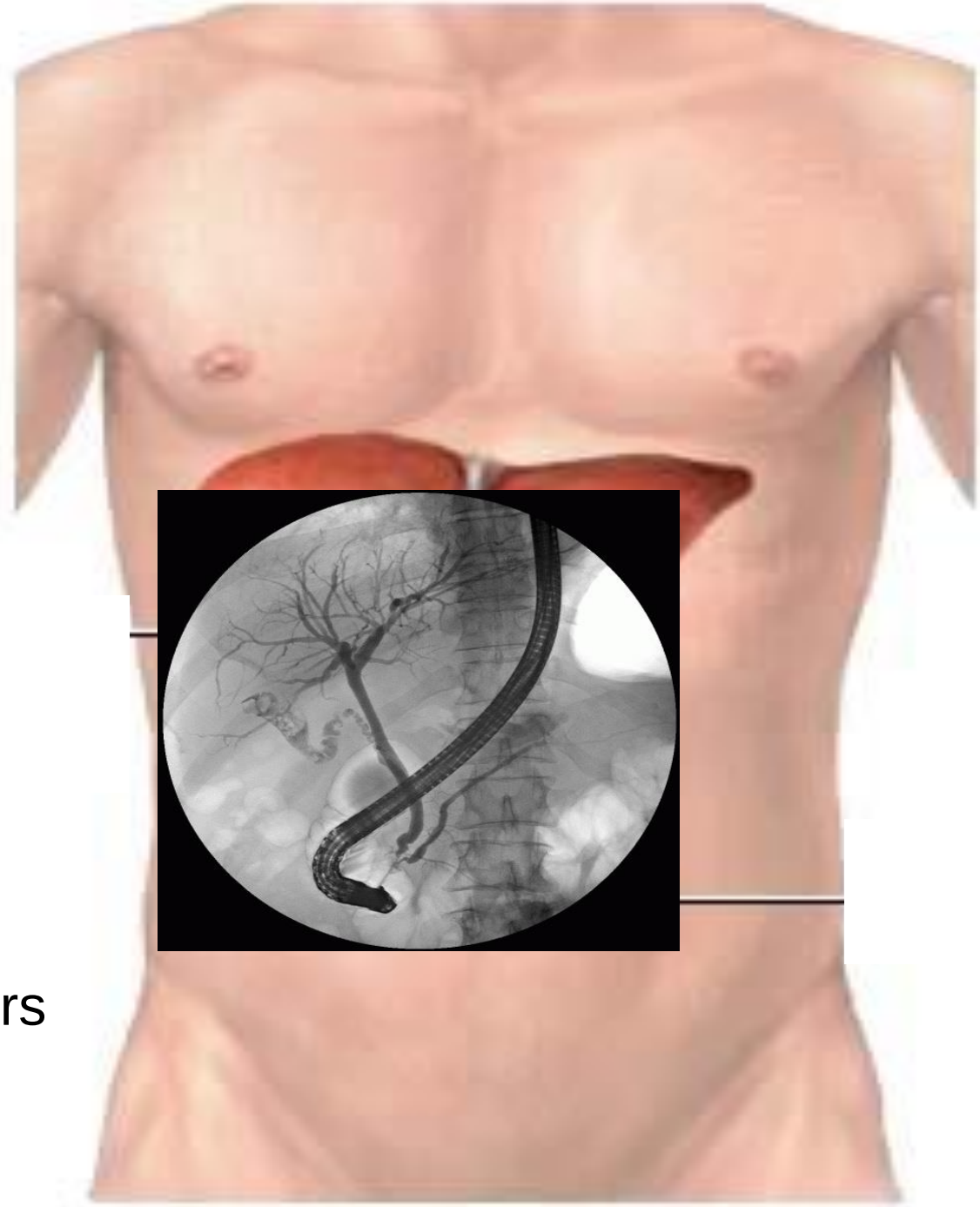
Networks

Female/male

Target organ

Novel biomarkers

Novel drugs



Geoepidemiology of PBC

Area	Year	Patients (No.)	Prevalence (per mln)	Incidence (per mln/yr)	Age (years)	Sex (M:F)
Europe	1984	569	23	54	54	1:10
Sweden	1985	111	151	13.3	55	1:6
Newcastle, UK	1989	347	154	19	58	1:9
Ontario, Canada	1990	225	22	3.3	59	1:13
Victoria, Australia	1995	84	19	-	-	1:11
Estonia	1995	69	27	2.3	-	1:22
Newcastle, UK	1997	160	240	22	66	1:10
Norway	1998	21	146	16	-	1:9
Minnesota, USA	2000	46	402	27	-	1:8
Newcastle, UK	2001	770	251	31	-	1:10
Victoria, Australia	2004	249	51	-	61	1:9
Japan	2005	9761	78	-	-	1:9
Canada	2009	137	227	30	53	1:5

Podda & Invernizzi. J Autoimmun 2013

Lombardia population

Inhabitants (about 10.000.000)

SEX	Number	Percentage
Female	2.073	69,8
Male	897	30,2
Totale	**2.970**	100,0

M:F PBC RATIO = **1:2**

Carbone et al. Scientific Report 2016

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M:F PBC RATIO = **1:2**

Denmark population

Inhabitants (about 5.500.000)

SEX	Number	Percentage
Female	584	80,9
Male	138	19,1
Totale	**722**	100,0

M:F PBC RATIO = **1:4**

Carbone et al. Scientific Report 2016

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Carbone et al. Scientific Report 2016

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Female/male

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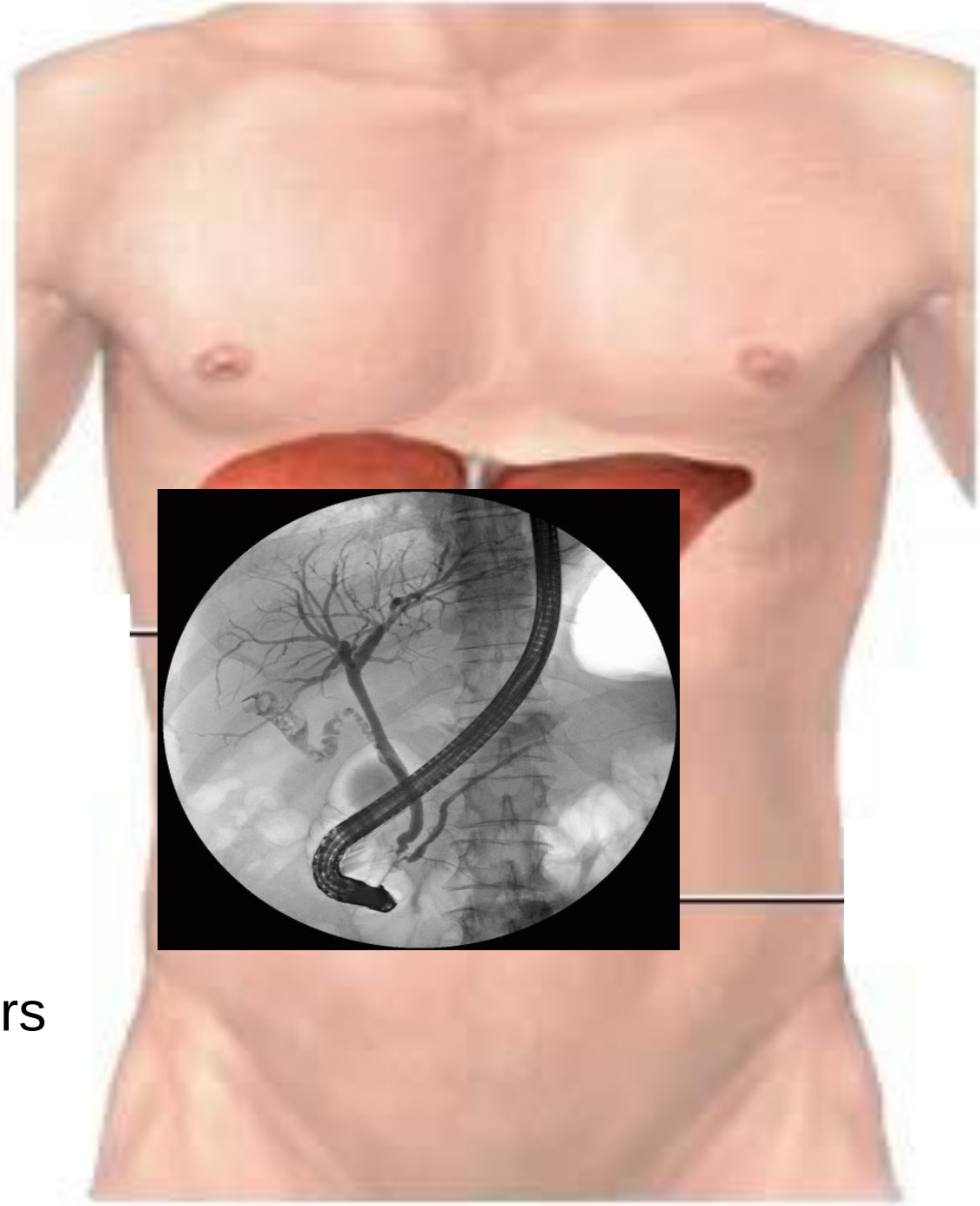
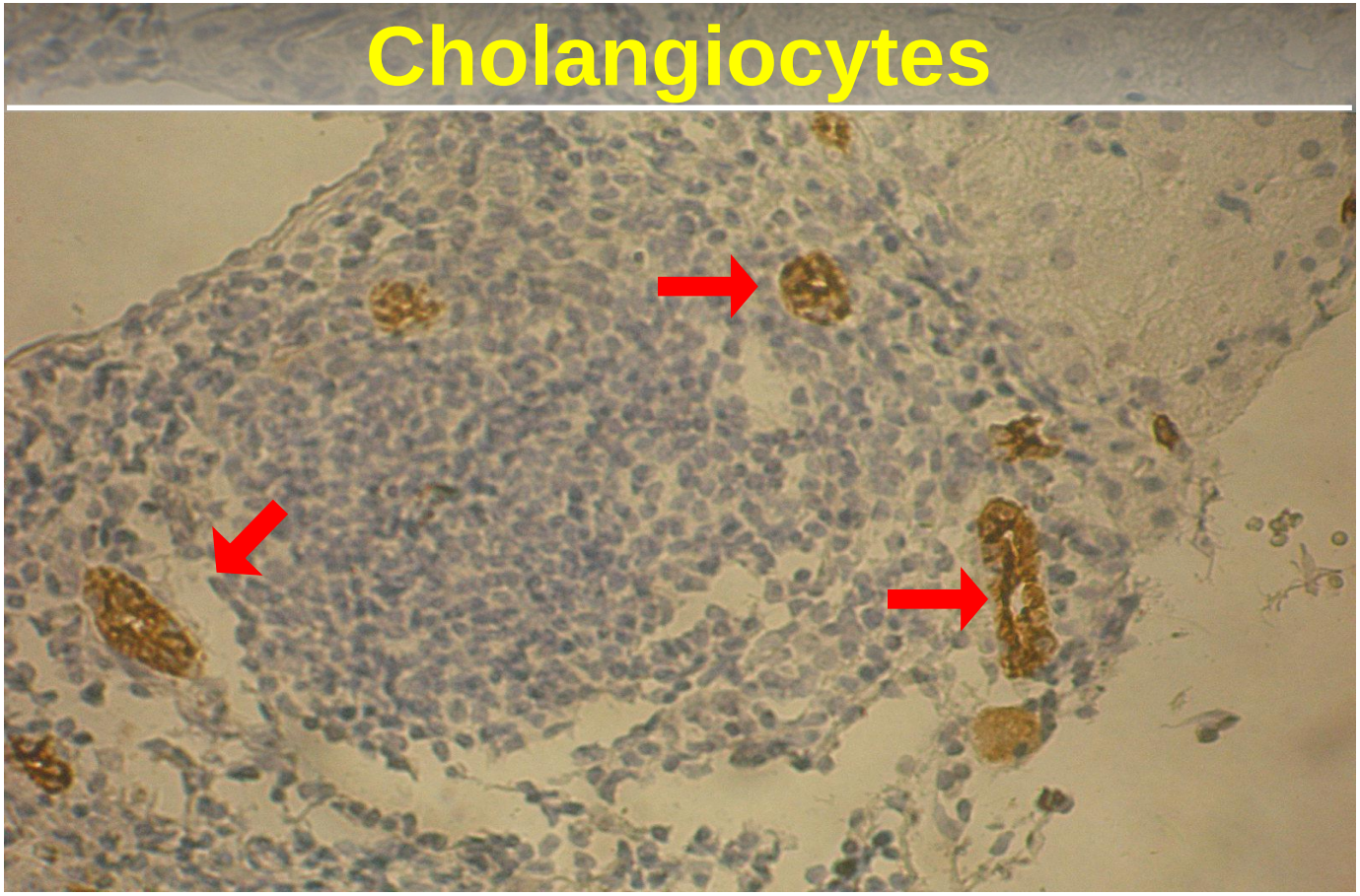
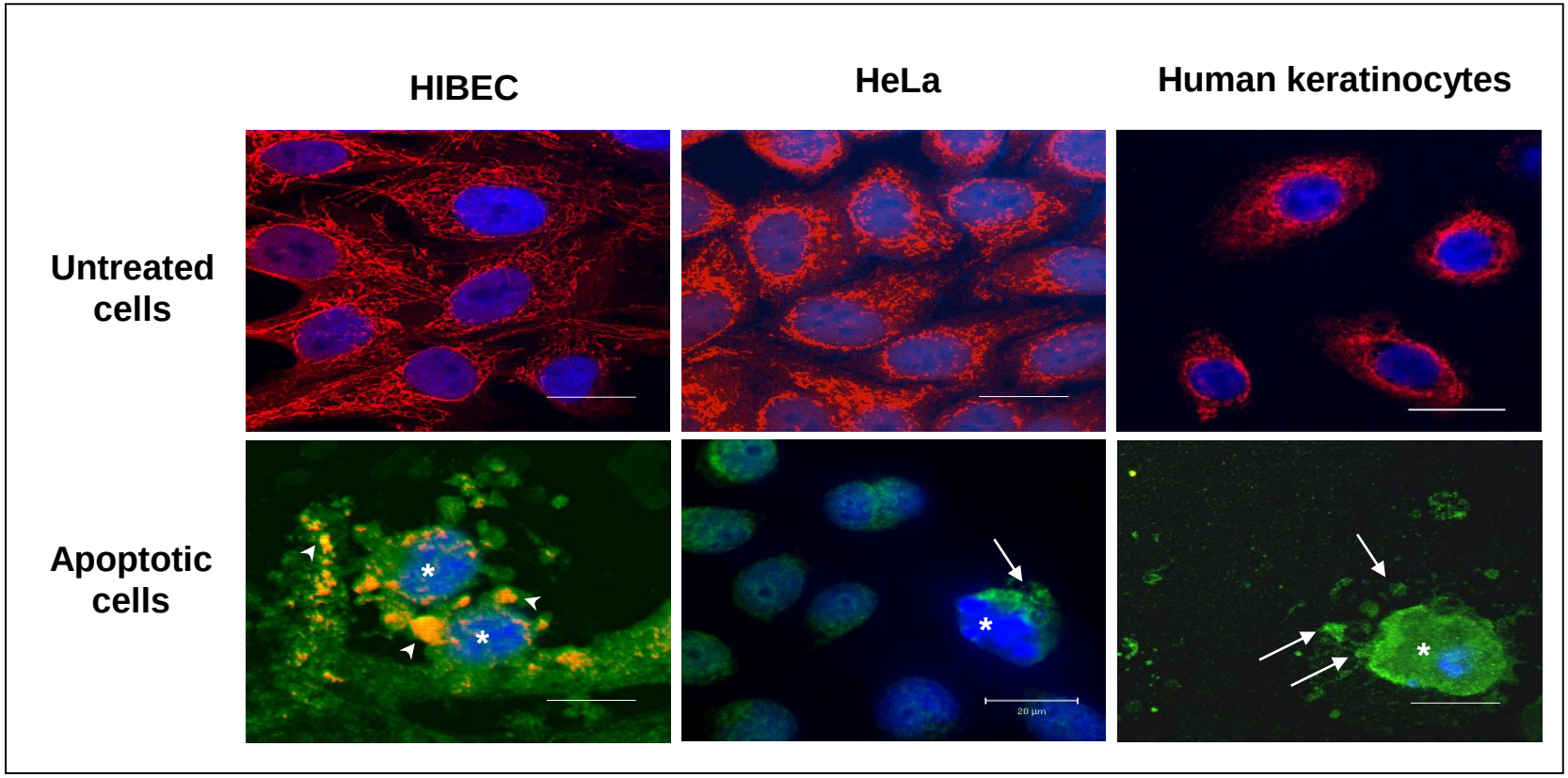


Target organ of autoimmune aggression in PBC

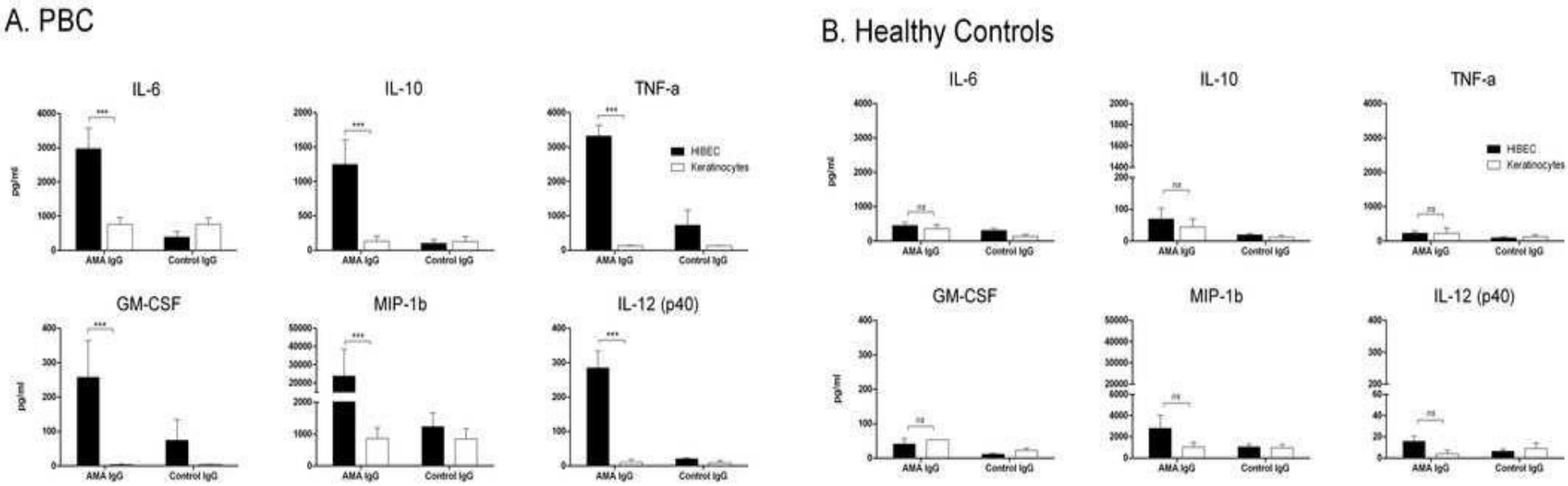


Mitochondrial PDC-E2 autoantigen of PBC localizes to apoptotic blebs of human intrahepatic biliary epithelial cells



Lleo A et al. Hepatology 2009

Apoptotic blebs of human cholangiocytes stimulate macrophages



Lleo A et al. Hepatology 2009

Lleo A et al. Hepatology 2010

Shotgun proteomics: Identification of unique protein profiles of apoptotic blebs of cholangiocytes

Protein	Gene	Description
CPN1	*CPN1*	Carboxypeptidase N catalytic chain
ITIH2	*ITIH2*	Inter-alpha (Globulin) inhibitor H2
C9	*C9*	Complement component C9
FGG	*FGG*	Fibrinogen gamma chain
FGA	*FGA*	Fibrinogen alpha chain
SERPINF2	*SERPINF2*	Alpha-2-antiplasmin
APOC2	*APOC2*	Apolipoprotein C-II
DRG1	*DRG1*	Developmentally-regulated GTP-binding protein 1

Lleo A et al. Hepatology 2009
Lleo A et al. Hepatology 2010
Lleo A et al. Hepatology 2014

Outline

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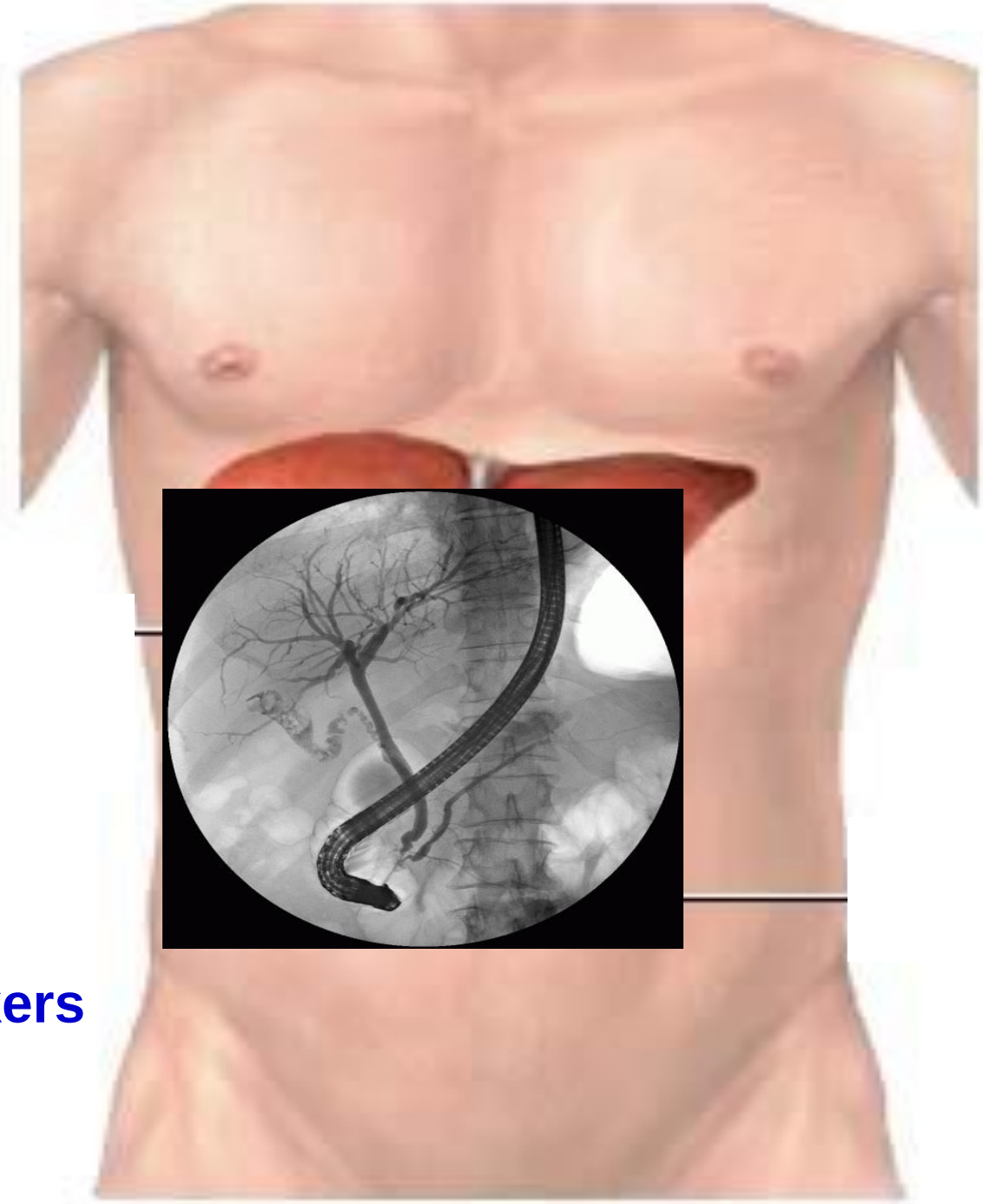
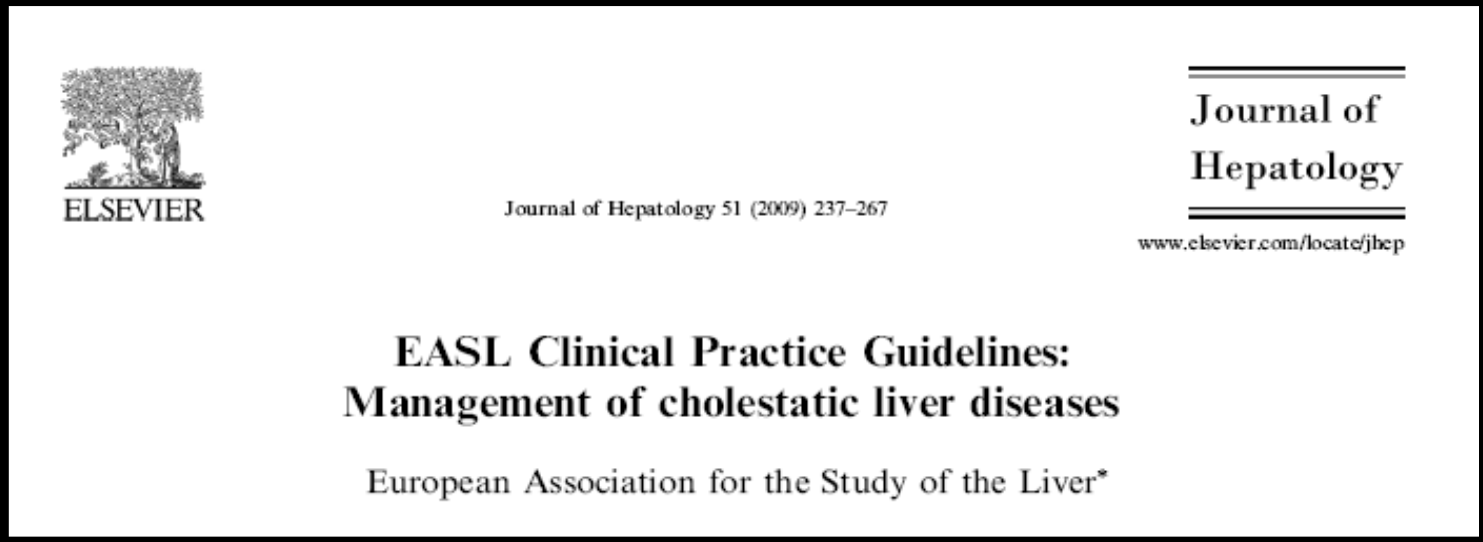




ELSEVIER

Journal of Hepatology 51 (2009) 237–267

Journal of Hepatology

www.elsevier.com/locate/jhep

EASL Clinical Practice Guidelines:
Management of cholestatic liver diseases

European Association for the Study of the Liver*

DIAGNOSIS OF PBC – Recommendations

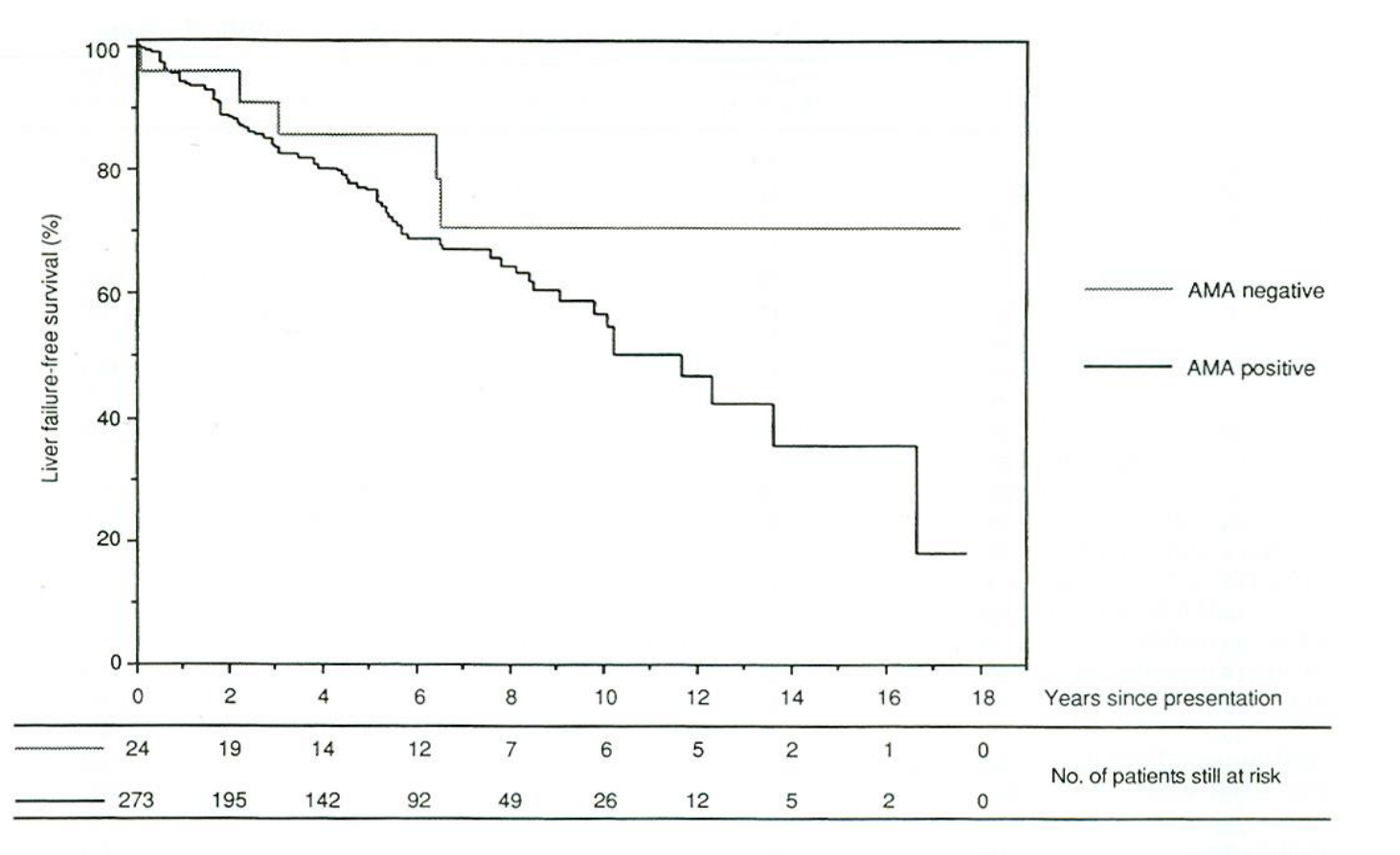
- A diagnosis of PBC can be made with confidence in adult patients with otherwise unexplained **elevation of AP** and presence of **AMA** (> 1:40) and/or AMA type M2
- A **liver biopsy** is needed for the diagnosis of PBC in the absence of PBC specific antibodies. A liver biopsy may also be helpful in the presence of disproportionally elevated serum transaminases and/or serum IgG levels to identify additional or alternative processes
- AMA-positive individuals with normal serum liver tests should be followed with annual reassessment of biochemical markers of cholestasis

Natural history models in PBC

- More widespread resort to laboratory examinations led to PBC being diagnosed with increasing frequency during the earliest disease stages, when patients are asymptomatic and have not yet entered a phase of progressive bilirubin or Mayo score elevation
- New prognostic indicators are needed for asymptomatic patients

Prognostic role of AMA in PBC

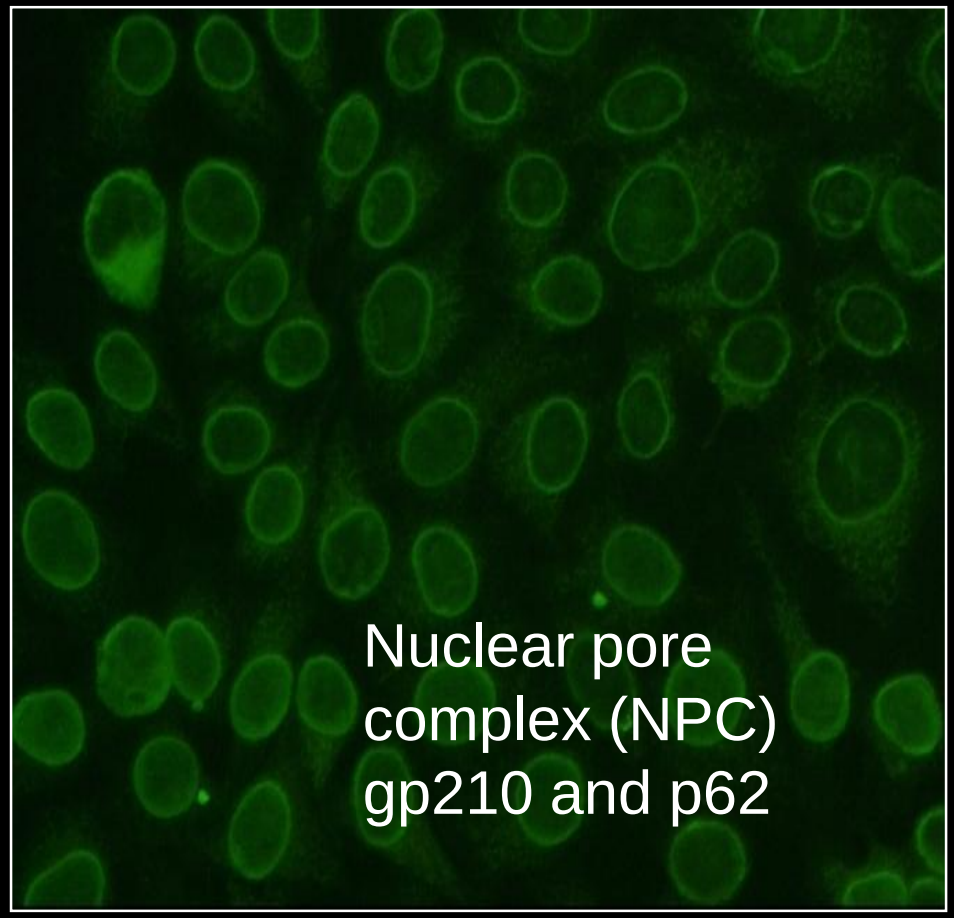
Liver failure-free survival



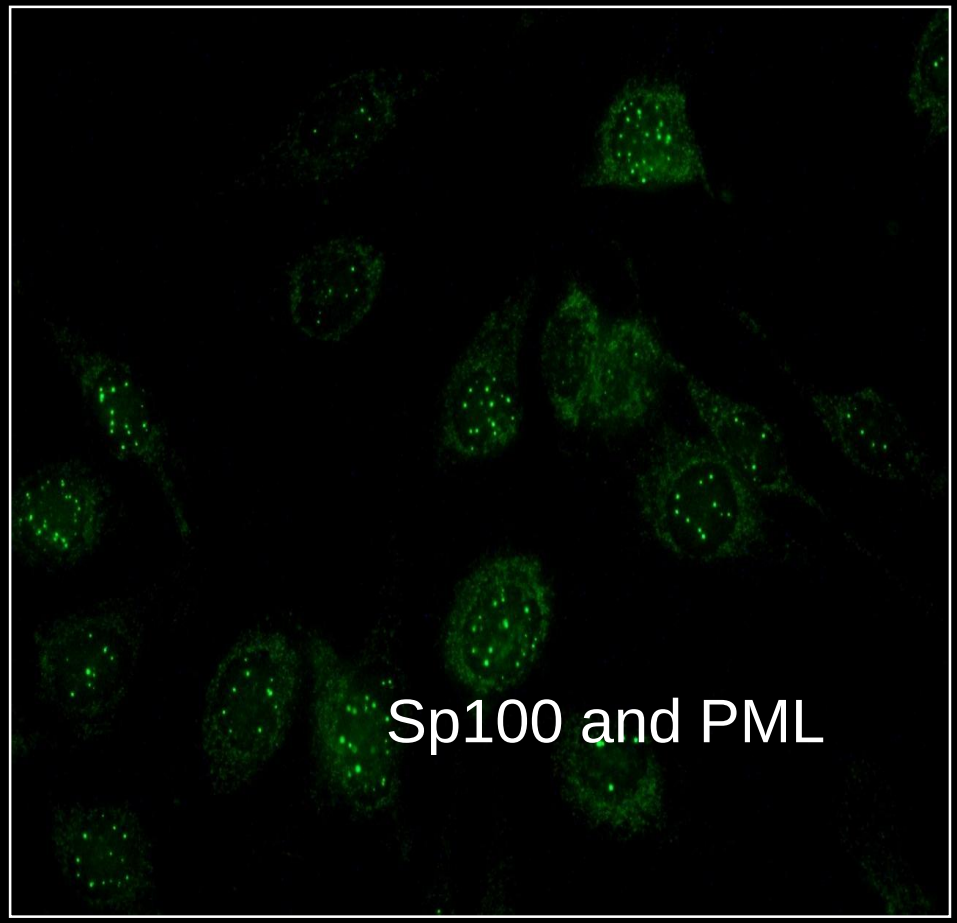
Invernizzi et al. Hepatology 1997

PBC-specific ANA

Rim like



Nuclear-dot



Invernizzi et al. Semin Liver Dis 2005

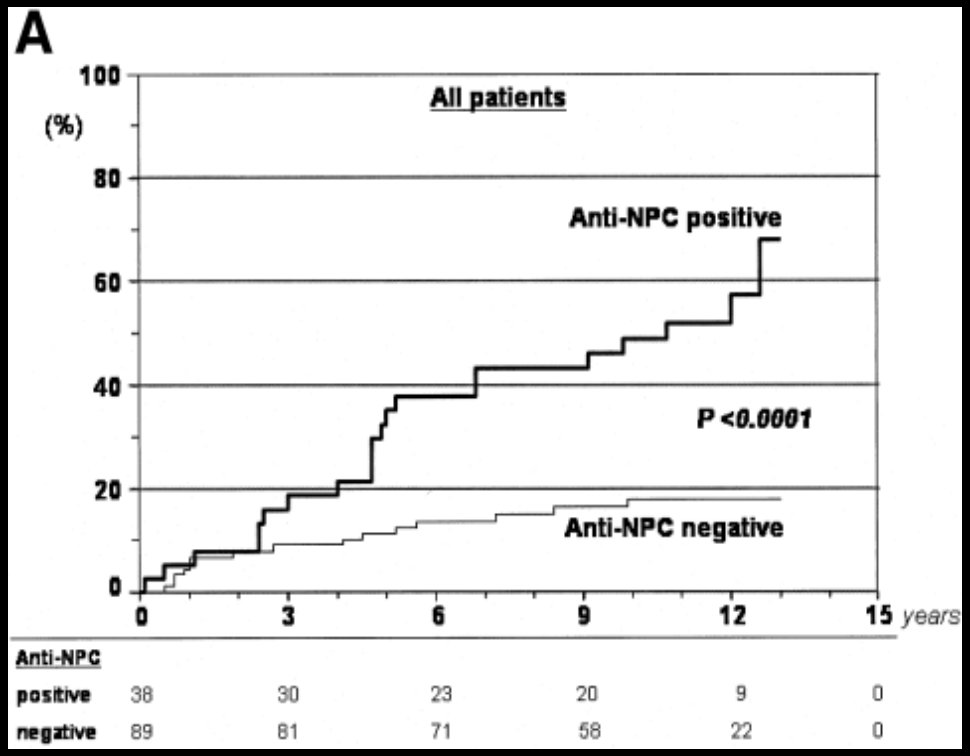
Clinical and biochemical features of PBC patients according to anti-NPCs status

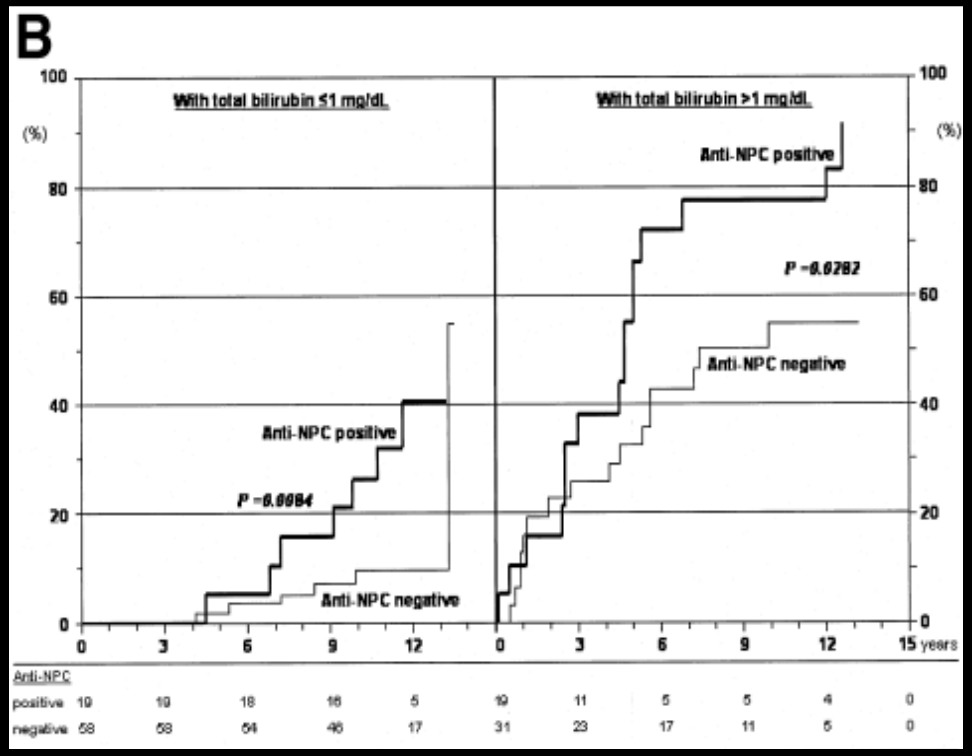
	NEGATIVE *(n = 125)*	POSITIVE *(n = 46)*
Age (years)	59±11	61±12
Asymptomatic patients	67 (54%)	13 (28%)
Cirrhosis	62 (50%)	36 (78%)
Major complications	10 (8%)	13 (28%)
Mayo score	5.0±1.0	5.8±1.1
Total Bilirubin (mg/dL)	1.0±1.1	2.2±3.7
Albumin (g/dL)	4.4±0.6	4.0±0.5
IgG (mg/dL)	1,736±538	1,921±673
IgA (mg/dL)	350±191	478±294

Invernizzi et al. J Hepatol 2001

Prognostic role of anti-NPC in PBC

Mortality rate





Wesierska-Gadek et al. Hepatology 2006

Outline

?

Genetics /environment

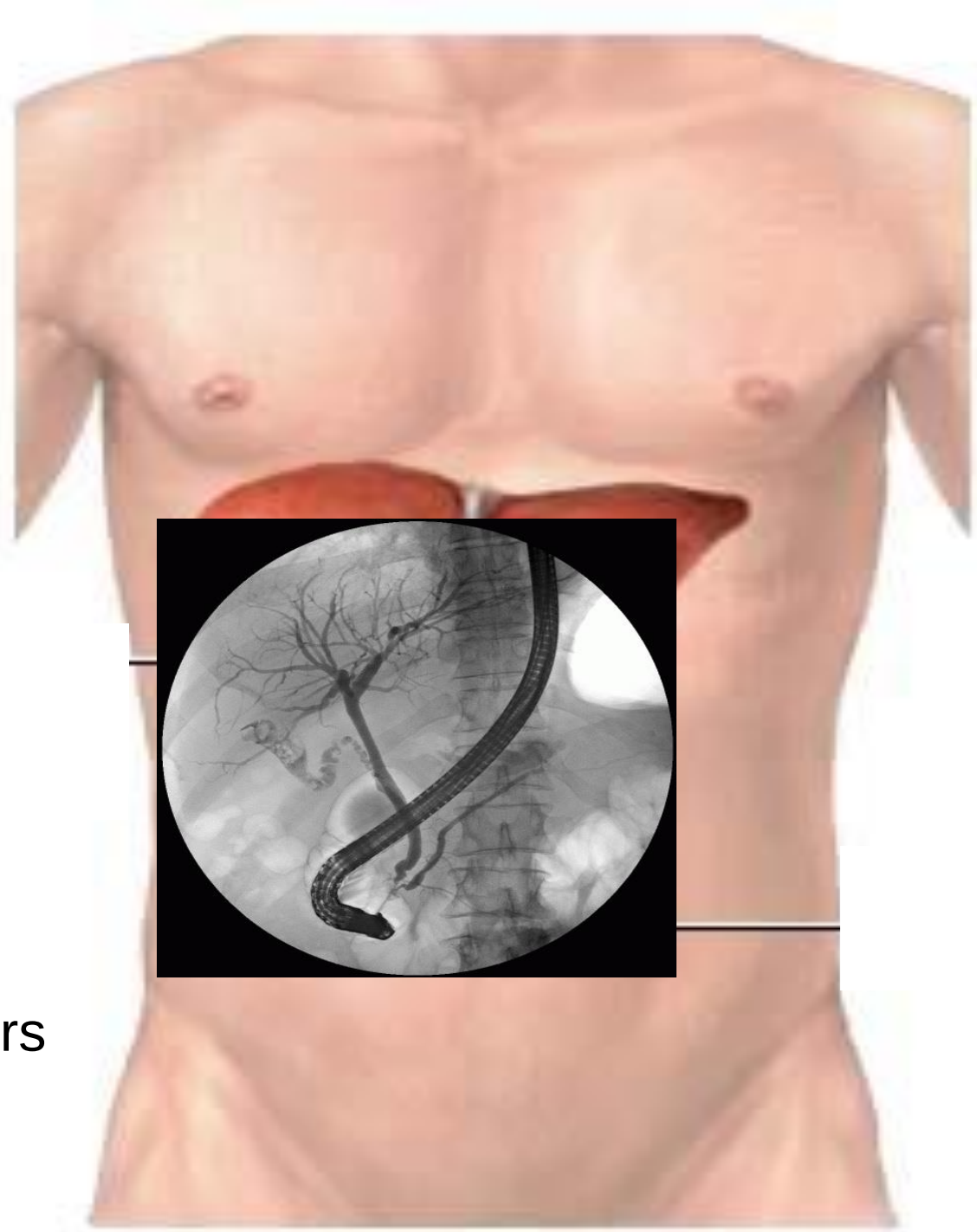
Networks

Female/male

Target organ

Novel biomarkers

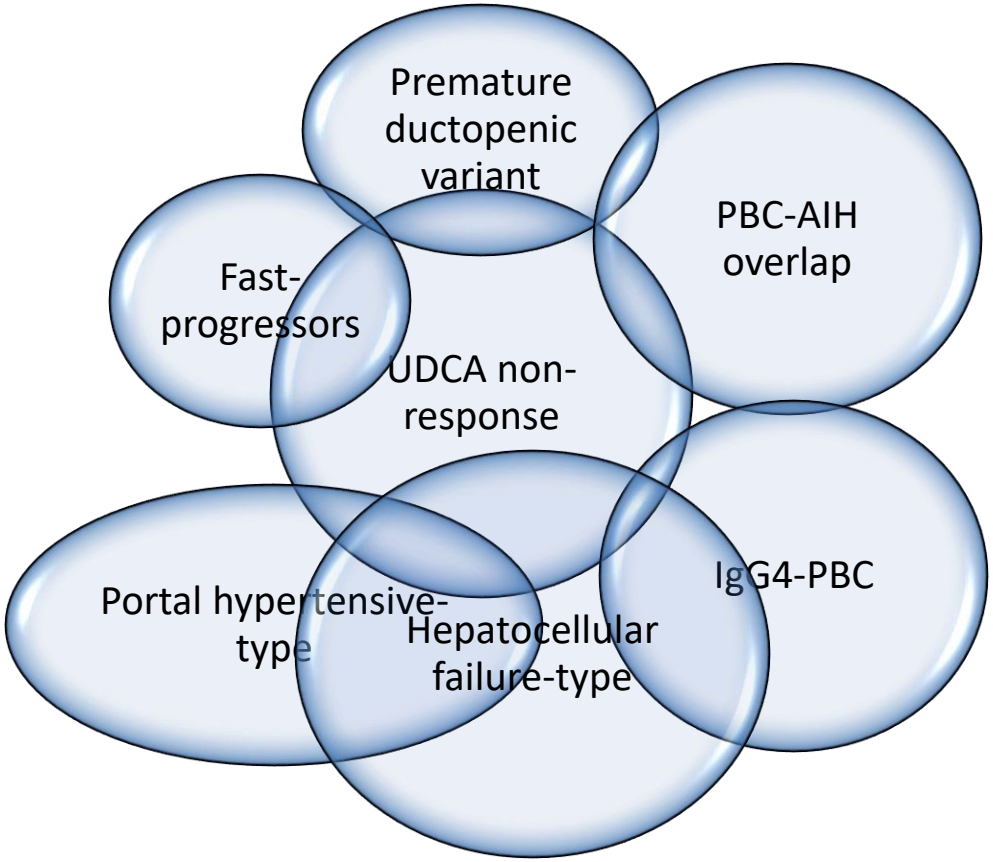
Novel drugs



- Recognise different disease phenotype and risk profiles
- Timely (early) treatment
- Second-line therapies

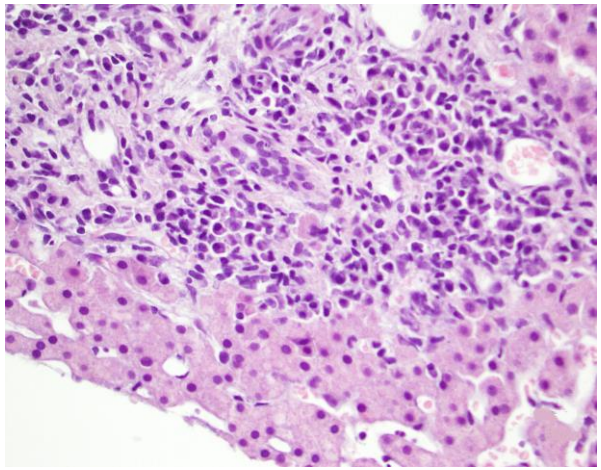
- **Recognise different disease phenotype and risk profiles**
- Timely (early) treatment
- Second-line therapies

PBC is an heterogeneous disease

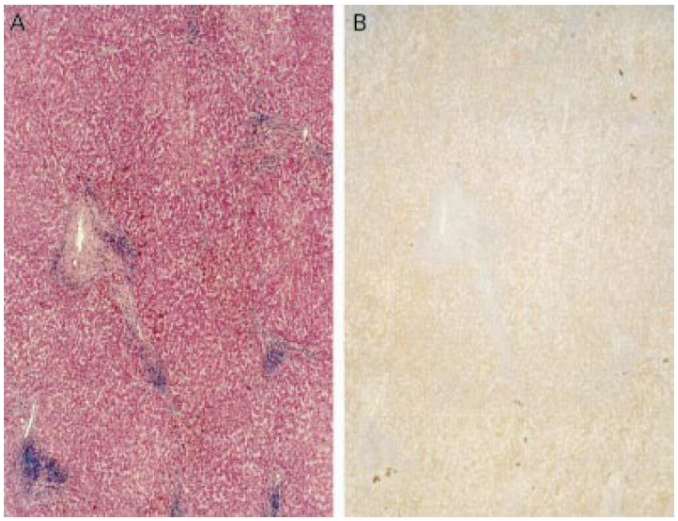


Disease sub-phenotypes in PBC

Overlap PBC+AIH

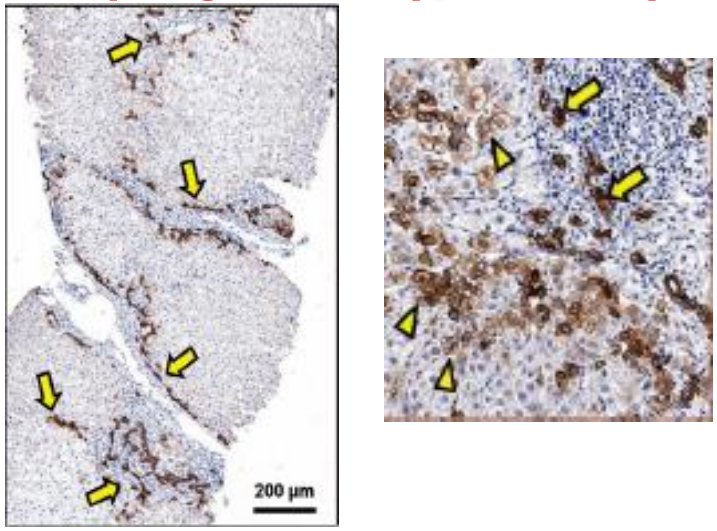


Premature ductopenic variant



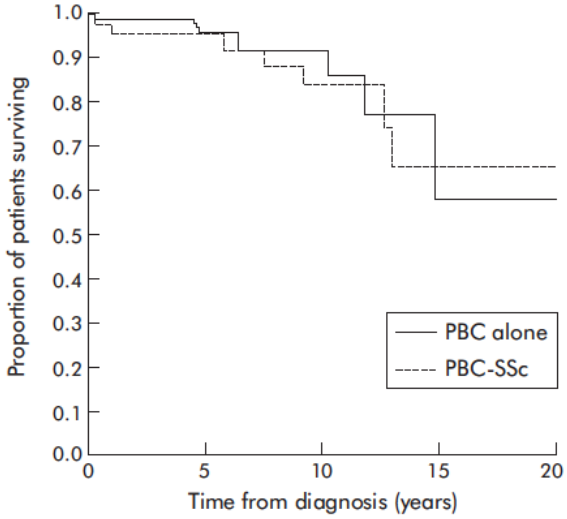
Vleggar FP et al. Gut 2001

Fast progressor (↑DR & IH)



Carbone M et al. **Lancet Gastro & Hepatol** 2018

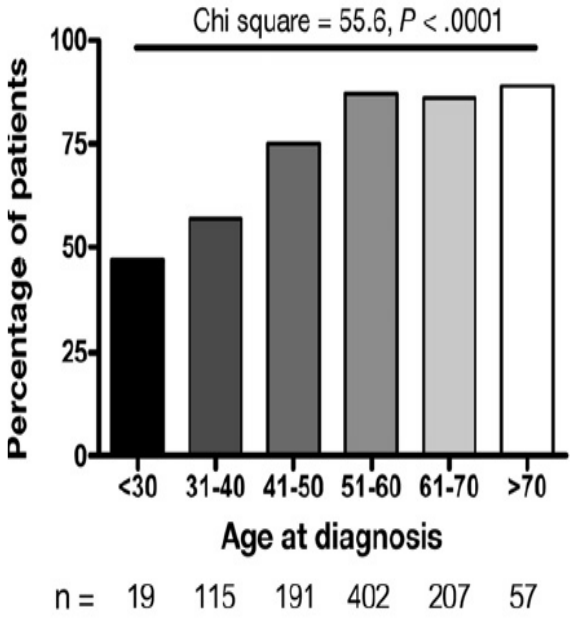
Slow progressor (SSc)



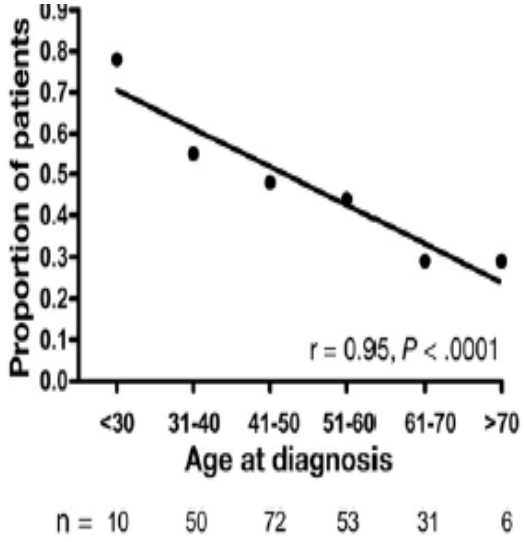
Rigamonti et al. **Gut** 2006

Predictors of response to UDCA Sex and Age (N=2353)

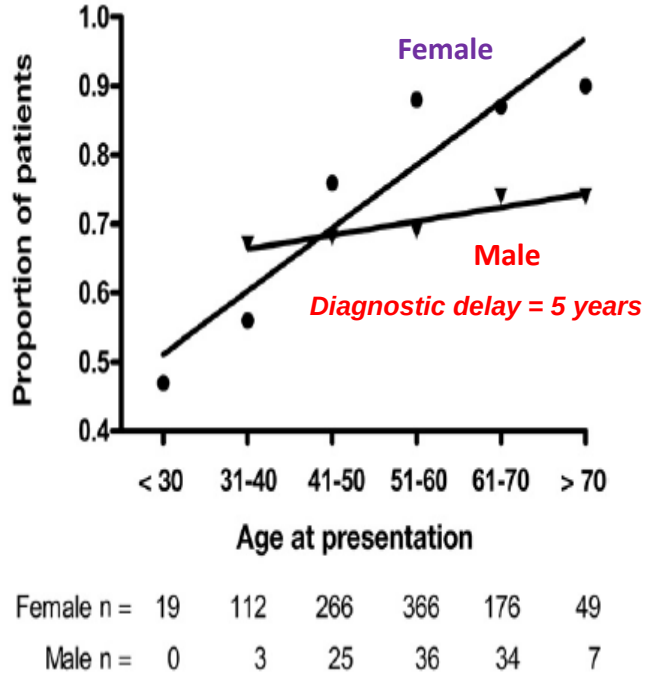
Patients who met the Paris I criteria of response categorized according to their age



Proportion of patients who did not meet the criteria for response to UDCA after a minimum of 2 years treatment because of the ALT/AST criterion (2 ULN) related to their age at diagnosis



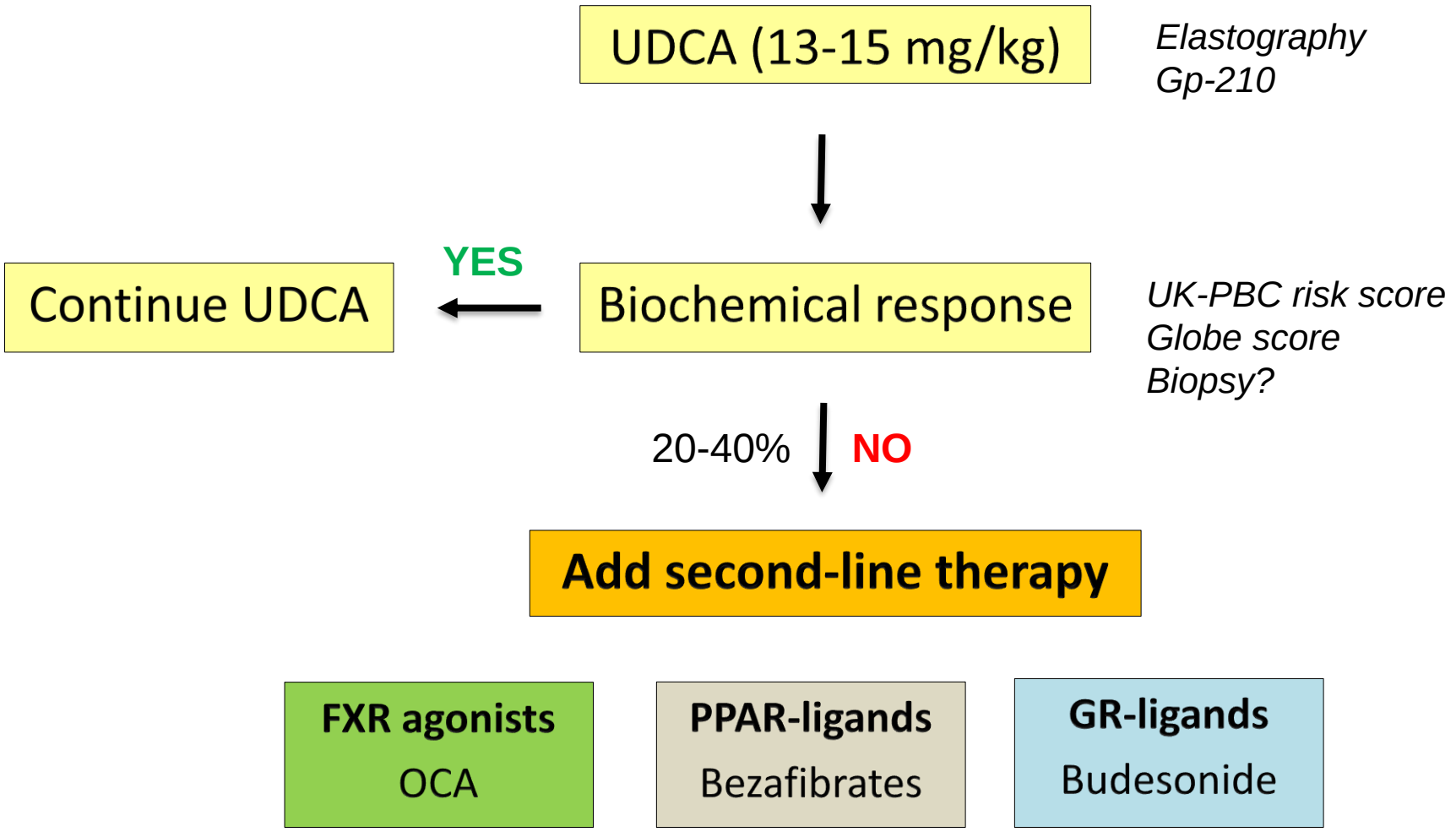
Gender differences in the age-related likelihood of achieving UDCA response criteria



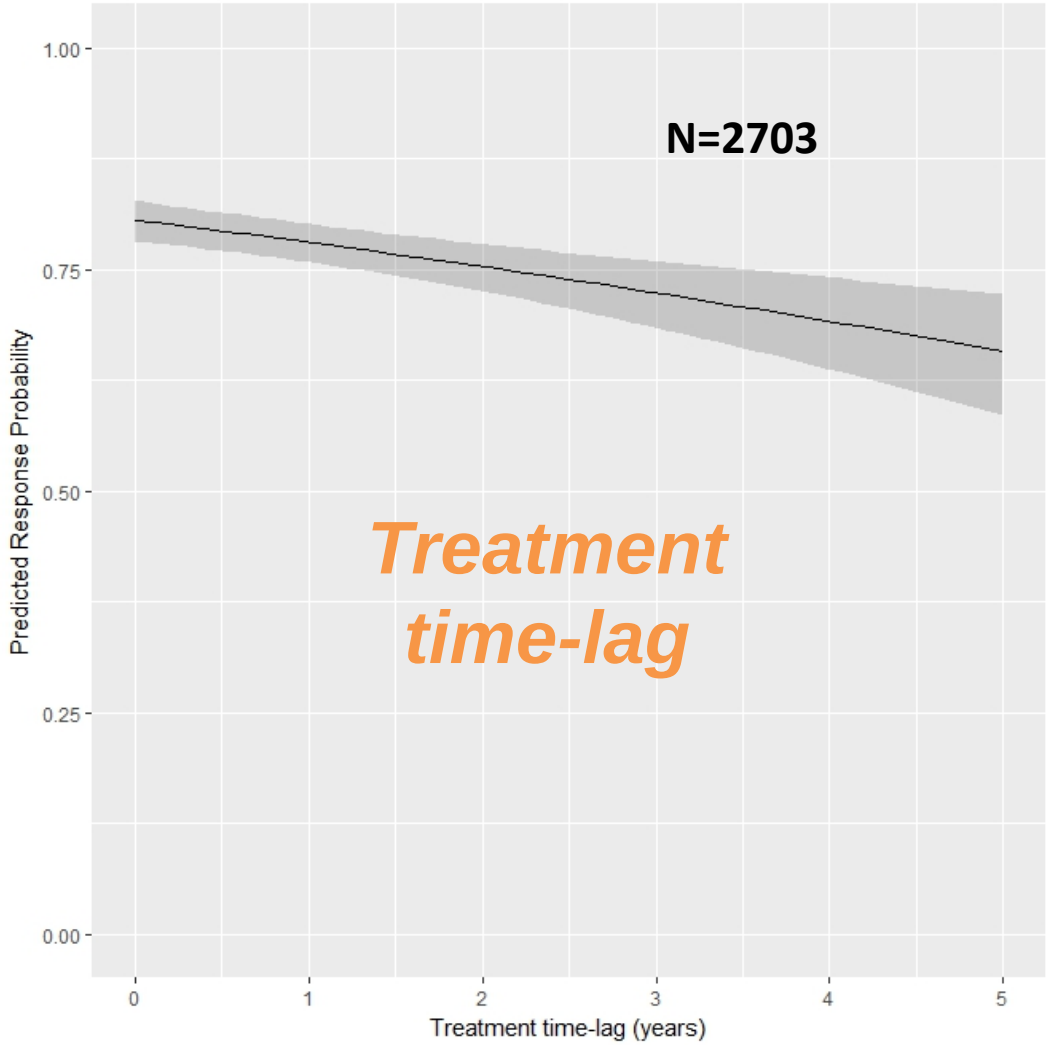
Carbone M, et al. ***Gastroenterology 2013***

- Recognise different disease phenotypes and risk profiles
- **Timely (early) treatment**
- Integrated care pathway

Response-guided approach



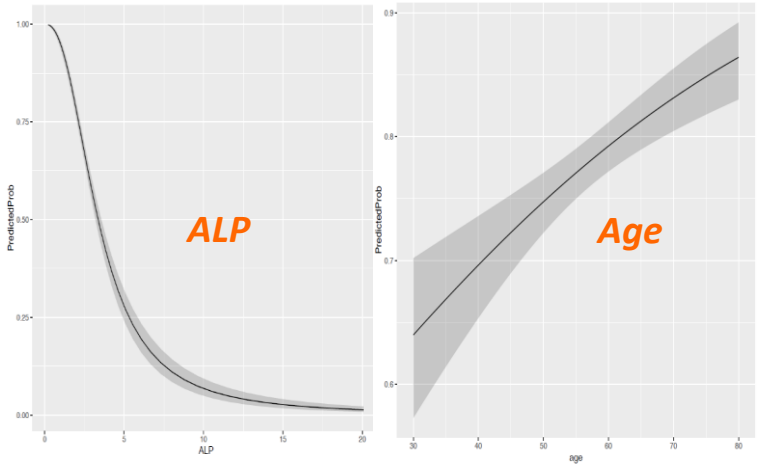
The more you wait, the lower is the response!



Carbone M, Nardi A et al. **Lancet Gastro & Hepatol 2018**

EARLY risk stratification

Predictors of UDCA response (n=2703)

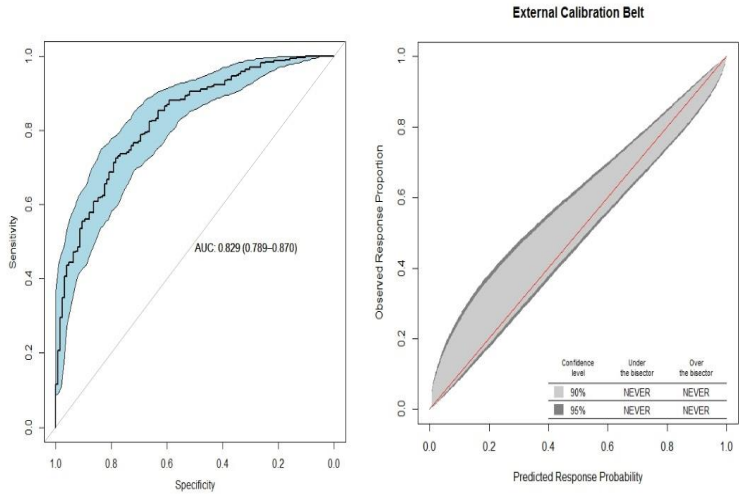


$$UDCA \text{ response score } (URS)$$

$$= 0.77 + 0.60 \times \left(\sqrt{TB_{diag}}\right)^{-1} - 2.73 \times \ln\left(ALP_{diag}\right)$$

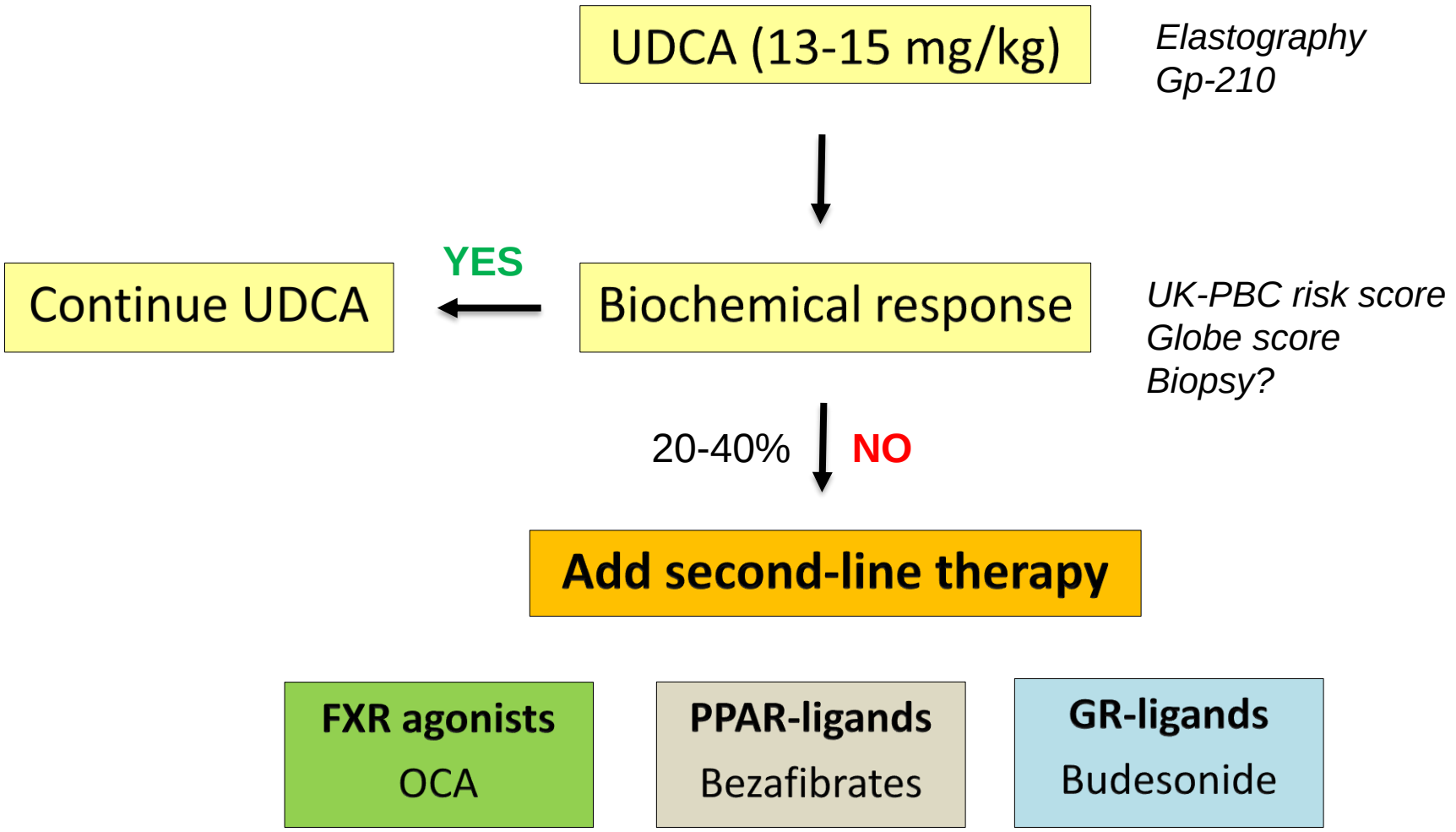
$$+ 0.35 \times \ln\left(TA_{diag}\right) + 0.03 \times age - 0.15 \times (treatment\ time\ lag)$$

$$- 0.56 \times \Delta \text{ALP}$$

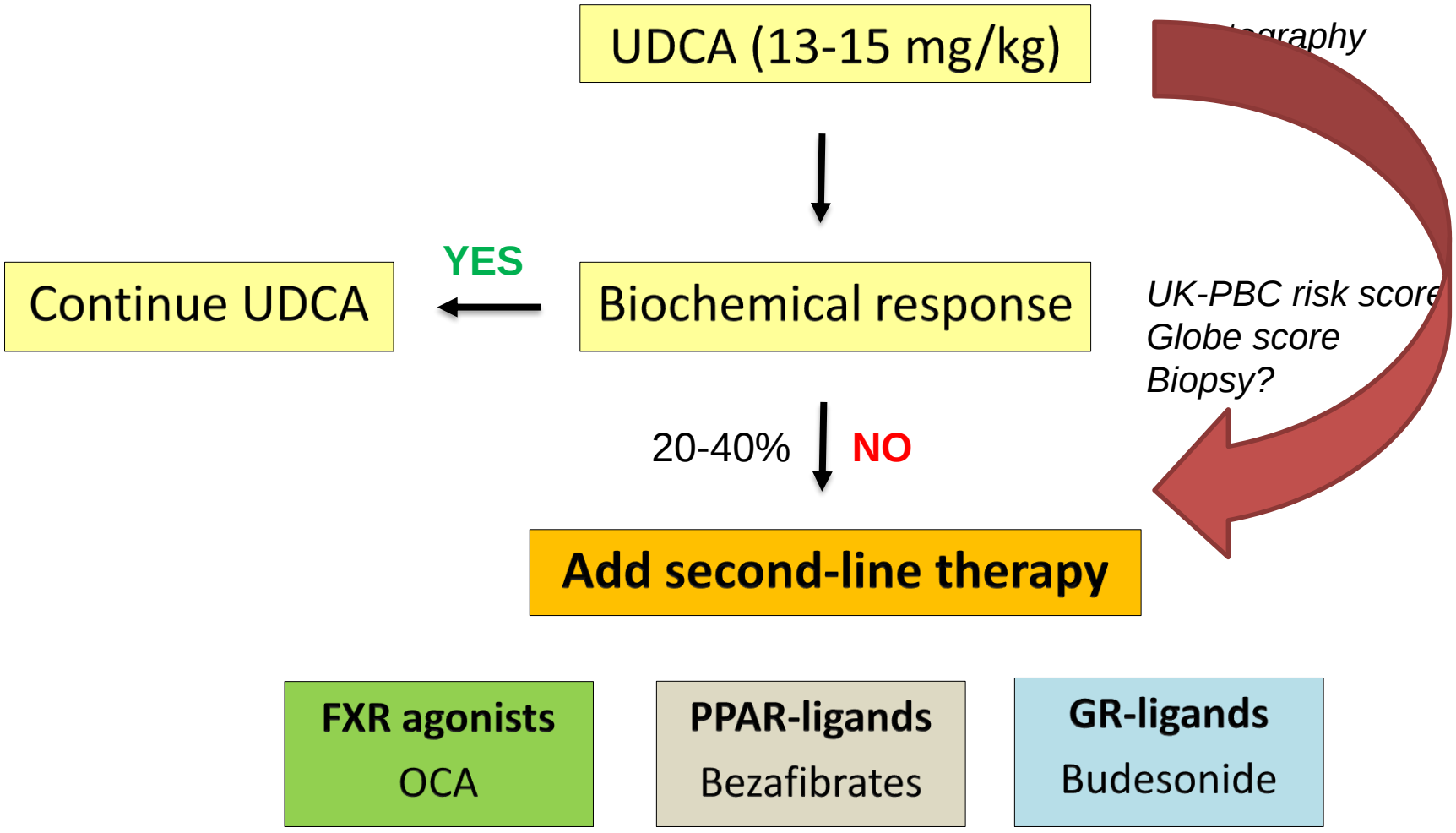


Carbone M, Nardi A et al. **Lancet Gastro & Hepatol 2018**

Response-guided approach



Baseline risk-guided approach



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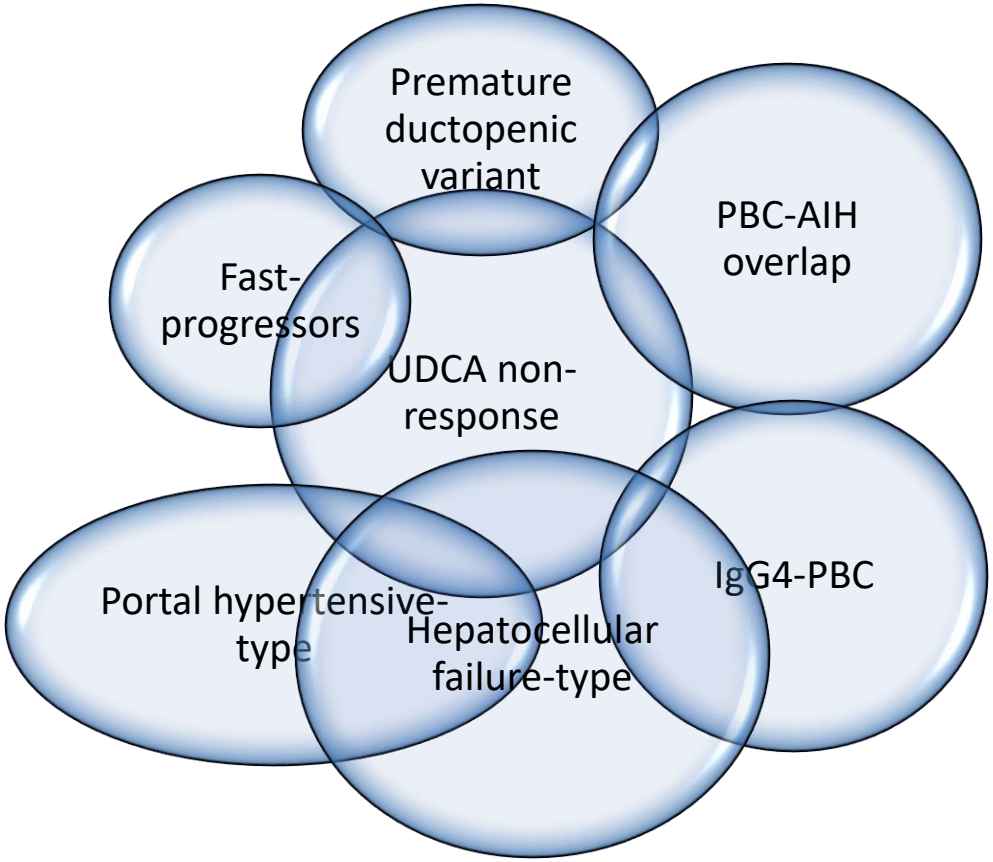
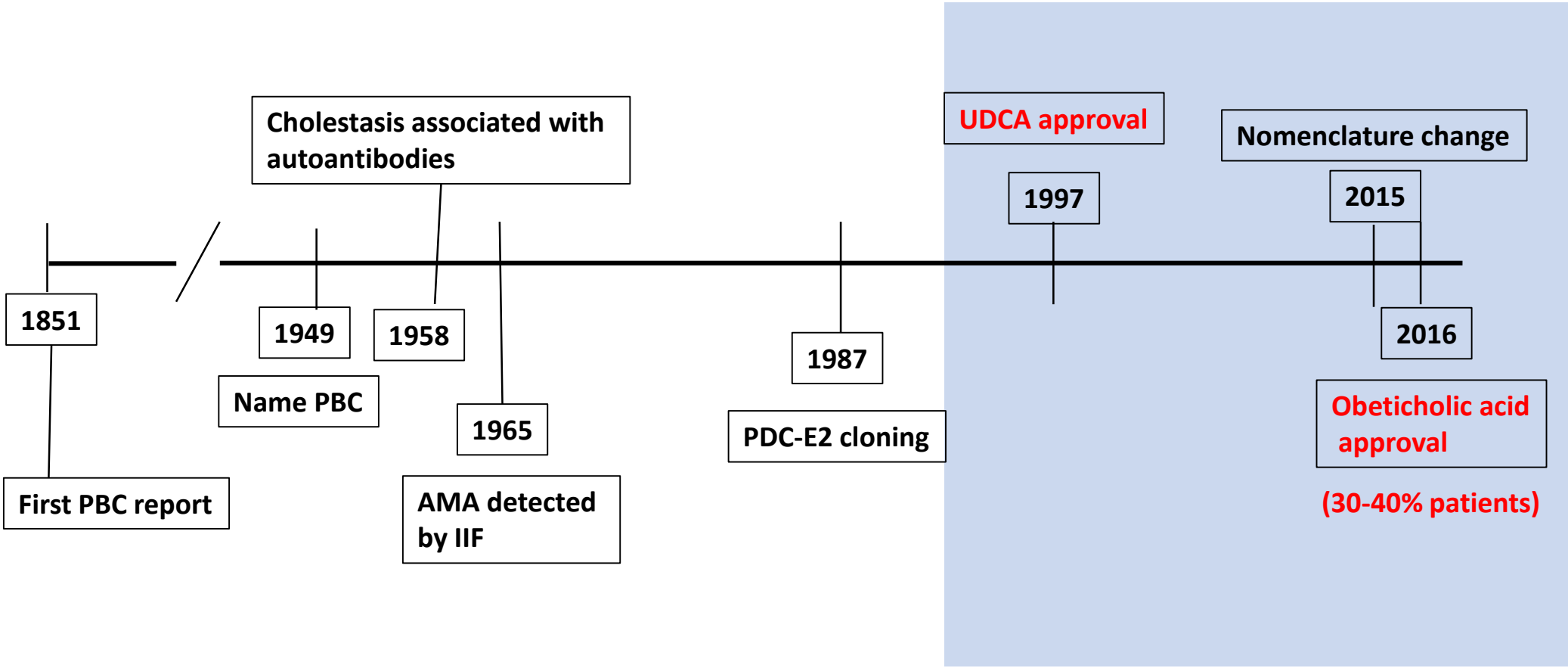


PBC story

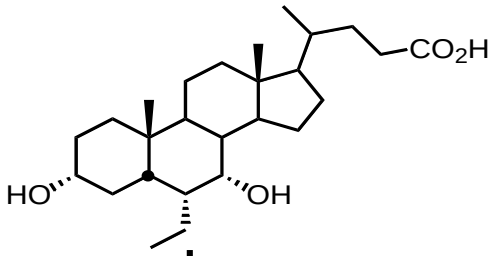


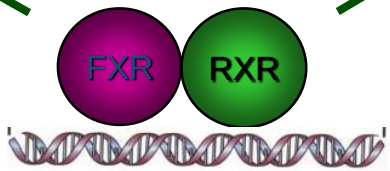
«Second PBC research meeting» J Autoimmun 2019;105:102328

Obeticholic acid

Bile Acid Metabolism

Controls bile acid biosynthesis, disposal and transport



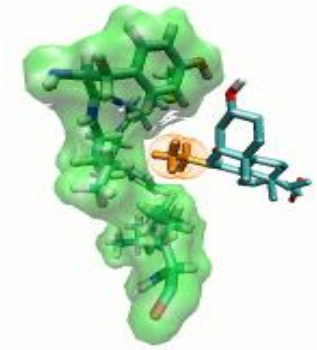


Lipid Metabolism

Downregulates hepatic fatty acid biosynthesis and VLDL formation

Inflammation & Fibrosis

Anti-inflammatory & anti-fibrotic effects in the liver, intestine and kidney

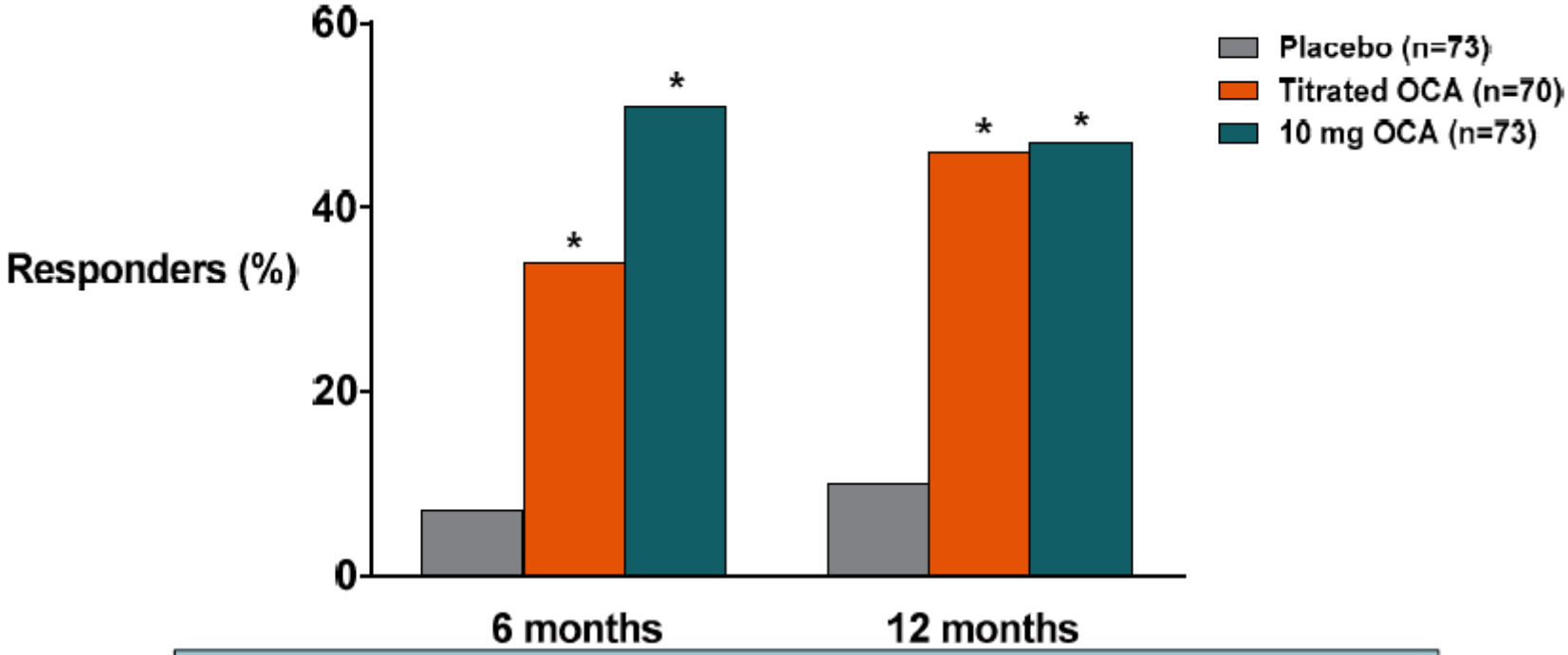


Carbohydrate Metabolism

Insulin signaling & sensitivity and hepatic gluconeogenesis

Obeticholic acid (Poise trial)

Biochemical response

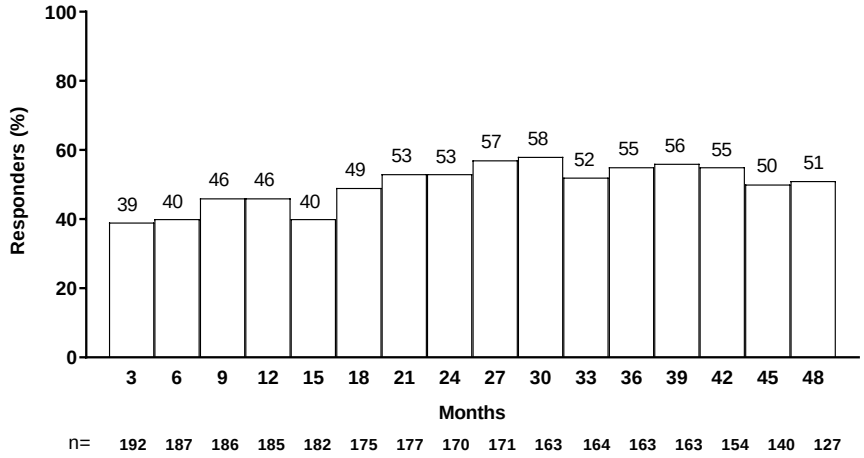


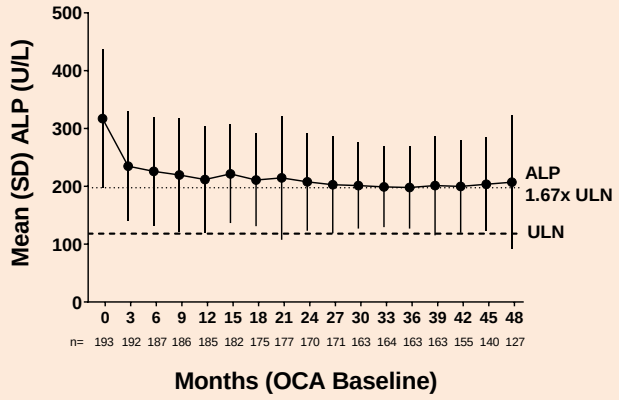
Primary Endpoint:
Proportion of subjects achieving ALP <1.67 x ULN with bilirubin ≤ ULN and ≥15% reduction in ALP

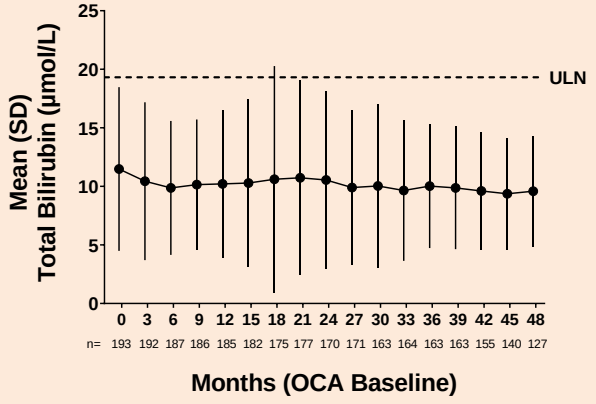
Nevens F et al. NEJM 2017

Obeticholic acid

Biochemical response (Open-Label Extension)







Trauner M et al, Lancet Gastroenterol Hepatol 2019

OCA adverse effects and caveats

Pruritus

- Common, dose related

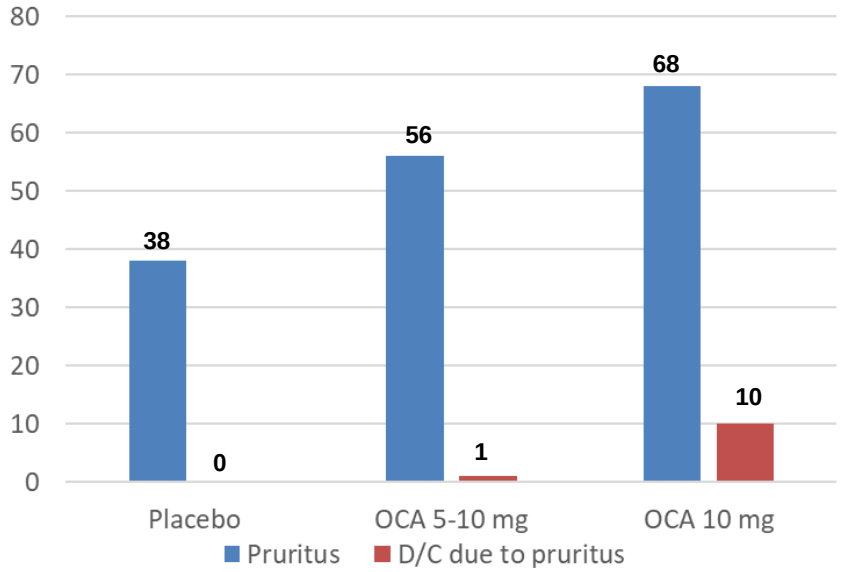
Cholesterol changes

- Decrease in HDL cholesterol

Potential risk of chronic increase in FGF19

- Increase risk of HCC in mouse models

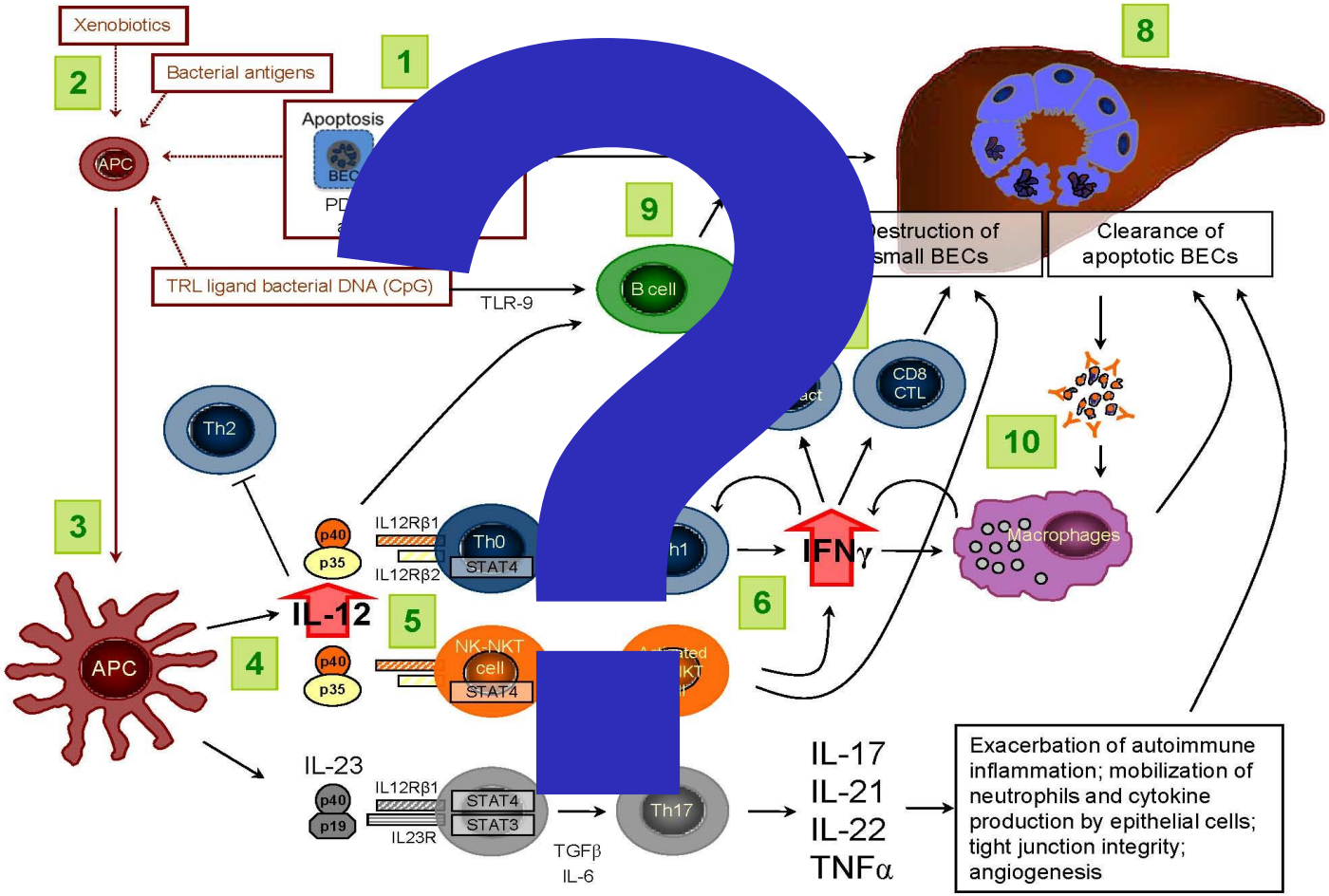
Warning in decompensated cirrhosis*



***FDA Post-Marketing Letter reporting 19 deaths, 8 with reported causes (7 cases of Child B or C cirrhosis and receiving 5 mg daily; 8 additional cases reported serious liver injury without death (3 cases with Child B or C cirrhosis and receiving 5 mg daily**

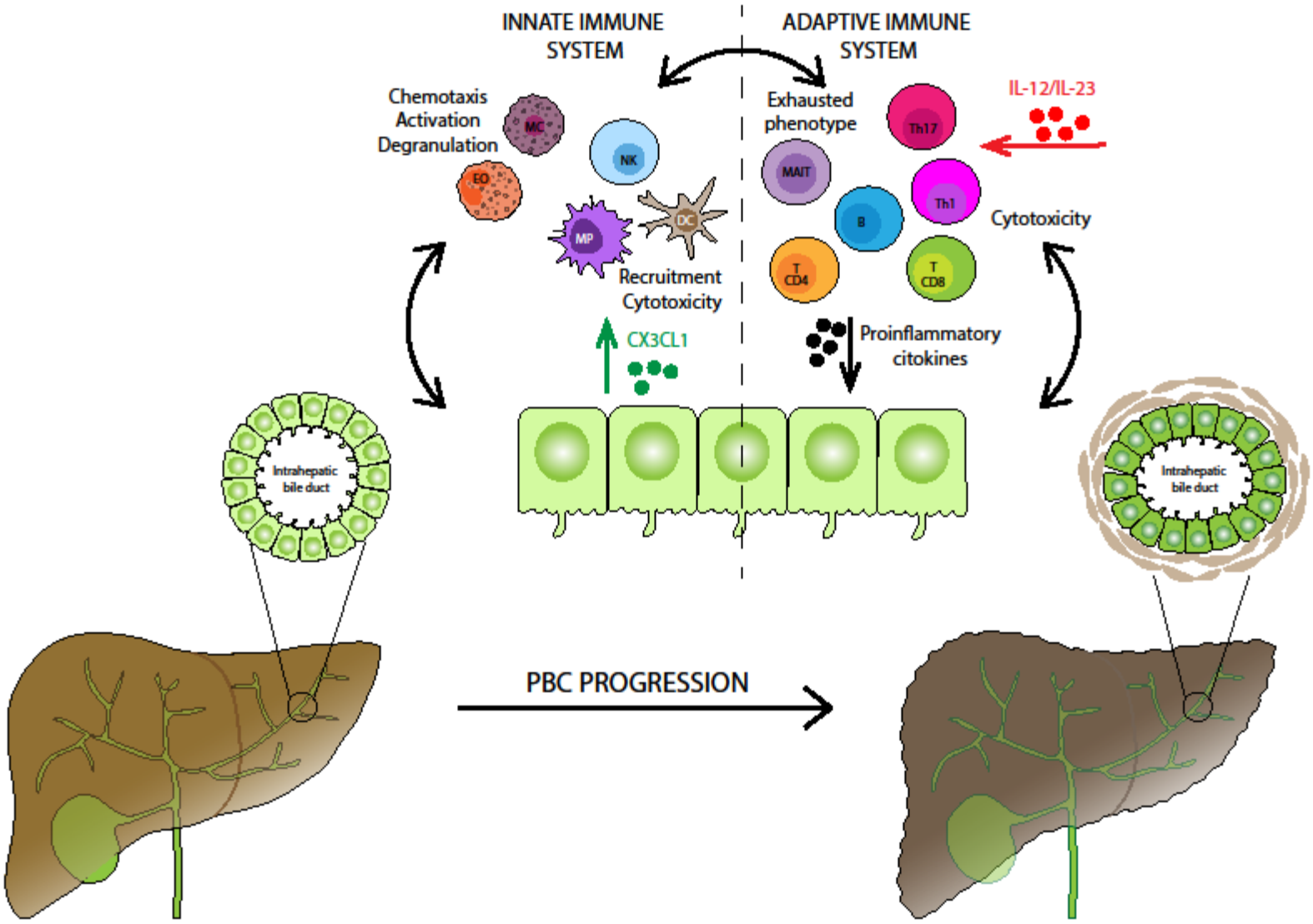
Nevens F et al. NEJM 2017

Pathogenesis of PBC

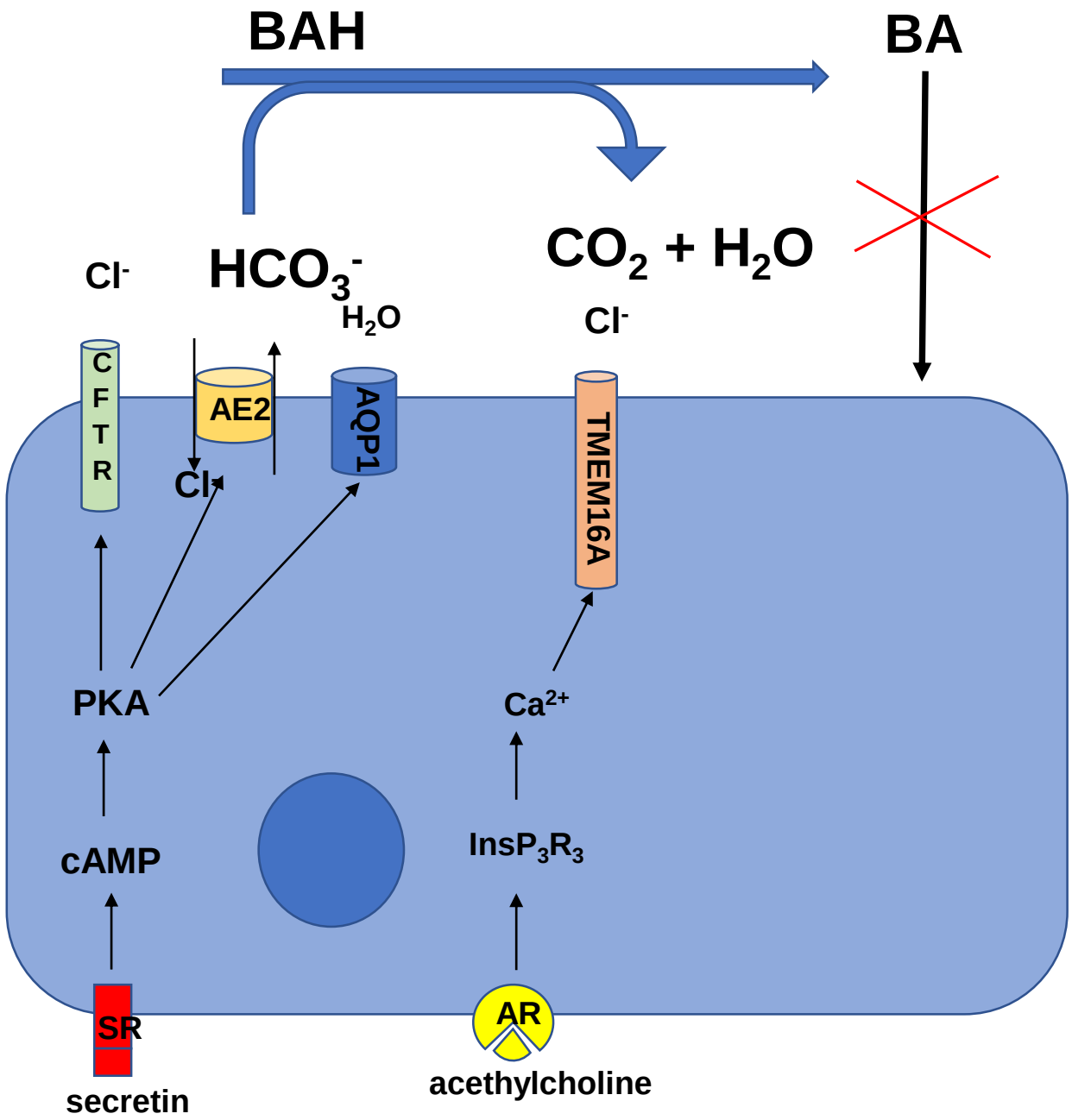


Lleo & Invernizzi. J Hepatol 2012

Immune system and cholangiocytes: a puzzling affair in PBC



Ronca V et al. Journal Leukocyte Biology 2020;108:659-671

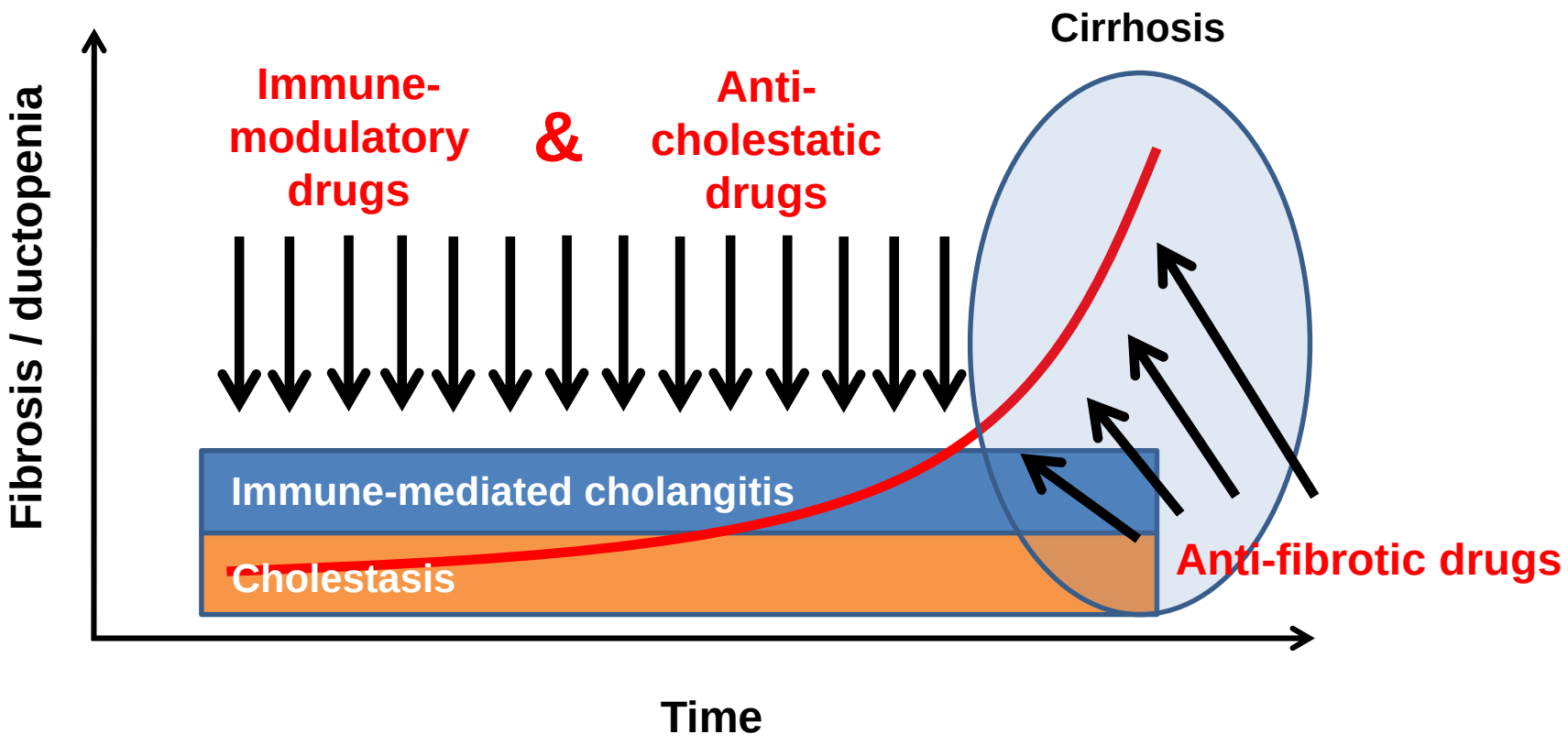


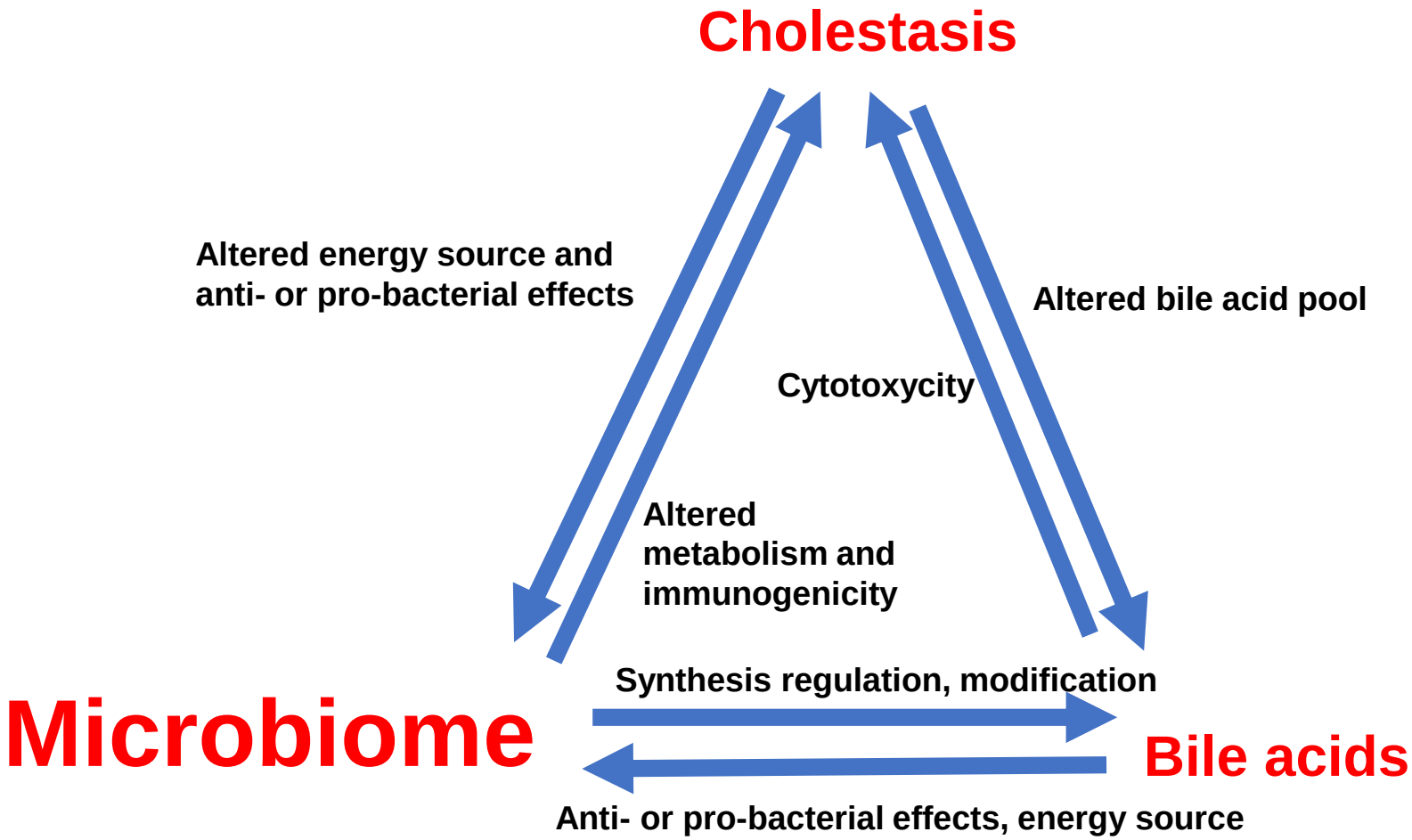
Schematic representation of cholangiocyte bicarbonate secretion regulated by secretin and acetylcholine.

Bicarbonate-rich bile leads to production of deprotonated bile acids, which cannot invade cells in the absence of transporters. In contrast, bicarbonate-poor bile leads to impaired deprotonation of the bile acids, which can invade cholangiocytes promoting apoptosis.

«Second PBC research meeting» J Autoimmun 2019;105:102328

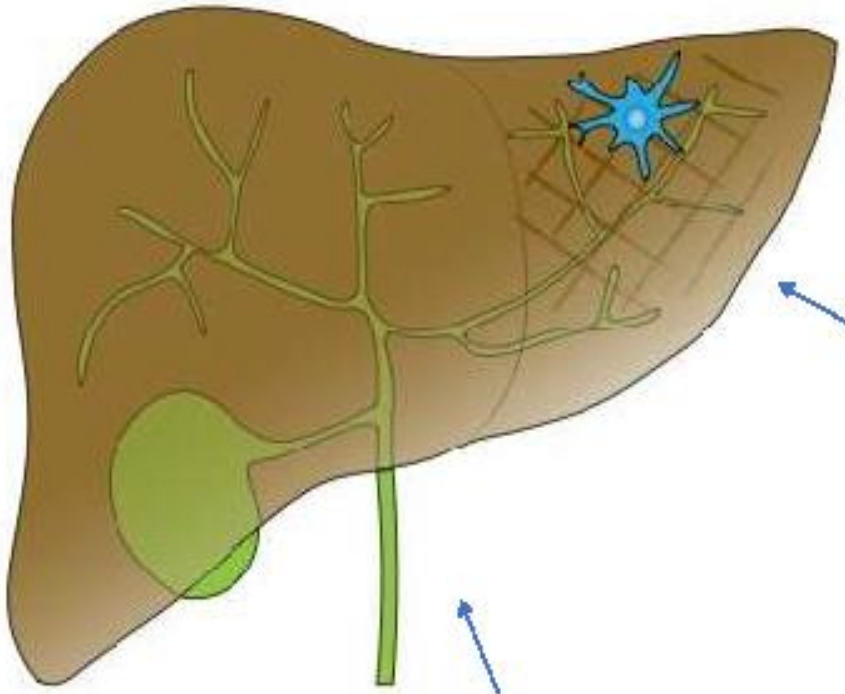
Immuno-pathogenesis of PBC / ideal therapies





«Second PBC research meeting» J Autoimmun 2019;105:102328

NOVEL DRUGS FOR PBC



Immunomodulants:
-JAK inhibitor: Baricitinib

Antifibrotic:
-Anti Nox 1-4: GKT831

Modulation of bile acids:
-FXR agonists (Tropifexor, EDP-305)
-FGF19 analogues (NGM282)
-PPAR agonists (Bezafibrate, Seladelpar, Elafibranor)

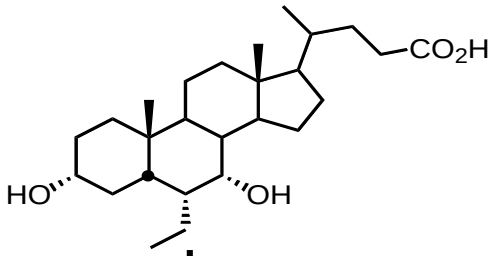
Gerussi A et al. Ann Hepatol 2020;19:5-16.

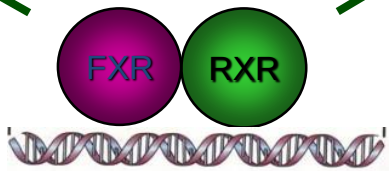
FXR agonists

Obeticholic acid

Bile Acid Metabolism

Controls bile acid biosynthesis, disposal and transport





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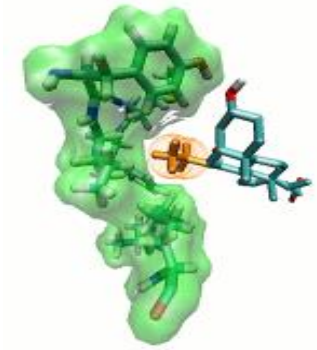


Carbohydrate Metabolism

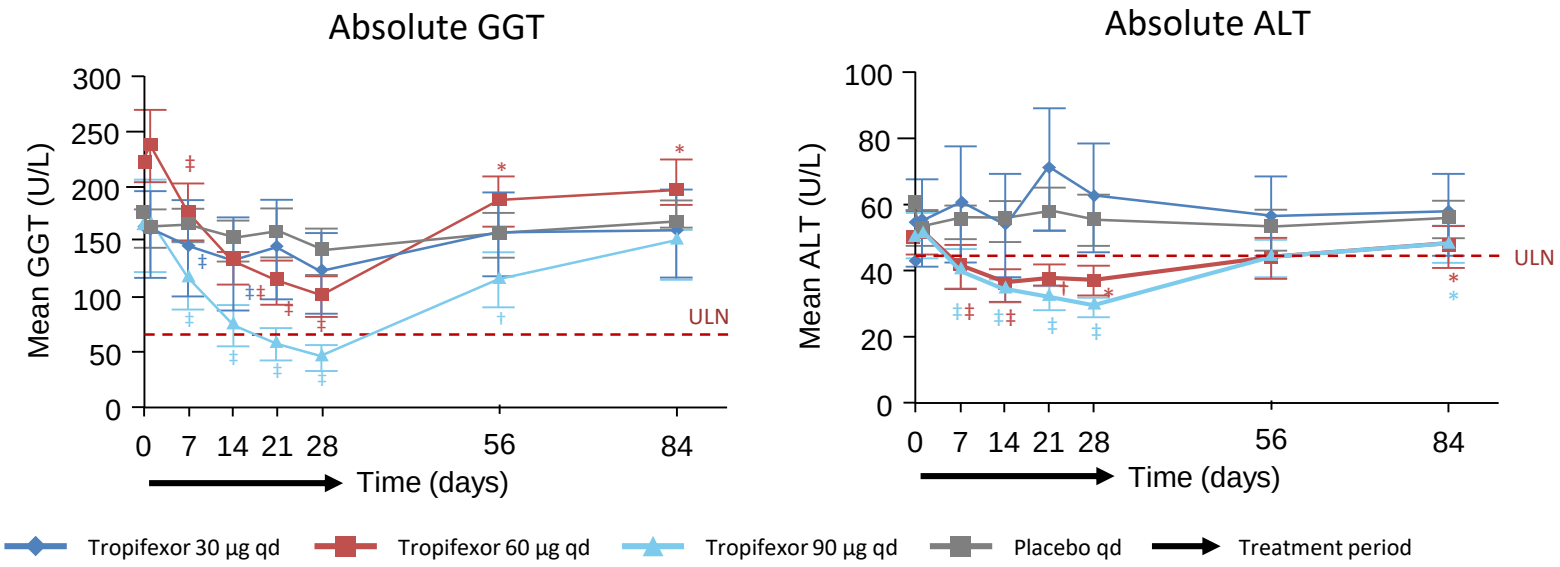
Insulin signaling & sensitivity and hepatic gluconeogenesis

Tropifexor

Non-bile acid FXR agonist

Phase 2 study (CLJN452X2201)

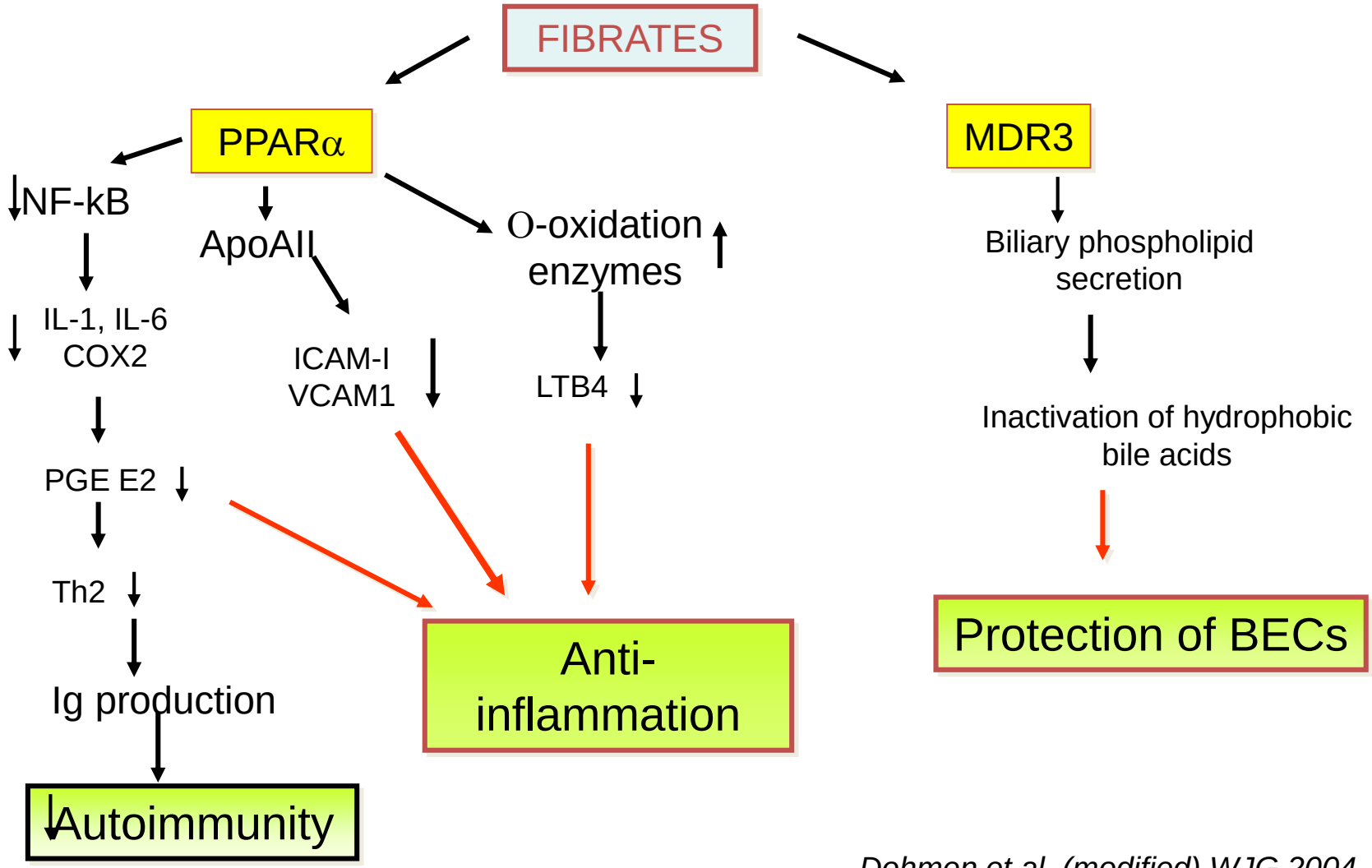
- Tropifexor improved cholestasis and fibrosis in preclinical trials
- Treatment with 30–90 µg tropifexor for 4 weeks
  - Significant dose-dependent reduction in GGT (primary endpoint), AP, ALT and AST over time
  - No discontinuations due to itch and no incidence of severe itch



*p<0.05, †p<0.01, ‡p<0.001 vs. placebo
Schramm C, et al. ILC 2018, #LBO-007

PPAR agonists

Use of PPAR agonists (fibrates) in PBC

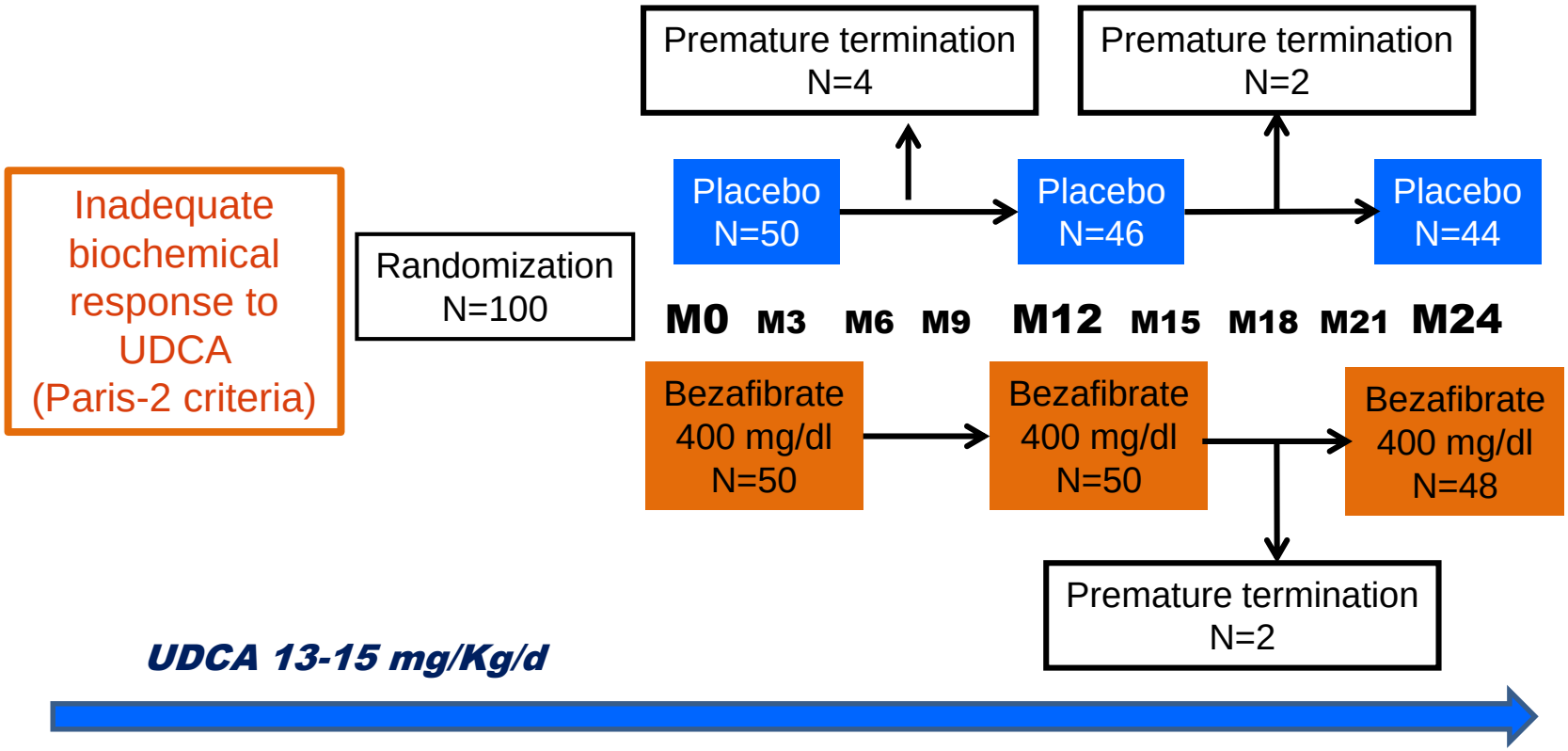


Dohmen et al. (modified) WJG 2004

	Author	Year	Country	Patients	Months FU	Improvement in
BEZA	Nakai	2000	Japan	10	12	ALP,ALT, GGT, IgM
	Kunhara	2000	Japan	12	12	ALP, ALT, GGT, IgM
	Kanda	2002	Japan	11	6	ALP
	Akbar	2005	Japan	10	12	ALP, ALT, GGT, IgM, Chol
	Kita	2006	Japan	22	6	ALP,GGT
	Hazzan	2010	Israel	8	4-12	ALP, GGT
	Honda	2013	Japan	19	3	ALP, GGT, ALT, Chol, TG, IgM
	Lens	2014	Spain	30	12	ALP, GGT, ALT, Chol TG
	Reig	2015	Spain	48	38	ALP, GGT, ALT, Chol, TG, pruritus
	Hosonuma	2015	Japan	12	96	ALP, Mayo Risk Score
FENO	Ohira	2002	Japan	7	6	ALP, GGT IgM
	Walker	2009	UK	16	3-4	ALP, IgM
	Liberopoulus	2010	Greece	6	2	ALP, GGT
	Levy	2011	USA	20	12	ALP. AST, IgM, IL-1, IL-6
	Cheung	2015	Canada	46	11	ALP
	Hegade	2016	UK	23	21	ALP, Not UK-PBC score

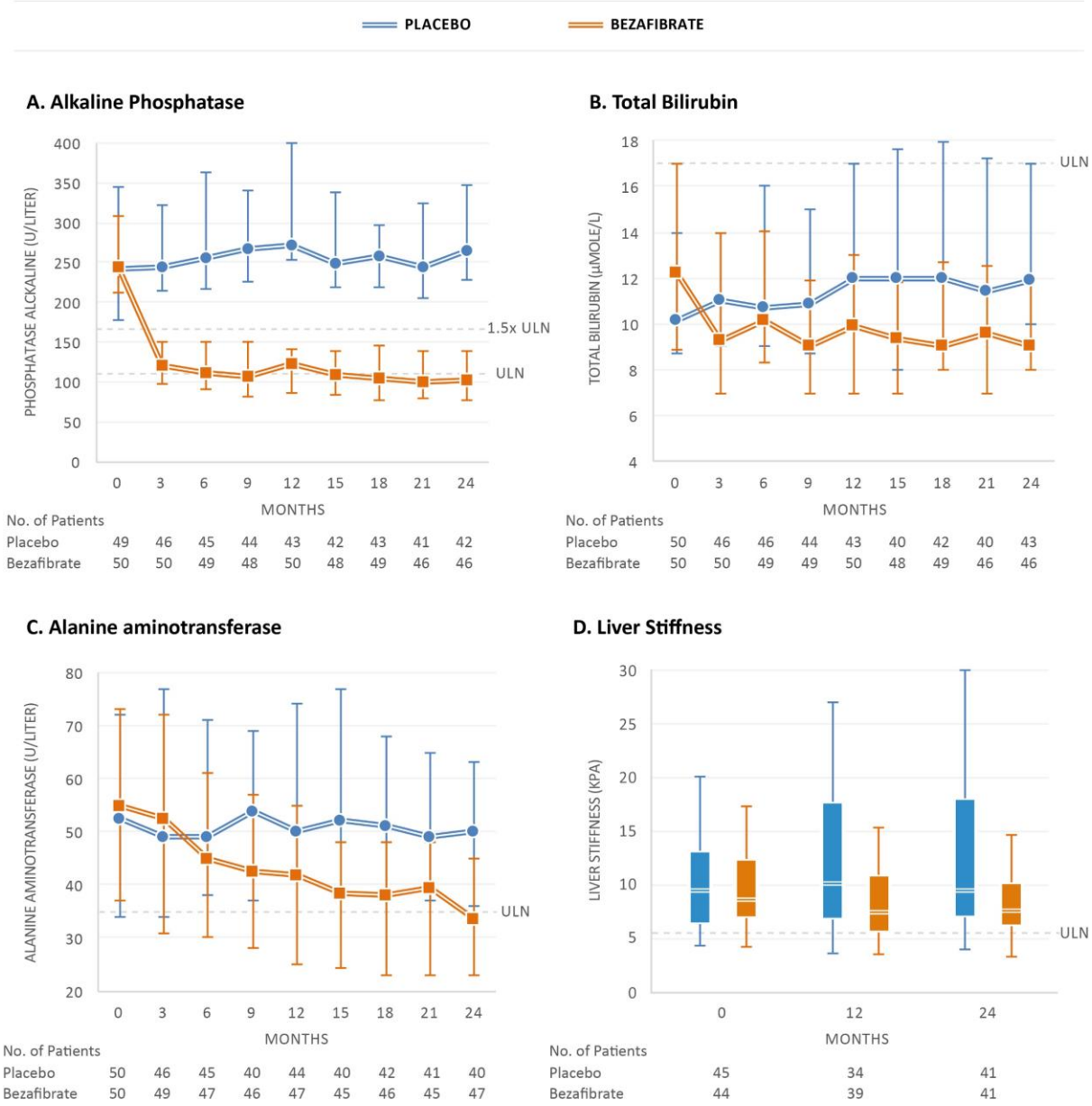
Bezofibrate (Bezurso trial)

2-year multicenter, double-blind, randomized, placebo-controlled trial of bezafibrate (400 mg/d) + UDCA



Corpechot et al ***NEJM 2018***

Figure 2.



Corpechot et al ***NEJM 2018***

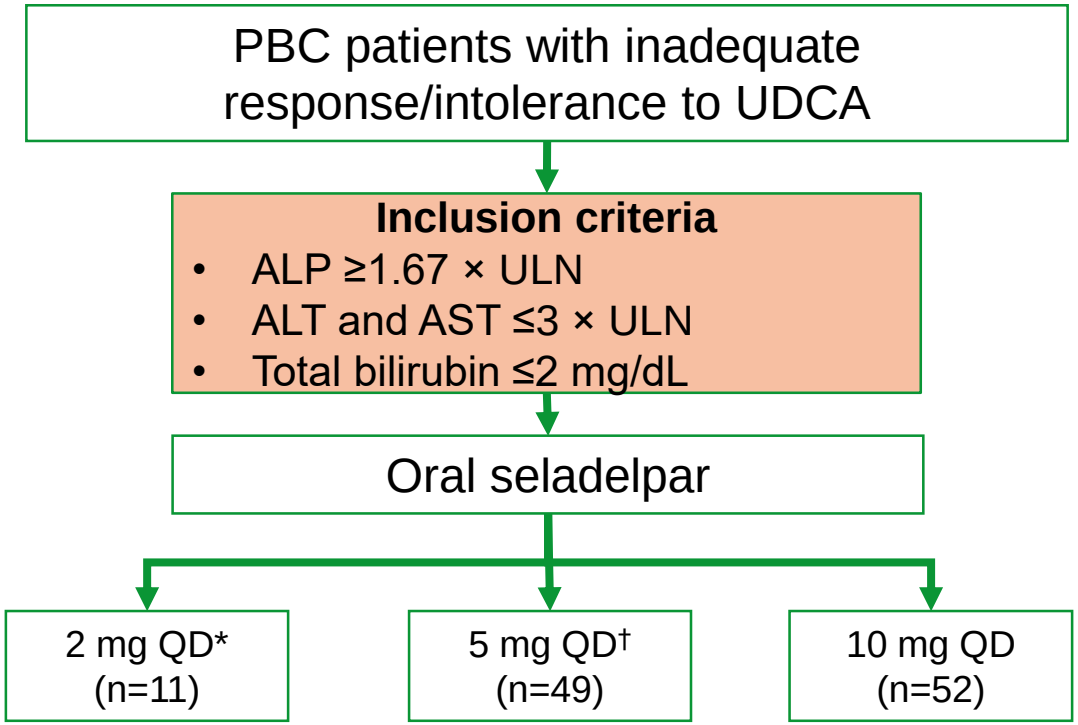
Seladelpar

Selective PPAR-δ agonist

Final results of an international phase 2 study

- Seladelpar is a potent PPARδ agonist that improved cholestasis markers in PBC

- To evaluate the efficacy, safety, and tolerability of seladelpar during 1-year, Phase 2, open-label, uncontrolled dose-finding study in PBC patients with an inadequate response or intolerance to UDCA
- **Primary endpoint**: % change in ALP at 1 year
- **Composite endpoint**: ALP <1.67 × ULN; ≥15% decrease in ALP; total bilirubin ≤ULN



112/119 patients evaluated for efficacy

At 1 year, no patients remained on 2 mg*

After 1 year, patients could enter a long-term study

†After 12 weeks, doses could be increased up to 10 mg based on biochemical response

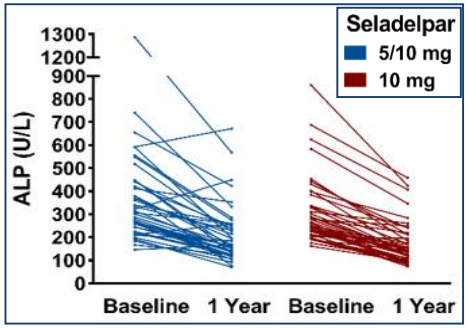
Cynthia Levy et al FRI133 EASL 2020

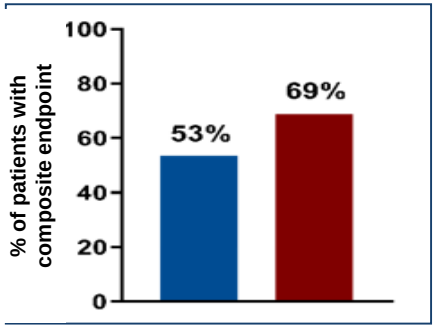
Seladelpar

Selective PPAR-δ agonist

Final results of an international phase 2 study

Mean (SD) values	5/10 mg (n=49)	10 mg (n=52)
% female	95	
Age, years	57	
Duration of PBC, years	10 (7)	
UDCA dose, mg/kg/day	15 (4)	
Baseline laboratory values		
ALP, U/L	353	301
Total bilirubin, mg/dL	0.76	0.83
GGT, U/L	244	239
ALT, U/L	46	46





Laboratory parameters at 1 year
ALP: ↓ 41% in 5/10 mg and ↓ 45% in 10 mg; total bilirubin: stable; GGT: ↓ 34% in 5/10 mg and ↓ 32% in 10 mg groups;ALT: ↓ 31% in both groups

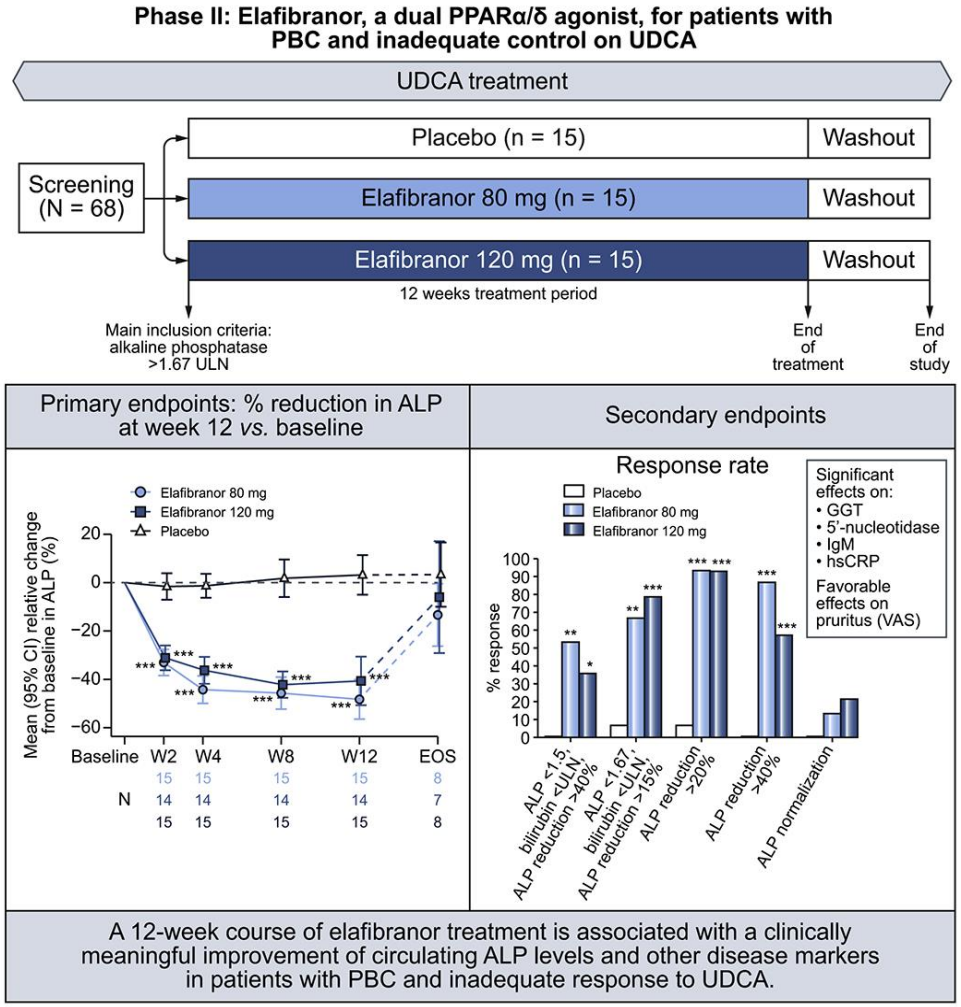
- ALP normalized in 14% in 5/10 mg and 33% in 10 mg groups
- 93% with moderate to severe pruritus in the 10 mg group experienced improvement in itch (VAS decrease ≥20 mm). SAEs in 14 patients were unrelated to the drug

Seladelpar resulted in a substantial and sustained biochemical response with a good tolerability and safety profile

Cynthia Levy et al FRI133 EASL 2020

Selective PPAR α/δ agonist
Elafibranor

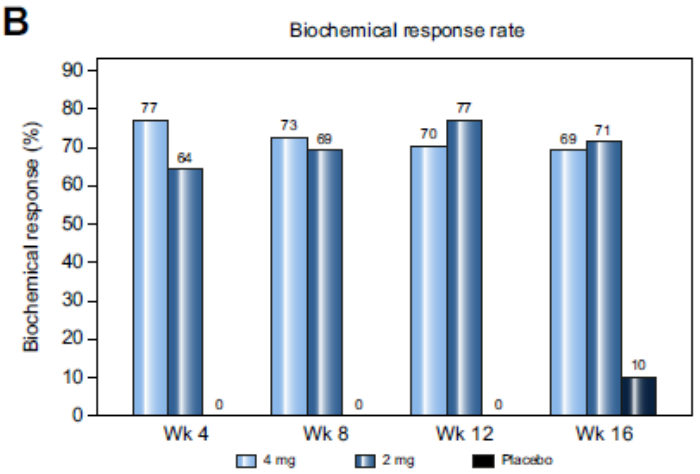
A randomized placebo-controlled trial of elafibranor in patients with primary biliary cholangitis and incomplete response to UDCA



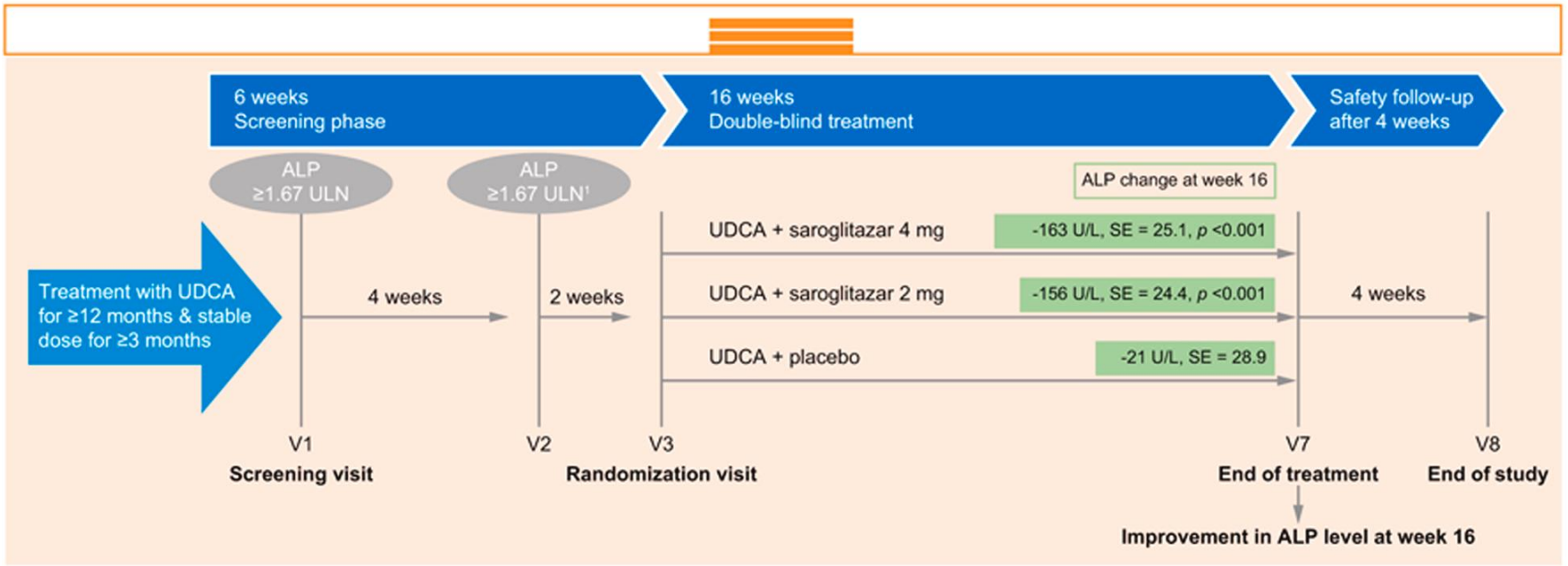
A 12-week course of elafibranor treatment is associated with a clinically meaningful improvement of circulating ALP levels and other disease markers in patients with PBC and inadequate response to UDCA.

Schattenberg JM et al.
J Hepatol. 2021 Jun;74(6):1344-1354.

Selective dual PPAR-α/γ agonist
Saroglitazar



Study ID	Phase	Country	Patient population	Comparator	No. patients[3]
EPICS-I	Phase II	USA	Primary biliary cholangitis	Placebo	37



1. <30% variance between the levels from Visit 1 to Visit 2
2. patients with <30% variance in AST, ALT, TB & INR from Visit 1 to 2 to proceed further
3. Actual

J Hepatol. 2021

Selective dual PPAR-α/γ agonist
Saroglitazar

- It is a potent and predominant PPARα agonist with moderate PPARγ agonistic activity

9 Dec 2020

FDA has granted FAST TRACK DESINATION to SAROGLITIZAR for PBC

Soon international phase 3 study

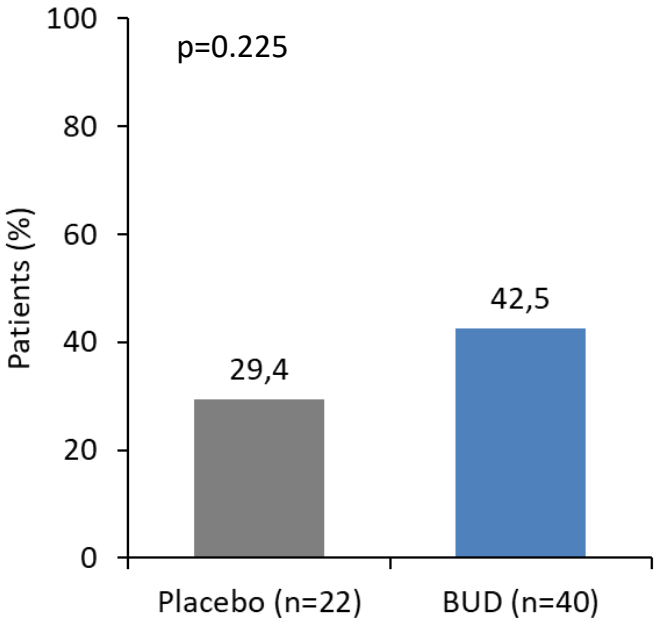
GR-Ligand

Budesonide

Phase 3 trial

- Randomized, double-blind, placebo-controlled trial (NCT00746486)
- Primary objective: efficacy, safety and tolerability of UDCA + BUD vs. UDCA + placebo
- 62 patients randomized and treated (ITT population) with 36 months of treatment with UDCA (12–16 mg/kg body weight/day) with or without BUD (3 mg tid*)

Improved liver histology†



Improved liver function

Mean change from baseline (SD)	Placebo (n=40)	BUD (n=22)
ALT (U/L)	–0.16 (46.8)	–12.1 (30.2)
AP (U/L)	–8.9 (176.94)	–94.5 (166.3)
Total bilirubin (mg/dL)	0.59 (2.2)	–0.02 (0.4)

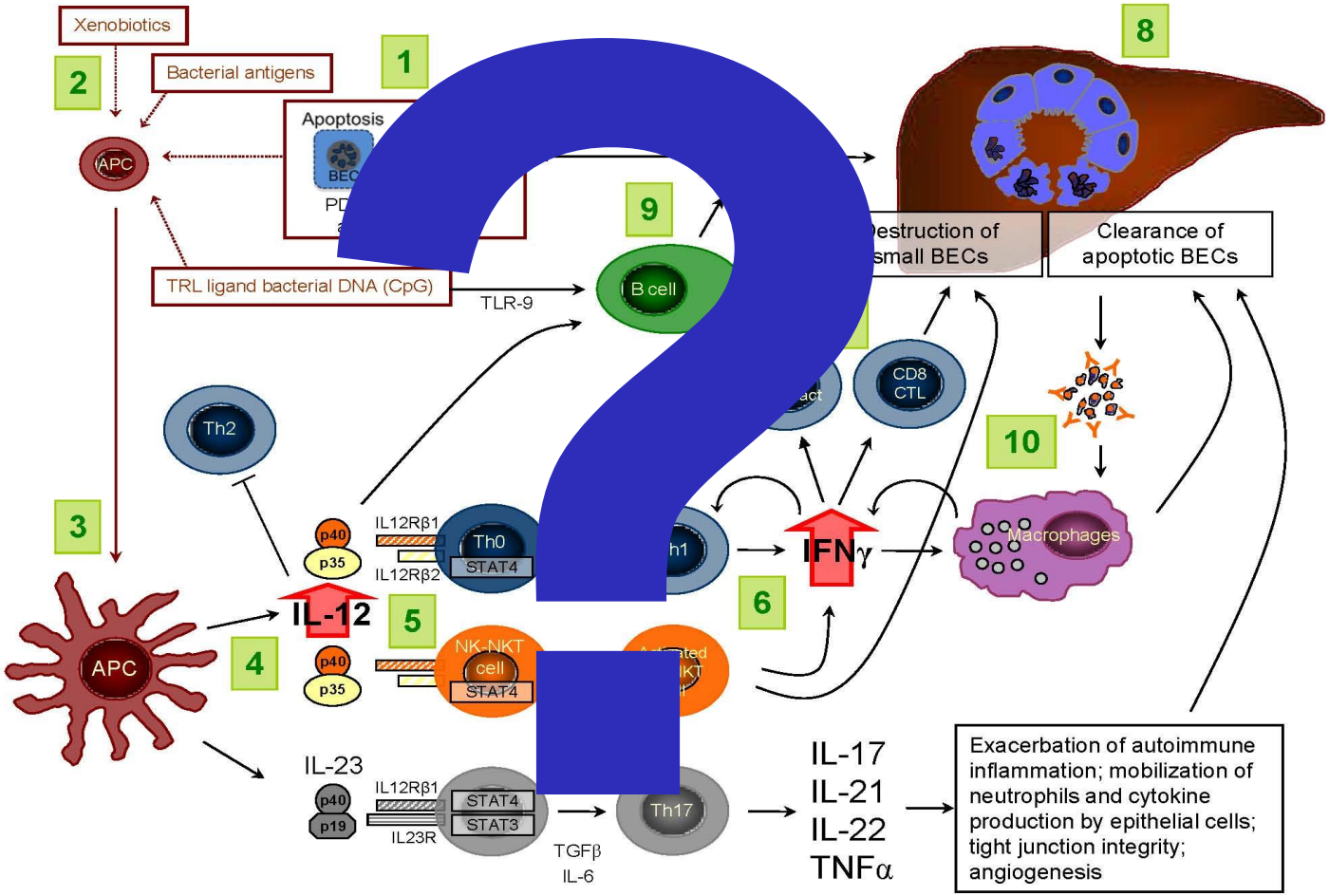
- Pruritus: 15% (6/40) in BUD group and 31.8% in placebo group (7/22)
- SAEs: 10 in BUD group and 7 in placebo group

CONCLUSION: Budesonide add-on therapy in patients with PBC, and insufficient response to UDCA, did not associate with improvement in liver histology; improvements in biochemical markers of disease activity were demonstrated in secondary analyses.

J Hepatol 2020

Biologics

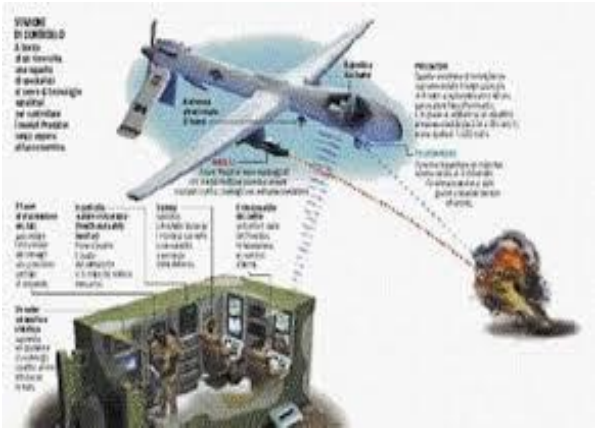
Pathogenesis of PBC



Lleo & Invernizzi. J Hepatol 2012

Precision drugs (weapons)

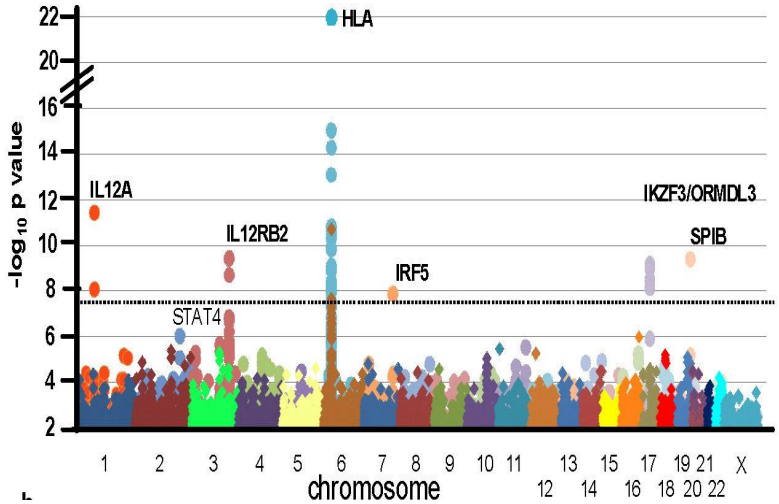
PRECISION DRUGS



- FXR
- PPAR
- ASBT
- Anti-IL 12
- Anti-CXCL10
- Anti-CD20
- Anti-CD40L
- Anti-NOX 1 & 4
- JAK inhibitor

Anti-IL12 for PBC

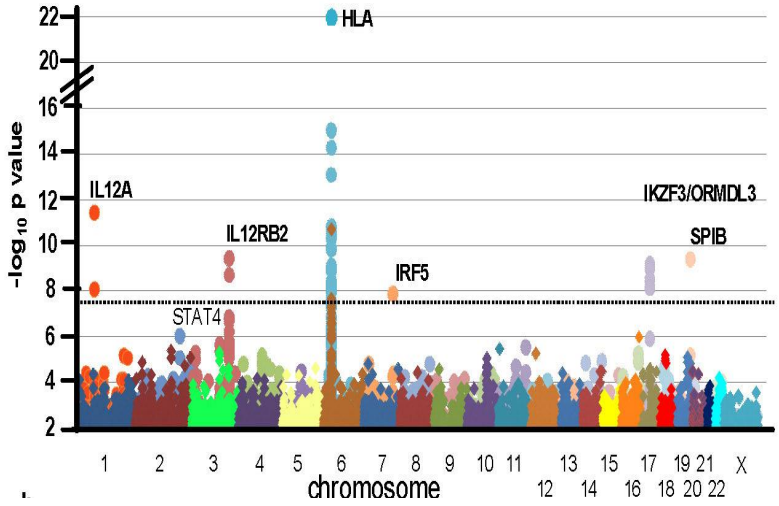
IL12
Genetic defect



Liu, Invernizzi, et al. Nature Genetics 2010

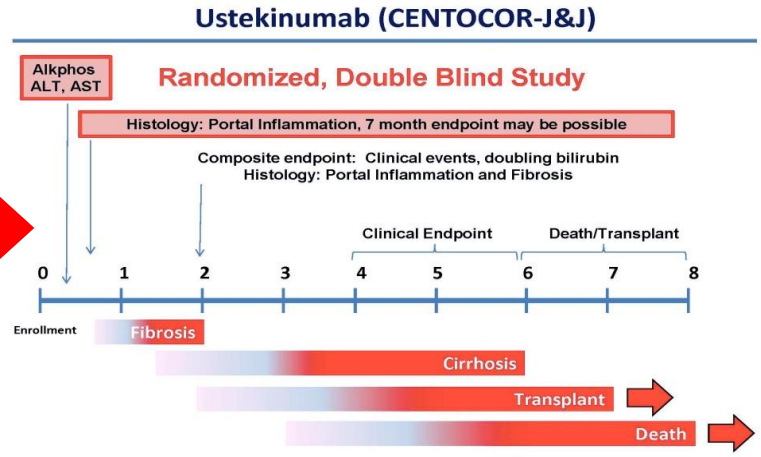
Anti-IL12 for PBC

IL12
Genetic defect



Liu, Invernizzi, et al. Nature Genetics 2010

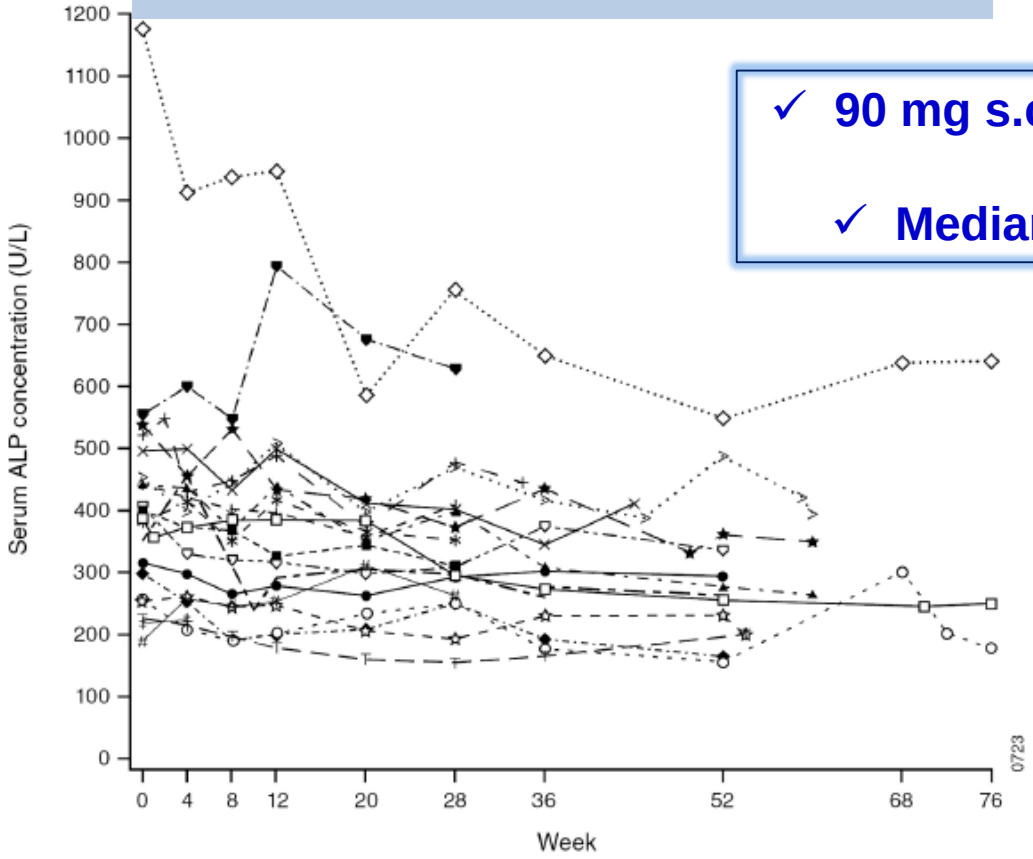
Anti-IL12
Clinical trial



Hirschfield, et al. Hepatology 2016

Anti-IL 12 for PBC

Reduction in ALP

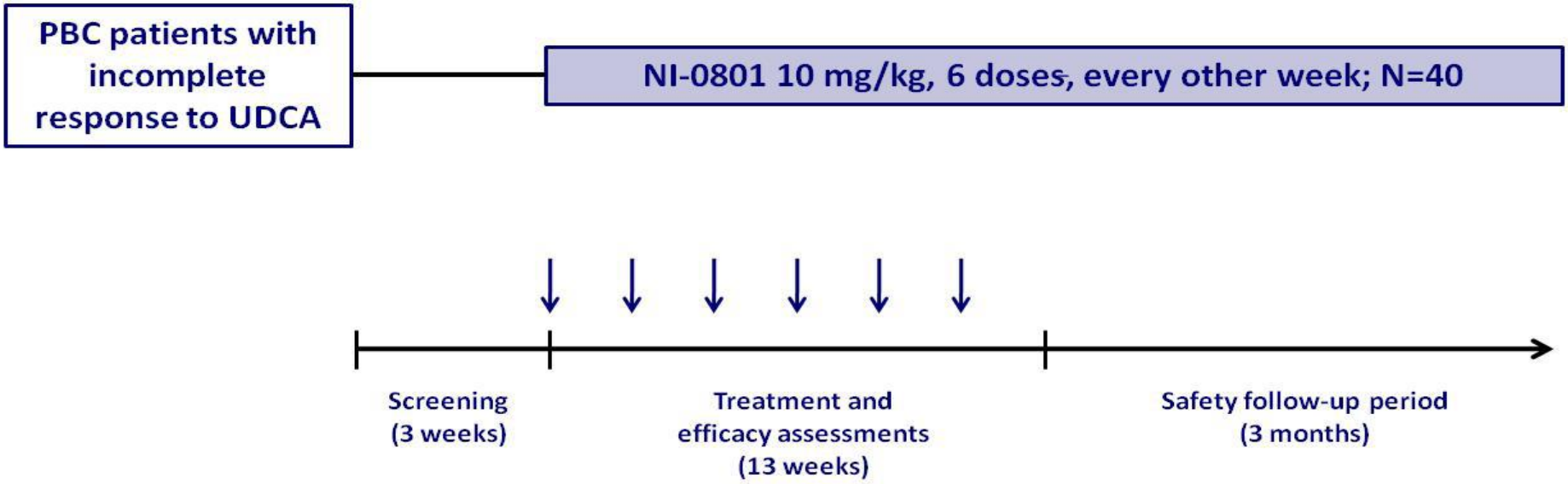


- ✓ 90 mg s.c. weeks 0,4, and 8 to 20 wks
- ✓ Median ALP 12% at 6 months

Hirschfield GH et al, Hepatology 2015

Anti-CXCL10 for PBC

PIANO Study design

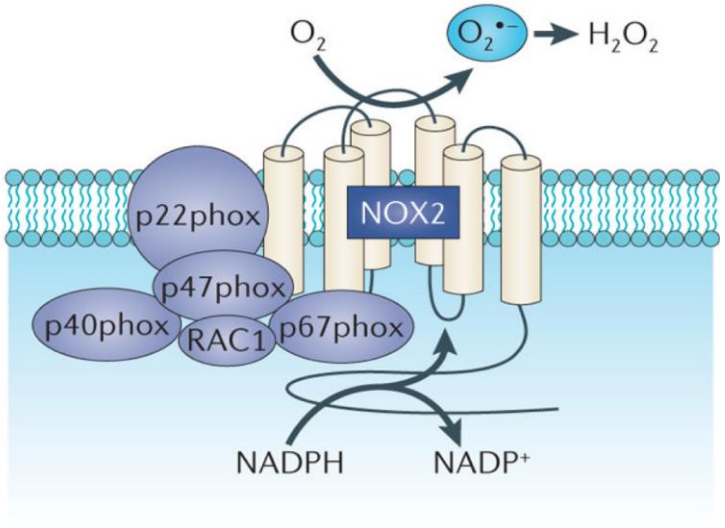


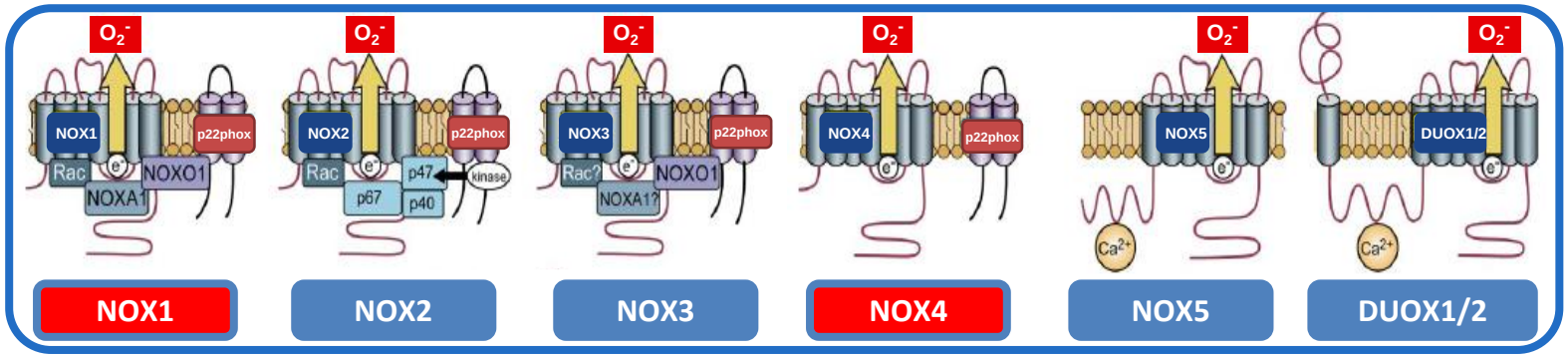
An open label single arm study to investigate the safety and efficacy of multiple administrations of NI-0801, **a fully human anti-CXCL10 monoclonal antibody** in PBC patients with an incomplete response to UDCA.

Invernizzi et al Hep Commun 2018;2:492-503

NADPH oxidases (NOX) inhibitors

Setanaxib

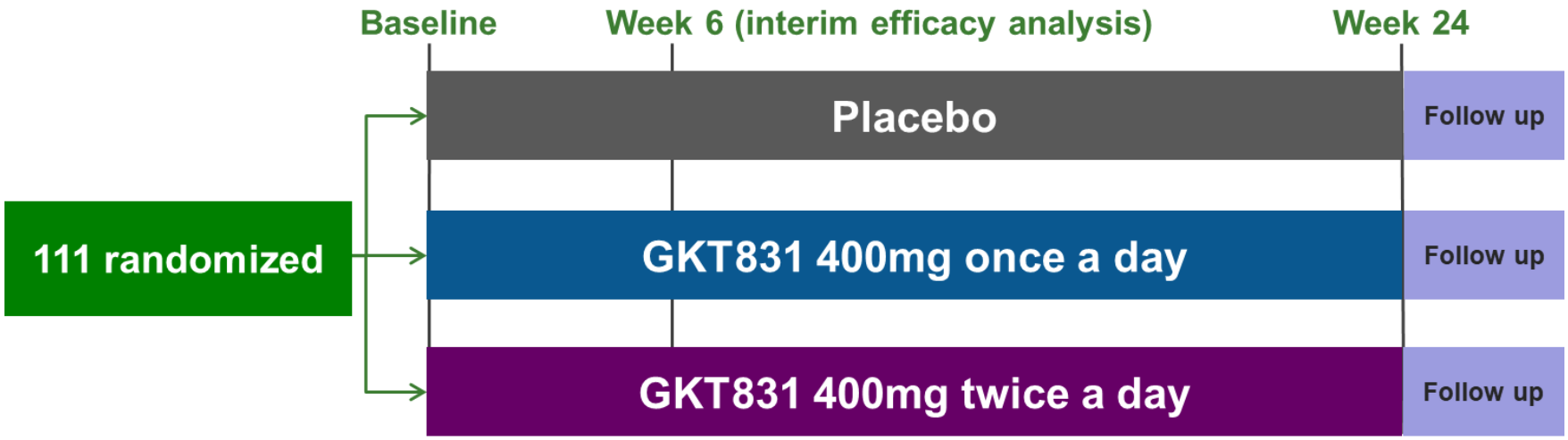




NADPH oxidases (NOX) inhibitors in PBC

Setanaxib

Trial design (phase 2)



ClinicalTrials.gov Identifier: NCT03226067

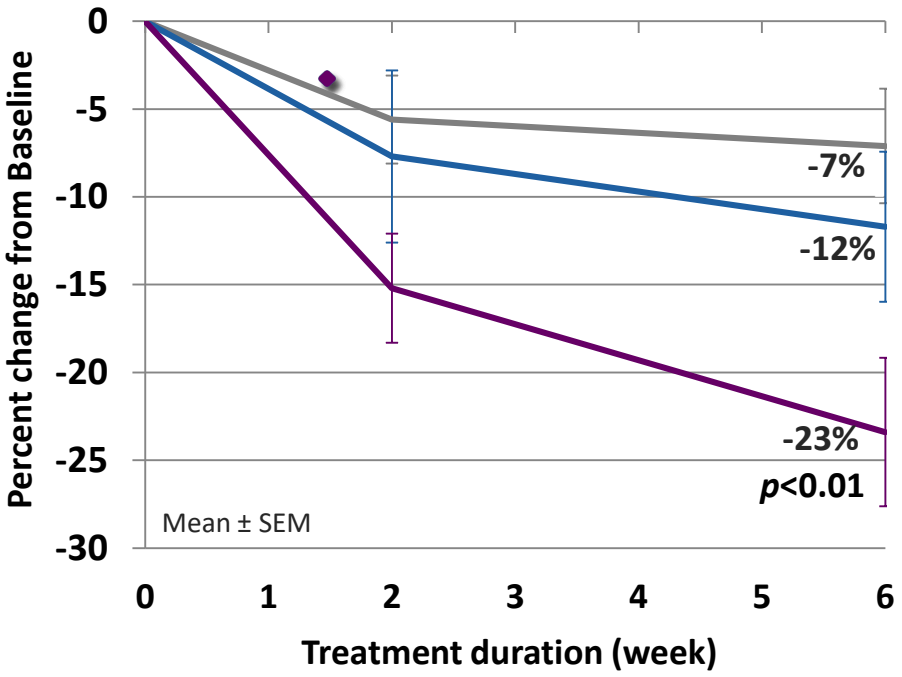
AASLD 2019 (Poster 1283)

NADPH oxidases (NOX) inhibitors in PBC

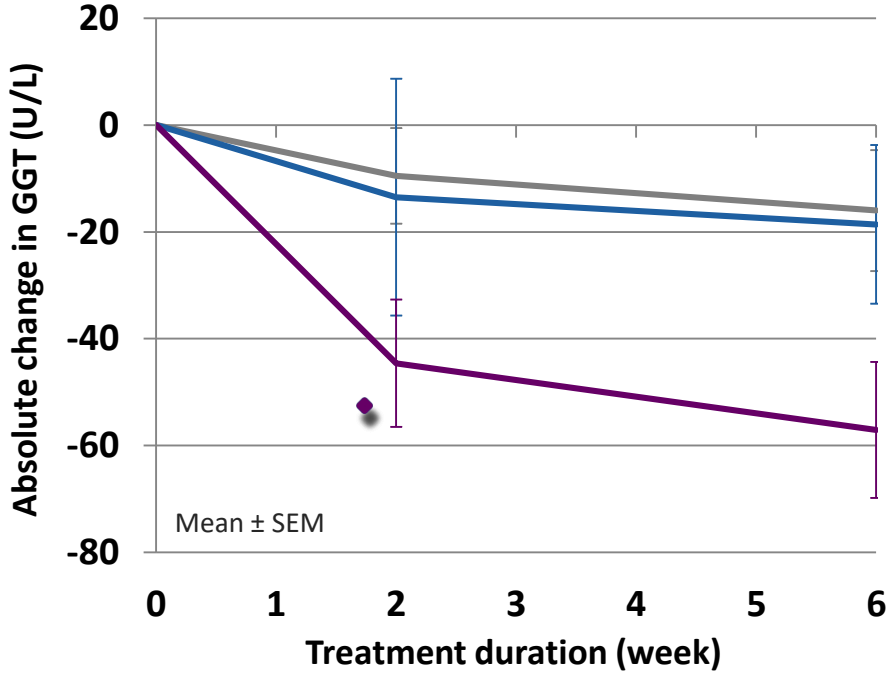
Setanaxib

Reductions in GGT at Week 6

Primary endpoint: percent change in GGT



Absolute change in GGT over time

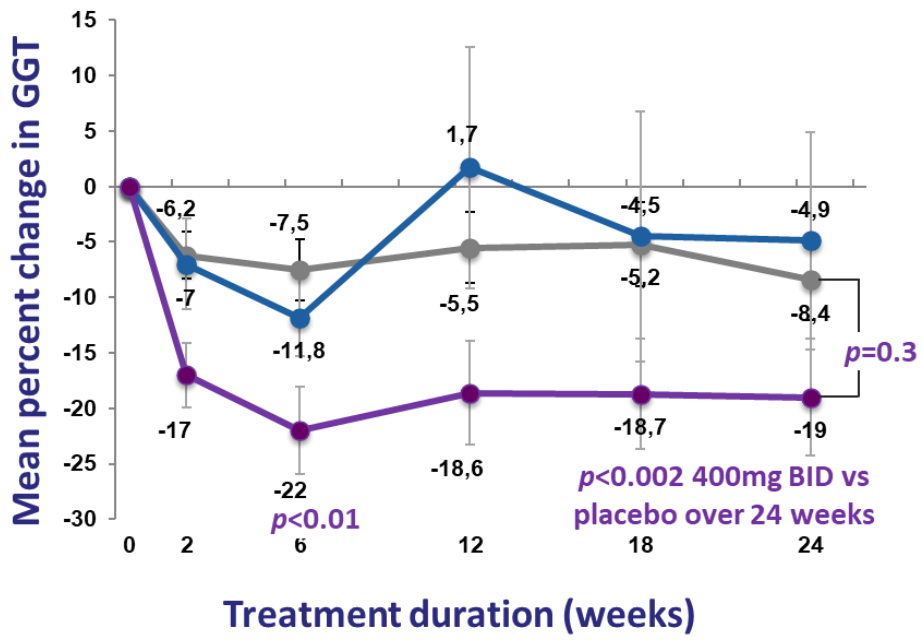


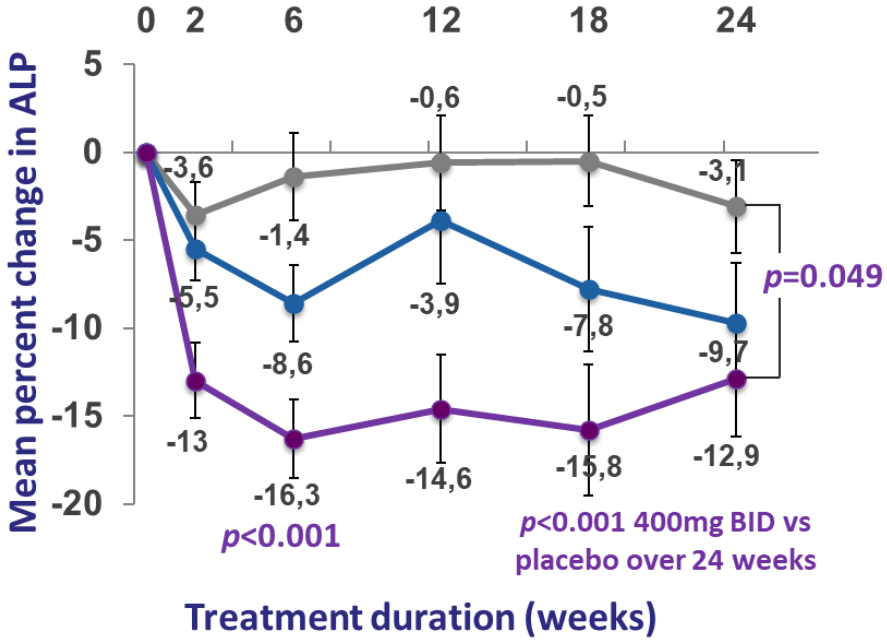
Placebo (n=31) GKT831 400mg OD (n=31) GKT831 400mg BID (n=30)

NADPH oxidases (NOX) inhibitors in PBC

Setanaxib

Reductions in GGT and ALP at Week 24





ClinicalTrials.gov Identifier: NCT03226067

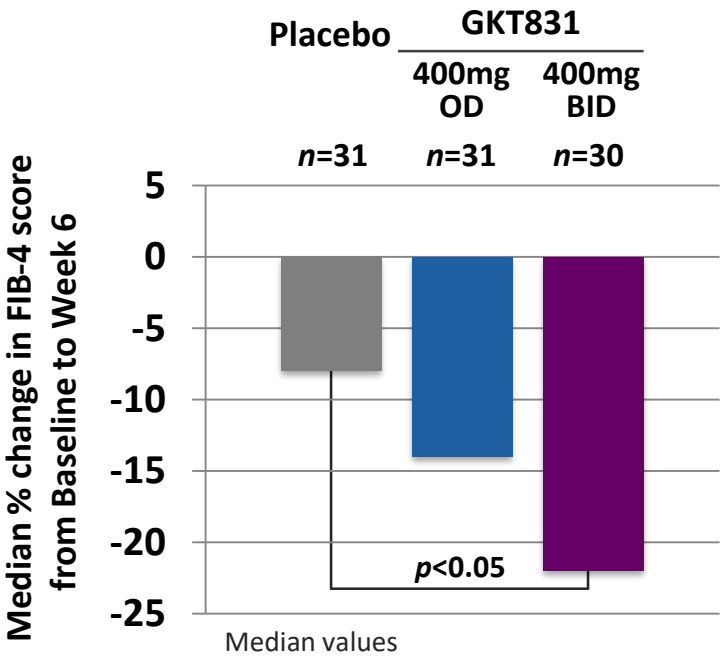
Manuscript submitted

NADPH oxidases (NOX) inhibitors in PBC

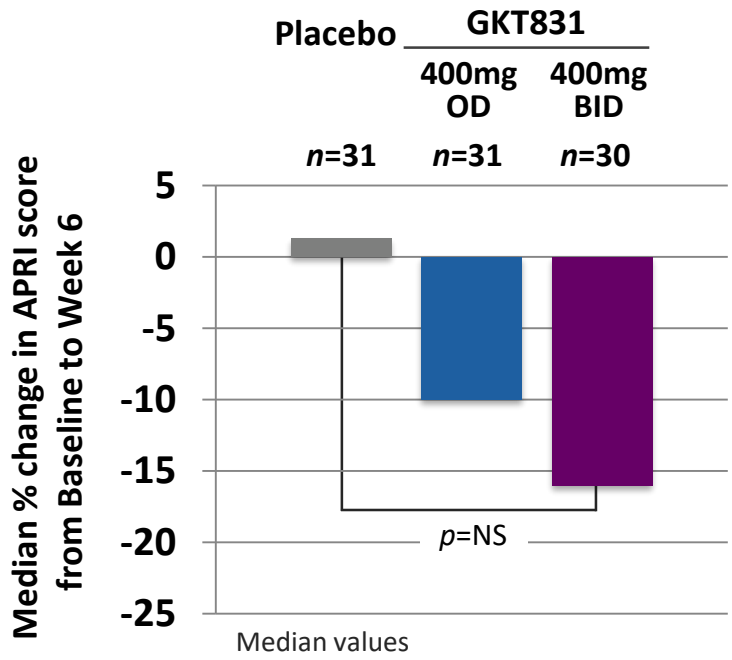
Setanaxib

(At Week 6)

Percent change in FIB-4 score



Percent change in APRI score



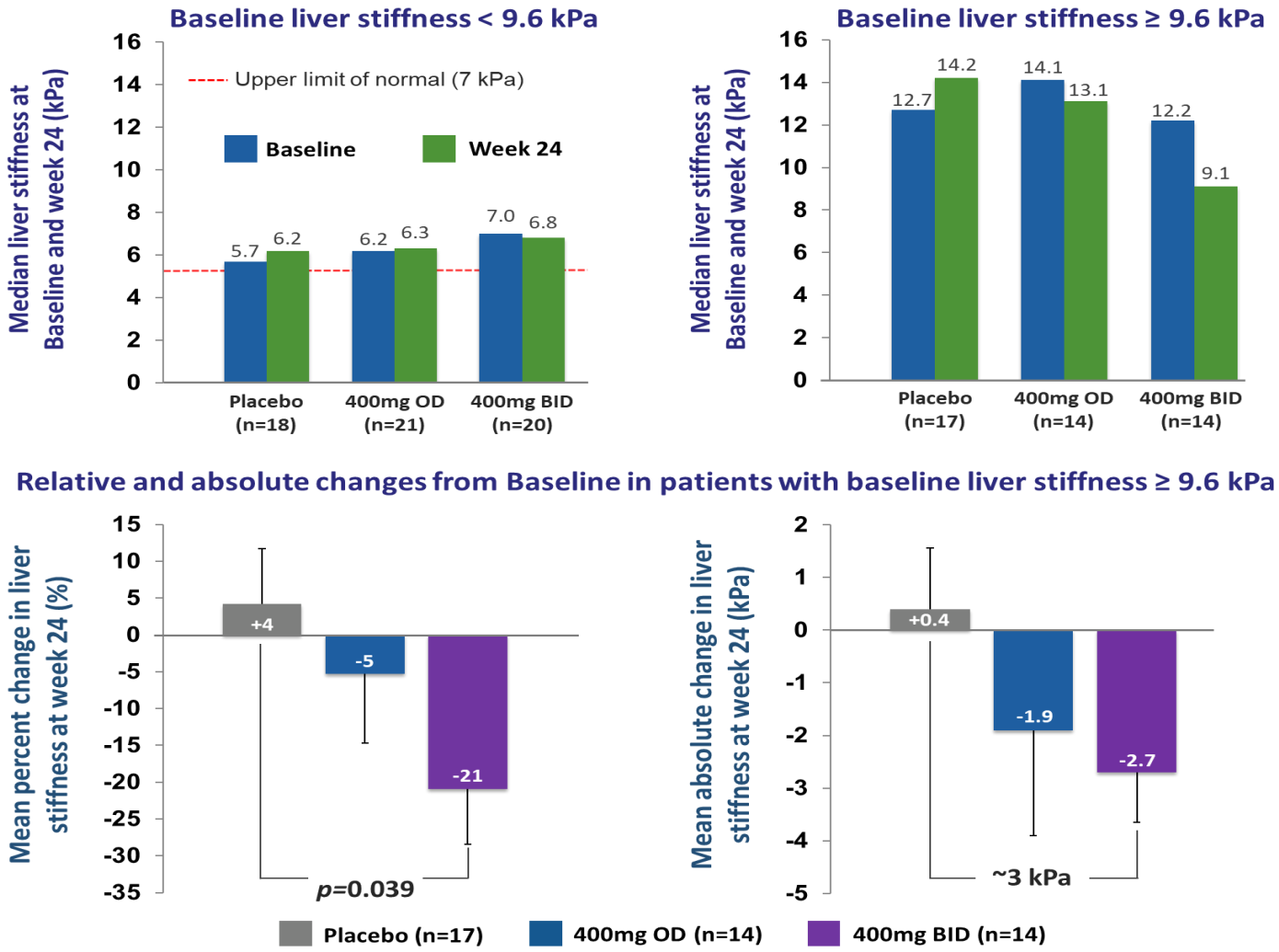
At weeks 12 & 24, assessments of liver fibrosis include Pro-C3 and the ELF score
Transient elastography (Fibroscan®) performed at week 24

ClinicalTrials.gov Identifier: NCT03226067

AASLD 2019 (Poster 1283)

NADPH oxidases (NOX) inhibitors in PBC

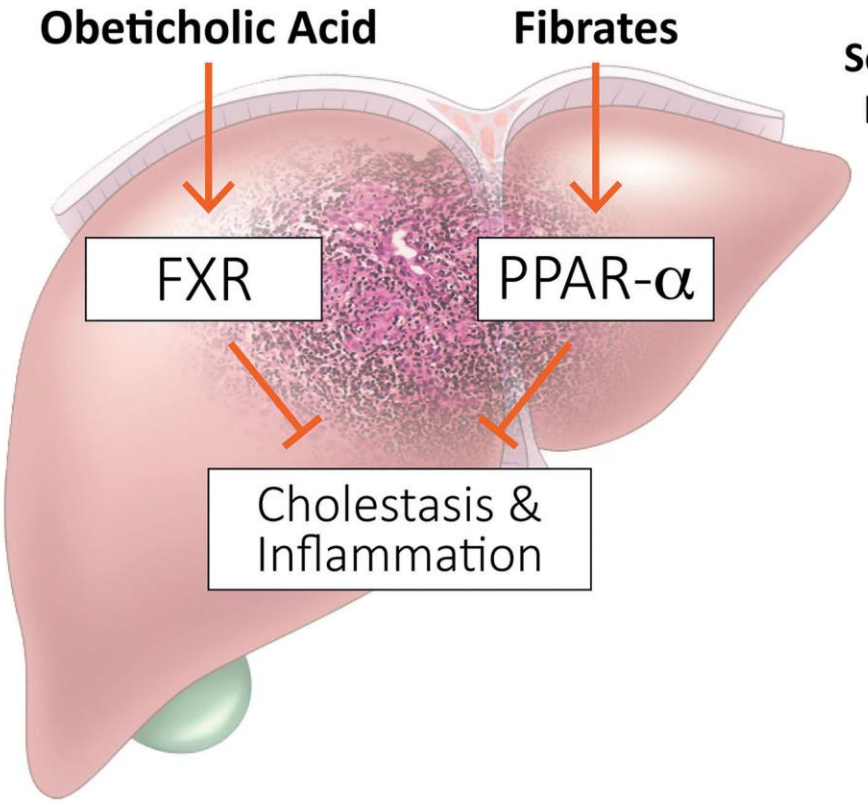
Setanaxib

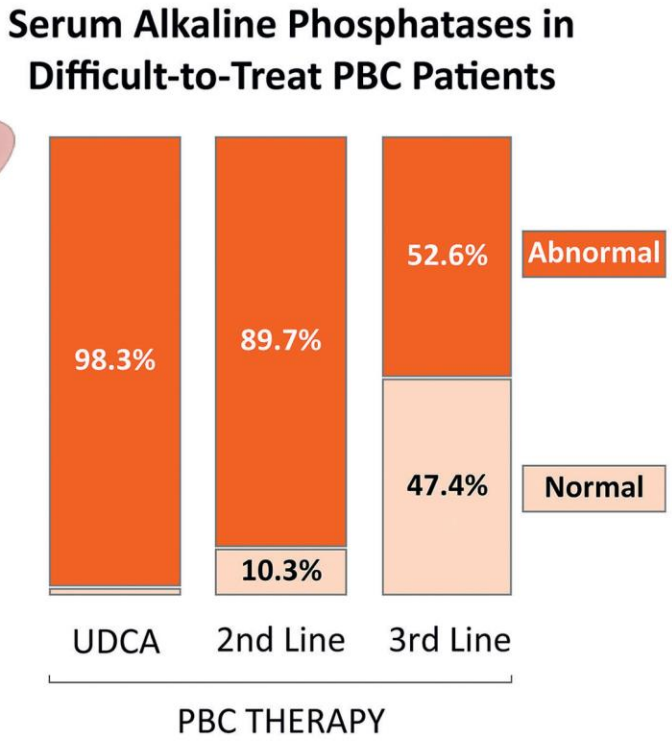


ClinicalTrials.gov Identifier: NCT03226067

Manuscript submitted

Additive beneficial effects of fibrates combined with OCA in the treatment of high-risk PBC

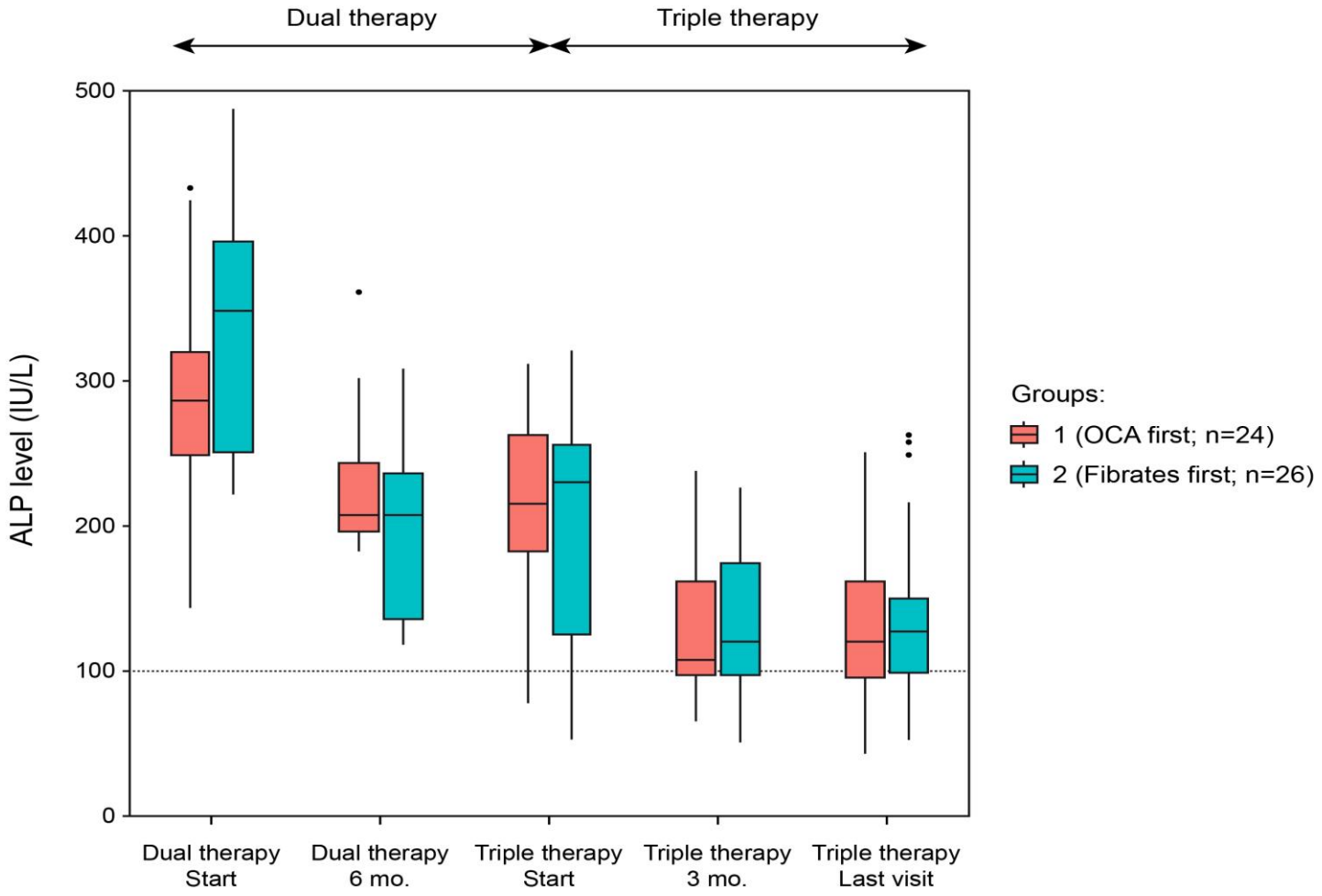




Soret et al, Am J Gastroenterol 2021

Additive beneficial effects of fibrates combined with OCA in the treatment of high-risk PBC

50 patients
From **16** centers
and **7** countries:
-France
-Belgium
-Germany
-Italy
-UK
-Spain
-USA



Soret et al, Am J Gastroenterol 2021

TISSUE REPAIR

Cholangiocyte organoids can repair bile ducts after transplantation in the human liver

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Organoid technology holds great promise for regenerative medicine but has not yet been applied to humans. We address this challenge using cholangiocyte organoids in the context of cholangiopathies, which represent a key reason for liver transplantation. Using single-cell RNA sequencing, we show that primary human cholangiocytes display transcriptional diversity that is lost in organoid culture. However, cholangiocyte organoids remain plastic and resume their in vivo signatures when transplanted back in the biliary tree. We then utilize a model of cell engraftment in human livers undergoing ex vivo normothermic perfusion to demonstrate that this property allows extrahepatic organoids to repair human intrahepatic ducts after transplantation. Our results provide proof of principle that cholangiocyte organoids can be used to repair human biliary epithelium.

Organoids have a particular potential for tissue repair as they retain key functions and characteristics of their tissue of origin. Nevertheless, their ability to repair native epithelia and restore their complexity after transplantation has not yet been established in humans, and organoid engraftment and survival in vivo has only been demonstrated in a limited number of animal studies (*1*).

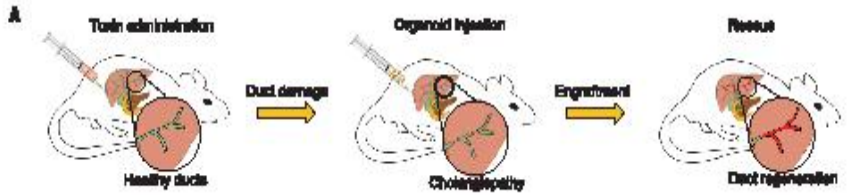
The bile duct epithelium presents an archediversity found in other hollow-lumen organs (*4*). In particular, different regions along the biliary tree display distinct transcriptional profiles and functional properties, such as the chemical modification of bile (*5*, *6*) as well as variation in disease susceptibility between the intrahepatic ducts, extrahepatic ducts, and the gallbladder. Nevertheless, the impact of this regional variation on the characteristics and regenerative potential of the organoids derived

stimuli. We subsequently use a biliary injury mouse model and a newly developed model for cell transplantation in human organs undergoing ex vivo normothermic perfusion (NMP) to prove that this plasticity allows cholangiocytes from one region to repair a different region of the biliary tree, thereby paving the way for cell-based therapy using organoids.

To characterize the cellular composition of the human biliary epithelium, cholangiocytes from different regions [intrahepatic bile ducts (IHD): 5 patients, 587 cells; common bile duct (CBD): 3 patients, 3006 cells; and gallbladder (GB): 3 patients, 3702 cells] were isolated using magnetic bead sorting, and their transcriptome was determined using droplet encapsulation single-cell RNA sequencing (scRNA-seq) (Fig. 1, A and B, and fig. S1, A to C). The isolated cells expressed key cholangiocyte markers, including KRT7, KRT19, SOX9, and GGT (fig. S2A). The transcriptomes of all three biliary cell populations shared a core transcriptional profile, illustrated by their proximity in uniform manifold approximation and projection (UMAP) space and high connectivity in partition-based graph abstraction (PAGA) analysis when compared with different liver cell types, such as stellate cells and liver sinusoidal endothelial cells (LSECs) (fig. S2, B to E). However, more detailed analysis after subclustering the cholangiocytes revealed nonoverlapping expression modules of the three populations (Fig. 1B), which suggests that, despite their similarities, cholangiocytes from different regions exhibit distinct gene expression signatures (*6*).

Differentially expressed gene (DEG) analysis (data S1) identified known region-specific mark-

Mouse



Humans

origin. Taken together, these results establish that cholangiocytes from different regions of the biliary tree are interchangeable, and they suggest that extrahepatic cells can be used to repair acute intrahepatic duct injury.

Cell transplantation experiments in mouse models are extremely useful but are not always predictive of therapeutic outcomes (*19*). Furthermore, the mouse liver microenvironment is different from that of humans, which raises the possibility that our results may not translate between species. To address these challenges, we developed a model for cell-based therapy in humans utilizing ex vivo organ perfusion (*20*). Ex vivo NMP was developed to improve organ preservation and reduce ischaemia-reperfusion injury by circulating warm oxygenated blood through liver grafts before transplantation. Notably, the biliary tree is particularly susceptible to ischaemia, which results in duct damage (*21*, *22*). Low bile pH (<*7.5*) during NMP is used as a predictor of this type of cholangiopathy (*23*).

To assess the therapeutic potential of our cells for repairing human bile ducts, RFP-expressing gallbladder organoids were injected in the intrahepatic ducts of deceased human transplant donor livers ($n = 3$) with a bile pH <*7.5* at the start of the experiment, which signified ischemic duct injury. The organs were

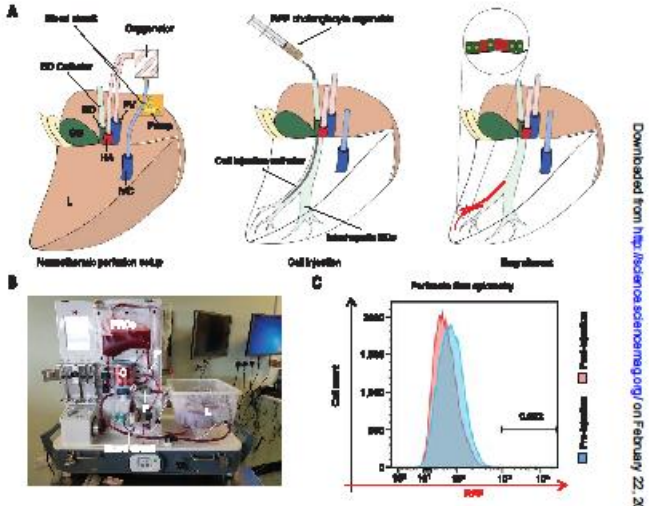


Fig. 4. Cholangiocyte organoids engraft in a human liver receiving NMP and improve bile properties. (**A** and **B**) Schematic representation of the technique for organoid injection (A) and photograph of the NMP circuit used (B). BD, bile duct; HA, hepatic artery; PV, portal vein; IVC, inferior vena cava; L, liver; P, pump; O, oxygenator; PRCs, packed red cells. (**C**) Flow cytometry revealing absence of RFP cells in the perfusate. (**D**) Immunofluorescence revealing the engraftment of RFP gallbladder organoids with up-regulation of intrahepatic markers (SOX4) and loss of gallbladder markers (SOX17). Scale bars, 50 μm. (**E**) Organoid injection improves bile pH and choleresis. ***$P < 0.001$. $n = 3$ NMP livers. Each measurement is represented by a different data point, and each organ is represented by a different symbol.

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