OBSERVATIONAL STUDY ABOUT HEPATIC TOXINIS KINETIC AND EVALUATION OF ORGAN DAMAGE IN ACUTE ON CHRONIC LIVER FAILURE (ACLF) PATIENTS (The Biliver Study)

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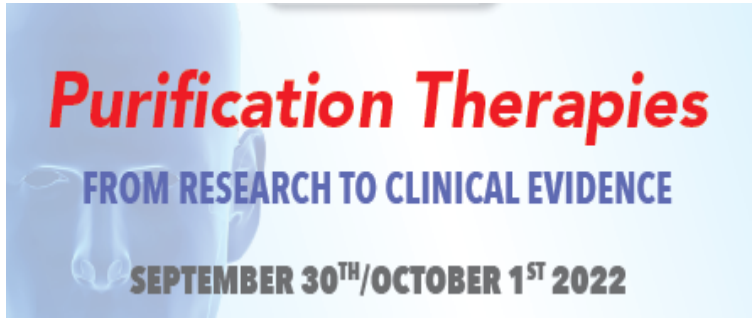
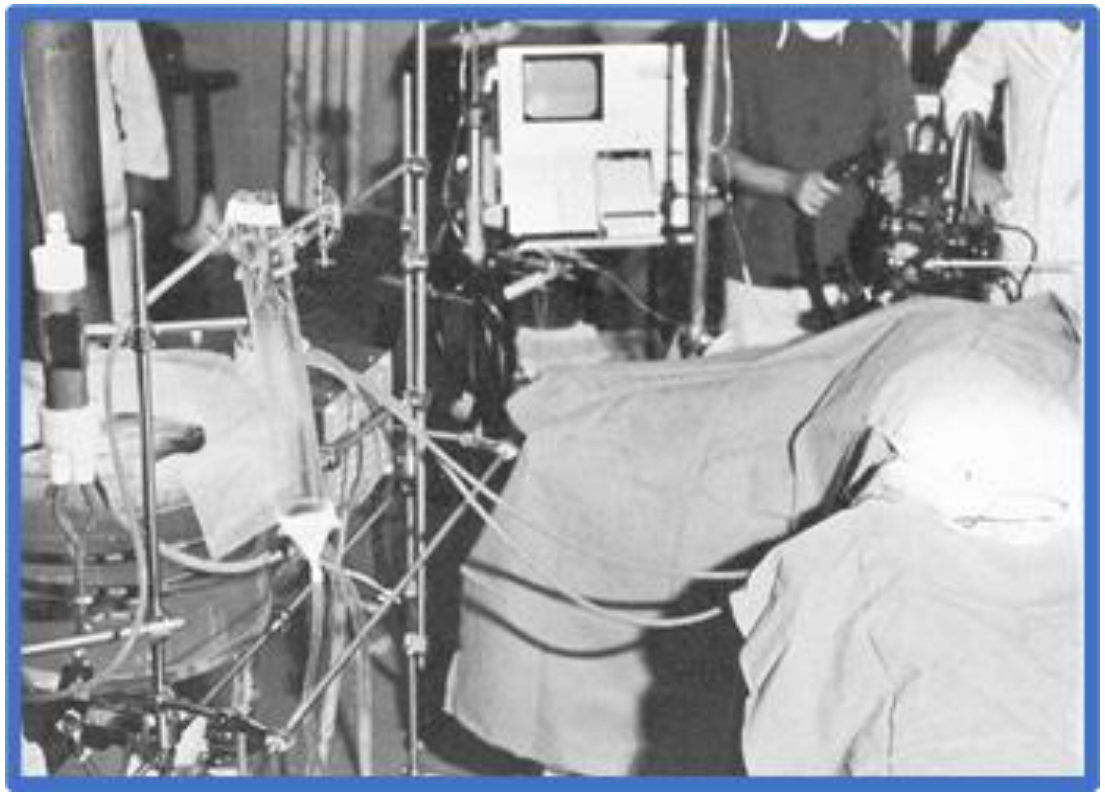
- What our clinical experiences are with purification therapies in Liver Failure
- Why a prospective observational study
- What the BILIVER Study can show us



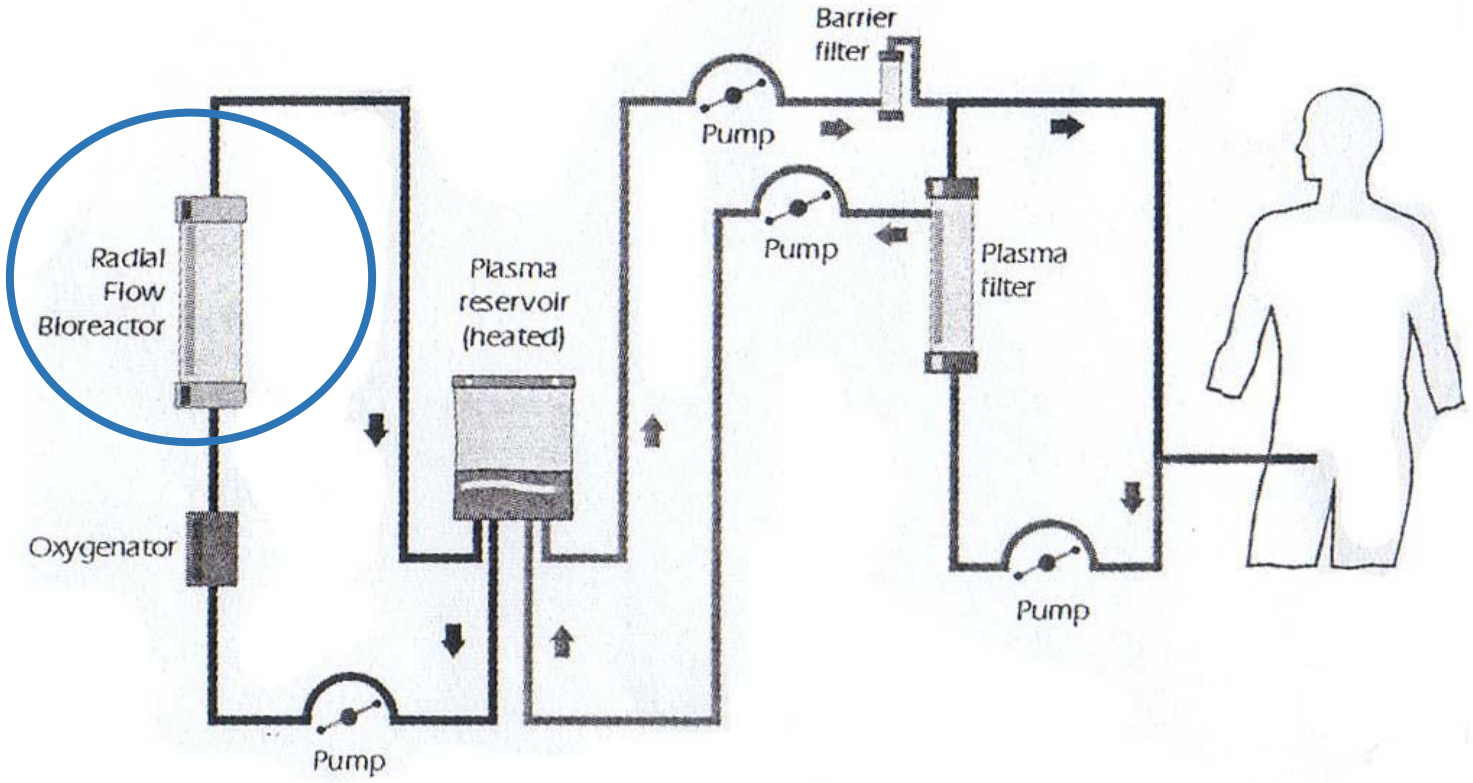
Extracorporeal liver support with pig/baboon livers

Our experience begins with this study that performed xenoperfusions of pig livers. This is an old photo, and this experiment was terminated for major immunology complications and high mortality as a result



Bioartificial Liver (BAL)

This complicated circuit with many pumps recirculates the patient's plasma through the **radial flow bioreactor** filled with high-density hepatocytes from pigs' cells. 6 out of 7 patients underwent orthotopic LT following BAL treatments. This initial experience confirmed the safety of this BAL configuration and suggested its clinical efficacy as a temporary liver support system in acute hepatic failure (AHF) patients. However, this was a limited experience and a clinical study has not yet been conducted.



Morsiani E, Faenza S et al. The International Journal of Artificial Organ 25 (3): 192-202, 2002

Our clinical experience in Liver Failure

ELSEVIER

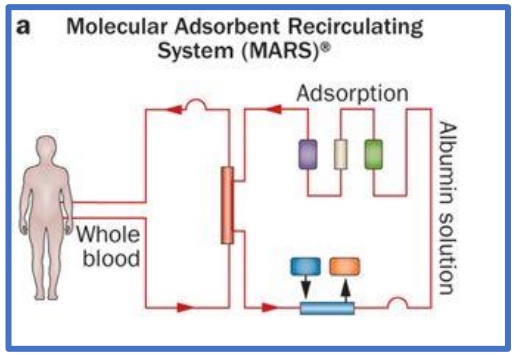
Transplantation Proceedings
Volume 40, Issue 4, May 2008, Pages 1169-1171

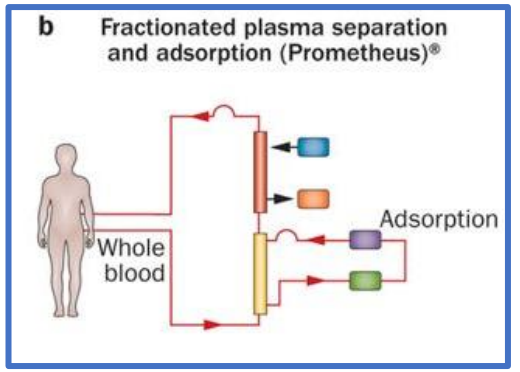
Liver anesthesia

MARS and Prometheus: Our Clinical Experience in Acute Chronic Liver Failure

S. Faenza [a] ✉, O. Baraldi [b], M. Bernardi [c], L. Bolondi [c], L. Coli [b], A. Cucchetti [a], G. Donati [b], F. Gozzetti [b], A. Lauro [a], E. Mancini [b], A.D. Pinna [a], F. Piscaglia [c], L. Rasciti [c], M. Ravaioli [a], G. Ruggeri [b], A. Santoro [b], S. Stefoni [b]

	Patients AoCLF	N° h per session	N° treatment session
MARS	41	6	191
Prometheus	16	6	87





Conclusion:

Recovery in dysfunction (3-month survival):
48.5% in the MARS group
33.5% in the Prometheus group.

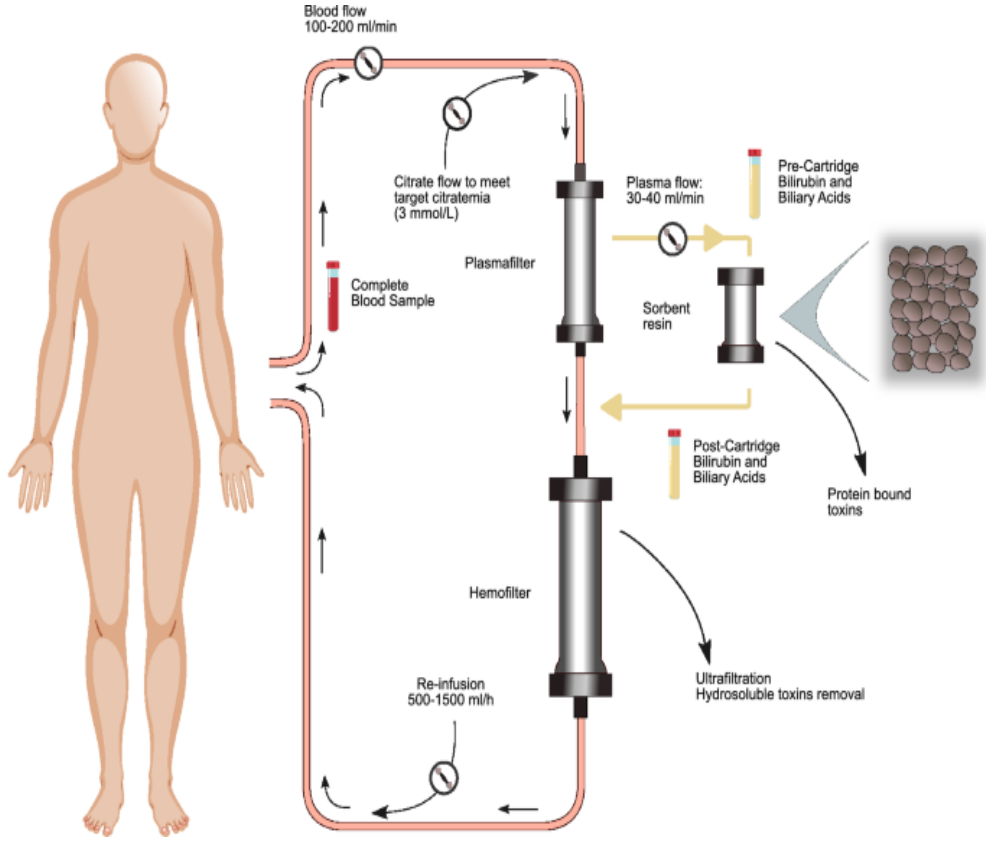
No results in PNF and secondary liver insufficiency

Our clinical experience in Liver Failure 2

Detoxification of bilirubin and bile acids with intermittent coupled plasmafiltration and adsorption in liver failure (HERCOLE study)

Gabriele Donati, Andrea Angeletti, Lorenzo Gasperoni, Fabio Piscaglia, Anna Laura Croci Chiocchini, Anna Scrivo, Teresa Natali, Ines Ullo, Chiara Guglielmo, Patrizia Simoni, Rita Mancini, Luigi Bolondi & Gaetano La Manna

Journal of Nephrology **34**, 77–88 (2021) | Cite this article



Prospective observational study

- 12 patients: 3 ALF 9 ACLF
- 31 CPFA treatments of 6 h each.

Rate Removals:

- 28.8% total bilirubin
- 32.7% direct bilirubin
- 29.5% indirect bilirubin
- 28.9% bile acids.

3 patients died:

- ➢ 2 during hospitalization
- ➢ 1 for a cardiac event at 4 months

Conclusions

Treatment with CPFA has been shown to be effective in removing bilirubin and bile acids in liver failure patients with a good safety profile, although it is a complex system in terms of technical application.

Removal of Bilirubin with a New Adsorbent System: In Vitro Kinetics

Claudia Gemelli[a] Aurora Cuoghi[a, b] Stefania Magnani[c] Mauro Atti[c]
Davide Ricci[d] Antonio Siniscalchi[e] Elena Mancini[d] Stefano Faenza[e]

[a]Science and Technology Park for Medicine, Mirandola, Italy; [b]Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia, Modena, Italy; [c]Aferetica S.r.l, Bologna, Italy; [d]Nephrology, Dialysis, Hypertension Unit, Teaching Hospital Policlinico S.Orsola-Malpighi, Bologna, Italy; [e]Anaesthesiology and Intensive Care Unit, Teaching Hospital Policlinico S.Orsola-Malpighi, Bologna, Italy

4 In-Vitro Experiments:

Experiments 1 & 2 (dynamic) with a solution **containing only unconjugated bilirubin, completely bound to the albumin** (1).
Objective: **To verify the removal of albumin-bound bilirubin**

Experiment 3 (dynamic) with a solution **with high bilirubin levels.**
Objective: **To study kinetics; to try to simulate in-vivo therapeutic treatment (24 h)**

Experiment 4 (static) pushing some beads charged with bilirubin into fresh albumin solution
Objective: **To verify the irreversibility of bilirubin-resin binding**

Table 1. Summary of experimental conditions designed for each in vitro experiment

	1st Exp.	2nd Exp.	3rd Exp.	4th Exp.
Bilirubin, mmol/L	0.4	0.8	0.8	–
Albumin, mmol/L	0.4	0.8	0.4	0.4
Time, h	8	8	24	24
Solution volume, L	6	6	6	0.06
Flow rate, mL/min	100	100	100	Static

Exp., experiment.

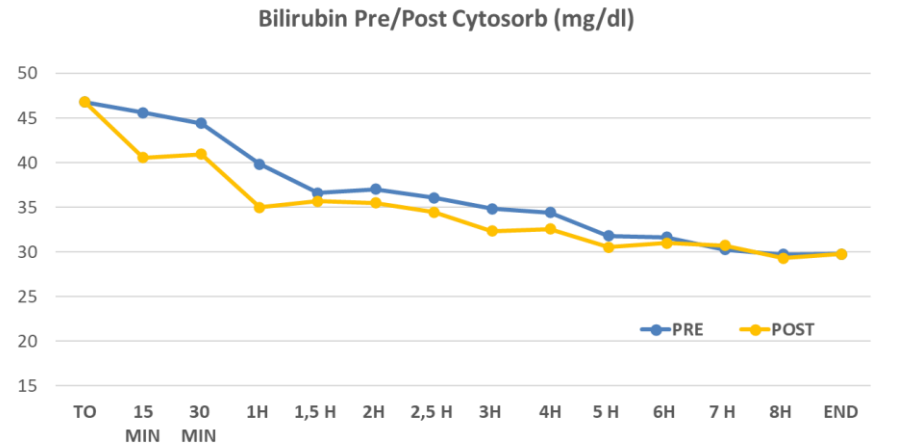
(1) On equimolar solution Bilirubin is strongly albumin-bound, with an association constant of $9{,}5x10/10^{7}M^{-1}$ corresponding to an unbound fraction in the equilibrium of less than 0,1%

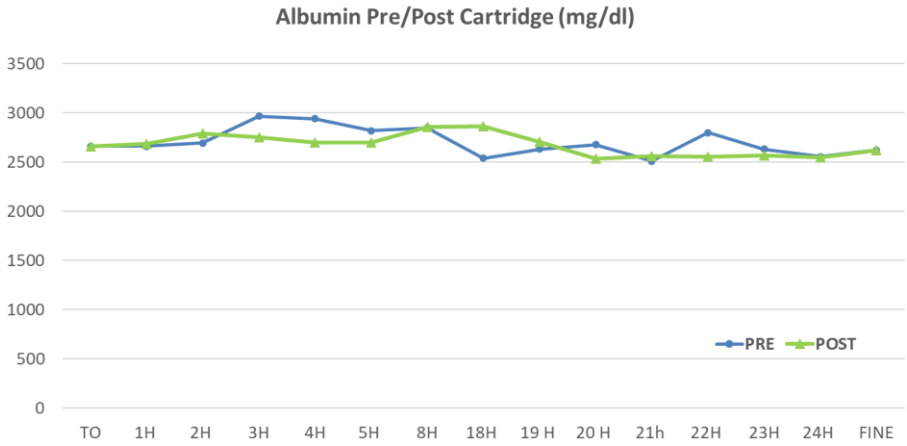
(Weber, Falkenhagen Biomacromolecules 2008, 9, 1322-1328).

Blood Purif. 2018 Sep 14:1-6. doi: 10.1159/000492378

Conclusions of the study

- Bilirubin:
  - Resin is able to adsorb **Unconjugated Bilirubin** breaking albumin-bilirubin complex (strongly bound).
  - Major amounts of Bilirubin Adsorption, increasing in condition «miming» the in-vivo condition
  - The cartridge does not release the adsorbed bilirubin, no evidence in 24H
  - The major adsorption is in the first hours of treatment but the system is actively removing bilirubin all along 24h
  - Bilirubin at high concentration is removed more efficiently than the one with lower concentration
- Albumin:
  - Minimal loss of Albumin (<5%).
  - Albumin is reinfused in the solution, while bilirubin is retained by the cartridge.





Our clinical experience in Liver Failure 3

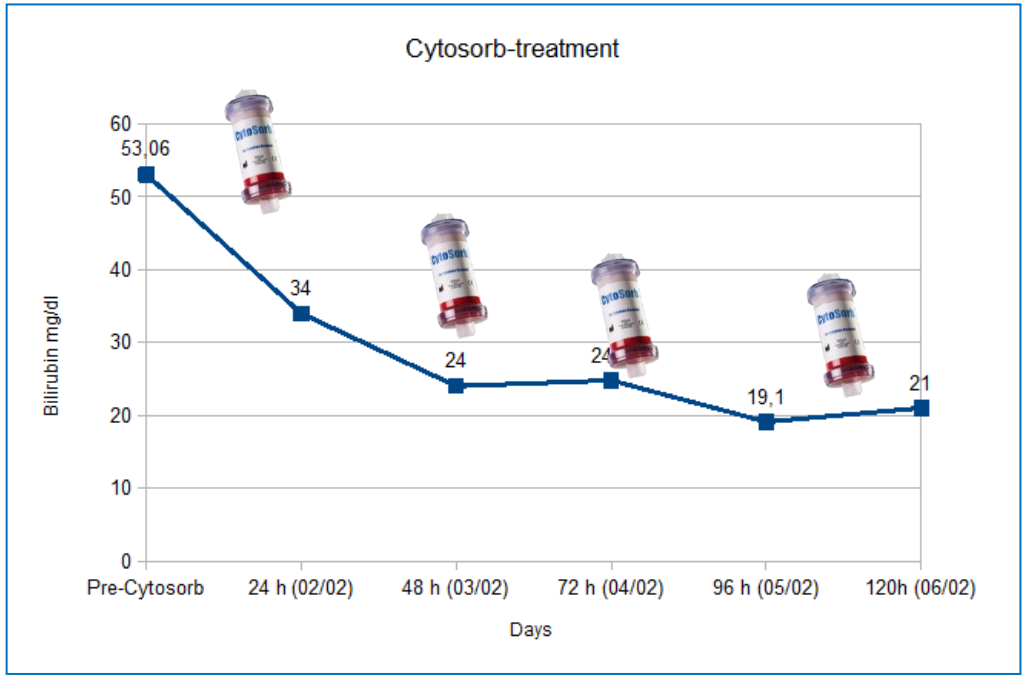
Male, 66 years old, **hepatitis C virus (HCV)-related cirrhosis, complicated by hepatocellular carcinoma (HCC)** receiving liver transplantation (MELD 10).

1. **First Liver Transplantation from a non heart-beating donor (NHBD)**.

Developed a post-reperfusion injury:

- High need of noradrenaline and adrenaline;
- Severe metabolic acidosis;
- Hyperlactatemia;
- Hypernatremia;
- Acute Renal Failure.

2. **Second Liver Transplantation after PNG of the first graft.**
   - Acute renal failure
   - Cytolysis (Myoglobin=23.118 ng/ml, CK=12.508 u/l)
   - Hyperbilirubinemia (Bilirubina=53,06 mg/dl)
   - Severe Sepsis (Enterobacter Cloache, PCT=70 ng/ml, PCR=11 mg/dl, GB=22000)



We used cytosorb therapy to treat sepsis after the 2nd transplant:

- **No more need of inotropes after 2nd treatment**
- **Dramatic Reduction of Bilirubin**
- **Normalization of Myoglobin level**
- **Normalization of Bilirubin level**
- **Functional recovery of the graft**
- **Dismission of the patient from ICU**

Extracorporeal Therapies in Liver Failure

- **Lots of techniques have been studied. All seem to be promising but some have contradictory results.**
- **The removal of hepatic toxins combined with inflammatory mediators appears to support liver function**

Device	Main Characteristics	Clinical Results
Charcoal hemoperfusion	Hemoperfusion through activated charcoal column	No survival benefit in ALF[7,58]
Molecular Adsorbent Recirculating System (MARS, Gambro Americas, Lakewood, CO)	Albumin dialysis against 20% albumin solution; membrane cutoff 50 kDa	Improvement in biochemical parameters in ALF and ACLF Beneficial effect on pruritus, encephalopathy, hemodynamics No survival benefit in ALF[21] and ACLF[23]
Fractional Plasma Separation Adsorption and Dialysis (Prometheus, Fresenius Medical Care, Bad Homburg, Germany)	Separation of patient's own albumin and detoxification through absorbers; membrane cutoff 250 kDa.	Good safety. Improvement in biochemical parameters No survival benefit in ACLF[28]
Single pass albumin dialysis (SPAD)	Albumin dialysis against 4% albumin solution, discarded after first pass; albumin impermeable membrane	Effective at removing bilirubin, ammonia, urea, creatinine; no RCT available
Selective plasma filtration therapy (SEPET)	Membrane pore size 100 kDa; small molecules including albumin cross the filter	Improved survival in animal models of ALF; no RCT in humans performed yet
High-volume plasmapheresis	Removal of patient plasma and replacement with fresh frozen plasma	Improvement in hepatic encephalopathy, hepatic and cerebral blood flow; improved transplant-free survival in ALF[35]

ACLF, acute-on-chronic liver failure; ALF, acute liver failure; RCT, randomized, controlled trial.

We need to evaluate the capabilities offered by extracorporeal therapies

Tritto, Giovanni et al. “Liver replacement therapy.” *Seminars in respiratory and critical care medicine* 33 1 (2012): 70-9 .

Multicenter Prospective Observational Study

Why a prospective observational study?

- Because it allows us to analyze the relationship between **“therapy”** and **“outcome”**
- It is important to categorize cases according to the therapy and, after adequate follow-up, to measure the outcome.

- Wider perspective than a single-center study.

Hypothesis of the study

The modulation of bilirubin and other toxic molecules and mediators, obtainable through the use of extracorporeal purification systems (CYTOSORB) may reduce the degree of organ failure in patients with ACLF

Acute on chronic liver failure *is a syndrome in patients with chronic liver disease with or without cirrhosis which is* ***characterized by acute hepatic decompensation*** *resulting in liver failure and one or more* ***extra-hepatic organ failures*** *that is associated with increased mortality within a period of 28 days from onset.*

World Congress of Gastroenterology 2014 ;147(1);4-10

BiLiver study

OBSERVATIONAL STUDY ABOUT HEPATIC TOXINS KINETIC AND EVALUATION OF ORGAN DAMAGE IN ACUTE-ON-CHRONIC LIVER FAILURE (ACLF) PATIENTS

Multicenter prospective observational study on the removal by **CytoSorb** of the main **toxins** involved in **Acute-on-Chronic Liver Failure** with the aim of evaluating its efficacy on the clinical outcome

- Primary Endpoint:

Study the adsorption of **bilirubin, bile acids** and **ammonia**

- Secondary Endpoint:

1. **Hepatic Function**
   - Hepatic enzymes
   - Lactate
   - Coagulation Parameters
   - MELD score- CLIF-OF score
2. **Inflammatory status**
   - PCT- PCR- WB
   - IL-6, IL-8, IL-10
3. **Neurological function**
   - Encephalopathy grade
4. **Renal Function**
   - Output diuresi
   - Increase sCr
5. **Cardiovascular Function**
   - Mean Arterial Pressione
   - Vasopressor need
   - Cardiac Indexes

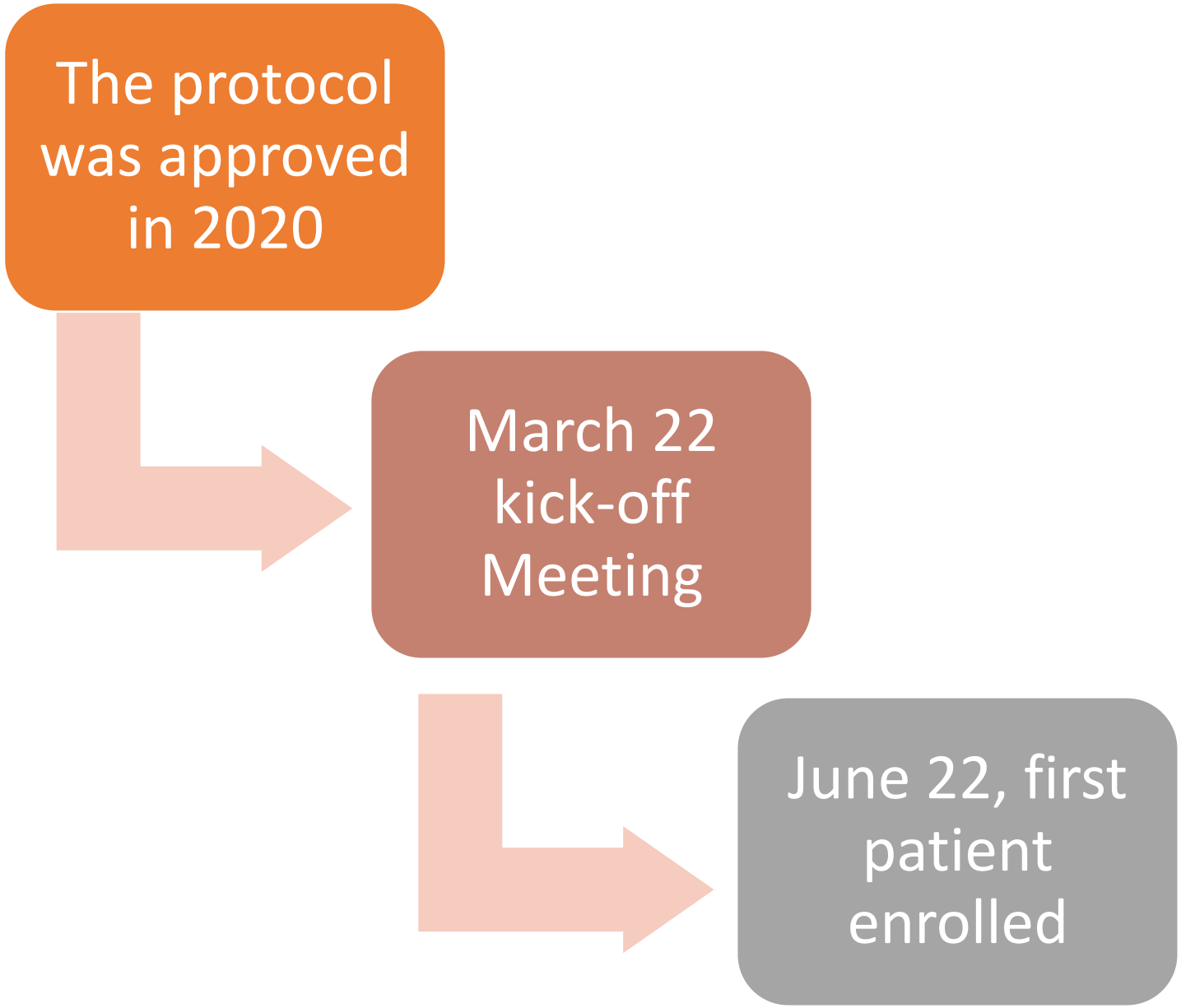
Study Structure

Opzione 2

Inclusion Criteria	▪ ACLF with Score ≥ 2 (CLIF-C OF Score) ▪ Use of CytoSorb
Data Collection During	• Admission • Pre-treatment • During treatments • At the end of treatments
Data collected	• Cytokines • Biochemistry • Clinical variables and blood gases • Demographics
Treatments	• Standard medical treatment + CRRT [1] (continuous renal replacement therapy) treatment + Hemoadsorption with CytoSorb • Standard medical treatment + Hemoadsorption with CytoSorb in Hemoperfusion
Follow-up	• Need for transplant • Length of stay • Hospital outcome
Statistical Analysis	▪ Made by the Coordinator Center (S. Orsola-Malpighi)

[1] According to KIDGO criteria CRRT if AKI ≥ 2: serum creatinine above the basal values of 2 to 2.9 times the basal value or diuresis <0.5 mL / kg / h ≥ 12h (renal dose 25-35 ml/kg/h Total effluent volume)

STUDY TIMELINE



State of the art

The study has been approved by ethics committees of:

Policlinico S.Orsola-Malpighi, Bologna, Italy (principal investigator)
Ospedali Riuniti, Ancona, Italy.
Niguarda Ca' Granda Hospital, Milan, Italy.
ASST Papa Giovanni XXIII, Bergamo, Italy.

Steering Committee:

- Bologna Sant'Orsola (Dr. A. Siniscalchi)
- Pisa Cisanello (Prof. G. Biancofiore)
- Torino Molinette (Dr. A. Ottobrelli)
- Padova AOU (Prof. P. Navalesi)
- Milano Niguarda (Prof. R. Fumagalli)

From June 2022
Already **5 patients** enrolled in the study

Expectations

From the study it will be possible to observe the ability of CytoSorb in the:

- Removal of **toxic liver molecules**
- Modulation of the **inflammatory response**
- Improvement of the **ACLF** level
- Prevention of **multi-organ dysfunction**

Conclusions

- Many **liver purification therapies** have been studied to support hepatic function. However, studies have been limited in scope with varying criteria.
- **Cytosorb** – which is a technically simple and long-lasting therapy— could have the ability to remove **bilirubin, bile acids, ammonia and cytokines** for patients with ACLF
- A **large, homogeneous Multicenter Prospective Observational Study** can provide an overview of the benefits and effects of purification therapies in liver failure.
- **The BILIVER** study evaluates CytoSorb adsorption of toxins involved in liver failure and inflammatory mediators and how this can influence the **degree of organ failure.**