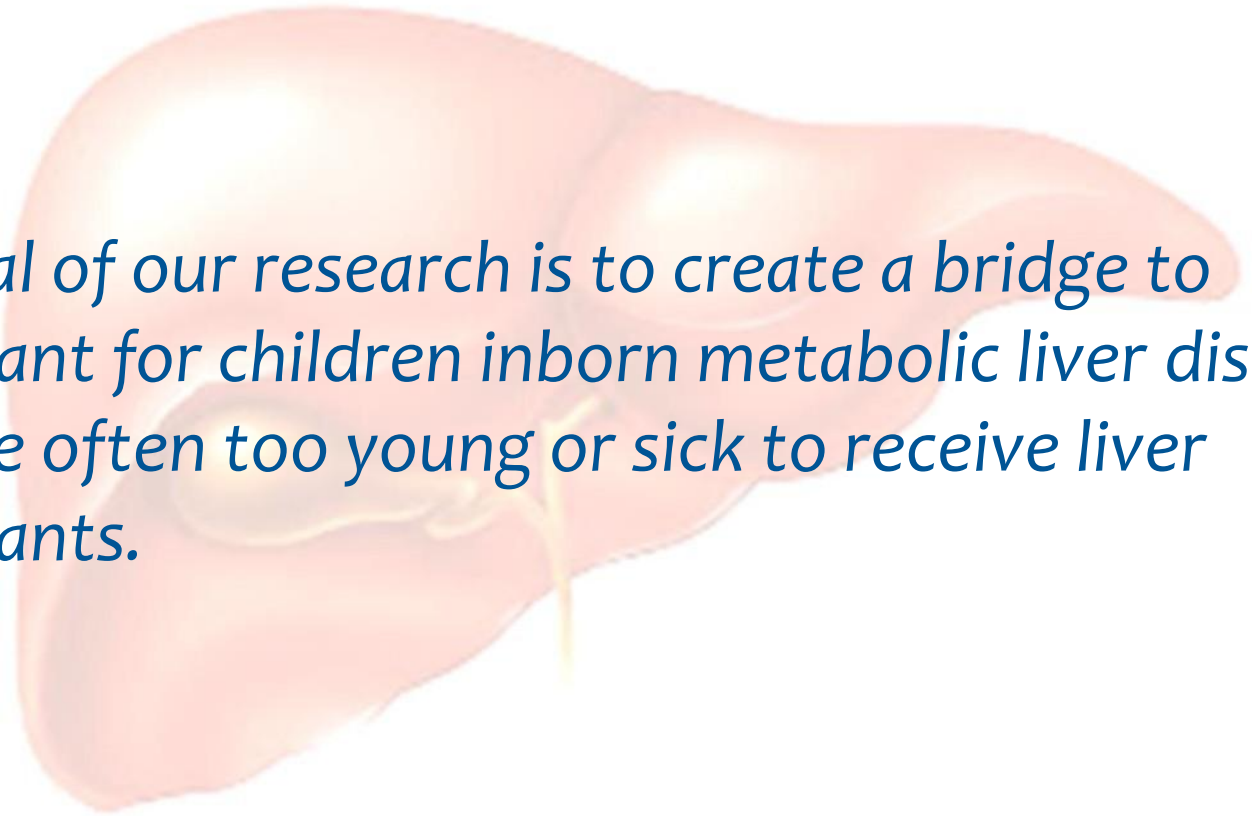
Cytonet, LLC

Cells for Life

AMAT 2015



Cytonet's Therapeutic Goal:



The goal of our research is to create a bridge to transplant for children inborn metabolic liver disease who are often too young or sick to receive liver transplants.

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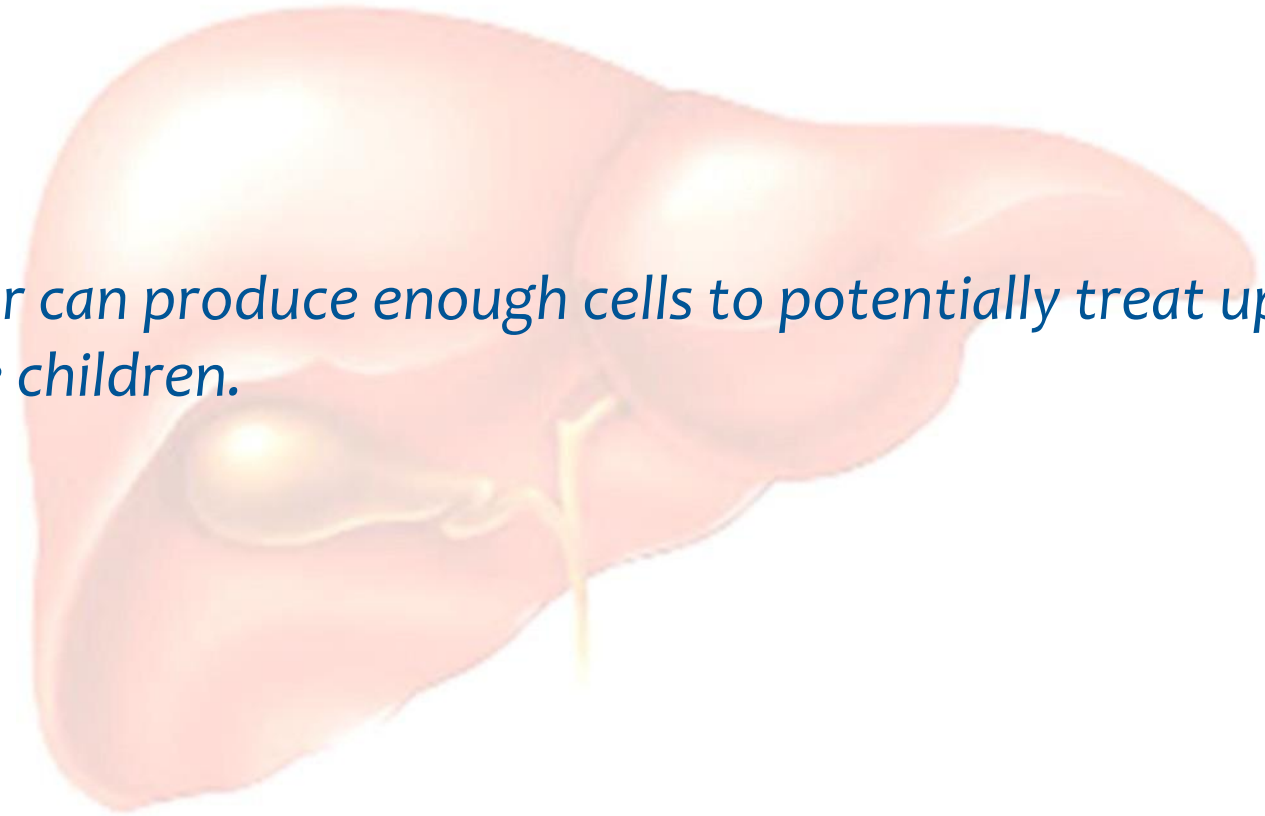
Cytonet's Therapeutic Goal:

We work solely with organ procurement organizations (OPO's) in the US and accept non-transplantable livers from brain dead, DCD and neonate donors. These livers are sent to our lab in Durham, North Carolina where liver cells are isolated, cryopreserved, tested for potency and safety, and held for use in our clinical trials.

Cytonet's Therapeutic Goal:

During the clinical trials, patients between and ages of 0-5 with liver metabolic disorders are enrolled into the program. The healthy donor liver cells are infused through the portal vein directly into the liver where they engraft and begin to function.

Cytonet's Therapeutic Goal:



One liver can produce enough cells to potentially treat up to 4 or more children.

Cytonet – Who We Are

Our Mission is to:

- Be a pioneer in the field of regenerative medicine
- Provide successful solutions for the treatment of rare, life-threatening diseases based on the use of human cells
- Worldwide leading position in the field of Liver Cell Therapy (in clinical stage)

Discussion

Overview of Cytonet

Urea Cycle Defect

Donor Criteria

The Donor Liver Arrives at Cytonet

Clinical Trials

Cytonet, Quickly…….

Non-Transplantable Liver

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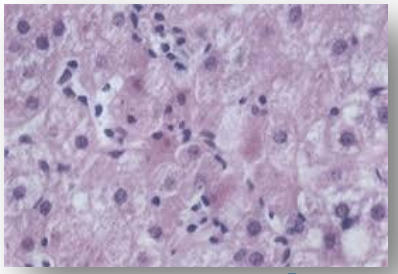

QUALITY QA ASSURANCE

Yale-New Haven Children's Hospital

Children's Healthcare of Atlanta

Lucile Packard Children's Hospital AT STANFORD

Ann & Robert H. Lurie Children's Hospital of Chicago

Alberta Children's HOSPITAL FOUNDATION

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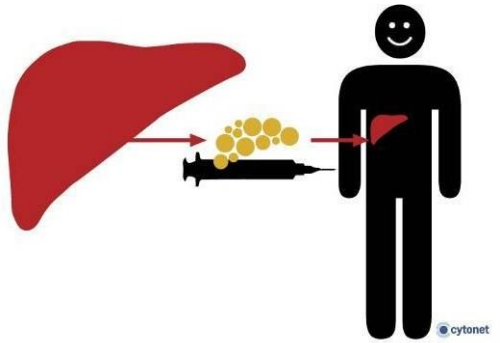
Advantages of Liver Cell Therapy (Allogenic Liver Cell Suspension)

- **Liver Cell based Pharma**
- Liver cell yield from **one non transplantable organ** is sufficient for the treatment of **multiple children** with severe metabolic liver disease, specifically Urea Cycle Defect.
- **Cryopreservation** (storage over years)
- Minimal medical risk (**infusion vs. organ transplantation**)
- Infusion of ABo compatible hepatocytes **initially requires immune-suppression**
- Liver cell infusion **can be repeated** as often as clinically needed
- Ready to use **whenever and wherever** needed



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Cytonet – Who we are

2000 Cytonet is founded

2004 1st therapeutic attempt on acute liver failure with human liver cells

2006 1st Therapeutic attempt on children with Urea Cycle Defects

2008 Start of the clinical trials phase II/III in Europe with EMA

2008 Acquired state of the art GMP cell production facility in Durham (NC)

2011 Began FDA Clinical Trials in North America

2014 Submitted Formal Application for Therapy to the EMA

Cytonet – Where we are

First US Treatment Dec 2010

clinicaltrials.gov

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What is Urea Cycle Disorder?

Initial Indication: Urea Cycle Disorder

Serious condition affecting newborns and infants…

- UCD is a genetic disorder affecting newborns
- Leads to inability to remove toxic ammonia from body through urine
- In severe cases, peaks in ammonia concentration lead to irreversible neurological damage or death in newborns and small infants

…with inadequate current therapeutic options

- Long term pharmacologic/ dietary management has poor prognosis
- Only "curative" therapeutic option for severe cases is liver transplant
- However, liver transplants for newborns & infants are infeasible or problematic
  - High failure rate of transplants
  - Infeasible for newborns
  - High risk for infants

Area of high unmet medical need

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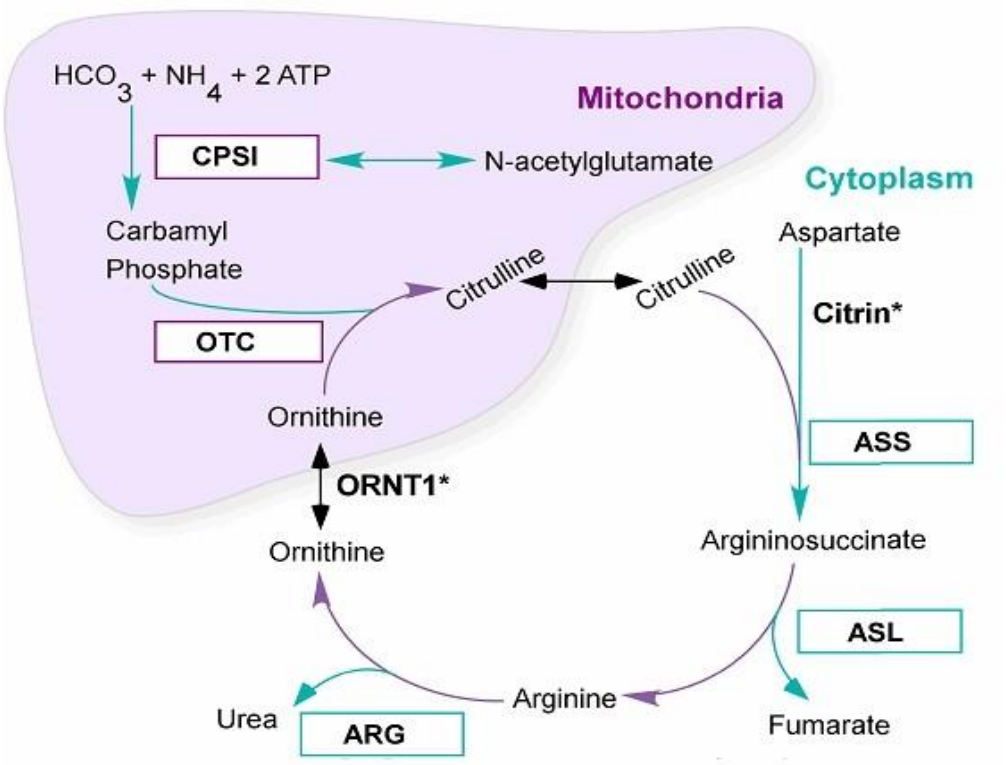
The Urea Cycle



Enzyme = Biological Catalyst

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Urea Cycle Defect in Newborns and Children

- UCDs represent a devastating class of inborn errors of metabolism
- Complete deficiency typically presents in neonatal period
- Even with best available medical treatment, very high morbidity and mortality
- Hyperammonia crises cause neurological damage
- Less than 20% of patients survive into teen years, most survivors suffer from developmental problems
- Approximately 1:50,000 neonates have full deficiency UCDs (USA)

- Mortality:
  - 30 - 50% within 1 years
  - 65 - 70% within 5 years
- Mean IQ of 47
- Mental Retardation: 79%
- Spastic Palsy: 46%
- Multiple Neurologic Deficits: 46%
- Organ transplantation in newborns is not possible

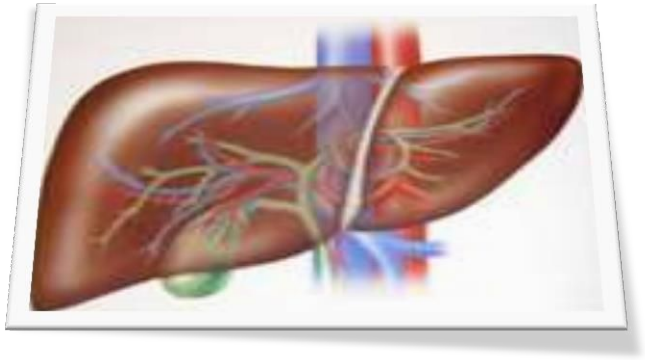
Current Treatment Methods for UCD

- Dietary Management - Specialized Amino Acid Formulations
- Drug Treatment – Buphenyl (ammonia scavengers)
- Liver Transplant
- Liver Cell Therapy

Cytonet Donor Criteria

Donor Criteria

- Brain Dead, Donation after Cardiac Death (DCD), and Neonate Donors
- Non-transplantable livers
- Informed consent for research
- Organ and DCD Donors
    - Neonatal – Birth (32 weeks) to 28 days > 2000 gms
    - 28 Days to 70 years of age (30 min WIT) (co-morbidity dependent)
    - Split Livers / Cut Downs - up to 40 years of age
- BMI taken into consideration
- Evaluate lab values and biopsy results
- Assessment of cold and warm ischemic times
- Medical history/co-morbidities
- Social history/high risk behavior
- Call **1-877-433-1916** for Donor Screening



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The Two Paths

- **Production Processing (GMP)** – Liver is processed for cell for potential infusion
- **Process Development (Development)** – Liver will be used for one of many on going research studies to improve processing, acceptance criteria, cryopreservation, recovery, transportation, etc

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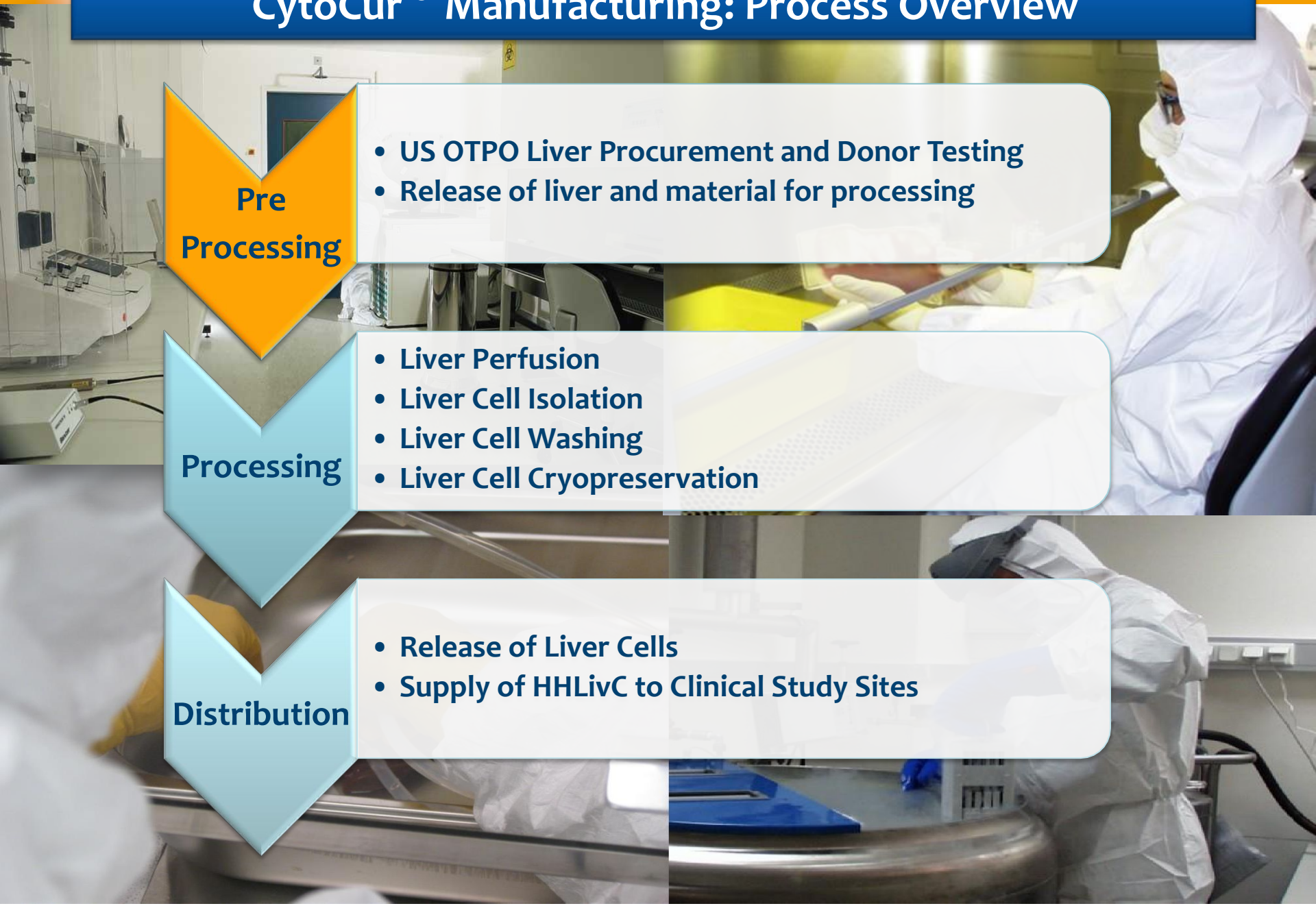
The Liver Arrives at Cytonet

CytoCur ® Manufacturing: Process Overview



Pre Processing

- US OTPO Liver Procurement and Donor Testing
- Release of liver and material for processing

Processing

- Liver Perfusion
- Liver Cell Isolation
- Liver Cell Washing
- Liver Cell Cryopreservation

Distribution

- Release of Liver Cells
- Supply of HHLivC to Clinical Study Sites

Cytonet Clinical Trials

Cytonet LCT – Excellent Fit with UCD

- Structurally normal liver allows safe and effective application (engraftment)
- Therapy effectively substitutes lacking enzyme activity to mitigate effects of disorder
- Intervention is viable and suitable for newborns and young children
  - Less invasive, lower risk, lower failure rate

- Outstanding efficacy and safety demonstrated in clinical trials (comprehensive data available under CDA)
  - Prevention of sequelae of UCD (brain damage, death), well tolerated

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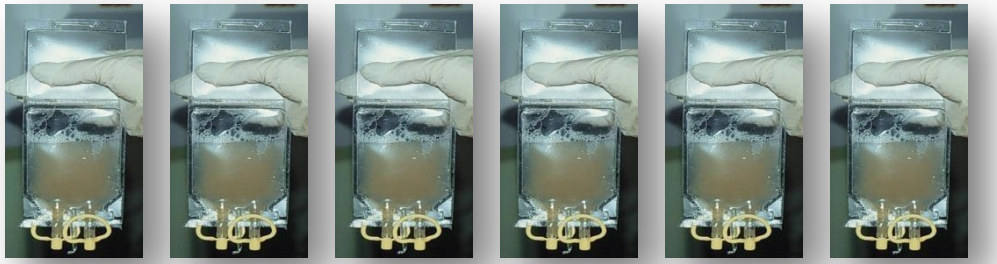
Dosage:

1 - 6 applications each with 0,05 x 10^9 viable cells per kg body weight



<u>Per patient:</u>

total viable cells:

0,3 x 10^9 per kg body weight

Planned number of sessions:

6 within 6 days

Observation:

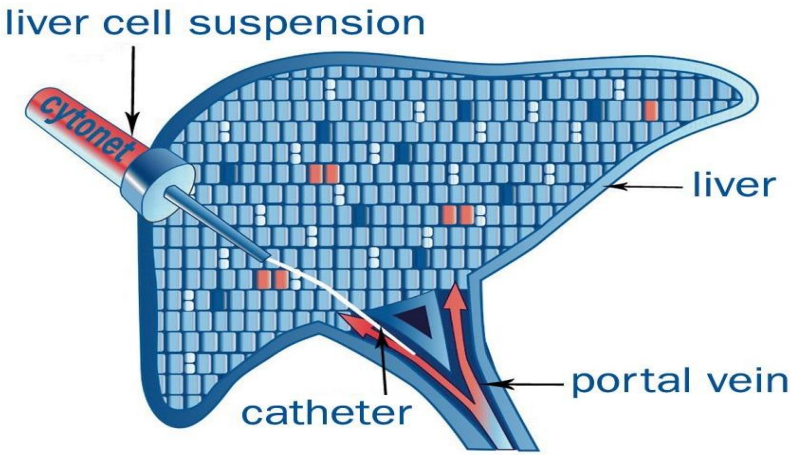
14 weeks including option for liver transplantation from week 7

Follow up:

24 month

HepaResc™ Cell Application

Clinical Cell Application

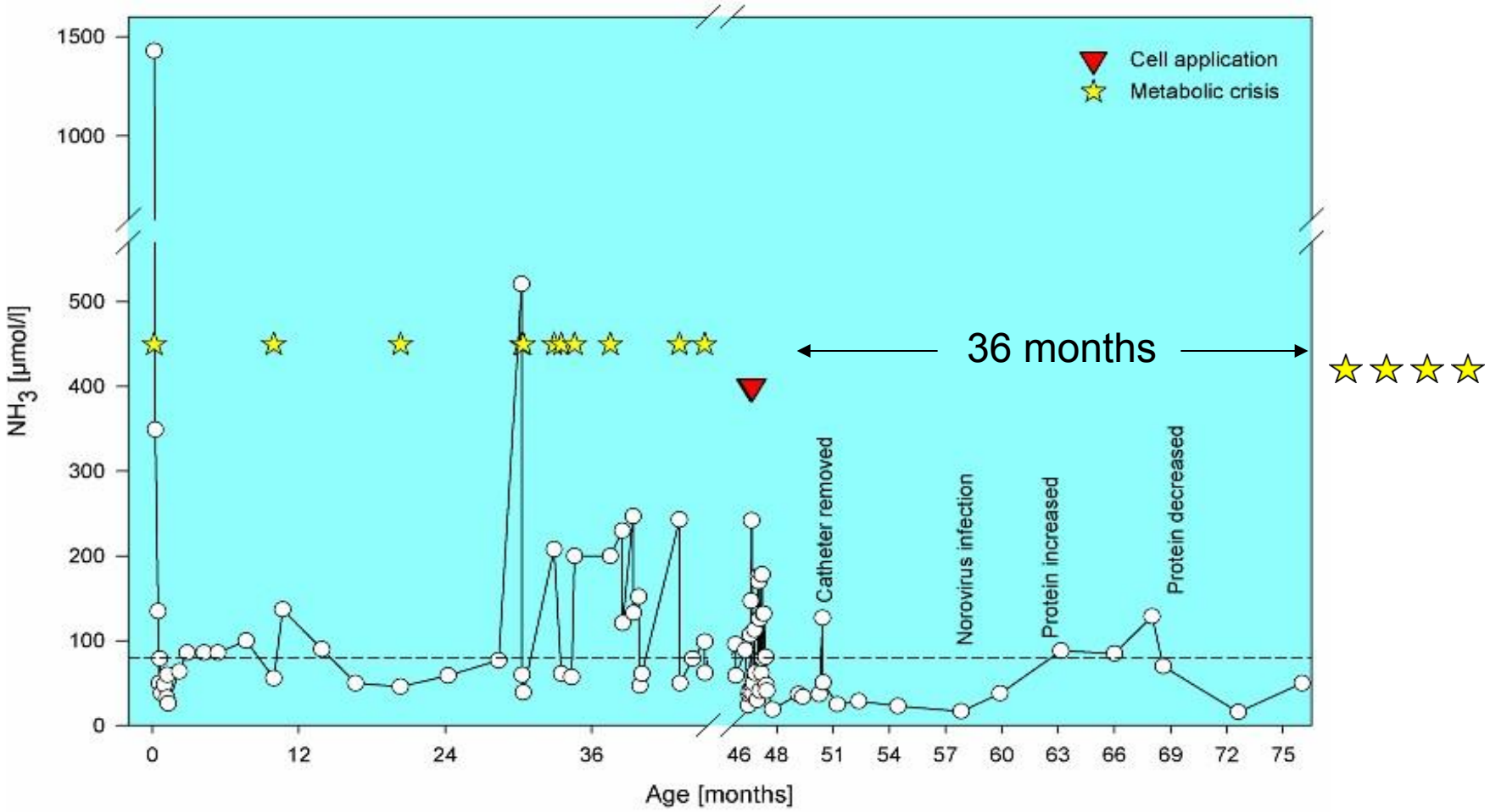


-Transfusion through portal vein
-monitored procedure
-Watch for portal HTN and blood clots, much like blood transfusion

HepaResc™ Cell Application

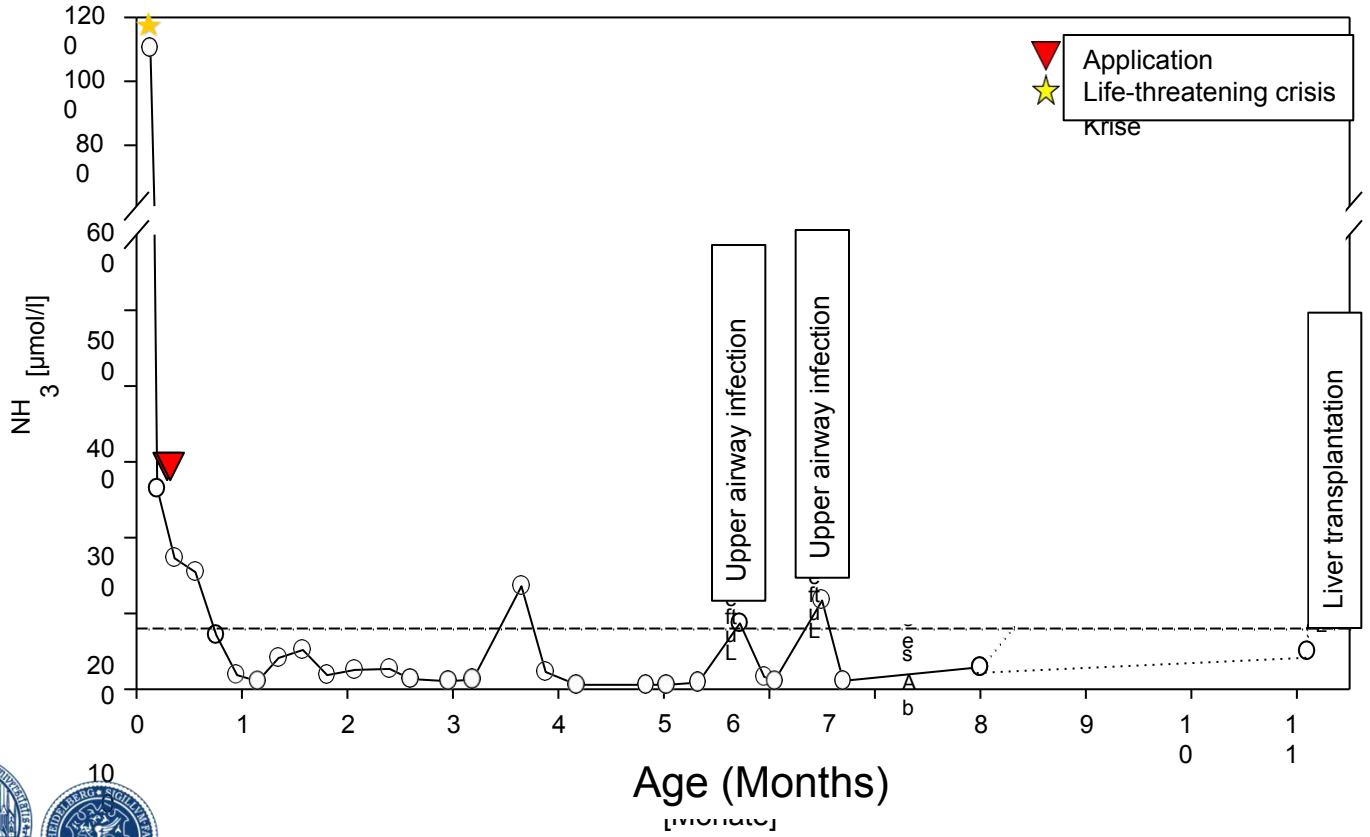
-Transfusion through portal vein
-monitored procedure
-Watch for portal HTN and blood clots, much like blood transfusion

Duration of Therapeutic Success



Kriegbaum and Meyburg, Eur Pediatr 3:20, 2009

OTC Deficiency



UniversitätsKlinikum Heidelberg

First Study Patient – SELICA III (USA)

When 8-month-old Abbas came to Yale-New Haven Children's Hospital with a life-threatening metabolic liver disease caused by a rare genetic defect, our pediatric transplant team knew he was a good candidate for a novel procedure that had proven successful in adults.

23 „Therapeutic Attempts“ using HHLivC-like cell preparations

				# of sessions	bio. viable cells		
1 (2003)	m	55 y	Acute-on-chronic (liver cirrhosis Child C, alcohol)	2	5,1	peritoneum	Hannover
2 (2003)	m	55 y	Acute-on-chronic (liver cirrhosis Child C, alcohol)	1	3,0	peritoneum	Hannover
3 (2004)	m	48 y	Acute-on-chronic (liver cirrhosis Child C, alcohol)	1	2,8	peritoneum	Hannover
4 (2004)	f	25 y	acute liver failure (Acute Hepatitis B, ALL)	2	3,0	peritoneum	Hannover
5 (2004)	m	65 y	other (Graft Dysfunction after OLT)	1	3,0	peritoneum	Hannover
6 (2004)	m	47 y	Acute-on-chronic (liver cirrhosis Child C, alcohol)	1	5,3	peritoneum	Hannover
7 (2004)	f	64 y	**acute liver failure (Amanita Phalloides Intoxication)**	3	4,5	liver/intraportal	Hannover
8 (2004)	f	55 y	Acute-on-chronic (liver cirrhosis Child C, alcohol)	2	5,4	peritoneum	Hannover
9 (2005)	m	52 y	other (Graft Dysfunction after OLT)	1	3,0	peritoneum	Hannover
10 (2005)	f	60 y	Acute-on-chronic (liver cirrhosis Child C, alcohol)	2	3,5	peritoneum	Hannover
11 (2005)	m	69 y	acute liver failure (Acute Hepatitis B)	4	5,4	liver/intraportal	Hannover
12 (2003)	f	48 y	Acute-on-chronic (liver cirrhosis Child C, alcohol)	1	0,7	peritoneum	Sofia
13 (2006)	f	4 weeks	Acute liver failure (HSV-2)	11	1,7	liver/intraportal	Heidelberg
14 (2006)	m	10 weeks	UCD: carbamoylphosphate synthetase I (CPS I-) deficiency	6	1,4	liver/intraportal	Heidelberg
15 (2007)	f	3 y	UCD: citrullinaemia	4	1,5	liver/intraportal	Heidelberg
16 (2007)	m	0 days	UCD: Ornithine transcarbamylase (OTC-) deficiency	3	0,6	liver/intraportal	Hannover
17 (2007)	m	8 days	UCD: Ornithine transcarbamylase (OTC-) deficiency	2	0,6	liver/intraportal	Padua
18 (2007)	m	3 y	Acute liver failure (Hepatitis E)	3	1,0	liver/intraportal	Budapest
19 (2007)	m	73 y	Acute liver failure (etiology unknown)	4	4,0	liver/intraportal	Hannover
20 (2008)	m	53 y	Acute-on-chronic liver failure (with Hep. B, MALT)	3	4.0	liver/intraportal	Hannover
21 (2009)	m	11 y	Crigler-Najjar Syndrome Type 1	6	7.3	liver/intraportal	Heidelberg
22 (2010)	w	16 mo	Primary Hyperoxaluria Type I	6	2.1	liver/intraportal	Köln
23 (2014)	m	7.5 mo	UCD: Ornithine transcarbamylase (OTC-) deficiency	6	2.9	liver/intraportal	Calgary

Hepatocyte Transplantation in an Acute Liver Failure Due to Mushroom Poisoning

Acute liver failure (ALF) remains a condition with substantial mortality (*1*, *2*). The only intervention of proven benefit for ALF is emergency liver transplantation (OLT) (*3*). The potential reversibility of ALF, the scarcity of donor organs, and the substantial mortality in patients, who have been transplanted in critical condition, have resulted in the development of techniques, which allow temporary support of liver function (*4*–*6*).

In this report, we document intraportal transplantation of cryopreserved hepatocytes into the portal vein of a 64-year-old woman who accidentally ingested liver toxin-producing amanita phalloides mushrooms. The patient was admitted to our hospital and transferred to the intensive care unit in stable condition. The clinical workup for OLT revealed considerable obesity, hypertension, and chronic heart failure. During counseling in the absence of encephalopathy the patient repeatedly refused to consent to OLT. At day 3 after ingestion of the mushrooms, International Normalized Ratio (INR) and factor V levels reached 4.2 and 9%, respectively, with no adequate increase after substitution. We decided to treat the patient with intraportal hepatocyte transplantation (HcTx) as hepatic coma progressed to level III. Under general anesthesia, the patient was mechanically ventilated and substituted with fresh frozen plasma, prothrombin complex concentrate, and antithrombin III. Under ultrasound and radiographic guidance, a peripheral portal vein branch was punctured transcutaneously and a 6-F catheter was placed into the central part of the portal vein. The procedure did not result in bleeding complications. Immunosuppression was started by intravenous application of steroids and cyclosporine A.

Blood group matched human hepatocytes were isolated from cadaveric livers according to Good Medical Practice guidelines as previously published and cryopreserved (*7*). Immediately prior to application, the cell suspensions were thawed rapidly and tested for viability by the trypan blue exclusion test. The average viability of the final transplant was 62%. The cells were infused through the portal vein catheter with continuous monitoring of the portal venous pressure. Altogether, 8×10^9 hepatocytes including $\sim5\times10^9$ viable cells were infused over a period of 30 hr. The catheter was removed without complications thereafter.

At the time of HcTx, the patient met the Clichy and King's College criteria for OLT (*8*, *9*). Then 36 hr after HcTx, the coagulation parameters remained at noncritical levels without further substitution of plasma proteins. Ammonia levels reached peak concentrations of 126 μmol/L immediately before HcTx. Eight hours after the last infusion of hepatocytes ammonia levels dropped to 45 μmol/L and remained stable. Bilirubin levels peaked one day after completion of HcTx and decreased continuously during the following days (Figure 1, A–C). Seven days after HcTx, the patient was extubated and transferred from the intensive care unit to the regular ward. Eight weeks after HcTx, an abdominal ultrasound scan showed normal liver architecture and a normal portal blood flow. Twelve weeks after HcTx, immunosuppressive therapy was stopped without signs of relapse. The patient finally recovered without concomitant extrahepatic organ damage. We conclude that the repeated application of primary human hepatocytes is safe and results in measurable benefit for patients with acute liver failure. Randomized studies will now be necessary to demonstrate a survival benefit for these patients.

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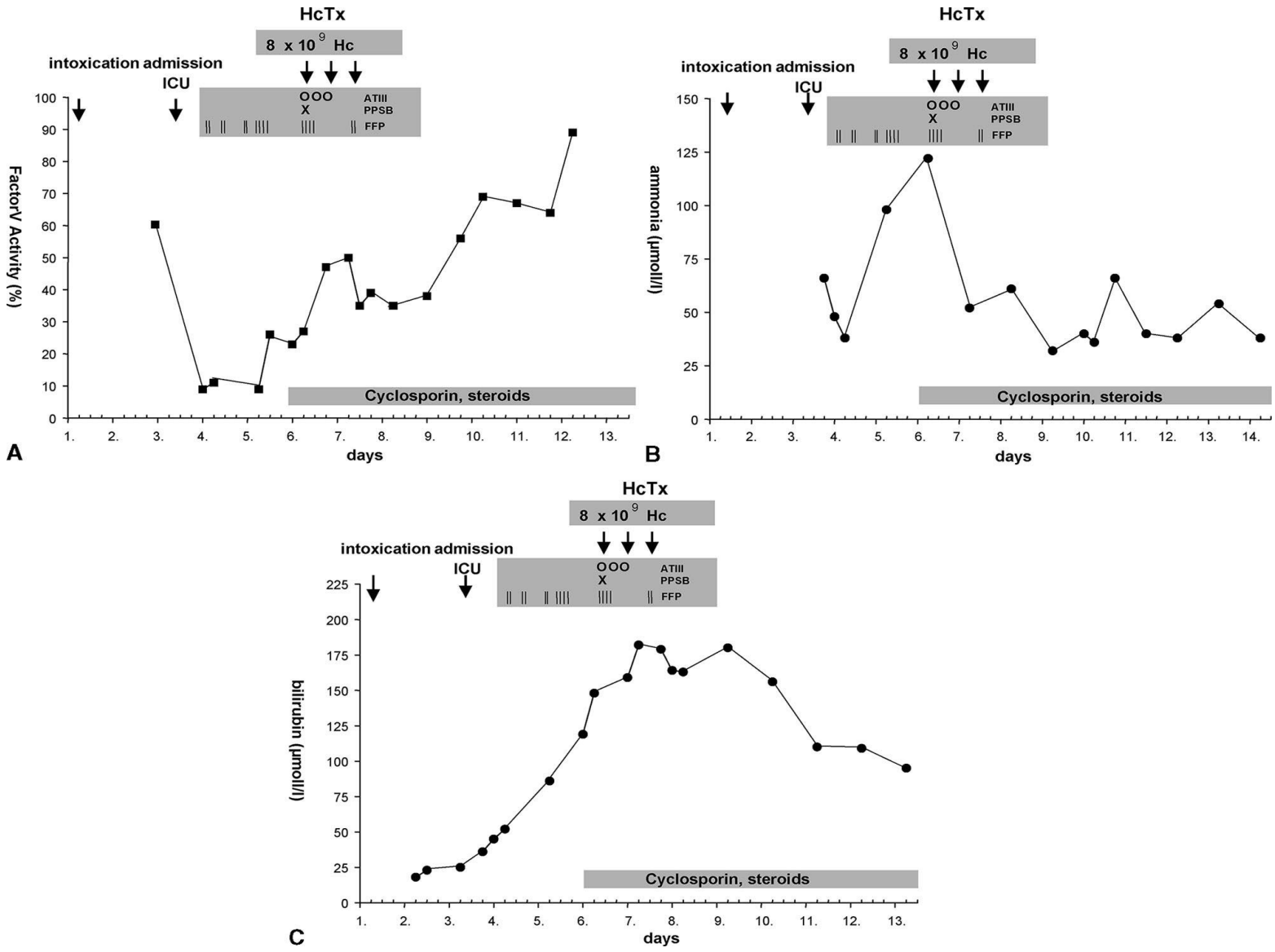
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Cyclosporin, steroids
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Liver Research Program

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Acute on Chronic Liver Failure

Definition

"acute deterioration of preexisting chronic liver disease usually related to a precipitating event and associated with increased mortality at three months due to multisystem organ failure."

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Multimodal definition of ACLF

- **Predisposition**
  - Aetiology/Age/ previous decompensation/comorbidities
- **Injury**
  - Precipitating illness
- **Response**
  - Inflammation/Infection
- **Organ**
  - Number and type of organ failures

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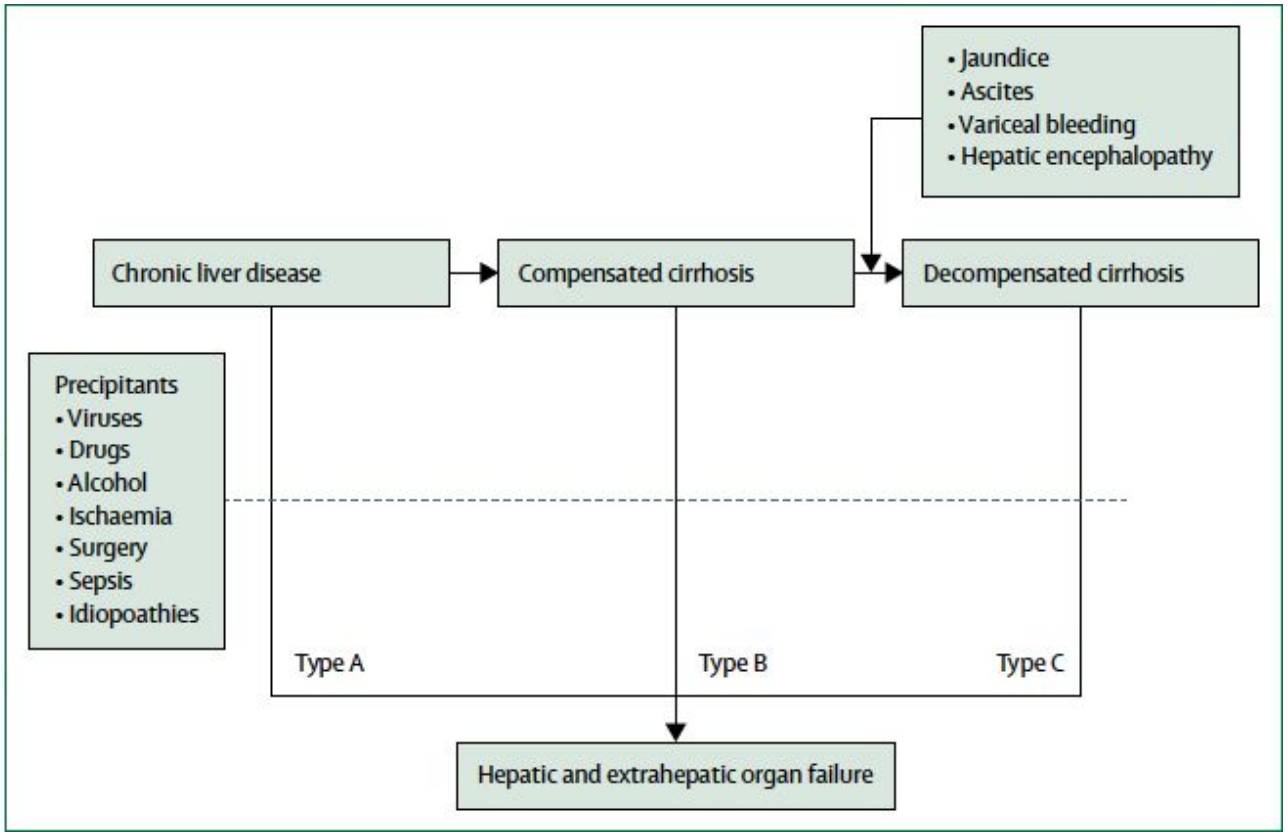
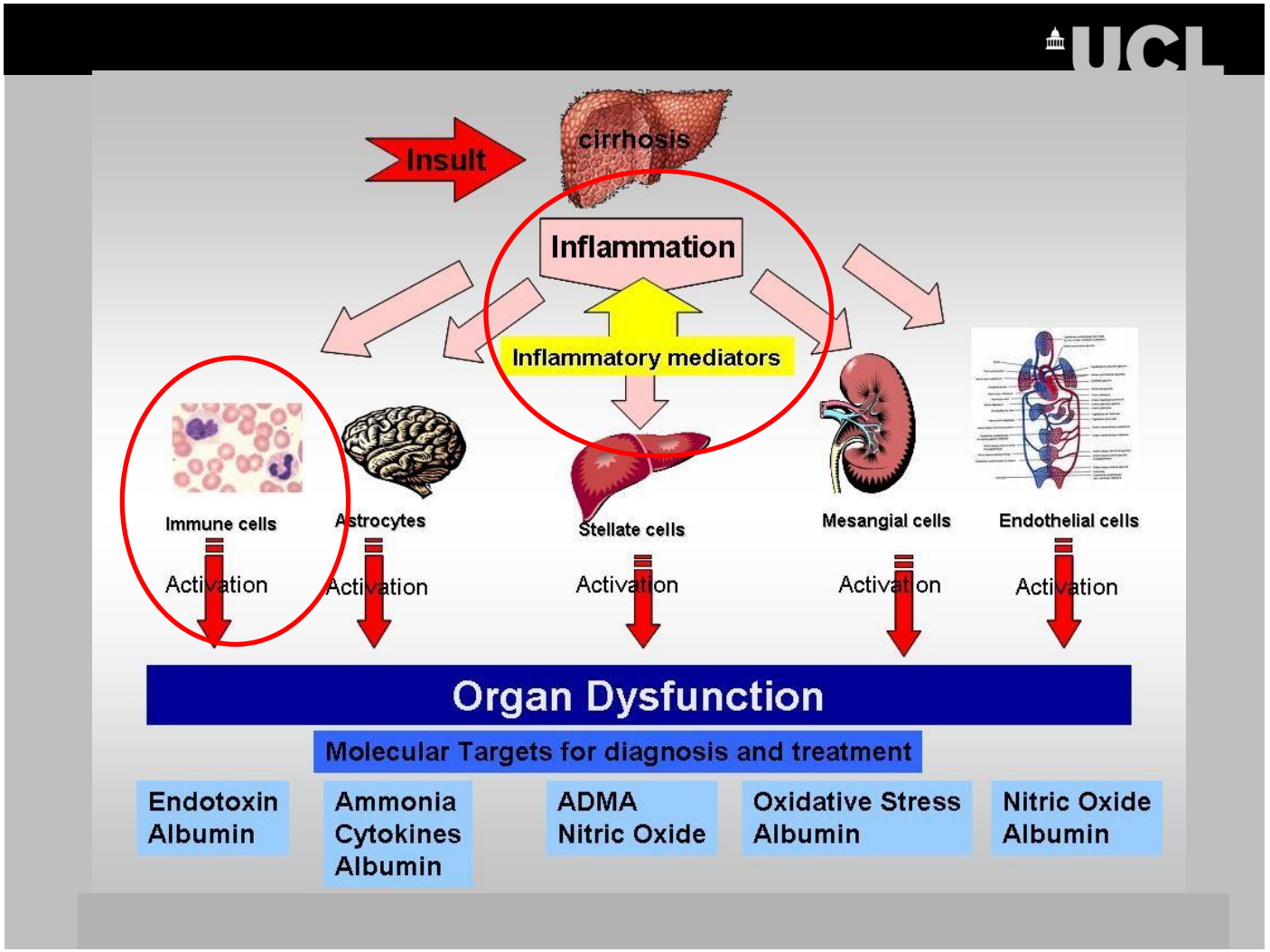
Acute-on-chronic liver failure

William Bernal, Rajiv Jalan, Alberto Quaglia, Kenneth Simpson, Julia Wendon, Andrew Burroughs

THE LANCET




UCL
Insult
cirrhosis
Inflammation
Inflammatory mediators
Immune cells
Astrocytes
Stellate cells
Mesangial cells
Endothelial cells
Activation
Activation
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Activation
Activation
Organ Dysfunction
Molecular Targets for diagnosis and treatment
Endotoxin
Albumin
Ammonia
Cytokines
Albumin
ADMA
Nitric Oxide
Oxidative Stress
Albumin
Nitric Oxide
Albumin

The CANONIC study

- Performed in 29 European hospitals to develop diagnostic and prognostic criteria for ACLF
  - Identify a patient group with a mortality of >15%
- 1353 patients included consecutively

- Hypothesis: Organ failure is important in pathophysiology of ACLF

Moreau, Jalan, Pavesi et al. Gastroenterology 2013

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The CLIF Organ Failure score for diagnosis

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine<2	Creatinine ≥2 <3.5	Creatinine ≥3.5 or renal replacement
Brain (West-Haven)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

Values at Study Enrolment. Highlighted area reflects the definition of each organ failure.

Diagnostic criteria and grades of ACLF

- **No ACLF**
  - Patients with no organ failure
  - Patients with single hepatic, coagulation, circulation or respiratory failure, serum creatinine <1.5 mg/dl and no HE
  - Patient with cerebral failure and serum creatinine <1.5 mg/dl
- **ACLF 1**
  - Patients with renal failure
  - Patients with other single organ failure with
    - serum creatinine ≥1.5 and<2 mg/dl and/or
    - HE grade 1-2.
- **ACLF 2**
  - Patients with 2 organ failures
- **ACLF 3**
  - Patients with 3 or more organ failures

Moreau, Jalan, Pavesi et al. Gastroenterology 2013

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Bacterial infection and active alcoholism are common but GI bleeding is not

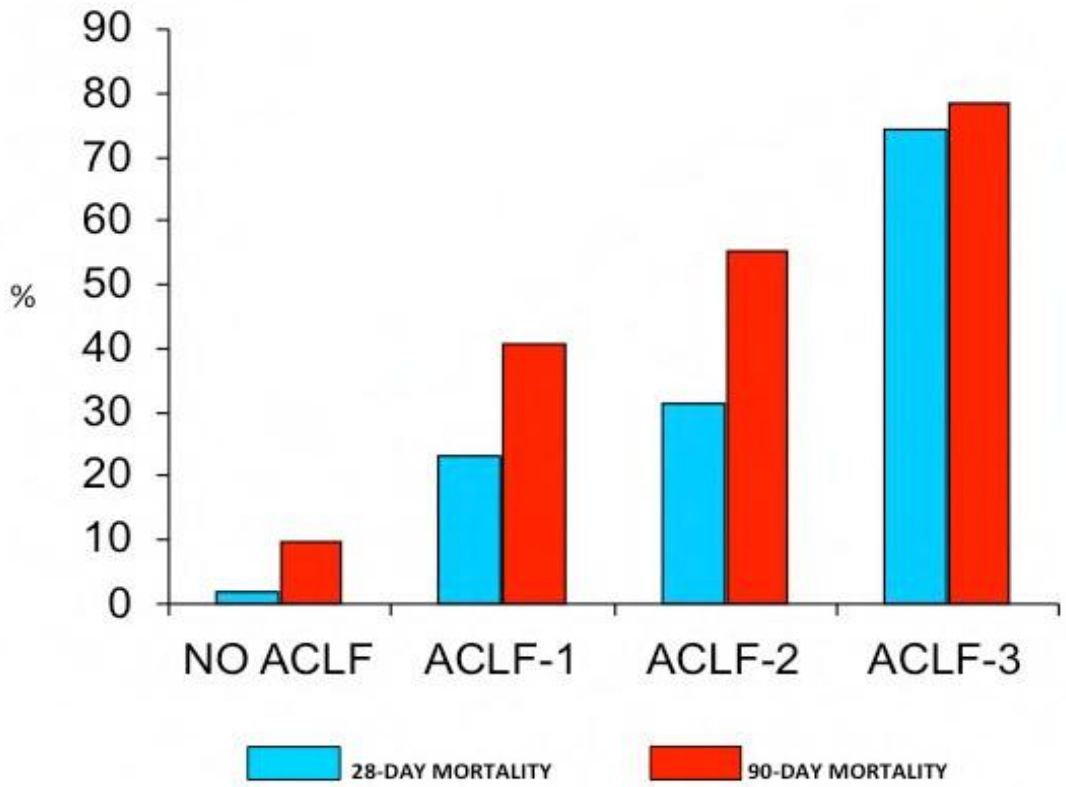
	NO ACLF (n=862)	ACLF-1 (n=213)	ACLF-2 (n=146)	ACLF 3 (n=56)
Bacterial Infection ‡	185 (21.5%)	61 (28.9%)	43 (29.7%)	23 (41.1%)
GI Bleeding	147	26 (12.2%)	21 (14.4%)	12
Active alcoholism* ‡	(17.1%)			(21.4%)
OtherPE †	113 (13.8%)	31 (15.8%)	36 (26.7%)	21 (37.5%)
	27 (3.3%)	16 (8.0%)	12 (8.5%)	3 (5.6%)

* Within 3 months prior to inclusion;
** Other PE: therapeutic paracentesis without albumin, TIPS, major surgery, acute hepatitis and acute alcoholic hepatitis.
*** Bacterial Infections, Active Alcoholism or Other PE' s ;
Overall comparison across ACLF categories. †: p<0.05; ‡: p<0.001

Demographics, etiology and previous decompensation

	NO ACLF (n=862)	ACLF (n=415)	p-value
Demographic Data and Etiology			
Age (SD)	58.2 (12.3)	55.8 (11.7)	0.01
Sex (male)	63.1%	64.1%	ns
Alcoholic cirrhosis	48.4%	58.2%	0.02
Hepatits C cirrhosis	22.4%	14.9%	0.01
Time from First Decompensation to study enrollment			
No prior decompensation	29.8%	26.5%	ns
<3 months	10.8%	15.4%	ns
3 months- 1 year	17.3%	17.0%	ns
<1 year	42.2%	41.1%	ns

28-day and 90-day mortality in ACLF



Moreau, Jalan, Pavesi et al. Gastroenterology 2013

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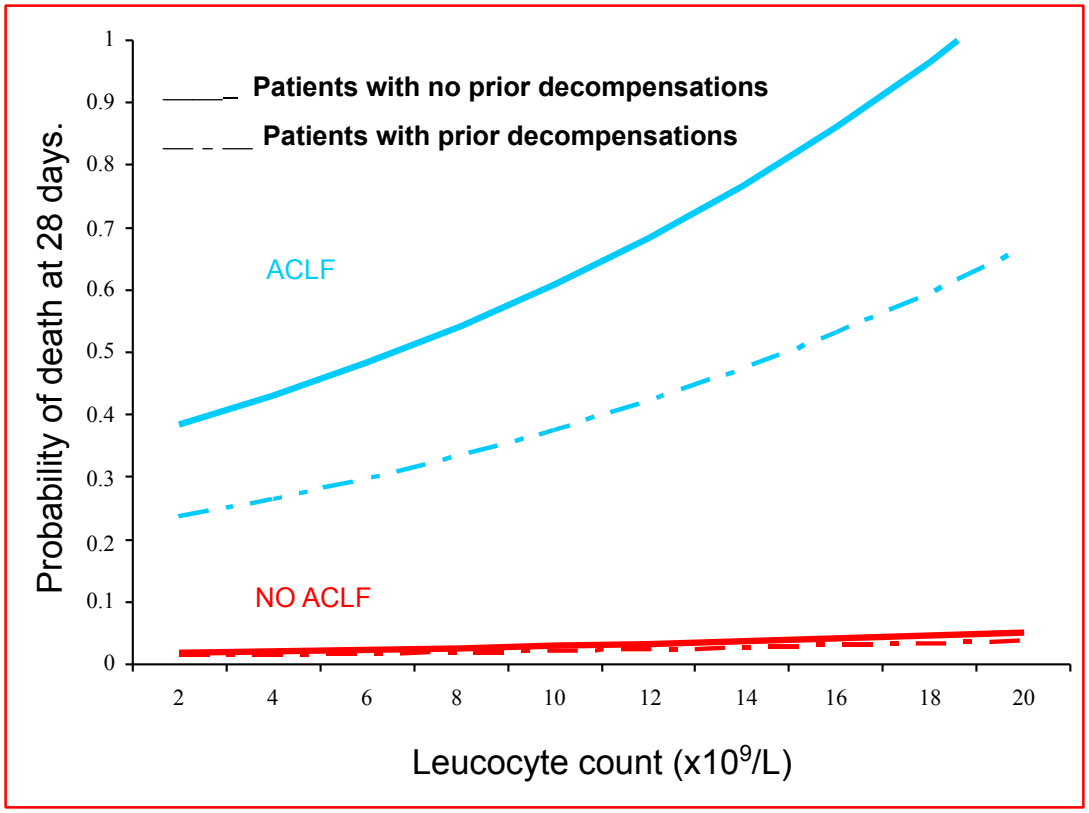
Immunological and Organ sensitization and Tolerance?

Moreau, Jalan, Gines et al. Gastroenterology 2013



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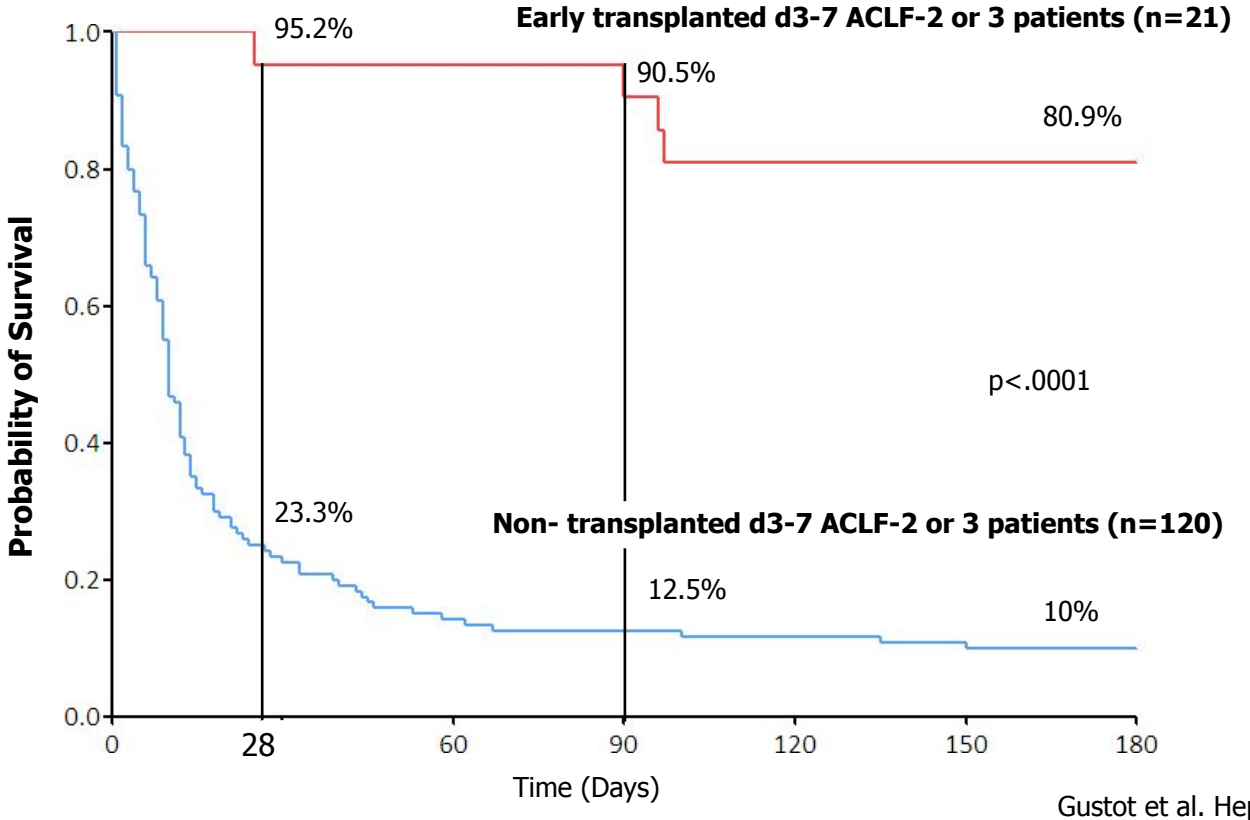
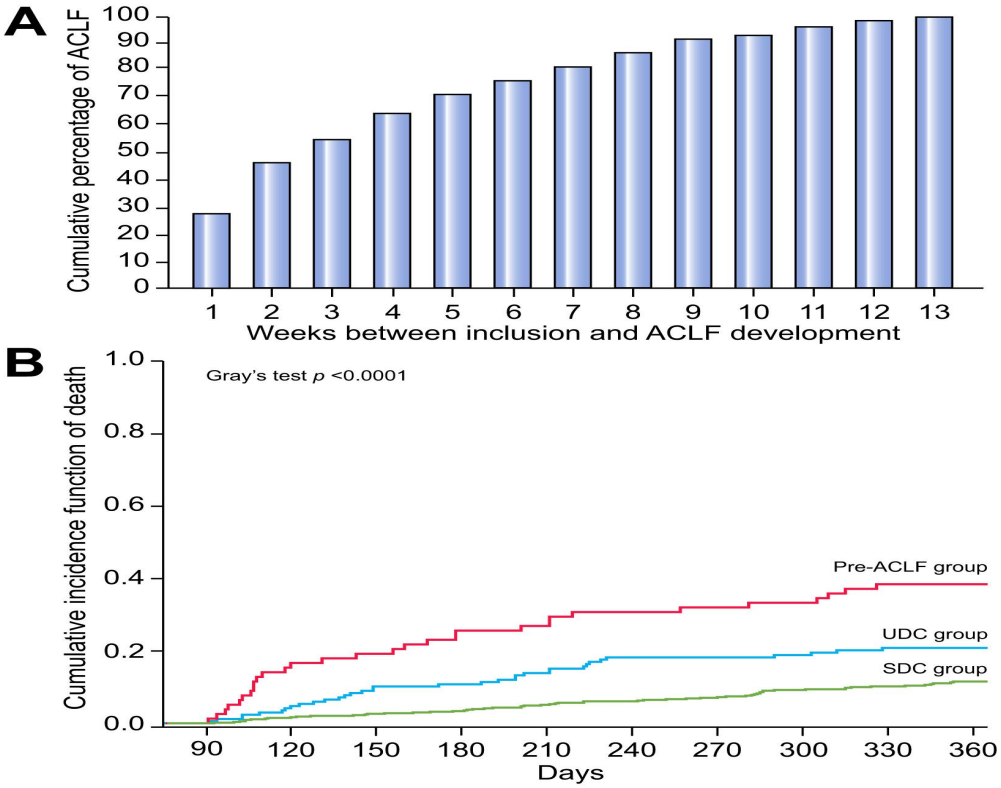
Utility of liver transplantation in ACLF



Gustot et al. Hepatology 2015

Cumulative rates of ACLF and death



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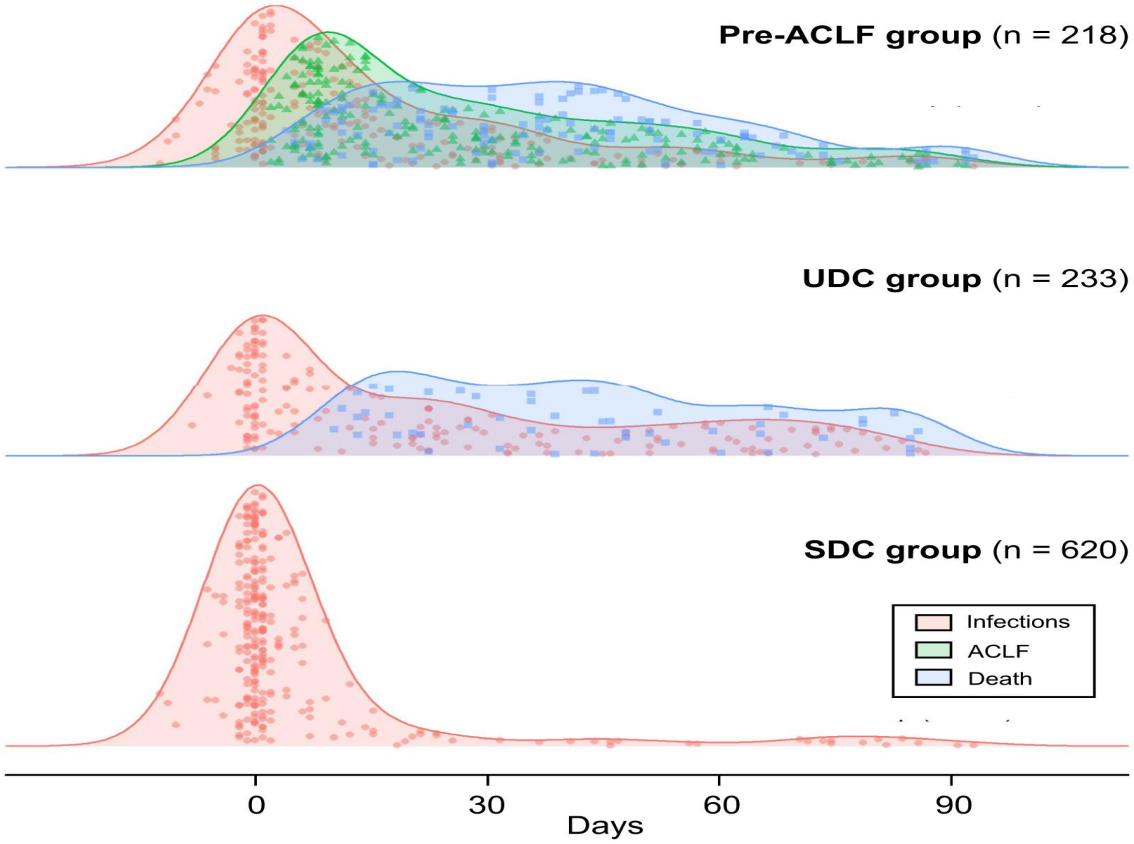
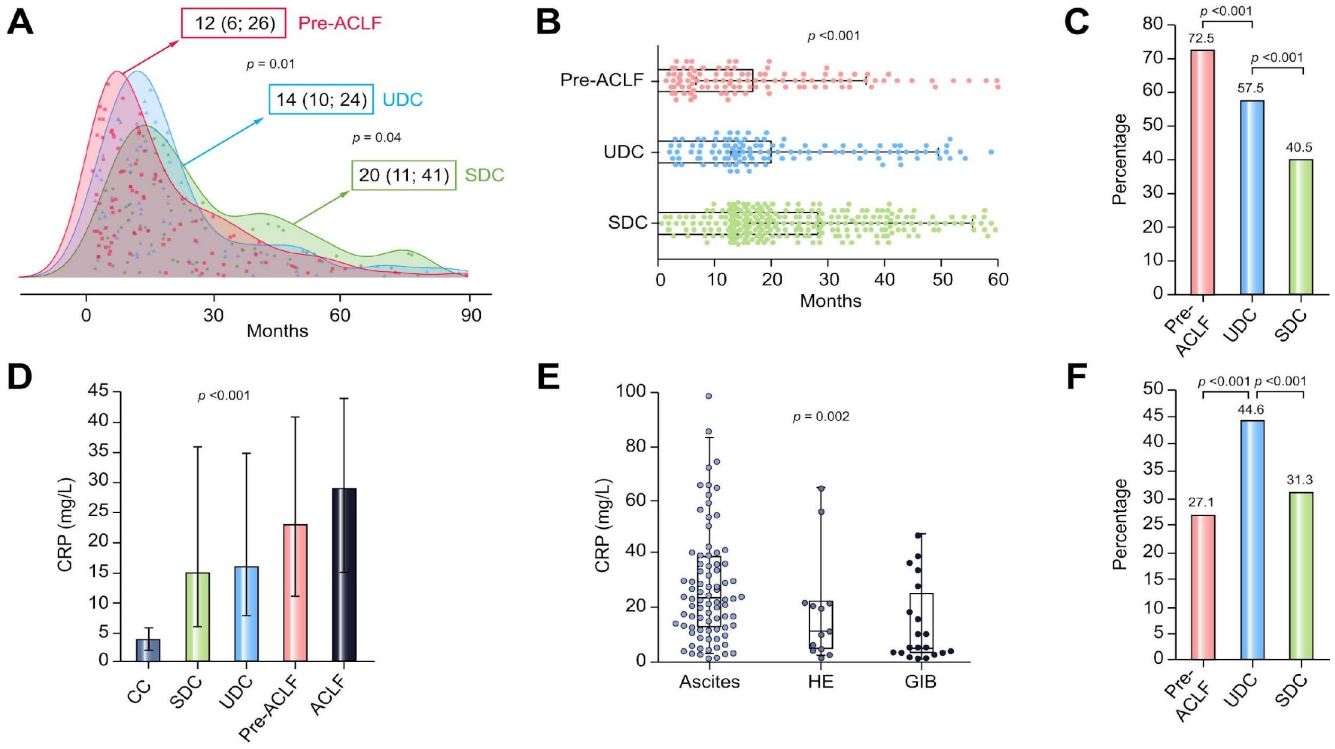
Density curves of events in pre-ACLF, UDC and SDC



Journal of Hepatology 2020 73842-854DOI: (10.1016/j.jhep.2020.06.013)

Liver transplantation, death and surrogates of systemic inflammation and portal hypertension in patients with pre-ACLF, UDC and SDC



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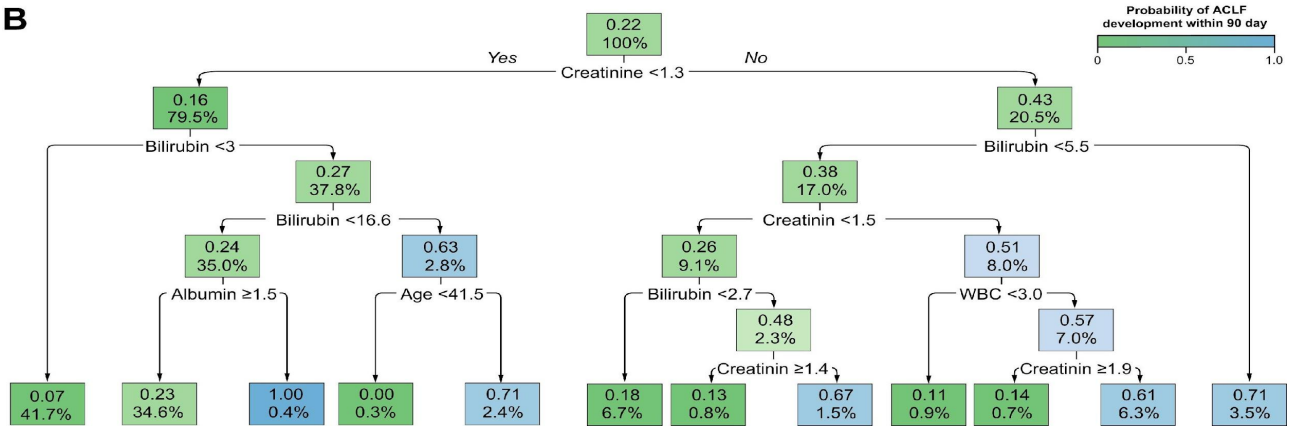
Predictive ability of different scoring systems

A

Severity scores	Derivation set (n = 707)	Validation set (n = 364)
	Harrel' C-index (95% confidence interval)	
CLIF-C ACLF-D	0.76 (0.72-0.80)	0.77 (0.72-0.82)
CLIF-C AD	0.70 (0.66-0.74)	0.75 (0.70-0.80)
MELD-sodium	0.70 (0.66-0.74)	0.74 (0.69-0.80)
MELD	0.70 (0.66-0.74)	0.73 (0.67-0.79)
Child-Pugh	0.64 (0.59-0.68)	0.67 (0.60-0.73)

B



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