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## Clinical Studies

# Extracorporeal Mass Exchange Technology Platform for Temporary Liver Support: A Clinical Feasibility Study on a Device and the Cell Source Primary Human Liver Cells

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ABSTRACT

Clinical feasibility phase-I study data are discussed on the use and the safety of a modular mass exchanger for temporary extracorporeal treatment of liver failure; and the use of the cell source primary human liver cells isolated from discarded transplant organs as a metabolic module in this mass exchanger. This technology platform can be compared with the mass exchange functions of a human placenta before giving birth. The "maternal blood side" can be used with various sources/modules of metabolic support including artificial (e.g. absorber) or biological elements (e.g. cells), separated by membrane compartments. These keep the source of metabolic support from contact with the patient, including the immune cells, while allowing exchange of soluble or protein-bound plasma components for therapy. Each of the multiple independent membrane compartments are bundled towards the in/outlets but interwoven to form a decentralized multi-compartment mass exchanger within an effector module compartment. The use of liver cells as a metabolic module in this compartment results in its function as a bioreactor. A combination with further modules outside of the mass exchanger was demonstrated through a continuous SPAD for detoxification. Nine patients (5 m, 4 f) with a median age of 43 years (range 11-55 years) were treated with a total of 11 metabolic modules in 12 sessions, with overall treatment times ranging from 11 to 216 hours. Patients suffered from acute-on-chronic liver failure (AoCLF, n=3), acute liver failure (ALF, n=3) and primary non-function graft after liver transplantation (PNF, n=3). Treatment resulted in a one-year survival of 78%. The results showed a significant decrease in thrombocytes and fibrinogen. No severe adverse effects were found. One patient (AoCLF) recovered without transplantation and remained alive for the one-year follow-up. Six patients (3 ALF, 2 PNF, and 1 AoCLF) were successfully bridged to transplantation, and two (1 AoCLF, 1 PNF) died within ten days after termination of therapy. Total and conjugated bilirubin, ammonia, urea and creatinine were significantly reduced by the end of therapy, compared to baseline. The MELD score decreased significantly, whereas no significant improvements were observed in APACHE-II, APACHE-III, SOFA and Child-Pugh scores.

**Conclusion:** The mass exchanger technology platform, the Core Module used with primary human liver cells as Metabolic Module, proved to be clinically feasible and safe. Further clinical studies are required to prove the efficacy of such therapies. However, the clinical impact of using human liver cells as a Metabolic Module is limited and a reliable, biocompatible and effective metabolic source is in need**.**



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## Introduction

Orthotopic liver transplantation (LTx) is the standard treatment in cases of severe acute liver failure and has reached 1-year and 5-year survival rates around 83% - 75% [1]. LTx represents major surgery and is associated with life-long immunosuppression and increased risks of infection and cancer, and also with high costs. Furthermore, despite newer approaches including split-liver and living donor liver transplantation, the disparity between the number of patients awaiting transplantation and the number of suitable donor organs is a significant problem. Alternatives to LTx are of interest, such as the development of extracorporeal liver assist devices that may be helpful in bridging to LTx in chronic liver failure, may bridge over surviving an acute-on-chronic episode, or even allow for recovery of the patient in acute liver failure. Ideally, liver support devices should be able to support or replace liver function in providing detoxification, biosynthesis and regulation of metabolic processes. Cell-free (artificial) liver support systems exclusively focus on the removal of accumulated water-soluble and albumin-bound toxins. The effective removal of toxins associated with liver failure has been demonstrated for albumin dialysis systems (Molecular Adsorbents Recirculation System (*MARS*), Single Pass Albumin Dialysis (*SPAD*), and for fractionated plasma separation (*PROMETHEUS*) [2-7]. However, neither of these artificial detoxification systems completely accomplish the complex tasks of synthesis and regulation of the liver.

Advancing the concept of Modular Extracorporeal Liver Support in the presented case of liver cells, the device combines a mass exchanger, as a Core Module, with a metabolic effector, the Metabolic Module. We describe a technology platform that can be compared with the mass exchange functions of a human placenta before a mother giving birth. This technology platform can be used with various sources of metabolic support (modules), instead of the maternal blood support of a placenta. Separated by membranes, various sources, or modules, of support can be incorporated: artificial (e.g. absorber), or biological elements (e.g. human cells). The use of liver cells in one compartment results in a function as a bioreactor. A combination with further modules in the circuit, but outside of the mass exchanger was demonstrated; a continuous *SPAD* was incorporated for additional artificial detoxification [8]. Given sufficient viable and biocompatible liver cells, as well as effective mass transfer conditions, cell-based bioartificial liver support with a mass exchanger that allows the separation of the patient from the system might be able to provide important metabolic functions of the liver. Bioartificial liver systems based on porcine liver cells have been evaluated in phase-I studies, and in one large prospective randomized multi-center-controlled trial, an improved 30-day survival in the subgroup of fulminant/sub-fulminant hepatic failure patients was demonstrated [9-11]. However, the risks of xenogeneic infections and unwanted effects due to metabolic incompatibility have limited the broader use of liver support systems based on porcine liver cells [12, 13]. Human-derived hepatoma cell lines were used in an extracorporeal liver assist device in a pilot-controlled trial in patients with acute liver failure, but no significant effect on survival was observed [14].

We demonstrate an alternative cell source, or Metabolic Module, for liver support: primary human hepatocytes isolated from explanted organs that are unsuitable for transplantation - a source that is available as approximately 10% of donor livers are discarded due to steatosis, cirrhosis, traumatic injury, or other reasons (eurotransplant.org, americantransplantfoundation.org).

The objective of this work was to gain data during clinical applications when evaluating the mass exchanger, the Core Module, with liver cells for extracorporeal treatment of liver failure in patients with ALF, AoCLF and PNF. Two aspects were evaluated, the feasibility and safety of using the mass exchanger device, and the use of the cell source primary human liver cells isolated from discarded transplant organs as a Metabolic Module in the mass exchanger. Concerns of using this cell source are briefly discussed.

## Patients and Methods

### Mass Exchanger Module

The investigated technology includes the Core Module, a hollow fiber membrane-based multi-compartment mass exchanger (*BR 600, StemCell Systems, Berlin, Germany*), providing integral oxygenation in case of using cells in the device. Separated by the membranes in this Core Module, we combined cells as a source, or module, for providing biological support. The core module, with housing made from two-component polyurethane (PUR 725A/B, Rohm&Haas, Frankfurt, Germany), accommodates a densely packed and interwoven array of hydrophilic microporous polyether sulphone hollow fiber capillary membranes (mPES, Membrana, Wupertal, Germany). These also serve as an immune-barrier and keep the source of metabolic support from contact with the patient´s cells, including the immune cells, while allowing exchange of soluble or protein-bound plasma components over the membranes. To form the multi-compartment mass exchanger, three independent capillary membrane bundles, each forming a mass exchange compartment, are interwoven. Two bundles for an "arterial" circulation, one for a "venous circulation", and (as we used viable cells in the Core Module) one made of hydrophobic oxygenation membranes (MHF200TL, Mitsubishi, Tokyo, Japan). All capillary fibers in the bundles are interwoven to give an evenly spaced 3D array of all compartments, achieving decentral mass exchange with low substance gradients between all membrane fibers in all compartments.

### Cells as a Metabolic Module

(n=11) Mass exchangers were charged with primary human liver cells obtained from organs explanted for LTx, but discarded due to steatosis (n=6), fibrosis/cirrhosis (n=2), steatosis combined with fibrosis/cirrhosis (n=3), and operated in a mass exchanger perfusion device (BR600 Perfusion System, *StemCell Systems, Berlin, Germany*). The median age of donors (4 female, 7 male) was 51 years with an interquartile range (IQR) of 49-64 years. The median cold ischemic time prior to isolation was 10 (IQR= 7.8-13.0) hours. Organs were preserved in *University of Wisconsin (UW)* solution (n= 6) or in Histidine-Tryptophan-Ketoglutarate (HTK) solution (n=5). Liver cells were isolated via a five-step perfusion procedure with collagenase digestion (Collagenase P, Roche Diagnostics, Mannheim, Germany), as described previously [15]. The digestion time ranged from 12 to 34 minutes with a median of 20 minutes (IQR= 18.5-30 minutes). The percentage of viable cells was determined by trypan blue exclusion test using a *Neubauer* hemocytometer and ranged from 45% to 70% with a median of 62%

(IQR= 55.1-65%). The median inoculated cell volume was 470 ml (IQR= 438-478 ml) and ranged from 340 ml to 765 ml. Cell modules were kept *in vitro*, perfused in a stand-by mode for a median of 9 days (IQR= 7-11 days) before clinical application. The culture conditions and long-term cell performance of hepatocytes have been described previously [16]. During stand-by times of cell modules, daily production of urea and ammonia, as well as sorbitol and galactose uptake, were determined.

**Device Configuration**

The mass exchanger Core Module with cells (primary human liver cell module) were connected to the patient`s blood circuit via a continuous plasma separation filter (Plasmaselect 0.4, Braun AG, Melsungen, Germany). The extracorporeal blood circuit had a total volume of approximately 110 ml and was perfused with a flow rate of 150-180 ml/min. The total volume of the plasma circuit with the mass exchanger was approximately 1200 ml, and circulated with a perfusion rate of 200-250 ml/min. The patient was connected to the blood circuit by a double-lumen dialysis catheter, into either the internal jugular or the femoral vein. Priming of the extracorporeal blood circuit was performed with isotonic saline solution, whereas 5% human albumin solution was used for priming the system plasma circuit prior to connecting the system to the patient. Anticoagulation was achieved by heparin infusion, and the activated clotting time (ACT) was adjusted to 160-180 s.

**Detoxification Module**

The plasma circuit was combined with a further module, providing additional detoxification; combining single-pass albumin-dialysis (*SPAD*) and continuous veno-venous hemodiafiltration (*CVVHDF*), using an HdF-100S highflux polysulfon membrane filter (*Fresenius medical care AG, Bad Homburg, Germany*) in the perfusion circuit, as described previously [17, 18].

**Study Protocol**

The study protocol was developed in accordance with the revised Helsinki declaration of 1975 and approved by the local Ethics Committee (Institutional Review Board) of the Charité - Medical Faculty of the Berlin Universities, Berlin, Germany. A written informed consent was signed by each patient or their legal representative. The inclusion criteria were ALF, AoCLF and PNF after transplantation. Additional inclusion criteria were hepatic encephalopathy (HE) ≥ II and INR ≥3.7, or the need for substitution of fresh frozen plasma (FFP) to maintain an INR < 3.7. Exclusion criteria were pregnancy, generalized neoplastic disease, pre-existing severe cardio-respiratory disorder, systemic or intracranial bleeding, brainstem herniation, irreversible brain damage, severe hypotension despite vasopressor agents, disseminated intravascular coagulation (DIC) or uncontrollable infections. According to the study treatment schedule, patients listed for LTx were treated with the device combination in the liver transplantation intensive care unit of the Department for Surgery Campus Virchow until LTx was performed (bridging to LTx). Patients not listed for LTx were treated with three therapy sessions (duration of 72 hours each) within a total period of 15 days. Each treatment session was followed by a treatment break period of 48 hours.

**Patient Monitoring, Data Analysis and Statistics**

The following parameters were determined at the start of each therapy and then at regular time intervals until the end of treatment: heart rate (HR), mean arterial pressure (MAP), body temperature, ventilation status, grade of hepatic encephalopathy (HE), serum electrolytes, urea, creatinine, total bilirubin, conjugated bilirubin, albumin, protein, glucose, ammonia, lactate, arterial blood gases, international normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen, antithrombin-III (AT-III), hemoglobin, hematocrit, erythrocytes, thrombocytes and leucocytes. Additionally, Child-Pugh score, sequential organ failure assessment (SOFA) score, model of end stage liver disease (MELD) score, acute physiology and chronic health evaluation (APACHE) score were determined.

**Data Analysis and Statistics**

SPSS software (Version 13.0, SPSS Inc., Chicago; Illinois) and R software (Version 2.8.1, The R Foundation for Statistical Computing) were used for statistical analysis. Due to small sample sizes, normal distribution could not be assumed, and consequently, descriptive parameters were expressed in terms of median, $25^{th}$, and $75^{th}$ percentiles. Biochemical parameters before and after therapy were tested with the Wilcoxon-test for non-parametric paired data. Significance was defined at p-values less than or equal to 0.05.

**Results**

**Patients**

Nine patients (5 male, 4 female) with a median age of 43.0 (IQR= 25.5-49.4) years were enrolled in the phase-I study. Liver failure was acute (n=3), acute-on-chronic (n=3) and caused by primary-non-function post liver transplantation (n=3). The etiology of ALF included M. Wilson (n=1), Hepatitis A in combination with valproat intoxication (n=1) and was unknown in one case. AocLF was based on Hepatitis B (n=1), Hepatitis C in combination with alcohol-intoxication (n=1), and acetaminophen and alcohol abuse (n=1). PNF after liver transplantation occurred due to initial non-function (n=1), autoimmune hepatitis (n=1) and chronic ductopenic rejection with fibrosis, chronic cholestasis and vasculopathy (n=1). The treatment of the patient with PNF after LTx due to chronic rejection (PAT-1) has been published in a detailed case report [16]. Six patients were listed for high urgency LTx.

In total, 12 sessions were performed, with treatment times ranging from 11 up to 79 hours with a median of 72 hours (IQR= 28-72 hours). The number of treatments per patient ranged from one to three sessions. Six treatments were aborted due to LTx, whereas the remaining 6 treatments were performed over 72 hours. The overall treatment time ranged from 11 to 216 hours.

**Characteristics of the mass exchanger Core Module used with human primary liver cells as Metabolic Module**

The performance of the primary human cell loaded mass exchangers before clinical use was evaluated by the measurement of urea, ammonia, galactose and sorbitol elimination. The daily urea production ranged from 50 mg to 861 mg with a median of 296 (IQR= 165-488) mg. The

median ammonia production per day was 172 (IQR= 125-244) mg ranging from 66 mg to 688 mg. Galactose uptake per day ranged from 3 mg to 679 mg and had a median of 540 (IQR= 234-626) mg. The daily consumption of sorbitol showed a median of 620 (IQR= 269-726) mg and ranged from 11 mg to 787 mg.

**Safety of the mass exchanger Core Module using primary human liver cells as Metabolic Module**

The treatment sessions were well tolerated by all patients and no severe adverse effects were observed. No significant increase of white blood cells, CRP or body temperature were seen. However, hypotension in the initial phase of treatment with a MAP < 60 mmHg was noted during three sessions (PAT-3, 4, 5a) but could be treated easily with the administration of norepinephrine over the initial phase of treatment. Furthermore, a significant decrease of thrombocytes and fibrinogen was noted, whereas aPTT was significantly elevated after treatment due to heparinization.

**Effects of treatment on hemodynamics**

The median MAP was 77 (IQR= 70-88) mmHg at the beginning of treatment and showed no significant difference when compared to MAP at the end of therapy (median, 75 mmHg; IQR= 66-83 mmHg). A reduction of dopamine/dobutamine dosage was observed during four treatments (PAT-1, 2, 3, 9), whereas, during one treatment (PAT-5b), the dosage of dobutamine had to be increased. Norepinephrine dosages could be reduced during two treatments (PAT-2, 9), whereas dosages had to be increased in five treatments (PAT-3, 4, 5b, 7b, 7c). The heart rate increased slightly when connecting all patients, but not significantly, from baseline 102 (IQR= 97-111) /min to 110 (IQR= 87-113) /min.

**Effects of treatment on hematologic and coagulation parameters**

A significant decrease of thrombocytes from 45 (IQR= 38-78) /nl to 26 (IQR= 17-37) /nl was observed during treatments, and, as a result, packed thrombocytes had to be transfused during eight treatments. Erythrocytes showed a non-significant decrease from 3.2 (IQR= 2.9-4.1) /pl to 3.1 (IQR= 2.8-3.7) /pl. An increase in the use of packed red blood cells was observed during six treatments (PAT-1, 2, 5a, 5b, 7b, 7c). INR increased non-significantly from 1.9 (IQR= 1.7-2.5) at baseline to 2.1 (IQR= 2-2.6) at the end of therapy. The administration of fresh frozen plasma had to be increased during six treatments (PAT-1, 2, 5b, 6, 7c, 9). Fibrinogen decreased significantly from 126 (IQR= 104-216) mg/dl to 93 (IQR= 75-145) mg/dl.

**Effects of treatment on liver function, renal function, electrolytes and metabolic parameters**

Total bilirubin showed a significant decrease of 18.2% (IQR= 10.1%-22.9%), whereas the reduction of conjugated bilirubin (median= 17.1%; IQR= 3.5%-26.3%) was slightly above the level of significance. Median ammonia decreased significantly from 112 (IQR= 92-163) µmol/dl to 62 (IQR= 55-112) µmol/dl resulting in a reduction of 38% (IQR= 26.2-45.1%). Initial median creatinine was 1 (IQR= 0.8-1.7) mg/dl and decreased significantly to 0.6 (IQR= 0.5-1.2) mg/dl, resulting in a reduction of 36.9% (IQR= 8.6%-44.6%). Hepatorenal syndrome with serum creatinine >1.5 mg/dl at the start of therapy was observed in three patients (PAT-1, 3, 9). The 24h-urinary-output did not change significantly, although a slight increase from 307 (IQR= 18-1589) ml at baseline to 670 (IQR= 25-2246) ml at the end of treatments was observed. Urea was reduced significantly from 87 (IQR= 78.5-125) mg/dl to 56 (IQR= 42-69) mg/dl, with a median reduction of 41.8% (IQR= 18.2%-51.7%). Electrolytes showed median reductions of 1.1% (sodium), 3.1% (potassium) and 8.8% (calcium) without reaching the level of significance.

**Effects of treatment on neurological function**

As most patients were analgo-sedated during ten treatments, an improvement of neurological function could only be judged in two patients. PAT-1 showed an improvement of Glasgow Coma Scale (GCS) from 3 to 14 points, whereas PAT-4 showed no change of GCS.

**Effects of treatment on ICU scores**

The initial APACHE-II score ranged from 4 to 34 with a median of 22 (IQR= 18-26) and showed no significant change compared to the end of therapy (median, 19; IQR= 14-24). A decrease was observed during seven treatment sessions, whereas in the remaining five treatment sessions, the APACHE-II score remained equal (n=2) or was elevated after treatment. The median initial APACHE-III score was 85 (IQR= 77-88) and decreased to a median of 69 (IQR= 61-87; p=0.167). The SOFA score showed a reduction in six treatment sessions, an increase in four sessions, and no change in the remaining two sessions. The median SOFA score at the start of therapy was 15 (IQR= 14-19) and showed no significant change compared to after therapy (median, 15; IQR= 12-21). The median Child-Pugh score was 11.5 (IQR= 11-12) before therapy and increased slightly to 11 (IQR= 11-11). The median MELD score decreased significantly from 25 (IQR= 24-28) to 23 (IQR= 19-26), resulting in a reduction of 16.2% (IQR= 0.9-20.0%).

**Effects of treatments on bridging to LTx/survival**

All patients with ALF (n=3), two patients with PNF, and one patient with AoCLF were successfully bridged to transplantation. A patient with AoCLF (PAT-9) recovered without transplantation. Two patients (AoCLF, n=1; PNF; n=1) died within ten days after therapy due to multi organ failure (PAT-5, PAT-7). Two patients (PAT-2, PAT-8) died within 30 days after LTx due to sepsis and heart failure resulting in a 30-day survival of 55.6% (5/9). Three patients (PAT-3, PAT-4, PAT-6) died within one year after LTx due to cerebral edema, heart failure and acute lymphomblastic leukemia. None of the deaths were related to treatment. One-year survival was achieved by two patients (PAT-1, PAT-9).

**Discussion**

The treatments with the mass exchanger as Core Module and primary human liver cells as a Metabolic Module were well tolerated by all patients. Signs of systemic inflammation or allergic reactions did not occur and no serious adverse events, or fatal, or life-threatening effects were observed. However, transitory hypotension in the initial phase of treatment was seen during three treatment sessions. This is a well-known and manageable effect that has often been described during treatments with conventional extracorporeal support systems. Treatments were associated with a significant decrease of thrombocytes (52%, p<0.01)

and fibrinogen (22%, $p<0.05$). A previous matched pair analysis with the mass exchanger charged with porcine hepatic cells revealed that a decrease of platelet count was also seen in most patients with liver failure under standard ICU treatment [9]. Furthermore, a decrease was observed for ions (sodium, 1.1%; potassium, 3.1%; calcium, 8.8%), protein (0.9%), hemoglobin (7%), hematocrit (8.7%) and ATIII (15.8%). These reductions can, presumably, be an effect of the plasma separation- and dialysis membrane filter or may be caused by dilution effects.

Therapy resulted in a significant reduction of total bilirubin (median 18%), conjugated bilirubin (median 17%), ammonia (median 38%), urea (median 42%) and creatinine (median 37%). These removal rates of protein-bound and water-soluble substances are in the range of other cell-based and non-cell-based liver support systems. An earlier phase I study of the mass exchanger Core Module charged with primary porcine liver cells as Metabolic Module resulted in an average reduction of 21% for total bilirubin and 29% for ammonia. In a phase I trial (n=7) of a primary porcine liver cells device from the Academic Medical Center (AMC) of Amsterdam, the average decrease of total bilirubin was 31% (range 3-62%), whereas for ammonia, an average decrease of 44% (range 9-66%) was observed [10]. The porcine cell based *HepatAssist* system used in a trial with 13 patients had reduction rates of 29% for bilirubin and 23% for ammonia (calculated from means) [19]. A retrospective analysis of MARS detoxification treatments of 15 patients revealed an average total bilirubin reduction of 18.2% and an ammonia reduction of 7.1% [20]. A more exact comparison of removal rates of devices can be achieved by analyzing the ratio of removal rates and pre-treatment values. The treatments showed a strong significant correlation ($R^2=0.9$; $p<0.001$) of bilirubin reduction and pre-treatment bilirubin levels. In comparison to a MARS study that analyzed the binding of bilirubin to albumin in liver failure patients, the regression line of the module combination had a higher slope indicating a somewhat higher removal rate of bilirubin [21]. In contrast, the correlation of the molar bilirubin/albumin ratio and the pre-treatment molar bilirubin/albumin ratio was not significant for our treatment sessions ($R^2=0.5$; $p=0.086$; slope=0.49), whereas MARS treatments showed a stronger correlation and a higher slope for these parameters ($r^2=0.76$, slope=0.64) [21]. Considering the small sample sizes, the performance of MARS and our system concerning the bilirubin removal appears to be similar. This comparison is in concordance with an *in vitro* comparison of MARS and SPAD that showed no significant differences for the removal of bilirubin, bile acids, urea and creatinine when MARS is performed in continuous veno-venous hemodialysis mode [22].

Treatment based on primary human liver cells in the present study resulted in a significant reduction of the MELD score, whereas the Child-Pugh score, APACHE-II score and SOFA score showed only a non-significant improvement. In a randomized controlled study of MARS treatments of AoCLF-patients, a significant reduction of the MELD score was observed in the MARS group (16.5 to 14.1, n=9), as well as in the group of patients with standard medical treatment (19.4 to 14.5, n=9) [23]. Also, a retrospective analysis based on the MARS-registry showed a significant reduction of the MELD score from 30.4±9.3 to 22.1±8.6 in 51 AoCLF patients [24]. A pilot-trial of the PROMETHEUS-System in eleven patients showed no significant changes of Child-Pugh and APACHE-II score [7]. No clinical scores were documented in a phase I study of the AMC system with twelve ALF-patients, but the authors reported that improved hemodynamics, diuresis and neurological state were observed [25].

Seven of the nine patients treated could be bridged to LTx (n=6) or recovered without LTx (n=1), resulting in a 30-day survival of 78%. To prove whether a device is associated with an improved outcome in liver failure is difficult, as potential confounders, e.g., the kind of liver failure, the clinical pre-treatment status of patients, or the timing of intervention, must be considered [26]. Meta-analyses have been performed to evaluate beneficial and harmful effects of artificial and bioartificial support systems for acute and acute-on-chronic liver failure. A *Cochrane Review* included twelve trials on artificial or bioartificial liver support systems versus standard medical treatment (483 patients), and two trials comparing different artificial support systems (105 patients) [27]. Mortality rate in the control group was 51% (123/239). In comparison to standard medical therapy, liver support systems showed no significant effect on mortality or bridging to liver transplantation, whereas a significant beneficial effect on hepatic encephalopathy was indicated. However, in subgroup analyses, artificial liver support systems appeared to reduce mortality by 33% only in acute-on-chronic liver failure but not in acute liver failure.

The role of a mass exchanger charged with primary human liver cells from discarded donor organs as a Metabolic Module remains controversial. Previous studies using artificial liver support systems showed that removal of certain substances during liver failure do not require cell containing systems. As the modular system in this feasibility study showed similar removal rates of bilirubin and ammonia in comparison to clinical studies with MARS, the detoxification capacity of the biological/bioartificial component has to be valued critically. However, the mass exchanger charged with primary human hepatic cells presumably provides important but largely unknown regulative processes and synthetic functions that might contribute to a better outcome to be studied in a large prospective randomized study. The efficacy of a cell-based bioartificial liver depends on the biocompatibility of the cell source, the mass of metabolic active hepatocytes and the mass exchange rate: Depending on theoretical considerations and on the volume of the inoculated cell mass, the cell viability, and the perfusion rate, it can be assumed that the functionality of the bioartificial liver achieved around 5-10% of the normal liver function [28].

Additionally, lower metabolic activity of hepatocytes may also be due to a reduced quality of the cell source, as cells were isolated from discarded donor organs and showed a high variance of urea synthesis; which has been proposed as a suitable parameter for evaluation of cell performance [16]. The use of primary human liver cells originating from discarded organs must be valued critically, since these cells show activity limitations and performance variability depending on donor organ quality. Thus, the cell source issue considering the use of porcine-, or primary human- versus other cells, e.g. *in vitro* expanded stem cell-derived hepatic cells must be discussed. Presumably, bioartificial liver support with liver cells will not succeed until highly metabolically active cells with human metabolism can be *in vitro* expanded in a sufficient amount. If a Metabolic Module avoiding *in vitro* cultured cells would be available, some of the discussed issues could be addressed. Alternatives to cultured cells could be discussed using extracorporeally-perfused livers, or a volunteer donating his time as a Metabolic Module on the mass exchanger, where the mass exchanger would also act as immune-

barrier preventing blood cell interactions.

However, the use of the mass exchanger as Core Module and primary human liver cells as Metabolic Module proved to be feasible and safe. Treatments were associated with a significant reduction of bilirubin, ammonia, urea and creatinine and resulted in a 30-day-survial of 78%. However, using primary human liver cells from discarded transplant organs remains controversial. The modular concept discussed provides a technical basis for new and innovative metabolic sources, modules, for extracorporeal liver support that should eventually be available. Identifying an appropriate metabolic source, prospective randomized controlled studies and multivariate analysis regarding important confounding parameters, as pre-treatment ICU scores, cause of hepatic failure and time of intervention, are then of interest to evaluate the efficacy of such liver support devices.

## Note

One of the cases analysed here was previously published as case report (J Hepatol. 2003; 39[4]: 649-653), details on this case can be found in this article.

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## Disclosures

J. Gerlach holds shares of StemCell Systems, Berlin, Germany, which prototyped the mass exchangers.

## Abbreviations

SPAD: single pass albumin dialysis;
CVVHDF: continuous veno-venous hemodiafiltration (CVVHDF);
AoCLF: acute-on-chronic liver failure;
MARS: Molecular Adsorbents Recirculation System;
ELAD: extracorporeal liver assist device;
ALF: acute liver failure;
PNF: primary non-function graft after liver transplantation;
LTx: liver transplantation;
IQR: interquartile range;
UW: University of Wisconsin;
HTK: Histidine-Tryptophan-Ketoglutarate;
ACT: activated clotting time;
FFP: fresh frozen plasma;
DIC: disseminated intravascular coagulation;
HR: heart rate;
MAP: mean arterial pressure;
HE: grade of hepatic encephalopathy;
INR: international normalized ratio;
aPTT: activated partial thromboplastin time;
CRP: C-reactive protein;
AT-III: antithrombin-III;
SOFA: sequential organ failure assessment;
MELD: model of end stage liver disease;
APACHE: acute physiology and chronic health evaluation;
AMC: Academic Medical Center;
ICU: intensive care unit;
PAT: patient;
BR: bioreactor

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