Extracorporeal Liver Support Devices for Listed Patients

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Abbreviations: ACLF, acute-on-chronic liver failure; ALF, acute liver failure; AMC-BAL, Academic Medical Centre Bioartificial Liver; ELAD™, Extracorporeal Liver Assist Device™; ELSD, extracorporeal liver support device; HE, hepatic encephalopathy; HSA, human serum albumin; HVP, high-volume therapeutic plasma exchange; ITT, intention to treat; Li-ALS, Li-Artificial Liver Support; MARS®, molecular adsorbents recirculating system; n.s., no significant difference between groups; PP, per protocol; SMT, standard medical therapy; SPAD®, single pass albumin dialysis; SRBAL, Spheroid Reservoir Bioartificial Liver; TPE, therapeutic plasma exchange; UCL-LDD, University College London-Liver Dialysis Device.

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ABSTRACT

An alternative to liver transplantation for patients with liver failure remains an unmet need. In acute liver failure, the ideal extracorporeal liver support device would replace the functions of the failing liver in order to permit spontaneous recovery, given the incredible regenerative potential of the liver, negating the need for transplantation. In acute-on-chronic liver failure, an extracorporeal liver support device would ideally support hepatic function until recovery to liver function prior to acute decompensation or until liver transplantation. In decompensated cirrhosis, an extracorporeal liver support device could again be used to support hepatic function until transplant. In addition, extracorporeal liver support devices may have potential to treat the multi-organ failure that accompanies liver failure including hepatic encephalopathy, renal failure and immune dysfunction or indeed potential to promote liver regeneration. Creation of an extracorporeal bioartificial liver able to completely replace liver function remains an unmet need. This review will describe a number of technologies suitable for clinical trials in man, which have resulted from decades of engineering and biological research to develop a bioreactor able to adequately sustain functional hepatocytes. In addition, this review will describe artificial liver support devices, primarily designed to replace the detoxifying functions of the liver and consider the current data available or studies required to support their use in liver failure patients on the transplant waiting list.

INTRODUCTION

Mortality in patients with liver failure who cannot be rescued with liver transplantation remains high despite improvements in supportive care (1). The fundamental thinking behind the use of extracorporeal liver support devices (ELSD) is the idea that if the patient's liver and extrahepatic organs can be supported long enough, recovery should be possible, because of the regeneration potential of the liver (2). Alternative aims of ELSD may be to 'bridge' liver failure patients to liver transplantation or to support patients with end-stage liver disease while on the waiting list for transplantation. Additional therapeutic goals may be to treat end organ dysfunction such as hepatic encephalopathy (HE), renal failure or immune

dysfunction (3). Finally, as one understands the pathophysiological basis of regeneration or its inhibition better, ELSD may be used to target particular molecules to enhance this regenerative process. Depending upon whether the liver failure occurs on the background of a previously healthy liver or in patients with underlying chronic liver disease, the conditions are referred to as acute liver failure (ALF) or acute-on-chronic liver failure (ACLF) respectively (Table 1).

ALF is a rare disease and is defined as the occurrence of HE in patients with severe acute liver injury within 6-months of the onset of symptoms (4). From a pathophysiological perspective, patients with ALF are the perfect group of patients likely to benefit from ELSD because recovery is likely to return the patient to their pre-liver failure state, in which there is no pre-existing liver pathology. It is becoming clear that in addition to providing support for hepatic function, modulation of hepatic and systemic inflammation will be important to prevent deaths either from an exaggerated inflammatory response or infection (3).

ACLF is much more common than ALF and typically occurs in patients with cirrhosis (5). The condition is characterized by acute deterioration of a cirrhotic patient with or without a recognized precipitating event, associated with organ failures and high mortality rates (6). Data from prospective studies are now available that allow accurate, sequential assessments of patients, which provide prognostic information. The CLIF Consortium organ failure score is used for diagnosis of the syndrome (Table 2) and the CLIF Consortium ACLF score for defining the prognosis (7). A pre-ACLF group has now been identified, which will allow studies of ELSD to prevent the occurrence of ACLF in susceptible patients (8). Systemic inflammation is the key pathophysiological factor that drives the syndrome making this a particular target of ELSDs (9). The aim of ELSDs in patients with ACLF is to support hepatic function during acute decompensation until recovery to baseline liver function and/or liver transplantation.

Decompensated cirrhosis is pathophysiologically different and typically represents patients that have end-stage cirrhosis with varying degrees of end-organ dysfunction. In this group of patients, ELSD is aimed at supporting them until liver transplantation.

This review describes the state of the art about the types of ELSDs that are available, the results of the large and important clinical trials and the new ELSDs that are in or about to enter clinical trials. The reported human, randomised, controlled, clinical trials of ELSDs, for which survival was the primary outcome, are given in Table 3 with selected survival data shown in Figures 1 to 3.

CURRENTLY AVAILABLE EXTRACORPOREAL LIVER SUPPORT DEVICES

The currently available artificial ELSDs are based on the principal of removal of protein bound and water soluble substances (blood purification) by albumin dialysis, by plasma separation and filtration or by therapeutic plasma exchange. Devices based solely on the removal of water soluble substances (blood detoxification) have not shown any benefit in survival, possibly because of the limited, non-specific absorptive capacity of chemical adsorbents (10).

The following artificial ELSDs are currently available:

(i) The Molecular Adsorbents Recirculating System (MARS®, Gambro, Sweden) was first described in 1993 (Supplementary Material Figure S1) (11). In MARS®, blood is dialyzed across an albumin-impermeable, approximately 50-60 kDa cut-off, membrane against 20% human serum albumin (HSA). HSA solution is continuously stripped of protein bound and water soluble toxins by passage through a secondary circuit containing a charcoal column, an anion exchange resin column and a low-flux dialyzer (12-14).

(ii) The Fractionated Plasma Separation, Adsorption and Dialysis device (Prometheus®, Fresenius Medical Care, Germany) separates the patient's

albumin/plasma from blood by passage across an approximately 300 kDa cut-off membrane (Supplementary Material Figure S2). Patient albumin/plasma is then passed directly over two columns containing different adsorbents. A high-flux dialyzer inserted into the blood circuit clears water-soluble substances (15, 16).

(iii) Single pass albumin dialysis® (SPAD®) can be carried out with a standard dialysis setup, by use of hollow fibres made of a high-flux albumin-impermeable membrane and the addition of HSA to the dialysis solution to enable solute transfer from the patient's blood to the dialysis solution (Supplementary Material Figure S3) (17, 18).

(iv) Therapeutic plasma exchange (TPE) involves extracorporeal separation and removal of patient plasma from blood and return of blood cells with a replacement fluid to the patient. Fresh frozen plasma is the typical replacement fluid, but HSA has also been reported (19).

MARS®, Prometheus® and SPAD® are all able to reduce serum bilirubin and bile acids. Studies comparing MARS® and Prometheus® in ACLF show higher efficiency of Prometheus® for removal of bilirubin and urea and equal efficiency for removal of bile acids (20, 21). However, an actual improvement of synthetic liver function has neither been expected nor observed. For patients awaiting liver transplantation improvement of systemic haemodynamics, renal function or HE might be able to “buy” valuable time until an organ becomes available, serve as a bridge to recovery and it can be hypothesized that this would also impact on prognosis after transplantation.

Molecular Adsorbents Recirculating System®

A meta-analysis (22) of 4 randomized (14, 23-25) and 2 selected non-randomized trials (26, 27) did not show any overall effect of MARS® on mortality. However, explorative analysis of the 2 non-randomized trials revealed a significant reduction in mortality in the MARS® group as compared to the standard medical treatment (SMT) group (22). Another randomized

controlled trial showed that MARS® therapy in patients with ACLF has a beneficial effect on circulating neurohormones, nitric oxide and free radical production, and reduces markers of oxidative stress (28). The clinical effects of these changes are reflected in individual organ function with temporal improvement in cholestasis, liver function, renal function, encephalopathy, and in some patients, mean arterial pressure (28). Indeed, one of the most consistent findings in studies of MARS® in ACLF is an improvement in portal and systemic haemodynamics (29-31). Furthermore a large randomized controlled trial revealed a significant effect of MARS® on the severity of HE (32). The largest study so far – the RELIEF trial – however could not show a benefit of MARS® on mortality in ACLF, but demonstrated safety, a dialysis effect and a modest effect on HE (33) (Figure 1, Table 3). Failure to show a survival benefit may have been due to the heterogeneous patient population. However, another large, randomized study in ALF – the FULMAR trial – also failed to show a survival benefit of MARS® (Table 3). In this study most patients were transplanted within a median of 16.2 hours, leaving little time for a liver support system to demonstrate its effect (34). A retrospective study of continuous MARS® treatment in critically ill patients listed for liver transplantation with ALF, ACLF or graft dysfunction, showed that MARS® may be of value as a bridge to transplant but also revealed severe side effects with respect to coagulation and electrolytes (35). Therefore, the use of MARS® in patients with liver failure waiting for an organ should be performed under close observation with treatment of coagulopathy and electrolyte disturbances (35). In another single centre observation from the Netherlands that included 20 children with ALF or graft dysfunction, MARS® could be successfully applied, but with similar coagulation side effects and the need for liver transplantation was not reduced (36). Another single centre experience from Mexico suggested that MARS® reduced the need for liver transplantation by contributing to native liver recovery (37). However, a retrospective cohort study is not the optimal study design to answer this question.

From the available data, it is not possible to conclude whether or not MARS® is beneficial for patients on the transplant waiting list. It is possible that efficiency of the device is not optimal.

Therefore, the development of a device with higher efficiency might be of value. Recently, the use of a double absorption unit in parallel has been tested (38).

Prometheus®

Initial and subsequent uncontrolled data for Prometheus® show high elimination of albumin bound toxins and good safety data (16, 39). Comparable to MARS®, Prometheus® can be used safely in patients awaiting an urgent liver transplantation (40), but severe coagulation disturbances have been reported (41). The largest cohort study of Prometheus® in ALF patients was performed in Turkey and demonstrated safety and efficacy: one third of patients survived without transplantation, leading the authors to suggest that Prometheus® may be effective as a bridge to recovery (42). However, as for MARS®, Prometheus® failed to improve survival of patients with ACLF in a large prospective randomized study (Figure 2, Table 3) (43). Therefore the current data does not allow us to conclude whether or not Prometheus® is of benefit to patients on the waiting list.

Single pass albumin dialysis®

SPAD® can be used with any dialysis setup, therefore there is no need to invest in an extra machine. However, the amount of HSA required is high. SPAD® has been mainly tested in vitro and reported in case reports. In vitro there is evidence that the detoxification capacity of SPAD® is greater than MARS®(18). In a retrospective study, MARS® and SPAD® showed equal efficacy (44). In ALF, SPAD® was well tolerated but failed to improve survival and did not change referral to liver transplantation (45). In a single-center experience from Germany, SPAD® did not have any impact on survival or transplantation rate in patients with ACLF listed for transplantation (46). However, patient numbers were small. Again, it is not possible to conclude whether or not SPAD® is beneficial for patients on the waiting list.

Therapeutic plasma exchange

Therapeutic plasma exchange has been reported in isolated ALF and ACLF patients since the 1960s. Rationale has been removal of all toxins, as well as harmful inflammatory mediators and replacement of beneficial plasma proteins normally synthesised by the liver. In liver failure, TPE has been shown to reduce serum bilirubin and ammonia and to increase coagulation factors improving coagulopathy. Hypocalcaemia and alkalosis occur due to anticoagulant use, but are easily corrected (19). A recent multi-centre open randomised controlled trial of high-volume TPE (HVP, exchange of approximately 8-12 litres or 15% body weight of plasma), on three consecutive days, in 182 ALF patients demonstrated increased survival to hospital discharge (Figure 3, Table 3) (47). In patients who fulfilled poor prognostic criteria, but were not listed for transplant, HVP (n=28) increased survival compared to SMT (n=36). This survival advantage was associated with immune modulation and improvement in renal function, cardiovascular status, SOFA score and CLIF-SOFA score (47).

EMERGING TECHNOLOGIES IN EXTRACORPOREAL LIVER SUPPORT DEVICES

Bioartificial ELSDs include a bioreactor that contains hepatocytes, which in the most ideal scenario, would replace the functions of the failing liver including: ammonia detoxification via the urea cycle; drug metabolism; protein synthesis; and carbohydrate and lipid metabolism (48). Bioartificial ELSD development has been limited by their requirement for primary hepatocytes, which demonstrate better hepatocyte functionality compared to immortalised cell lines, but with the accompanying disadvantage of reduced cell viability and limited availability (48). Moreover, bioreactor design has been challenged with maintaining large hepatocyte cultures for effective patient treatment, whilst simultaneously acting as an effective interface between bioreactor hepatocyte function and patient plasma (48). Nevertheless progressive evolution of bioreactor design and hepatocyte biology has resulted in bioreactors with considerable hope for ALF and ACLF treatment. These include: Extracorporeal Liver Assist Device™ (ELAD™); Academic Medical Centre Bioartificial Liver (AMC-BAL); and Spheroid Reservoir Bioartificial Liver (SRBAL). ELAD has entered human

clinical trials. SRBAL and the latest version of AMC-BAL have shown efficacy in animal experiments, but data from human clinical trials are currently unavailable.

A number of artificial ELSDs are in development that may either improve detoxification compared to current ELSDs or combine detoxification with techniques to attenuate liver injury. These include: Hepa Wash®; Li-Artificial Liver Support (49) and University College London-Liver Dialysis Device (UCL-LDD). All three of these devices have shown efficacy in animal experiments, but data from human clinical trials are currently unavailable.

Extracorporeal Liver Assist Device

ELAD™ has been trialled in animal models of ALF and human liver failure patients since the 1990s (50-52). Its key component is a quartet of hollow fibre dialysis cartridges containing HepG2/C3A cells, a human hepatoblastoma cell line, within the extra-fibre spaces (Supplementary Material Figure S4) . HepG2/C3A cells remain viable throughout the recommended 3-10 day treatment (53). HepG2/C3A cells demonstrate albumin synthesis and cytochrome P450 activity, but functionality is significantly less than primary hepatocytes with failure to detoxify ammonia via the urea cycle (48, 54). Early phase I pilot studies in limited numbers of human ALF patients have demonstrated safety, but no improvement in survival and biochemical and clinical parameters (51, 52). Preliminary results of a trial in patients with acute decompensation of chronic hepatitis B or C reported significant extension of 30 day transplant free survival and biochemical improvement (Table 3) (55). Clinical trials of ELAD™ in ACLF, ALF, severe acute alcoholic hepatitis and alcoholic-induced liver failure are currently ongoing (56). In a recent press release, the results of the large randomised trial of ELAD™ in alcohol-related ACLF patients were reported to be negative (57). The full report is awaited.

Academic Medical Centre Bioartificial Liver

The AMC-BAL has been in development since the 1990s (58). Key bioreactor features are: a non-woven matrix for 3-D hepatocyte cultures; spiralling of this 3-D matrix around oxygen carrying capillaries; and direct exposure of hepatocytes to patient plasma (Supplementary Material Figure S5). Primary hepatocyte viability has been reported to be 90% on day three. The first phase I clinical trial of AMC-BAL in man used a device containing primary porcine hepatocytes. In this trial 12 ALF patients were treated for 4 to 35h: eleven were successfully bridged to liver transplantation and one recovered spontaneously. AMC-BAL treatment was associated with improvement in neurological and haemodynamic status in all patients; improvement in renal function in those with renal insufficiency and reduction in hyperbilirubinaemia and lactic acidosis (58). Porcine endogenous retrovirus DNA was found in patient plasma directly after treatment, but was undetectable thereafter. Nevertheless clinical use of this device was restricted due to ethical, immunological and zoonotic concerns.

Recently the HepaRG human hepatoma cell line has been cultured in the AMC-BAL instead of primary porcine hepatocytes. HepaRG cells approximate primary hepatocyte cultures more than any other human hepatocyte cell line (48). Culture within the AMC-BAL: 1) increased hepatic functionality with respect to ammonia elimination, the urea cycle and cytochrome P450 activity and 2) revealed lactate consumption, amino acid metabolism, drug metabolism and bile acid production similar to that of primary hepatocytes (59). In a rat ALF model, the HepaRG-AMC-BAL resulted in a 50% increase in survival and delay in progression of HE, kidney failure and hyperammonaemia (60).

Spheroid Reservoir Bioartificial Liver (SRBAL)

SRBAL has been in development since the early 2000s. Its key component is a bioreactor containing primary porcine hepatocytes in suspension, which when exposed to an oscillation frequency of 0.25Hz cluster into spheroids with stable cell viability (Supplementary Material Figure S6) (61, 62). Hepatocyte spheroids demonstrate good hepatocyte function in terms

of: phase I and phase II drug metabolism; ammonia conversion to urea via the urea cycle; and albumin synthesis (61). A trial using a pig ALF model has been reported (63). Pigs were treated either with two 6-hour treatments (intermittent) or one 24 hour treatment (continuous). Both SRBAL treatments improved survival and reduced hyperammonaemia and continuous SRBAL reduced intracranial hypertension and brain water.

Hepa Wash®

Hepa Wash® is an artificial ELSD that detoxifies blood by albumin dialysis against a 2% albumin dialysate (64). The albumin dialysate is recirculated via a 'Hepa Wash' circuit, which contains two parallel conventional haemofilters, in which albumin bound toxins are released through exposure to an alkaline or acid environment and subsequently removed by filtration. This design aims to maintain clearances of protein bound toxins through the treatment period (64). This is contrary to MARS®, where a decline in clearance of protein bound toxins is seen throughout the recommended 7hr treatment (65).

In a pig liver ischaemia ALF model, Hepa Wash® resulted in improvement in survival, cerebral perfusion pressure, haemodynamic status and kidney function. Moreover, Hepa Wash® resulted in reduction in azotaemia, hyperammonaemia, and blood nitrate/nitrite levels (64). Clinical trials in humans with ALF and ACLF were initiated in 2010, but have since been terminated for unknown reasons (NCT01079104, NCT01079091).

Li-Artificial Liver Support (Li-ALS)

Li-ALS is an artificial ELSD that combines a low-volume TPE (exchange of approximately 2.5% body weight of plasma) circuit with a modified MARS secondary circuit, in which high-flux hemofiltration replaces low-flux haemodialysis (49). This approach seeks to benefit from the more comprehensive detoxification achieved by TPE compared to MARS, without need for a supply of exogenous fresh frozen plasma, as patient plasma is returned post-detoxification to the patient. In a D-galactosamine pig model of ALF, Li-ALS resulted in an

improvement in survival compared to treatment with low-volume TPE alone and to treatment with the modified MARS circuit alone (49).

University College London-Liver Dialysis Device (UCL-LDD)

UCL-LDD is an artificial ELSD, in which blood is filtered across a high-cut off membrane (nominal cut-off of 60kDa) and then passed over a selective endotoxin adsorption membrane. Filtration across a high-cut off membrane results in albumin loss, which is replaced by HSA infusion (66). The resultant albumin exchange is proposed to correct irreversible loss of detoxifying function of albumin reported in liver failure. Reduction in endotoxaemia aims to reduce innate immune response, which worsens liver injury. Moreover high-cut off filters reduce circulating pro- and anti-inflammatory cytokines and correct immune dysfunction in septic patients with acute renal failure (67), so the same may apply to ALF. In a pig model of paracetamol-induced ALF, UCL-LDD improved survival and cardiovascular and respiratory function and reduced circulating dysfunctional albumin, endotoxaemia and immune system activation (66).

CONCLUSION

An ELSD that is able to bridge patients with liver failure either to recovery or to the state they were in, prior to the present deterioration, remains an unmet medical need. The main impediments to the development of an effective device can be thought of as being either patient related or device related. It is clear that once multiorgan failure is established, it is probably too late for an ELSD to be effective: in this situation the sole aim of ELSD treatment should be a bridge to transplant. Therefore, clinical trials need to include patients at risk of progression to multiorgan failure. The number of patients that will be required to attain adequate power will be high. It is also clear that the currently available devices show improvements in pathophysiological variables known to be associated with liver failure, but only one, TPE, has demonstrated survival benefit. The deficiencies of the currently available devices have inspired the newer devices, which are currently in clinical trials or due to enter

trials shortly. As ACLF has now been defined and the pathophysiology of both ALF and ACLF becomes clearer, it is very likely that an effective ELSD will emerge. Moreover further indications for ELSD may become evident. Indeed, new opportunity has arisen following the discovery of the new directly acting anti-viral drugs for Hepatitis C virus infection, which have been shown to reverse the severity of cirrhosis in many patients (68). One can envisage a situation whereby, Hepatitis C patients with decompensated cirrhosis are treated with ELSDs as out-patients for weeks and months, while the new directly acting anti-viral drugs take effect, negating the need for liver transplantation.

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Table 1: Types of Liver Failure

	Hyperacute/ Acute	**Sub acute**	**ACLF underlying cirrhosis**	**Decompensated cirrhosis**
Time from symptoms to failure	Weeks	Months	Weeks	Years
Common aetiology	Toxic	?Viral	Variable	Variable
Precipitating event	Liver injury	Liver Injury	Infection Alcohol Unknown	Unknown Infection (others)
Prognostic score	Kings	Kings	CLIF C score	MELD
Potential for regeneration	High	Poor	Unknown	Poor

Adapted from Jalan et al. Gastroenterology 2014 (1).

Table 2: The CLIF Consortium organ failure score for the diagnosis of acute on chronic liver failure

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2.0	Creatinine ≥2.0 or <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

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; or patient with cerebral failure and serum creatinine <1.5 mg/dl.

ACLF 1: Patients with renal failure or patients with other single organ failure with either 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.

Adapted from Jalan et al. Journal of Hepatology 2014 (7)

Table 3: Reported human randomised controlled clinical trials for ELSDs with survival as the primary outcome measure.

Data from intention to treat (ITT) and per protocol (PP) analyses are included where reported separately. (ALF, acute liver failure; ACLF, acute-on-chronic liver failure; n.s., no significant difference between groups; SMT, standard medical therapy; HVP, high-volume therapeutic plasma exchange)

Liver support device	**Study name or identifier**	**Type of trial**	**Patient type**	**Number of patients randomised (patients excluded after randomisation given in brackets)**	**Primary outcome**	**Secondary outcomes (only significant outcomes described)**	**Safety profile**
MARS®	The RELIEF Trial (33)	Multi-centre open randomised controlled trial	ACLF	Total=189 MARS® =95 SMT=94 (-ITT analysis: 5 exclusions per group -PP analysis: 24 MARS® and 9 SMT exclusions)	28-day ITT survival: MARS®, 61%; SMT, 59% (n.s.). 28-day PP survival: MARS®, 60%; SMT, 59% (n.s.).	At day 4, MARS® resulted in a significant reduction in serum creatinine, bilirubin and hepatic encephalopathy scores compared to SMT.	Incidence of severe adverse events was similar in MARS® and SMT groups

MARS®	The FULMAR Trial (34)	Multi-centre open randomised controlled trial	ALF	Total=110 MARS® =57 SMT=53 (-ITT analysis: 4 exclusions per group -PP analysis: 18 MARS® and 4 SMT exclusions)	6-month ITT survival: MARS®, 85%; SMT, 76% (n.s.). 6-month PP survival: MARS®, 82%; SMT, 76% (n.s.).		Incidence of severe adverse events was similar in MARS® and SMT groups
Prometheus®	The HELIOS trial (43)	Multi-centre open randomised controlled trial	ACLF	Total=145 Prometheus®=77 SMT=68 (-ITT analysis: 0 exclusions -PP analysis: 22 Prometheus® and 14 SMT exclusions)	28-day ITT survival: Prometheus®, 66%; SMT, 63% (n.s.). 28-day PP survival: Prometheus®, 71%; SMT, 67% (n.s.). 90-day ITT survival: Prometheus®, 47%; SMT, 38% (n.s.). 90-day PP survival: Prometheus®, 41%; SMT, 39% (n.s.). (Figure 2)	At day 28, Prometheus® resulted in a significant reduction in serum bilirubin compared to SMT.	Incidence of severe adverse events was similar in Prometheus® and SMT groups

High-volume therapeutic plasma exchange (HVP)	ClinicalTrials .gov number NCT00224705 (47)	Multi-centre open randomised controlled trial	ALF	Total=183 HVP=92 SMT=91 (1 SMT excluded after randomisation)	Survival to hospital discharge: HVP, 59%; SMT, 48% (P=0.008). (Figure 3)	On day 1 to day 7, HVP resulted in significant reduction in international normalised ratio, bilirubin, ALT, SOFA-score and CLIF-score.	Incidence of severe adverse events was similar in HVP and SMT groups
ELAD™	(55)	Multi-centre open randomised controlled trial	Chronic hepatitis B or C with acute decomp-ensation	Total=60 ELAD™=40 SMT=20 (-ITT analysis: 0 exclusions -PP analysis: 5 ELAD™ and 1 SMT exclusions)	30-day ITT transplant-free survival: ELAD™, 80%; SMT, 50% (P=0.03). 30-day PP transplant-free survival: ELAD™, 86%; SMT, 47% (P=0.004).		ELAD™ was associated with significant thrombocytopenia, whilst SMT was not.

1

2

FIGURE LEGENDS

Figure 1: Survival data from the RELIEF trial.

28-day survival for MARS® (light grey line) compared to standard medical therapy, SMT (dark grey line) with intention to treat analysis on the left and per protocol analysis on the right. Number of survivors at each time point is inserted into the graphs. See Table 3 for study details. (Reproduced with permission from Hepatology by John Wiley and Sons (33))

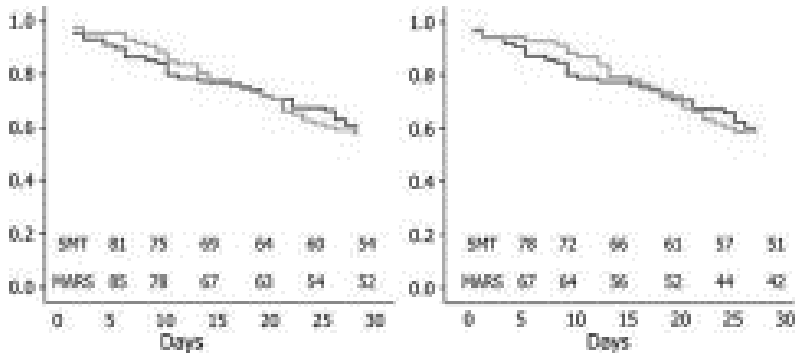
Figure 2: Survival data from the HELIOS trial.

90-day intention to treat survival for Prometheus®, FPSA+SMT, compared to standard medical therapy, SMT. See Table 3 for study details. (Reproduced with permission from Gastroenterology by Elsevier (43))

Figure 3: Survival data from the high-volume plasma exchange trial.

90-day intention to treat survival for high-volume plasma exchange, HVP, compared to standard medical therapy, SMT. See Table 3 for study details. (Reproduced with permission from Journal of Hepatology by Elsevier (47))

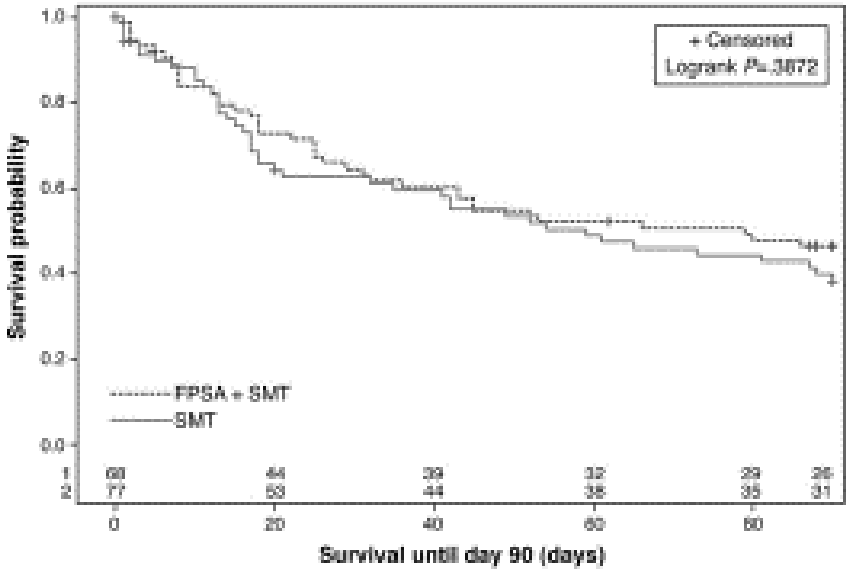
Figure 1


1.0
0.8
0.6
0.4
0.2
0.0
SMT 81 75 69 64 60 54
PARS 85 78 67 63 54 52
0 5 10 15 20 25 30
Days
1.0
0.8
0.6
0.4
0.2
0.0
SMT 78 72 66 61 57 51
PARS 67 64 56 52 44 42
0 5 10 15 20 25 30
Days

1

2

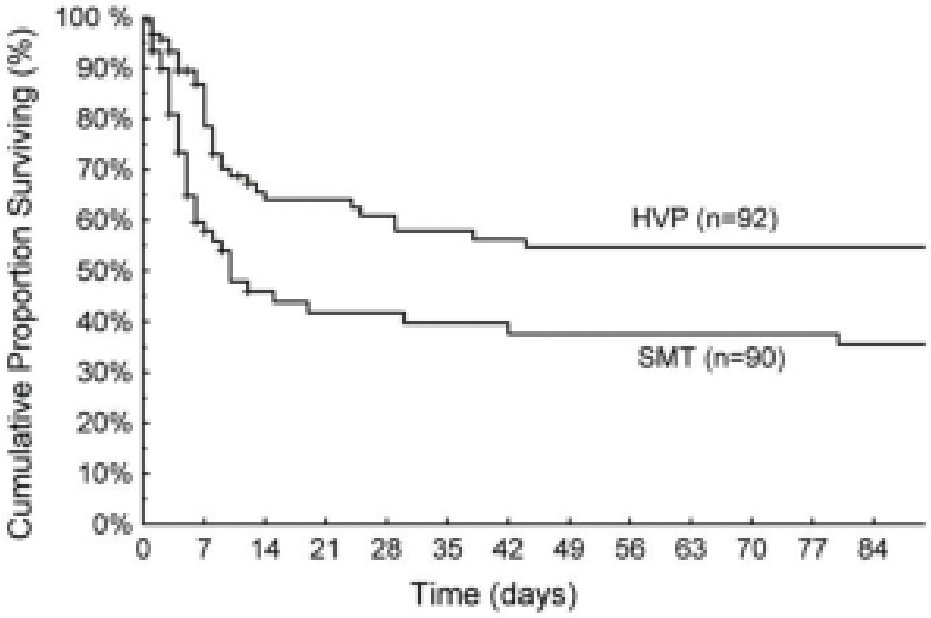
Figure 2


+ Censored
Logrank P=.3872
Survival probability
1.0
0.8
0.6
0.4
0.2
0.0
FPSA + SMT
SMT
1 68 44 39 32 29 26
2 77 53 44 38 35 31
0
20
40
60
80
Survival until day 90 (days)

1

2

Figure 3


Cumulative Proportion Surviving (%)
100 %
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
7
14
21
28
35
42
49
56
63
70
77
84
Time (days)
HVP (n=92)
SMT (n=90)

1