

1 **Extracorporeal Liver Support Devices for Listed Patients**

2

3 Karla C L Lee^{1‡}, Vanessa Stadlbauer^{2‡}, Rajiv Jalan^{3*}

4

5 ‡Joint first authors

6

7 ¹Department of Clinical Science and Services, The Royal Veterinary College, Hertfordshire,

8 UK; ²Department of Internal Medicine, Division of Gastroenterology and Hepatology, Medical

9 University of Graz, Graz, Austria; ³Liver Failure Group, Institute of Liver and Digestive

10 Health, University College London Medical School Royal Free Campus, London, UK

11

12 **Keywords (5 not in title):** albumin dialysis, bioartificial liver, acute liver failure, acute-on-

13 chronic liver failure, plasma exchange

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

9

10 **Conflicts of Interest:** The authors have nothing to declare with the following exceptions.

11 Rajiv Jalan has research collaborations with Ocera, Grifols, Norgine and Gambro, consults
12 for Ocera and Conatus and has received speaking fees from Norgine and Grifols. Rajiv
13 Jalan is the inventor of University College London-Liver Dialysis Device, which has been
14 patented by UCL and licensed to Yagrit Limited.

15

16 ***Corresponding author:** Professor Rajiv Jalan, MD PhD. Liver Failure Group, Institute of
17 Liver and Digestive Health, University College London Medical School Royal Free Campus,
18 Roland Hill Street, London, NW3 2PF, UK. Tel: +442074332795. Fax: +442074332775.
19 Email: r.jalan@ucl.ac.uk.

20

21

1 **ABSTRACT**

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

19

20 **INTRODUCTION**

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

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

7

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

15

16 ACLF is much more common than ALF and typically occurs in patients with cirrhosis (5). The
17 condition is characterized by acute deterioration of a cirrhotic patient with or without a
18 recognized precipitating event, associated with organ failures and high mortality rates (6).

19 Data from prospective studies are now available that allow accurate, sequential
20 assessments of patients, which provide prognostic information. The CLIF Consortium organ
21 failure score is used for diagnosis of the syndrome (Table 2) and the CLIF Consortium ACLF
22 score for defining the prognosis (7). A pre-ACLF group has now been identified, which will
23 allow studies of ELSD to prevent the occurrence of ACLF in susceptible patients (8).

24 Systemic inflammation is the key pathophysiological factor that drives the syndrome making
25 this a particular target of ELSDs (9). The aim of ELSDs in patients with ACLF is to support
26 hepatic function during acute decompensation until recovery to baseline liver function and/or
27 liver transplantation.

28

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

4

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

10

11 **CURRENTLY AVAILABLE EXTRACORPOREAL LIVER SUPPORT DEVICES**

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

18

19 The following artificial ELSDs are currently available:

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

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

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

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

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

14
15 MARS[®], Prometheus[®] and SPAD[®] are all able to reduce serum bilirubin and bile acids.

16 Studies comparing MARS[®] and Prometheus[®] in ACLF show higher efficiency of
17 Prometheus[®] for removal of bilirubin and urea and equal efficiency for removal of bile acids
18 (20, 21). However, an actual improvement of synthetic liver function has neither been
19 expected nor observed. For patients awaiting liver transplantation improvement of systemic
20 haemodynamics, renal function or HE might be able to “buy” valuable time until an organ
21 becomes available, serve as a bridge to recovery and it can be hypothesized that this would
22 also impact on prognosis after transplantation.

23 24 **Molecular Adsorbents Recirculating System[®]**

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

1 controlled trial showed that MARS[®] therapy in patients with ACLF has a beneficial effect on
2 circulating neurohormones, nitric oxide and free radical production, and reduces markers of
3 oxidative stress (28). The clinical effects of these changes are reflected in individual organ
4 function with temporal improvement in cholestasis, liver function, renal function,
5 encephalopathy, and in some patients, mean arterial pressure (28). Indeed, one of the most
6 consistent findings in studies of MARS[®] in ACLF is an improvement in portal and systemic
7 haemodynamics (29-31). Furthermore a large randomized controlled trial revealed a
8 significant effect of MARS[®] on the severity of HE (32). The largest study so far – the RELIEF
9 trial – however could not show a benefit of MARS[®] on mortality in ACLF, but demonstrated
10 safety, a dialysis effect and a modest effect on HE (33) (Figure 1, Table 3). Failure to show a
11 survival benefit may have been due to the heterogeneous patient population. However,
12 another large, randomized study in ALF – the FULMAR trial – also failed to show a survival
13 benefit of MARS[®] (Table 3). In this study most patients were transplanted within a median of
14 16.2 hours, leaving little time for a liver support system to demonstrate its effect (34). A
15 retrospective study of continuous MARS[®] treatment in critically ill patients listed for liver
16 transplantation with ALF, ACLF or graft dysfunction, showed that MARS[®] may be of value as
17 a bridge to transplant but also revealed severe side effects with respect to coagulation and
18 electrolytes (35). Therefore, the use of MARS[®] in patients with liver failure waiting for an
19 organ should be performed under close observation with treatment of coagulopathy and
20 electrolyte disturbances (35). In another single centre observation from the Netherlands that
21 included 20 children with ALF or graft dysfunction, MARS[®] could be successfully applied, but
22 with similar coagulation side effects and the need for liver transplantation was not reduced
23 (36). Another single centre experience from Mexico suggested that MARS[®] reduced the
24 need for liver transplantation by contributing to native liver recovery (37). However, a
25 retrospective cohort study is not the optimal study design to answer this question.
26
27 From the available data, it is not possible to conclude whether or not MARS[®] is beneficial for
28 patients on the transplant waiting list. It is possible that efficiency of the device is not optimal.

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

3

4 **Prometheus[®]**

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

15

16 **Single pass albumin dialysis[®]**

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

26

27 **Therapeutic plasma exchange**

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

14

15 **EMERGING TECHNOLOGIES IN EXTRACORPOREAL LIVER SUPPORT DEVICES**

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

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

3

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

9

10 **Extracorporeal Liver Assist Device**

11 ELAD™ has been trialled in animal models of ALF and human liver failure patients since the
12 1990s (50-52). Its key component is a quartet of hollow fibre dialysis cartridges containing
13 HepG2/C3A cells, a human hepatoblastoma cell line, within the extra-fibre spaces
14 (Supplementary Material Figure S4) . HepG2/C3A cells remain viable throughout the
15 recommended 3-10 day treatment (53). HepG2/C3A cells demonstrate albumin synthesis
16 and cytochrome P450 activity, but functionality is significantly less than primary hepatocytes
17 with failure to detoxify ammonia via the urea cycle (48, 54). Early phase I pilot studies in
18 limited numbers of human ALF patients have demonstrated safety, but no improvement in
19 survival and biochemical and clinical parameters (51, 52). Preliminary results of a trial in
20 patients with acute decompensation of chronic hepatitis B or C reported significant extension
21 of 30 day transplant free survival and biochemical improvement (Table 3) (55). Clinical trials
22 of ELAD™ in ACLF, ALF, severe acute alcoholic hepatitis and alcoholic-induced liver failure
23 are currently ongoing (56). In a recent press release, the results of the large randomised trial
24 of ELAD™ in alcohol-related ACLF patients were reported to be negative (57). The full report
25 is awaited.

26

27 **Academic Medical Centre Bioartificial Liver**

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

14
15 Recently the HepaRG human hepatoma cell line has been cultured in the AMC-BAL instead
16 of primary porcine hepatocytes. HepaRG cells approximate primary hepatocyte cultures
17 more than any other human hepatocyte cell line (48). Culture within the AMC-BAL: 1)
18 increased hepatic functionality with respect to ammonia elimination, the urea cycle and
19 cytochrome P450 activity and 2) revealed lactate consumption, amino acid metabolism, drug
20 metabolism and bile acid production similar to that of primary hepatocytes (59). In a rat ALF
21 model, the HepaRG-AMC-BAL resulted in a 50% increase in survival and delay in
22 progression of HE, kidney failure and hyperammonaemia (60).

24 **Spheroid Reservoir Bioartificial Liver (SRBAL)**

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

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

6

7 **Hepa Wash[®]**

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

15

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

21

22 **Li-Artificial Liver Support (Li-ALS)**

23 Li-ALS is an artificial ELSD that combines a low-volume TPE (exchange of approximately
24 2.5% body weight of plasma) circuit with a modified MARS secondary circuit, in which high-
25 flux hemofiltration replaces low-flux haemodialysis (49). This approach seeks to benefit from
26 the more comprehensive detoxification achieved by TPE compared to MARS, without need
27 for a supply of exogenous fresh frozen plasma, as patient plasma is returned post-
28 detoxification to the patient. In a D-galactosamine pig model of ALF, Li-ALS resulted in an

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

3

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

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

16

17 **CONCLUSION**

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

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

9

10 REFERENCES

- 11 1. Jalan R, Yurdaydin C, Bajaj JS, Acharya SK, Arroyo V, Lin HC, et al. Toward an
12 improved definition of acute-on-chronic liver failure. *Gastroenterology* 2014;147:4-10.
- 13 2. Jalan R, Sen S, Williams R. Prospects for extracorporeal liver support. *Gut*
14 2004;53:890-8.
- 15 3. Antoniadis CG, Berry PA, Wendon JA, Vergani D. The importance of immune
16 dysfunction in determining outcome in acute liver failure. *J Hepatol* 2008;49:845-61.
- 17 4. O'Grady JG, Williams R. Classification of acute liver failure. *Lancet* 1993;342:743.
- 18 5. Jalan R, Gines P, Olson JC, Mookerjee RP, Moreau R, Garcia-Tsao G, et al. Acute-
19 on chronic liver failure. *J Hepatol* 2012;57:1336-48.
- 20 6. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic
21 liver failure is a distinct syndrome that develops in patients with acute decompensation of
22 cirrhosis. *Gastroenterology* 2013;144:1426-37, 37 e1-9.
- 23 7. Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Gines P, et al. Development and
24 validation of a prognostic score to predict mortality in patients with acute-on-chronic liver
25 failure. *J Hepatol* 2014;61:1038-47.
- 26 8. Jalan R, Pavesi M, Saliba F, Amoros A, Fernandez J, Holland-Fischer P, et al. The
27 CLIF Consortium Acute Decompensation score (CLIF-C ADs) for prognosis of hospitalised
28 cirrhotic patients without acute-on-chronic liver failure. *J Hepatol* 2015;62:831-40.

- 1 9. Arroyo V, Moreau R, Jalan R, Gines P. Acute-on-chronic liver failure: A new
2 syndrome that will re-classify cirrhosis. *J Hepatol* 2015;62:S131-43.
- 3 10. Rozga J. Liver support technology--an update. *Xenotransplantation* 2006;13:380-9.
- 4 11. Stange J, Mitzner S, Ramlow W, Gliesche T, Hickstein H, Schmidt R. A new
5 procedure for the removal of protein bound drugs and toxins. *ASAIO J* 1993;39:M621-5.
- 6 12. Mitzner SR, Stange J, Klammt S, Risler T, Erley CM, Bader BD, et al. Improvement
7 of hepatorenal syndrome with extracorporeal albumin dialysis MARS: results of a
8 prospective, randomized, controlled clinical trial. *Liver Transpl* 2000;6:277-86.
- 9 13. Stange J, Ramlow W, Mitzner S, Schmidt R, Klinkmann H. Dialysis against a
10 recycled albumin solution enables the removal of albumin-bound toxins. *Artif Organs*
11 1993;17:809-13.
- 12 14. Heemann U, Treichel U, Looock J, Philipp T, Gerken G, Malago M, et al. Albumin
13 dialysis in cirrhosis with superimposed acute liver injury: a prospective, controlled study.
14 *Hepatology* 2002;36:949-58.
- 15 15. Falkenhagen D, Strobl W, Vogt G, Schrefl A, Linsberger I, Gerner FJ, et al.
16 Fractionated plasma separation and adsorption system: a novel system for blood purification
17 to remove albumin bound substances. *Artif Organs* 1999;23:81-6.
- 18 16. Rifai K, Ernst T, Kretschmer U, Bahr MJ, Schneider A, Hafer C, et al. Prometheus--a
19 new extracorporeal system for the treatment of liver failure. *J Hepatol* 2003;39:984-90.
- 20 17. Peszynski P, Klammt S, Peters E, Mitzner S, Stange J, Schmidt R. Albumin dialysis:
21 single pass vs. recirculation (MARS). *Liver* 2002;22 Suppl 2:40-2.
- 22 18. Sauer IM, Goetz M, Steffen I, Walter G, Kehr DC, Schwartlander R, et al. In vitro
23 comparison of the molecular adsorbent recirculation system (MARS) and single-pass
24 albumin dialysis (SPAD). *Hepatology* 2004;39:1408-14.
- 25 19. Stenbog P, Busk T, Larsen FS. Efficacy of liver assisting in patients with hepatic
26 encephalopathy with special focus on plasma exchange. *Metab Brain Dis* 2013;28:333-5.

- 1 20. Stadlbauer V, Krisper P, Beuers U, Haditsch B, Schneditz D, Jung A, et al. Removal
2 of bile acids by two different extracorporeal liver support systems in acute-on-chronic liver
3 failure. *ASAIO J* 2007;53:187-93.
- 4 21. Krisper P, Haditsch B, Stauber R, Jung A, Stadlbauer V, Trauner M, et al. In vivo
5 quantification of liver dialysis: comparison of albumin dialysis and fractionated plasma
6 separation. *J Hepatol* 2005;43:451-7.
- 7 22. Khuroo MS, Farahat KL. Molecular adsorbent recirculating system for acute and
8 acute-on-chronic liver failure: a meta-analysis. *Liver Transpl* 2004;10:1099-106.
- 9 23. El Banayosy A, Kizner L, Schueler V, Bergmeier S, Cobaugh D, Koerfer R. First use
10 of the Molecular Adsorbent Recirculating System technique on patients with hypoxic liver
11 failure after cardiogenic shock. *ASAIO J* 2004;50:332-7.
- 12 24. Mitzner SR, Klammt S, Peszynski P, Hickstein H, Korten G, Stange J, et al.
13 Improvement of multiple organ functions in hepatorenal syndrome during albumin dialysis
14 with the molecular adsorbent recirculating system. *Ther Apher* 2001;5:417-22.
- 15 25. Schmidt LE, Wang LP, Hansen BA, Larsen FS. Systemic hemodynamic effects of
16 treatment with the molecular adsorbents recirculating system in patients with hyperacute
17 liver failure: a prospective controlled trial. *Liver Transpl* 2003;9:290-7.
- 18 26. Chen S, Zhang L, Shi Y, Yang X, Wang M. Molecular Adsorbent Recirculating
19 System: clinical experience in patients with liver failure based on hepatitis B in China. *Liver*
20 2002;22 Suppl 2:48-51.
- 21 27. Hessel FP, Mitzner SR, Rief J, Guellstorff B, Steiner S, Wasem J. Economic
22 evaluation and 1-year survival analysis of MARS in patients with alcoholic liver disease.
23 *Liver Int* 2003;23 Suppl 3:66-72.
- 24 28. Sen S, Davies NA, Mookerjee RP, Cheshire LM, Hodges SJ, Williams R, et al.
25 Pathophysiological effects of albumin dialysis in acute-on-chronic liver failure: a randomized
26 controlled study. *Liver Transpl* 2004;10:1109-19.

- 1 29. Catalina MV, Barrio J, Anaya F, Salcedo M, Rincon D, Clemente G, et al. Hepatic
2 and systemic haemodynamic changes after MARS in patients with acute on chronic liver
3 failure. *Liver Int* 2003;23 Suppl 3:39-43.
- 4 30. Sen S, Mookerjee RP, Cheshire LM, Davies NA, Williams R, Jalan R. Albumin
5 dialysis reduces portal pressure acutely in patients with severe alcoholic hepatitis. *J Hepatol*
6 2005;43:142-8.
- 7 31. Laleman W, Wilmer A, Evenepoel P, Elst IV, Zeegers M, Zaman Z, et al. Effect of the
8 molecular adsorbent recirculating system and Prometheus devices on systemic
9 haemodynamics and vasoactive agents in patients with acute-on-chronic alcoholic liver
10 failure. *Crit Care* 2006;10:R108.
- 11 32. Hassanein TI, Tofteng F, Brown RS, Jr., McGuire B, Lynch P, Mehta R, et al.
12 Randomized controlled study of extracorporeal albumin dialysis for hepatic encephalopathy
13 in advanced cirrhosis. *Hepatology* 2007;46:1853-62.
- 14 33. Banares R, Nevens F, Larsen FS, Jalan R, Albillos A, Dollinger M, et al.
15 Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-
16 on-chronic liver failure: the RELIEF trial. *Hepatology* 2013;57:1153-62.
- 17 34. Saliba F, Camus C, Durand F, Mathurin P, Letierce A, Delafosse B, et al. Albumin
18 dialysis with a noncell artificial liver support device in patients with acute liver failure: a
19 randomized, controlled trial. *Ann Intern Med* 2013;159:522-31.
- 20 35. Olin P, Hausken J, Foss A, Karlsen TH, Melum E, Haugaa H. Continuous molecular
21 adsorbent recirculating system treatment in 69 patients listed for liver transplantation. *Scand*
22 *J Gastroenterol* 2015;50:1127-34.
- 23 36. Lexmond WS, Van Dael CM, Scheenstra R, Goorhuis JF, Sieders E, Verkade HJ, et
24 al. Experience with molecular adsorbent recirculating system treatment in 20 children listed
25 for high-urgency liver transplantation. *Liver Transpl* 2015;21:369-80.
- 26 37. Cisneros-Garza LE, Munoz-Ramirez Mdel R, Munoz-Espinoza LE, Ruiz Velasco JA,
27 Moreno-Alcantar R, Marin-Lopez E, et al. The molecular adsorbent recirculating system as a
28 liver support system: summary of Mexican experience. *Ann Hepatol* 2014;13:240-7.

- 1 38. Marangoni R, Bellati G, Castelli A, Romagnoli E. Development of high-efficiency
2 molecular adsorbent recirculating system: preliminary report. *Artif Organs* 2014;38:879-83.
- 3 39. Evenepoel P, Laleman W, Wilmer A, Claes K, Maes B, Kuypers D, et al. Detoxifying
4 capacity and kinetics of prometheus--a new extracorporeal system for the treatment of liver
5 failure. *Blood Purif* 2005;23:349-58.
- 6 40. Nyckowski P, Skwarek A, Zieniewicz K, Patkowski W, Alsharabi A, Wroblewski T, et
7 al. Orthotopic liver transplantation for fulminant hepatic failure. *Transplant Proc* 2006;38:219-
8 20.
- 9 41. Meijers BK, Verhamme P, Nevens F, Hoylaerts MF, Bammens B, Wilmer A, et al.
10 Major coagulation disturbances during fractionated plasma separation and adsorption. *Am J*
11 *Transplant* 2007;7:2195-9.
- 12 42. Senturk E, Esen F, Ozcan PE, Rifai K, Pinarbasi B, Cakar N, et al. The treatment of
13 acute liver failure with fractionated plasma separation and adsorption system: Experience in
14 85 applications. *J Clin Apher* 2010;25:195-201.
- 15 43. Kribben A, Gerken G, Haag S, Herget-Rosenthal S, Treichel U, Betz C, et al. Effects
16 of fractionated plasma separation and adsorption on survival in patients with acute-on-
17 chronic liver failure. *Gastroenterology* 2012;142:782-9.
- 18 44. Kortgen A, Rauchfuss F, Gotz M, Settmacher U, Bauer M, Sponholz C. Albumin
19 dialysis in liver failure: comparison of molecular adsorbent recirculating system and single
20 pass albumin dialysis--a retrospective analysis. *Ther Apher Dial* 2009;13:419-25.
- 21 45. Karvellas CJ, Bagshaw SM, McDermid RC, Stollery DE, Bain VG, Gibney RT. A
22 case-control study of single-pass albumin dialysis for acetaminophen-induced acute liver
23 failure. *Blood Purif* 2009;28:151-8.
- 24 46. Umgelter A, Lange K, Kornberg A, Buchler P, Friess H, Schmid RM. Orthotopic liver
25 transplantation in critically ill cirrhotic patients with multi-organ failure: a single-center
26 experience. *Transplant Proc* 2011;43:3762-8.

- 1 47. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, et al.
2 High-volume plasma exchange in patients with acute liver failure: An open randomised
3 controlled trial. *J Hepatol* 2015;Aug 29. pii:S0168-8278(15)00590-5.
- 4 48. van Wenum M, Chamuleau RA, van Gulik TM, Siliakus A, Seppen J, Hoekstra R.
5 Bioartificial livers in vitro and in vivo: tailoring biocomponents to the expanding variety of
6 applications. *Expert Opin Biol Ther* 2014;14:1745-60.
- 7 49. Zhou N, Li J, Zhang Y, Lu J, Chen E, Du W, et al. Efficacy of coupled low-volume
8 plasma exchange with plasma filtration adsorption in treating pigs with acute liver failure: A
9 randomised study. *J Hepatol* 2015;63:378-87.
- 10 50. Sussman NL, Chong MG, Koussayer T, He DE, Shang TA, Whisennand HH, et al.
11 Reversal of fulminant hepatic failure using an extracorporeal liver assist device. *Hepatology*
12 1992;16:60-5.
- 13 51. Sussman NL, Gislason GT, Conlin CA, Kelly JH. The Hepatix extracorporeal liver
14 assist device: initial clinical experience. *Artif Organs* 1994;18:390-6.
- 15 52. Ellis AJ, Hughes RD, Wendon JA, Dunne J, Langley PG, Kelly JH, et al. Pilot-
16 controlled trial of the extracorporeal liver assist device in acute liver failure. *Hepatology*
17 1996;24:1446-51.
- 18 53. Vital. Therapies Inc. Vital Therapies® targeting liver disease.
19 <http://vitaltherapies.com/>. Accessed July 2015.
- 20 54. Nyberg SL, Rimmel RP, Mann HJ, Peshwa MV, Hu WS, Cerra FB. Primary
21 hepatocytes outperform Hep G2 cells as the source of biotransformation functions in a
22 bioartificial liver. *Ann Surg* 1994;220:59-67.
- 23 55. Duan Z-P, Zhang J, Xin S, Chen JM, He D, Brotherton JD, et al. Interim results of
24 randomized controlled trial of ELAD™ in acute and chronic liver disease. *Hepatology*
25 2007;46:247A.
- 26 56. US. National Institutes of Health. ClinicalTrials.gov.
27 https://www.clinicaltrials.gov/ct2/results?term=ELAD%E2%84%A2+liver&no_unk=Y.
28 Accessed July 2015.

- 1 57. Vital Therapies Inc. Vital Therapies announces that topline results of VTI-208 fail to
2 achieve primary or secondary endpoints of improvement in overall survival pre-specified
3 exploratory subset analyses suggest efficacy trends.
4 <http://ir.vitaltherapies.com/releasedetail.cfm?ReleaseID=928472>. Accessed August 2015.
- 5 58. van de Kerkhove MP, Poyck PP, Deurholt T, Hoekstra R, Chamuleau RA, van Gulik
6 TM. Liver support therapy: an overview of the AMC-bioartificial liver research. *Dig Surg*
7 2005;22:254-64.
- 8 59. Nibourg GA, Hoekstra R, van der Hoeven TV, Ackermans MT, Hakvoort TB, van
9 Gulik TM, et al. Increased hepatic functionality of the human hepatoma cell line HepaRG
10 cultured in the AMC bioreactor. *Int J Biochem Cell Biol* 2013;45:1860-8.
- 11 60. Nibourg GA, Chamuleau RA, van der Hoeven TV, Maas MA, Ruiters AF, Lamers WH,
12 et al. Liver progenitor cell line HepaRG differentiated in a bioartificial liver effectively supplies
13 liver support to rats with acute liver failure. *PLoS One* 2012;7:e38778.
- 14 61. Nyberg SL, Hardin J, Amiot B, Argikar UA, Remmel RP, Rinaldo P. Rapid, large-
15 scale formation of porcine hepatocyte spheroids in a novel spheroid reservoir bioartificial
16 liver. *Liver Transpl* 2005;11:901-10.
- 17 62. McIntosh MB, Corner SM, Amiot BP, Nyberg SL. Engineering analysis and
18 development of the spheroid reservoir bioartificial liver. *Conf Proc IEEE Eng Med Biol Soc*
19 2009;2009:5985-8.
- 20 63. Glorioso JM, Mao SA, Rodysill B, Mounajjed T, Kremers WK, Elgilani F, et al. Pivotal
21 preclinical trial of the spheroid reservoir bioartificial liver. *J Hepatol* 2015;63:388-98.
- 22 64. Al-Chalabi A, Matevossian E, AK VT, Luppia P, Neiss A, Schuster T, et al. Evaluation
23 of the Hepa Wash(R) treatment in pigs with acute liver failure. *BMC Gastroenterol*
24 2013;13:83.
- 25 65. Evenepoel P, Laleman W, Wilmer A, Claes K, Kuypers D, Bammens B, et al.
26 Prometheus versus molecular adsorbents recirculating system: comparison of efficiency in
27 two different liver detoxification devices. *Artif Organs* 2006;30:276-84.

- 1 66. Lee KC, Baker LA, Stanzani G, Alibhai H, Chang YM, Jimenez Palacios C, et al.
2 Extracorporeal liver assist device to exchange albumin and remove endotoxin in acute liver
3 failure: Results of a pivotal pre-clinical study. *J Hepatol* 2015;63:634-42.
- 4 67. Morgera S, Haase M, Rocktaschel J, Bohler T, Vargas-Hein O, Melzer C, et al.
5 Intermittent high-permeability hemofiltration modulates inflammatory response in septic
6 patients with multiorgan failure. *Nephron Clin Pract* 2003;94:c75-80.
- 7 68. Pawlotsky JM, Feld JJ, Zeuzem S, Hoofnagle JH. From non-A, non-B hepatitis to
8 hepatitis C virus cure. *J Hepatol* 2015;62:S87-99.
- 9
10

1 **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

2

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

4

1 **Table 2: The CLIF Consortium organ failure score for the diagnosis of acute on**
 2 **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:	>300	≤300 - > 200	≤200
PaO₂/FiO₂	>357	>214- ≤357	≤214
or SpO₂/FiO₂			

3 No ACLF: Patients with no organ failure; patients with single hepatic, coagulation, circulation
 4 or respiratory failure, serum creatinine <1.5 mg/dl and no HE; or patient with cerebral failure
 5 and serum creatinine <1.5 mg/dl.

6 ACLF 1: Patients with renal failure or patients with other single organ failure with either
 7 serum creatinine ≥ 1.5 and < 2 mg/dl and/or HE grade 1-2.

8 ACLF 2: Patients with 2 organ failures.

9 ACLF 3: Patients with 3 or more organ failures.

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

11

12

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

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

1 **FIGURE LEGENDS**

2 **Figure 1: Survival data from the RELIEF trial.**

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

7

8 **Figure 2: Survival data from the HELIOS trial.**

9 90-day intention to treat survival for Prometheus[®], FPSA+SMT, compared to standard
10 medical therapy, SMT. See Table 3 for study details. (Reproduced with permission from
11 Gastroenterology by Elsevier (43))

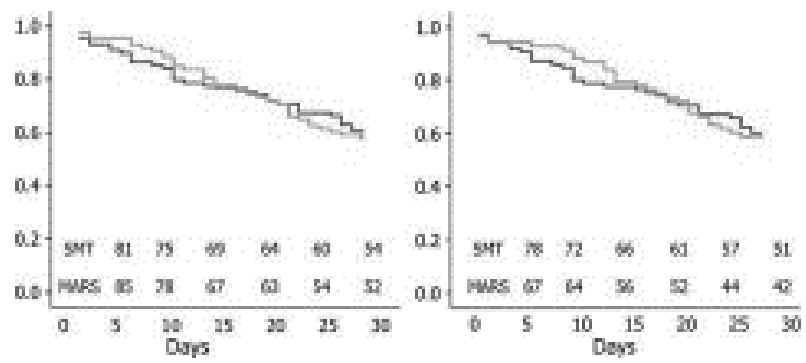
12

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

14 90-day intention to treat survival for high-volume plasma exchange, HVP, compared to
15 standard medical therapy, SMT. See Table 3 for study details. (Reproduced with permission
16 from Journal of Hepatology by Elsevier (47))

17

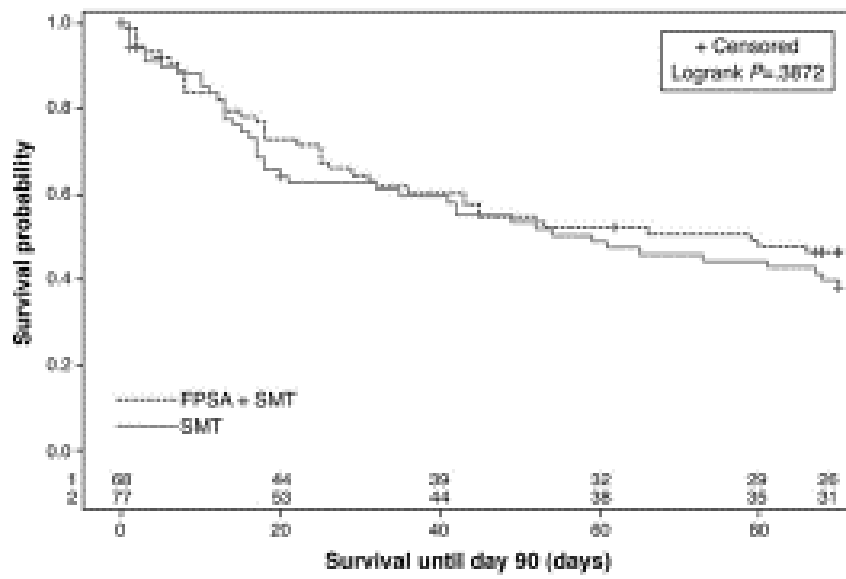
Figure 1



1

2

Figure 2



1

2

Figure 3

