# Chapter 17
# Looking Past Orthotopic Liver Transplantation: A Review of Emerging Strategies for Managing Acute and Acute-on-Chronic Liver Failure

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**Key Points**
- Several artificial liver support devices that have been tested in patients with Acute and Acute-On-Chronic Liver Failure, but as of yet no clear survival benefit noted based on current evidence with any of these devices in clinical setting.
- Hepatocyte transplantation and stem cell therapy could serve as potential treatments for ACLF with limited but promising supportive data.
- Semisynthetic organs may offer novel therapeutic management of ALF and ACLF in future.

## Introduction

Acute liver failure (ALF) and acute-on-chronic liver failure (ACLF) are both characterized by an acute insult leading to dysfunction of the liver and often other organs. Acute liver failure is defined by coagulopathy (INR > 1.5) and hepatic encephalopathy in the context of a new hepatic insult within the past 26 weeks [1]. It is relatively rare, with approximately 2000 cases per year diagnosed in the United

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**Table 17.1** Commonly used ACLF definitions

| Definition | Characteristics |
|---|---|
| APASL [3] | Bilirubin >5.0 and either INR > 1.5 or prothrombin activity (PTA) < 40% with ascites and/or encephalopathy in patients with chronic liver failure |
| EASL-CLIF [4] | Defined by organ failure, with severity increasing with number of organs failing. Organ failure is measured on a modified sequential organ failure assessment (SOFA) scale:<br>• Liver failure: Bilirubin ≥12.0 mg/dL<br>• Renal failure: Creatinine ≥2.0 mg/dL OR patient requiring renal replacement therapy<br>• Cerebral failure: Hepatic encephalopathy grade 3 or 4<br>• Coagulopathy: INR ≥2.5 OR platelet count ≤20,000/μL<br>• Circulatory failure: Requirement for vasopressors<br>• Respiratory failure: $P_{aO2}/F_{IO2} \leq 200$ OR $Sp_{O2}/F_{IO2} \leq 214$ OR need for mechanical ventilation<br>**Grade 1:** Renal failure OR creatinine 1.5–1.9 in the context of another single organ failure and hepatic encephalopathy<br>**Grade 2:** Two organ failures<br>**Grade 3:** Three or more organ failures |

States [2]. ACLF refers to an acute decompensation of chronic liver disease, but lacks a single clear definition [1]; the definitions used the most in studies discussed herein are reviewed in Table 17.1. Orthotopic liver transplant (OLT) is a valuable therapy for both conditions, but as with chronic liver failure, the demand for organs exceeds the supply. In the absence of liver transplant, the mortality of ALF has been estimated at >80% in some studies [1]. While the inconsistent definition makes ACLF mortality difficult to determine, a review based on the Asian Pacific Association for the Study of the Liver (APASL) and the European Foundation for the Study of Chronic Liver Failure (EASL-CLIF) definitions found a 90-day transplant-free mortality of about 50% [5].

With this high mortality in the absence of transplant, there is a need for alternative medical strategies that can support these patients—either until they recover sufficiently or until they receive a liver graft. One category of treatment comes in the form of hepatic assist devices, which are designed to exogenously support liver function through artificial or bioartificial means. Artificial devices such as the Molecular Adsorbent Recirculating System (MARS®) mostly target metabolic detoxification through dialysis techniques, while bio-artificial devices like HepatAssist® make use of human or animal cell lines to provide some synthetic capacity. There are also hybrid devices combining dialysis techniques and bioartificial support. Several of these hepatic assist devices have been tested in patients with ALF and/or ACLF, with mixed results.

Transplantation of hepatocytes allows cells to be prepared from livers that are suboptimal for transplantation and enables the treatment of multiple patients with cells from a single donor organ. There are also techniques under development to generate new and usable donor organs, though these remain at the preclinical stage of research. Decellularizing and repopulating organs allows for the transformation of a suboptimal or even porcine liver into one populated with the recipient's cells,

while 3-D printing and stem cell organoids are promising techniques for the *de novo* production of liver tissue. We will discuss each of these strategies and, when available, the evidence regarding its use in patients with ALF or ACLF.

## Artificial Liver Support Systems

The artificial liver support systems apply dialysis techniques to filter protein-bound and water-bound metabolites from the bloodstream. There are three major modalities that have been tested in patients: the Molecular Adsorbent Recirculating System (MARS®), the Prometheus® system, and single-pass albumin dialysis (SPAD®) [6]. These systems all operate on the principle of filtering whole blood or plasma against a dialysate containing albumin, which may or may not be then regenerated and recycled.

### *Molecular Adsorbent Recirculating System*

The MARS system was developed in Germany in 1993, and was commercialized and available for clinical use by 1998 [7]. A stream of whole blood is filtered against a stream of albumin-enriched dialysate via a high flux membrane filter, allowing the passage of hydrophobic, albumin-bound metabolites. The albumin-rich stream is then dialyzed against a stream of normal dialysate and regenerated with an adsorption column and an ion exchanger. This regenerated stream is then again passed against whole blood from the patient (Fig. 17.1). The MARS system has been tested in a number of clinical scenarios, including ALF, ACLF, severe hepatic encephalopathy (grade > II), elevated intracranial pressure, acute hypoxic hepatitis with bilirubin >8 mg/dL ("shock liver"), hepatorenal syndrome, progressive intrahepatic cholestasis, and graft dysfunction after liver transplant [9–11]. In 2005, it was approved by the FDA for use in drug overdose and poisonings so long as the agent is dialyzable and bound by charcoal [12]. It was also approved in 2012 for use in hepatic encephalopathy caused by a decompensation of chronic liver disease [13]. However, the device is not indicated by the FDA as a bridge to liver transplant, and it is additionally not approved for use in patients who are sedated [13].

MARS has been shown to reduce elevated bilirubin and creatinine levels in the case of ALF or ACLF, but no significant mortality benefit has been demonstrated. The RELIEF trial was a multi-center randomized controlled trial that compared MARS plus standard medical therapy (SMT) to SMT alone in patients with ACLF [14]. In this study, ACLF was defined as a known insult in the setting of chronic liver disease causing an increase in serum bilirubin >5 mg/dL and either hepatorenal syndrome, hepatic encephalopathy Grade > II, or bilirubin >20 mg/dL at the time of admission. There was no observed difference in short-term or long-term transplant-free survival in this trial. However, a more recent retrospective cohort study in a

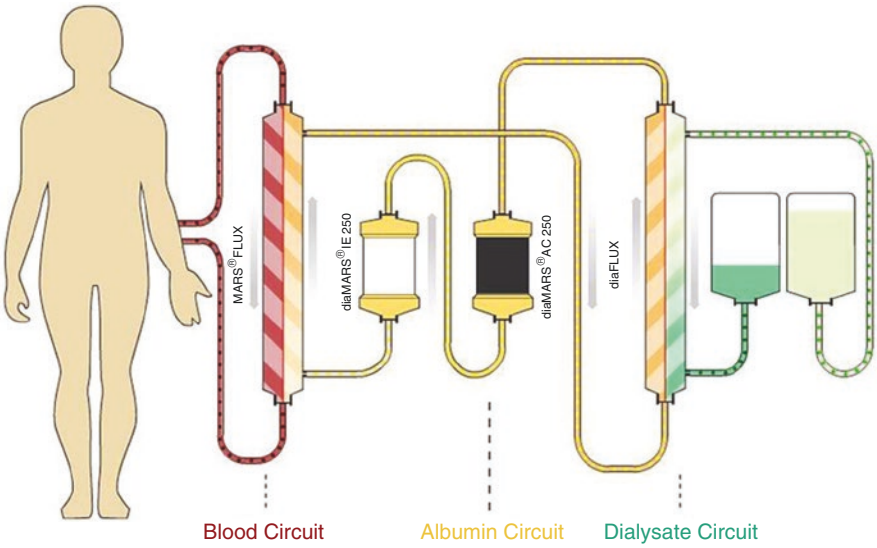



**Fig. 17.1** Molecular adsorbent recirculating system (MARS). Whole blood is filtered against a dialysis solution containing albumin. This solution is then filtered against a traditional dialysis solution to remove water-bound solutes, regenerated online with a neutral resin, filter, and anion exchanger to remove the protein-bound solutes, and finally recycled against the whole blood stream. Image is open access, reproduced via the Creative Commons License [8]

similar-sized population found that MARS + SMT had a survival benefit compared to SMT alone in patients with more severe ACLF as defined by Grade 2 or greater on EASL-CLIF criteria for ACLF [15]. Additionally, this group re-analyzed the patient data from the RELIEF trial and found an increase in 14-day survival among patients with ≥Grade 2 EASL-CLIF ACLF, but no difference in longer-term survival. These data suggest that MARS may in fact play a therapeutic role in the sickest patients with ACLF and may in fact be valuable as a bridge to OLT.

In the setting of ALF, however, there is less convincing evidence. A multi-center randomized controlled trial found no benefit in 6-month survival after using MARS in patients with ALF, including a subgroup analysis based on the etiology of liver failure [16]. A case-control study by Gerth et al. examined patients with ALF or graft failure and found no survival benefit after using MARS [17]. Although there were improvements in laboratory values—decreased bilirubin, blood urea nitrogen, creatinine, and lactate—the lack of mortality benefit makes the efficacy of this device in ALF questionable. Additionally, there are some adverse effects associated with MARS, similar to those seen with hemodialysis: an increase in bleeding risk and decrease in platelet count and fibrinogen, both of which may be clinically concerning in liver patients with an underlying coagulopathy [18].

### *Prometheus Device*

Another artificial liver support system that has received attention in clinical trials is the Prometheus device, which operates by generating an albumin-rich ultrafiltrate from the patient's own blood and cleansing this stream with an adsorption column and anion exchanger. This is combined with the retentate to reconstitute the patient's blood, which is then filtered through a traditional hemodialysis system to remove water-soluble toxins.

The only large multi-center randomized controlled trial evaluating Prometheus ACLF was performed by the HELIOS group and found that there was no significant difference in 28-day or 90-day survival in patients treated with Prometheus + SMT vs. SMT alone [19]. However, in subgroup analysis there was a significant improvement in survival in patients with a MELD score of >30, indicating severe failure. There are limited data surrounding the use of this device in ALF, and only cohort studies rather than randomized controlled trials. One larger cohort study showed that 33% of ALF patients treated with Prometheus were downgraded from needing a transplant [20].

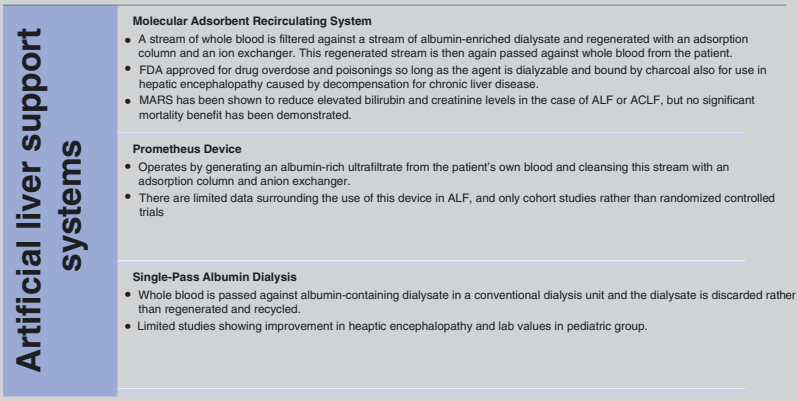
### *Single-Pass Albumin Dialysis*

The last major methodology of artificial hepatic support device is single-pass albumin dialysis (SPAD), wherein whole blood is passed against albumin-containing dialysate in a conventional dialysis unit and the dialysate is discarded rather than regenerated and recycled. There are very limited data regarding the use of SPAD in liver failure, and no randomized controlled trials. There is a crossover study comparing the effect of SPAD and MARS on laboratory values in patients with ALF and ACLF, which showed no difference in mortality between the two devices, but there is no comparison to a control [21]. Although there are some retrospective studies, including a recent one in pediatric patients showing a significant improvement in hepatic encephalopathy and laboratory values [22], these are also uncontrolled.

**Summary**

There are several artificial liver support devices that have been tested in patients with ALF and ACLF, but as of yet there is no clear survival benefit with any of these devices in either clinical setting. Tsipotis et al. published a systematic review and meta-analysis of albumin dialysis strategies that evaluated the efficacy of MARS and Prometheus in patients with liver failure across several trials; there were no studies of SPAD compatible with the analysis [23]. With 239 patients given MARS + SMT vs. 222 given SMT alone, there

was no significant survival benefit (OR 0.97, 95% CI 0.85–1.11). There were 91 patients treated with Prometheus + SMT vs. 82 treated with SMT alone, and no significant survival benefit (OR 0.87, 95% CI 0.66–1.14). It is apparent that the data do not support the systematic use of these devices in patients with liver failure. However, data from large randomized controlled trials suggest that the patients with the most severe disease (defined by MELD score or the EASL-CLIF grade of ACLF) may benefit from albumin dialysis [14, 15, 19]. These artificial liver support devices may eventually offer a method to support the sickest patients either as a bridge to transplant or as supportive therapy until the patient recovers on their own.




## Bioartificial Liver Support Systems

Bioartificial liver support systems combine aspects of the artificial liver support devices, but the addition of hepatocytes allows for some replacement of the liver's synthetic functionality. There are two systems that have received the most research: The Extracorporeal Liver Assist Device (ELAD®), which uses human cells, and the HepatAssist®, which uses porcine hepatocytes. There are several other bioartificial liver systems in development, but these have yet to be tested with an RCT.

### *Extracorporeal Liver Assist Device (ELAD)*

The bioreactor in the ELAD consists of human hepatoblastoma cells (cell line C3A) in hollow cartridges. These cells serve a synthetic role, synthesizing proteins normally produced by hepatocytes, and their functional CYP450 system allows for some detoxification of metabolites [24]. The initial device passed whole blood

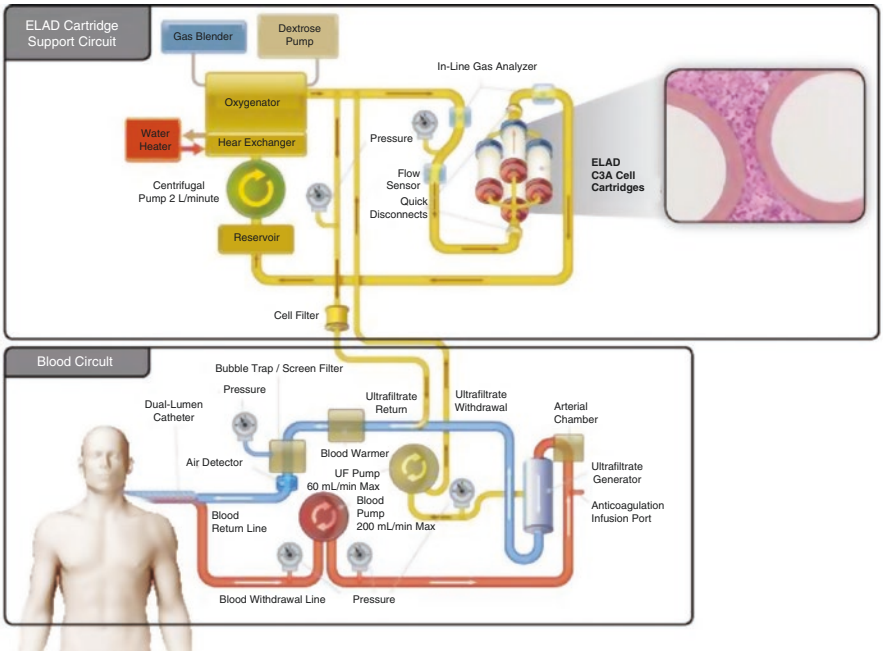



**Fig. 17.2** Extracorporeal liver assist device. Ultrafiltrated plasma is generated from the patient's whole blood and passed into the ELAD circuit. Within the device, plasma is heated and oxygenated before passing through the four cartridges containing C3A human hepatoblastoma cells. Ultrafiltrate is passed through a cell filter before being mixed back with the retentate to reconstitute the patient's blood, which is returned to the patient. This image is reproduced from open access literature via the Creative Commons License [25]

through the bioreactor, but most applications have instead generated an ultrafiltrate of plasma which is then oxygenated and warmed and exposed to the cells (Fig. 17.2). There is theoretically a risk of these hepatoblastoma cells making it back to the patient, but a number of membranes and valves make this unlikely.

An RCT by Duan et al. in China compared ELAD + SMT to SMT alone in patients with ACLF and found a significant benefit to transplant-free survival with ELAD, with the length of treatment time correlating to survival benefit [26]. Conversely, a phase III clinical trial evaluating the device in patients with severe alcoholic hepatitis—liver failure secondary to alcohol use, which is tacitly considered ACLF due to the history of alcohol abuse—found no effect of ELAD on overall survival [25]. Although subgroup analysis in this trial suggested a survival benefit in patients with less severe disease (MELD < 28), a phase III pivotal trial of the device in patients with alcoholic hepatitis was recently terminated due to failure to achieve the primary endpoint of increased overall survival [27]. As for ALF, the original pilot study of ELAD found no survival benefit regardless of transplant listing status [24]; per a recent systematic review, this remains the only RCT of ELAD in the setting of ALF [28]. From these results, the only survival benefit from ELAD was shown in the trial by Duan et al., in which 65% of the patients had ACLF secondary

to chronic hepatitis B; it is possible that the varying etiology of ACLF may contribute to the difference in outcomes.

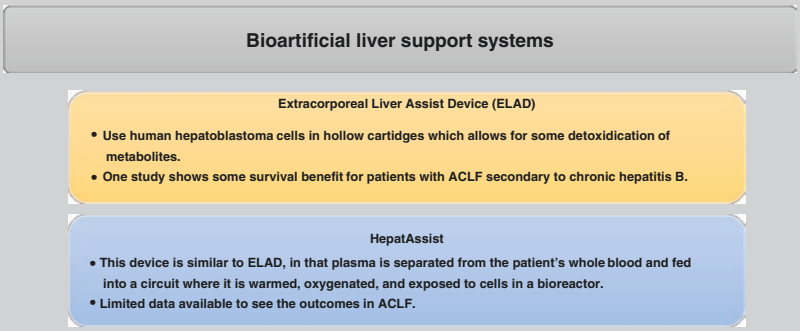
## *HepatAssist*

There are several bioartificial liver support systems based on the use of porcine hepatocytes in a bioreactor. Although some of these are being tested in phase 1 clinical trials, the only such device that has been evaluated with an RCT in human patients is HepatAssist [28]. This device is similar to ELAD, in that plasma is separated from the patient's whole blood and fed into a circuit where it is warmed, oxygenated, and exposed to cells in a bioreactor.

In the RCT by Demetrious et al. patients with ALF or post-OLT graft failure were treated with either HepatAssist + SMT or SMT alone and there was no significant difference in 30-day survival; this resulted in early termination of the trial [29]. However, in subgroup analysis of patients with ALF (excluding the graft failures), there was a significant reduction in mortality (RR 0.56, $p = 0.048$) and a significantly longer time to death within the first 30 days. Although immune reactions and zoonosis are major concerns when animal tissue is used in human patients, these adverse events were not reported. Based on these findings, further research into the application of HepatAssist in ALF could be promising. Additional trials should be performed to determine whether this device is helpful in improving outcomes in ACLF.

**Summary**

The limited available data suggest that bioartificial liver support systems may have value in the treatment of ALF and ACLF. Although it is based on a retrospective subgroup analysis, a survival benefit was observed in ACLF patients with a low MELD treated with ELAD and in ALF patients treated with the HepatAssist device. The potential role of these devices in the treatment of liver failure can only be determined by further trials assessing their efficacy in patients.



## Cell Transplantation

The transplantation of hepatocytes or stem cells into patients with liver failure aims to aid in liver regeneration to facilitate recovery from the acute insult. Using primary human hepatocytes allows many recipients to receive cells from a single donor, while the use of stem cells is not limited by the availability of donor organs. In addition, transplantation of cells into the spleen or portal vein is a much less traumatic procedure than OLT, reducing the risk of surgical complications and the high cost associated with transplant.

### *Hepatocyte Transplantation*

There have been numerous trials testing the efficacy of human hepatocyte transplantation (HT) across a spectrum of liver disease [30]. Primary human hepatocytes are harvested from a donor organ with collagenase and cryo-preserved until they are ready to be used. This allows for the immediate availability of cells—therapy can be given as needed without the need for waiting on a transplant list. There are numerous inborn errors of metabolism that result in failure of the liver to fulfill its metabolic role. While some of these conditions can be treated with diet and/or medical therapy, the morbidity and mortality is usually high unless these children receive an OLT. There are case reports describing the use of HT in patients with a variety of metabolic defects.

Ornithine transcarbamylase (OTC) deficiency is an X-linked urea cycle defect that can present in the first several days of life as severe hyperammonemia with metabolic encephalopathy [31]. In one case series, children with OTC deficiency who were poor candidates for OLT were treated with HT and showed a marked reduction in hyperammonemia [32]. Two of the children were treated neonatally with the goal of avoiding the developmental defects associated with the disease. Out of four children treated in the series, three survived until the end of the study period and showed sustained clinical improvement. Two of these three were on the list for OLT at the time the series was published. Crigler-Najjar syndrome is a defect in UDP-glucuronosyltransferase (UTG), the enzyme required to conjugate bilirubin [33]. One form of the disease presents neonatally as severe unconjugated hyperbilirubinemia that can cause kernicterus, resulting in permanent brain damage. Several cases have been reported of these patients receiving HT, resulting in a significant reduction of bilirubin and increased UGT activity; however, these patients inevitably require OLT [30, 34].

In the settings of ALF and ACLF, there are also only case reports describing the therapeutic use of HT. There is great disparity in the number of hepatocytes transplanted to patients (from $10^6$ cells to $10^{10}$ cells transplanted) and the reports include liver failure from a variety of etiologies [35]. In the setting of ALF caused by drugs or viral infection or idiopathic ALF, outcomes following HT were either OLT or death. The only report of HT as therapy for ACLF treated 7 patients with an expected survival of 8 weeks via intrasplenic injection of donor hepatocytes [36]. Of these

patients, 3 died, 1 required OLT, and 3 survived without the need for transplant. In surviving patients, viable hepatocytes were observed in the spleen 48 months after HT. The limited data available suggest that HT may be a viable therapy for patients with ACLF, but there is currently insufficient evidence to determine this clearly. The available data do not suggest that HT is a potential replacement for OLT in ALF, although it is possible that it may be useful as a bridging therapy as with the metabolic diseases.

A major limitation of HT is the availability of donor hepatocytes. The most viable organs are allocated for OLT, so hepatocytes in these studies were almost universally isolated from poor quality livers; this may have affected the outcomes [30]. Additionally, further study is needed regarding the immune response to these transplanted cells and the immunosuppressive strategies needed to prevent their destruction. Although cells were detected 4 years post-HT in the ACLF case series, most reports indicated that cells were no longer detectable after 6–9 months [35]. Current work aims to enhance the engraftment and proliferation of transplanted cells, optimize immunosuppression, and circumvent the supply issues by generating functional hepatocyte-like cells from stem cells [30]. HT may be a promising technique for treating ACLF or bridging ALF patients to OLT, and further study is required.

## *Stem Cell Transplantation*

There are numerous ongoing clinical trials testing the efficacy of stem cell transplantation in liver failure. Although most of these studies focus on chronic liver failure, there have been several RCTs testing stem cell transplantation in the setting of ACLF [37]. Most studies apply either bone marrow or umbilical cord blood derived mesenchymal stem cells (BMSCs and UMSCs, respectively). These multipotent progenitor cells can self-renew and differentiate into cells from the mesenchymal lineage. Administration of these cells through the hepatic artery or portal vein aims to repopulate damaged tissue and facilitate recovery.

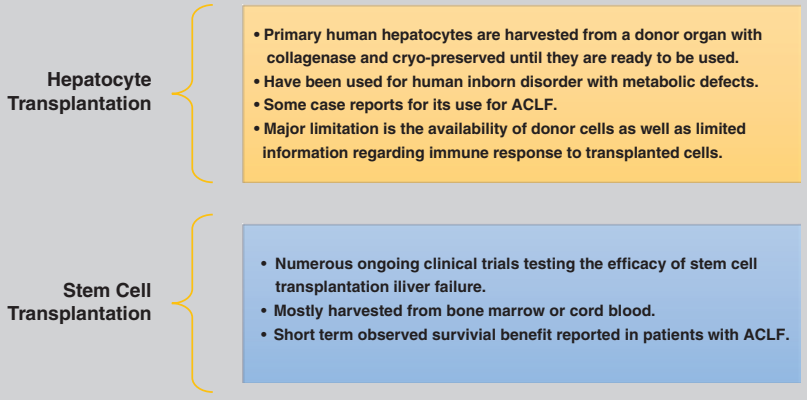
A recent meta-analysis by Xue et al. characterized the efficacy of stem cell transplantation in ACLF [38]. The survival analysis was performed on a pooled data set from trials using different cell types, with different etiologies of ACLF, and administering the cells through either the hepatic artery or portal vein. Most of these trials were performed in China, and as a result about 75% of all ACLF patients had chronic hepatitis B. On this heterogenous data set, stem cell therapy was found to significantly improve survival up to 6 months after the therapy. Between 9 months and 2 years, there was a non-significant trend towards improved survival after stem cell therapy. Improvement in clinical markers was variable, but significant improvements in total bilirubin, albumin, and MELD score after stem cell transplant were reported in another meta-analysis [39]. However, the observed survival benefit indicates that stem cell transplantation has promise as therapy for ACLF.

As for ALF, only animal data are currently available to support the efficacy of stem cell therapy, but the results are promising. In rat models of liver failure, injection of BMSCs significantly improved serum ALT and AST levels and reduced hepatocyte apoptosis [40, 41]. A mouse model of acetaminophen-induced liver injury had significantly improved survival and reduced liver damage following adipose tissue-derived stem cell (ASC) transplantation [42]. The application of human BMSCs to a pig model of ALF showed a significant survival benefit and reduction in serum cytokine levels [43]. Numerous other studies have shown benefits of stem cell therapy in animal models [44], but a translational effort is needed to determine whether this treatment will be valuable for humans with ALF.

There are limitations to stem cell therapy that must be overcome before it can be possible used routinely in human disease. A sustainable and consistent source of cells is necessary, and there are ethical concerns surrounding stem cell acquisition and research [45]. Additionally, a great deal of study is needed to determine the long-term safety and efficacy of this therapy. The available data suggest that stem cell transplantation is a promising strategy for the treatment of ACLF, and possibly for ALF.

**Summary**

There is a limited amount of evidence regarding cell transplantation as therapy for ALF or ACLF, but the available data support both hepatocyte transplantation and stem cell therapy as potential treatments for ACLF. The efficacy of HT in ALF is questionable, and there are currently no reported trials of stem cell therapy for ALF. These therapies offer great potential for life saving therapy that can defer the need for OLT or replace transplant entirely. More research into these therapies will hopefully continue to yield positive results and lead to the routine application in patients with liver failure.



## Other Medical Therapies

### *Granulocyte Colony-Stimulating Factor*

Similar to the direct administration of mesenchymal stem cells aimed to support liver functionality through regeneration, the administration of granulocyte colony-stimulating factor (G-CSF) to patients with liver failure aims to mobilize these stem cells from the patient's own bone marrow. There is not a great deal of evidence regarding its application, but a meta-analysis of two trials showed a significant improvement in overall survival when patients with ACLF were treated with G-CSF + SMT vs. SMT alone [46]. Additionally, several trials of GCSF in patients with severe alcoholic hepatitis—which is tacitly ACLF—have showed a survival benefit without major adverse events [47–49]. These data support the administration of GCSF in patients with ACLF. There are no clinical data available supporting the use of GCSF in ALF, but there have been animal models showing its efficacy. In a rat model of ALF, administration of GCSF significantly improved survival and reduced hepatic injury [50]. A study with a pig model of ALF had a dramatic survival benefit—6/6 control animals died, while 5/6 animals given GCSF survived indefinitely [51]. Based on the demonstrated benefit of G-CSF in ACLF, the supportive animal data, and the relative tolerability of G-CSF therapy, it would be reasonable to trial G-CSF in human patients with ALF. This may eventually offer a readily available medical therapy that provides some of the benefit of stem cells without as many limitations.

### *Plasma Exchange*

High volume plasma exchange (HVP) operates on the same principle as the artificial liver support systems, removing toxic metabolites from the patient's bloodstream. One trial found that HVP significantly improves biomarkers such as INR, total bilirubin, and albumin and significantly boosts transplant-free survival in patients with ALF [52]. Based on the patient population in this study, it is likely that the most benefit came from patients in severe condition with ALF caused by acetaminophen toxicity [53]. A nested cohort study within this trial found that HVP reduced serum levels of proinflammatory cytokines, including TNF-α and IL-6. This suggests that HVP may act to suppress the systemic inflammatory response that is characteristic of ALF. Although there are no data regarding the use of HVP in ACLF, the apparent anti-inflammatory effect of this therapy may provide a benefit. More extensive trials should be performed to assess the efficacy of HVP in liver failure.

## Semisynthetic Organs

The construction of a synthetic organ from a patient's own cells is a major target in transplant research, as it will provide a means to acquire organs independent of human donors. There is still much work to be done before organ synthesis will be a viable and readily applicable methodology, but that work is ongoing. A major limitation in the generation of solid organs has been maintaining nutrient and oxygen delivery within the structure [54], and efforts to synthesize a vascular tree have been unsuccessful [55]. One strategy involves removing the cells and immunogenic particles from an animal organ and repopulating it with stem cells from the patient. Another possibility is using 3D-printed organ scaffolds that can be populated with stem cells. There is also work being done on the implantation of organoids produced from stem cells, in which the cells produce their own vascular and biliary trees.

### *Decellularized and Repopulated Organs*

Decellularization techniques clear the organ of cellular contents and leave behind a scaffold of extracellular matrix including an intact vascular and biliary tree [56]. This scaffold can be used to reconstruct a functional organ using stem cells, hepatocytes, or hepatocyte-like cell lines. If stem cells from a patient awaiting liver transplant were used, it may be possible to produce an organ that can be implanted without the need for immunosuppression. Proof of concept was first obtained using a rat model, with repopulated organs successfully implanted into animals to rescue them from hepatectomy [56]. Since then, the technique has been employed in a wide variety of animal livers, including porcine livers that are of comparable size to human [57, 58]. The proteins making up the extracellular matrix are well conserved between species [59], meaning that a scaffold produced from pig livers and repopulated with human cells could possibly be used in human patients.

Currently, the biggest limitation of this technique lies in the recellularization of the organ scaffolds. It is necessary to line the organ's vasculature with endothelial cells and to reconstruct the organ's parenchyma. Earlier attempts using hepatocytes and cell lines were met with limited success [57], but better results were obtained with the use of mesenchymal stem cells, with transplantation of repopulated organs rescuing mice from acute liver failure [60]. Another possible method is the direct implantation of the decellularized scaffold, as in situ repopulation with liver parenchymal cells has been reported in a rat model [61]. If development continues on this technique, it is foreseeable that porcine livers—which are in much greater supply than human livers—could be used to provide scaffolding for large-scale production of semisynthetic human livers.

## *3D Printing of Organs*

The technique of 3D printing relies on a computer-controlled nozzle that extrudes material in layers to form a 3-dimensional shape and has been widely used with thermoplastics. Advances in this technology have allowed for the development of bioprinters that can extrude viable biological matter, offering a new route for organ engineering [62]. Production of artificial liver tissue via 3D bioprinting is has been approached from several different angles [63]. Some techniques are based on artificial scaffolds, wherein a bio-ink containing cells and support proteins is extruded into tissue. One group used a human cell line with alginate, gelatin, and human extracellular matrix proteins to produce liver tissue that produces albumin and has a functional CYP450 system [64]. This has been applied in animal models, with another group reporting that 3D printed liver tissue produced from a human cell line and a hydrogel significantly improved survival in a mouse model of acute liver damage [65]. Scaffold-free techniques have also been employed, with one group reporting that hepatocyte spheroids constructed from primary hepatocytes can be assembled into liver tissue that produces glucose and bile and detoxifies drugs [66]. Another group engineered different lines of liver cells to express linking proteins on their surface that allow cells to stay adhered long enough to secrete their own extracellular matrix, producing functional 3-dimensional tissue [67].

These advances are promising, but there are still major limitations, including the production of vasculature to deliver nutrients to cells in a 3D structure. The source of cells is also a concern, as primary human hepatocytes have the same limitations as organ transplant while immortalized cell lines do not adequately reproduce hepatocyte functionality. One possible inroad is the usage of stem cells from the patient, which would allow for the avoidance of immunosuppression, and there are many groups working with induced pluripotent stem cells to produce 3D liver tissue [68]. Optimization of this technique may eventually allow for the widespread production of artificial liver tissue.

## *Organoid Implantation*

Targeted differentiation of induced pluripotent stem cells (iPSCs) allows for the *in vitro* production of organ-like structures called organoids. One group differentiated iPSCs into hepatic endoderm cells, which they then co-cultured with endothelial cells and mesenchymal stem cells [69]. These cells organized themselves into a 3D liver-like structure that the group called an organ bud. These buds produced their own vasculature and biliary trees and became natively perfused after implantation into mice. This same group later reported scalable mass production of liver organoids entirely from human iPSCs, and found that implantation of these organoids significantly improved survival in a mouse model of ALF [70]. There is much more preclinical research to be done on this topic, but the available results suggest that it

is an encouraging avenue for synthesizing organs. The self-organization of vascular and biliary trees overcome one of the major difficulties of tissue engineering, and the ability to use iPSCs produced from the patient's own fibroblasts avoids the need for immunosuppression.

**Summary**
Organ engineering, when optimized and fully deployed, will change the field of transplant medicine. Production of an organ from a patient's own cells removes the need for a transplant wait list and the reliance on cadaveric transplantation. It is also likely that it would significantly reduce the incidence of rejection, as the new organ should be detected as the patient's own tissue. There is a great deal of progress that needs to be made before this can become a reality, but the preclinical research that is available suggests that these techniques may 1 day significantly improve survival of patients with liver failure.

## Early Prevention of ACLF

Preventing ACLF before it can fully develop has the potential to significantly improve mortality. ACLF is thought to be present in 24–40% cirrhotic patients admitted to the hospital [71] and increases the risk of mortality almost 20-fold [4]. The Asian Pacific Association for the Study of the Liver identified a "golden therapeutic window," a period between the acute onset of liver failure and the onset of multi-organ failure, during which preventative measures can reverse the pathology in a patient [3]. It is important to clinically identify patients at risk for ACLF by taking proactive measures. Bacterial infection is a common cause of ACLF, which is part of why diagnostic paracentesis is commonly performed in cirrhotic patients who are hospitalized. In the absence of a known etiology, therapies that can control the systemic inflammatory response associated with ACLF may also prove beneficial [71].

**Summary**
The best therapy to improve survival in ALF and ACLF is OLT, but there are not enough livers available to meet the demand. We have discussed therapies that are under development for these disease states that largely aim to support the functionality of the liver to either allow full recovery or bridge a patient to transplant. The extracorporeal liver support systems offer some hepatic functionality, and the current data support a survival benefit for some of these systems in subsets of patients with ALF and ACLF. Administration of G-CSF has also been shown to have a survival benefit for ACLF and has not been tested in patients with ALF.

More novel strategies such as hepatocyte transplantation and stem cell therapy have limited evidence surrounding their use but may show promise. There are also efforts being made in tissue engineering that may eventually allow for the production of semisynthetic livers. There is still much work to be done to improve the survival of these patients, but it is possible that one of these avenues will prove successful.

**Questions**

1. Which of the following artificial liver support systems has been approved by FDA for management of hepatic encephalopathy due to decompensation of chronic liver disease?

   A) MARS
   B) Prometheus Device
   C) Single-Pass Albumin Dialysis

2. Based on current available data which of the following statements is true:

   A) ELAD would improve the survival in patients with ACLF due to alcoholic hepatitis
   B) HepatAssis has 30 days survival benefit in patients with post-OLT graft failure.
   C) A survival benefit was shown from ELAD in patients with ACLF with chronic hepatitis B infection.

**Answers**

Question 1—The answer is A.
Question 2—The answer is C.

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