Liver Support Systems

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Abstract

In recent years liver transplantation was shown to be the only clinically effective method of treating acute or chronic hepatic failure due to various causes. However, this ultimate therapeutic approach is limited by the growing disparity between organ donation and the number of patients on the waiting list. Factors such as high cost, morbidity, and the need for lifelong immunosuppression accelerated the research on alternative methods to support the failing liver. Recently, new technologies incorporating hepatocytes and extracorporeal circulation devices were introduced for liver support. This review presents current knowledge on liver support systems and their role in the treatment of acute liver failure.

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Acute liver failure is a devastating syndrome that results from massive hepatocyte death, leading to jaundice with rapidly progressive encephalopathy, coagulopathy and eventually multi-organ dysfunction [Table 1]. The main etiologies of ALF are viral infection and drug-induced liver injury [Table 2]. ALF can develop either in the absence of previous liver disease or as an acute exacerbation of chronic liver dysfunction. The loss of synthetic, metabolic and detoxification processes of the liver initiates a systemic response that results in multi-organ involvement and death. The rapid appearance of jaundice, coagulopathy and encephalopathy denotes a bad prognosis. Cytotoxic brain edema, leading to increased intracranial pressure and eventually brain herniation, is often the immediate cause of death. While improved intensive care technologies enable better support of various failing organ systems, in the case of ALF intensive care alone does not reduce the mortality rate, which still remains unacceptably high without transplantation [1,2].

ALF = acute liver failure

Table 1. Main involvement of organ system in acute liver failure

Organ system involvement	Pathophysiology
Nervous system Reduced consciousness	Encephalopathy, increased intracranial pressure and brain edema
Hematopoietic system Bleeding tendency, anemia, Thrombocytopenia	Coagulopathy, hypersplenism and gastrointestinal bleeding
Kidneys Reduced function, hepatorenal syndrome	Reduced renal blood flow
Cardiovascular system Hyperdynamic state, reduced oxygen consumption	Reduction in systemic vascular resistance due to increased peripheral shunting
Lungs Reduced oxygenation	Increased ventilation-perfusion mismatch
Metabolic Hypoglycemia, hypokalemia and hyponatremia	Reduced liver gluconeogenesis, increased urinary potassium secretion, alkalosis and increased total body free water

Table 2. Common etiologies of ALF

Etiology	Cause
Viral infection	Viruses: A, B, C, D, E, non A-E, cytomegalovirus, herpes, Ebstein-Barr, adenovirus
Drugs	Acetaminophen, halothane, ketoconazole, sodium valproate, Ecstasy
Toxins	*Amanita phalloides*, carbon tetrachloride
Ischemia	Veno-occlusive disease, ischemic hepatitis
Miscellaneous	Wilson's disease, fatty liver of pregnancy, Reye's syndrome, heat stroke

Liver transplantation is considered the only effective treatment of this entity. Advances in surgical techniques, perioperative management and immunosuppression have contributed to the encouraging survival of 65 to 90% one year following orthotopic liver transplantation [3,4]. However, liver transplantation is a costly procedure and has several limitations:

- ALF is a potentially reversible clinical condition and 14–30% of the patients are expected to recover with medical treatment alone [2,4]. Early performance of transplantation eliminates the chances of spontaneous liver regeneration, as recently reported with the technique of auxiliary partial orthotopic liver transplantation [5].

- A growing disparity between the number of organ donors and liver transplants that are performed every year and the limited window-of-time opportunities for transplantation are responsible for the fact that only 10% of patients with ALF are being transplanted [4]. The other patients are put on the waiting list, and many die while still waiting.
- Following transplantation, patients require lifelong immunosuppression and are subject to inevitable infectious complications and a higher rate of secondary malignancy.
- These patients may experience continuous slow functional deterioration of the transplanted liver and chronic rejection.
- Patients with a morbid psychological profile, or active drug abusers, are not expected to comply with the post-transplant lifelong immunosuppression and are thus not considered suitable for transplantation.
- The presence of active sepsis, multi-organ system failure or irreversible brain damage contraindicates liver transplantation.

For the above stated reasons, a tremendous effort has been directed in recent years at developing liver support systems for use as a temporary measure – either bridging patients to transplantation or helping to keep them alive until the recovery of native liver function. The development of such systems presents a challenge: they have to replace the complex metabolic functions of the liver and help to reverse the pathological multi-organ involvement that leads to patient death. To that end, improving coagulation and reducing brain edema (the leading cause of death in these patients) are targets to be met when a liver assist device is tested for efficiency.

Initially, attempts at developing artificial hepatic support were focused on various forms of dialysis or hemoperfusion, using cartridges and membranes that are able to remove low molecular weight toxins. Recently, advances in hepatocyte isolation and cell culture have contributed to the creation of a new technology of liver assist devices incorporating various forms of hepatocytes that provide both detoxifying and synthetic hepatic functions.

Liver support systems based on extracorporeal blood purification techniques

In a desperate attempt to purify the blood of a 13 year old boy in hepatic coma, two cycles of blood exchange transfusion were implemented by Lee and Tink in 1958 [6]. Elevated blood bilirubin levels fell to normal and the patient regained full consciousness. Later, Trey et al. [7] used the blood exchange method to treat seven patients with hepatic coma caused by viral hepatitis. There was an improvement in mental status in all patients. However, this method was rarely used and has never been tested in a controlled trial. Later, a total body washout technique was tried, involving exsanguination combined with rapid infusion of an albumin and electrolyte solution followed by fresh blood transfusion. However, this method was applied in a small number of patients with only mild improvement [8].

These first attempts to detoxify the blood of patients with ALF gave impetus to the installation of more sophisticated and less harmful methods of blood purification. Blood detoxification methods based on dialysis or hemadsorption constituted the next endeavors to support the failing liver. The rationale for these methods was based on the presumption that hepatic encephalopathy is caused by the accumulation of small molecular weight toxins that can be dialysed [9]. The accumulation of toxins that are normally metabolized by the liver are thought to have a pivotal role in causing the multi-organ dysfunction that develops in patients with liver failure and stems from their cytotoxic effects [10]. However, most of the toxins are bound to albumin, whose binding sites are limited in the presence of liver failure, leading to increased fractions of the unbound toxins. This is the reason why methods such as hemofiltration or hemodialysis were found to be insufficiently effective in detoxifying the blood of patients with hepatic coma [11]. In a further attempt to improve blood purification, hemadsorption using resins was employed in patients with hepatic failure in order to remove the protein-bound toxins. But these methods were met with only partial success [12] and were even blamed for the possible worsening of hepatic function due to their non-selectivity and their ability to adsorb hepatic growth factors responsible for liver regeneration [13]. In addition, the direct contact between the blood and the adsorbents resulted in a continuous loss of platelets and leukocytes as well as clotting factors.

Charcoal hemoperfusion has been known for many years as the most widely used mode of therapy for liver failure [14]. The effectiveness of charcoal stems from its ability to adsorb a wide range of water-soluble molecules (up to 5 kDa) and many of the toxins that are accumulated in serum of patients with liver failure such as mercaptanes or aromatic acids. In contrast, protein-bound compounds are not adsorbed by charcoal. Despite initial reports of improvement in the neurological status of ALF patients treated with this method, this was not confirmed by a controlled clinical study [15].

Sabin and Merritt [16] introduced plasma exchange as a treatment for the failing liver in 1968. In their report on three patients' with hepatic coma they noted a short-lived reduction in plasma bilirubin levels and mild neurological improvement, but these effects were transitory and all the patients died [16]. Other reports similarly showed only short-term improvement in patients status without any effect on outcome [17]. In an attempt to improve the efficiency of plasma exchange, Kondrup et al. [18] used high volume plasma exchange to treat 11 ALF patients, which led to recovery in 5 patients with acetaminophen-induced liver injury. Another study used combined plasma exchange and hemodiafiltration in 67 ALF patients, resulting in a 55% survival rate [19]. It should be noted that plasma exchange can be associated with several complications, including chemical toxic reaction, viral infections and multi-organ damage, as reported by Brunner and Losgen [20].

Recently, a new blood purification system based on dialysis of blood with a double-sided, albumin-impregnated hollow fiber

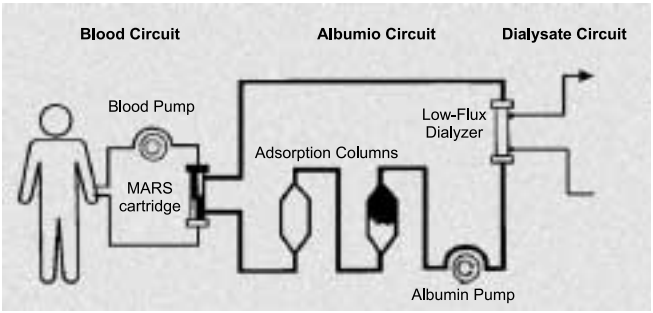

Figure 1. Scheme of the Molecular Adsorbent Recycling System (MARS). The patient is connected to a blood circuit containing the MARS membrane. A double-sided, albumin-impregnated hollow fiber dialysis membrane acts as molecular adsorbent in a closed-loop dialysis circuit containing albumin. The dialysis solution is purified online from albumin-bound toxins by ion echange resin and active charcoal. A dialysis module is incorporated to the secondary circuit to add all the benefits of hemodialysis.

dialysis membrane as a molecular adsorbent in a closed loop dialysis circuit was described by Stange and colleagues [21]. In contrast to the regular methods of hemodiafiltration, this system was designed to remove both low and middle molecular weight water soluble substances as well as albumin-bound molecules. The system is based on the dialysis of blood against a special membrane coated with albumin but impermeable to it [Figure 1]. The albumin compounds with the free binding sites in the membrane compete with the carrier proteins for the toxins in the blood. The membrane transiently adsorbs and holds the toxins that are normally carried on the blood albumin (i.e., bilirubin and bile acids) but are released from blood albumin upon contact with the membrane according to the concentration gradient. The toxins are trapped and then carried to the other side of the membrane, where dialysis against a fluid rich in albumin completely separates the toxins. The dialysate is recirculated against a charcoal column (for the removal of lipophylic toxins such as bile acids) and an anion exchange resin for the removal of bilirubin. The albumin-containing dialysate is then completely renewed by dialysing it against a normal dialysis solution. In that way, water-soluble toxins are removed. At the end of the process the albumin-containing dialysate solution is ready for re-use, thereby reducing the need to replenish the costly albumin solution. Since the treatment is based on the dialysis of blood against a solution containing albumin, which functions as a *molecular adsorbent recycling system,* the system was named MARS [21]. The MARS blood purification method minimizes the loss of vital compounds like growth factors, hormones and vitamins, which are carried by proteins other than albumin and are not dialyzed by this process. This prevents the negative side effects of plasma-sorbent contact as in plasmapheresis [22]. In addition, the dialysis step of the MARS system enables the removal of water-soluble toxins and maintenance of acid-base and electrolytes balance, which are disturbed in ALF patients due to concomitant loss of renal function [22]. In addition, ammonia – which is not removed by charcoal because, at physiological pH, it is ionized – is efficiently removed by the MARS system. The utility of the MARS system was studied in 13 patients suffering from life-threatening hepatic failure who had not responded to state-of-the-art therapy. The overall survival rate was 69% [23]. All patients showed a positive response to therapy (reduction in encephalopathy). Furthermore, improvement in biochemical profile (reduction in blood levels of bilirubin, creatinine and bile acids) and liver synthetic function (elevation of coagulation factors level) was also noted [23]. It seems that the MARS membrane separation is a protein-impermeable safety barrier between the patient's blood and any cellular or chemical agent of detoxification [22]. In addition, the system is effective in reducing the blood levels of the protein-bound toxins accumulating in ALF [23]. However, the utility of this system must be further tested in randomized controlled trials in order to decide which complications of ALF it can most effectively treat.

Another blood purification system called "the liver dialysis unit" was recently introduced for the treatment of hepatic failure. This compact mobile device is able to remove a wide range of toxins from the blood. Moreover, it can balance the patient's electrolytes and fluid status. This new technology incorporates a unique vacuum-pressure propulsion system that uses the motion of the dialyzer membranes to propel blood through the circuit, thus obviating the use of roller pumps and virtually eliminating hemolysis. Also, use of the liver dialysis unit does not mandate additional anticoagulation. An initial report supports the effectiveness of the system to improve the blood biochemistry profile of patients with hepatic coma [24]. Nonetheless, more data are needed for better evaluation of the optimal use of this system.

Biological liver support systems

The poor outcome in the management of ALF with the various detoxification systems described above is understandable in view of the fact that physical methods are not sufficient to correct the complex metabolic disorders accompanying liver failure. This prompted extensive research on developing bioartificial liver support systems. These methods combine a biological component (hepatocytes) and a synthetic milieu that is responsible for the close contact with the patient's blood. These hybrid devices expose the blood of the ALF patient to hepatocytes before being returned to the patient. As such, these bioartificial devices were designed to replace not only the excretory function of the liver but also the synthetic and biotransformatory functions. The first methods that included biological components for supporting the failing liver were trials in human and animal cross-circulation. However, these methods were abandoned because of severe side effects, such as infection of the healthy partner, sepsis, allergic reactions and shock. Later experiments using livers of baboons, pigs and dogs in *ex vivo*

MARS = molecular adsorbent recycling system

liver were reported. Although transient improvement in encephalopathy was noted, most patients died shortly after being connected to the *ex vivo* liver preparation [25]. Moreover, the preparation was noted to rapidly lose its function, preventing continuous treatment. The use of an xenogenic method to support the failing liver as a bridge to transplantation resulted in only a limited and transitory effect, and a severe humoral rejection reaction against the pig liver xenograft was reported [26]. Nevertheless, various methods for modulation of the immunological reaction to the xenograft are being examined in experimental models, so far without significant success [26].

Recently, human livers that were not suitable for transplantation were used as *ex vivo* support systems for ALF patients [27]. But due to shortage of organs and the need for exact timing for organ availability it is unlikely that this method will be widely available. In addition, complications such as rejection, rapid decay of function and the complicated logistics involved in the methods of *ex vivo* liver perfusion will probably preclude this method from gaining a prominent role in future liver support strategies.

Bilir et al. [28] recently reported the use of percutaneous hepatocyte transplantation in a salvage attempt among adults with ALF. In their work, cryopreserved human hepatocytes from a liver cell bank were injected into the spleen of six patients suffering from severe encephalopathy and multi-organ dysfunction due to liver failure. Although they showed a transient improvement in encephalopathy and biochemical profile, all the patients died, albeit after a longer period than expected according to their corresponding moribund situation. In contrast, when injected into three patients with chronic liver failure and cirrhosis, a substantial improvement in encephalopathy and better control of ascites were noted [28]. All patients were reported to be alive 7 months after the treatment. Another report revealed encouraging results when human hepatocytes were injected into the portal vein of children suffering from ALF [29]. The role of this new interesting technology in the treatment of patients with ALF is awaiting further confirmation.

The idea of combining hepatocytes with a synthetic assembly to create a bioreactor was based on the assumption that these techniques will be able to support both the metabolic and the excretory functions of the liver. The sources for hepatocytes considered suitable to be incorporated in the bioartificial liver are animals such as pigs, and primary or replicating human hepatocyte cell lines transformed by oncogenes or immortalized in cultures. Bioreactors were designed on the basis of countercurrent blood flow, as used in conventional hemodialysis over a semi-permeable membrane containing functional hepatocytes on the other side. The development of these devices was feasible due to improved understanding of the complex interactions of isolated hepatocytes and the artificial matrix containing them, and the improvement of hollow-fiber technology [30].

Sussman and co-workers [31] developed the extracorporeal liver assist device (ELAD). In this system, blood is perfused through a dialysis cartridge containing cells in the extracapillary space derived from a hepatoblastoma cell line and cultured within the dialysis cartridge. The ELAD treatment can be continued for long periods [32], and proved to be life-saving in a canine model of ALF induced by acetaminophen, in which 80% of the animals survived as compared to 100% mortality in the control group [33]. In addition, using the ELAD in animals demonstrated a reduction in the extent of liver injury assessed biochemically and histologically in hemoperfused animals [31]. When this method was used in humans, 8 of 11 ALF patients showed improved mental status, and 4 of them were transplanted although only one survived [32]. A pilot controlled study in which patients with ALF were randomly allocated to control therapy or ELAD perfusion for a median period of 72 hours showed some evidence of neurological improvement, although no difference in the survival rate was demonstrated between the groups [34].

Kamohara et al. [30] developed the bioartificial liver. In this system blood is removed from the patient and the plasma is separated and perfused through a charcoal column to reduce its toxicity before undergoing perfusion in a bioreactor, which is a hollow fiber module containing viable hapatocytes attached to a matrix. The use of the bioartificial liver was reported initially by Chen et al. [35], whose 12 ALF patients were successfully bridged to transplantation with a mean time to transplantation of 39 hours. A reduction in the grade of encephalopathy and intracranial pressure was also noted. In a phase I clinical trial presented by Watanabe's team [36], the bioartificial liver system was used for a median of 45 hours on 18 ALF patients of whom 16 survived until transplantation. These encouraging results favor continued research in phase II and III trials.

A common problem of all liver support systems is finding the ideal technique for efficient hepatocyte isolation and cultivation, both costly and lengthy processes. Since hepatocytes are anchorage dependent, tissue engineering technology based on cell surface interaction studies has designed microcarriers or microcapsules, to which free hepatocytes could attach, thus prolonging their life span [37]. It is estimated that between 20 and 30% of normal liver mass is needed for survival. As the normal liver weighs 1,500 g, a support system should be based on 400 g of hepatocytes in order to adequately support metabolism in an adult patient. Theoretically, human hepatocytes should serve as an ideal biological component in liver support systems, but they are in short supply and survive for only a few hours in cultures. Furthermore, they do not replicate and their biochemical activity deteriorates rapidly with time [38]. As such, if a continuous treatment is planned, a new supply of cells for the bioreactor is needed every 6–7 hours [38]. A proposed solution was to use the relatively easy-to-grow hepatoma cells, which have a short population-doubling time; but the transformed human hepatoma-derived cell lines have reduced metabolic activity compared with primary hepatocytes [39]. Additionally, leakage of cells through the semi-permeable

ELAD = extracorporeal liver assist device

membrane into the patient's circulation can present a major hazard in view of the patient's immune-suppressed state [22]. The development of immortalized human hepatocyte lines without the use of oncogenes enabled hepatocytes to be maintained for a very long period in cultures. However, their biological metabolic activity could be undesirably low in these conditions [39].

The porcine hepatocytes used in most of the biological liver support devices were chosen for their similar physiology to human cells. Another advantage of the porcine hepatocytes is the unlimited supply. Disadvantages of porcine hepatocytes include immunological reactions related to porcine protein exposure [22] and the potential for transmission of porcine viruses, such as retroviruses [40]. The latter mandates safety measures such as pig isolation and repeated serological examinations. This is a costly procedure.

Another intriguing problem concerning liver support systems is the difficulty to assess their efficiency, since no biochemical parameter has yet been identified as a reliable prognostic marker of patient survival. Although coagulation parameters might give some indication as to prognosis [2], the chances for recovery from ALF are related to the delicate equilibrium between the amount of damaged liver tissue and the regeneration rate of the liver. This equilibrium can only be assessed histologically by a liver biopsy, a dangerous procedure in the presence of coagulopathy. Having said that, the timing of transplantation versus continued artificial support is hard to determine. In this regard, the development of safe and reliable liver support systems will undoubtedly provide greater flexibility to the clinical team regarding therapeutic decision-making.

Conclusion

While acute liver failure continues to have a high mortality rate, the development of artificial liver support systems may offer new opportunities. By providing essential liver functions, these systems may keep patients alive until orthotopic liver transplantation with a suitable donor is successfully accomplished, or the native liver regenerates. However, success with the current liver support systems has been demonstrated only for relatively short-term periods of treatment with a paucity of controlled data. The goal for the future should be the development of a liver support technology that can be used on a long-term basis with the convenience and safety of dialysis in end-stage renal disease. This could also be used to treat patients with chronic liver disease, albeit not on an emergency basis, thus improving their chance of survival until liver transplantation becomes available.

References

1. Schiodt FV, Atillasoy E, Shakil AO, Schiff ER, Caldwell C, Kowdley KV, Stribling R, Crippin JS, Flam S, Somberg KA, Rosen H, McCashland TM, Hay JE, Lee WM. Etiology and outcome for 295 patients with acute liver failure in the United States. *Liver Transpl* 1999;5:29–34.
2. Shakil AO, Kramer D, Mazariegos GV, Fung JJ, Rakela J. Acute liver failure: clinical features, outcome analysis, and applicability of prognostic ctiteria. *Liver Transpl* 2000;6(2):163–9.
3. Ascher NL, Lake JR, Emond JC, Roberts JP. Liver transplantation for fulminant hepatic failure. *Arch Surg* 1993;128:677–82.
4. Cattral MS, Levy GA. Artificial liver support – pipe dream or reality? *N Engl J Med* 1994;331:268–9.
5. Chenard-Neu MP, Boudjema K, Bernuau J, Degott C, Belghiti J, Cherqui D, Costes V, et al. Auxillary liver transplantation: regeneration of the native liver and outcome in 30 patients with fulminant hepatic failure: a multicenter European study. *Hepatology* 1996;23:1119–27.
6. Lee C, Tink A. Exchange transfusion in hepatic coma. Report of a case. *Med J Austr* 1958;1:40–2.
7. Trey MB, Burns DG, Saunders SJ. Treatment of hepatic coma by exchange blood transfusion. *N Engl J Med* 1996;274:473–81.
8. Cooper GN, Karlson KE, Clowes GH, Martin H, Randall HT. Total body washout and exchange. A valuable tool in acute hepatic coma and Reye's syndrome. *Am J Surg* 1977;133:522–30.
9. Kiley JE, Welch HF, Pender JC. Removal of blood ammonia by hemodialysis. *Proc Soc Exp Biol Med* 1956;91:489–90.
10. Trewby PN, Warren R, Contini S, Crosbie WA, Wilkinson SP, Laws JW, Williams R. Incidence and pathophysiology of pulmonary edema in fulminant hepatic failure. *Gastroenterology* 1978;74:859–65.
11. Kiley JE, Pender JC, Welch HF. Ammonia intoxication treated by hemodialysis. *N Engl J Med* 1958;259:1156–61.
12. Bihari D, Hughes RD, Gimson AE, Langely PG, Ede RJ, Eder G, Williams R. Effect of serial resin hemoperfusion in fulminant hepatic failure. *Int J Artif Organs* 1983;6:299–302.
13. Malchesky PS. Nonbiological liver support: historic review. *Artif Organs* 1994;18:342–7.
14. Yatzidis H, Oreopoulos D. Early clinical trials with sorbents. *Kidney Int* 1976;7(Suppl):S215–17.
15. O'Grady JG, Gimson AES, O'Brien CJ, Pucknell A, Huges RD, Williams R. Controlled trials of charcoal hemoperfusion and prognostic factors in fulminant hepatic failure. *Gastroenterology* 1988;94:1186–92.
16. Sabin S, Merritt JA. Treatment of hepatic coma in cirrhosis by plasmapheresis and plasma infusion. *N Engl J Med* 1966;274:473–81.
17. Freeman JG, Matthewsson K. Plasmapheresis in acute liver failure. *Int J Artif Organs* 1986;9:433–8.
18. Kondrup J, Almdal T, Vilstrup H, Tytgstrup N. High volume plasma exchange in fulminant hepatic failure. *Int J Artif Organs* 1992;15:669–76.
19. Yoshiba M, Sekiyama K, Iwamura Y, Sugata F. Development of reliable artificial liver support (ALS) – plasma exchange in combination with hemofiltration using high performance membranes. *Dis Sci* 1993;38:469–76.
20. Brunner G, Losgen H. Benefits and dangers of plasma exchange in patients with fulminant hepatic failure. In: Oda T, Shiokawa Y, Inoue N, eds. Therapeutic Plasmapheresis. VI. Therapeutic Plasmapheresis. Cleveland: ISAO Press, 1990:187–91.
21. Stange J, Mitzner S, Ramlow W, Gliesche T, Hikstein H, Schmidt R. A new procedure for the removal of protein bound drugs and toxins. *ASAIO J* 1993;39:621–5.
22. Stange J, Mitzner S. A carrier-mediated transport of toxins in a hybrid membrane. Safety barrier between a patient's blood and a bioartificial liver. *Int J Artif Organs* 1996;19: 677–91.
23. Stange J, Mitzner SR, Risler T, Erly CM, Lauchart W, Goehl H, Klammt S, Peszynski P, Freytag J, Hickstein H, Lohr M, Liebe S, Schareck W, Hopt UT, Schmidt R. Molecular adsorbent recycling system (MARS): clinical results of a new membrane-based blood purification system for bioartificial liver support. *Artif Organs* 1999;23:319–30.
24. Mazariegos GV, Ash SR, Patzer JF. Preliminary results: randomized clinical trial of the Biologic-DT in treatment of acute hepatic failure (AHF) with coma. *Artif Organs* 1997;21:529.
25. Chari RS, Collins BH, Magee JC, DiMaio JM, Kirk AD, Harland RC, McCann RL, Platt JL, Meyers WC. Treatment of hepatic failure with ex vivo pig-liver perfusion followed by liver transplantation. *N Engl J Med* 1994; 331:234–7.

26. Harland RC, Platt JL. Prospects for xenotransplantation of the liver. *J Hepatol* 1996;25:248–58.
27. Fox IJ, Langhans AN, Fristooe LW, Shaefer MS, Vogel JE, Antonson Dl, Donovan JP, Heffron TG, Markin RS, Sorrell MF, Shaw BW. Successful application of extracorporeal liver perfusion for the treatment of fulminant hepatic failure. A technology whose time has come. *Am J Gastroenterol* 1993;88:1876–81.
28. Bilir BM, Guenette D, Ostrowska A, Durham J, Kumpe D, Krysl J, Trouillot T, Teitelbaum I, Everson GT. Percutaneous hepatocyte transplantation (PHT) in liver failure. *Hepatology* 1997;26:252A.
29. Soriano HE, Wood RP, Kang DC. Hepatocellular transplantation in children with fulminant liver failure. *Hepatology* 1997;26:239A.
30. Kamohara Y, Rozga J, Demetriou AA. Artificial liver: review and Cedars-Sinai experience. *J Hepatobiliary Pancreat Surg* 1998;5:273–85.
31. Sussman NL, Chong MG, Koussayer T, He D, Shang TA, Whisennand HH, Kelly JH. Reversal of fulminant hepatic failure using an extracorporeal liver assist device. *Hepatology* 1992;16:60–5.
32. Sussman NL, Gislason GT, Conlin CA, Kelly GA. The hepatic extracorporeal liver assist device: initial clinical experience. *Artif Organs* 1994;18:390–6.
33. Sussman NL, Kelly GA. Extracorporeal liver assist in the treatment of fulminant hepatic failure. *Blood Purif* 1993;11:170–4.
34. Ellis AJ, Hughes RD, Wendon JA, Dunne J, Langley PG, Kelly JH, Gislason GT, Sussman NL, Williams R. Pilot-controlled trial of the extracorporeal liver assist device in acute liver failure. *Hepatology* 1996;24:1446–51.
35. Chen SC, Hewitt WR, Watanabe FD, Eguchi S, Kahaku E, Middleton Y, Rozga J, Demetriou AA. Clinical experience with a porcine hepatocyte-based liver support system. *Int J Artif Organs* 1996;199:664–9.
36. Watanabe FD, Mullon CJ, Hewitt WR, Arkadopoulos N, Kahaku E, Eguchi S, et al. Clinical experience with a bioartificial liver in the treatment of severe liver failure: a phase I clinical trial. *Ann Surg* 1997;225:484–91.
37. Gerlach J, Kloppel K, Schauwecker HH, Tauber R, Muller C, Buherl ES. Use of hepatocytes in adhesion and suspension cultures for liver support bioreactors. *Int J Artif Organs* 1989;12:788–92.
38. Rozga J, Podesta L, LePage E, Morsiani E, Moscioni AD, Hoffman A, Sher L, Villamil F, Woolf G, McGrath M. A bioartificial liver to treat severe acute liver failure. *Ann Surg* 1994;219:538–46.
39. Nyberg SL, Remmel RP, Mann HJ, Peshwa MV, Hu WS, Cerra FB. Primary hepatocytes outperform HepG2 cells as the source of biotransformation functions in a bioartificial liver. *Ann Surg* 1994;220:59–67.
40. Patience C, Takeuchi Y, Weiss RA. Infection of human cells by an endogenous retrovirus of pigs. *Nat Med* 1997;3:282–6.

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