Cell Medicine, Part B of Cell Transplantation, Vol. 1, pp. 47–54, 2010
Printed in the USA.

2155-1790/10 $90.00 + .00
DOI: 10.3727/215517910X516646
www.cognizantcommunication.com

Review

New Strategies for Acute Liver Failure: Focus on Xenotransplantation Therapy

Luiz Anastácio Alves,* André Bonavita,* Kátia Quaresma,* Elenilde Torres,* Paulo Anastácio Furtado Pacheco,* Vinícius Cotta-de-Almeida,† and Roberto Magalhães Saraiva‡

*Laboratório de Comunicação Celular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil
†Laboratório de Inovações em Terapias, Ensino e Bioprodutos, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil
‡Instituto de Pesquisa Evandro Chagas (IPEC), Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Acute liver failure (ALF) has a poor prognosis and, despite intensive care support, reported average survival is only 10–40%. The most common causes responsible for ALF are viral hepatitis (mainly hepatitis A and B) and acetaminophen poisoning. Hepatic transplantation is the only appropriate treatment for patients with unlikely survival with supportive care alone. Survival rates after transplantation can be as high as 80–90% at the end of the first year. However, there is a shortage of donors and is not uncommon that no appropriate donor matches with the patient in time to avoid death. Therefore, new technologies are in constant development, including blood purification therapies as plasmapheresis, hemodiafiltration, and bioartificial liver support. However, they are still of limited efficacy or at an experimental level, and new strategies are welcome. Accordingly, cell transplantation has been developed to serve as a possible bridge to spontaneous recovery or liver transplantation. Xenotransplant of adult hepatocytes offers an interesting alternative. Moreover, the development of transgenic pigs with less immunogenic cells associated with new immunosuppressor strategies has allowed the development of this area. This article reviews some of the newly developed techniques, with focus on xenotransplant of adult hepatocytes, which might have clinical benefits as future treatment for ALF.

Key words: Xenotransplantation; Acute liver failure; Cell therapy; Survival

INTRODUCTION

The liver is the major metabolic organ and has a central role in the homeostasis of the organism, as it is responsible for the synthesis of a great variety of proteins and factors, metabolism of glucose, amino acids, cholesterol, and drugs, and blood purification from metabolites and external toxins. The human liver has about 250×10^9 cells organized in about 1 million liver lobules. Hepatocytes represent the main cell population, contributing 65–70% of the total liver cells. On the other hand, many different factors, such as toxins, microorganisms, ischemia, and neoplasia, may harm the liver (39).

Acute liver failure (ALF) is a clinical syndrome characterized by rapid loss of liver function and with high mortality. ALF occurs when a patient without previous liver disease develops evidence of hepatic failure with encephalopathy within 8 weeks from the onset of the symptoms. The most common causes responsible for ALF are viral hepatitis (mainly hepatitis A and B) and acetaminophen poisoning. Other causes include toxins (e.g., *Amanita phalloides*), drugs (e.g., halothane), ischemia, occlusion of large (Budd-Chiari syndrome) or small hepatic veins, malignant infiltration, Wilson's disease, and Reye's syndrome, among others (49). ALF presents with metabolic acidosis (lactic acidosis), coagulopathy with prolonged prothrombin time, hypoglycemia, renal failure, sepsis, and, the most ominous, encephalopathy with cerebral edema (60). The encephalopathy degree can vary from confusion to coma and is associated to the cerebral edema intensity. The intracranial pressure rises and intracranial hypertension is one of the most

Received June 8, 2009; final acceptance Spetember 11, 2009.
Address correspondence to Luiz Anastácio Alves, M.D., Ph.D., Laboratório de Comunicação Celular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz-FIOCRUZ, Av. Brasil, 4365 Manguinhos, Rio de Janeiro, Brazil, 21045-900. E-mail: alveslaa@ioc.fiocruz.br

important causes of death in ALF, together with sepsis and multiple organ failure (13,20–22).

Mortality rate in ALF is high, ranging from 60% to 90%, depending on the studied series (8) even with the modern life support therapies available in intensive care units. Estimates indicate that 1–2 million persons worldwide die each year from hepatic failure, with more than 50,000 deaths per year in the US (47). The prognosis of patients with ALF is poor, and the outcome depends on many characteristics such as the patient's age, disease etiology, metabolic parameters, as well as the severity of encephalopathy and others complications. In this context, the majority of patients who progress to ALF die, unless they undergo intervention (47).

Because there is no satisfactory treatment for hepatic failure, the only recommended therapy is the substitution of the hepatic function, which can only be successfully accomplished nowadays by liver transplantation. Survival rate of patients submitted to this technique can be as high as 80–90% at the end of the first year post-transplantation (31). However, the applicability of liver transplantation is limited in patients with ALF by shortage of organ donors, such that many patients die of rapidly progressive multiorgan failure before a liver donor becomes available (17). Even for patients with chronic liver dysfunction, according to the "United Network for Organ Sharing," there were more than 17,000 patients on the waiting list for just 5,435 liver transplantation donors in the US in 2005 (31). In this article, we review the new strategies for the treatment of ALF, giving focus on xenogeneic cell therapy.

TEMPORARY LIVER SUPPORT TECHNIQUES

Alternatives to liver transplantation are blood purification therapies such as plasmapheresis, hemodiafiltration, and bioartificial liver support. Plasmapheresis implies removal of the patient's plasma with reposition of fresh plasma. Although it results in immediate transitory clinical improvement, it has a high cost and all the risk associated with transfusion of great amount of blood products. In addition, the liver has more than 500 different functions that cannot be replaced just by plasma exchange. Therefore, development of extracorporeal systems to replace liver function has been the focus of many researchers. The bioartificial liver uses liver tissue or adult hepatocytes to replace the synthesis and detoxification functions. However, clinical studies demonstrated that humans can develop immune response against swine proteins, and that anaphylactic reactions did occur when patients were subjected to extracorporeal perfusion using animal livers (1). Later, bioreactors containing artificial networks where hepatocytes were allocated in the extrafiber spaces were developed (16, 36,59). These are still highly complex structures that make their widespread use in clinical settings unlikely. An alternative was whole-liver extracorporeal perfusion but using a hemoperfusion column capable of absorbing leukocytes and immunoglobulin in order to avoid hyperacute reaction against the xenogeneic liver (37,38). However, risks remained for transmission of xenogeneic protein from the extracorporeal liver to patient blood stream and consequent immune reactions (e.g., immunocomplex deposition), and also of zoonosis. The most common method used in clinical trials to treat ALF is the combination of plasmapheresis with hemodiafiltration using human albumin. Use of albumin dialysis is necessary because most toxins removed by the liver are not hydrosoluble, and usually circulate in the cardiovascular system bound to albumin. The more recent methods that follow this principle are MARS (Molecular Adsorbents Recirculating System) and Prometheus (27). However, these methods must still be considered experimental as they have not improved survival in patients with ALF, despite biochemical improvement (27). The reader is invited to consult recent reviews that discuss the development of and the clinical experience with these devices (38,47,51).

CELL THERAPY FOR ALF

Cell transplantation has been proposed as a promising method to support patients with liver insufficiency. As the liver structure usually is preserved in ALF, and the liver has potential to regenerate, cell transplantation provides an attractive approach to support metabolic functions to buy time for liver recovery (17,39). In this view, many investigators are evaluating the transplantation of stem cells from many tissues or adult allogeneic or xenogeneic liver cells as a bridge for patients who are waiting for a donor liver. Moreover, cell transplantation has some advantages over whole organ transplantation: it is a less invasive procedure and can be performed repeatedly. Identification of suitable additional sources of cells that could be transplanted into the liver should be effective for cell therapy applications.

Stem Cells: A Solution or a Problem?

Cell-based therapy and tissue bioengineering development allowed a possible treatment for several degenerative diseases (46). As current methods to support life in ALF are of limited efficacy, cell transplantation, using adult hepatocytes or stem cells, has emerged as a possible bridge to liver transplantation or spontaneous recovery (2,39).

Human adult hepatocytes are hard to obtain and manipulate, and the experience using these cells is very limited, which makes this method unlikely to be widespread in the near future (39). Nevertheless, the experience reported to date in ALF is encouraging. Most pa-

tients who received intrasplenic (6,15), intraperitoneal (18,25), or direct liver infusion (15,50) of adult or fetal human hepatocytes presented improvement in encephalopathy and biochemical tests, and some were able to be brought successfully to liver transplantation or experienced spontaneous recovery. However, randomized clinical trials including higher numbers of patients are needed in order to prove a survival benefit. Even so, as adult or fetal human hepatocytes are hard to obtain, the clinical results obtained to date with these cells stimulate the research with other cell types.

The development of stem cell therapy allowed the study of other cell types, including mesenchymal, hematopoietic, fetal, and liver stem cells (2,39). However, many questions remain to be addressed before these cells can be used to treat ALF in humans. Among these points, the most crucial is the question if these stem cells can differentiate in vivo into adult hepatocytes capable of performing all the functions that a normal liver does.

Embryonic stem cells have also been tested in acute liver failure models. It seems possible to obtain functional hepatocyte-like cells from embryonic stem cells in vitro (9). Furthermore, transplantation of embryonic stem cells (28), or adult cells obtained from in vitro differentiation of embryonic stem cells (54), into animal models of liver failure resulted in survival improvement. Additionally, in a mouse model of lethal fulminating hepatic failure, bone marrow stem cells were able to differentiate into functional hepatocytes, increase animal survival, and facilitate endogenous hepatocytes to repopulate liver tissue (29). Interestingly, fetal hepatocytes have also been regarded as potentially suitable to be used as donor cells in ALF. In contrast to adult hepatocytes, fetal hepatocytes are thought to be highly proliferative, which may facilitate engraftment and expansion of transplanted cell population (3). Recent work demonstrated that rat fetal liver cells repopulate two thirds of a liver after partial hepatectomy in normal rats, and that transplanted cells are morphologically and functionally integrated into the host liver and indistinguishable from host hepatocytes, except for the marker gene (40,41).

Nonetheless, some researchers have presented controversial data. Recently, Sharma and coworkers (52) demonstrated that hepatic stem cells have a limited repopulation capacity associated with an immature hepatic phenotype. In fact, stem cells transplanted to liver form "clusters," but not a real hepatic tissue with normal architecture (39). In addition, spontaneous cell fusion was observed between hepatocytes and hematopoietic or embryonic stem cells. These cells adopt the phenotype of the recipient cells, which, without detailed genetic analysis, might be interpreted as differentiation into adult tissue cells (57,65). It is important to point out that cells expanded and differentiated into adult hepatocytes in vitro must keep their new phenotype in order to be capable of performing all functions of a normal organ when injected in vivo (19). Furthermore, despite the functional recovery obtained after cell transplantation between rodents in acute liver failure models (23), no significant improvement in hepatic function was noted when human stem cells were used in a similar mouse model (39).

Another limitation with the use of uncommitted cells is their putative capacity to generate tumors in the patient (34). Comparative proteomic analysis of embryonic and tumor liver cells demonstrate that both cell populations share certain oncofetal markers, suggesting that fetal liver stem cells could grow as a hepatocellular carcinoma cell (30). Besides, these totipotent cells could enter on systemic circulation and potentially populate any other organ, which might lead to unexpected results. Finally, the timing for cell differentiation in vitro or in vivo into adult hepatocytes might be incompatible with the urgent need for hepatic function replacement required for ALF patients.

Xenotransplantation: From Organ to Cell

The use of cells, tissues, and organs from other species for transplantation, called xenotransplantation, has being pointed as a potential solution to the problem of severe shortage of transplant donors for liver diseases as ALF and other liver-based inherited metabolic disorders. Moreover, xenotransplantation offers two additional advantages that might be explored to enlarge the application of this transplantation modality. First, xenotransplantation might not be susceptible to certain human viruses, like hepatitis and HIV. The second benefit is that xenotransplantation might offer a way for "gene delivery" that could overcome some current hurdles to gene therapy, because genetic changes could be carried out to egg embryo genome (24). For many years, liver support systems have been developed to supply the liver functions, mostly as a bridge to transplantation, but these systems are not yet clinical standard therapy, as discussed before. The therapeutic option of swine organ xenotransplantation to humans has being investigated (10). However, the clinical application of this practice is limited by severe immune response of the recipient against the donor organ, resulting in immunological destruction of the graft. This response toward a xenograft is complex and comprises initially two antibody-mediated processes, termed hiperacute rejection (HAR) and acute humoral xenograft rejection (AHXR) (64). The xenoreactive natural antibodies that mediate both HAR and AHXR are directed against the ubiquitous terminal carbohydrate epitope Galα1-3Galβ1-4GlcNAc (α1,3Gal) that is present on the glycoproteins and glycolipids of porcine vascular endothelium. As many microorganisms also express α1,3Gal, humans are constantly exposed to

these antigenic determinants. For this reason, humans present these natural antibodies in the sera (10,64).

The development of transgenic pigs was an important step towards the control of hyperacute and vascular rejections. Xenotransplants to animal models using livers obtained from pigs expressing human complement regulatory proteins (5,11), whose organs are protected against the recipient's complement system, or from pigs that have the α1,3-galactosltransferase gene disrupted and, therefore, do not express α1,3Gal (26), demonstrated longer xenograft survival (10). Moreover, a recent study demonstrated that transgenic pigs that expresses HLA-E/human β2-microglobuin are protected against NK cell-mediated cytotoxity and this new approach has important implications for the generation of transgenic pigs as organ or tissue donors for clinical xenotransplantation (62). Modulation of the recipient immune response and anticoagulation therapy prolong the survival of the xenograft and avoid intravascular thrombosis of the graft (10).

The risk of cross-species infection or zoonosis (also called xenozoonosis or direct zoonosis) is another hurdle to xenotransplantation (14). Nevertheless, this risk can be minimized by housing the animals in an ambient environment with efficient barrier methods and stringent screening for possible exogenous microorganisms. Although the risk of swine endogenous retrovirus transmission still exists (56), among 160 patients treated with tissues of swine origin no swine retrovirus infection was detected even 10 years after exposure (43). Another study with 13 recipients of embryonic porcine mesencephalic tissue or extracorporeal perfusion with transgenic pig livers also found no evidence of porcine endogenous retroviruses (PERV) sequences by PCR testing peripheral blood mononuclear cells (44).

Transplantation of isolated xenogeneic hepatocytes instead of whole liver has been proposed and would constitute an unlimited supply of cells to treat severe diseases (55). Besides that, the use of adult hepatocytes is an interesting option because there is no endothelium related with xenogeneic whole organ transplantation (7). In 1995, an original work demonstrated that rat hepatocytes could reconstitute the liver of hepatectomized mice (45). In the same way, highly differentiated human adult hepatocytes could repopulate the immunotolerant RAG-2 knockout mouse liver (12), confirming the possible use of adult hepatocytes for xenotransplant. Mai and coworkers (32) demonstrated that the injection of human hepatocytes in mice with ALF was able to provide life-supporting and liver-specific function requiring no im-

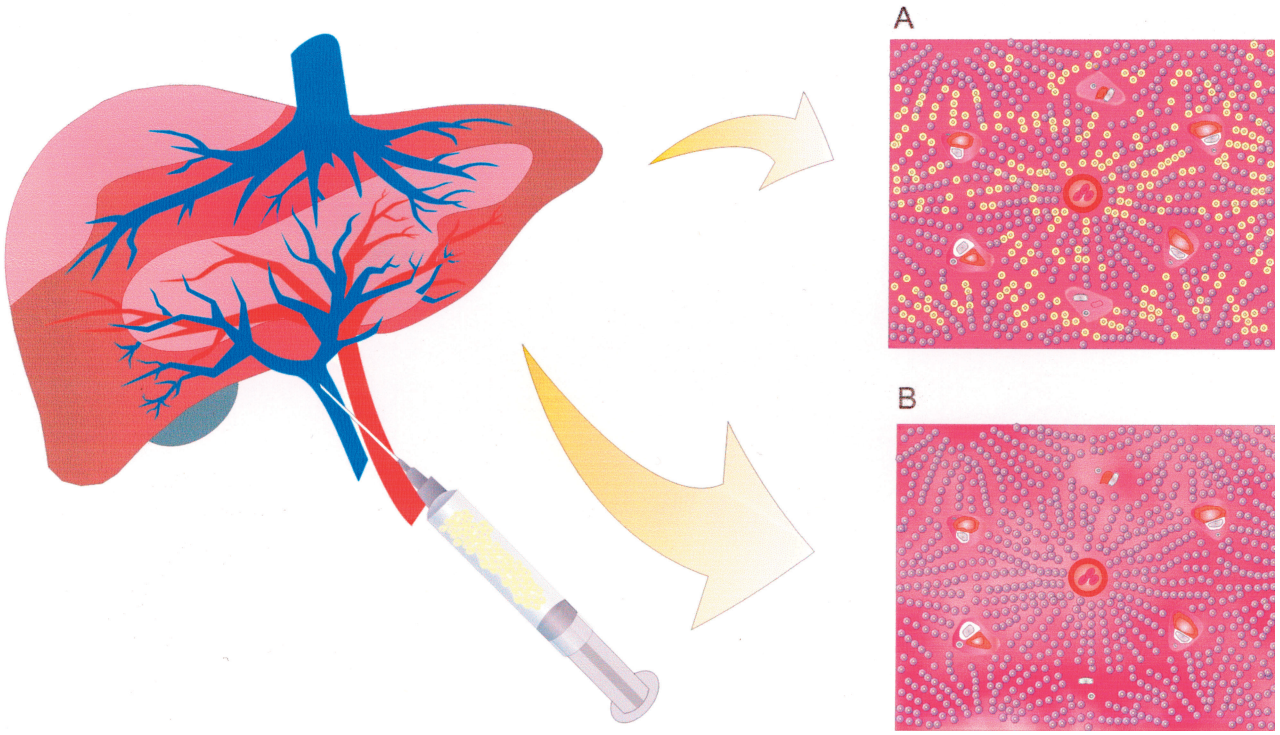

Figure 1. The xenotransplant of hepatocytes in acute liver failure. Acute liver failure patients will be subjected to knock-out pig hepatocyte transplantation. As illustrated, xenotransplanted hepatocytes will live together with normal cells (A) or at least have a transient survival allowing the multiplication of host cells (B). In both cases a better survival is expected.

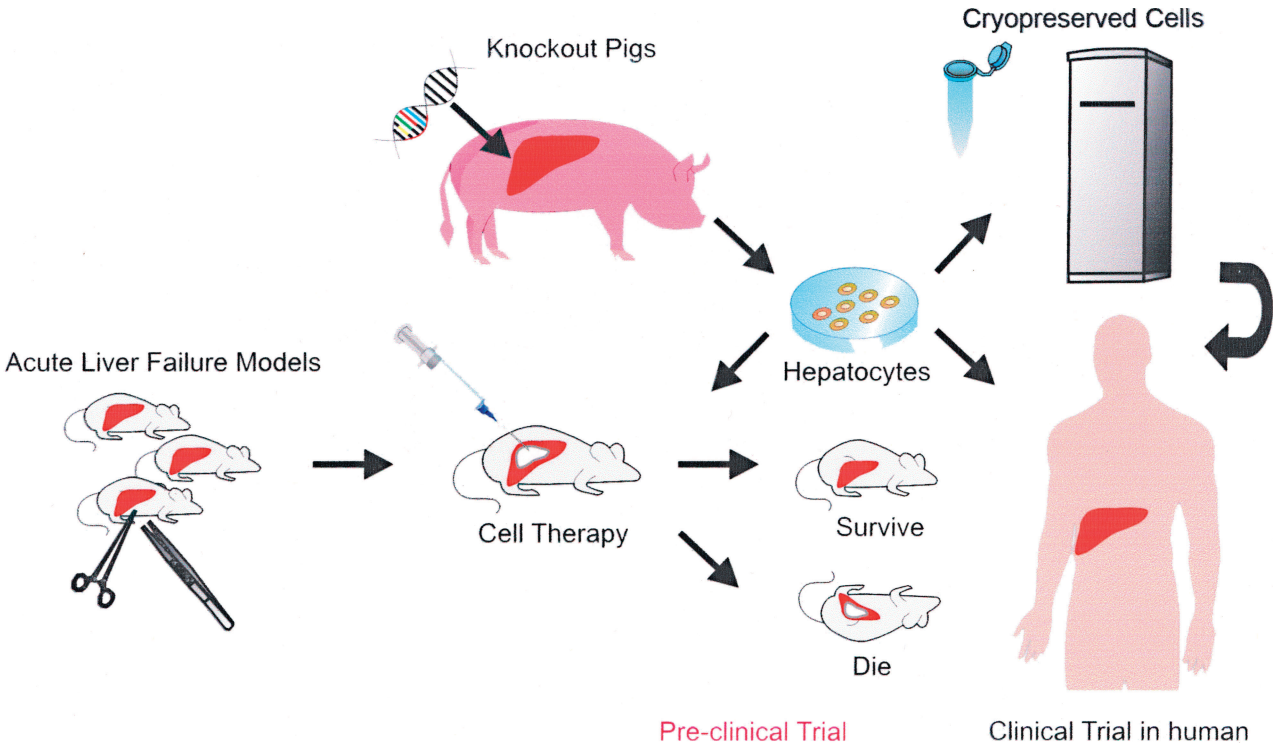

Figure 2. Development and application of xenogeneic transplantation in acute liver failure. Adult hepatocytes will be isolated from porcine livers obtained from α1,3-galactosyltransferase knockout pigs. These cells will be transplanted into the liver of acute liver failure animal models where we expect a higher survival (preclinical trials). The ideal number of injected cells will be experimentally determined. The porcine cells may be frozen or directly transplanted to human with acute liver failure (clinical trials).

munosuppressive therapy. Similarly, the transplantation of immortalized human hepatocytes into the spleens of severe combined immunodeficiency mice with acetaminophen-induced ALF resulted in increased survival (58). However, the use of immunoregulators could have applicability on xenogeneic implantation on the liver. A recent work using a mouse model of ALF treated with anti-Fas antibody transplanted with rat hepatocytes suggested that the induction of cell proliferation blockade in recipient livers could promote sufficient engraftment of transplanted hepatocytes to allow transient or definitive treatment of liver failure (61). In addition, the administration of daclizumab, a humanized anti-IL2 receptor α chain antibody, in rats with ALF contributed to improve the survival of animals that received rabbit hepatocytes (42).

Porcine hepatocytes may also be a source for cell xenotransplantation. Encapsulated porcine hepatocytes transplanted into the rat peritoneum remained viable and functional 1 month after the procedure (4), and porcine hepatocytes directly injected into the spleen of monkeys were able to engraft and survive (35), suggesting that these cells could be used for the treatment of ALF. In fact, transplantation of either encapsulated (48) or non-encapsulated (33) adult porcine hepatocyte transplantation into the peritoneum of rats with ALF resulted in significant reduction in the mortality rate. The use of porcine hepatocytes for cell xenotransplantation has also been tested in chronic liver failure models where xenogeneic hepatocytes were able to integrate the recipient liver and produce albumin for more than 50 days (53). The use of hepatocytes obtained from pigs expressing human complement regulatory proteins or not expressing antigen on their surface may confer additional advantages to the xenogeneic transplanted cells. The use of siRNA on porcine hepatocytes to the α1,3-galactosyltransferase gene result in cells resistant to natural antibodies in human serum (63), indicating that they could be resistant to HAR during an interspecies transplantation. In addition, cell transplantation would be a far more simple technique than the transplant of a whole organ, a principle that should be taken into account in the treatment of critical patients.

To date, however, there are few data about cellular xenotransplantation of knockout pigs in ALF models, and more studies in this area are necessary. The expectation is that xenotransplantation of swine adult hepatocytes to failing livers may improve their function and

act as a bridge to liver transplant or even to spontaneous recovery of the native liver (Fig. 1). The transplanted cells might be functional and result in improvement of liver function with consequent improvement in the survival. The xenotransplanted cells will eventually be rejected by the host, but the use of cells from transgenic animals, possibly combined with the use of immunosuppressors, would allow a prolonged survival of these cells in the host.

Other potential use for cell xenotransplantation is the treatment of chronic liver failure with acute deterioration. Besides correcting the factor that contributed to the acute deterioration, the hepatocyte xenotransplantation could give the necessary metabolic support while waiting for spontaneous recovery or liver transplantation.

CONCLUSION

Liver transplantation is currently the only concrete hope for patients with ALF. However, liver transplantation is not available to the majority of patients, and new techniques to replace liver function are needed. Cell transplantation may be a great option as the hepatocytes are capable of performing the complex tasks normally carried out by the whole liver. For the reasons outlined, transplant of xenohepatocytes may represent an interesting alternative and further studies with animal models are needed to provide the basis for future studies involving humans with ALF, as illustrated in Figure 2. Additionally, swine hepatocytes can be readily available in enough number to change the clinical status of the patient on a short-term basis. The research in this field may result in a new reality for patients in ALF that wait in critical conditions for a liver transplantation.

REFERENCES

1. Abouna, G. M.; Boehmig, H. G.; Serrou, B.; Amemiya, H.; Martineau, G. Long-term hepatic support by intermittent multi-species liver perfusions. Lancet 2(7669):391–396; 1970.
2. Aleem, K. A.; Parveen, N.; Habeebs, M. A.; Habibullah, C. M. Journey from hepatocyte transplantation to hepatic stem cells: A novel treatment strategy for liver diseases. Indian J. Med. Res. 123(5):601–614; 2006.
3. Allen, K. J.; Soriano, H. E. Liver cell transplantation: The road to clinical application. J. Lab. Clin. Med. 138(5): 298–312; 2001.
4. Baldini, E.; Cursio, R.; De Sousa, G.; Margara, A.; Honiger, J.; Saint-Paul, M. C.; Bayer, P.; Raimondi, V.; Rahmani, R.; Mouiel, J.; Gugenheim, J. Peritoneal implantation of cryopreserved encapsulated porcine hepatocytes in rats without immunosuppression: Viability and function. Transplant. Proc. 40(6):2049–2052; 2008.
5. Bhatti, F. N.; Schmoeckel, M.; Zaidi, D.; Cozzi, E.; Chavez, G.; Goddard, M.; Dunning, J. J.; Wallwork, J.; White, D. J. Three-month survival of HDAFF transgenic pig hearts transplanted into primates. Transplant. Proc. 31(1–2):958; 1999.
6. Bilir, B. M.; Guinette, D.; Karrer, F.; Kumpe, D. A.; Krysl, J.; Stephens, J.; Mcgavran, L.; Ostrowska, A.; Durham, J. Hepatocyte transplantation in acute liver failure. Liver Transpl. 6(1):32–40; 2000.
7. Cattan, P.; Zhang, B.; Braet, F.; Atia, N.; Conti, F.; Conjeaud, H.; Weill, B.; Chereau, C.; Houssin, D.; Calmus, Y. Comparison between aortic and sinusoidal liver endothelial cells as targets of hyperacute xenogeneic rejection in the pig to human combination. Transplantation 62(6):803–810; 1996.
8. Chamuleau, R. A.; Deurholt, T.; Hoekstra, R. Which are the right cells to be used in a bioartificial liver? Metab. Brain Dis. 20(4):327–335; 2005.
9. Cho, C. H.; Parashurama, N.; Park, E. Y.; Suganuma, K.; Nahmias, Y.; Park, J.; Tilles, A. W.; Berthiaume, F.; Yarmush, M. L. Homogeneous differentiation of hepatocyte-like cells from embryonic stem cells: Applications for the treatment of liver failure. FASEB J. 22(3):898–909; 2007.
10. Cox, A.; Zhong, R. Current advances in xenotransplantation. Hepatobiliary Pancreat. Dis. Int. 4(4):490–494; 2005.
11. Cozzi, E.; Tucker, A. W.; Langford, G. A.; Pino-Chavez, G.; Wright, L.; O'Connell, M. J.; Young, V. J.; Lancaster, R.; McLaughlin, M.; Hunt, K.; Bordin, M. C.; White, D. J. Characterization of pigs transgenic for human decay-accelerating factor. Transplantation 64(10):1383–1392; 1997.
12. Dandri, M.; Burda, M. R.; Török, E.; Pollok, J. M.; Iwanska, A.; Sommer, G.; Rogiers, X.; Rogler, C. E.; Gupta, S.; Will, H.; Greten, H.; Petersen, J. Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus. Hepatology 33(4):981–988; 2001.
13. Detry, O.; Arkadopoulos, N.; Ting, P.; Kahaku, E.; Margulies, J.; Arnaout, W.; Colquhoun, S. D.; Rozga, J.; Demetriou, A. A. Intracranial pressure during liver transplantation for fulminant hepatic failure. Transplantation 67(5):767–770; 1999.
14. Fishman, J. A.; Patience, C. Xenotransplantation: Infectious risk revisited. Am. J. Transplant. 4(9):1383–1390; 2004.
15. Galvão, F. H.; de Andrade Júnior, D. R.; de Andrade, D. R.; Martins, B. C.; Marson, A. G.; Bernard, C. V.; dos Santos, S. A.; Bacchella, T.; Machado, M. C. Hepatocyte transplantation: State of the art. Hepatol. Res. 36(4):237–247; 2006.
16. Gerlach, J. C.; Encke, J.; Hole, O.; Muller, C.; Ryan, C. J.; Neuhaus, P. Bioreactor for a larger scale hepatocyte in vitro perfusion. Transplantation 58(9):984–988; 1994.
17. Gewartowska, M.; Olszeski, W. L. Hepatocyte transplantation—biology and application. Ann. Transplant. 12(1): 27–36; 2007.
18. Habibullah, C. M.; Syed, I. H.; Qamar, A.; Taher-Uz, Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. Transplantation 58(8):951–952; 1994.
19. Hengstler, J. G.; Brulport, M.; Schormann, W.; Bauer, A.; Hermes, M.; Nussler, A. K.; Fandrich, F.; Ruhnke, M.; Ungefroren, H.; Griffin, L.; Bockamp, E.; Oesch, F.; von Mach, M. A. Generation of human hepatocytes by stem cell technology: Definition of the hepatocyte. Expert Opin. Drug. Metab. Toxicol. 1(1):61–74; 2005.
20. Jalan, R. Intracranial hypertension in acute liver failure: Pathophysiological basis of rational management. Semin. Liver Dis. 23(3):271–282; 2003.
21. Jalan, R.; Davies, N. A.; Damink, S. W. Hypothermia for the management of intracranial hypertension in acute liver failure. Metab. Brain Dis. 17(4):437–444; 2002.

22. Jalan, R.; Olde Damink, S. W.; Deutz, N. E.; Davies, N. A.; Garden, O. J.; Madhavan, K. K.; Hayes, P. C.; Lee, A. Moderate hypothermia prevents cerebral hyperemia and increase in intracranial pressure in patients undergoing liver transplantation for acute liver failure. Transplantation 75(12):2034–2039; 2003.
23. Jang, Y. Y.; Collector, M. I.; Baylin, S. B.; Diehl, A. M.; Sharkis, S. J. Hematopoietic stem cells convert into liver cells within days without fusion. Nat. Cell Biol. 6(6):532–539; 2004.
24. Kanazawa, A.; Platt, J. L. Prospects for xenotransplantation of the liver. Semin. Liver Dis. 20(4):511–522; 2000.
25. Khan, A. A.; Habeeb, A.; Parveen, N.; Naseem, B.; Babu, R. P.; Capoor, A. K.; Habibullah, C. M. Peritoneal transplantation of human fetal hepatocytes for the treatment of acute fatty liver of pregnancy: A case report. Trop. Gastroenterol. 25(3):141–143; 2004.
26. Kolber-Simonds, D.; Lai, L.; Watt, S. R.; Denaro, M.; Arn, S.; Augenstein, M. L.; Betthauser, J.; Carter, D. B.; Greenstein, J. L.; Hao, Y.; Im, G. S.; Liu, Z.; Mell, G. D.; Murphy, C. N.; Park, K. W.; Rieke, A.; Ryan, D. J.; Sachs, D. H.; Forsberg, E. J. J.; Prather, R. S.; Hawley, R. J. Production of alpha-1,3-galactosyltransferase null pigs by means of nuclear transfer with fibroblasts bearing loss of heterozygosity mutations. Proc. Natl. Acad. Sci. USA 101(19):7335–7340; 2004.
27. Krisper, P.; Stauber, R. E. Technology insight: Artificial extracorporeal liver support—how does Prometheus compare with MARS? Nat. Clin. Pract. Nephrol. 3(5):267–276; 2007.
28. Kuai, X. L.; Cong, X. Q.; Du, Z. W.; Bian, Y. H.; Xiao, S. D. Treatment of surgically induced acute liver failure by transplantation of HNF4-overexpressing embryonic stem cells. Chin. J. Dig. Dis. 7(2):109–116; 2006.
29. Kuo, T. K.; Hung, S. P.; Chuang, C. P.; Chen, C. T.; Shih, Y. R.; Fang, S. C.; Yang, V. W.; Lee, O. K. Stem cell therapy for liver disease: Parameters governing the success of using bone marrow mesenchymal stem cells. Gastroenterology 134(7):2111–2121.e3; 2008.
30. Lee, N. P.; Leung, K. W.; Cheung, N.; Lam. B. Y.; Xu, M. Z.; Sham, P. C.; Lau, G. K.; Poon, R. T.; Fan, S. T.; Luk, J. M. Comparative proteomic analysis of mouse livers from embryo to adult reveals an association with progression of hepatocellular carcinoma. Proteomics 8(10): 2136–2149; 2008.
31. Lopez, P. M.; Martin, P. Update on liver transplantation: Indications, organ allocation, and long-term care. Mt. Sinai J. Med. 73(8):1056–1066; 2006.
32. Mai, G.; Nguyen, T. H.; Morel, P.; Mei, J.; Andres, A.; Bosco, D.; Baertschiger, R.; Toso, C.; Berney, T.; Majno, P.; Mentha, G.; Trono, D.; Buhler, L. H. Treatment of fulminant liver failure by transplantation of microencapsulated primary or immortalized xenogeneic hepatocytes. Xenotransplantation 12(6):457–464; 2005.
33. Makowka, L.; Rotstein, L. E.; Falk, R. E.; Falk, J. A.; Nossal, N. A.; Langer, B.; Blendis, L. M.; Phillips, M. J. Allogeneic and xenogeneic hepatocyte transplantation in experimental hepatic failure. Transplantation 30(6):429–435; 1980.
34. Mendez-Otero, R.; de Freitas, G. R.; Andre, C.; de Mendonca, M. L.; Friedrich, M.; Oliveira-Filho, J. Potential roles of bone marrow stem cells in stroke therapy. Regen. Med. 2(4):417–423; 2007.
35. Nagata, H.; Nishitai, R.; Shirota, C.; Zhang, J. L.; Koch, C. A.; Cai, J.; Awwad, M.; Schuurman, H. J.; Christians, U.; Abe, M.; Baranowska-Kortylewicz, J.; Platt, J. L.; Fox, I. J. Prolonged survival of porcine hepatocytes in cynomolgus monkeys. Gastroenterology 132(1):321–329; 2007.
36. Naruse, K. Artificial liver support: Future aspects. J. Artif. Organs 8(2):71–76; 2005.
37. Naruse, K.; Nagashima, H.; Sakai, Y.; Kokudo, N.; Makuuchi, M. Development and perspectives of perfusion treatment for liver failure. Surg. Today 35(7):507–517; 2005.
38. Naruse, K.; Sakai, Y.; Guo, L.; Natori, T.; Shindoh, J.; Karasawa, Y.; Iida, Y.; Kojima, K.; Michishita, K.;Makuuchi, M. Development of a new extracorporeal whole-liver perfusion system. J. Artif. Organs 6(3):211–217; 2003.
39. Nussler, A.; Konig, S.; Ott, M.; Sokal, E.; Christ, B.; Thasler, W.; >Brulport, M.; Gabelein, G.; Schormann, W.; Schulze, M.; Ellis, E.; Kraemer, M.; Nocken, F.; Fleig, W.; Manns, M.; Strom, S. C.; Hengstler, J. G. Present status and perspectives of cell-based therapies for liver diseases. J. Hepatol. 45(1):144–159; 2006.
40. Oertel, M.; Menthena, A.; Chen, Y. Q.; Shafritz, D. A. Properties of cryopreserved fetal liver stem/progenitor cells that exhibit long-term repopulation of the normal rat liver. Stem Cells 24(10):2244–2251; 2006.
41. Oertel, M.; Menthena, A.; Dabeva, M. D.; Shafritz, D. A. Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells. Gastroenterology 130(2):507–520; 2006.
42. Papagoras, D.; Papalois, A.; Tsaroucha, A.; Lytras, D.; Kyriazanos, J.; Giannakou, N.; Laftsidis, P.; Simopoulos, C. Beneficial effect of an antibody against interleukin-2 receptor (daclizumab) in an experimental model of hepatocyte xenotransplantation. World J. Gastroenterol. 13(9): 1435–1437; 2007.
43. Paradis, K.; Langford, G.; Long, Z.; Heneine, W.; Sandstrom, P.; Switzer, W. M.; Chapman, L. E.; Lockey, C.; Onions, D.; Otto, E. Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. The XEN 111 Study Group. Science 285(5431):1236–1241; 1999.
44. Perico, N.; Benigni, A.; Remuzzi, G. Xenotransplantation in the 21st century. Blood Purif. 7(4):338–346; 2002.
45. Rhim, J. A.; Sandgren, E. P.; Palmiter, R. D.; Brinster, R. L. Complete reconstitution of mouse liver with xenogeneic hepatocytes. Proc. Natl. Acad. Sci. USA 92(11): 4942–4946; 1995.
46. Risbud, M. V.; Shapiro, L. M.; Vaccaro, A. R.; Albert, T. J. Stem cell regeneration of the nucleus pulposus. Spine J. 4(6 Suppl.):348S–353S; 2004.
47. Rozga, J. Liver support technology—an update. Xenotransplantation 13(5):380–389; 2006.
48. Sarkis, R.; Benoist, S.; Honiger, J.; Baudrimont, M.; Delelo, R.; Balladur, P.; Capeau, J.; Nordlinger, B. Transplanted cryopreserved encapsulated porcine hepatocytes are as effective as fresh hepatocytes in preventing death from acute liver failure in rats. Transplantation 70(1):58–64; 2000.
49. Schiodt, F. V.; Lee, W. M. Fulminant liver disease. Clin. Liver Dis. 7(2):331–349; 2003.
50. Schneider, A.; Attaran, M.; Meier, P. N.; Strassburg, C.; Manns, M. P.; Ott, M.; Barthold, M.; Arseniev, L.; Becker, T.; Panning, B. Hepatocyte transplantation in an acute liver failure due to mushroom poisoning. Transplantation 82(8):1115–1116; 2006.
51. Sgroi, A.; Serre-Beinier, V.; Morel, P.; Bühler, L. What clinical alternatives to whole liver transplantation? Current status of artificial devices and hepatocyte transplantation. Transplantation 87(4):457–466; 2009.

52. Sharma, A. D.; Cantz, T.; Vogel, A.; Schambach, A.; Haridass, D.; Iken, M.; Bleidissel, M.; Manns, M. P.; Schöler, H. R.; Ott, M. Murine embryonic stem cell-derived hepatic progenitor cells engraft in recipient livers with limited capacity of liver tissue formation. Cell Transplant. 17(3):313–323; 2008.
53. Stefan, A. M.; Coulter, S.; Gray, B.; Lamorte, W.; Nikelaeson, S.; Edge, A. S.; Afdhal, N. H. Xenogeneic transplantation of porcine hepatocytes into the CCl_4 cirrhotic rat model. Cell Transplant. 8(6):649–659; 1999.
54. Tabei, I.; Hashimoto, H.; Ishiwata, I.; Tachibana, T.; Akahori, M.; Ohi, S.; Kubo, H.; Satou, K.; Yamazaki, Y.; Yanaga, K.; Ishikawa, H. Characteristics of hepatocytes derived from early ES cells and treatment of surgically induced liver failure rats by transplantation. Transplant. Proc. 37(1):262–264; 2005.
55. Tackaberry, E. S.; Ganz, P. R. Xenotransplantation: Assessing the unknowns. CMAJ 159(1):41–43; 1998.
56. Takeuchi, Y.; Patience, C.; Magre, S.; Weiss, R. A.; Banerjee, P. T.; Le Tissier, P.; Stoye, J. P. Host range and interference studies of three classes of pig endogenous retrovirus. J. Virol. 72(12):9986–9991; 1998.
57. Terada, N.; Hamazaki, T.; Oka, M.; Hoki, M.; Mastalerz, D. M.; Nakano, Y.; Meyer, E. M.; Morelm, L.; Petersen, B. E.; Scott, E. W. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 416(6880):542–545; 2002.
58. Tsuruga, Y.; Kiyono, T.; Matsushita, M.; Takahashi, T.; Kasai, H.; Matsumoto, S.; Todo, S. Establishment of immortalized human hepatocytes by introduction of HPV16 E6/E7 and hTERT as cell sources for liver cell-based therapy. Cell Transplant. 17(9):1083–1094; 2008.
59. Uchino, J.; Tsuburaya, T.; Kumagai, F.; Hase, T.; Hamada, T.; Komai, T.; Funatsu, A.; Hashimura, E.; Nakamura, K.; Kon, T. A hybrid bioartificial liver composed of multiplated hepatocyte monolayers. ASAIO Trans. 34(4):972–977; 1988.
60. Vaquero, J.; Chung, C.; Cahill, M. E.; Blei, A. T. Pathogenesis of hepatic encephalopathy in acute liver failure. Semin. Liver Dis. 23(3):259–269; 2003.
61. Vidal, I.; Blanchard, N.; Alexandre, E.; Gandillet, A.; Chenard-Neu, M. P.; Staedtler, F.; Schumacher, M.; Bachellier, P.; Jaeck, D.; Firat, H.; Heyd, B.; Richert, L. Improved xenogenic hepatocyte implantation into nude mouse liver parenchyma with acute liver failure when followed by repeated anti-Fas antibody (Jo2) treatment. Cell Transplant. 17(5):507–524; 2008.
62. Weiss, E. H.; Lilienfeld, B. G.; Müller, S.; Müller, E.; Herbach, N.; Kessler, B.; Wanke, R.; Schwinzer, R.; Seebach, J. D.; Wolf, E.; Brem, G. HLA-E/human beta2-microglobulin transgenic pigs: Protection against xenogeneic human anti-pig natural killer cell cytotoxicity. Transplantation 87(1):35–43; 2009.
63. Yan, Q. J.; Zhang, Y. F.; Yang, J.; Ding, Q.; Wang, J. G.; Jiang, H. W.; Zhao, H.; Xu, K.; Gong, J.; Li, L. J.; Liu, C. H.; Guo, J. L.; Liu, E. J. The porcine alpha1, 3 galactosyltransferase gene siRNA targeted heterozygous hepatocyte negative express GT. Zhonghua Gan Zang Bing Za Zhi 12(8):482–484; 2004.
64. Yang, Y-G.; Sykes, M. Xenotransplantation: Current status and a perspective on the future. Nat. Rev. Immunol. 7(7):519–531; 2007.
65. Ying, Q. L.; Nichols, J.; Evans, E. P.; Smith, A. G. Changing potency by spontaneous fusion. Nature 416(6880):545–548; 2002.