Bridging to Allotransplantation—Is Pig Liver Xenotransplantation the Best Option?

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Abstract

In the past 20 y, the number of patients in the United States who died while waiting for a human donor liver totaled >52 000. The median national wait time for patients with acute liver failure and the most urgent liver transplant listing was 7 d in 2018. The need for a clinical “bridge” to allotransplantation is clear. Current options for supporting patients with acute liver failure include artificial liver support devices, extracorporeal liver perfusion, and hepatocyte transplantation, all of which have shown mixed results with regard to survival benefit and are largely experimental. Progress in the transplantation of genetically engineered pig liver grafts in nonhuman primates has grown steadily, with survival of the pig graft extended to almost 1 mo in 2017. Further advances may justify consideration of a pig liver transplant as a clinical bridge to allotransplantation. We provide a brief history of pig liver xenotransplantation, summarize the most recent progress in pig-to-nonhuman primate liver transplantation models, and suggest criteria that may be considered for patient selection for a clinical trial of bridging by genetically engineered pig liver xenotransplantation to liver allotransplantation.
INTRODUCTION

“It’s certain to go forward,” said liver transplant pioneer Thomas Starzl in discussing xenotransplantation in 1992. “But everything that has ever happened in this field has been very tough and paid for with tears. This isn’t going to be any different. I don’t actually relish the prospect, but I think it’ll get done…”

In 1992, after the death of the first human recipient of a baboon liver (with graft survival of 70 d), Starzl et al moved forward with plans for a series of 3 more orthotopic baboon-to-human liver transplants. His team performed a baboon liver transplant in 1 more patient in 1993. This patient expired after 26 d from a widespread infection. Realizing his team needed to learn more before progressing further, he put a hold on his clinical research program.

In the same year (1993), Makowka et al performed the world’s only clinical pig liver xenotransplant in a 26-y-old woman with autoimmune hepatitis, admitted with grade III encephalopathy. The aim had been to keep the patient alive with the heterotopically placed pig liver until a liver from a deceased human donor became available. As measured by active bile production, reduction in liver enzymes (AST and ALT), as well as reduction in lactic acid, the liver xenograft functioned. However, graft rejection was detected as early as 3 h posttransplantation, progressing to ischemic necrosis 34 h posttransplantation. Unfortunately, the patient expired within 2 d from irreversible brain damage.

Experimental results of liver xenotransplantation, especially up to 2016, had done little to sway doubters of its potential as a therapeutic option (Table 1). Compared with kidney and heart xenotransplantation, where survival of life-supporting pig grafts in nonhuman primates (NHPs) is now measured in months, advances in liver xenografting have proven difficult.

However, some progress has been made, and clinical application of pig liver xenotransplantation has become an attractive goal. Survival of genetically engineered pig liver grafts in NHPs has been extended to almost 1 mo (Table 1). Further progress may justify consideration of a pig liver transplant as a clinical bridge to allotransplantation (as planned by Makowka). It is therefore perhaps time to give further consideration to patient selection in future clinical trials.

There are some in the field of transplantation who do not support the concept of bridging by a liver xenograft, as it does not increase the number of organs available for transplantation. However, bridging by any method, for example, a ventricular assist device for heart transplantation, ECMO for lung transplantation, or even chronic dialysis for kidney transplantation, does not increase the number of organs. Patients awaiting a liver graft may have no alternative to a xenograft if they are to be bridged successfully for a realistic period of time.

In this review, we first discuss the problems of acute liver failure (ALF), acute-on-chronic liver failure (ACLF), and primary allograft failure. We then detail progress as well as challenges in pig-to-NHP liver transplantation models. After explaining the current,
mostly experimental, alternatives for bridging to allotransplantation, we conclude with the feasibility of clinical trials and patient selection.

The Problem of Acute or Acute-on-Chronic Liver Failure

Today, >13,000 patients are on the United Network for Organ Sharing waiting list for liver transplantation. In 2019 alone, 2,421 patients were removed from the waiting list due to death (n = 1,218) or because they became too sick for the surgical procedure (n = 1,203). Over the last 20 years, the number of patients who died while waiting for a human donor liver in the United States totaled >52,000.

We suggest that initial clinical trials of pig liver xenotransplantation should focus on patients who would otherwise die due to lack of organ availability and for whom no realistic alternative therapy is available. These patients generally have ALF (or fulminant liver failure), ACLF, or primary allograft failure. Assuming that initial clinical trials of bridging to allotransplantation are successful, with further genetic modifications to the organ-source pig and additional experimental experience, destination therapy of pig liver xenotransplantation may be justified.

Acute Liver Failure—ALF is a relatively rare clinical syndrome referring to rapid loss of liver function, that is, over days to weeks, in a patient with no preexisting liver disease. Its current incidence in the United States is unclear, though prior data approximate 2000 cases diagnosed each year. Before the introduction of liver allotransplantation, patients with ALF suffered an 80% mortality rate. Today, 40%–50% of adult ALF patients die during their index hospitalization because of progressive multiorgan failure. In the United States, in 2018, about 200 patients were listed for the most urgent liver transplant listing (Status 1A) with a median national wait time of 7 days.

Although viral infections (hepatitis A, B, and E) are likely responsible for the majority of cases of ALF in the developing world, drug-induced liver injury is responsible for about 50% of cases in the United States. Local hepatic tissue injury and necrosis is thought to be responsible for the cascade of events leading to inflammatory mediators, neuroinflammation, and progression to multiorgan failure. These patients may deteriorate unexpectedly and rapidly.

While spontaneous recovery occurs in approximately 45% of patients with ALF in the United States, death without transplantation occurs in approximately 30%, and death with liver transplantation occurs in approximately 25% or fewer patients. With the use of emergency liver allotransplantation, up to 75% of patients with ALF can now be expected to survive. We lack recent data regarding patients with ALF who die because a deceased human donor organ is not readily available.

Acute-on-Chronic Liver Failure—ACLF is a syndrome characterized by acute and severe hepatic abnormalities (from different types of precipitating events) in patients with underlying chronic liver disease. The occurrence of complications in patients with cirrhosis, such as jaundice, ascites, and variceal bleeding, increases mortality to 40%–50%. Alcoholic and chronic viral hepatitis are the most common underlying liver diseases,
with 40%–50% of cases of ACLF having no identifiable trigger.\textsuperscript{32} Insults that lead to the host acute inflammatory response in ACLF include active infection (HBV, HCV, etc), major surgery, or transjugular intrahepatic portosystemic shunt insertion (usually used in an attempt to reduce internal bleeding and ascites).\textsuperscript{33}

Heise et al\textsuperscript{34} described a “golden window” of time in which a liver transplant is the optimal therapeutic option, after which transplants for patients with high-grade ACLF are futile. In patients with ACLF undergoing urgent liver allotransplantation, survival rates at 1 and 5 y are reported to be 87% and 82%, respectively.\textsuperscript{35} This is a similar outcome to that in patients undergoing liver transplantation for other indications.

The definition of ACLF is now extended by 2 or more extrahepatic organ failures, including circulatory failure, grade III/IV hepatic encephalopathy, renal failure requiring renal replacement therapy, and respiratory failure requiring mechanical ventilation.\textsuperscript{36} A patient with ACLF is generally felt to be too sick to receive a liver transplant.\textsuperscript{37} These patients, with no alternatives available, may in the future be appropriate candidates for a clinical trial of pig liver xenotransplantation.

**Primary Allograft Failure**—The incidence of primary graft nonfunction, a severe manifestation of early allograft dysfunction, ranges from 0.9% to 8.5%.\textsuperscript{38} Post–liver transplant insults, such as hepatic artery and portal vein thrombosis, can sometimes be managed conservatively.\textsuperscript{39-41} However, primary non-function is characterized by massive necrosis that may cause hemodynamic compromise. Removal of the necrotic liver may be necessary to improve hemodynamic stability. A recent study indicated that urgent liver retransplantation is an excellent option for patients with early primary graft failure. Improved second graft survival has been reported by several groups, and includes 1-, 5-, and 10-y survival of 85%, 75%, and 64%, respectively.\textsuperscript{39}

Potential conditions for which initial clinical trials of pig liver xenotransplantation might therefore be indicated include ALF, ACLF, and primary allograft failure. Prompt availability of a transplantable liver for patients with ALF or ACLF is extremely important.\textsuperscript{42} Given that these patients spend an average of only a few days on the waiting list before their demise, bridging a patient to allotransplantation may require a transplanted pig liver graft to support the patient for only a few days or a week or 2.\textsuperscript{43} Successful pig liver xenotransplantation as a bridge to allotransplantation could have the potential to save many lives.\textsuperscript{42,44}

**Progress in Pig-to-NHP Liver Transplantation Models**

Hyperacute rejection of a pig organ graft has been largely averted through the use of genetically modified pigs with (i) deletion of expression of the 3 known xenoantigens against which humans have natural preformed antibodies (Table 2) and (ii) transgenic expression of human genes that offer some protection from the effects of activation of the recipient complement and coagulation cascades\textsuperscript{43,44} (Table 3). (After pig liver transplantation, the liver will produce pig complement, and this is less damaging to a pig graft than primate complement).\textsuperscript{45} Indeed, there is evidence suggesting that a pig liver may be less susceptible to antibody-mediated injury than other pig organs (reviewed in Hara et al).\textsuperscript{46} Survival of a pig liver xenograft in NHPs, however, continues to be limited by
a number of factors, including coagulation disorders and inflammation, which are more important than rejection, which can be controlled by immunosuppressive therapy.\textsuperscript{47} Pigs with 9 or more genetic manipulations are now available but have not yet been tested in a model of pig liver transplantation in NHPs.\textsuperscript{48-50} Such a study is urgently needed if progress is to be made.

The pioneering studies by Calne et al\textsuperscript{4,5} in 1968 raised the potential of liver xenotransplantation (Table 1). Seven wild-type (ie, genetically unmodified) pig-to-baboon orthotopic liver xenotransplants were performed with survival up to 3.5 d.

Survival subsequently improved through the use of livers from genetically modified pigs. However, it remained limited due to the development of profound thrombocytopenia that occurred within minutes of reperfusion of the pig liver. In a series of α-1,3-galactosyltransferase gene-knockout (GTKO) pig-to-baboon liver transplants in 2010, thrombocytopenia resulted in spontaneous hemorrhage at multiple sites, preventing survival beyond 7 d (Figure 1).\textsuperscript{10} If the GTKO pig liver was inserted as an auxiliary graft and the native baboon liver was not excised, thrombocytopenia still occurs, but bleeding was reduced.\textsuperscript{12} Nevertheless, recipient survival remained at 8 d or less. From 1968 to 2016, survival of pig-to-NHP liver transplants had not exceeded 9 d.

Human and NHP platelets (and to some extent red blood cells) are recognized as “foreign” by the pig liver graft and are phagocytosed by the pig Kupffer cells and liver sinusoidal endothelial cells (LSECs).\textsuperscript{10,51-56}

There is evidence to suggest that this phagocytosis is associated with an incompatibility between primate CD47 (expressed on the primate platelets) and signal-regulatory protein-alpha (SIRP-α; expressed on the pig LSECs). This incompatibility may be corrected by genetic engineering of the organ-source pig to express human SIRP-α. This interspecies incompatibility with respect to CD47/SIRP-α self-signaling is believed to be a major factor in phagocytic dysregulation.\textsuperscript{57,58} These genetically engineered pigs are not yet available.

Other factors that may play a role include (i) upregulation of tissue factor expression on activated donor LSECs triggered by the immune response (demonstrated by the measurement of endothelial cell activation markers, such as P-selectin, E-selectin, and CD106), (ii) tissue factor expression on recipient platelets and peripheral blood mononuclear cells (demonstrated by immunohistochemistry, polymerase chain reaction, and flow cytometry),\textsuperscript{52,59} and (iii) formation of platelet aggregates with leukocytes in the microvasculature.\textsuperscript{53,59}

In a separate study of GTKO pig-to-baboon liver xenotransplantation, survival was again limited to 7 d, but the development of thrombocytopenia was ameliorated by the continuous administration of human coagulation factors.\textsuperscript{13} When combined with costimulation blockade, the continuous infusion of a human prothrombin complex concentrate in the recipients led to the longest recipient survivals to date (25 and 29 d).\textsuperscript{14} There were again no features of rejection. Furthermore, in addition to a resolution of the thrombocytopenia, there was a decrease in overall transfusion requirements and an avoidance of thrombotic microangiopathy within the hepatic xenografts.
Although relatively successful, this approach of treating the recipient continually with coagulation factors is difficult and not without risk of thrombosis. Acute portal vein thrombosis developed late in the posttransplant course, the baboon surviving for 29 d, indicating that the balance shifted to a procoagulant state in the setting of exogenous coagulation factor infusion. This, along with resolution of thrombocytopenia, suggests that the need for exogenous coagulation factor infusion may only be transient. An alternative, or, perhaps, complemental, approach would be to genetically engineer the organ-source pig to overcome this dysfunction.

Human platelet phagocytosis by porcine Kupffer cells and LSECs has been investigated ex vivo and in vitro. Knockout of the gene for the asialoglycoprotein receptor-1 was associated with improvements in the extent of thrombocytopenia. There was some association between the loss of platelets and the extent of binding of anti-pig antibody to the pig antigens. Thrombocytopenia would therefore appear to be partially antibody-dependent. Knockout of the porcine carbohydrate genes may therefore limit the consumption of human platelets by the pig liver (Figure 2).

In summary, these experiments demonstrated that limiting the humoral response, as well as strong expression of human coagulation-regulatory proteins, may be important in decreasing human platelet aggregation and phagocytosis. We therefore anticipate that specific genetically engineered pig livers may be associated with significantly reduced thrombocytopenia by (i) the amelioration of antibody-mediated phagocytosis, (ii) the high expression of human coagulation-regulatory transgenes (e.g., thrombomodulin and endothelial protein C receptor [EPCR]), and (iii) the transgenic expression of human SIRP-α in the pig graft.

**Synthetic and Coagulation Function of Genetically Engineered Pig Livers**

Normal hematologic, biochemical, and coagulation values have been reported in healthy pigs with different genetic backgrounds, but there are some differences in liver function between GTKO pigs and primate species. Nevertheless, although follow-up was limited in most cases, after GTKO pig liver transplantation, pig proteins and coagulation factors maintained relatively normal liver function in recipient baboons (Figure 3). Adequate pig liver function was demonstrated by the production of (i) pig proteins (albumin, fibrinogen, haptoglobin, plasminogen confirmed by Western blot) and (ii) several pig coagulation factors (FI, FV, FVII, FVIII, FIX, FX, FXI).

**The T Cell–dependent Adaptive Immune Response**

Current experimental data from pig kidney transplantation in NHPs suggest that conventional immunosuppressive therapy, that is, based on calcineurin inhibitors, may be insufficient to suppress the recipient’s T cell–dependent adaptive immune response to a xenograft and that a regimen based on blockade of the CD40/CD154 costimulation pathway is required. Such regimens have successfully maintained life-supporting genetically engineered pig kidneys and hearts for periods of several months or even >1 y.
Alternatives Methods of Bridging to Allotransplantation

Current options for supporting patients with ALF are not comparable with those used to support a patient with either kidney failure (eg, hemodialysis) or heart failure (eg, a ventricular assist device). The present alternatives for patients with ALF include (i) artificial liver support devices (biologic or nonbiologic), (ii) extracorporeal liver perfusion (ECLP), and (iii) hepatocyte transplantation (Table 4). With the exception of artificial liver support devices and therapeutic plasma exchange (described next), which are used in some centers, these methods are generally not used in everyday practice.

Artificial Liver Support Devices—The goal of liver support systems is to provide temporary functional support until a donor liver becomes available or the failing liver spontaneously recovers and regenerates. The published selection criteria for the use of artificial liver support as a bridge to allotransplantation or spontaneous recovery may form the basis for selection of patients for a pig xenograft.

Most attempts to find an effective extracorporeal liver support device have been based on nonbiologic dialysis techniques that aim to remove hepatotoxic metabolites and inhibitors of hepatic regeneration. Various devices have been developed, including those based on (i) albumin dialysis (eg, Molecular Adsorbent Recirculating System [MARS], Prometheus [Fractionated Plasma Separation and AdsorptionSystem], Single-Pass Albumin Dialysis system), or (ii) therapeutic plasma exchange (Table 4). However, clinical trials have generally been disappointing.

MARS therapy is one of the most studied methods. Although there are proven beneficial effects on hepatic encephalopathy, hepatorenal syndrome, and hyperbilirubinemia, a survival advantage for MARS for ALF or ACLF has not yet been demonstrated. As with MARS therapy, the use of Prometheus is well tolerated and improves parameters of liver function, including circulating levels of serum bilirubin, bile acids, and ammonia, in patients with ACLF. However, Prometheus therapy does not improve systemic hemodynamics or neurological status. Furthermore, the only randomized controlled trial evaluating the effect of Prometheus in patients with ACLF showed no survival benefit. With MARS or Prometheus, the nonspecific removal of toxic compounds, as well as the severity of liver failure, likely accounts for the limited clinical effect.

Acute hepatic necrosis leads to release of ammonia and proinflammatory cytokines. Plasma exchange uses a filter to remove the plasma of the patient and replace it with fresh frozen plasma. This therapy may normalize the biochemistry. Most recently, a prospective randomized controlled trial using high-volume plasma exchange in patients with ALF demonstrated a statistically significant benefit in transplant-free hospital survival of approximately 10%. While these results are encouraging, this beneficial effect was primarily found in patients who were not transplant candidates. Any benefit to transplant candidates remains uncertain.

Extracorporeal Bioartificial Liver and Liver Assist Device Support—Given the clear limitations of nonbiologic liver support systems in adequately replicating liver function, live cells that possess hepatic function have been incorporated into the
development of bioartificial liver (BAL) support systems. BAL systems differ from each other in both the type of cell and cell housing used. Examples include the HepatAssist device, which uses cryopreserved porcine hepatocytes, and the Vitagen extracorporeal liver assist device, which incorporates human hepatoblastoma cells.\textsuperscript{85}

Demetriou et al\textsuperscript{72} showed a statistically significant improved survival in fulminant/subfulminant hepatic failure patients treated with the HepatAssist device compared with control therapy. This study demonstrated that porcine cells can support the synthetic and immunologic roles of the liver. However, their limits in performing fully differentiated metabolic functions, as well as design constraints, appear to have dampened further clinical trials using BAL systems.\textsuperscript{86}

**Extracorporeal Liver Perfusion**—ECLP involves a circuit in which the patient’s blood is circulated through a whole liver (that may be of human or animal origin). Initial experience in the 1960s and 1970s using human or pig livers has been reported and summarized by others.\textsuperscript{87–93}

In 1994, Chari et al\textsuperscript{91} treated 4 patients with intermittent pig liver perfusion, only 1 of whom survived to liver allotransplantation. In 2000, Horslen et al\textsuperscript{92} used human or wild-type pig livers to successfully bridge 9 of 14 patients with ALF to successful liver allotransplantation. Although the numbers are small, ECLP using pig livers in patients with ALF (cause mainly hepatitis) showed evidence for a survival benefit.\textsuperscript{91,92} For the short periods of these studies, the pig livers appeared to sustain life as well as human livers. The selection criteria (Table 5) may help provide the basis for patients appropriate for a clinical trial of pig liver xenotransplantation.

In the period during which spontaneous recovery might occur in patients with ALF, for example, those with acetaminophen toxicity, therapy in the form of daily ECLP through an isolated genetically engineered pig liver may provide life support. This would not require significant immunosuppressive therapy or a liver transplant.\textsuperscript{94}

The Liver Advisory Group (National Health Service) of the United Kingdom outlines a set of super urgent selection criteria (of which at least 1 must be met) for patients with ALF (Table 6).\textsuperscript{95} We propose these criteria may be considered for patient selection for a clinical trial of bridging to liver allotransplantation with an orthotopic pig liver graft in patients who may not survive until an allograft becomes available.

Today, ECLP using the advanced genetically engineered pigs now available might prove to be much more successful than in previous studies. ECLP has the advantage that, if the pig liver is rejected, it can be replaced by a second (or third or fourth) pig liver. However, if costimulation blockade immunosuppressive therapy is administered to the patient, livers from the genetically engineered pigs available today are unlikely to be rejected, and so transplantation might be preferred (as long as thrombocytopenia can be prevented). Furthermore, if the native liver is necrotic, it might be essential to remove it.

**Hepatocyte Transplantation**—With hepatocyte transplantation, a fraction of liver cells, the hepatocytes—that are considered the metabolic “engines” of the liver—are infused.\textsuperscript{96}
The procedure is performed by using a perfusion pump to deliver hepatocytes into the portal vein.97 The number of hepatocytes necessary to provide adequate hepatic function in a patient with ALF has been estimated to be slightly higher than for metabolic disorders and is approximately 10%–15% of the total liver cell mass.98

Similar to artificial support devices, hepatocyte transplantation in patients with ALF or ACLF has resulted in clinical improvement by reduction of ammonia, bilirubin, and hepatic encephalopathy. However, without randomized controlled trials, the evidence for the use of hepatocyte transplantation in ALF or ACLF remains weak.98 Other obstacles to implementing this therapy include (i) an insufficient supply of human donor cells, (ii) a lack of direct monitoring (elevations of serum concentrations of aspartate transaminase [AST] and alanine transaminase [ALT] are markers of liver rejection after liver allotransplantation, but these assays are of limited value when the number of donor cells is few), and (iii) difficulties in optimizing immunosuppressive protocols.99

Buhler et al100 have explored the transplantation of hepatocytes over many years.101-104 Working with microencapsulated pig hepatocytes, Machaidze et al101 established a large animal (baboon) model for ALF. Although there was variability in the outcome in the control group, 3 of 4 animals in the treatment group completely recovered normal liver function. The model suggests potential temporary liver function support with porcine cells would be successful. In contrast, Iwase et al17 had no success with the transplantation of nonencapsulated hepatocytes in immunosuppressed baboons.

CONCLUSIONS—PIG LIVER XENOTRANSPLANTATION AS A BRIDGE AND AS DESTINATION THERAPY

If inserted heterotopically, bridging with a pig liver would potentially allow survival until recovery of the native liver or until a suitable allograft could be obtained. Xenografting 6 GTKO pig livers using 2 different heterotopic surgical techniques into NHP recipients, Zhang et al reported near-normal hepatic function and coagulation parameters. Elevation of certain cytokines preceded the onset of hepatic damage. Heterotopic xenotransplantation may potentially avoid the severe systemic consumptive coagulopathy and thrombocytopenia that continue to remain obstacles to orthotopic liver xenotransplantation.15

In contrast, if inserted orthotopically, thus allowing removal of the native liver, the pig liver may limit the systemic inflammation seen in patients with necrotic native or allograft livers. Patients with ALF would appear to be ideal recipients of bridging liver xenografts, having not suffered the effects of chronic liver disease.

If initial trials of bridging to allotransplantation are successful, destination therapy may become an achievable goal. Patients with end-stage liver disease have only a 50% chance of receiving an allograft, with consistently >1000 patients on the United Network for Organ Sharing liver waiting list dying each year.105

Although data are limited, there is evidence that good graft function can be obtained by a pig liver graft (see previous). The major challenge now is the issue of coagulopathy, which can
be partially prevented by selected medical therapy. However, it would be greatly preferable for it to be completely prevented by further genetic-engineering to the organ-source pig.

Although coagulopathy remains an immense challenge, recent studies in pig-to-NHP liver xenotransplant models have demonstrated survival of almost 30 d.\textsuperscript{14} Further, Starzl et al\textsuperscript{2,3} and Makowka et al\textsuperscript{2,3} have already shown the feasibility of transplanting liver xenografts into humans, with graft survival as long as 70 d (though this was achieved after transplanting a baboon liver, not a pig liver).

The choice of the first patients will be crucial to the success of clinical trials involving bridging by pig liver transplantation. The most ethical approach would involve selecting patients who would otherwise die due to lack of a deceased human donor organ and for whom no alternative therapies are available. These are patients who do not fulfill the criteria for receiving an allograft and are judged to be “too sick to transplant.” Conditions that may render a patient potentially inappropriate for a human liver transplant include advanced/advancing age (>65 y of age), sarcopenia, ACLF, and those with significant medical comorbidities.\textsuperscript{37} The notion of a “futile liver transplant” has been defined as survival <3 mo or in-hospital post–liver transplant mortality. Around 2.9% of all patients with cirrhosis are transplanted each year while already in an ICU.\textsuperscript{106}

There remain, however, unresolved questions with regard to the identification of “too sick to transplant” patients.\textsuperscript{36} Artru et al\textsuperscript{106} showed patients with grade 3 ACLF (the most severe) who underwent a human liver transplant have similar survival to that of patients with lower grade of ACLF. This is in contrast to earlier studies that have shown increased mortality in patients transplanted for severe cirrhosis.\textsuperscript{107-109}

Bridging by pig liver transplantation may potentially lengthen survival and prolong the “transplantation window,” successfully lead to a human liver transplant, and decrease mortality in these patients.

Patients with ALF are another potential target population. In specialist units, the management of these patients has improved, providing potential benefit from high-volume plasma exchange, with rapid access to transplantation.\textsuperscript{110} Liver transplantation remains the standard of care for patients with ALF with poor prognosis. There are limited recent data regarding patients with ALF who die waiting for a transplant.

Selection criteria by Horslen et al, as well as the super urgent selection criteria proposed by the United Kingdom National Health Service, focus on clinical and laboratory parameters, for example, a pH <7.24, progression to grade IV coma, and a lack of availability of a human liver (Tables 5 and 6). These benchmarks could be used to guide enrollment in initial clinical trials. With increasing experience, criteria could then be adjusted for cause and type of liver insult (ALF, ACLF, or primary nonfunction). However, using a pig liver xenograft as a bridge or as destination therapy may require further genetic modifications before any clinical trial can be contemplated.
Acknowledgments

Work on xenotransplantation at the University of Alabama at Birmingham is supported in part by NIH NIAID U19 Grant AI090959, and in part by a grant to UAB from United Therapeutics, Silver Spring, MD. Work on xenotransplantation in the Xenotransplantation Research Laboratory at Indiana University has been supported by (i) internal funds of the Department of Surgery, (ii) a Board of Directors of the Indiana University Health Values Fund for Research Award (VFR-457-Ekser), (iii) the Indiana Clinical and Translational Sciences Institute, and (iv) Grant # UL1TR001108 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award. Work on this project was also supported by the Washington University DDRCC (NIDDK P30 DK052574).

REFERENCES


FIGURE 1.
Platelet counts in baboons after genetically engineered pig liver xenotransplantation (n = 11). Reprinted with permission from Ekser et al.¹⁰
FIGURE 2.
Ex vivo perfusion of pig livers with human platelets, showing the percentage of human platelets remaining during ex vivo perfusion of WT, GTKO (GGTA1−/−), ASGR1-KO, and GTKO/Neu5Gc-KO (GGTA1−/−.CMAH−/−) pig livers. Means are shown (n = 3). Absence of expression of Gal and Neu5Gc was associated with higher numbers of platelets remaining in circuits. Reprinted with permission from Butler et al. ASGR1-KO, asialoglycoprotein receptor-1 gene-knockout; CMAH, CMP-N-acetylneuraminic acid hydroxylase; GTKO, α-1,3-galactosyltransferase gene-knockout; Neu5Gc-KO, N-glycolylneuraminic acid knockout; WT, wild type.
FIGURE 3.
Evidence of genetically engineered pig liver function in baboons (by testing detoxification, protein synthesis, complement activity, and coagulation parameters. A, ALT pre– and post–liver xenotransplantation. B, INR pre– and post–liver xenotransplantation. C, Factor V in pre– and post–liver xenotransplantation with pig (P), baboon (B), and human (H) average values. D, Western blot assay to demonstrate pig proteins in baboon blood. Reprinted with permission from Ekser et al. ALT, alanine transaminase; INR, international normalized ratio.
**TABLE 1.**
Progress in pig-to-NHP liver xenotransplantation (1968–2017)

<table>
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<th>Recipient</th>
<th>Pig type</th>
<th>n</th>
<th>Survival (d)</th>
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<td>11</td>
<td>&lt;1, &lt;1, &lt;1, &lt;1, 1, 4, 5, 6, 6, 6, 7</td>
<td>Eksel/2010⁶⁰</td>
</tr>
<tr>
<td>Baboon</td>
<td>GTKO</td>
<td>3</td>
<td>6, 8, 9</td>
<td>Kim/2012⁴¹</td>
</tr>
<tr>
<td>Baboon</td>
<td>GTKO</td>
<td>3</td>
<td>6, 9, 15</td>
<td>Yeh/2014⁴²</td>
</tr>
<tr>
<td>Baboon</td>
<td>GTKO</td>
<td>7</td>
<td>1, 3, 5, 5, 6, 6, 7</td>
<td>Navarro-Alvarez/2016⁴⁵</td>
</tr>
<tr>
<td>Baboon</td>
<td>GTKO</td>
<td>4</td>
<td>5, 8, 25, 29</td>
<td>Shah/2017¹⁴</td>
</tr>
<tr>
<td>Tibetan monkey</td>
<td>GTKO ± hCD47</td>
<td>6</td>
<td>3, 5, 6, 11, 12, 14</td>
<td>Zhang/2017¹⁵</td>
</tr>
</tbody>
</table>

*Heterotopic liver transplantation (all others were orthotopic liver transplantation).

GTKO, α-1,3-galactosyltransferase gene-knockout; hCD46, expression of the human complement-regulatory protein, CD46; hCD47, expression of the human transgene CD47; hCD55, expression of the human complement-regulatory protein, CD55; hCD59, expression of the human complement-regulatory protein, CD59; HT, transgenic expression of H-transferase; WT, wild type (ie, genetically unmodified).
**TABLE 2.**

Known carbohydrate xenoantigens expressed on pig cells

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Responsible enzyme</th>
<th>Gene-knockout pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal</td>
<td>α-1,3-galactosyltransferase</td>
<td>GTKO</td>
</tr>
<tr>
<td>Neu5Gc</td>
<td>CMAH</td>
<td>CMAHKO</td>
</tr>
<tr>
<td>Sd⁺</td>
<td>β-1,4 N-acetylgalactosaminyltransferase</td>
<td>β-4GalNT2KO</td>
</tr>
</tbody>
</table>

CMAH, CMP-N-acetylneuraminic acid hydroxylase; CMAHKO, CMP-N-acetylneuraminic acid hydroxylase gene-knockout; Gal, galactose-α1,3-galactose; GTKO, α1,3-galactosyltransferase gene-knockout; Neu5Gc, N-glycolyneuraminic acid; β-4GalNT2-KO, β-1,4 N-acetylgalactosaminyltransferase gene-knockout.
### TABLE 3.
Selected genetic modifications in pigs produced for xenotransplantation research

<table>
<thead>
<tr>
<th>Complement regulation (transgene insertion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD46 (membrane cofactor protein)</td>
</tr>
<tr>
<td>CD55 (decay-accelerating factor)</td>
</tr>
<tr>
<td>CD59 (protectin or membrane inhibitor of reactive lysis)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deletion of expression of known pig xenoantigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTKO</td>
</tr>
<tr>
<td>CMAH gene-knockout</td>
</tr>
<tr>
<td>β-4GalNT2-KO</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suppression of cellular immune response (gene expression or downregulation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIITA-DN (MHC CIITA knockdown, resulting in swine leukocyte antigen class II knockdown)</td>
</tr>
<tr>
<td>Class I MHC-knockout (MHC-I-KO)</td>
</tr>
<tr>
<td>HLA-E/human β-2-microglobulin (inhibits human natural killer cell cytotoxicity)</td>
</tr>
<tr>
<td>Human FAS ligand (CD95L)</td>
</tr>
<tr>
<td>Human GmT-III gene</td>
</tr>
<tr>
<td>Porcine CTLA4-Ig (CTLA4 or CD152)</td>
</tr>
<tr>
<td>Human TRAIL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coagulation regulation (gene deletion or transgene insertion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF-deficient (natural mutant)</td>
</tr>
<tr>
<td>Human TFPI</td>
</tr>
<tr>
<td>TBM</td>
</tr>
<tr>
<td>Human EPCR</td>
</tr>
<tr>
<td>Human CD39 (ectonucleoside triphosphate diphosphohydrolase-1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-inflammatory and antiapoptotic (transgene insertion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human A20 (tumor necrosis factor-alpha–induced protein 3)</td>
</tr>
<tr>
<td>HO-1</td>
</tr>
<tr>
<td>Human CD47 (species-specific interaction with SIRP-α inhibits phagocytosis)</td>
</tr>
<tr>
<td>Porcine ASGR1-KO (decreases platelet phagocytosis)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prevention of PERV activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERV siRNA (prevents activation)</td>
</tr>
<tr>
<td>PERV-KO</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deletion of expression of growth hormone receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHRKO</td>
</tr>
</tbody>
</table>

ASGR1-KO, asialoglycoprotein receptor-1 gene-knockout; β-4GalNT2-KO, β-1,4 N-acetylgalactosaminyltransferase gene-knockout; CIITA, class II transactivator; CMAH, CMP-N-acetylmuramylpeptide amidohydrolase; CTLA4, cytotoxic T-lymphocyte antigen 4; EPCR, endothelial protein C receptor; GHRKO, growth hormone receptor knockdown; GnT-III, N-acetylgalactosaminyltransferase III; GTKO, α-1,3-galactosyltransferase gene-knockout; HO-1, human hemeoxygenase-1; PERV, porcine endogenous retrovirus; SIRP-α, signal-regulatory protein-alpha; TBM, human thrombomodulin; TFPI, tissue factor pathway inhibitor; TRAIL, tumor necrosis factor-alpha–related apoptosis-inducing ligand; vWF, von Willebrand factor.
### TABLE 4. Methods of bridging to liver allotransplantation

<table>
<thead>
<tr>
<th>Method</th>
<th>Types/description</th>
<th>Advantages/benefits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS devices</td>
<td>1 Albumin dialysis (MARS, Prometheus, SPAD).</td>
<td>1 Improvements in hepatic encephalopathy, hepatorenal syndrome, and hyperbilirubinemia.</td>
<td>1 No statistically significant survival benefit shown in liver transplant patients.</td>
</tr>
<tr>
<td></td>
<td>2 Therapeutic plasma exchange.</td>
<td>2 Increase in-hospital survival in patients with ALF (high-volume plasma exchange).</td>
<td>2 Minimal evidence for improvement in hemodynamic status.</td>
</tr>
<tr>
<td></td>
<td>3 Biologic (extracorporeal bioartificial liver, liver assist device).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECLP</td>
<td>Perfusion circuit through human or animal (porcine) origin.</td>
<td>1 Clinically significant evidence for bridging to allotransplantation.</td>
<td>1 Few clinical trials.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Can provide metabolic support in patients with ALF</td>
<td>2 Limited supply (human origin).</td>
</tr>
<tr>
<td>Hepatocyte</td>
<td>Liver cells (human or animal) infused into native liver.</td>
<td>1 Improvements in ammonia, bilirubin, and hepatic encephalopathy (human hepatocytes).</td>
<td>1 Insufficient supply of donor cells (human).</td>
</tr>
<tr>
<td>transplantation</td>
<td></td>
<td>2 Potential liver support shown in baboon model using porcine cells.</td>
<td>2 Weak evidence.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 Difficulty in immune suppressive protocols.</td>
</tr>
</tbody>
</table>

ALF, acute liver failure; ALS, artificial liver support; ECLP, extracorporeal liver perfusion; MARS, Molecular Adsorbent Recirculating System; SPAD, Single-Pass Albumin Dialysis.
### Table 5.

Selection criteria for bridging to allotransplantation

<table>
<thead>
<tr>
<th></th>
<th>Selection criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Patients with ALF with further deterioration despite ICU care</td>
</tr>
<tr>
<td>2</td>
<td>Patients who are candidates for liver allotransplantation (with no exclusion criteria)</td>
</tr>
<tr>
<td>3</td>
<td>Encephalopathy with progression to grade IV coma, despite conventional supportive care</td>
</tr>
<tr>
<td>4</td>
<td>Endotracheal intubation</td>
</tr>
<tr>
<td>5</td>
<td>Marginal cerebral perfusion pressure on intracranial pressure monitoring</td>
</tr>
<tr>
<td>6</td>
<td>No available human liver suitable for implantation</td>
</tr>
</tbody>
</table>

\[a\] Modified from rHorslen et al.\textsuperscript{92}

ALF, acute liver failure; ICU, intensive care unit.
<table>
<thead>
<tr>
<th>Cause</th>
<th>Inclusion and exclusion criteria</th>
</tr>
</thead>
</table>
| Paracetamol poisoning | **Category 1:**  
| | pH < 7.24 ≥24 h after overdose and fluid resuscitation  
| | **Category 2:**  
| | Coexisting prothrombin time >100 s or INR >6.5, and serum creatinine > 300 μmol/L or anuria, and grade 3–4 encephalopathy  
| | **Category 3:**  
| | Significant liver injury and coagulopathy following exclusion of other causes of hyperlactatemia after adequate fluid resuscitation: arterial lactate > 5 mmol/L on admission and > 4 mmol/L 24 h later in the presence of clinical hepatic encephalopathy  
| | **Category 4:**  
| | Two of the 3 criteria from category 2 with clinical evidence of deterioration (increased ICP, increasing inotrope requirements) in the absence of clinical sepsis  
| Favorable nonparacetamol causes (acute viral hepatitis or ecstasy/cocaine-induced ALF) | **Category 5:**  
| | Presence of clinical hepatic encephalopathy is mandatory and prothrombin time >100 s, or INR > 6.5, or any 3 from the following: age > 40 or < 10 y; prothrombin time > 50 s or INR > 3.5; any grade of hepatic encephalopathy with jaundice to encephalopathy time > 7 d; serum bilirubin > 300 μmol/L  
| Unfavorable nonparacetamol causes (seronegative or idiosyncratic drug reactions) | **Category 6:**  
| a. | Prothrombin time > 100 s, or INR > 6.5, or b) in the absence of clinical hepatic encephalopathy then INR > 2 after vitamin K repletion is mandatory and any 2 from the following: age > 40 or 50 s or INR > 3.5; if hepatic encephalopathy is present then jaundice to encephalopathy time > 7 d; serum bilirubin > 300 μmol/L  
| Acute presentation of Wilson’s disease, or Budd-Chiari syndrome | **Category 7:**  
| | A combination of coagulopathy and any grade of encephalopathy  

ALF, acute liver failure; ICP, intracranial pressure; INR, international normalized ratio; NHS, National Health Service.