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PIG LIVER XENOTRANSPLANTATION AS A BRIDGE TO ALLOTRANSPLANTATION: WHICH PATIENTS MIGHT BENEFIT?

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Abstract

Acute liver failure is a potentially devastating clinical syndrome that, without liver transplantation (Tx), is associated with high mortality. Rapid deterioration in clinical status and a shortage of deceased human organs prohibits liver Tx in many patients. Bridging to liver Tx has been attempted by various approaches, e.g., bioartificial liver support, extracorporeal pig liver perfusion, hepatocyte Tx, but none of these approaches has convincingly improved patient survival.

The orthotopic Tx of a genetically-engineered pig liver could theoretically provide successful bridging. Immediate availability, perfect metabolic condition, adequate size-match and hepatocyte mass, and freedom from potentially pathogenic microorganisms could be assured. The advantages and disadvantages of bridging by pig liver Tx compared to other approaches are discussed. The selection of patients for an initial clinical trial of pig liver Tx would be similar to that for various prior trials in patients experiencing rapid and severe deterioration in liver function. The ability to give truly informed consent for a pig bridging procedure at the time of listing for liver Tx renders the patient with acute-on-chronic liver failure or primary allograft failure a preferable candidate for this procedure than a patient who is admitted urgently with acute (fulminant) liver failure in whom consent may not be possible.

Although several barriers to successful pig organ xenoTx remain, e.g., coagulation dysfunction between pig and primate, if these can be resolved by further genetic engineering of the organ-source pigs, a pig liver may prove life-saving to patients dying rapidly of liver failure.

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INTRODUCTION

Starzl introduced liver transplantation (Tx) as a treatment for end-stage liver disease in 1967. Since that time, refinements in surgical technique, organ preservation, immunosuppression, and management of complications (surgical and infectious) have resulted in improved patient and graft survival (now 80% and 71% at 5 years, respectively) (1). Today, more than 16,000 patients are on the United Network for Organ Sharing (UNOS) waiting list for liver Tx (2). The lack of sufficient numbers of donor livers resulted in the deaths of 1,483 patients in the waiting list only in 2008 and 22,898 patients during the past 13 years. In 2008, 958 patients were removed from the liver Tx waiting list because they became too sick to undergo Tx; since 1995 this number has totaled 7,113. In summary, during the past 13 years 30,000 patients have died waiting for a liver Tx (2). The shortage continues despite the fact that surgeons have liberalized their acceptance criteria for suitable deceased donor livers, have exploited the use of ABO-incompatible and marginal deceased donors, and have also used partial liver grafts from living donors. It is clear that an alternative source of donor organs must be pursued if we are ever going to offer liver Tx to all patients who could benefit from this form of therapy.

Cross-species Tx, i.e., xenotransplantation (xenoTx) using pig organs, could resolve the shortage of suitable donor organs (3,4). If pig organs could be transplanted successfully into human patients, the advantages would be numerous. The supply of organs would be unlimited, they would be available electively when needed, and the organ-source pig would be known to be free of specific microbes that might cause morbidity in the recipient. Clinical application of xenoTx has been held back because it has proved difficult to prevent graft injury by immunosuppressive therapy for a prolonged period of time.

There is a need for xenoTx to be used in the clinical setting where the patient may benefit and yet the surgical team would learn how xenografts function in the human body; this might allow identification of new targets for genetic engineering in the organ-source pig. XenoTx will move toward routine clinical application in a stepwise fashion as improvements in organ-source 'donor' pigs allow for longer and longer periods of xenograft survival. Liver xenoTx used as a bridge to alloTx represents a situation that meets the requirement of offering potential benefit to the patient while providing information that would be of critical value in leading to improvement in survival and function of a pig xenograft in a human being.

The ability to genetically engineer pigs to protect their organs from the primate's immune response has resulted in survival of heterotopic pig hearts in baboons for up to 6 months (5, 6) and of life-supporting pig kidneys for almost 3 months (7,8). Experience with pig liver xenoTx is sparse, with maximum graft survival in the nonhuman primate of only 8 days (reviewed by Hara et al, 2008, and by Ekser et al, 2009) (9,10).

LIVER XENOTRANSPLANTATION

There are few reports of xenogeneic liver Tx in large animal models (reviewed in Hara, 2008) (9). Dog-to-pig (11) sheep-to-pig (12,13), monkey-to-baboon (14,15,16), baboon-to-monkey (17), and pig-to-nonhuman primate (18,19,20,21) models have all been investigated. The pig-to-nonhuman primate model is currently of most clinical relevance.

Pig-to-nonhuman primate liver xenotransplantation

The only previous experience of the Tx of livers from genetically-engineered pigs is by Ramirez et al in 2000 (20). This group transplanted livers from wild-type (unmodified) pigs or pigs transgenic for the human complement-regulatory protein, human decay-accelerating factor (hDAF), into immunosuppressed baboons. The two baboons that received hDAF organs survived for 4 and 8 days, respectively, whereas the three that received wild-type pig livers survived for shorter periods of time. There are no published data reporting the Tx of livers from $\alpha 1,3$ -galactosyltransferase gene-knockout pigs (that do not express the important Gal $\alpha 1,3$ Gal antigens against which primates have natural antibodies) into nonhuman primates.

Clinical pig liver xenotransplantation

The only clinical attempt at liver xenoTx involved the liver from an unmodified pig transplanted in an unsuccessful effort to bridge to alloTx in a patient with fulminant liver failure, which was reported in 1995 by Makowka et al (22). The patient was a 26-year-old woman with long-standing autoimmune hepatitis and hepatitis C. She developed grade III encephalopathy and deteriorated rapidly. Following plasmapheresis and *ex vivo* perfusion of the donor pig kidneys to adsorb anti-pig antibodies, a pig liver was transplanted heterotopically. There was evidence that the pig liver functioned, but the patient deteriorated and suffered brain stem herniation and died 34h after xenografting before she could receive an allograft.

THE CASE FOR BRIDGING TO ALLOTRANSPLANTATION WITH A PIG LIVER

The lack of truly successful alternative therapies for patients with terminal hepatic failure, particularly for those with acute or fulminant liver failure, necessitates urgent liver Tx. Although some benefits have been obtained using bioartificial liver (BAL) support devices (reviewed by Rozga, 2006, and by van de Kerkhove et al, 2004) (23,24), some of which contain porcine hepatocytes as the cellular component, these devices have to date failed to address the significant problems that orthotopic liver xenoTx could resolve. Similarly, extracorporeal liver perfusion (ECLP), sometimes with pig livers (25), and hepatocyte Tx (26) have not proved entirely successful. The majority of patients die unless a suitable allograft becomes available, and this has led to efforts to use ABO blood type-incompatible and 'marginal' organs, and partial grafts from living donors (27,28,29,30).

There are three types of patient who could potentially benefit from receiving a bridging pig liver xenograft:-

1. Patients with acute decompensation of chronic liver disease (acute-on-chronic liver failure [ACLF]).
2. Patients with primary allograft failure as the result of primary non-function or hepatic artery thrombosis within 7 days of Tx.
3. Patients with acute or fulminant liver failure (ALF) unrelated to intentional drug overdose. On the grounds of emotional instability, we would exclude patients who had attempted suicide as this might complicate follow-up, which is likely to be much more rigorous in patients who have been recipients of a xenograft. In any case, these are often patients with acetaminophen-induced ALF and are less likely to require liver Tx (31).

A potential fourth group is those in whom ALF is anticipated to recover spontaneously (that would include most of those with acetaminophen-induced ALF, of whom only 8–13% require liver Tx [31]), although it is not always possible to identify them correctly. These patients might benefit from an auxiliary pig liver graft, the pig liver subsequently being removed after recovery of the native liver. Alternatively, they might be candidates for ECLP or BAL support.

As they present different management problems and respond more readily to medical therapy, we have excluded such patients from the subsequent discussion.

Acute-on-chronic liver failure (ACLF)

Acute-on-chronic liver failure (ACLF), defined as acute deterioration in liver function over a short period, occurs in patients with well-compensated liver disease following a precipitating event, and is characterized by jaundice, hepatorenal syndrome, and hepatic encephalopathy. Liver Tx is the treatment of choice for type-1 or type-2 hepatorenal syndrome (32,33,34). The hemodynamic and neurohumoral abnormalities associated with hepatorenal syndrome disappear within the first month post-Tx, and patients regain the ability to excrete sodium and free water. The survival of patients with hepatorenal syndrome who undergo liver Tx is 62% at 5 years (35). The problem with liver Tx in hepatorenal syndrome, particularly for type-1, is that most patients die before a donor liver becomes available. The overall mortality in ACLF is 50–90% (36,37), and the need to develop bridge-to-Tx therapy for such patients is obvious.

Extracorporeal liver perfusion with pig livers, initially carried out in the late 1960s and early 1970s (25,38,39,40,41) for patients with ACLF prior to the development of liver Tx, provides strong supporting evidence that the pig liver can be used to improve the patient's clinical condition in this population. Acute decompensation of chronic liver disease is frequently caused by variceal hemorrhage secondary to long-standing portal hypertension that can be relieved by removing the native liver. Orthotopic pig liver xenoTx would allow for the removal of the diseased liver and provide the necessary hepatocyte mass to keep the patient intact until he/she can receive a human liver transplant. Informed consent regarding bridging liver xenoTx could be obtained at the time of listing for liver Tx as a precautionary measure.

Primary allograft failure

The incidence of primary non-function in liver Tx is 6% (42,43,44). Hepatic artery thrombosis occurs in 3% of adult liver transplants and 8.3% in children (45). These complications may result in as many as 10–15% of recipients requiring urgent reTx. UNOS data (2) and a recent systematic review (45) suggest that approximately 50% of these adult patients and 62% of children undergo reTx within 14 days of being listed as status 1, while 10–33% die within 14 days without the benefit of reTx, and 32% are removed from the waiting list as they become too sick to undergo the procedure. Long-term outcomes for early reTx for hepatic artery thrombosis or primary non-function can be quite good if performed within a month of the initial transplant, with graft and patient survival approaching 75% at 3 years (2,45).

Patients with primary allograft failure have necrotic hepatocytes that cause hemodynamic compromise, and removal of the necrotic liver is necessary to improve hemodynamic stability. Unlike ECLP and other forms of hepatic assist, e.g., BAL support, orthotopic pig liver xenoTx would allow for removal of the necrotic liver. The pig liver xenograft may also be able to diminish the decline in renal function that accompanies primary allograft failure and the need for and morbidity associated with perioperative dialysis could be avoided. Patients with primary allograft failure could have discussed the potential bridging procedure when listed for their initial transplant, and would have thus been able to provide informed consent.

Acute (fulminant) liver failure (ALF)

Acute liver failure (ALF) is potentially devastating and is associated with a high mortality from cerebral edema (encephalopathy), hemodynamic instability, and coagulopathy. The etiologies of ALF vary greatly by country and have evolved over time. Acetaminophen toxicity accounts for almost 50% of all cases (46), and its incidence in the USA is increasing. Additional causes include certain other drugs, viral hepatitis (mainly hepatitis A and B), autoimmune hepatitis, Budd-Chiari syndrome, complications of pregnancy, Wilson's disease, neonatal liver failure

(47), as well as miscellaneous other causes, such as mushroom poisoning and heat stroke; the cause in some cases remains unknown (46,48).

Prior to the successful introduction of liver Tx for ALF, the mortality rate was 80–85% (50). Currently in the US, there are approximately 1,600 patients with ALF each year (49). In adults, recovery with medical therapy occurs in approximately 45%, liver Tx is required in 25%, and death without Tx occurs in 30%. Higher rates of recovery (56%) and Tx (31%) are reported in children, with a concomitant reduced number of deaths (13%) (48,51,52). Thus, successful pig liver bridging may be life-saving.

There are specific criteria for urgent listing of these patients (e.g. UNOS category 1-A (Table 1) (2) and the United Kingdom Super Urgent Liver Scheme (Table 2) (53). Despite the availability of several prognostic scoring systems for ALF, such as those of Clichy, King's College, APACHE II, and MELD, the American Association for the Study of Liver Diseases does not recommend reliance on any one (54,55). However, in the setting of the donor organ shortage, it is important to identify patients who are too ill to benefit from liver Tx and remove them from the waiting list.

Unfortunately, clinical deterioration may develop rapidly, and many patients are removed from the urgent waiting list prior to a donor organ becoming available. This problem was demonstrated in patients with acetaminophen-induced ALF who fulfilled the King's College Hospital Criteria (56). Thirty percent (30%) could not be listed for Tx because of the rapid onset of complications, and 35% of those who were listed were eventually “delisted” because of rapid clinical deterioration. The majority (90%) who initially met the criteria for liver Tx did not undergo the procedure and died (57). In the largest US study, 29% of patients with ALF underwent liver Tx, but 25% of those listed (10% of the entire group) died prior to receiving an organ (58).

At first glance, patients with ALF seem to be the most ideal recipients of bridging liver xenografts; they have not suffered the effects of long-standing liver disease and are poised to make a full recovery after Tx. The problem is that these patients become ill unexpectedly, and it would be difficult to obtain truly informed consent since they are usually intubated and in coma at the time of Tx.

Proposed inclusion and exclusion criteria for an initial clinical trial of bridging by pig liver xenoTx are outlined in Table 3.

Alternatives to bridging to allotransplantation with a pig liver

Extracorporeal artificial liver devices—In recent years there has been renewed interest in techniques for providing temporary liver support to bridge the patient with rapidly deteriorating liver function to orthotopic liver Tx or to allow time for liver regeneration. Such liver support can be non-biologic or biologic, and both methods of support are in clinical use (reviewed in van de Kerkhove et al, 2004 and in Sgroi et al, 2009) (24,59). The published selection criteria for use of BAL support as a bridge to alloTx (or spontaneous recovery) (Tables 4 and 5) may provide a basis for selection of patients for bridging by a pig xenograft.

The most promising non-biologic liver support therapies combine detoxification of water-soluble and protein-bound toxins in a dialysis system (e.g., the Molecular Adsorbents Recirculating System [MARS]) or an albumin dialysis system (the Artificial Liver Support System). Some beneficial effects on plasma toxin levels have been reported in uncontrolled trials of the albumin dialysis system in patients with ALF (60). However, in ALF patients, these systems have led to only limited or no improvement in patient survival (24,59); the

nonspecific removal of toxic compounds and the lack of capacity of the device to synthesize specific proteins and other hepatotrophic factors probably account for their limited effect.

Biologic approaches exploit hepatocytes of human or xenogeneic origin to support the patient with a BAL (bioartificial liver) device. Examples are the Extracorporeal Liver Assist Device that uses human hepatoblastoma cells (61) and the HepatAssist device that uses cryopreserved porcine hepatocytes (62). No clinical trial to date has shown convincing evidence of increased patient survival. Although several benefits have been reported during BAL support, no study has demonstrated significant improvement in plasma ammonia level and metabolic function. Several adverse events have been reported, such as tachypnea, tachycardia, hyperpyrexia, disseminated intravascular coagulation, thrombocytopenia, hypotension, hypoxia, sepsis, and, when porcine hepatocytes were used in the device, features of cell rejection (24,61,62).

Hepatocyte isolation and preservation is expensive, and long-term BAL support is likely to require a large number of cells. The cost-effectiveness of these procedures is therefore questionable. Furthermore, the number of hepatocytes in a BAL is far fewer than in a whole pig liver. The HepatAssist BAL contains an estimated 7 billion porcine hepatocytes (though the viability of these after cryopreservation is uncertain), whereas an average adult human liver may contain as many as 15–30 billion hepatocytes (63).

In summary, the use of a BAL for bridging patients with ALF to liver Tx is promising, but its clinical value awaits further improvement in the devices, and further assessment in controlled clinical trials.

Extracorporeal liver perfusion (ECLP)—Initial experience using human livers was documented in the 1960s and 1970s (38,39). At least 141 ECLPs were performed to treat 87 patients with liver failure (40). The method was then largely abandoned because of the successful development of orthotopic liver alloTx. However, with the increasing shortage of available donor organs, there has been renewed interest. Neurologic improvement to at least hepatic coma grade III or II has been documented in most patients. The results do not correlate with the underlying cause of the hepatic failure.

In 1994, Chari et al (41) reported their clinical experience with four patients treated by intermittent porcine liver perfusion, but only one survived to Tx. In 2000, Horslen et al used human or unmodified (wild-type) miniature swine livers to bridge 14 patients to alloTx (25). Patient selection is summarized in Table 6. Nine patients were supported successfully to orthotopic liver Tx, the longest period of support being for >4 days, with seven surviving. ECLP maintained low ammonia levels for only 48h, and did not lower bilirubin levels.

In a small clinical trial by Levy et al (64), livers from pigs transgenic for the human complement-regulatory proteins, CD55 (hDAF) and CD59, were extracorporeally perfused in two patients with ALF for 6.5h and 10h, respectively, as bridging to successful alloTx.

Hepatocyte transplantation—Hepatocyte Tx could theoretically support the patient until the native liver recovers or could bridge to liver Tx (reviewed by Fisher et al, 2006 [26], by Strom et al, 2006 [65], and by Sgroi et al, 2009 [59]). It is doubtful, however, whether a sufficient number of donor hepatocytes could be infused to support a patient with severe liver failure, who may have increased metabolic needs through the presence of encephalopathy, coagulation, and toxic cytokine release from the necrotic native liver. Furthermore, there can be a 48–72h delay in function of the transplanted hepatocytes (66), which could be a major problem.

DISCUSSION

The ready availability of a perfect pig liver would avoid (i) delay during which the patient's clinical condition may deteriorate further, (ii) prolonged cold ischemia, (iii) size mis-match between liver and patient, (iv) the Tx of an inadequate number of hepatocytes, and (v) the harmful effects of leaving a necrotic native liver *in situ*. Patients would be able to receive a xenotransplant at the time of greatest clinical need, helping to avoid prolonged hospitalization and exposure to nosocomial pathogens.

Advantages over bridging by BAL, ECLP, or hepatocyte Tx

For several reasons, xenoTx of a whole pig liver as a bridge to alloTx may be preferable to BAL support:- (i) isolation of hepatocytes and preparation of the device may take time during which the clinical status of the patient may deteriorate, (ii) cryopreservation of porcine hepatocytes may impair their subsequent function, (iii) the number of hepatocytes in a BAL is far fewer than in a whole liver; metabolic function of a whole liver is likely to be superior to that of a relatively small number of hepatocytes, (iv) the BAL provides hepatocellular function, but without the structure of the liver or the other cell types, both of which are important in the function of the liver, and (v) use of a pig liver may be more cost-effective when compared with porcine hepatocyte isolation and utilization in a BAL. Many of these concerns apply equally to hepatocyte Tx.

In comparison to ECLP, the Tx of a pig liver would (i) enable removal of the patient's necrotic liver, and thus avoid harmful cytokine release and prevent further clinical deterioration (although it is perhaps possible that removal of the diseased native liver would not be necessary in the presence of a well-functioning extracorporeal pig liver), (ii) reduce the risk of infectious complications associated with prolonged ECLP, and (iii) possibly maintain greater hemodynamic stability than can be achieved with ECLP.

Immunological considerations

Although pig liver xenoTx might be ideal in many respects, there are several immunologic problems that currently prevent its use (67) (reviewed in Hara et al 2008) (9). The recent availability of $\alpha 1,3$ -galactosyltransferase gene-knockout pigs (68,69), however, particularly if additionally transgenic for a human complement-regulatory protein (70), offers protection from hyperacute rejection, and could possibly allow survival for a sufficient number of days for the graft to act as a successful bridge. Pigs with multiple genetic modifications are currently becoming available (71).

Currently, coagulation dysregulation between pig and primate remains an important challenge (4). The breeding of pigs transgenic for an 'anticoagulant' or 'anti-thrombotic' gene, such as human tissue factor pathway inhibitor, hirudin, or CD39, or lacking the gene for the prothrombinase, fibrinogen-like protein-2 (fgl-2), is anticipated to inhibit the change from an anticoagulant to a procoagulant phenotype that takes place in the porcine vascular endothelium after Tx into a primate (4,72).

Importantly, in both *in vitro* experiments and in animal models, it has been demonstrated that a potential recipient with severe liver failure would be less capable of initiating hyperacute rejection of a xenograft than a healthier recipient (73,74). Furthermore, there is currently no evidence that a prior pig xenograft will sensitize a patient to a subsequent allograft (reviewed in Cooper et al, 2004) (75).

Metabolic and physiologic considerations

Relatively few data are available with regard to the function of a pig liver in a primate; what data are available have been reviewed by Ibrahim et al (76). However, although follow-up was limited to 7 days, a recent study of ours indicated surprisingly good hepatic function, including parameters of coagulation, after the orthotopic transplantation of livers from genetically-engineered pigs into baboons (77, *Ekser B et al, submitted for publication*). These data would suggest that, from this perspective, a pig liver would be able to support a patient adequately until an allograft could be obtained.

The potential for infectious complications

Concerns have been raised about the potential transmission of infectious porcine agents to the recipient and, subsequently, possibly to his/her human contacts (78,79). Porcine endogenous retroviruses (PERV) have been of particular concern (80,81). The evidence that PERV will be a problem remains minimal. In 1999, Paradis et al (82) demonstrated in 160 patients who had undergone various forms of exposure to porcine tissues that no PERV could be detected in any recipient tissues, with follow-up for up to a decade after exposure. Until today, no formal evidence has been presented from studies in nonhuman primates or from humans transplanted with pig organs, tissues, or cells that PERV infects primate cells *in vivo* (79).

In any case, careful selection and/or genetic-engineering of pig herds should aid in minimizing the risk of PERV infection and/or pathogenicity (83,84). RNAi technology can inhibit activation of PERV (85), and PERV have been reported to be susceptible to currently used anti-viral agents (79).

A major advantage of pig xenoTx may be the potential resistance of the pig liver to infection by certain human pathogens, including HIV, HTLV, hepatitis viruses (HBV, HCV), and herpes viruses (including CMV) (79,86). For example, porcine cytomegalovirus does not appear to infect baboon tissues *in vivo* (87). This 'species specificity' may reflect the absence of receptors or of cellular 'machinery' necessary for viral replication in human cells (79).

Ethical considerations

Perhaps the most important ethical aspect of bridging with a pig xenograft is the question of informed consent. We believe there is a significant difference between obtaining consent for 'experimental' bridging liver xenoTx and life-saving definitive liver alloTx. National regulatory authorities, such as the US Food and Drug Administration, will require much more intensive follow-up of patients who have received a xenograft, even if this was present in the patient only temporarily. It is for this reason that we believe a bridging xenotransplant should only be carried out in patients who have given consent 'in the cold light of day', i.e., when they have had plenty of time to consider all aspects of such a procedure, which would ideally be at the time they are listed for alloTx. We do not believe it is sufficient to suggest xenoTx to them under the conditions surrounding the development of ALF.

Some patients with ALF will be semi-comatose or even comatose at the time bridging is being considered, and will be unable to make an informed decision on whether to proceed with such an experimental form of therapy. The decision will therefore rest with the patient's family or legal guardian. In view of the patient's critical state, the decision will of necessity have to be made urgently. This will allow little time to fully consider the implications of the Tx of a pig organ. As the patient has a high risk of dying if bridging is not attempted, the pressure on the patient's legal representative may be extreme. (The decision to be made by the patient or legal representative will be even more difficult and complex if ALF is a result of intentional drug overdose [suicide attempt], and we believe these patients should be excluded.)

The decision-maker will be required to agree on behalf of the patient to life-long monitoring, as outlined by the guidelines suggested by the Ethics Committee of the International Xenotransplantation Association (88). This agreement will include the avoidance of blood donation, the need for informing close contacts about the xenoTx, the potential risk of infection, and other modifications in life-style that may be required. Requiring the patient to agree to life-long monitoring effectively denies him/her the right to withdraw from the clinical trial at any time in the future, a fundamental right which is delineated in the Declaration of Helsinki and the US Code of Federal Regulations.

Furthermore, family members who have been in contact with the patient after xenoTx may be asked to agree to be monitored even if the patient dies.

(Whether consent for life-long monitoring could be enforced or be legally binding is, of course, questionable. However, even if the patient subsequently decided to withdraw from the trial, consent at least for data collection may be possible.)

In contrast to the patient with ALF, patients with ACLF and primary allograft failure can provide informed consent electively at the time they consent for alloTx and are placed on the waiting list. Consent for a bridging liver xenotransplant will, for most listed patients, be just a precautionary measure, but for those listed patients who develop ACLF or undergo Tx and experience primary allograft failure, it may be life-saving.

It is the ability to obtain truly informed consent that identifies patients who experience acute decompensation of chronic liver disease or primary allograft failure as the most likely candidates to participate in bridge-to-allograft pig liver xenoTx trials. A policy of obtaining consent for a bridging pig xenograft at the time of listing for alloTx would avoid the many ethical problems associated with obtaining informed consent after ALF has developed.

Conclusions

Undoubtedly, liver alloTx is the preferred treatment in patients with ACLF, primary allograft failure, or ALF when medical therapy proves insufficient to prevent encephalopathy or coagulopathy (89). However, the ready availability of a deceased human donor liver continues to be a major problem. In such cases, due to the limited time before encephalopathy or coagulopathy develops, selection of the best alternative treatment for the patient is crucial (Figure 1). To date, none of the currently-available options has shown consistent success. If the remaining immunologic problems can be overcome, bridging with a pig liver may provide a solution. The experience gained may provide essential information that enables future advances in xenoTx until it becomes a therapeutic option for all patients with end-stage organ failure.

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ABBREVIATIONS

ACLF, acute-on-chronic liver failure; ALF, acute liver failure; BAL, bioartificial liver; ECLP, extracorporeal liver perfusion; hDAF, human decay-accelerating factor; PERV, porcine endogenous retroviruses; Tx, transplantation; UNOS, United Network for Organ Sharing.

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Figure 1.

Treatment decision algorithm for patients with rapidly deteriorating liver function when conventional medical treatment proves insufficient

Table 1

UNOS inclusion (and potential exclusion) criteria for liver Tx in patients with ALF*

UNOS Inclusion Criteria for Adults (Status 1A)	
1) Age ≥18 years	
2) Life expectancy without liver Tx of <7 days	
3) Onset of encephalopathy within 8 weeks of the first symptoms of liver disease	
4) Absence of pre-existing liver disease (except for fulminant Wilson's disease)	
5) Residence in the intensive care unit	
6) At least one of the following:	
- ventilator-dependence,	
- renal replacement therapy	
- INR (international normalized ratio) >2.0	
7) Complication of a transplanted liver within 7 days of implantation	
- primary non-function of a transplanted liver	
- hepatic artery thrombosis in a transplanted liver with evidence of severe liver injury	
UNOS Inclusion Criteria for Children (Status 1A)	
Same criteria as in adults except:	
- A PELD (Pediatric End-Stage Liver Disease) score of >25 in patients <12 years-old or a MELD (Model for End-Stage Liver Disease) score of >25 in patients 12–17 years-old.	
- Hepatic artery thrombosis in a transplanted liver within 14 days of implantation	
Potential Exclusion Criteria	
1) Age >70 years old (relative)	
2) Certain malignancies outside of the liver	
3) Severe cardiac, lung, or multiple organ failure	
4) Uncontrolled septic shock / severe infection	
5) Uncontrolled bleeding	
6) Evidence of irreversible brain damage	

* Source: UNOS (2) and Lee WM (46) and Lee WM et al. (48)

Table 2
Selection criteria for urgent liver transplantation in the United Kingdom and Republic of Ireland*

At least one of the following criteria must be met:-

Paracetamol poisoning	
Category 1: pH <7.25 >24h after overdose and after fluid resuscitation	
Category 2: co-existing PT >100 sec or INR>6.5, and serum creatinine >17.5 mg/dL (>300 µmol/L) oranuria, and Grade III-IVencephalopathy	
Category 3: serum lactate >3.5 mmol/L on admission >24h after overdose or >3.0mmol/L after fluid resuscitation	
Category 4: two of the 3 criteria from category 2 with clinical evidence of deterioration e.g. increased intracranial pressure, FiO2 >50%, increasing inotropic requirements) in he absence of clinical sepsis	
Seronegative hepatitis, Hepatitis A or B, or an idiosyncratic drug reaction	
Category 5: PT >100 sec or INR >6.5, and any grade of encephalopathy	
Category 6: any grade of encephalopathy and any 3 from the following;	
a) unfavourable etiology (idiosyncratic drug reaction, seronegative hepatitis)	
b) age >40 years	
c) jaundice to encephalopathy time >7 days	
d) serum bilirubin >17.5 mg/dL (>300 µmol/L)	
e) PT >50 sec or INR >3.5	
Acute presentation of Wilson's disease, or Budd-Chiari Syndrome	
Category 7: a combination of coagulopathy, and any grade of encephalopathy	
Post-liver transplantation complications	
Category 8: hepatic artery thrombosis on days 0 to 14	
Category 9: early graft dysfunction on days 0 to 7 with at least 2 of the following: AST >10,000 IU/L, INR >3.0, serum lactate >3.0 mmol/L, absence of bile production	
Acute liver failure in children <2 years of age	
Definition: multi-system disorder in which severe acute impairment of liver function with or without encephalopathy occurs in association with hepatocellular necrosis (in the absence of recognized underlying chronic liver disease)	
INR >4.0 or Grade III/IV encephalopathy	
paracetamol overdose - adult criteria apply	
exclude leukemia/lymphoma, hemophagocytosis, disseminated intravascular coagulation	
exclude ALF associated with conditions that will not be cured by liver Tx : a) mitochondrial disorder with neurological involvement, b) Niemann-Pick disease type C, c) Giant-cell hepatitis with Coombs' positive hemolytic anemia	
AST = aspartate transaminase, INR = international normalized ratio	

* **Source:** UK Transplant (53)

Table 3

Proposed inclusion and exclusion criteria for a clinical trial of bridging to liver allotransplantation by an orthotopic pig liver graft in patients with rapidly deteriorating severe liver failure

Inclusion Criteria	
1) Criteria for adult or pediatric urgent liver Tx (e.g., UNOS Status IA [Table 1] or foreign equivalent [e.g., Table 2])	
2) Grade III or IV encephalopathy	
3) Primary non-function after orthotopic liver Tx:-	
a) >48h but <7 days after liver Tx	
b) listed as highest priority for reTx	
c) lack of bile production (if a T-tube is present)	
d) presence of blood flow through the liver by ultrasound/Doppler	
e) elevated aspartate serum transaminase (AST) (>2500 IU/L)	
Exclusion Criteria	
1) Age >70 years old (relative)	
2) Pregnancy	
3) Known hypersensitivity to porcine products	
4) Other contraindications for liver allotransplantation	
a) uncontrolled sepsis, shock / severe infection	
b) severe cardiac, lung, or multiple organ failure	
c) uncontrolled bleeding	
d) evidence of irreversible brain damage	
e) certain malignancies outside of the liver	
f) suicide attempt	

Table 4

Selection criteria in a pilot controlled trial of an extracorporeal liver assist device in patients with ALF*

Group 1: Patients considered to have a 30–50% chance of spontaneous recovery (Divided into 2 arms, second arm to receive ELAD support)	
1) Acetaminophen hepatotoxicity (admitted within 72h of overdose) with:	
a) International normalized ratio (INR) >7	
b) Prothrombin time (PT) >98 seconds	
2) Acetaminophen hepatotoxicity (admitted after 72h) with:	
a) INR >2.5	
b) PT >35 seconds	
c) presence of Grade III encephalopathy and renal failure (serum creatinine >17.5 mg/dL (>300 µmol/L))	
3) For nonacetaminophen causes – 2 of the 5 Tx criteria (see below)	
Group 2: Patients who fulfill the criteria for liver Tx at enrolment (Divided into 2 arms, second arm to receive ELAD support)	
1) Acetaminophen hepatotoxicity with:	
a) Arterial blood pH of <7.3 following volume repletion, or	
b) Grade III encephalopathy in the presence of an INR >8, or	
c) PT >100 seconds, or	
d) Serum creatinine >17.5 mg/dL (>300 µmol/L)	
2) For nonacetaminophen causes – 3 of the 5 Tx criteria (see below)	

Tx criteria:

1. age >40 years;
2. presumed cause of non-A, non-B hepatitis or drug-related liver disease;
3. jaundice to encephalopathy >7 days;
4. serum bilirubin of >17.5 mg/dL (>300 µmol/L);
5. INR >3.6 (PT >50 seconds)

* **Source:** Ellis et al 1996 (61)

Table 5

Inclusion and exclusion criteria for a randomized, multicenter, controlled trial of a bioartificial liver (BAL, the HepatAssist device) in patients with ALF *

Inclusion Criteria	
1) Weight >40 kg	
2) Grade III or IV encephalopathy	
3) Primary non-function after orthotopic liver Tx:	
a) >48h and <7 days after liver Tx	
b) listed as highest priority for reTx	
c) lack of bile production if a T-tube is present	
d) presence of blood flow through the liver by ultrasound/Doppler	
e) elevated aspartate serum transaminase (AST) (>2500 IU/L)	
f) prothrombin time >16.5 seconds	
g) lack of post-transplant improvement in liver function	
Exclusion Criteria	
1) Pregnancy	
2) Known hypersensitivity to porcine products	
3) Other definitive contraindications for liver Tx (e.g., sepsis, severe cardiac disease, uncontrolled bleeding, evidence of irreversible brain damage)	

* **Source:** Demetriou et al 2004 (62)

Table 6

University of Nebraska inclusion criteria for extracorporeal liver perfusion using a human or pig liver in a patient with ALF *

Inclusion Criteria	
1) Patients who are candidates for liver Tx	
2) Encephalopathy with progression to Grade IV coma despite conventional supportive care	
3) Endotracheal intubation	
4) Marginal cerebral perfusion pressure on intracranial pressure monitoring	
5) No human liver suitable for implantation	
Exclusion Criteria	
No exclusion criteria were listed	

* Source: Horslen SP et al 2000 (25)