Long-term correction of urea cycle disorders

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Long-term correction of urea cycle disorders is achieved by correction of the enzymatic defect in hepatocytes. Currently, orthotopic liver transplantation is the primary means of achieving this correction. In the United States most liver transplantations for urea cycle disorders have been restricted to patients with ornithine transcarbamylase deficiency and argininosuccinic aciduria. However, patients with citrullinemia have also received transplants, but more so in Europe and Japan. Recent advances in organ procurement, surgical technique, and immunosuppression have significantly decreased morbidity and mortality. However, unique short-term complications associated with surgery and long-term complications associated with chronic immunosuppression have spurred continued efforts to develop gene replacement therapies for management of acute metabolic decompensations as intercurrent therapy until liver transplantation, and ultimately, for long-term correction. The pathophysiology of urea cycle disorders requires gene vector delivery systems that are highly efficient for liver transduction and transgene expression. To date, adeno viral vectors are unique in fulfilling these criteria, and significant data have been gained in both animal and human studies with early versions of adeno viral vectors. Ultimately, the development of helper-dependent adeno viral vectors may offer the long-term expression and increased margin of safety necessary for adjunctive therapies. (J Pediatr 2001;138:S62-S71)

Even with aggressive nutritional and dietary management of severe urea cycle deficiencies, patients with urea cycle disorders are at risk for intercurrent hyperammonemic episodes resulting from peripheral mobilization of nitrogen stores caused by catabolic stress. Long-term correction is required to reduce cumulative life-time morbidity and mortality, especially for those patients with antenatal or rapid neonatal diagnosis, followed by normalization of hyperammonemia by pharmacotherapy, renal dialysis, or both. It is in this cohort that prevention of future hyperammonemia will have the greatest impact on quality of life, because significant neurologic morbidity and severe mental retardation may be prevented with rapid diagnosis and treatment. Higher risk therapy aimed at long-term correction is also indicated for patients who have severe urea cycle deficiencies such as carbamyl phosphate synthetase and ornithine transcarbamylase deficiencies early in the urea cycle. In these patients metabolic control may be more difficult, for their positions in the metabolic pathway do not afford intermediate compounds such as citrulline in citrullinemia and argininosuccinic acid in argininosuccinic aciduria to serve as sinks for nitrogen disposal. In some patients with argininosuccinic aciduria, chronic hepatic dysfunction results in cirrhosis and liver failure, and liver transplantation is often indicated.

Until recently, the sole prospect for long-term correction of UCDs was orthotopic liver transplantation. However, limited organ availability and the significant morbidity and mortality associated with the perioperative period and with chronic immunosuppression negatively affected the risk/benefit ratio. Advances in these areas have reduced the overall morbidity and mortality rate during the past 10 years, but significant risks are still associated with hyperammonemia during the waiting period for transplantation. For this reason, and the continued morbidity after transplantation, gene replacement therapy is required to manage...
neonatal hyperammonemia, to prevent hyperammonemia during the transplan-
tation waiting period, and ultimately to provide long-term correction in
these disorders. Although it is unlikely that neither gene replacement
therapy nor orthotopic liver transplantation will supersede the other, they
may be used in complementary fashion to achieve long-term correction in sev-
eral UCDs, with the ultimate outcome being reduced cumulative life-time
morbidity and mortality.

ORTHOTOPIC LIVER TRANSPLANTATION

Indications for Liver Transplantation

Liver transplantation has successfully been performed on multiple infants
with urea cycle defects.1-3 According to past reports5,6 and our own experi-
ence, serum ammonia levels are controlled promptly after liver transplan-
tation, negating the need for protein restriction or medications. Previously,
liver transplantation was limited by the ability to perform successful transplant-
tion in the neonatal period before irreversible nervous system damage oc-
curred. Many have believed that patient survival was lower in patients
who underwent transplantation at <1 year of age6 and therefore have avoid-
ed transplantsations in this age group. However, dramatic improvements have been achieved in the technical as-
pects of liver transplantation and in the application of immunosuppression in the very young patient.6 The timing of liver transplantation is determined by weighing the relative risks and benefits of medical therapy, the anticipated availability of a donor, and the likely outcome of liver transplantation. Therefore early evaluation, listing, and transplantation to avoid neurologic im-
pairment are indicated for patients such as hemizygous male children with
severe OTC deficiency.4 In addition, liver transplantation should be consid-
ered in the child affected with hyperammonemia caused by CPS I deficien-
cy, argininosuccinic aciduria, or citrulinenia, that cannot be controlled medically. The female patient with OTC who exhibits skewed X-inactiva-
tion must receive special consideration, because a review of the literature de-
scribes multiple girls and young women who remain at risk of having recurrent hyperammonemia despite optimal medical management.7-9 Pa-

tients with argininemia are not usually candidates for transplantation, because recurrent hyperammonemia is not the primary feature of this disease.

In the UCD patient group we suggest that liver transplantation be con-
sidered for any patient who cannot follow the necessary dietary restrictions or who has recurrent episodes of hyperammonemia despite optimal medical management.4 In the subgroup of patients with severe CPS I and OTC deficiency, early evaluation and listing is indicated because the natural history of these diseases is usually character-
ized by more difficult control. In con-
trast, with increasing age most children with citrullinemia or argininosuccinic acid lyase deficiency have an increased protein tolerance and a decreased fre-
quency of hyperammonemia. These medical indications must ultimately be weighed against the medical-social sit-
uation: can the family maintain the rigid dietary and pharmacotherapy regimen required for medical treat-
ment of a patient with a severe UCD, especially in the first years of life? Can they better adhere to an immuno-
suppression regimen without strict dietary restrictions? Does the patient have rapid access to a tertiary care facility equipped to manage acute hyperam-
monemia? This last point is critical, for if acute control of hyperammonemia in a tertiary care center is not available, the likelihood of neurologic morbidity is enhanced even in the best family sit-
uations. The more chronic management issues of immunosuppression may be deemed to require less immediate acute

intervention and hence be associated with less short-term morbidity.

Contraindications to Liver Transplantation and Preoperative Assessment

In general, it is unusual to exclude a pediatric patient from liver transplan-
tation; however, there are absolute con-
traindications to liver replacement in children with UCDs including positive human immunodeficiency virus culture or severe irreversible neurologic injury. Conditions that may present as relative contraindications and require evaluation individually include advanced or partially treated systemic infections, grade IV hepatic encephalopathy, and severe psychosocial abnormalities.

Early referral and careful preopera-
tive assessment are essential to select suitable candidates and to recognize conditions amenable to medical therapy (Table I).

Donor Options

The true potential of pediatric liver transplantation is currently not being realized because of the limited availability of suitable donor organs. Brain death primarily affects school-age children and adults.10 By contrast, most children requiring liver transplantation for urea cycle defects will benefit most if the procedure can be performed at the earliest age possible. Initial at-
ttempts at liver transplantation used only size-matched donor organs. Ex-
cessive waiting list mortality and in-
creased risks of primary nonfunction stimulated the development of surgical techniques for donor allograft size re-
duction,11 living-donor liver transplan-
tation,12 split-liver transplantation,13
and auxiliary liver transplantation.14

These techniques have produced im-
mediate benefits, for the mortality in potential recipients <20 kg awaiting liver transplantation has been reduced from 25% to 50% before these surgical
innovations to approximately 2% to 5%.15,15 With increased experience these innovative surgical techniques
have been able to provide additional liver allografts with results similar to whole organ liver allografts. Despite these surgical attempts at increasing the donor pool of liver allografts, additional efforts with other innovative techniques such as hepatocyte transplantation or gene therapy are needed.

**Outcomes of Liver Transplantation**

The over-riding goal of liver transplantation in the child with a urea cycle defect is complete preservation of neurologic function, rehabilitation, and a relatively normal quality of life. Factors contributing to the attainment of this goal include improved pretransplant control of hyperammonemia, early liver transplantation, and improved pretransplant nutritional status. The overall patient survival rates for children with metabolic liver disease at 3 months, 1 year, 3 years, and 5 years are 93.5%, 91.9%, 83.9%, and 81.5%, respectively (Table II).16 In addition, patient survival is negatively affected by increasing United Network of Organ Sharing pretransplant numerical status, with the lower the number having the higher priority.16 At present, patients with OTC deficiency are listed as the highest status, 1, only when hospitalized.

Liver transplantation morbidity is associated with early complications related to graft quality, surgical procedure, acute immunosuppression, and acute rejection (Table III). In general, the rate of early complications has fallen, with improved donor graft quality attributable to new procurement techniques such as in situ split grafts. This improvement and improvements in the preoperative nutritional status and weight of patients have contributed to a general decrease in early complications. In contrast, long-term complications are directly related to chronic immune suppression. Suppressed immunity and the side effects of medications such as cyclosporin contribute to complications that include infection,
malignancy, and nephrotoxicity (Table III). One of the most challenging aspects of post-transplant management is the high incidence of viral-related disease in the child, especially Epstein-Barr virus. EBV infection in the post-transplant period presents with a wide spectrum of disease ranging from a mononucleosis-type illness to post-transplant lymphoproliferative disease. The high incidence of EBV-related post-transplant lymphoproliferative disease in pediatric liver transplantation is influenced by the intensity of the immunosuppression required, its duration, and the absence of previous EBV infection.17 Currently, EBV-related disease is initially managed with an incremental decrease in immunosuppression. Approximately 60% of affected patients will respond to this protocol, which can be complicated by the development of allograft rejection.18 A more recent novel approach to the treatment of patients with EBV involves the in vitro development of recipient-derived EBV-specific cytotoxic T-lymphocytes. This approach has produced promising results in pediatric patients undergoing bone marrow transplants19 and is now being studied in patients receiving liver transplants.

Post-transplant medical management should include monitoring of serum amino acids, specifically arginine levels. Because liver transplantation will not correct urea cycle enzymes in the rest of the body, for example, OTC in the intestine or ASS in the kidneys, there is still net deficiency of arginine biosynthesis. After transplantation, patients with OTC deficiency continue to have low citrulline levels, whereas patients with ASS deficiency will continue to have elevated citrulline levels.5 Although a normal dietary intake containing approximately 7% arginine will prevent arginine deficiency, episodes of prolonged fasting such as during illness should be evaluated for citrulline or arginine deficiency, and arginine supplementation should be considered.

**GENE REPLACEMENT THERAPY**

Gene replacement therapy for genetic diseases requires different vector delivery systems, each with qualities suited to the pathogenesis of the specific disease. Features of vector delivery systems include ease of large-scale production, DNA cloning capacity, tissue tropisms depending on routes of delivery, efficiency of target tissue transduction, status of genome within the cell nucleus (episome vs integration), and tendency for host immune response. Ultimately these factors help to determine the duration and level of transgene expression and hence clinical efficacy. The pathophysiology of UCDs requires a gene delivery system that can efficiently transduce hepatocytes, preferably by an intravascular delivery route. Moreover, unlike disorders such as hemophilia, studies have shown a requirement for transducing a significant proportion of hepatocytes for even a partial clinical effect. In preclinical animal studies, this has ranged from 10% to 30%.20 This is partly due to the cell-autonomous nature of the basic defect: there is little established metabolite communication between cells and theoretically, to re-establish normal nitrogen flux through the urea cycle in the liver, the defect must be corrected in each hepatocyte.

Although retroviral vectors have been evaluated in “proof of concept” studies in vitro, adenoviral vectors have offered the best hope for attaining a clinically relevant effect21,22 because of the unique hepatotropic nature of Ad vectors after a single intravenous injection. In rodent and primate studies, 95% to 98% of Ad is found in liver after intravenous injection. Ad vectors can also be produced in large quantities for clinical use according to Good Manufacturing Guidelines, and only Ad vectors can transduce up to 100% of hepatocytes in vivo. Therefore treatment of genetic diseases requiring a moderate to high level transduction of hepatocytes in conjunction with a high level of transgene expression have focused primarily on the use of Ad vectors.23 Wild-type Ad is a 38-kb double-stranded DNA virus that encodes early and late transcription units important in the replication life cycle of the virus. Several early proteins including the E1 open reading frames are required for transactivation of late genes and hence productive viral replication. First-generation replication-deficient Ad vectors were deleted in the E1 region to render them replication-deficient. Production was achieved in 293 cells that complemented the E1 function in trans. While replication-incompetent, late viral gene expression continued to occur in cells transduced with this vector. Hence, later-generation Ad vectors were designed with deletion of additional early genes such as E2 or E4 in an attempt to decrease viral gene expression and immunogenicity.24,25 Although animal studies initially suggested increased safety profiles for these multiply deleted vectors, these have not been shown to be clinically significant.26-28

There have been significant obstacles in translating the preclinical studies in animals into clinical successes (Table IV). The major roadblock has been the narrow therapeutic index associated with these Ad vectors. Significant toxicity has been associated with high levels of hepatocyte transduction.26,29 This toxicity may be divided into 3 phases.26 The first, innate immunity, is related to viral entry and processing of vector coat proteins and occurs in the first few hours of transduction.30,31 The second occurs over the next several days and can be associated with de novo expression of adenoviral late proteins and foreign transgene proteins.32 This results in a recruitment of cell-mediated and humoral immune responses against Ad-transduced cells.33-36 This may lead to activation of cytotoxic T-lymphocytes and clearance of transduced cells with associated tissue inflammation. The last phase of toxicity
evolves over several weeks and is associated with hepatocyte hypertrophy and fibrosis and may be mediated by both cell-mediated mechanisms and chronic production of late viral proteins. This systemic toxicity translates into limited expression of therapeutic genes and hence transient correction of the clinical phenotype. Moreover, signs of localized hepatitis characterized by inflammatory cell infiltrates and elevated liver function tests may be seen. Systemically, the inflammatory response may be indicated by fever, myalgia, thrombocytopenia, and signs of disseminated intravascular coagulation. 

**Table IV. Traditional adenoviral vectors**

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<th>Advantages</th>
<th>Limitations</th>
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<td>Preparation of high titer stock</td>
<td>Dose-related toxicity</td>
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<td>Efficient hepatic transduction</td>
<td>Innate immunity</td>
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<td>Tropism for hepatocytes</td>
<td>Cell-mediated and humoral immunity</td>
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<td>Lack of vector integration</td>
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<td>Limited duration of transgene expression</td>
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**Gene Transfer in the Urea Cycle Disorders**

Despite these problems, the need for efficient hepatocyte transduction and high-level gene expression has focused efforts on improving adenoviral vectors for treating urea cycle disorders. Most preclinical studies in animal models have focused on OTC and ASS deficiency. Correction of the metabolic phenotype has been demonstrated in the sparse fur mouse of partial OTC deficiency by E1-deleted and E1/E2-deleted Ad vectors. Intravenous administration of these vectors into animals resulted in increased urea synthesis, decreased orotic aciduria, and increased tolerance to challenges with ammonium chloride. In a similar manner, treatment with an E1-deleted Ad vector expressing human ASS in a large bovine model of citrullinemia rescued the neonatal lethality with correction of hyperglutaminemia and evidence of de novo urea cycle activity as measured by stable isotope precursor-product studies. However, in all cases ubiquitously expressing promoters were used to regulate the therapeutic transgene, and only transient correction of the phenotype was achieved. In most cases evidence of gene expression decreased by the end of the first month after treatment. In addition, species differences were noted between the bovine and murine models. For example, doses (1 × 10^15 vector particles/kg) that transduced 100% of hepatocytes in mice transduced only 30% of bovine hepatocytes. Moreover, the same doses that caused elevations of liver function and transient thrombocytopenia in mice had minimal toxicities in calves. The only human clinical trial to date in this arena has been performed at the University of Pennsylvania, where in a phase I study, E1/E4-deleted Ad vectors expressing human OTC were administered systemically into one lobe of the liver by intrahepatic artery infusion. In this dose escalation study, adults with partial OTC received doses ranging from 2 × 10^9 to 6 × 10^11 viral particles/kg. They were evaluated for signs of local and systemic toxicity, vector persistence, and evidence of de novo urea cycle activity by stable isotope studies. Patients receiving the lower doses tolerated the vector with only nonspecific signs of fever, backache, nausea, and vomiting. At higher doses, however, elevations of liver function tests were noted, but these were not clearly dose-related. Transient thrombocytopenia was found to be dose-related. The last patient treated in this trial received a dose of 6 × 10^11 viral particles/kg and had acute toxicity, culminating in death. The clinical picture was consistent with that of systemic inflammatory response syndrome with disseminated intravascular coagulation, multiorgan failure, and acute respiratory distress syndrome. Unlike in preclinical studies, a significant amount of vector was found in the subject’s bone marrow. The dose at which this adverse event occurred was also lower than that which caused similar toxicities in nonhuman primates.

**Obstacles to Successful Gene Replacement Therapy**

Several conclusions may be drawn from these preclinical and clinical studies. It is clear that delivery with an adenoviral-based vector can transduce sufficient proportions of the liver and achieve high-level gene expression required for correcting the clinical phenotype of these enzymatic deficiencies. However, the Ad vectors used in these studies exhibited limiting toxicities that will prevent their successful use in the clinic. The presence of viral capsid proteins, the continued low level of viral gene expression even in multiply deleted vectors, and the likely pharmacogenetic variation in the human population, both to susceptibility and response to systemic Ad vectors, together contribute to the very narrow therapeutic index of this treatment modality. One of these pharmacogenetic determinants may be the distribution and affinity of the Coxsackie adenovirus receptor and the required associated integrins that mediate Ad attachment and cell entry. Moreover, retreatment with these vectors is prevented by the development of serotype-specific neutralizing antibodies and the potential for and consequences of an immune response to the therapeutic transgene in patients who have null mutations and hence might respond to the therapeutic transgene as a neo-antigen.
These obstacles do not mean that Ad vectors should be discarded. Instead, they demonstrate the need for additional developments to increase the therapeutic index of vectors and a critical evaluation of risk versus benefit in specific clinical trials and applications. To date, no other vector type has been shown to be capable of the high level of hepatocyte transduction required for the management of these diseases. At the same time, the great risk of neurologic morbidity and potential death in severely ill patients suggests that this patient cohort, that is, neonates with OTC or CPS awaiting liver transplantation, might be appropriate for early attempts with new therapies.

**New Developments Improving Ad Vector Persistence and Safety**

A recent development that holds promise for improving the therapeutic index of Ad vectors is one involving the deletion of all viral coding sequences (Table V). Based on the rationale that viral gene expression is a significant contributing factor to the host immune response and cellular toxicity and hence limited transgene expression, several groups have generated Ad vectors devoid of all viral genes. The viral genes are replaced by human genomic DNA, usually derived from completely sequenced intron regions of characterized genes, for example, hypoxanthine phosphoribosyl transferase. To produce this vector, the factors required for viral replication must be provided in trans by a helper virus. The feasibility of helper-dependent vectors was first demonstrated in the mid 1990s. However, because of production yield limitations and helper virus contamination, experiments in animal models were difficult. With the recent development of a novel method for eliminating helper virus based on Cre recombinase deletion of the helper virus packaging signal, these vectors can now be produced in sufficient quantities and with minimal helper virus contamination to allow for animal experiments (Fig).

Several groups have now demonstrated persistent transgene expression after both intravenous and intramuscular delivery of HDAd vectors. These include dystrophin, cystic fibrosis transmembrane conductance, human α-1 antitrypsin, leptin, erythropoietin, and human ApoAI. No significant hepatotoxicity has yet been observed with these vectors at doses that were lethal in mice with first-generation vectors. An added benefit is the increased cloning capacity of these vectors. Early generation Ad vectors could accommodate transgenes up to 8 kb. Because the entire Ad vector may now be replaced with a therapeutic cassette, up to 56 kb can be used for cloning. This has enabled the use of endogenous promoter and gene structure in therapeutic transgenes. For example, the use of the entire hAAT gene locus (19 kb) and human ApoAI gene locus has resulted in supraphysiologic levels of both these secreted proteins in mice.

Despite these impressive data, there are still limitations to be overcome before human clinical trials can start. With the current reagents, the production of HDAd vectors is still less efficient than for first-generation vectors. However, recent data suggest that modification of the helper virus and vector backbone may overcome this. In addition, some HDAd vectors are prone to recombination irrespective of vector size. This and the variability of helper virus contamination in different preparations require strict quality assurance procedures to evaluate the vector genome integrity, helper virus contamination, and vector infectivity. Because these vectors do not form plaques in tissue culture cells, infectivity must be evaluated by methods such as fluorescent in situ hybridization or quantitation of transgene protein production. Ultimately, the advantages of HDAd vectors derive from the absence of viral gene expression and hence the avoidance of the host cell-mediated immune response and the direct hepatotoxicity of viral gene products. Although these features avoid the second and third phases of Ad vector toxicity, HDAd vectors may not circumvent the immediate toxicity attributable to processing of capsid proteins. The degree that this may limit its clinical application is yet to be determined.

Another vector that deserves mention is the adenovirus-associated vector. The use of adenovirus-associated vector has produced encouraging preliminary data in clinical trials in patients with hemophilia. Its major advantages are minimal vector-associated toxicity and prolonged transgene expression caused by genomic integration. Limitations include the allowable size of the therapeutic transgene (<5 kb) and, at least for UCDS, the relatively low (5%) efficiency of hepatocyte transduction with direct intrahepatic vascular delivery. It is unclear whether this level of liver transduction will be clinically beneficial. Although it is unlikely that this method will completely correct the enzyme deficiency, it may ameliorate the clinical severity of the disorder by providing enough de novo urea synthesis to make dietary management and pharmacotherapy more effective.

Although modification of vector type offers hope for improving the therapeutic index of these treatments, re-
cent data suggest that modifying the tissue specificity of transgene expression may also be important in avoiding the immune clearance of transduced cells. From original studies on erythropoietin and β-galactosidase, the host immune response to transgene has been shown to contribute to clearance of the transgene product and of transduced hepatocytes. At least in the case of a secreted protein, liver-specific expression from an albumin promoter in mice has been reported to confer long-term expression, whereas expression from an ubiquitous promoter resulted in characteristic development of antibodies to hAAT and clearance from the blood in 3 weeks. In support of these data, high-level and long-term expression of hAAT and ApoAI (>1 year in baboons and mice) has been achieved from the respective genomic promoters that restrict gene expression to a few tissues including the liver. Whether prevention of transgene expression in immune cells such as antigen-presenting cells might blunt the host humoral or cellular immune response remains to be determined. Finally, strategies aimed at readministration of Ad vectors have shown some promise. Because neutralizing antibodies to Ad vectors are serotype-specific, the use of different serotype vectors such as type 5 versus type 2 has been shown to enable readministration. Alternatively, immune modulation strategies at the time of vector administration such as inhibition of costimulatory molecules important in B cell antigen presentation may prevent or blunt neutralizing antibody production. Given the natural history of severe urea cycle disorders, gene replacement lasting several years followed by several rounds of successful retreatment would be a significant benefit in the risk/benefit assessment for experimental treatments.

**Figure.** Generation of helper-dependent adenoviral vectors. Therapeutic transgene with genomic stuffer DNA is inserted into vector backbone consisting of Ad5 inverted terminal repeats (ITR) with endogenous packaging signal (Ψ). Coinfection of vector with helper virus results in vector and helper Ad DNA replication. E1 function is provided by producer cell line, whereas Ad early and late proteins are provided by the helper virus. Only vector is packaged, because helper virus packaging signal is flanked by lox P sites. Excision of lox P flanked sequence is mediated by Cre recombinase produced by producer cell line. Final purification from residual helper is by physical separation by high-speed centrifugation.

**Conclusions**

The long-term cumulative neurologic morbidity and mortality associated with UCDs make the development and implementation of higher risk therapies appropriate. Ten years ago the mortality after liver transplantation was considerable. However, with improvements in organ procurement, pretransplantation nutrition, surgical techniques, and post-transplant immune suppression, both short- and long-term complications of pediatric liver transplantation have dropped dramatically. In the United States, liver transplantation for severe neonatal OTC and CPS deficiency, liver failure and cirrhosis in ASL deficiency, and failed medical-pharmacologic treatment should be considered when contraindications such as severe neurologic injury are absent. From a medical management perspective, optimizing pretransplant care by aggressively managing intercurrent hyperammonemia, ensuring vaccinations and prophylaxis are given against infectious agents such as respiratory syncytial virus and pneumococcus, and optimizing growth with appropriate caloric intake are all critical for minimizing peritransplant morbidity.

Because of limited availability of organs, morbidity during the pretransplant waiting period, and short- and long-term complications of transplantation and immune suppression, the development of adjunctive therapies such as gene replacement is warranted in this population and may ultimately be extended to all patients with UCD. The pathophysiologies of OTC and ASS deficiencies require gene replacement strategies enabling efficient transduction of hepatocytes. Studies with early generations of Ad vectors show that clinical correction can be achieved, at least in rodent and bovine models. However, studies in humans and nonhuman primates show that toxicity caused by Ad vector is limiting. Developments to increase the therapeutic index of these treatments
include use of helper-dependent Ad vectors, adenovirus-associated vectors, and use of liver-specific gene expression. Long-term gene expression by these methods in conjunction with strategies aimed at readministration should be implemented in the clinical arena first in patients with appropriate risk/benefit profiles such as patients with severe UCD awaiting liver transplantation. In the future, gene replacement therapy and liver transplantation may be complementary therapies that together will reduce the cumulative morbidity and mortality seen in these diseases.

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Liver Transplantation

Continued follow-up of patients with UCDs by metabolic experts after liver transplant is important, because correcting the liver enzyme problem does not correct the total body enzyme deficiency, and patients are still arginine deficient and may need supplementation, especially if they become ill and cannot eat and thereby do not consume arginine present in protein.

There is unlikely to be a role for lipid-mediated delivery of corrective genes for UCDs, because the amount of enzyme and the number of cells that must be transduced are currently beyond the efficiency of this gene therapy vehicle.

For example, to correct citrullinemia, animal studies suggest that 15% to 30% of normal liver enzyme activity is needed. However, this number may be lower for OTC deficiency.

Is there any leak into the circulation of the recombinant protein? Arginase is used as an indicator of inflammation because its activity is high. In a similar fashion, OTC was used as an indicator of liver cell damage; thus it is presumed that there is leakage of many enzymes from cells into the lymphatics and bloodstream.

**LONG-TERM CORRECTION**

**DISCUSSION**

**Patient Register**

The creation and maintenance of a register to track idiosyncratic responses and complications related to treatment was considered important, although it was recognized that these are expensive to set up and run.