Feasibility of Gene Therapy for Late Neuronal Ceroid Lipofuscinosis

Dolan Sondhi, PhD; Neil R. Hackett, PhD; Robin L. Aplet, BA; Stephen M. Kaminsky, PhD; Robert G. Pergolizzi, PhD; Ronald G. Crystal, MD

Late infantile neuronal ceroid lipofuscinosis (LINCL; Batten disease) manifests between the ages of 2 and 4 years with seizures, ataxia, myoclonus, impaired vision, and delayed speech as the primary symptoms. The disease is characterized by cerebral and cerebellar atrophy, with progressive loss of neurons and retinal cells. The central nervous system (CNS) and retinal cells show characteristic autofluorescent curvilinear lysosomal storage bodies. The main component of this storage material is the mature form of subunit c of mitochondrial adenosine triphosphate synthetase, suggesting a defect in the turnover of this protein. Afflicted children develop blindness and become chair bound by the age of 4 to 6 years, with death by the age of 8 to 12 years.1

Late infantile neuronal ceroid lipofuscinosis is caused by mutations in the CLN2 (ceroid lipofuscinosis, neuronal 2) gene, a 6.7-kilo-base pair (kbp) gene with 13 exons and 12 introns mapped to 11p15 (Figure, A). The gene expresses 2.5- and 3.5-kbp messenger RNA transcripts. Three mutations account for most cases of LINCL, including an intron G→C transversion in the invariant AG of the 3' splice junction of intron 5; an exon 6 C→T causing a premature stop; and an exon 10 G→C missense mutation.2 The CLN2 gene product is tripeptidyl peptidase I (TPP-I), a lysosomal enzyme that progressively removes groups of 3 amino acids from the amino terminus of proteins (Figure, B). Tripeptidyl peptidase I is normally secreted into the extracellular milieu in a “pro–TPP-I” form that is then taken up via 2 mannose-6-phosphate high-affinity receptors, and shunted to lysosomes. The secreted pro–TPP-I protein is enzymatically inactive, but when acidified in the lysosomes it is autocatalytically converted to its active form.1,3

NEW TREATMENT OPTIONS FOR LINCL

The goal of therapy for LINCL is to deliver active TPP-I to CNS and retinal cells in a sufficient amount and in time to prevent the cell loss. Theoretically, this goal could be achieved by enzyme augmentation therapy, allogeneic stem cell transplantation, and various forms of gene therapy. These strategies are all based on the concept that the pro–TPP-I protein is taken up via mannose-6-phosphate receptors on both the producer and the neighboring cells.4,5 The late emergence of an LINCL phenotype associated with mild mutations of the CLN2 gene suggests that a therapy that would provide 5% to 10% of normal intracellular TPP-I activity would be sufficient to prevent progression of LINCL.2

It is uncertain what duration of therapy will be needed to impact the clinical phenotype. Because LINCL is a
hereditary disorder, it has been assumed that therapy must be continuous. However, because the clinical phenotype is not manifested until the age of 2 to 4 years, effective clearance of this material might “reset the clock” and delay progression of the disease, ie, transient augmentation of extracellular pro–TPP-I levels may provide protection of CNS and retinal cells for a few years. Contrary to this view is the hypothesis that TPP-I has critical functions other than the degradation of proteins accumulating in lysosomes, ie, the accumulation of metabolic by-products is not central to disease pathogenesis. This question will likely not be answered until clinical studies are carried out, but for now, it is prudent to have continuous augmentation of extracellular pro–TPP-I levels (and, hence, active intracellular TPP-I) in the target tissues as the highest priority.

Enzyme augmentation therapy is a strategy in which purified, recombinant pro–TPP-I would be infused into affected individuals. In addition to the challenge of producing and purifying the recombinant protein, the blood-brain barrier precludes effective TPP-I therapy for the CNS from being administered systemically. This could be circumvented by direct intracerebral administration of recombinant pro–TPP-I, but this is a difficult challenge given the diffuse nature of the CNS disease and the requirement for continuous augmentation.

Allogeneic stem cell therapy is based on the knowledge that bone marrow–derived stem cells can differentiate into blood monocytes that migrate to the brain to further differentiate into microglial cells. For LINCL, stem cell transplantation from a matched or partially matched donor would theoretically provide a cell source in the CNS for the normal TPP-I protein. It is unlikely, however, that this approach will work. In addition to the risk of long-term immunosuppression therapy, one attempt with stem cell therapy was unsuccessful in correcting the CNS pathology in a patient with LINCL, and the CNS abnormalities of mice with a related lysosomal storage disease are not corrected by stem cell therapy administered in the postnewborn period.

Gene therapy entails delivery of the CLN2 complementary DNA (cDNA) to the target tissues as the source of TPP-I. Theoretically, if the transferred CLN2 cDNA persists and continues to express TPP-I, there would be a continual local supply of the therapeutic protein. There are several approaches to consider for gene therapy for LINCL.

Systemic administration of autologous, genetically modified cells involves ex vivo modification of CD34+ stem cells (bone marrow or blood) to express the CLN2 cDNA and intravenous administration of those modified cells to the patient. This strategy has the same logic as allogeneic therapy, but would not require long-term immunosup-

---

A, The CLN2 (ceroid lipofuscinosis, neuronal 2) gene. The positions of the 3 most common mutations associated with late infantile neuronal ceroid lipofuscinosis are indicated: 3556G→C indicates an intron G→C transversion in the variant AG of a 3′ splice junction; 3670C→T (Arg208Stop), an exon C→T transversion that prematurely terminates translation at amino acid 208 of 563; and 5271G→C (Gln422His), missense mutation. UTS indicates untranslated sequence. B, Tripeptidyl peptidase I (TPP-I) protein. The open reading frame of the CLN2 gene encodes pre-pro–TPP-I (563 amino acids). The signal sequence (amino acids 1-16) directs the nascent protein to the secretory pathway with insertion into the endoplasmic reticulum and glycosylation at the sites indicated. The 547-residue pro–TPP-I protein is inactive until it is retaken up by the cell via the mannose-6-phosphate receptor system and until exposure to low pH in the lysosome, resulting in proteolytic cleavage at residue 196 to yield the 46-kd active mature product of 367 residues. Based on data from Sleat and colleagues.
pression, and by using a strong constitutive promoter, the differentiated microglial cells would theoretically express large amounts of TPP-I. Unfortunately, gene transfer to CD34+ cells in humans has been successful only in conditions with positive selection pressure in vivo for the infused, transduced stem cells. This, together with the low rate of migration of monocytes into the CNS, diminishes the chances of success for this strategy.

Transplantation of genetically modified cells directly to the CNS has the advantage of ensuring high levels of local production of TPP-I. Most experimental systems have used autologous fibroblasts or allogeneic fetal neurons as the cells to be modified and transplanted. In addition to the social/political issues that may limit the use of fetal cells, this approach is not easily applicable to LINCL because of the diffuse nature of the CNS disease, requiring multiple sites of administration. This approach also has the risk of transplanting a large number of cells to a closed space. This safety issue, the challenge of obtaining large numbers of genetically modified autologous cells, and the necessity for ex vivo manipulation assign a low priority to this approach.

In vivo gene therapy is a strategy in which the CLN2 cDNA would be directly delivered to cells throughout the CNS and retina, enabling the modified cells to produce TPP-I. This direct approach is the strategy most likely to succeed. The major challenge for in vivo gene therapy is whether the available gene transfer vectors can modify cells in the target tissues to produce sufficient amounts of TPP-I in the appropriate anatomic regions to stabilize and/or reverse the disease.

**IN VIVO GENE THERAPY FOR LINCL: VECTORS AND EXPERIMENTAL STUDIES**

Given the biological challenge and the available technology, we conclude that in vivo gene therapy is the most viable short-term option for treating the CNS and retinal manifestations of LINCL. Before discussing the practical aspects of initiating human gene therapy trials, it is useful to review the technology available to accomplish this and the experience of in vivo gene therapy in relevant models.

**Gene Transfer Vectors**

To efficiently transfer a gene to the nucleus of target cells of the CNS and retina, it is necessary to put the gene in a “vector,” a carrier that helps to circumvent biological barriers to nucleic acid internalization to the nucleus, where it can use the normal cellular machinery to transcribe the exogenous gene. Viral gene transfer vectors capitalize on the property of viruses to efficiently transfer their genome to the nucleus of cells. These vectors are designed to be “replication deficient” by removal of critical genetic information. A viral gene vector used to treat LINCL would have the addition of an “expression” cassette containing the TPP-I cDNA controlled by an appropriate promoter. There are 3 types of gene transfer vectors, adeno-associated virus (AAV), lentivirus (LV), and adenovirus (Ad), that have the biological characteristics appropriate for effective in vivo gene transfer for LINCL. Other vectors do not fit the needs of this clinical target (nonviral vectors have low efficiency and transient gene transfer/expression, murine retroviruses have low transduction efficiency in vivo and require target cells to be proliferating to transfer genes to the nucleus, and human herpes simplex virus has not been proved to be sufficiently safe for this application).

Adeno-associated viruses are small nonenveloped icosahedral paroviruses with a 4.7-kbp single-stranded DNA genome. All viral genes in AAV vectors are replaced by an expression cassette, leaving intact essential cis elements, including the inverted terminal repeats, the DNA packaging signal, and the replication origin. Adeno-associated vectors are effective in long-term gene transfer to the CNS and retina, and there is a good safety record in humans using AAV serotype 2.

Lentiviruses are a family of complex retroviruses that include the human immunodeficiency viruses. Lentivirus gene transfer vectors have the useful features of retroviral vectors (efficient integration in the chromosome, absence of viral genes from the genetic information transferred, and limited host responses to the vector), and the important added ability to infect nondividing cells such as those found in the CNS. However, there are concerns about the safety of LV vectors, given the serious nature of the human diseases attributable to members of this family.

Adenoviruses are nonenveloped icosahedral viruses with a double-stranded 36-kbp DNA genome that causes transient mild infections of the upper respiratory tract and intestine. Deletion of essential genes, typically E1, renders Ad replication deficient and leaves room for insertion of an expression cassette. Most clinical experience with Ad vectors is with serotype 5. Because the Ad genome does not replicate or integrate into the genome, and these vectors initiate antivector host responses, Ad vectors mediate only transient expression. Because the brain is partially immunoprivileged, transgene expression from Ad vectors may persist for longer periods in the CNS than in other tissues. The critical questions for use of Ad for LINCL are as follows: (1) How long and at what levels is the protein expressed? (2) At a therapeutic dose, is readministration possible and at what intervals? (3) If expression of the CLN2 gene can clear storage granules and prevent neuronal death, how long does the enzyme persist and how quickly do storage granules reaccumulate?

Following the death of a patient involved in a clinical trial using an Ad vector at the University of Pennsylvania, Philadelphia, in late 1999, there have been safety concerns about the intravascular use of high doses of Ad vectors. However, extensive assessment of the record by the Food and Drug Administration and the National Institutes of Health has shown that Ad is well tolerated at moderate doses, if administered directly to the target.

**Lessons From Experimental Models**

There is no animal model for LINCL. However, data from other experimental models support the use of AAV, LV, and Ad vectors to treat the CNS and retinal manifestations of LINCL. Most relevant are the data from mice with...
mucopolysaccharidosis (MPS) VII (β-glucuronidase deficiency or Sly syndrome, a related lysosomal disorder). Mice with MPS VII are characterized by the accumulation of storage granules in CNS neurons and photoreceptor cell degeneration\(^{24,25}\) (untreated, these mice live 5 months). Like TTP-I, β-glucuronidase is normally secreted and taken up by neighboring cells using the mannose-6-phosphate receptor pathway. Studies of mice with MPS VII have established the following principles: (1) direct gene transfer to the CNS is required for correction of the storage defect in the adult brain\(^{26-28}\), (2) there is cross correction, with transplantation of wild-type cells correcting mutant cells over an area much greater than the region of transplantation\(^{29}\); and (3) AAV, LV, and Ad vectors can reverse storage and behavioral defects after direct CNS administration.\(^{30-34}\)

**Summary**

Adeno-associated virus, LV, and Ad vectors appear to be suitable to treat the CNS and retinal manifestations of LINCL.

**HUMAN GENE THERAPY FOR LINCL: PRACTICAL CONSIDERATIONS**

The objective of clinical gene therapy trials for LINCL is to reverse the progress of the disease. Whether this can be achieved using current technology is not known, but if the preclinical efficacy and toxicology studies are sufficiently robust to support a clinical trial, the available scientific evidence, together with an overwhelming medical need for this rare, universally fatal disorder for which there is no therapy, strongly argues that resources should be devoted toward this end. The following are practical considerations to achieve this goal.

**Vectors**

Comparing the biological characteristics of the vectors, their known safety profiles, and theoretical risks with the biological features of LINCL, we conclude that the priorities for vector development for clinical use should be as follows: AAV is greater than LV, which is greater than Ad. Adeno-associated virus merits the highest priority because LINCL is a hereditary disorder, and there is good experimental evidence that AAV can mediate gene transfer with long-term expression in the CNS and retina. While LV has many of the same characteristics, it has to be given a lower priority because there is far less experience with LV compared with AAV in human trials, and because of the theoretical safety issue of the vector and the TPP-I protein in the CNS of a large experimental animal. This variable is governed by the volume injected (a buffered sugar-salt solution is used as a vehicle for the vector) and by the diffusion characteristics of the vector in the target tissue. Because the CNS is a closed space, there is a limit to the volume that can be administered per unit time. A reservoir could be used to slowly administer the vector, but this will require studies of safety and vector stability, and their use may be associated with a risk of infection. Despite the diffuse nature of LINCL, there are some CNS regions (eg, the cortex and the cerebellum) that may play a dominant role in the

term survival, but many of the treated mice later developed hepatocellular carcinoma.\(^{35}\) The origin of the hepatocellular carcinoma may be a consequence of the underlying pathology of the mice with MPS VII, rather than of the AAV vector therapy.

While Ad vectors may only provide transient expression, if the abnormal lysosomal storage is fundamental to the loss of neurons in patients with LINCL, then transient (1-2 weeks) expression of the CLN2 cDNA in the CNS may provide sufficient TPP-I to clear the storage granules and “set the clock back,” delaying the morbidity in children with LINCL by years. This concept, plus human safety data for Ad vector administration to the CNS for glioma,\(^{36}\) suggests that Ad vectors should be developed for possible clinical use, albeit at a lower priority.

**Expression Cassette**

For gene therapy of LINCL, the expression cassette will be the CLN2 human cDNA controlled by an active promoter. With the knowledge that gene transfer technology can only deliver genes to a limited percentage of cells in a target, the requirement to deliver the CLN2 protein product in a diffuse fashion argues that high-level constitutive expression is necessary, ie, regardless of the vector used, the assumption of cross correction of neighboring cell types makes production of a high level of TPP-I protein of paramount consideration. Examples of such promoters include the Rous sarcoma virus long terminal repeat, the cytomegalovirus early/intermediate promoter/enhancer, and the chicken β-actin promoter with cytomegalovirus enhancer.\(^{36-38}\)

**Administration**

There are 3 possible routes of delivery of vectors to the CNS: intravascular, intrathecal, or intracranial. For the retina, the vector can be administered directly to the subretinal or the vitreal space.

Theoretically, intravascular or intrathecal delivery could be used, but the substantial technology (intravascular) and doses (intrathecal) required for these strategies when balanced against the urgent clinical need for a therapy suggest this should be a low priority. The most direct approach to administration of vectors to the CNS is direct injection into the brain parenchyma. The requirement for diffuse administration necessitates injection into several sites, something achieved only with multiple Burr holes. It is unknown how many of these will be required without knowing the diffusion characteristics of the vector and the TPP-I protein in the CNS of a large experimental animal. This variable is governed by the volume injected (a buffered sugar-salt solution is used as a vehicle for the vector) and by the diffusion characteristics of the vector in the target tissue. Because the CNS is a closed space, there is a limit to the volume that can be administered per unit time. A reservoir could be used to slowly administer the vector, but this will require studies of safety and vector stability, and their use may be associated with a risk of infection. Despite the diffuse nature of LINCL, there are some CNS regions (eg, the cortex and the cerebellum) that may play a dominant role in the
phenotype; these areas might be the primary targets for the initial studies. Alternatively, the vector could be administered repetitively. This has the advantage of being able to assess each single administration for adverse events before proceeding, but assumes that host defenses against the vector will not limit the safety and effectiveness of repeated administration.

For the retina, the most common effective procedure for vector administration is a single injection through a sclerotomy into the subretinal space. Alternatively, the vector can be administered with a single injection through the front of the eye, to the vitreous, or continuing through the vitreous into the superior subretinal space.

Dose Response
The administration of a gene transfer vector has the potential of inducing toxicity from the vector and/or transgene product. Because the CNS and retina may be sensitive to such adverse events, it would be prudent to limit the intensity and spatial distribution of any toxicity by initiating a gene transfer trial to these sites with low doses of the vector, and to administer the vector to a limited (local) region of the brain or to one eye. The development of gene therapy for LINCL must balance ethical issues (eg, using a low dose may not be efficacious for this fatal disorder) against safety issues (eg, persistent high-dose vector expression causing toxicity). If a conservative approach is taken using a low dose and/or administration to a limited region of the brain, the only solution is to consider readministration (if possible) after sufficient time passes to assess the toxicity from the first administration.

Repeat Administration
For most drugs, repeat administration is the norm. However, for LINCL, each administration to the CNS will require burr holes. Systemic immunity against a viral vector may preclude effective gene transfer on readministration, and host defenses against the vector may induce inflammation that may be unacceptable. Despite the fact that the CNS is a partially immunoprotected site, we predict that repeated administration of viral vectors to the CNS and retina may be safe but may not be progressively efficacious.

Preclinical Data
Without the availability of a suitable model for LINCL, critical preclinical studies must address the following issues: (1) Will the TPP-1 product diffuse throughout the brain in sufficient levels to provide efficacy? (2) What toxic effects are associated with administration of the vector at doses within and above the range likely to be used in the clinical studies? For each vector and each site, these studies need to be carried out with naive and antivector immune-positive animals, and with single and repeat administration. As another assessment of toxicity, the creation of transgenic mice with the CLN2 cDNA will be useful to know if there is a phenotype associated with overexpression.

Regulatory and Ethical Issues
In the context that LINCL is a rare, fatal disease, it may be necessary to develop gene therapy for it by compressing the studies into a combined phase 1, 2, and 3 study that simultaneously assesses safety and efficacy parameters. The initial human studies will likely require a lower than therapeutic dose. While local delivery will be the safest way to start, it will likely not treat all aspects of the CNS disease. Furthermore, the parameters that measure clinical outcome in the CNS are relatively insensitive and may provide little information about the efficacy of gene transfer, ie, CLN2 gene therapy studies in humans involving small numbers of patients may yield ambiguous data in posttherapy tests. Depending on the final inclusion/exclusion criteria, there will be a limited number of patients eligible for the therapeutic development pathway. There is an ethical dilemma regarding the recruitment of children with far advanced disease, but restrictive inclusion/exclusion criteria could radically reduce the number of available subjects for a trial. Because families are highly motivated to participate in a gene therapy trial for LINCL, clear consent materials will be necessary, as will provisions for extensive discussions before and during the study.

SUMMARY
There are many unanswered questions that will require assessment before proposing a clinical gene therapy trial for LINCL. Many decisions will require data generated from experimental animals. Objective, quantitative parameters will need to be developed before assessment of subjects with LINCL in a gene therapy protocol can be achieved. However, there is sufficient information available to conclude that if our assumptions regarding the biology are correct, the resources are available, and the regulatory climate is supportive, it is rational to initiate the preclinical safety and toxicity studies directed toward mounting clinical trials for the CNS and retinal manifestations of LINCL.

Accepted for publication April 23, 2001.
This study was funded in part by Nathan’s Battle Foundation, Indianapolis, Ind.
We thank R. Boustany, MD, K. Wisniewski, MD, PhD, N. Zhong, MD, M. Sands, PhD, P. Lobel, PhD, P. Gutin, MD, M. Soueidane, MD, F. Marshall, MD, J. Bennet, MD, PhD, P. Leopold, PhD, and R. Zalaznick, BA, for helpful discussions and advice; and B. Charlot, T. Virgin-Bryan, and N. Mohamed, MPH, for preparation of the manuscript.
Corresponding author and reprints: Ronald G. Crystal, MD, Institute of Genetic Medicine, Weill Medical College of Cornell University, 515 E 71st St, Suite 1000, New York, NY 10021 (e-mail: geneticiainmedicine@med.cornell.edu).

REFERENCES