

## Clinical Protocol

# Gene Therapy of Canavan Disease: AAV-2 Vector for Neurosurgical Delivery of Aspartoacylase Gene (*ASPA*) to the Human Brain

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### ABSTRACT

This clinical protocol describes virus-based gene transfer for Canavan disease, a childhood leukodystrophy. Canavan disease, also known as Van Bogaert–Bertrand disease, is a monogenic, autosomal recessive disease in which the gene coding for the enzyme aspartoacylase (*ASPA*) is defective. The lack of functional enzyme leads to an increase in the central nervous system of the substrate molecule, *N*-acetyl-aspartate (NAA), which impairs normal myelination and results in spongiform degeneration of the brain. No effective treatment currently exists; however, virus-based gene transfer has the potential to arrest or reverse the course of this otherwise fatal condition. This procedure involves neurosurgical administration of ~900 billion genomic particles (~10 billion infectious particles) of recombinant adeno-associated virus (AAV) containing the aspartoacylase gene (*ASPA*) directly to affected regions of the brain in each of 21 patients with Canavan disease. Pre- and post-delivery assessments include a battery of noninvasive biochemical, radiological, and neurological tests. This gene transfer study represents the first clinical use of AAV in the human brain and the first instance of viral gene transfer for a neurodegenerative disease.

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## BACKGROUND

CANAVAN DISEASE is associated with mutations in the gene for aspartoacylase (*ASPA*), an enzyme that converts *N*-acetyl-aspartate (NAA) into acetate and aspartate. The resulting enzymatic deficiency causes accumulation of NAA in brain, lungs, kidneys, and liver (Matalon *et al.*, 1989), although significant pathology appears to be limited to the central nervous system. Levels of NAA in brain white matter may approach 15–20 mM in untreated Canavan patients (Barker *et al.*, 1992; Bluml, 1999; Leone *et al.*, 2000) compared to the normal range of 5–10 mM (Michaelis *et al.*, 1993; Lam *et al.*, 1998; Wang *et al.*, 1998; Keevil *et al.*, 1998) and mean cerebrospinal fluid levels of NAA are elevated 10-fold over normal at ~0.3 mM (Dr. P. Leone, unpublished data). The plasma NAA concentration in Canavan disease may reach as high as 1 mM, compared to trace amounts in normal individuals (Baslow, 1997), and urine NAA is grossly elevated over normal, often at >1000  $\mu\text{mol}/\text{mM}$  creatinine (Matalon *et al.*, 1993). The deleterious effect of high levels of NAA is probably multi-factorial and osmotic, metabolic, and cytotoxic hypotheses have been proposed.

Canavan disease is characterized by dysmyelination and gross morphological changes of white matter (cf. Leone *et al.*, 1999). Neurons are not thought to be directly affected, although neuron-glia interactions may be important in the pathology. Patients present with macrocephaly, poor head control, truncal hypotonia, visual impairment, and failure to meet developmental milestones. As the disease progresses, limb spasticity and hyperreflexia predominate and patients become increasingly incapacitated. Patients never develop normally and exhibit severe neurological deficits, ultimately progressing to complete immobility, mental retardation, blindness, and death. Depending on the extent and quality of supportive care available, which usually includes gastric feeding tubes, orthotics, and full-time nursing care, affected children may survive in a chronic vegetative state into their teenage years or in rare cases into adulthood (Zelnik *et al.*, 1994; Zafeiriou *et al.*, 1999).

In a pilot study of two Canavan patients in 1996, neurosurgical administration of the aspartoacylase gene (*ASPA*) to the cerebroventricular space of the brain was shown to be safe (Leone *et al.*, 2000). In addition, *ASPA* gene transfer was associated with regionally lowered NAA as measured by proton magnetic resonance spectroscopy ( $^1\text{H}$  MRS) and mild neurological and radiologic improvements. Starting in 1998, a second clinical trial was conducted with a larger group of patients using the same approach (I.N.D.-7307). Both original studies used a non-viral gene transfer vector known as LPD, composed of a recombinant plasmid in conjunction with a condensing

agent (poly-L-lysine or protamine sulfate) and a liposomal formulation (DC-CHOL/DOPE). Clinical changes in most participants were not pronounced and were relatively transient, thought to be due primarily to inadequacies of the vector or delivery system. The current protocol aims to improve upon the previous methodology with a new vector system and new method of delivery. We anticipate that I.N.D.-9119 will provide the basis for Phase II/III studies, and will be a useful reference for future clinical trials using AAV in the human brain.

## RATIONALE

The rationale of this clinical protocol is to supplement the gene for an enzymatic pathway in the brain. The study is designed with two primary objectives, (1) to follow the natural history of untreated Canavan disease, and (2) to examine the physiological effects following gene transfer using a variety of outcome measures. Study participants are assessed longitudinally (i.e., before and after gene transfer) to determine the safety of AAV gene transfer to the human brain and the effects of AAV-*ASPA* in modifying the progression of the disease. The general hypothesis to be tested is that AAV-*ASPA* has appreciable effects on the natural history of Canavan disease. Effects of *ASPA* gene transfer in the Canavan population will be determined using magnetic resonance (MR) imaging and spectroscopy at Children's Hospital of Philadelphia (CHOP). Quantitative data will be collected on NAA levels (brain, blood, urine, CSF) and magnetic resonance signal changes (white matter myelination, brain water content, morphology). Other hypothesized effects such as improved clinical and neurological status will be measured at Cooper Hospital-UMDNJ.

There is currently no viral vector that preferentially targets glial cells. Even with strong glial promoters, cellular transduction and gene expression with AAV-2 occurs predominantly in neurons (Bartlett *et al.*, 1998; Xu *et al.*, 2001), though oligodendrocytes, astrocytes, or microglia may be transduced to a limited extent. Despite the fact that Canavan disease is commonly thought of as a disease of white matter, there are reasons for targeting a variety of cells, particularly neurons. As described in the multi-compartmental model of Canavan disease (Baslow, 1997, 1999), the site of NAA production is primarily inside neurons, while the site of pathological action after it is released from neurons is in the vicinity of glial cells. NAA is generated in the mitochondria of neurons and transported to the interstitial space where it is metabolized by membrane-bound aspartoacylase on the cell surface of oligodendrocytes (Baslow, 1999) and taken up by astrocytes (Sager *et al.*, 1999). A recent study suggested that NAA may be metabolized inside astrocytes as well as inside myelinating cells (Bhakoo, 2001). To a lesser degree, NAA may be broken down or modified by enzymes contained within neurons. NAA is ordinarily maintained at a high concentration in neurons, but does not appear to be essential for neuronal survival. For example, cerebral NAA was reported to be completely lacking in one child (Martin *et al.*, 2001). We suggest that it may not be necessary, or even desirable, to produce aspartoacylase exclusively in oligodendrocytes in the Canavan brain. In fact, it may be adequate to express the *ASPA* gene in neurons at the source of production of NAA. Whether or not the enzyme normally exists within neurons, we

propose that when it is expressed at sufficiently high levels, *ASPA* will help to lower whole-brain NAA levels and will have a demonstrable effect on the progression of the disease. In support of the safety of this plan, we have found that expression of aspartoacylase in neuronal culture does not elicit any obvious toxic effects, a finding also supported by *in vivo* toxicology.

### SUMMARY OF PRECLINICAL EXPERIENCE WITH AAV-2

AAV-2 vectors have been used in numerous models of neurological disease and are currently in clinical trials for hemophilia (Kay *et al.*, 2000) and cystic fibrosis (Wagner *et al.*, 1998). Although I.N.D.-9119 represents the first clinical use of AAV in the human brain, AAV was shown to be effective in driving gene expression in rodent and primate brain (Kaplitt *et al.*, 1994; During *et al.*, 1998; Bankiewicz *et al.*, 2000), and neurons from resected human hippocampal tissue also have been transduced *ex vivo* with AAV (Freese *et al.*, 1997). Previous experience with human gene transfer using LPD-*ASPA* has provided the main proof-of-principle for clinical use of AAV-*ASPA*. At first, no animal model was available specifically for Canavan disease. Therefore, despite a strong rationale for viral-based gene transfer, initially it was not possible to predict the functional effects of *ASPA* gene transfer outside of cell-based assays. Recently, a transgenic Canavan mouse was developed (Matalon *et al.*, 2000) and a naturally occurring rat model of Canavan disease has been characterized. The Tremor rat (Kitada *et al.*, 2000) has a deletion in the *ASPA* gene with elevated NAA and symptoms closely resembling Canavan disease. Preliminary data using gene transfer in this genetic model with the same AAV-*ASPA* construct as I.N.D.-9119 suggests biochemical and phenotypic normalization, in further support of our rationale (McPhee *et al.*, 2001).

We began pre-clinical testing of AAV-2 by demonstrating production of full-length *ASPA* transcript and aspartoacylase protein. Clinical-grade plasmid (pNSE-*ASPA*) was transfected in Neuro-2A cells and *ASPA* gene expression was detected with RT-PCR. The clinical reagent AAV-*ASPA* was then used to transduce C17.2 neuronal cells and expression was confirmed with RT-PCR and Western blot. AAV-*ASPA* is a recombinant AAV-2 construct containing human *ASPA* cDNA, a human neuron-specific enolase (NSE) promoter element, and regulatory elements (see below). In order to confirm that functional aspartoacylase protein is produced with AAV-*ASPA* in Canavan cell lines, fibroblasts from Canavan patients were transduced with an AAV-*ASPA* construct containing an elongation factor (EF) promoter; assays showed mean aspartoacylase activity to be ~30% of the positive control values from mock-transduced normal fibroblasts at the one-week time point. To demonstrate a lack of cytotoxicity in cells transduced with AAV-*ASPA*, an MTT cell viability assay was conducted in primary neuronal culture, which did not suggest treatment-related effects.

Gene expression and toxicology studies were conducted in naive animals using AAV-*ASPA*, AAV-*ASPA/GFP* (fusion gene), and AAV-*GFP* (reporter gene). Quantitative PCR and fusion or reporter genes were used to measure gene expression. AAV-*ASPA* vector (or vehicle) was delivered via the intraparenchymal (IP) route in 72 rats and via the intracerebroven-

tricular (ICV) route in 20 rats. IP delivery of AAV was found to afford significantly higher expression than ICV delivery. This direct approach also obviates the need for an indwelling Ommaya reservoir in human patients, which carries an increased risk of infection and other complications. Expression of *ASPA* mRNA was demonstrated with RT-PCR for up to 7 months following AAV-*ASPA* administration in the hippocampus, striatum, parietal/occipital cortex, and other regions. There was no vector DNA or *ASPA* mRNA detectable by PCR and RT-PCR in other sampled organs (liver, kidney, lungs, heart). In related work aimed at quantifying the relative activities of AAV-2 expression cassettes, 110 rats received AAV-2 reporter gene constructs (Xu *et al.*, 2001). Altogether, these studies in over 200 rats suggest that AAV-2 vectors efficiently express a variety of genes in the brain and appear to have a favorable toxicity profile. Standardized locomotor assays in rats treated with AAV-*ASPA* were indistinguishable from control animals. TUNEL for apoptosis was negative in 25 rats sectioned throughout the brain in the sagittal plane. Contracted toxicology studies were performed by Primedica Corporation (Worcester, MA) on 20 rats, to assess the effects of AAV-*ASPA* and AAV-*GFP* when administered to the brain at four sites per animal, bilaterally into the parietal and occipital lobes at 1  $\mu$ l/min flow rate. Intracranial administration of  $2 \times 10^{10}$  genomic particles (g.p.) of AAV-2 was found to be well-tolerated. No adverse effects were observed in cage behavior, body weights, temperatures, food consumption, physical examination, hematology and clinical chemistry, or gross and microscopic pathology. One death occurred, which was attributed to the surgical procedure. The unit dose of ~10 billion AAV-*ASPA* g.p./g of brain was determined to be below the "no observable adverse effect level" (NOAEL) of the rat.

To supplement data collected in rodents, *in vivo* toxicology and gene expression studies were performed in young primates using clinical grade AAV-*ASPA*, AAV-*GFP*, or PBS control. Each administration contained a volume of 70 or 150  $\mu$ l and was injected using a multichannel microperfusion pump at 4  $\mu$ l/min through flexible fused silica capillary tubing. Twelve male African green monkeys were injected bilaterally in the frontal, parietal, and occipital lobes at parenchymal depths of 1.0, 1.0, and 1.5 cm, respectively. The first six doses of AAV-*ASPA* vector were standardized at  $4 \times 10^{11}$  genomic particles (g.p.) per ml; at this concentration, 70  $\mu$ l per injection site equals a total dose of  $1.7 \times 10^{11}$  g.p. or  $\sim 1.7 \times 10^9$  g.p./g brain. The last six doses of vector were standardized at a genomic titer of  $2.7 \times 10^{12}$  g.p./ml; at this concentration, 150  $\mu$ l per site is equivalent to a total dose of  $2.4 \times 10^{12}$  g.p. or  $\sim 2.4 \times 10^{10}$  g.p./g brain. This final dose is >30-fold greater than the unit dose in humans under this protocol ( $\sim 7 \times 10^8$  g.p./gram brain). All monkeys treated with AAV-*ASPA* and AAV-*GFP* tolerated the procedure. One animal (of 12) died from surgical complications prior to the infusion of vector. All animals were awake within 1 hr after surgery, and most were eating within the second post-surgical hour. Following surgery, each animal was observed closely for 5 days. During this period, body temperature and neurological status were carefully monitored. All body temperatures recorded for the post-surgical period were within the baseline range (99.4–104.1°C). There was no vomiting, seizures, or ataxia. Each animal had a neurological examination with videotaping at 2 and 7 days post-surgery, which

showed normal reflexes, normal motor function, and normal response to auditory, visual, and tactile stimuli. A hematological and clinical chemistry screening was performed prior to surgery and at 3 and 8 days post-surgery; all values were within the normal range. Therefore, it was concluded that intracerebral administration of AAV-2 containing either *ASPA* or *GFP* to monkeys produced little or no acute change in behavior or general health.

Histopathology was obtained post-mortem on primate brains and other body organs, with staining for the astrocytic marker glial fibrillary acidic protein (GFAP) and the immune markers CD45, CD8, and CD3 (markers for activated microglia, T cells, and leukocytes). Out of eight monkeys sacrificed, all but one showed a complete absence of immunohistochemical or morphological evidence of damage. One specimen showed changes consistent with freezing artifact. Brain specimens from monkeys were also analyzed for gene expression by quantifying levels of *ASPA* mRNA using quantitative RT-PCR, or by using GFP protein as a surrogate index of gene expression. In order to detect *ASPA* transgene selectively from endogenous *ASPA*, probes were used which did not target endogenous primate *ASPA*. Brain sections prepared for GFP and mRNA quantification showed gene expression in a variety of brain regions at the 3-month and later time points. To test immune response to AAV-*ASPA*, we assayed sera for anti-AAV antibodies following administration of AAV-2 at a time point of 2 months. Total IgG from monkeys injected with AAV-*ASPA* or PBS was purified from serum using a protein-G/IgG purification kit. Three separate naive monkey serums and three treated serums were assayed, with triplicates run for each individual sample. The mean values for each group were compared to values obtained for wells incubated with anti-AAV VP-1,2,3 antibody at a concentration of 1  $\mu\text{g/ml}$ . The data were expressed as a percentage of the ELISA values obtained using anti-AAV antibody, and were not found to be statistically significant between controls and AAV-injected animals.

## SPECIFIC HYPOTHESES AND GOALS

1. We hypothesize that gene transfer will have appreciable effects on brain chemistry, myelination, brain water content, and brain morphology in the Canavan population. We will assess the extent of these effects using non-invasive magnetic resonance (MR) spectroscopy, T1 measurements, and imaging with novel scanning protocols. These MR techniques are complementary and together will provide information that is unavailable from a single type of test alone. MR spectroscopy is particularly useful because it allows us to quantify levels of brain NAA, a surrogate marker for gene transfer which we believe is the most robust and clinically relevant end point. We will also collect quantitative data on additional brain metabolites (choline, creatine, *myo*-inositol).

2. We hypothesize that changes in brain chemistry will enhance functioning on a systems level in the brain. Therefore, we will assess changes in the clinical status of the patients. Tests will include neurological and cognitive evaluations conducted by a neurologist and a behavioral psychologist. To improve standardization, the same neurologist will assess all patients. We have developed a parental assessment tool for at-home mon-

itoring. Because the primary outcome measure is safety, we have developed a comprehensive clinical monitoring plan that will detect and document any adverse events following gene transfer.

3. We hypothesize that the anticipated decrease in brain NAA will lead to a drop in peripheral levels of NAA. Therefore, we will assess changes in NAA acidemia and aciduria through biochemical assays of blood and urine. Although results from the previous clinical trial suggested that urine and serum NAA were not particularly sensitive indices of CNS status, because our vector is more potent we believe that it is worthwhile to assess peripheral as well as central levels of NAA by direct means. These tests will help us to validate our MR spectroscopy techniques. Patients who participated in I.N.D.-7307 were implanted with a reservoir and will have cerebrospinal fluid (CSF) removed, to verify NAA levels by biochemical assay. We will measure the persistence of the *ASPA* transgene from cells in CSF from these patients, using sensitive PCR and RT-PCR analyses. Finally, we will determine if neutralizing antibody is present in blood or CSF after gene transfer, an important safety consideration.

4. We hypothesize that complex molecular derangements may exist in Canavan disease due to compensatory changes associated with the primary defect. For example, intersecting biosynthetic and catabolic pathways such as NAA synthetase are likely to be abnormally regulated. Because one of the key limitations to molecular and cellular work on Canavan disease is the lack of human tissue and cell lines, we have developed a plan for collecting and banking tissue specimens through The Coriell Center for Medical Research, an NIH-funded repository of valuable cell lines. Also we have formulated a life-long surveillance plan for all patients who enroll, which includes post-mortem analyses that will facilitate a better understanding of secondary molecular and cellular defects.

## OUTCOME MEASURES

The primary response variable is the change in regional brain NAA levels from baseline to 12 months, as measured by single-voxel proton magnetic resonance spectroscopy ( $^1\text{H}$  MRS). Quantitative biochemical analysis of blood and urine will be used to confirm trends in NAA levels. The secondary response variable will be the patients' clinical status, which will include formal neurological exams and psychometric tests. MRI will be used to supplement information gained from clinical examination. Safety is the primary end point, and all response variables are designed to help assess the safety of the clinical reagent. Clinical end points pertaining specifically to safety are considered separately below, under patient monitoring and adverse events reporting. Without performing serial brain biopsies, direct measurement of *ASPA* gene expression is not possible in humans. Therefore, evidence of gene transfer will rely on surrogate markers. The most robust surrogate marker is cerebral NAA levels. Grossly elevated NAA is pathognomonic for Canavan disease, and will be followed non-invasively with serial  $^1\text{H}$  MRS. The results of spectroscopy will be validated using biochemical methods to quantify NAA concentration in the blood and urine. It is conceivable that neuronal cell death could lead to decreased brain NAA in the absence of gene expression; how-

ever, we have not observed cytopathic effects in pre-clinical studies, which suggests that this possibility is unlikely. The use of other outcome measurements including a variety of MR techniques (e.g., diffusion tensor imaging, quantitative T1), neurological examination, and clinical chemistry will help to control for the possibility of NAA decreasing due to causes other than an increase in aspartoacylase following successful gene transfer. It is important to note that natural history studies performed to date indicate that mean NAA levels do not decrease as Canavan disease progresses, but rather appear to increase linearly over the age range of patients in this study. Therefore, a reversal in the slope of brain NAA levels would constitute strong evidence of a treatment effect.

#### *Primary outcome measure: magnetic resonance spectroscopy (MRS) and imaging (MRI)*

Of particular interest to our study is the fact that *N*-acetyl-aspartate (NAA) levels may be measured non-invasively and accurately. <sup>1</sup>H MRS provides a method of performing quantitative and semi-quantitative measurements of the brain (Kreis *et al.*, 1993). Levels of NAA, *myo*-inositol, choline-containing compounds, total creatine, and water content will be quantified using <sup>1</sup>H MRS in four brain areas: frontal cortex, parietal cortex, occipital cortex, and basal ganglia. The scan regions will be in proximity to areas targeted for gene transfer. Investigators will use modeling software to obtain NAA metabolite levels and water content, which will be measured in response to gene transfer. The main disadvantage of MRS is that measurements are quite slow and have low spatial resolution; measurement of a single volume element (voxel) 2 cm on a side can take 10–15 min to measure. Thus the combination of three separate approaches, namely <sup>1</sup>H MRS, conventional T1- and T2-weighted images, and quantitative T1 will provide a comprehensive MR study with which to follow the progression of the patient during treatment. Conventional T1-weighted and T2-weighted images have been used by many groups to study the development of the brain during infancy (Barkovich, 1988). The relative change in contrast between white and gray matter gives rise to characteristic appearances of these images. The study radiologist will be responsible for determining anatomically important changes in MRI signal, including myelination patterns. While such conventional images do provide exquisitely high resolution images with which to assess general development of brain anatomy, they do not provide a quantitative assessment needed for longitudinal studies of brain condition. For such purposes, quantitative measurement of the longitudinal relaxation time (T1) is the preferred method (cf., Kingsley, 1999; Steen, 1997). The longitudinal relaxation time (T1) is influenced by a variety of factors, of which the dominant ones in the brain of young children are myelination and water content (McArdle *et al.*, 1987). While it is not appropriate to use the relaxation time as a measure of myelin content per se, it is common practice to use it to track the changes in the physiological status of the brain in situations where myelin content is believed to be changing, such as brain development following birth. Recently, a protocol was developed by which T1 may be measured with >1% accuracy and about 4% precision over the whole brain (i.e., 15 slices) in less than 2 min (Haselgrove, 2000). The resolution of 2 × 2 mm in-plane is slightly less than that of the conventional

clinical images but is still sufficient to analyze different regions of the brain. MR studies are performed at CHOP using a 1.5T whole body Siemens MR system using a conventional head coil. Since 1996, the scanner at CHOP has been used for patients affected by Canavan disease, and a database of over 30 patients has been generated on age-matched normals and untreated Canavan patients (Lam *et al.*, 1998). This clinical protocol will follow patients over time prior to their gene transfer, and will increase our knowledge of the natural history of the disease.

#### *Secondary outcome measure: clinical evaluations*

The clinical exam has two components, neurodevelopmental or psychometric tests (Mullen Scales of Early Learning, Pediatric Evaluation of Disability Index, Canavan HELP-135) and neurological exam with quantitative tests of motor function (Gross Motor Function Measure, Ashworth modified spasticity scale). In addition, all physicians will complete clinical evaluation sheets as part of safety monitoring. Because the primary end point is safety, parents and physicians are required to document any abnormal findings, which will be recorded in adverse event reports. The clinical coordinator is responsible for monitoring these data, including required laboratory results and physical exams. Safety assessments will require periodic collection of blood and urine for hematology and clinical chemistry screening tests as well as specialized biochemical assays. Although NAA is generated in the mitochondria of neurons, the level of this metabolite in serum reflects the large excess that is transported continually from the brain and targeted for excretion. Direct biochemical assays using HPLC will be performed on serum (mM units) and urine (mM NAA/mM creatinine) in order to quantify changes in NAA outside the brain. Any enrolled patients with a pre-implanted reservoir will have CSF aspirated for NAA and *ASPA* transcript quantification. The reason that only these patients will have CSF drawn is in order to avoid complications associated with spinal fluid collection. Moreover, it is likely that *ASPA* transcript will not be homogeneously spread throughout the CSF and levels in the lumbar spinal cord are likely to be different from those in the lateral ventricles. Earlier studies (Leone *et al.*, 2000) indicated that the *ASPA* transgene can persist in cells isolated from the cerebrospinal fluid of patients up to 12 months after delivery of LPD-*ASPA* in the lateral ventricle, and similar levels may be detectable following AAV-*ASPA* treatment. We will also assay for the presence of antibodies to AAV.

### STANDARD CLINICAL TESTING REGIMEN

Clinical testing is broken down into three phases. In addition to testing done over the time course of the initial study, long-term follow-up will be done with the patient's referring physician on a yearly basis for the remainder of the patient's life. The clinical tests are designed to assess clinical status and NAA levels.

#### *Pretreatment phase (2–6 assessments)*

1. Magnetic resonance spectroscopy (MRS)
2. Magnetic resonance imaging (MRI)

3. Neurological examination (GMFM, Ashworth modified spasticity scales)
4. Psychometric tests (Mullen Scales, PEDI, HELP-135)
5. CSF analysis—only in patients with pre-existing reservoir
6. Quantitative blood and urine analysis for NAA
7. Hematology, clinical chemistry, anti-AAV2 neutralizing antibody profile

#### *Surgery and gene delivery phase*

Single-arm, open label clinical study with standard dose of 900 billion AAV-NSE-ASPA genomic particles to each of 21 patients by intraparenchymal injections to the brain.

#### *Post-treatment phase: Serial assessments at 1, 3, 6, 9, 12 (18, 24 . . . ) months*

Patients will be closely monitored before discharge from Cooper Hospital. At the 2-week time point, a complete outpatient physical and neurological exam will be conducted, including hematology and clinical chemistry. Brain MRI will be obtained in order to look for any postoperative signal changes and to definitively rule out edema and bleeding. All tests are otherwise identical to pre-treatment at the intervals listed above.

## STATISTICAL CONSIDERATIONS

### *Overview of research design*

This study will evaluate the safety of AAV as a vector for gene transfer to the brains of children with Canavan disease. It is our intention to treat all patients following identical procedures. There is no plan in the current protocol for varying dosage by design. The key statistical issues are the estimation of the treatment-associated toxicity rate and the preliminary assessment of response to gene transfer. The relevant study variables are demographics (e.g., age, sex, ethnicity), clinical outcomes, and adverse events. In recording adverse events, clinical assessments will be essentially continuous for the 1-2 weeks following gene transfer, with daily physicians' progress reports. All adverse events will be rated as to seriousness, severity, expectedness, and relatedness to the treatment. In recording clinical outcomes, the primary end points are NAA spectroscopy, magnetic resonance T1 values, and neurodevelopmental scores, all of which are quantitative. Because there are no untreated or sham-treatment controls, clinicians and patients will be unblinded. Nevertheless, spectroscopy, standardized psychometric tests, and neurological examinations are objectively measured and are performed independently from the principal investigators and sponsor. The secondary end points include behavioral assessments, neurological examinations, and radiologic impressions, all of which are semi-quantitative. We will assess participants at early time points (15 and 30 days) even though we do not anticipate the response to treatment to be maximal, since safety assessment at those time points is critical.

### *Safety analysis*

As a Phase I study, this trial will make use of a relatively small sample size (21) and will provide limited power to de-

tect treatment-related toxicities. Because relatively rare toxicities have a low probability of occurring, the search for toxicities should continue with larger, Phase II/III trials. Should adverse events occur, we will record them and compute appropriate estimates of risk and associated confidence intervals. Because toxicities are assumed to be rare, we will use exact methods for confidence intervals rather than a large-sample asymptotic method. Risk rates will be broken down by the type of event, relative severity, and patient characteristics, as warranted. A standard case report form will be used. A modified National Cancer Institute (NCI) "Common Toxicity Criteria" table will classify the severity. Power calculations are used to determine the toxicity rates that could be detected, assuming that 21 subjects are enrolled and evaluated. The 95% two-sided confidence bounds for the true toxicity rate (%) are calculated for an observed number of subjects (out of 21) experiencing a toxicity of Grade 3 or higher.

### *Sample size estimation*

In order to judge whether the sample size proposed is reasonable for detecting clinically important changes, we analyzed spectroscopy data from the previous human study. The standard error of the pre-post contrast was 0.1485 on 11 degrees of freedom. This corresponds to a standard deviation of 0.493. Using 0.5 as the standard deviation of differences in a two-tailed, 5% paired *t* test, a sample size of 15 gives power exceeding 99% for differences of 2-3 mmol in NAA, the difference we think will be important. While this could be taken as suggesting that a smaller sample size is sufficient for this study, we also want a reasonable sample size for comparing brain regions, etc., all of which will benefit from a larger *N*. The total sample size for this study will be *N* = 21, sufficiently large to allow for patient attrition without compromising study results. Every effort will be made to retain subjects in the study through the completion of all follow-up visits, especially for the critical 1-month and 3-month safety assessments. It will be emphasized to the subjects and their parents/guardians the importance of compliance with the evaluation schedule.

### *Pre-post comparisons and efficacy analysis*

It is expected that this trial will help to determine the best definitive outcome measures to use in later trials. We begin our analyses with descriptive presentation of the data including means and standard deviations by time and brain region, supplemented with graphs to examine the time trend. Particularly with clinical information that is semiquantitative we will organize the results in tabular and graphical form in order to look for patterns across domains of motor and cognitive performance or across anatomical regions. For each end point, the hypothesis we will test is that the average post-treatment values differ from the average pre-treatment, averaging over times and brain region for NAA spectroscopy and quantitative T1, or over times for neurodevelopmental score. We do the analysis this way to make best use of the relatively small sample size (as viewed statistically) and our belief that the response to treatment will occur fully by 3 months and then be retained. Thus, we will not use the 15-day and 1-month assessments, as they are likely to be prior to the time of full response.

### *Longitudinal modeling of treatment effects*

Secondary analyses will examine the time trend in responses (all end points) and the consistency of responses for NAA spectroscopy and quantitative T1 across various brain regions. To examine time trends, in addition to graphing, we will enter time as a continuous variable rather than a categorical factor and include the 15-day and 1-month measurements. For consistency across brain regions, we will examine the region by pre-post interaction. For longitudinal modeling, because our primary end points are quantitative, the approach to analysis will be repeated measures (mixed model) analysis of variance. The within-subject factors will be time and brain region. For all analyses, we will check the residuals for approximate normality and transform the data, if needed, to better satisfy the normality assumption. We will use SAS Proc Mixed, which allows for missing data. Because we are collecting quantitative NAA levels in untreated Canavan patients over different age ranges, we will use those data to develop a general model of NAA levels by brain region and age in the Canavan population, similar to previous work done in the normal pediatric population (Lam *et al.*, 1998; Horska *et al.*, 2002).

### *Exploratory analyses*

We seek to learn what we can from these data to generate hypotheses for further work. To this end, we will conduct exploratory analyses. These analyses will focus on the consistency of response of the various end points. We are interested here in whether the responses of the various end points showing response occur at the same time or whether some are delayed relative to the others, and in whether there is consistency of response among the end points. Our approach will be graphical (i.e., time trends) and analytical (i.e., correlations).

## PROTECTION OF HUMAN SUBJECTS

This study follows all the procedures and principles for the "Protection of Human Subjects" outlined in 45 CFR 46, and meets criteria outlined in "Additional Protections for Children Involved as Subjects in Research," Subpart D, Sections 401-409. The clinical protocol and consent form were reviewed and approved by Institutional Review Committees at Cooper Hospital and Children's Hospital of Philadelphia. An Appendix M document was submitted to the Office of Recombinant DNA Activities at the NIH on January 15, 2000 and publicly presented to the RAC on March 16, 2000. The investigator's brochure was submitted to the FDA on June 6, 2000 and assigned I.N.D.-9119. The final clinical protocol was approved by the FDA on April 11, 2001. The protocol follows all guidelines for Good Clinical Practice. All clinical research performance sites adhere to these stated criteria for protection of subjects and conduct of research. There are no conflicts of interest to disclose on the part of the study sponsor or investigators. Procedures will be strictly followed to maintain privacy & confidentiality. All patient samples collected for research purposes are stored in secure -70°C freezers. All paper and digital files will be kept confidential and standard practices of patient confidentiality such as the use of coded identifications of research subjects will be applied by the clinical coordinators and study investigators.

## CONSENT FORMS

The consent form gives information in non-technical language, in addition to an outline of the study protocol and the risks and potential benefits of the intervention. The investigators will be responsible for contacting interested parties and describing the study to them. All relevant points in the protocol will be discussed with the parents of prospective participants in language that is understandable to them. It is not possible to obtain consent directly from the participants, because (1) they are minors and (2) they suffer from severe cognitive impairment. This study involves only pediatric subjects, therefore parental permission is necessary for informed consent. Both parents will be contacted if possible and will be allowed sufficient time following discussions with the investigators to make a decision about the participation of their child in the study. The requirement for the child's assent is waived. The prospective enrollees are likely to be familiar with some aspects of the study through other sources. The level of sophistication will vary among the enrollees, and the discussion will be tailored to the background and needs of each family. If the child of a non-English speaking family is considered for enrollment, the consent form will be prepared in his/her native language and a translator will be present at all conversations between the family and the investigators. Parents will be free to choose whether to enroll or not to enroll in the study at any point, assuming the study has not been filled.

## INCLUSION OF WOMEN, MINORITIES, AND CHILDREN AS RESEARCH SUBJECTS

Female and male patients will be included in this study. Canavan disease is a genetic disease that is most common in people of (Ashkenazi) Jewish descent; however, there will be inclusion of various ethnic minorities and subjects from other ethnic backgrounds. We plan to enroll Americans as well as patients from Europe, South America, and the Middle East. Canavan disease is a childhood leukodystrophy, in which patients are developmentally impaired from infancy. Because the disease is relentlessly progressive, most patients die while still in childhood. Therefore, only children will be enrolled.

## SUBJECT RECRUITMENT AND SELECTION

Subjects will be recruited with the help of non-profit research foundations, following eligibility criteria described below. These charitable trusts have reliable databases of Canavan patients. No advertisements will be used to recruit patients. Institutionally approved consent forms will be provided to parents of the children. Based upon inclusion/exclusion criteria, we will enroll 21 patients. The first cohort ( $N = 3$ ) was enrolled in 2001. For purposes of safety monitoring and the identification of adverse events, 18 new study participants will be divided into three cohorts ( $N = 6$ ). Enrollment will be closed after 18 new patients have been selected. At that point, additional patients who desire to participate will be kept on a "short list" in the event that another patient drops out of the study before treatment. These short-list patients will be randomly assigned to the

protocol if the selected patients suddenly withdraw or are unable to participate. In a worst-case scenario where 25% of patients are lost to follow-up, we hope to retain at least 15 patients. Each cohort will be filled following a review of interim safety data by the investigators, study monitor, and independent data monitoring board. Each cohort will include a mixture of male and female patients, but otherwise will be randomly selected from among the available pool of candidates.

### STUDY ELIGIBILITY: INCLUSION CRITERIA

1. A definitive diagnosis of Canavan disease, based upon biochemical criteria and genetic (mutation) analysis. The *sine qua non* of CD is elevated brain NAA levels, which is correlated with NAA acidemia and aciduria. These may be established by HPLC analysis of blood or urine. Spectroscopy is another method that may be used to establish grossly elevated levels of NAA. Biochemical studies will be followed by documentation of a specific mutation in the *ASPA* gene as assessed by Southern blot and/or RT-PCR with sequencing of the patient's defective gene. Enzyme activity assay will also be performed using fibroblasts collected from skin biopsy. The patient must demonstrate clinical or radiologic findings consistent with Canavan disease, such as macrocephaly, developmental delay, seizures, or other positive findings.

2. The patient must be between the age of 3 and 96 months (preferably, within the range of 0.5 to 4 years of age).

3. At least 50% of enrollees must be naive subjects. (Previous participation in gene therapy for Canavan disease is not required, nor is it an exclusion criterion. Previously treated patients may be enrolled at the discretion of the investigators.)

4. The parents of study participants must agree to comply in good faith with the conditions of the study, including attending all of the required baseline and follow-up assessments. The principal investigators will make every possible effort to assist the parents in the accomplishment of these tasks. At this point in time, we do not believe that follow-up will present a problem for patients currently awaiting enrollment, but we do ask for a written commitment to the study as part of an "intention to enroll" letter.

5. Both parents or guardians must understand and sign their child's informed consent forms and may be asked to meet with members of an independent safety monitoring and advisory board.

### STUDY ELIGIBILITY: EXCLUSION CRITERIA

1. Other significant medical or neurological conditions may disqualify the patient from participation in this study, particularly those which would create an unacceptable operative risk. Examples include brain cancer; congenital heart failure; liver or renal failure. Each case will be individually reviewed and the final decision shall rest with the Independent Data Monitoring Committee.

2. Concurrent participation in any other FDA clinical protocol is disallowed, although the study physicians will work with other doctors to accommodate specific requests (e.g., a study of nutritional supplements probably would not be a disqualification).

3. Patients with a documented severe allergy to certain antibiotics may be excluded from the study, due to the fact that the vector may be produced with small quantities of these drugs. However, the trace quantities present are not likely to be clinically relevant, unless the patient has a severe reaction to antibiotics.

### DELIVERY OF THE GENE VECTOR TO THE HUMAN BRAIN (SURGICAL PROTOCOL)

The method of vector delivery is critical in obtaining efficient transduction of target cells in the brain. This protocol requires administration of a standard dose of AAV-*ASPA* vector to each patient's brain at six sites, chosen to maximize spread of the vector through the affected regions. The precise injection sites are determined on a case-by-case basis by the neurosurgeon, but will be located bilaterally in the frontal, parietal, and occipital regions. The neurosurgeon will identify unique clinical features of each patient. To standardize the sites of burr-hole entry among patients and to ensure that the target sites are in the vicinity of the MRS voxel ( $2 \times 2 \times 2$  cm) in the frontal, parietal, and occipital lobes and away from poor surgical access sites (e.g., cortical sulci, instrumentation, heavily vascularized regions), we will use pre-operative MRI to guide the choice of sites. Anatomical landmarks will be used to plan the burrhole sites. More specifically, the coronal suture will be used along with bony landmarks of the skull base (odontoid process) to draw a line in the sagittal plane on MRI which will then be used to measure three arcs in that plane for standardization of the frontal, parietal, and occipital sites. The target site will be a cortical gyrus in the frontal, parietal, and occipital regions. There will be six target sites per brain, with three in each hemisphere. In the sagittal plane, the frontal site will be 15–25° anterior to the reference line; the parietal site will be 0–10° posterior to the reference line; the occipital site will be 30–40° posterior to the reference line. In the coronal plane, all sites will lie within 15–25° from the midline. We will mark the range of angles (as specified) on the images and choose the ideal target sites within that range. The measurements of the chosen sites will be entered in advance on the surgical Case Report Form. We will approximate  $s$ , the arc length subtended by the angle  $\Theta$ , by using  $s = r\Theta$ , where  $r$  is the distance from the odontoid to scalp and  $\Theta$  is the angle in radians ( $=$  angle in degrees  $\times \pi/180$ ) between the reference line (i.e., odontoid to coronal suture) and the target site. For example, for the frontal lobe site, if the distance from the odontoid to the scalp at the target site is 10 cm and the angle  $\Theta$  is 20° (0.349 rad) then the arc is  $\sim 3.5$  cm. Because the head shape is irregular and ellipsoid rather than perfectly spherical, this will probably be a slight overestimate of the desired distance. The burrholes (2.0–2.5 cm) will be large enough to make appropriate visual adjustments prior to the insertion of the catheter. The "sagittal view measurements" column on the surgical Case Report Form record the anterior-posterior (AP) distances, as measured from the coronal suture in centimeters. The "coronal view measurements" column record the medio-lateral (ML) distances, as measured from the midline in centimeters. We will check these estimated distances using a flexible measuring ruler along the apparent edge of the scalp on MR images; the ruler will be marked with the calibration scale lines (cm) from each MR image. The depth for



the cannula insertion also will be estimated from MR images, measuring from the apparent surface of the brain. As mentioned above, there are scaling measurements (cm) on standard MRI which allow for calibration of distance. Due to intraoperative brain shifting once the dura is opened, the insertion distance as measured from the inner calvarial table is likely to be a slight underestimate of the true distance required. Therefore, the insertion distance is best judged from the surface of the brain. The distance from the brain surface to the calvarial inner table will be estimated from MRI and confirmed intraoperatively. Determination of injection site during followup spectroscopy and MRI will be evident from bony signal void from the burrholes as well as neuroanatomical features.

Each site in the brain will receive 150  $\mu\text{l}$  of vector at the standard viral titer. Our reasoning in doing the surgery this way is that subcortical injection of AAV vector at a depth of  $\sim 2$  cm will maximize the spread of vector along tissue planes and allow for absorption into cortical and subcortical cells (superficial and deep), where it may be spread by axonal transport along white matter tracts. The standard viral dose was developed based on our criterion of generating a viral titer  $\geq 10^{12}$  genomic particles per milliliter. At this concentration, 1  $\mu\text{l}$  of vector has a genomic titer of  $\geq 10^9$  particles. The infectious titer is several orders of magnitude lower than the genomic titer, and in a cell-based assay this typically corresponds to  $10^6$ – $10^7$  i.p./ $\mu\text{l}$ . We have found, in an *in vivo* assay involving direct injection to the rodent brain, that 1  $\mu\text{l}$  of vector at this genomic titer of  $1 \times 10^{12}$  can transduce approximately  $2 \times 10^5$  cells (Xu *et al.*, 2001). Thus the volume of 900  $\mu\text{l}$  of vector per brain corresponds to  $\sim 900$  billion genomic particles or approximately  $1.8 \times 10^8$  (or 180 million) transduced cells per brain under optimal conditions. Although this number of cells is much greater than we were able to transduce using the previous gene vector (LPD), it still represents less than 1% of the cells in the human brain. Although we would like to use a higher dose in order to transduce perhaps 10% or more of the cells, we are starting at a conservative dose in order to establish safety. We have tested an equivalent viral load and volume of fluid vehicle in young monkeys, with a brain mass 10% or less of that of humans, to determine wide safety margins. The rationale for the dosage we have chosen is to introduce adequate vector in order to create measurable changes, but also to maintain safety.

The protocol calls for direct cannular infusion of AAV-ASPA into the brain parenchyma, as part of a single-arm study design. The dose will be uniform for all patients and will be based upon a measured viral genomic titer of  $1 \times 10^{12}$  genomic particles/ml. A secondary criterion is that the cell-based assay for infectious titer must be  $\geq 1 \times 10^9$  i.p./ml. The range of usable viral titers will be between  $1 \times 10^{12}$  and  $5 \times 10^{12}$  genomic particles/ml. If the titer is  $> 1 \times 10^{12}$ , then it will be diluted to the standard concentration of  $1 \times 10^{12}$  genomic particles/ml. We have decided, based on practical considerations which limit the length of surgery, that the volume to be delivered will be no more than 900  $\mu\text{l}$ , or 150  $\mu\text{l}$  per injection site. The total dose at  $1 \times 10^9$  genomic particles/ $\mu\text{l}$  will be  $\sim 900$  billion particles.

Neurosurgical administration of AAV-ASPA involves drilling six small burrholes in the calvarium under general anesthesia in order to gain access to defined subcortical regions of the brain with a fine borosilicate catheter attached to a Hamilton-type injection syringe and multi-syringe micropfusion pump. A neurosurgeon will be responsible for placement of the

burrholes and delivery of vector at a rate of 2  $\mu\text{l}/\text{min}$ . We calculate this rate of vector delivery based upon our observation that optimal expression in healthy animals occurs at a rate of  $< 0.5 \mu\text{l}/\text{min}$ . However, balancing our desire to maximize the expression of the vector is our need to keep operative time to a minimum. To maximize safety, we would like to limit the time that the patient is under general anesthesia to 2–3 hr. With this goal in mind, we consider the 150  $\mu\text{l}$  volume per injection site and the time limit for the entire procedure. A much faster flow rate is probably feasible in CD patients due to the increased porosity of their brains, but we do not want to exceed the rate of 4  $\mu\text{l}/\text{min}$  that was performed in animals. The micropfusion pump is adjustable and can accommodate up to six syringes simultaneously. Intravenous mannitol (1.0 g/kg) will be given in order to reduce brain free water and aid in delivery of the vector. The six burrholes will be drilled at the same time before placing any of the catheters, which will be pre-attached to each syringe and pre-loaded with vector. Extra syringes will be kept on hand in case of equipment failure. The vector will be injected continuously at the specified rate, and the catheter will then be left in place for a further 10 min in order to assure adequate tissue penetration. The catheters will be tested immediately before and after withdrawal to ensure proper flow. Each patient will be monitored post-operatively in a recovery room or intensive care unit until he/she is stable to be transferred to the pediatrics or general neurosurgical unit. The total length of hospitalization will be 2–4 days, assuming no post-operative complications occur. At a later time, repeat or escalated dosing may be considered as part of a phase II/III continuation study.

#### ADVERSE EFFECTS SCALES

The safety and tolerability of the study treatment will be monitored in real-time by study investigators by means of adverse event reporting and clinical reports of laboratory and clinical data. Guidelines for toxicity used by the National Cancer Institute (NCI) will be followed to assign a level or severity to any serious adverse event. These data are entered into the database within 48 hr of the time at which results of laboratory tests or clinical examinations become available. The NCI rating scale has been modified to take into account the characteristics of the patient population under consideration. We have made changes to standard NCI grading criteria in view of the subjects' age and underlying neurological status. We have developed a case report form (CRF) for "Modified NCI Toxicity Criteria," subdivided on the basis of the time at which a SAE occurs. There are separate criteria for grading acute ( $< 4$  weeks from gene transfer) and long-term ( $> 4$  weeks from gene transfer) events. We will use the Medical Dictionary for Regulatory Activities (MedDRA) for online adverse events reporting, when available. This reporting system will facilitate the transition to Phase II trials.

##### Level 0 No adverse effects

Level 1 Mild symptoms: responsive to drugs and appropriate supportive measures. For example, mild fever, headache, nausea, diarrhea, seizures, mildly increased intracranial pressure, urticaria or other immune reaction (well-controlled), mild renal complications such as decreased urine output or mild proteinuria, mild cardiovascular complications such as mild hy-

pertension or hypotension, mild respiratory complications including decreased  $PO_2$  and/or mild respiratory distress, other mild symptoms that respond clinically to treatment.

**Level 2 Moderate symptoms:** responsive to drugs and appropriate supportive measures. Moderate signs/symptoms are similar to level 1, except they are moderate in nature.

**Level 3 Severe symptoms:** nonresponsive to drugs and persisting for >24 hr. These signs/symptoms are criteria for pausing the study. For example, cardiac arrhythmia, certain infections, sustained fever with febrile complications, severe intracranial hypertension, severe immunological or anaphylactic reactions, severe seizures, and other severe but non-life-threatening systemic complications.

**Level 4 Life-threatening events.** These signs/symptoms are criteria for stopping the study. For example, high fever for >72 hr after gene delivery that is insensitive to antipyretics and not attributable to other causes; increased intracranial pressure for >48 hr with major sequelae; intractable status epilepticus; coma and other extreme symptoms not attributable to other etiology.

### SAFETY ENDPOINTS

For the purpose of safety monitoring, we have assembled an Independent Data Monitoring Committee (IDMC), an Independent Study Monitor (ISM), and a Medical Safety Monitor (MSM). The IDMC is equivalent to the "Study Monitoring Committee" described in the NIH clinical monitoring guidelines. The safety and tolerability of the study treatment will be monitored in real time by the study investigators by means of adverse event reports and reports presenting laboratory and clinical data. It is required that these data are reviewed and entered into the database within 48 hr of the time at which the results of the laboratory tests or clinical examinations become available. Accrual and toxicity data will be made available to study investigators in concert with the ISM and the study biostatistician on a monthly basis or as needed. All toxicities will be evaluated with the IDMC on scheduled conference calls. Per FDA requirements, all serious, related, unexpected adverse events will be reported to the Institutional Review Committee within 24 hr. The primary safety end point is the development of toxicities of Grade 3 or higher, attributable to study treatment. Secondary safety end points are the development of toxicities of Grade 3 or higher, regardless of attribution, or examination indicating clinically significant deterioration in performance, as defined under NCI criteria (e.g., blood chemistry and hematology tests).

### INTERIM SAFETY ANALYSES

The following analyses will be performed once each cohort has completed the 3-month follow-up visit. Demographic variables (including the proportion of subjects who are gene therapy naive) will be summarized using descriptive statistics. The proportion of subjects experiencing life-threatening toxicities will be tabulated. All diagnoses and signs and symptoms of Grade 2 or greater will be listed. The listings will include each occurrence of the event, when it was reported (calendar date and study week), the severity, and duration. The results of blood, urine, and CSF analysis will be listed and reviewed for

each subject. Vital signs will be listed for each subject and clinically significant changes will be noted. Descriptive statistics reflecting change in NAA levels will be presented by study visit.

### STOPPING GUIDELINES

To ensure that risks to subjects are minimized, subjects will be enrolled into the study in separate cohorts of no more than six subjects each. Following gene transfer on each cohort, further gene transfer procedures on other enrolled patients (cohorts) will not occur until the previous cohort has completed at least 4 weeks of follow-up, or until the primary safety evaluation is done and examined by the IDMC. In this study, a Grade 3 event for a single patient will serve as a pausing criterion for further treatment within a cohort, while a Grade 4 event for a single patient will serve as a stopping criterion if the event occurs within 4 weeks after gene transfer. Any serious adverse event occurring beyond four weeks will be immediately reported to the FDA, IRC, and NIH. If the event is Grade 3 or 4, the investigators in consultation with the above agencies will determine if the episode warrants pausing or stopping the study. For example, infectious meningitis two weeks after gene transfer would qualify as a pausing criterion. However, infectious meningitis six months after gene transfer is most likely environmentally acquired rather than related to the gene transfer surgical procedure, and will be reported and discussed with the FDA, NIH, and IRC, but most likely would not serve as a pausing criterion for the study.

### RISKS OF THE STUDY

The greatest risks of this procedure are those related to the surgical procedure, which include hemorrhage, stroke, anesthetic complications, infection, paralysis, coma, or death. Children with Canavan disease are at increased risk for anesthesia and sedation because of their difficulty in handling secretions, and may have a greater than normal risk of respiratory arrest. Therefore, the risks of surgery may be slightly increased in these patients as a part of their baseline condition. Although routine magnetic resonance testing itself involves no more than minimal risk, there is some risk associated with sedation, which is standard for imaging tests in which patient motion must be minimized while inside the magnet. In addition, the introduction of AAV-ASPA (the gene and gene transfer vector) to the brain carries some risks. Because this is a new experimental treatment, these risks are largely unknown. One such risk is the possibility of an adverse immune reaction, particularly if the gene vector should leak into blood vessels in the brain. None of the patients treated to date have had any serious adverse effects, apart from mild post-operative fever, but an idiosyncratic immune reaction is possible. Pre-clinical animal data in rats and monkeys suggest a lack of toxicity. However, long-term adverse effects are possible. In summary, there are a number of potential risks associated with this study. These risks include post-operative pain or discomfort; nausea; vomiting; fever; infection; hemorrhage; allergic or other immune reaction; and the possibility of permanent neurological damage including seizures, coma, or death. There is also the risk that the procedure may prolong the

patient's life without significantly improving his/her quality of life, or that the procedure may not lead to the anticipated clinical benefit.

### ALTERNATIVE AND FUTURE APPROACHES

There is no accepted treatment for Canavan disease and current management is entirely symptomatic. Gene transfer has the advantage of being the most direct and well-tested experimental approach, which is rationally based upon our understanding of the molecular defect. One possible alternative to gene transfer would be to infuse recombinant enzyme into the brain; however, based on the results of enzyme therapy in children with Tay-Sachs disease (von Specht *et al.*, 1979), such an approach is impractical and unlikely to be successful. More recent results with Fabry Disease (Eng *et al.*, 2001; Schiffmann *et al.*, 2001) suggest that enzyme therapy is feasible for a systemic disease, but remains problematic in the brain due to delivery and diffusion limitations, protein instability, and immune reaction, among other problems. A related approach is the use of recombinant enzyme bioreactors in conjunction with hemodialysis to lower whole-body levels of a toxic metabolite, as has been tried in phenylketonuria and other inborn errors of metabolism (cf. Ambrus *et al.*, 1987; Rutledge *et al.*, 1989); this approach is very expensive, demanding on patients, and may not appreciably affect NAA levels in the brain. Another approach is pharmacologic targeting of the biosynthetic pathways for NAA. Some substances have been shown to decrease NAA synthesis in healthy animals (Baslow *et al.*, 2000); however, these substances are themselves toxic (e.g., ethanol, LSD) or of unproven efficacy (e.g., lithium). Moreover, NAA biosynthesis appears to be downregulated in CD patients as a result of feedback inhibition (Moreno *et al.*, 2001), and further inhibition may not reduce the steady-state levels of NAA unless the degradative pathway is also targeted. Another approach would be to neurosurgically deliver human stem cells to replace the oligodendrocytes that have died as a result of excessive cerebral NAA. However, the process of stem cell differentiation is still poorly understood, and these cells would be prone to cell death unless they were engineered (using *ex vivo* gene transfer) to express high levels of recombinant *ASPA*. Depending on the magnitude of effects observed following viral gene transfer, other approaches may be considered in the future, including modification of AAV to target a greater number of glial cells, concomitant use of *ex vivo* techniques in conjunction with stem cell technologies, or hemofiltration/enzyme bioreactors.

### IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

Previous clinical trials as well as the most recent animal and human data suggest that clinical benefit is possible. Similar gene transfer methods may eventually be utilized in the treatment of other neurodegenerative diseases. We will be looking primarily at safety measures for this study. The collection of baseline clinical data on untreated Canavan disease patients will help us to determine the best efficacy end points for future trials. This is a unique opportunity to collect longitudinal natural history

data on a group of patients affected by this rare leukodystrophy, and to offer them a treatment option at the same time. The implications for gene therapy in the brain are highly significant.

### RISK-BENEFIT ANALYSIS

Although the risks are considered (by most people) to be above minimal, pre-clinical and human data suggest that there is a prospect for direct benefit to the study participants. Patients with Canavan disease will die in the absence of any intervention and arguably, the risks associated with gene transfer are equivalent to the risks which these unfortunate children face in their daily lives. For example, patients with Canavan disease frequently have epilepsy and are at risk for status epilepticus and many serious complications including respiratory arrest. This gene transfer procedure may help with symptomatic relief of seizures and other sequelae of pathologically elevated NAA in the brain. Canavan patients also have difficulty handling secretions and are at risk for aspiration pneumonia as a part of their underlying condition. Because of their difficulty with feeding, they will eventually require a gastrostomy tube and are at risk for possible complications from anesthesia at that time, as well as surgical risks associated with placement of the feeding tube. Therefore, the surgical risk of this procedure could be considered no more than the usual and customary risk that subjects will face in the normal course of events, with the potential in this case for long-term benefit. There may be benefits to other Canavan patients and to society in general, as we learn more about gene therapy through this study. In summary, the risk-benefit ratio clearly favors proceeding with this Phase I study.

### STANDARD OPERATING PROCEDURES (SOP) FOR CLINICAL MONITORING

#### *General considerations*

This section briefly describes the Clinical Standard Operating Procedures (CSOP), the safety monitoring and oversight plan. Because the period of hospitalization presents the highest risk from a safety point of view, we have taken particular attention to ensuring that proper monitoring and oversight is in place during this critical period. While patients are hospitalized, they will be followed daily by the departments of neurosurgery, pediatrics, and neurology. The principal investigators and clinical coordinator will help to coordinate medical staff for the duration of the hospitalization. The clinical coordinator will assure that Source Documents and Case Report Forms are properly documented. The Independent Study Monitor (ISM), Medical Safety Monitor (MSM), and Independent Data Monitoring Committee (IDMC) will provide additional oversight. We have developed an outpatient surveillance system to ensure that long-term adverse events after gene transfer are recognized and documented. After subjects are discharged from the hospital, their progress will be carefully monitored. All subjects will have a post-operative visit at 2 weeks; 1, 3, 6, 9, and 12 months; and at regular intervals thereafter for the life of the subject. The clinical coordinator is responsible for regular communications with families and local physicians in the intervals between

checkups, at which time the subjects will receive full neurological exams, imaging, and other testing. Unlike later follow-up appointments, the 2-week visit will be at home with the subject's local physician. All other follow-up visits are in Camden, NJ and Philadelphia, PA. Follow-up monitoring is described in the "study checklist" Case Report Form, which lists the complete tests and timetable.

#### *Inpatient monitoring plan*

The clinical coordinator(s) will contact the child's local neurologist prior to gene transfer to obtain updated information about the clinical status of the child. The local physician will receive and review the research protocol. The principal investigator is responsible for the organization and guidance of all medical personnel prior to the gene transfer procedure, including obtaining all required pre-operative assessments. Pre-operative orders will include a pediatric consult (i.e., attending physician to see patient prior to surgery and follow daily) and a neurology consult. The clinical coordinator(s) will be responsible for ordering the appropriate laboratory tests. When subjects are hospitalized, the clinical coordinator(s) will contact staff on the pediatrics, neurology, and neurosurgery services in order to confirm that the necessary studies have been performed. Following surgery, patients will stay overnight in the Pediatric ICU with vital signs q15 min by nursing staff with neurological checks. The patient also may be taken directly to the floor, at the neurosurgeon's discretion. The patients will be transferred to the Pediatric floor on post-operative day (POD) # 1 if stable. Routine monitoring will take place by pediatrics, neurosurgery, and nursing including vital signs and neurological checks q4–8h. The clinical coordinator and principal investigators will be responsible for extracting the data from source documents in the patients' charts and completing all necessary Case Report Forms (CRFs). The clinical coordinator will attend the procedures at which blood, urine, and CSF are drawn. He/she will bring the required samples to the Cell and Gene Therapy Center for storage. The principal investigator will conduct daily meetings with the clinical coordinator(s) and study investigators while each study subject is hospitalized. All investigators and the clinical coordinators will carry digital pagers so that they can be reached at any time. If a problem arises, the principal investigator is responsible for informing the co-investigators, clinicians, and relevant regulatory agencies in a timely fashion. E-mail, digital paging, and telephone/fax will be used for this purpose. Patients will be discharged when study data and parameters are met, probably on POD#4.

#### *Outpatient monitoring plan*

After discharge, subjects will be monitored through regular follow-up appointments and by evaluation by their physicians. Close cooperation among study investigators, clinical coordinator(s), study biostatistician, and IDMC will be necessary in order to evaluate and respond to any occurrences of toxicity in a timely manner. Information about the child's hospitalization will be forwarded to his/her personal physicians within 72 hr of discharge. His/her doctor will receive by fax a clinical summary of the hospital course for gene transfer, including surgical notes, summary discharge notes, and other relevant materials. The clinical coordinator and principal investigator will

telephone and email the family and outpatient physicians to assess the continuing clinical status of the patient and to ensure that he/she receives scheduled post-gene transfer studies.

A. After the child is discharged, the clinical coordinator will maintain contact by telephone and/or electronic mail, in order to ensure that appropriate follow-up studies are done. If the child's course is uncomplicated (i.e., no inpatient adverse events), telephone contact with the family will occur at least at the following time points: 7 days, 14 days, and 1, 2, 3, 6, 9, 12 months after gene transfer. Contacts with family will be more frequent if clinical problems arise. These contacts will occur at least 1 week before the patients are scheduled for follow-up testing.

B. At 2 weeks post-procedure, the child will be seen by his/her physician for routine safety monitoring. He/she will receive a complete neurological examination and a brain MRI to rule out edema and occult intracranial bleeding. Routine blood tests will be performed. The clinical coordinator will contact the local doctor at this time in order to obtain information about the outcome of the appointment. Relevant source documents (i.e., neurological report, MRI report, lab results) will be forwarded by e-mail or fax to the clinical coordinator and principal investigators.

C. If the child is hospitalized for any reason after gene transfer, the parents are instructed to have the child's physician contact the principal investigators at once. The clinical coordinator will obtain a copy of the patient's hospital notes and discharge summary and the physician caring for the patient will be contacted directly by the clinical coordinators and principal investigator.

D. When the subjects return for repeat testing, the clinical coordinator will prepare lab slips and provide them to the patients; he/she will accompany the patients while the specimens are obtained and bring specimens to the clinical laboratory for storage. The clinical coordinator will centrifuge blood specimens and will collect sera and plasma in sterile tubes for cold storage.

#### *Measures to facilitate the required follow-up studies at the appropriate intervals*

An electronic calendar will be created for each patient. Each patient's calendar will indicate the time at which the child's parents and physicians must be contacted, when follow-up studies are scheduled, and when results from the follow-up study results should be tracked down in the event that they are not received. The clinical coordinator will be responsible for maintaining the calendar and ensuring that follow-up studies are performed and the results are received. We will assist families with follow-up by scheduling in advance the MRS/MRI studies and the neurological and psychometric testing. We will help families to make necessary travel and lodging arrangements. Regular telephone contacts with families and physicians will provide the opportunity to keep them updated on the study schedule. All follow-up studies will be obtained in accordance with the clinical protocol. The clinical coordinator will keep a digital record of studies performed and when the results were received. A back-up hard copy will be archived and regularly updated. The ISM will ensure that follow-up studies are obtained as stipulated in the clinical protocol.

### *Protocol for review, data entry, and storage of source documents and case report forms*

All source documents (SDs) will be reviewed by the clinical coordinator within 24 hr of their receipt at the Cell and Gene Therapy Center. If the report warrants immediate attention, the Principal Investigator (PI) will be contacted. Otherwise, the data will be abstracted from the SD by the clinical coordinator and entered into an electronic database. The clinical coordinator will place his/her initials and date at the bottom of the source document, then forward the document in a timely fashion to the PI. After review of the SD, the PI will initial and date the bottom of the document, then return the form to the clinical coordinator for placement in the patient's confidential study file. An identical procedure will be followed for monitoring of each Case Report Form (CRF). These will be completed by either the clinical coordinator or PI. The summary CRF log indicates the person designated to complete each form. If the form is completed by the clinical coordinator, he/she will initial and date each form before forwarding the form to the PI for review prior to placement in the patient's confidential study file. The Independent Study Monitor (ISM) will audit SDs and CRFs. The monitor will ensure that all CRFs have been completed and that all SDs have been received according to the schedule outlined in the protocol. The ISM will verify that information from the SDs has been reviewed by the appropriate personnel and that the data have been accurately abstracted and entered into the electronic database. The ISM will review CRFs for completeness, accuracy, presence of appropriate initials and dates.

### *Reporting of Serious Adverse Events (SAEs)*

All SAEs will be reported within 24 hr to the Principal Investigator, the FDA, NIH/OBA (Office of Biotechnology Activities), the IRC/IBC, and OPRR (Federal Office of Protection from Research Risks). All SAEs will be reported, regardless of whether or not they are thought to be related to the gene transfer. The immediate telephone report to the above agencies will be made by the PI. A follow-up written report will be prepared in a timely manner by the clinical coordinator, in consultation with the PI and physicians caring for the child. An SAE Case Report Form has been developed for reporting, following OBA and FDA guidelines. Follow-up reports will be forwarded to the above agencies, as appropriate.

### *Criteria for evaluating patient status after gene transfer*

The criteria to be used in evaluating the research subjects are severalfold. To begin with, acute toxicity will be assessed on the basis of the "Modified NCI Common Toxicity Criteria." On an inpatient basis, the patients are assessed by study clinicians in the hospital. On an outpatient basis, the Principal Investigator and clinical coordinator are responsible for continued care, in coordination with outpatient physicians.

### *Independent Data Monitoring Committee (IDMC)*

This group will provide oversight and counseling on whether the dual study goals of safety monitoring and data collection are being met. IDMC shall consist of clinicians, scientists, ethicists, and data management experts. The committee is orga-

nized by the principal investigator, who will sit ex officio on the committee. IDMC will receive periodic updates at the same time as the FDA and IRC safety reports, and will confer on the proper conduct of the clinical trial. IDMC will review and approve source documents and case report forms, as well as any protocol amendments or other significant changes, and will make recommendations for additional auditing procedures. IDMC will receive reports from the clinical coordinator and principal investigator on a regular basis, in order to assess the adequacy of the study protocol, including data collection and safety reporting features.

The IDMC will be independent of oversight from the study investigators, but will work closely with them to modify the protocol, should the need arise. Reports summarizing clinical and laboratory toxicities, along with all adverse event forms for events of Grade 3 or higher, will be sent to IDMC members. Each member will review toxicity data and complete and submit a statement indicating whether he/she has concerns or questions. Following report submissions by the PI, if there is a quorum then the committee is convened by conference call or web conferencing (or other means) to discuss relevant issues concerning the case report forms or the study protocol, and will make suggestions. This body has the power to consult with the study sponsor and co-investigators and any other relevant parties to make recommendations. The IDMC will be convened at least twice yearly by internet conferencing and all hearings will be open to members, investigators, and the Sponsor.

The IDMC will make recommendations on whether the study should proceed as planned or be modified or terminated. In their review of interim safety data, the IDMC will be guided by the following principles regarding toxicities possibly or definitely attributable to study treatment. The IDMC will be promptly consulted by the Principal Investigator if (1) any subject in a cohort experiences a Grade 4 or fatal toxicity; (2) two subjects within a cohort experience a Grade 3 toxicity of any attribution. Additionally, although the primary safety analyses will occur at three months, toxicities will be monitored after three months and throughout the study period. If the IDMC determines that there are treatment-related toxicities (occurring at any time) that may compromise subject safety, it will recommend whether the study should be modified or terminated. The IDMC will also consider the completeness of the data set in deciding whether to recommend the modification or termination of the study, i.e., if one or more subject in any cohort has failed to complete all the protocol-specified follow-up evaluations, or if the follow-up data are missing or uninterpretable for any reason, the IDMC will be consider whether the study should be modified or terminated. Because this is primarily a safety study, the safety outcomes should form the basis for modification or early termination of the trial. Since lack of evidence of treatment activity may itself be a safety concern, the IDMC may also consider exploratory response variables.

### *Responsibilities of the study sponsor*

Following the 3-month review period for a given cohort and at regular intervals thereafter, the FDA will receive from the Sponsor a complete report on SAEs and safety monitoring. The Sponsor will submit periodic updates, will inform the FDA of any serious adverse event (SAE), and in the event of any un-

foreseen complications, will communicate with FDA officials after patients undergo gene transfer. The Sponsor will inform co-investigators and regulatory bodies of any departures from protocol in a timely fashion, and shall maintain proper oversight and direction of the clinical trial at all times. The Sponsor is responsible for the safe and ethical conduct of the clinical trial, including meeting all of the reporting guidelines of the IRC, FDA, and NIH. The Sponsor shall appoint a Medical Safety Monitor (MSM) who shall be independent of the physicians who provide care to the patients; this person will meet with the Sponsor to ensure that Good Clinical Practice is being met. If this monitor has concerns, it is the responsibility of the Sponsor to contact appropriate regulatory bodies and the IDMC.

#### *Communications with the Institutional Review Committee (IRC)*

The IRC will receive periodic updates from the Principal Investigator and will be informed of any protocol modifications. The IRC will be informed immediately of any SAEs or events that could affect patient safety or the integrity of the clinical trial. These events include, but are not limited to, changes in medical knowledge or clinical practice, changes in study personnel (e.g., clinical coordinator, co-investigators), or any other unforeseen complications.

#### *Independent Study Monitor (ISM) and Medical Safety Monitor (MSM)*

The ISM is appointed by the Principal Investigator (PI) to audit the study and document any problems with the protocol or with clinical monitoring. Prior to the gene transfer procedure, the ISM will ensure that eligibility criteria (as defined in the Investigator's Brochure) are met; that consent forms are completed and signed appropriately; and that CRFs prior to gene transfer are appropriately completed and the required pre-gene transfer clinical evaluation studies have been performed. During the hospitalization, the ISM will audit patient records with the clinical coordinator to ensure that the clinical protocol is followed. The ISM will ensure that any serious adverse events have been reported according to the study serious adverse event reporting protocol, based on FDA and NIH guidelines. After gene transfer and the child's discharge from the hospital, the monitor will review source documents and case report forms, ensuring adherence to the protocol in terms of follow-up studies at the appropriate time intervals; accuracy in the completion of the case report forms; and accuracy of data entry into the study database. The ISM will ensure that any changes in the protocol receive approval of the IRC and FDA before implementing the modification, with the exception of a modification necessitated by an emergency life-threatening situation.

The MSM is appointed by the Sponsor in order to maintain independent clinical oversight over the conduct of the trial and to advise on any additional measures that need to be taken; he/she will receive copies of all adverse events reports and also will receive copies of detailed hospital discharge notes from the clinical coordinator. The MSM is a physician who will oversee the serious adverse event reports and other case report forms for submission to the FDA, the IRC, the NIH, and other bodies; will help to identify trends in preliminary data; and will raise safety concerns as appropriate. He/she will communicate regularly with the PI and the Sponsor.

## PRODUCTION OF THE CLINICAL GENE VECTOR

### *Production of the orphan drug product—background*

The gene vector (Fig. 1) will be produced at the UNC Gene Therapy Center, Human Applications Laboratory. Dr. R.J. Samulski will oversee production of clinical lots of AAV for I.N.D.-9119. The UNC Gene Therapy facility was established in 1993 and participates in a number of grant-supported collaborative efforts. The Human Applications Laboratory was constructed in 1996 and is dedicated to the production of gene vectors and other biological reagents for investigators with FDA-approved clinical protocols.

### *Production of the orphan drug product—raw materials*

Recombinant AAV is produced by transfection of a human embryonic kidney 293 (HEK 293) cell line that is stably transfected with the adenovirus serotype 5 E1A gene. Vials of HEK 293 cells will be obtained from a certified Master Cell Bank. Large-scale plasmid production and purification will be performed by the NIH-sponsored Coriell Institute for Medical Research (Camden, NJ) in facilities directly neighboring the Sponsor at UMDNJ. Coriell provides many preparative and diagnostic nucleic acid and molecular biology services, all subject to extensive quality controls. The QA/QC unit at UMDNJ and Coriell will closely communicate with the production facility staff at UNC in the selection and shipping of all raw materials to ensure that only materials suitable for rAAV production enter the human applications laboratory.

### *Production of the orphan drug product—methods*

We use a modified version of previously described protocols for generating clinical grade rAAV vectors (Ferrari *et al.*, 1997; Xiao *et al.*, 1998).

### *Quality control assays*

Quality Control (QC) testing includes an assessment of vector purity and identity. A silver stain protein gel will be run to assess AAV purity and cellular protein contamination. As an identity test, a Western blot will be run using anti-VP1,2,3 antibodies to verify the presence of the viral capsid proteins. Quantitative PCR will be performed to assess the quantity of DNA. Other standard testing performed on the final product will include tests for sterility, endotoxin content, and assays to detect adventitious viruses, including replication competent AAV and adenovirus. Bulk harvest from each production lot will be tested for sterility, mycoplasma, and adventitious virus assay, as well as *in vivo* and *in vitro* assays for viral contami-

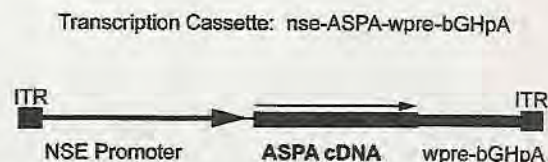


FIG. 1. AAV transcription cassette: The components of each ssDNA unit that is packaged inside the AAV capsid.

nation. The physical particle, genomic particle, and infectious particle titers will be estimated for each production lot using dot blot, quantitative PCR, and Ad-coinfection PCR assays, respectively. All final stocks will be standardized to a titer of  $10^{12}$  genomic particles/ml in PBS. Lot release specifications require the infectious titer to be at least within  $10^3$  of the genomic titer, which in this study corresponds to an infectious titer of  $>10^9$  infectious particles/ml.

#### QA/QC procedures

The QA/QC unit will inspect the production facility on a regular basis to ensure production and QC procedures are being correctly performed. Audits of CROs will also be performed on an annual basis.

#### Shipment of final product

After production and purification of the AAV vector, aliquots are stored in a  $-70^{\circ}\text{C}$  freezer in a quarantined storage lock box. Only lots meeting release criteria will be shipped to the clinical site. Shipment of sealed final product vials will be undertaken by a company specialized in the transportation of biopharmaceuticals. The monitored shipment will be sent with dry ice and temperature monitoring validation. Certificate of Analysis (CoA) and relevant SOPs will accompany each vector shipment for inclusion in patient study files. A vector inventory will be kept at the clinical site. A completed vector CoA and full results of all lot release tests will be submitted to the FDA prior to patient administration. All unused, empty or waste vials from each lot will be returned from the operating room to the QA/QC Unit and retained for the duration of the proposed study.

#### ACKNOWLEDGMENTS

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**INVESTIGATIVE CONSENT FORM**

Patient: \_\_\_\_\_

Department: Surgery (Division of Neurosurgery)

Principal Research Investigator:

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*Institutional Review Committee*

Approval date: May 30, 2002

**CLINICAL TRIAL: AAV-ASPA FOR BRAIN GENE TRANSFER IN CANAVAN DISEASE***Invitation to participate and description of project*

Your child is being asked to participate in a clinical research study. Before you can make an informed decision on whether your child will be allowed to participate, you should understand the possible risks and benefits associated with this study. This process is known as informed consent and means that you will:

- Receive detailed information about this research study;
- Be asked to read, sign and date this document, assuming that you wish to have your child participate. If you do not understand something about the study or if you have any questions, please be sure to ask for an explanation before you sign this form.
- Be given a copy of this signed and dated form to keep for your own records.

Please be aware that your relationship with the clinical investigators bears important differences from your relationship with your child's personal physician. Your child's personal physician individualizes the treatment of your child's specific problem, with the expectation of a benefit to your child. The research physicians treat all subjects under a protocol to obtain generalizable knowledge, and your child may or may not benefit from his/her participation in the study. Please ask the study investigators if you want further clarification of this relationship.

This gene transfer project, an experimental treatment for Canavan disease, involves introducing missing genetic material into the brain that is needed to make the human protein aspartoacylase. The aspartoacylase enzyme, or "ASPA" for short, does not function normally in patients with Canavan disease. The "gene vector" is defined as the *ASPA* gene packaged inside a specially modified adeno-associated virus or "AAV." The procedure will involve direct injections of this gene vector into the brain of each patient. Once in the brain, we expect that the *ASPA* gene will be used by brain cells to make functional protein, using the body's natural mechanisms of protein production.

As you are aware, your child suffers from Canavan disease. This disease is a progressive destruction of the "white matter" of the brain, which leads to disability and premature death. This disease is caused by an error in the *ASPA* gene, which causes the *ASPA* enzyme not to work. There is evidence that the injury to the brain and the neurological impairments are caused by the abnormal build-up of a naturally occurring brain chemical, *N*-acetyl-aspartate (NAA), which is broken down and metabolized by the enzyme *ASPA* in people with a functioning gene.

This consent form gives you detailed information about the study, which members of the research team will discuss with you. The discussion will review all aspects of the research covered in this consent form including its purpose, the pre-surgery requirements, the clinical procedures and list of clinical follow-ups, risks and benefits of the procedure, and alternative treatment options. Once you fully understand the study, you will be asked if you will permit your child to participate. If the answer is yes, then you will be asked to sign this form.

Subject's initials: \_\_\_\_\_

Date: \_\_\_\_\_

### Purpose

Our plan is to deliver the ASPA gene to cells in the brain so that the cells may then produce functional enzyme. This research project is referred to as a Phase I clinical trial, which means that it aims simply to test the safety of the procedure. If sufficient delivery of the gene occurs, the resulting increase in function of the protein made may lower the levels of NAA and thereby slow down the progression of the disease. Previous studies have demonstrated a decrease in NAA after gene transfer. However, this decrease only persisted for several months. Therefore, even if levels of NAA do decrease in your child after gene transfer, the possibility of permanent benefit is by no means assured. Due to the experimental nature of this treatment, the delivery of the gene may not have any beneficial effects. Although this treatment is popularly referred to as "gene therapy," we cannot guarantee that any therapeutic or beneficial result will follow, and it is best to consider this trial a gene transfer experiment.

### Study procedures

The study is designed as an experimental treatment for 18 newly enrolled patients and is divided into three parts: pre-treatment, treatment/surgery, and post-treatment.

*Pre-treatment phase.* Non-invasive brain scans will be performed at Children's Hospital of Philadelphia (CHOP). All magnetic resonance imaging (MRI) and spectroscopy (MRS) will be done at CHOP to facilitate the interpretation of the data. Psychometric testing will be done at Cooper-UMDNJ by a developmental neuropsychologist. Other clinical evaluations will be undertaken by medical staff at Cooper Hospital and by your child's physician.

- a) *Non-invasive brain scans.* MRI is done to provide anatomical information about your child's brain. MRS is done to measure NAA and other chemicals in your child's brain. At least two separate evaluations will be carried out prior to gene transfer. The duration will be approximately 3 hours per session.
- b) *Psychometric testing.* To evaluate motor, cognitive, social abilities. The Mullen Scales of Early Learning and Pediatric Evaluation of Disability Inventory (PEDI) will be used. The duration will be approximately 1 hour per session.
- c) *Neurological evaluation.* To assess baseline neurological ability. A Canavan clinical exam will be performed, with emphasis on the neurological component. The Gross Motor Function Measure (GMFM) and Ashworth spasticity scale will be used. The duration will be approximately 1 hour per session.
- d) *Cerebrospinal fluid (CSF) analysis.* To measure levels of NAA and to demonstrate absence of antibodies against the gene transfer vector. Note that this study will be performed only in those patients who have pre-existing reservoirs in the brain.
- e) *Urine analysis.* The urine will be collected and tested for NAA.
- f) *Blood analysis.* The blood will be tested for NAA and antibodies to the gene transfer vector. These samples also will be routinely screened with clinical chemistry & hematology tests, for drug safety purposes.

Subject's initials: \_\_\_\_\_ Date \_\_\_\_\_

The pre-treatment phase will be longer for some subjects than others because there is a waiting list and only a few subjects at a time can enter the gene delivery phase. For all patients, the pre-treatment tests described above will be performed at least twice but no more than six times. The frequency of testing will be at least two months in between assessments. We will use the information from tests during the pre-treatment phase to help us understand how Canavan's disease affects children who are receiving standard supportive care.

*Surgical phase and gene delivery phase.* This part of the study will be undertaken with 18 newly enrolled patients at Cooper Hospital-UMDNJ. Your child will undergo a surgical procedure in which 6 small holes, each less than 2 centimeters (1/4 inches) in diameter, will be drilled in the skull under general anesthesia in order to gain access to defined regions of the brain (Fig. 2). A neurological surgeon (Dr. Mark Testaiuti) will be responsible for placement of these burrholes and for the delivery of the gene vector through special tubing that is approximately as thin as a coarse human hair. The procedure involves cutting an incision in the scalp to expose the skull, followed by the creation of the holes and the insertion of the tubes. The tubes are connected to a pump that will infuse the vector into the brain at a slow and steady rate. The incision will be sewed up and the holes will be covered by the scalp. The entire procedure should take no longer than 3 hours. The dosing will be standardized for all patients, according to a predetermined standard viral titer and a range of volumes which shall not exceed 0.150 milliliters per burrhole. See the schematic diagram below, showing the locations of the holes in the skull. The total volume of the gene vector to be injected will be less than 1 milliliter (10 drops), and the total dose will be no more than 900 billion gene vector particles. Appropriate postoperative care will be ensured in the recovery room and neurosurgical unit. Your child will recover for 4-6 days at the Children's Hospital at Cooper.

Subject's initials: \_\_\_\_\_ Date \_\_\_\_\_

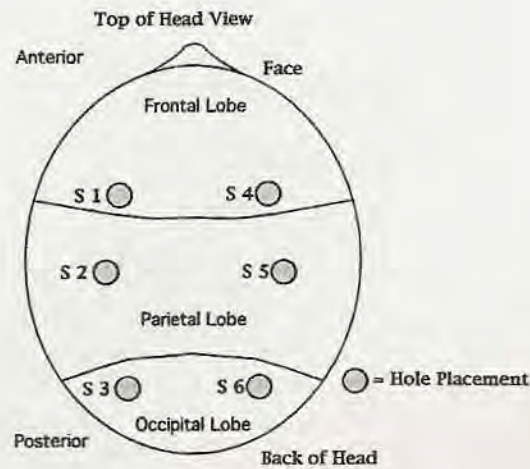


FIG. 2. Schematic diagram: surgical sites of delivery.

*Post-gene delivery phase.* The post-gene transfer follow-up studies will take place over a 24-month period. Please refer to the flow chart of experimental design (see below). This phase will involve the same tests and will take the same amount of time per day as with the pre-testing phase, on a number of separate days. The clinical procedures will be performed in the same location as in the pretreatment phase until the completion of the study. Post-procedure testing will entail all of the tests enumerated above. MRI, neurological assessments, and serum chemistry & hematology will be carried out at 2 weeks post gene transfer. In addition, all assessments done during the pre-gene transfer phase (i.e., MRI/MRS, psychometric testing, neurological examination, CSF levels of NAA, serum levels of NAA, serum chemistry & hematology) will be carried out at 1, 3, 6, 9 and 12 months following the procedure. In addition, MRI, MRS, psychometric testing, and neurological examinations will be performed at 18 and 24 months post gene transfer. The number of procedures carried out at the later time points may be modified if recommended by the principal investigators or study physicians.

### Overview of Experimental Design:

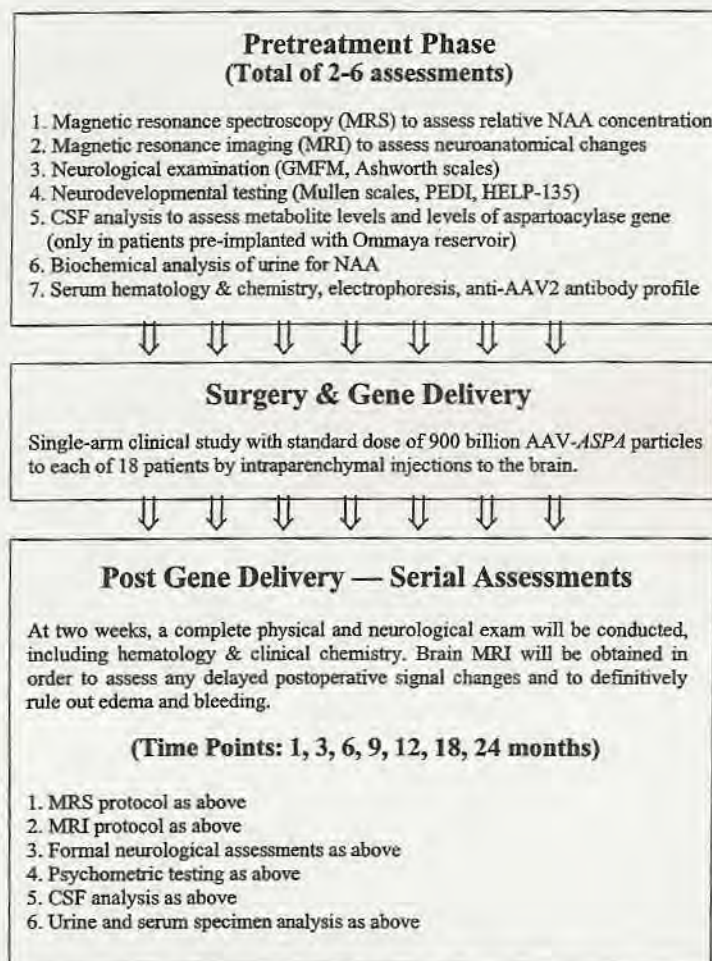
An overview of the experimental design for gene therapy for Canavan disease is shown in Fig. 3.

#### *Risks and inconveniences*

The greatest risks of this procedure are primarily related to the surgical procedure, which may include hemorrhage, brain ischemia, stroke or other permanent neurological injury, anesthetic complications, infection, paralysis, coma, or death. Children with Canavan disease appear to be at increased risk for anesthesia and sedation because of their difficulty handling secretions, and may have a slightly greater than normal risk of respiratory arrest. Therefore, the risks of surgery may be increased in these patients as a part of their baseline condition.

Although routine MRI testing itself involves no more than minimal risk, there is some risk associated with the sedative medicine given to make your child sleep during MRI. Sedation is standard for imaging tests in which patient motion must be minimized while inside the magnet. Oral or intravenous drugs commonly used for deep sedation are chloral hydrate or pentobarbital. The specific sedation regimen will be discussed with you in advance by the nurse or anesthetist. Your child may sleep for up to three hours after these drugs are administered. Although it is very unlikely, there is a small risk of an allergic or other reaction to the sedatives. Only trained personnel will administer the sedative medications and emergency personnel and equipment will be available in the event of a serious adverse reaction. Prior to the gene transfer procedure at Cooper Hospital, a pediatric anesthesiologist also will examine your child and discuss in detail the general anesthesia which will be administered during your child's surgery. As stated above, although it is very unlikely, there is a small risk of an adverse reaction to anesthesia.

In addition, the introduction of the gene and gene transfer vector to the brain carries some risks. Because this is a new experimental treatment, these risks are largely unknown and are difficult to predict. One such risk is the possibility of an adverse immune reaction, which could manifest as fever, rash, swelling, or difficulty breathing, particularly if the gene vector should leak into blood vessels in the brain. None of the patients in the first cohort (of 3 patients) had any serious adverse side effects, apart from mild post-operative fever, but an unusual immune reaction is possible. Pre-clinical animal data in rats and monkeys suggest a lack of acute toxicity, and the FDA approved this study with the expectation that it is reasonably safe to proceed in humans. However, the FDA has informed us of animal studies carried out by another group, which suggest that long-term adverse effects such as tumors are possible. Those pre-clinical experiments were different from this clinical study in a number of im-



**FIG. 3.** An overview of the experimental design for gene therapy for Canavan disease is shown in Fig. 3.

portant respects: the scientists used AAV expressing a gene other than *ASPA*; the AAV vector was administered using different methods and to a different anatomical location; and experimental animals were treated with continuous, very high doses of AAV. Some of the mice from that study developed tumors. It should be noted that the mice were injected with AAV through a systemic vein, and none of the mice in question received AAV injections in the brain. While it is unclear whether this tumor development was related to the use of AAV, you should be aware of these findings.

We have tested the AAV-*ASPA* gene vector in a group of 11 young monkeys. These one-year old monkeys showed no evidence of acute toxicity. One additional primate died at surgery due to hemorrhage. The remaining primates were followed for three or more months, and demonstrated no abnormalities on their clinical examination. Three monkeys were kept alive for long-term analysis. Full autopsy was conducted on 8 animals and was essentially normal, although one had enlargement of the spleen and pelvic lymph nodes, suggesting an inflammatory reaction. This reaction may have resulted from many causes unrelated to gene transfer, but it is possible that it is related to gene transfer. Microscopic examination of the brain showed mild pathological changes in some animals (atypical cells or "astrogliosis"). This finding was also found in the control (untreated) animal. It is thought that astrogliosis was related to inflammation caused by the surgical procedure, and is not an unexpected finding. One animal (out of 8 autopsied) did demonstrate an atrophy or loss of brain tissue. The pathologist attributed these findings to freezing artifact, or structural changes that occurred when the brain tissue was prepared for study. However, it is possible that these changes were partly a result of gene transfer.

The AAV vector has gone through extensive sterility testing. It has been tested for the presence of contamination by all known viruses including herpes simplex virus and HIV. The FDA has approved the sterility testing used in the first cohort of patients and for subsequent groups of patients. Because the gene vector is devoid of viral genes and also requires a helper virus for replication, there is no chance of reversion to a self-replicating or "live" virus. Moreover, the AAV vector is non-pathogenic and is not associated with any human disease.

As discussed above, your child will have several teaspoons (approximately 4–6 cc) of blood withdrawn at the stipulated time-points. There are nominal risks associated with routine drawing of blood. These risks include pain as the needle is inserted, bleeding, and bruising around the site of the blood draw. If your child has a pre-existing Ommaya reservoir, you have been told that your child also will have cerebrospinal fluid (CSF) drawn at the stipulated time points. The amount of CSF collected will be approximately 3 teaspoons. Certain risks may be associated with the CSF collection, which include discomfort as the needle is inserted, bleeding, and infection.

In preliminary studies in two children in New Zealand and in 14 children in the USA using a different gene vector, the *ASPA* gene and the previous delivery method were relatively well-tolerated. The adverse effects associated with the delivery of the first-generation vector were transient fever, febrile seizures, and nausea. Based on additional data we have collected in humans, it is thought that the AAV vector causes less side effects than the previous vector. In summary, there are a number of risks associated with this study that you should be particularly aware of. These risks include post-operative pain or discomfort; nausea; vomiting; seizures; fever; infection; hemorrhage; allergic or other immune reaction; and the possibility of permanent neurological damage including seizures, coma, or death.

### *Benefits*

This gene transfer procedure may result in no benefit, meaning a complete absence of improvement in your child. There is also the risk that the procedure may prolong your child's life without significantly improving the quality of his/her life. It is possible that your child may not experience any positive effects at all. Apart from any benefit that may be imparted to your child, there may be a benefit to society in expanding our knowledge about gene transfer, or to other Canavan patients who may benefit from knowledge gained. Any information obtained during this study that may be important to the health of your child will be shared with you and at your request, it will be shared with your physician.

### *Concomitant medications*

The subjects enrolled in this trial may continue their standard course of medications.

### *Alternative treatments*

At present, there is no accepted treatment or cure for Canavan disease. This study is the only approved clinical trial in the U.S. or Europe for Canavan disease. Current management is to treat symptoms, rather than the underlying cause of the disease. Such palliative treatment is only temporary. Canavan disease has always been a fatal childhood disease.

### *Confidentiality*

Care will be taken to preserve the confidentiality of all medical information. A record of your child's progress during this study will be kept in a confidential file at Cooper Hospital. The confidentiality of any computer records will be carefully guarded and no information by which your child can be identified will be released or published. Your child's records, with regard to participation in this study, may be subject to review by the appropriate offices of Cooper Hospital, and by your insurance carrier if necessary. In addition, the following governmental agencies or organizations may request and will be given access to records of the participants in this study: Food & Drug Administration (FDA) and National Institutes of Health (NIH).

### *Compensation in case of injury*

In the event of physical injury or illness of your child as a direct result of the experiments, treatment(s) and/or procedure(s) used in this investigation, comprehensive medical and/or surgical care (including hospitalization) is available to your child. Cooper Hospital cannot assure that this medical care will be provided without charge. All or some of the costs incurred for the care of your child may ultimately be your responsibility. You may be responsible for the costs related to any illness of your child or unanticipated complications as a result of the surgery or hospitalization. Where applicable, the hospital reserves the right to bill third party payers for services your child receives. Cooper Hospital will not provide you or your child with any compensation for such injuries. Please contact Dr. Carolyn Bekes for further information on compensation in the event of injury.

### *Economic considerations*

Neither you nor your child will receive any financial benefits or inducements to participate in this study. The on-going expenses particularly as they relate to the costs of travel, accommodation, hospitalizations, surgical procedure, pre- and post-treatment MRI, spectroscopy, neurological examination, psychometric testing, laboratory studies, and other possible costs may be your responsibility. Please note that these costs may be substantial. The surgeon and anesthesiologist will waive their customary fees for the surgical procedure, and some of the costs may be covered by your health insurance. However, any costs that are not covered by your insurance are your responsibility. There is also the possibility of funding from public grant money and private research foundations. However, we cannot guarantee that this funding will be available. In summary, you are responsible for the full cost of the procedure, hospitalization, pre-gene transfer and post-gene transfer studies, and the costs of travel and accommodations necessary to obtain these studies.

### *Questions*

We have strived to make this form as accessible and easy to understand as possible. However, we have used some technical terms in this form. Please inquire about anything that you do not fully understand. Please review and consider this research and consent form carefully, for as long as necessary, before you agree to participate. If you have specific questions about the participation of your child as a research subject, you may call Dr. Mark Testaiuti at (856) 342-2270. If you have any questions about the rights of research subjects, you may call the IRC office at Cooper Hospital.

If you believe that your child has suffered any injury as a result of participation in this study you can contact Dr. Mark Testaiuti (856) 342-2270 or Dr. Carolyn Bekes (856) 963-3835. They can review the matter with you, identify resources that may be available to you, and provide further information as to how to proceed.

### *Voluntary permission and subject withdrawal*

You voluntarily permit to your child's participation in this research investigation. You have been told what your child's participation will involve, including the possible risks & benefits of his/her participation. The participation of your child in this research project may be terminated at any time at the study physicians' discretion, for any medical reason(s).

You have been told that you may refuse your child's participation in this investigation or withdraw your permission and discontinue your child's participation in this study without penalty and without affecting your child's future care or you're his/her ability to receive alternative medical treatment at Cooper Hospital.

In the event that you withdraw your child from the study after gene transfer, the study physician will ask your permission to continue to follow-up the health status of your child, including obtaining records from your child's private physician and records of any hospitalizations that your child may have had after gene transfer surgery. This will enable the study physician and FDA to monitor the health status of all subjects after they have received gene transfer.

### *Autopsy*

In the event of your child's death during the course of the study, you will be asked to permit his/her autopsy in order to determine the cause of death. In the event of your child's death after the formal conclusion of the study, we also request that you make your child's remains (brain) available for autopsy, so that we may learn more about the effects that the gene transfer had on your child. This information on the effects of gene transfer will be made available to you.

### *Waiver of legal rights statement*

By your agreement to your child's participation in this study, and by signing this consent form, you also have been told that you are not waiving any legal rights. You also affirm that you have read and received a copy of this signed consent form.

### **SIGNATURES:**

\_\_\_\_\_  
Name of subject)

\_\_\_\_\_  
Signature (Mother)/Date

\_\_\_\_\_  
Signature of Clinical Investigator/Date

\_\_\_\_\_  
Telephone Number

\_\_\_\_\_  
Witness/Date

\_\_\_\_\_  
Signature (Father)/Date