

NIH OBA-RAC

Update and Discussion of Human Gene Transfer Protocol #0312-619

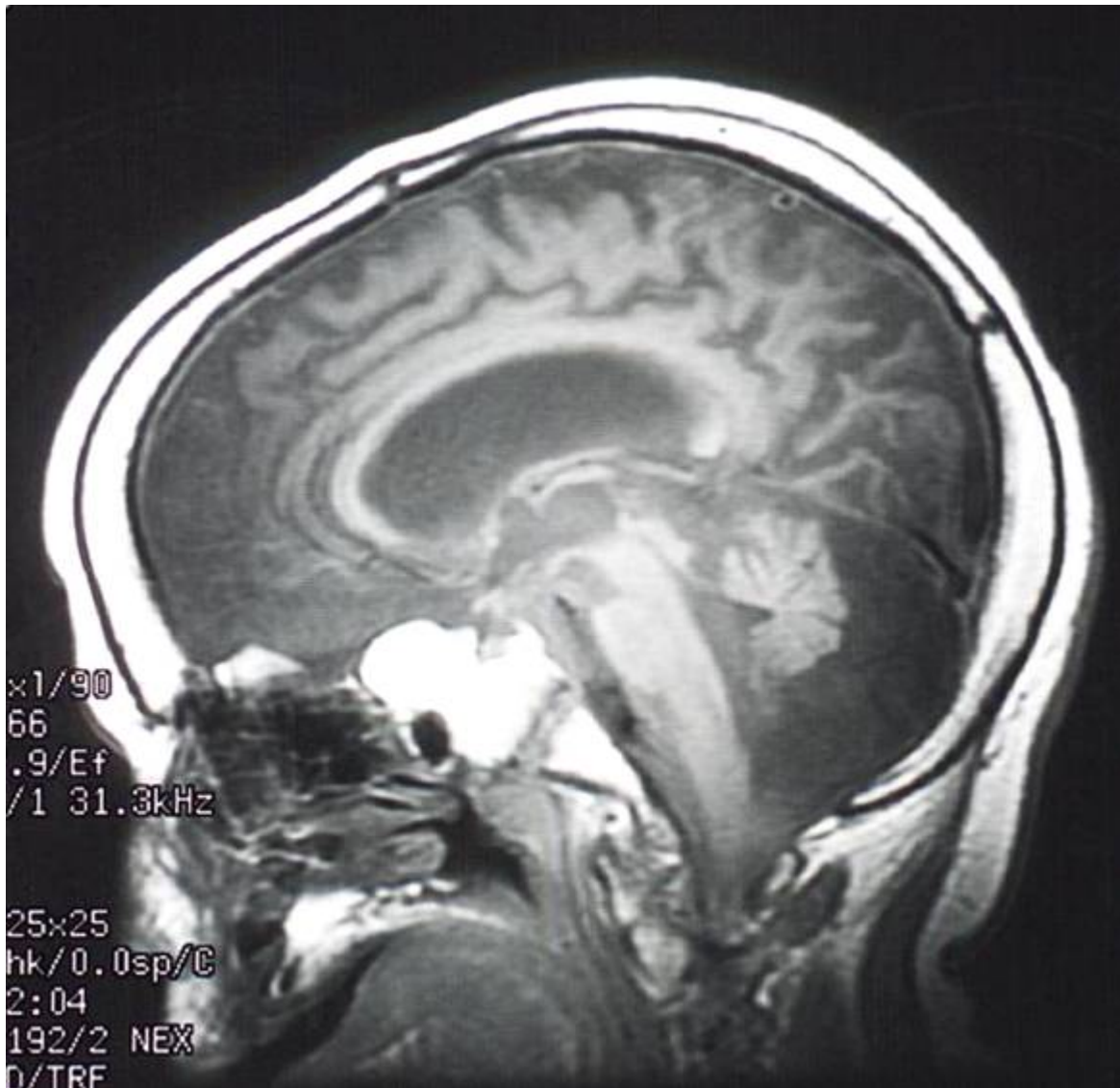
Administration of a Replication-Deficient Adeno-Associated Virus Gene Transfer Vector Expressing the Human CLN2 cDNA to the Brain of Children with Late Infantile Neuronal Ceroid Lipofuscinosis

R. Crystal

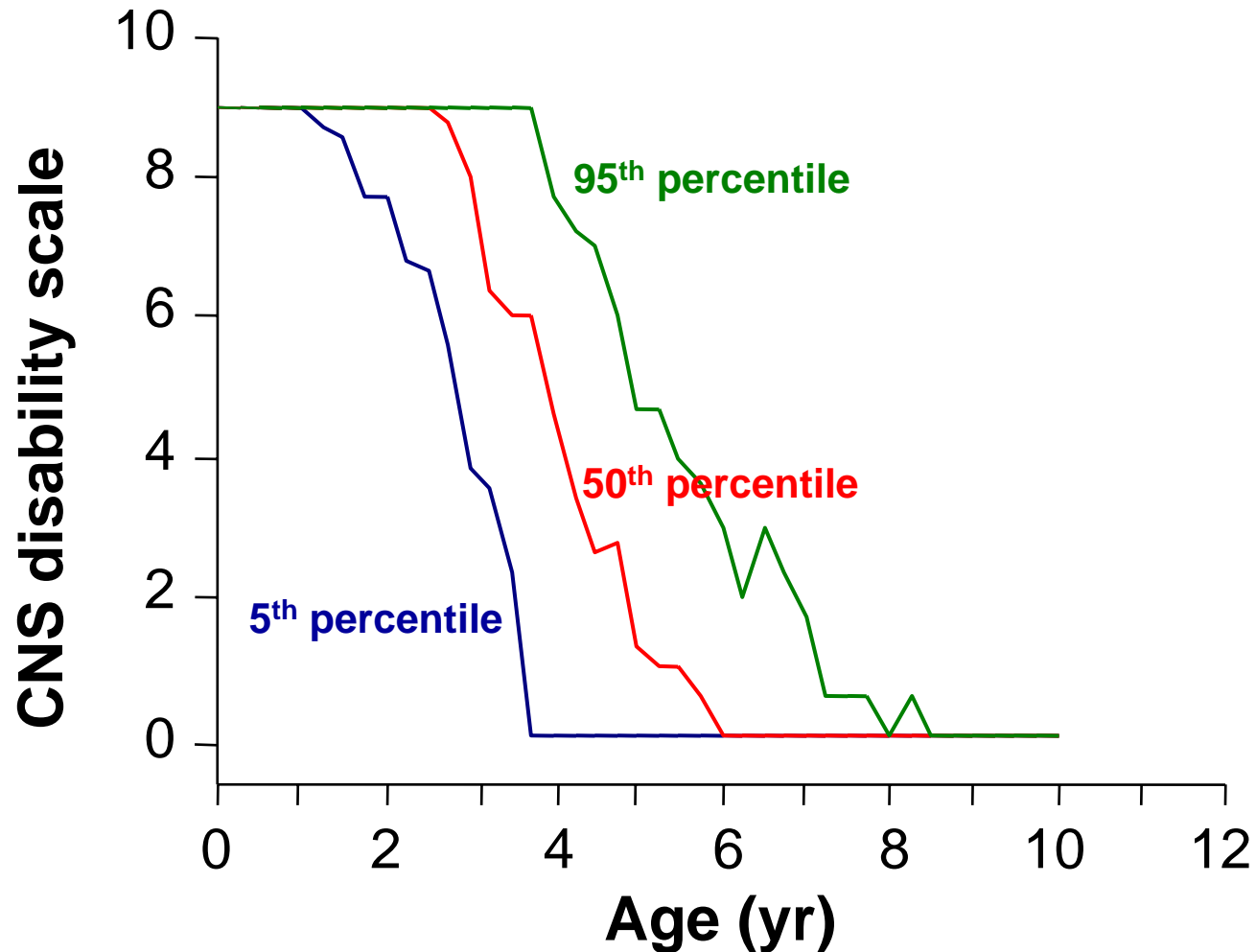
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Late Infantile Neuronal Ceroid Lipofuscinoses (LINCL)

- Lysosomal storage disorder
- Affects 1 in every 2 million births
- Autosomal recessive
- Children are healthy at birth and develop normally until the onset of the disease at ages 2-4
- Cognitive impairment, visual failure, seizures, and deteriorating motor development which leads to a vegetative state and early death by ages 8-12

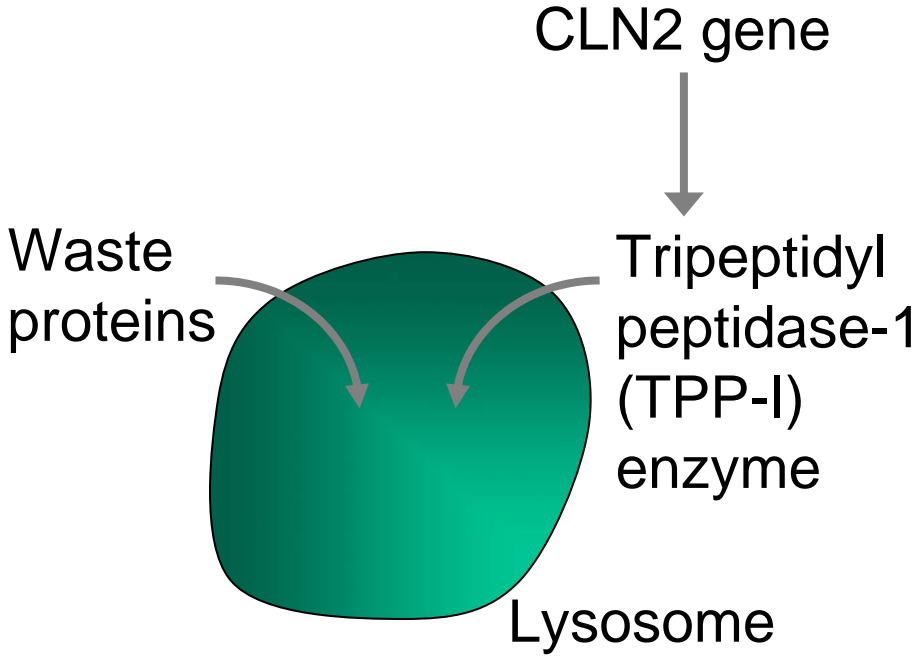
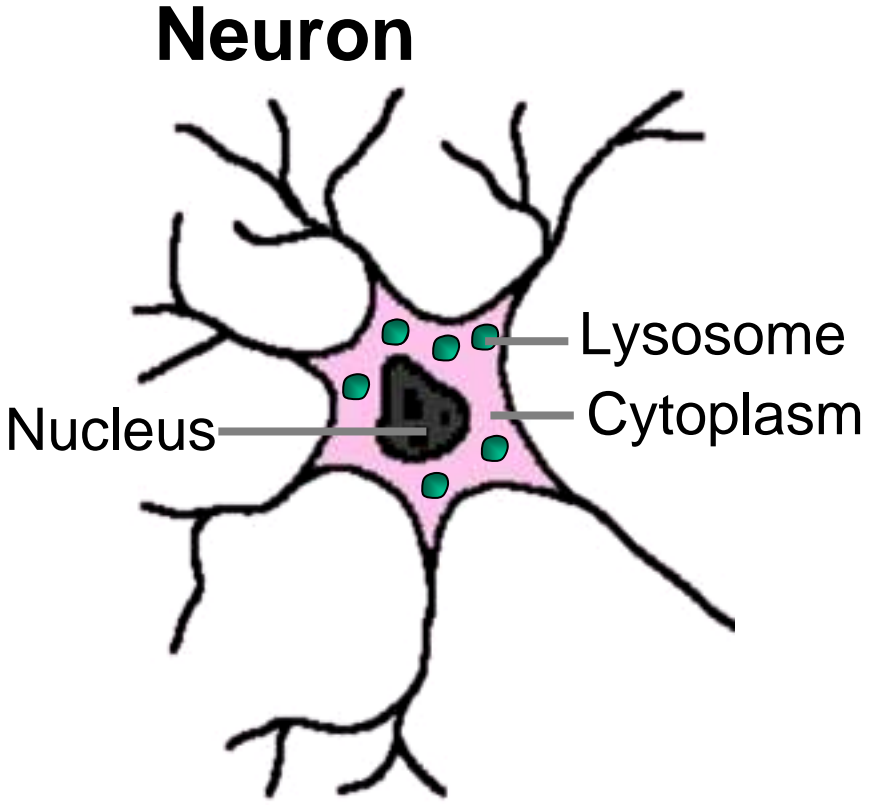


Progression of LINCL Based on the CNS Clinical Rating Scale

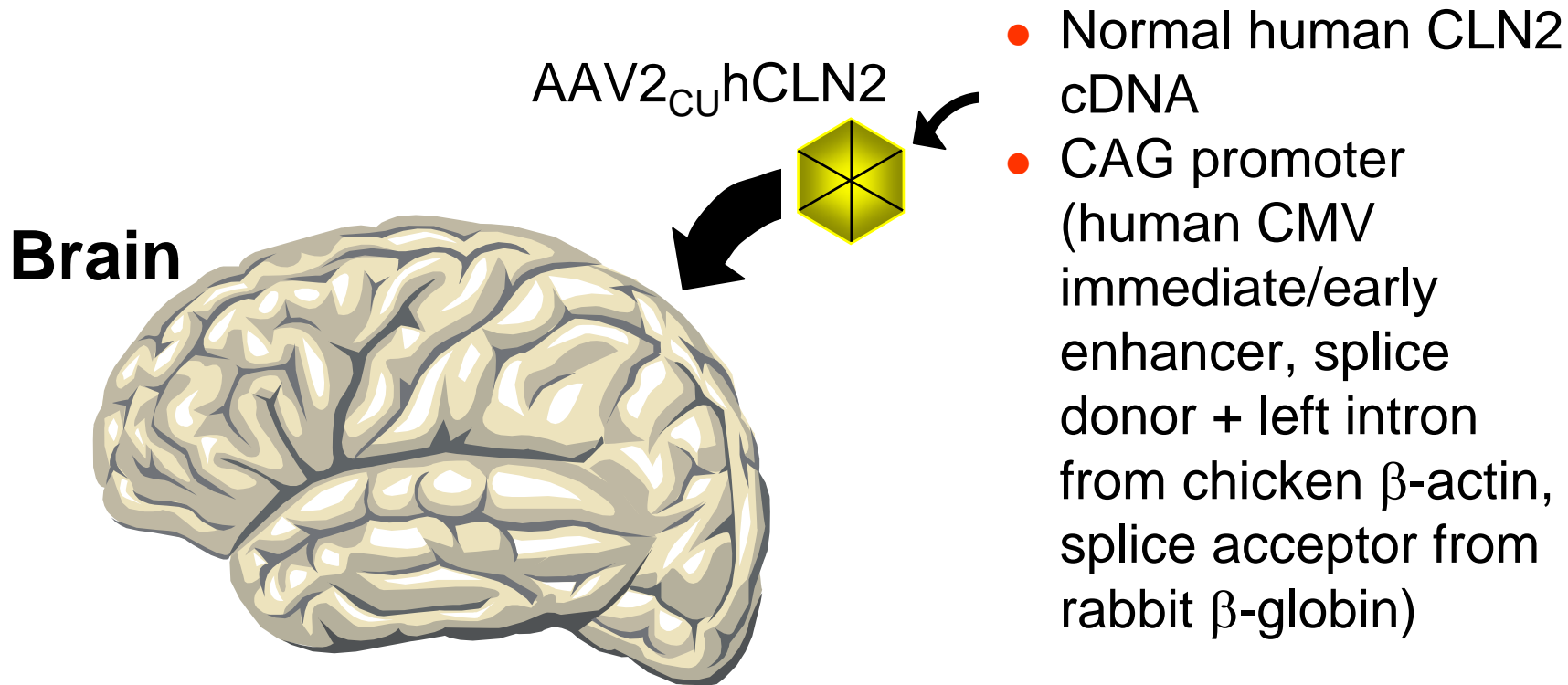


* Modified from Steinfeld et al, Am J Med Gen 2002; 112: 347-354

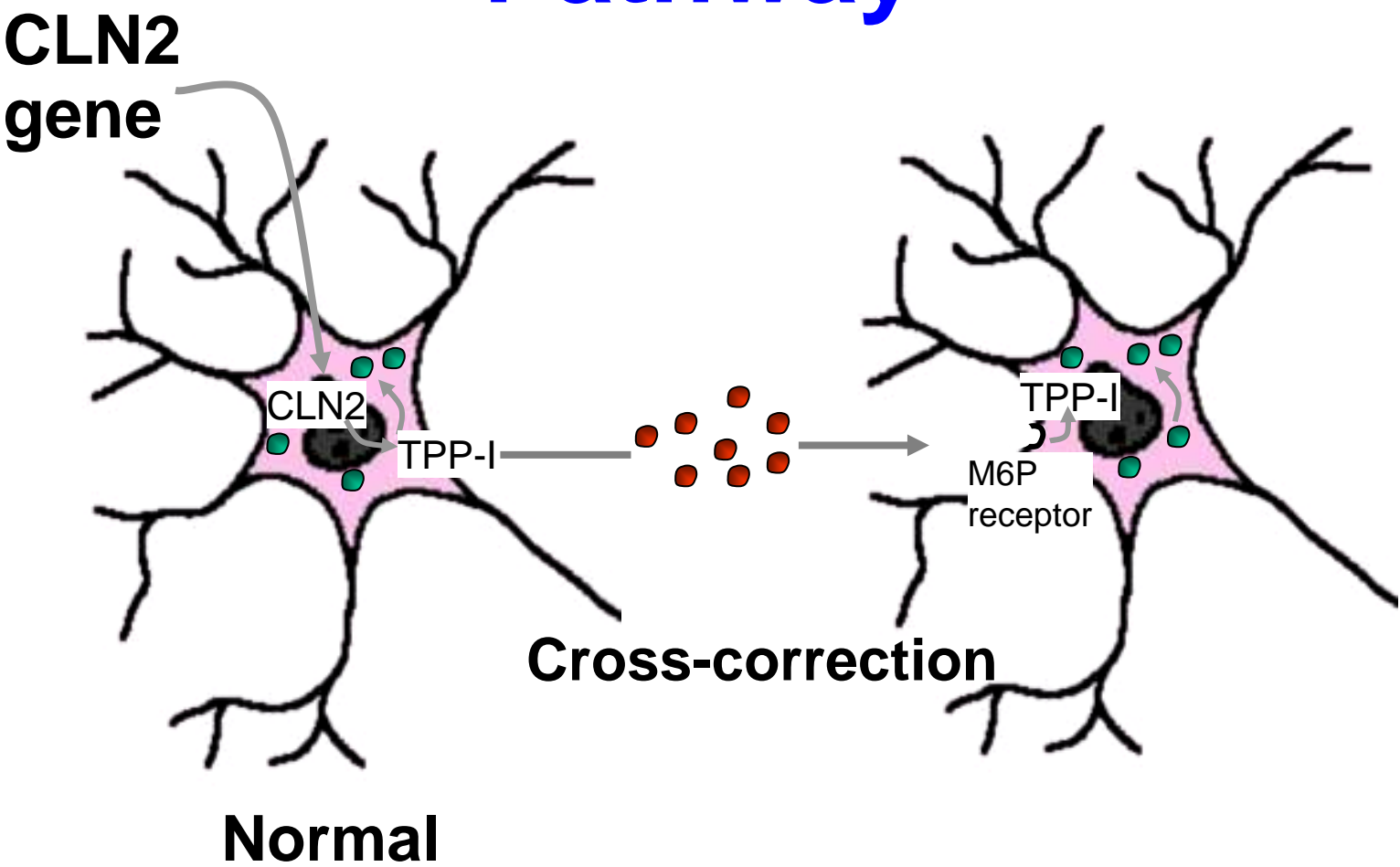
LINCL Is Caused by Mutations in the CLN2 Gene



Gene Transfer Strategy to Treat LINCL

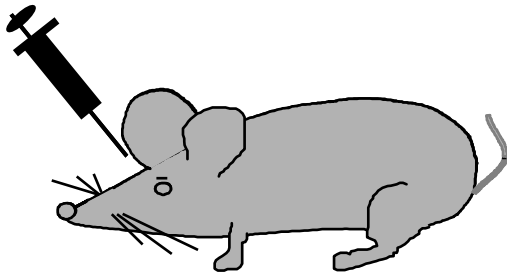


Cross-correction of CNS Cells via the Mannose-6-phosphate Pathway



Time Course of Gene Product Detected in Rat Striatum Following AAV2_{CU}hCLN2 Gene Transfer

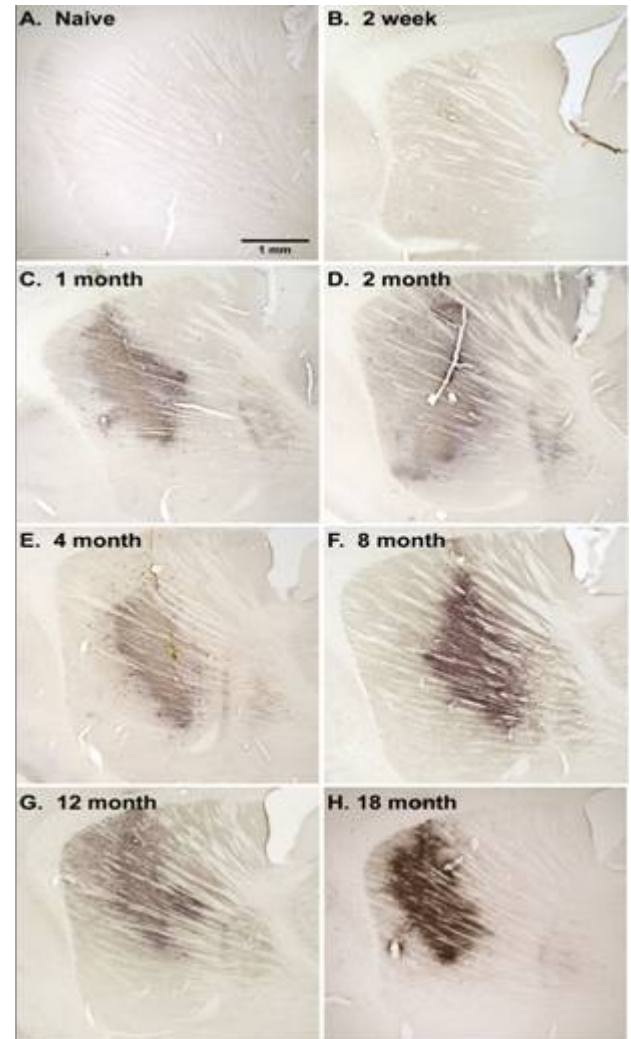
- AAV2_{CU}hCLN2
10¹⁰ particle units in 1 μ l



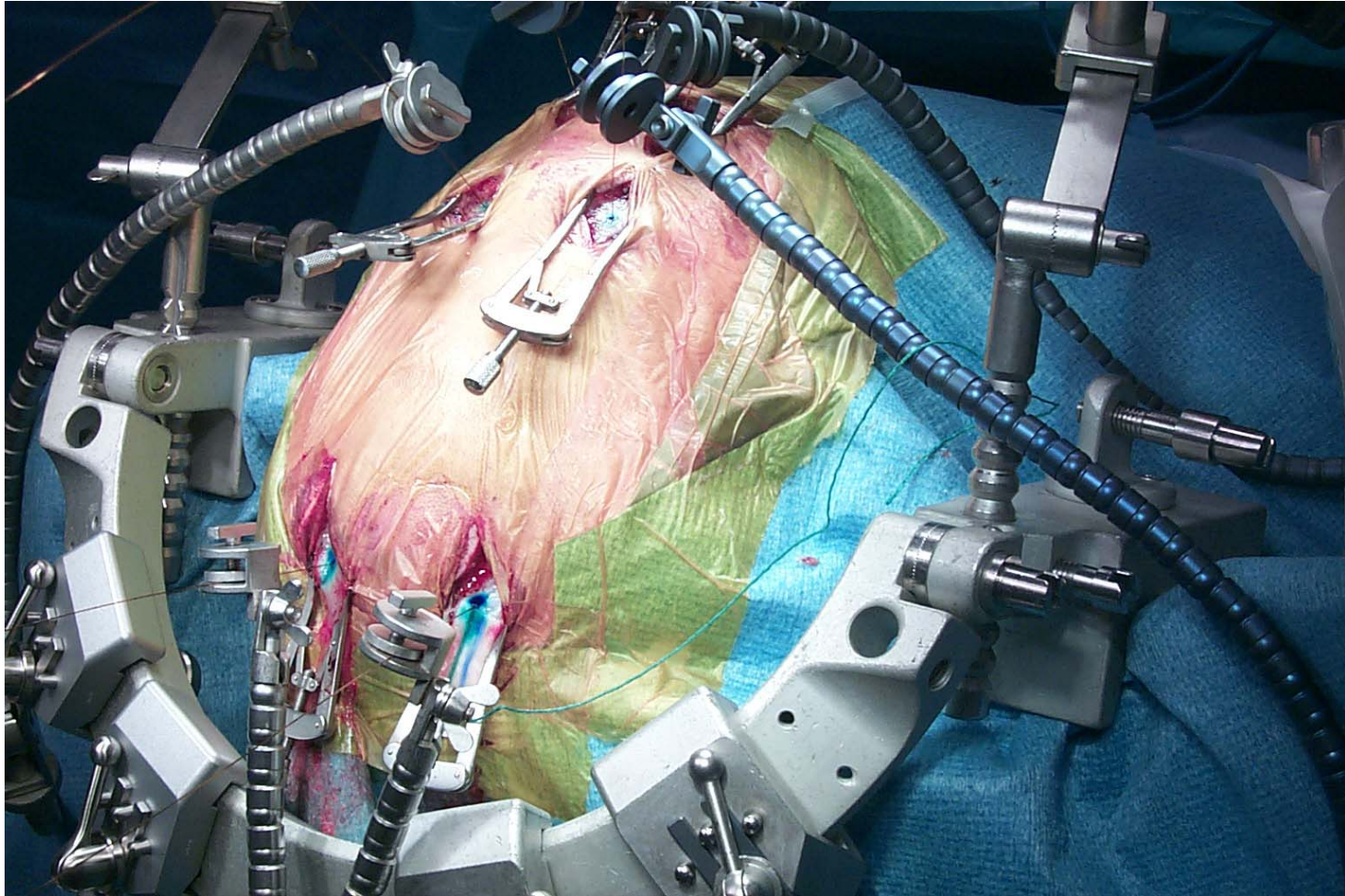
0 – 18 mo

Evaluate

- TPP-I expression by immunohistochemistry



Burr Holes, Frame and Catheters



Current Status of Clinical Study

Subject (screening study #)	Age at therapy	Sex	Genotype	LINCL rating	AAV2 _{CU} hCLN2 dose (particle units)	Status
1	9	M	G3556C/ T3016A	3 (severe)	3.6x10 ¹²	Stable, 9.5 months
2	9	M	G3556C/ G3085A	3 (severe)	3.6x10 ¹²	Stable, 8.5 months
3	6	M	G3556C/ G3085A	3 (severe)	3.6x10 ¹²	Stable 7.5 months
6 ¹	8	F	IVS5-1 G>C homozygote	3 (severe)	3.6x10 ¹²	Discharged 7 days post-therapy, developed status epilepticus day 14, died day 49

¹ Screening study # 6; therapy study # 4

II. Specific Questions Relating to AEs and Death of Subject 4

- Epilepsy scores at enrollment and comparison with others
- Timing of last generalized seizure
- Comparison of sites of administration
- Operative and post-op management (intubation/ventilation, post-op medications/doses)
- Use of mannitol

Batten Screening Study Subject Characteristics

Subject	Age	Sex	Genotype	Epilepsy score	LINCL rating ²	Screen date	Eligible?	Vector	Note
1	9	M	G3556C/ T3016A	3	3 (S)	3-18-04	Y	Y(6-1-04)	Stable 9.5 months post vector
2	9	M	G3556C/ G3085A	3	3 (S)	3-21-04	Y	Y(6-22-04)	Stable 8.5 months post vector
3	6	M	G3556C/ G3085A	3	3 (S)	3-21-04	Y	Y(7-27-04)	Stable 7.5 months post vector
4	13	F	C3670T/ T4396G	3	3 (S)	3-28-04	N	—	Kyphoscoliosis, restrictive lung disease; died 8-28-04
5	6	M	G3556C/ C3670T	3	5 (Mod)	4-4-04	Y	—	—
	7			2	2 (S)	3-6-05	TBD	—	—
6 ¹	8	F	IVS5-1G>C homozygote	3	3 (S)	8-18-04	Y	10-5-04	Status epilepticus day 14 post-vector, died day 49 post-vector
7	6	F	IVS5-1G>C homozygote	3	3 (S)	8-19-04	Y	—	—

¹ Screening subject 6 = therapy subject 4.

² Rating scale: 0-4 severe (S), 5-6 moderate (Mod), 7-8 mild (Mi), 9 normal (N).

Batten Screening Study Subject Characteristics (2)

Subject	Age	Sex	Genotype	Epilepsy score	LINCL rating ¹	Screen date	Eligible?	Vector	Note
8	6	M	IVS5-1G>C Homozygote	3	4 (S)	9-12-04	Y	—	—
9	6	M	G3556C/ T4383C	3	4 (S)	1-6-05	Y	—	—
10	5	M	C3670T ²	3	5 (Mod)	2-10-05	TBD	—	—
11	5	F	IVS5-1G>C Homozygote	3	5 (Mod)	2-24-05	TBD	—	—

¹ Rating scale: 0-4 severe (S), 5-6 moderate (Mod), 7-8 mild (Mi), 9 normal (N).

² 80% of mutations assessed; likely a rare mutation on the other allele; TPP-I levels are low, diagnostic of LINCL.

Epilepsy Score of Vector Subject 4 (Screening Study Subject 6) and Timing of Last Generalized Seizure

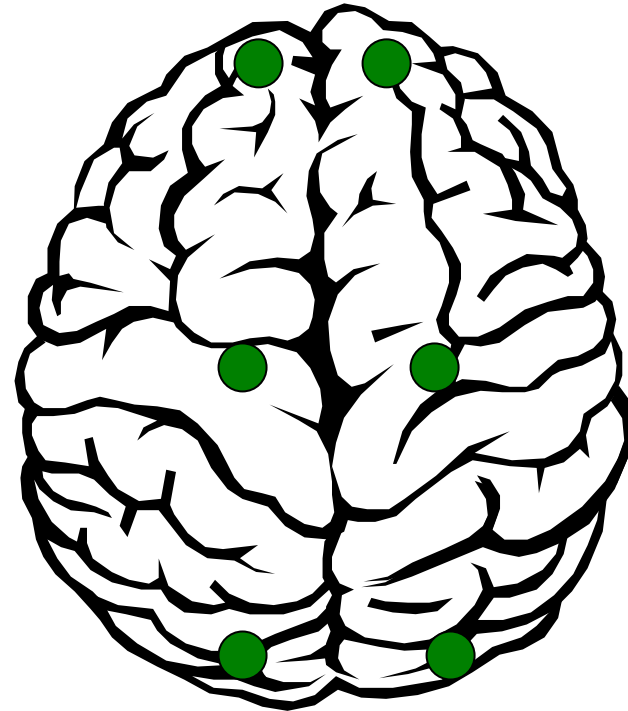
- Seizure score 3 (no seizures in 3 months prior to enrollment), identical to other 3 subjects receiving vector
- Last seizure 9-03 (not 9-04; typo in summary), 11 months prior to screening, 13 months prior to vector administration
- Occurred at “school” (UK medical facility where handicapped children receive daily care) – resolved rapidly with rectal midazolam

Comparisons of Sites of Administration

- Determined on a case-by-case basis by the neurosurgeons on the basis of the pre-op (within 24 hr) MRI
- 6 burr holes, 3 bilateral, 2 depths, maximum 2 cm from brain surface
- Criteria
 - safety – avoid blood vessels, cysts, malformations, white matter tracts, Broca's area etc
 - broadest distribution possible
 - protect functional regions of the brain with “salvageable” tissue

Sites of Administration

- Six sites per patient
- 2 frontal, 2 pre-motor,
2 parieto-occipital
- Minor adjustments based on patient anatomy (avoid sulci, vessels)



Comparison of Operative and Post-operative Management

Subject	Total length of anesthesia time in operating room (hr:min)	Total time of operative + post-op intubation (hr)	Differences in medication / doses	Use of mannitol¹
1	8:03	56	No	No
2	7:35	38	No	No
3	8:03	27	No	No
4	7:40	53	No	No

¹ Use of mannitol at discretion of anesthesiologist

III. Inclusion/Exclusion Criteria and Management of Risks to Subjects

- How were initial criteria set up?
- Are there non-medical criteria?
- Why was there a change in the prescreen from 6 months to 8 months ? Should it be shorter?
- Are there concerns about enrolling children with very low neurologic scores?
- Should epilepsy score be considered a possible basis for excluding potential study subjects?
- Details regarding proposed EEG assessment and use of this data? (e.g., if the baseline EEG reveals occult seizure activity, will this serve as the basis for exclusion?)

III. Inclusion/Exclusion Criteria and Management of Risks to Subjects (2)

- Are there MRI findings that could be used as inclusion/exclusion criteria?
- Will the EEG be used to map seizure foci in potential subjects, and will this be used to guide/avoid regions of vector administration? Will there be an attempt to avoid areas associated with seizure susceptibility (e.g., mesial temporal lobe?)
- Why 12 injections though 6 burr holes? Are there other delivery strategies that could decrease risk?

III. Inclusion/Exclusion Criteria and Management of Risks to Subjects (3)

- Should group B be postponed, and more studies carried out in group A?
- Will the death of subject 4 have any impact on the proposed continuation of the study into group B? Should this group be postponed pending evaluation in more children with severe disease?

Establishment of Inclusion/Exclusion Criteria

- Eligibility criteria developed based on a meeting on 7-18-03 with the Weill-Cornell Batten team + outside experts

LINCL Clinical Protocol Development Meeting

July 18, 2003, 9:00 AM

S-10 Conference Room, Gene Therapy Core Facility
Weill Medical College of Cornell University (WMC)
515 East 71st Street, NY, NY 10021

Invitees: Jonathan Mink (University of Rochester), Krystyna Wisniewski (Institute for Basic Research, Staten Island), Fredrick Marshall (University of Rochester, by telephone), Leon Dure (University of Alabama, by telephone), Syed Hosain (WMC), Mark Souweidane (WMC), Ronald Crystal (WMC), Dolan Sondhi (WMC), Neil Hackett (WMC), Stephen Kaminsky (WMC), Charleen Hollmann (WMC), Philip Leopold (WMC)

Meeting Agenda:

1. Summary of meeting goals (R Crystal) - 9:00 - 9:10 am
2. Summary of the LINCL preclinical data (D Sondhi) - 9:10 - 9:30 am
3. Proposed clinical trial (R Crystal) - 9:30 - 9:45 am
4. Clinical characteristics of LINCL (K Wisniewski) - 9:45 - 10:00 am
5. Rating Scale Development (J Mink) - 10:00- 10:15 am
6. Group discussion of possible clinical study design 10:15 am -12:00 pm
 - Inclusion / exclusion criteria
 - Injection - number, sites, volume, rate, time of procedure
 - Primary outcome parameters
 - Secondary outcome parameters

Endpoint Variables

Primary

- Neurologic assessment – LINCL clinical rating scale

Secondary

- CNS imaging – MRI/MRS

LINCL CNS Clinical Rating Scale*

Functional categories

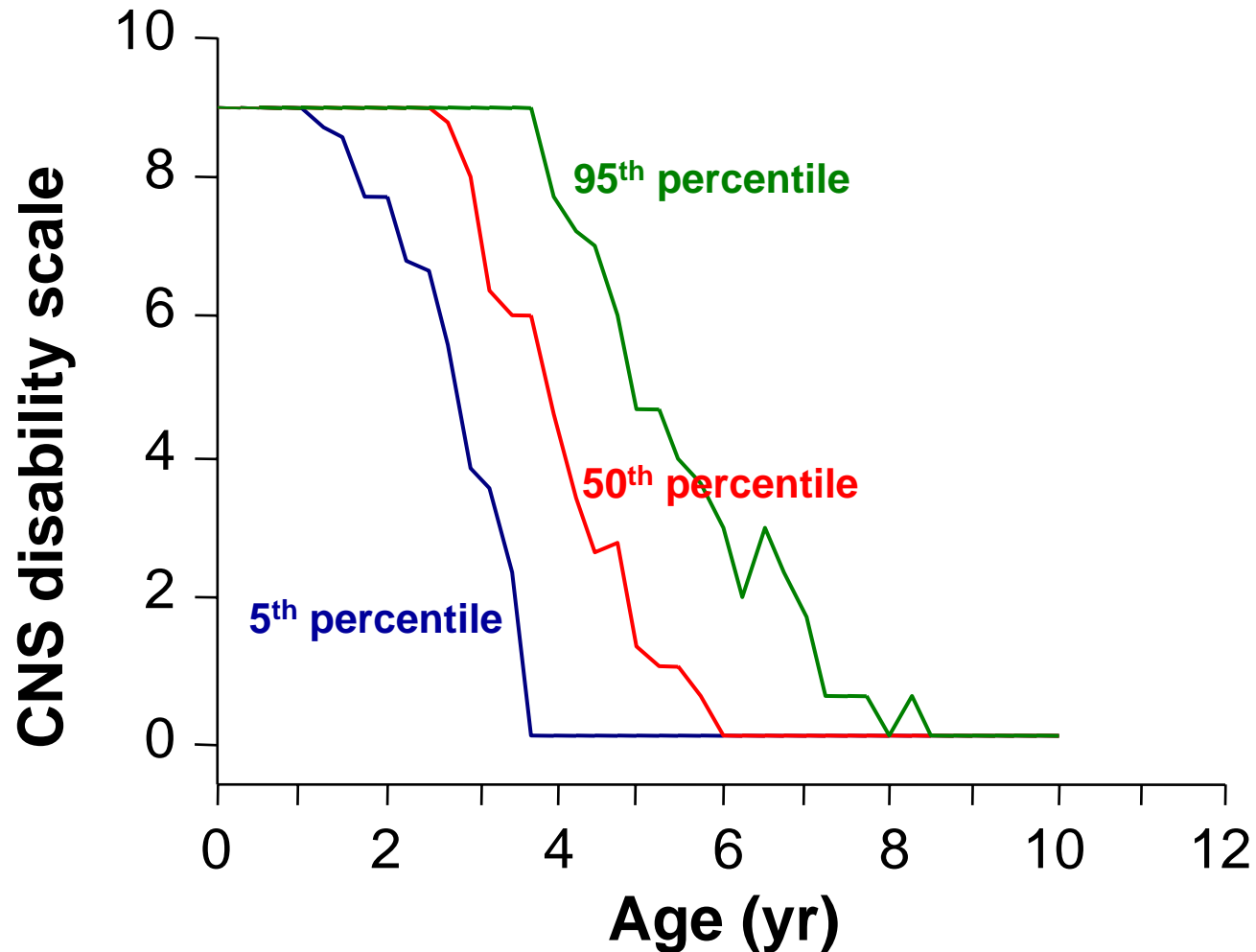
- Motor function (0–3)
- Seizures (0–3)
- Language (0–3)

CNS disability score

- Normal (9)
- Mild (7–8)
- Moderate (5–6)
- Severe (0–4)

* Modified from Steinfeld et al, Am J Med Genet 2002; 112: 347-54 (deleted vision; broadened “severe” category because seizures usually = 3)

Progression of LINCL Based on the CNS Clinical Rating Scale



Seizure Part of LINCL Rating Scale

- Seizure score breakdown
 - 3 = no seizures per 3 month period
 - 2 = 1 to 2 seizures per 3 month period
 - 1 = 1 seizure per month
 - 0 = >1 seizure per month
- All 4 subjects receiving the vector had a seizure rating of 3
- No future subjects will be enrolled with a rating <3, i.e., only subjects with adequate control of seizures

Non-medical Eligibility Criteria

Informed consent

- Both parents or guardians must sign the child's informed consent form
- 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

Non-Medical Eligibility Criteria (2)

Informed consent

- “No payment will be provided. You and your families will bear the costs of travel to and from the NYPH-WMC. You will also bear the costs of the accommodations and other expenses outside of the NYPH-WMC.”

Why Was There a Change in the Prescreen from 6 months to 8 Months? Should it be Shorter?

- In the screening protocol, the only procedure judged to be above-minimal risk is the MRI/MRS with anesthesia
- The change to 8 months was to accommodate scheduling variations beyond our control
- The additional time provides more opportunity to study genotype-phenotype correlations
- The pre-therapy screen just prior to vector administration repeats everything (except for the ophthalmologic assessment which is not part of the inclusion/exclusion criteria)

Are There Concerns About Enrolling Children with Very Low Neurologic Scores? Should Epilepsy Score be Considered a Possible Basis for Excluding Potential Study Subjects?

- Severe category has range of 0-4
- All 4 subjects had a score of 3
- We agree that scores of <3 should be excluded
- We agree that a seizure score of <3 (3 is the maximum score in this category) should be excluded

Details Regarding Proposed EEG Assessment and Use of this Data?

- 24 hr EEG monitoring pre-therapy and post-therapy, 1st 24 hr and 14 days
- These children all have abnormal EEGs with diffuse global dysfunction and interictal epileptiform activity
- If pre-therapy EEG reveals evidence of sub-clinical status epilepticus, this will exclude the subject
- Post-therapy, EEG evidence of increased seizure activity compared to pre-therapy will be treated appropriately

Are There MRI Findings That Could be Used as Inclusion /Exclusion Criteria?

Exclusion

- If there is evidence on MRI of infarct, hemorrhage or significant mass

Will the EEG be Used to Map Seizure Foci in Potential Subjects, and Will This be Used to Guide/Avoid Regions of Vector Administration?

Will There be an Attempt to Avoid Areas Associated with Seizure Susceptibility (e.g., Mesial Temporal Lobe?)

Yes

- All subjects will have diffuse global dysfunction and may have interictal epileptiform activity
- If a subject demonstrates a subclinical EEG seizure focus during the 24 hr monitoring period, attempts will be made to map the focus to a specific anatomical region and avoid that region in selecting vector administration sites
- The mesial temporal lobe and other areas associated with seizure susceptibility will be avoided

Why 12 Injections Though 6 Burr Holes? Are There Other Delivery Strategies that Could Decrease Risk?

- 6 burr holes decided as the maximum in consultation with the FDA
- 2 sites per injection expands the use of each burr hole/catheter insertion without significantly increasing risk
- This strategy maximizes targeting to greatest areas of function bilaterally while protecting key motor and cognitive areas
- No other CNS administration strategies have been shown in humans to be safe and provide broader distribution

Should Group B be Postponed, and More Studies Carried Out in Group A? Will the Death of Subject 4 Have Any Impact on the Proposed Continuation of the Study into Group B? Should This Group be Postponed Pending Evaluation in More Children with Severe Disease?

- From the data available, it is not possible to determine if the status epilepticus was due to the natural history of the disease, the surgical procedure, and/or drug administration in the setting of this subject's advanced underlying disease, the biologic vector *per se*, or some combination thereof
- Two MRI scans post-development of the status epilepticus (on days 21 and 44 post-vector) showed no inflammation in the areas of vector administration or elsewhere
- Assessment of cerebral spinal fluid on admission with status showed no evidence of inflammation

More Group A or B (2)?

- All 4 subjects – 24 hr post MRI scans showed no inflammation and no loss in the parenchyma relative to pre-therapy
- There is no data to suggest that subjects in group B (moderate) would have a greater risk of developing seizures
- Theoretically, there is likely a greater risk of developing seizures subsequent to surgery in group A (severe)

More Group A or B (3)?

Plan

- In agreement with the FDA, we plan to continue the subject recruitment as in the protocol
- As per agreement with the FDA, the patients will be staggered by 1 month (rather than 2 wk)

IV. Monitoring and Follow-up of Current and Future Subjects

- Are there concerns about dilutions of medications with IV fluids? How will the potential need for changes in seizure medications be handled?
- Should parents be requested to keep a seizure diary prior to screening and between screening and vector administration?
- How will follow-up EEGs be used in the management of patients?
- Have any alternative options for medical management of the disease changed since the study was initiated?

Total IV Fluids per Subject on Vector Administration Day

Subject	Fluid administered	Rate (cc/hr)	Subject weight (kg)	Total fluid per day (L)
1	½ NS	75	34.1	1.80
2	D5 ½ NS	80	37.0	1.92
3	D5 ½ NS	65	20.2	1.56
4	D5 ½ NS	70	30.9	1.68

Are There Concerns About Dilutions of Medications with IV Fluids? How Will the Potential Need for Changes in Seizure Medications be Handled?

- Anti-seizure medications will be monitored pre-therapy, and post-vector day 7, day 14, 1 month, 6 months, 18 months
- These children receive multiple anti-seizure medications which likely act in the aggregate, the doses of which have been “tweaked” by the physicians caring for the subject over years
- It takes days-wk to get back the levels for some of the seizure medications
- Clinical seizures will be treated as per standard practice
- Sub-clinical (EEG monitoring) seizure activity will be treated as per the attending neurologist/epilepsy expert

Seizure Medications (2)

Anti-seizure drugs	Subject										
	1	2	3	4	5	6	7	8	9	10	11
Clobazam								●	●		●
Clonazepam			●		●	●	●				
Clorazepate	●	●	●	●							
Ethosuximide											●
Lamotrigine	●										
Levetiracetam						●	●				
Topiramate						●	●	●		●	
Valproate		●			●	●	●		●		
Zonisamide			●	●	●						

● Subject received vector, ● subject screened only

Should Parents be Requested to Keep a Seizure Diary Prior to Screening and Between Screening and Vector Administration?

- The protocol SOP requires an extensive history, including a history of seizure activity
- There are two in-patient hospitalization opportunities to obtain the history, screening and pre-therapy, prior to a decision regarding therapy
- The anticipatory guidance to parents in the consent form (Group A and B, page 3, paragraph 1) states
“IF YOU DETECT ANY CHANGES IN YOUR CHILD’S CONDITION AFTER DISCHARGE FROM THE HOSPITAL, WE RECOMMEND THAT YOU CONTACT YOUR HEALTH CARE PROVIDER IMMEDIATELY.”

Details Regarding Proposed EEG Assessment and Use of This Data?

- 24 hr EEG monitoring pre-, and post- 1st 24 hr and 14 days
- These children all have abnormal EEGs with diffuse global dysfunction and interictal epileptiform activity
- If pre-therapy EEG reveals evidence of sub-clinical status epilepticus, this would exclude the subject
- Post-therapy EEG sub-clinical evidence of increased seizure activity compared to pre-therapy will be treated as appropriate

How Will Follow-up EEGs Be Used in the Management of Patients?

- Post-therapy, evidence of increased seizure activity compared to pre-therapy will be treated as appropriate
- If increased seizure activity is observed, attempts will be made to identify the responsible anatomic region and determine if it correlates with regions of administration

Have Any Alternative Options for Medical Management of the Disease Changed Since the Study was Initiated?

- No

V. FDA Concerns

- Amendment 13 addressed the FDA concerns based on their 2-10-05 letter; were there any other concerns in the letter not addressed in amendment 13?
 - All FDA issues were addressed in amendments 12, 13

Changes to Protocol Based on Discussions with the FDA and the FDA 2-10-05 Letter

- The time interval between product administration for consecutive subjects is increased from 2 wk to 1 month
- The day 14 follow-up visit will be performed at the Investigator's site (NYPH-WMC)
- 24 hr EEG monitoring will be performed and reviewed at the following timepoints: at pre-therapy (defined as within 1 wk prior to vector administration), at 24 hr following vector administration, and at day 14 (± 2 days) post-vector administration
- Serum drug levels for all anti-epileptic medications will be measured and reviewed (assuming such assays are available) both prior to vector administration and at the following post-vector timepoints:
 - Day 7 (± 2 days), day 14 (± 2 days), month 1 (± 5 days)
 - Month 6 (± 30 days), and month 18 (± 30 days)

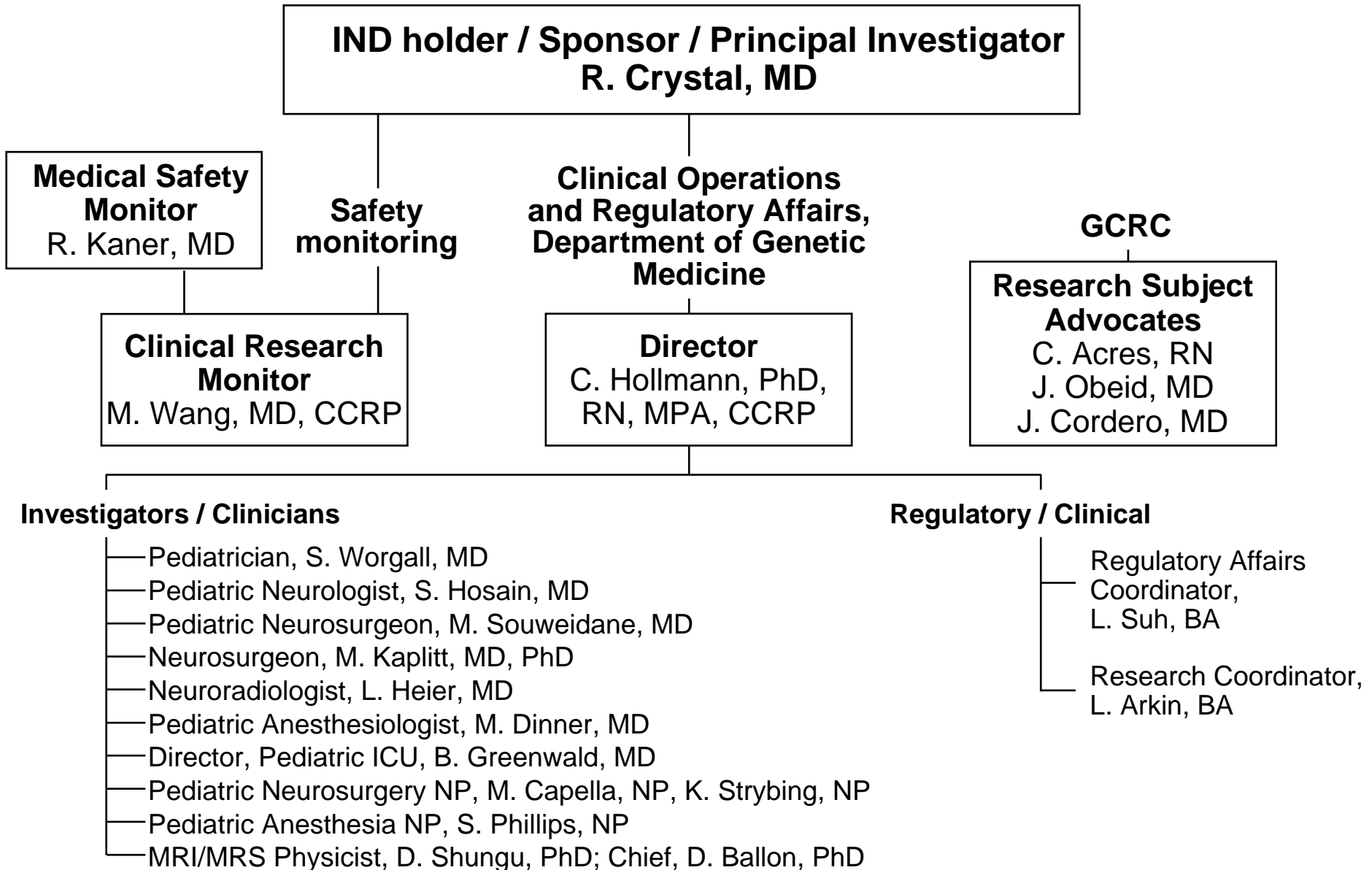
VI. IRB Review and Ethics Issues

- What were the concerns raised by the IRB? Were there questions about therapeutic misconception, about subject recruitment, criteria for enrollment, or other issues? How were these issues addressed?
- Was there additional IRB review subsequent to the death of subject 4? What issues were raised?
- Subsequent recruitment – how were potential subjects contacted? What role did the foundation play? How many of the subject's families were personally known to the investigators prior to enrollment?
- Should the informed consent address financial risks in the case of serious adverse events?

Overall Strategy Regarding Consent, Enrollment and Foundation Issues

- Consent addresses “therapeutic misconception”
- Structure of donation – divorce Foundation from any control
- Handling of enrollment inquiries
- Two studies – screening, gene transfer
- Enrollment decided by committee whose primary appointments are outside of the PI’s department
- Divorce PI from decisions regarding enrollment or clinical care
- Monitoring is done by a clinical research monitor separate from regulatory affairs and clinical operations
- Independent medical safety monitor that reports to IRB/DSMB
- GCRC research subject advocates independent of investigators

Clinical Organizational Chart for LINCL Protocol (BB IND 11481)



Consent Issues

Therapeutic misconception issues

- **Page 1, paragraph 2**

“This research study involves a high-level of risks to your child, which includes the risk of death.”

- **Page 1, paragraph 3**

“It is important for you to know that participation of your child in this research study is voluntary. We cannot and do not guarantee that your child will receive any benefits from this study. However, the knowledge gained in this research study will benefit others in the future. We hope AAV2_{CU}hCLN2 (an adeno-associated virus vector) may prevent the disease from getting worse. However, this is an experimental research study, and there is no proof that this will occur.”

Consent Issues (2)

- Protocol is emailed to the families of potential subjects and they are encouraged to review it with their physicians and others that can help in their decision process
- At the time of enrollment, the consent process is carried out by
 - S. Worgall, MD, Pediatrician, Co-investigator
 - M. Kaplitt, MD, PhD, Neurosurgeon, Co-investigator or M. Souweidaine, MD, Neurosurgeon, Co-investigator
 - L. Arkin, BA, Research Coordinator
 - C. Acres, RN, J. Cordero, MD or J. Obeid, MD
Research Subject Advocate (Weill Cornell NIH GCRC)
- The PI is not involved in the consent process

Enrollment Issues

Funding

- The clinical study is funded, in part, by Nathan's Battle Foundation, the Weill Cornell NIH GCRC and funds from the Department of Genetic Medicine
- How to separate a Foundation (funded in part, by families with children with LINCL) from influence regarding the clinical study, and specifically, enrollment?

Separation of Funding Influences on Clinical Research



Enrollment Issues (2)

- The funds from Nathan's Battle Foundation are a gift to the University
- The Foundation has no control over its use in pre-clinical studies, the clinical study, nor in subjects that are enrolled

Enrollment Issues (3)

How are subjects found?

- No advertisements are used
- Lists of subjects have been obtained from Nathan's Battle Foundation and the Batten Disease Support and Research Association
- Presentations by the PI and other faculty
 - 9th International Congress on Neuronal Ceroid Lipofuscinosis (Batten Disease) 2003
 - American Society of Gene Therapy, 2002, 2003, 2004, 2005
 - Society for Neuroscience 2002, 2003, 2004
- Word of mouth led to many emails from throughout the world that continue at 1 to 2/wk
- The PI does not respond to the emails; all correspondence is by the Clinical Operations and Regulatory Affairs group in the Department of Genetic Medicine

Enrollment Issues(4)

How are decisions made regarding enrollment?

- Two studies
 - Screening
 - Gene transfer
- There is no guarantee that enrollment in the screening study will automatically mean enrollment in the gene transfer study

Enrollment Issues (5)

Selection of subjects for the gene transfer study

- Assessed in order of screening (with caveat of personal scheduling of the families)
- No outside input
- PI is not involved
- Decisions made by 3 co-investigators (1 of the 2 neurosurgeons, pediatric neurologist, general pediatrician), none of whom have a primary appointment in the PI's Department of Genetic Medicine

Was There Additional IRB Review Subsequent to the Death of Subject 4? What Issues Were Raised?

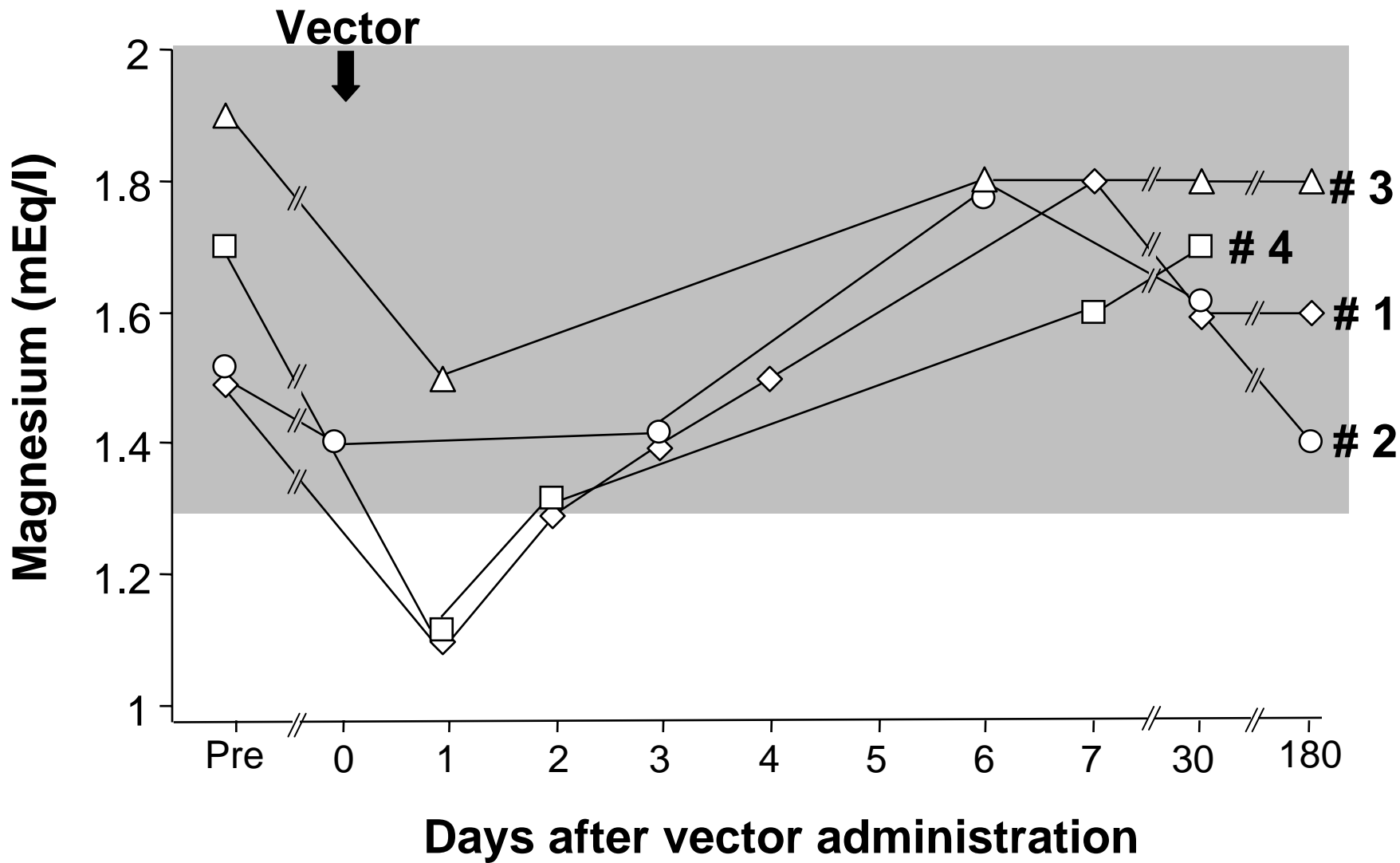
Timeline and actions

- Status epilepticus occurred on day 14 post-vector administration
- All relevant regulatory bodies informed of serious adverse event within 3 days (10-22-04) as per regulations
- Day 6 post-status epilepticus(10-25-04) we informed the FDA, IRB, IBC, DSMB and OBA-RAC that we were placing the study on clinical hold
- Day 31 (11-18-05) the FDA formally informed us of their clinical hold
- Day 115 (2-10-05) the FDA released the clinical hold

Was There Additional IRB Review Subsequent to the Death of Subject 4? What Issues Were Raised?

Combined IRB/DSMB issues post-death of subject 4

- **Insure that an epilepsy expert is involved**
S. Hosain, MD, full time faculty member of the Dept of Neurology, a co-investigator in the study, is Director of Pediatric Epilepsy, Weill Cornell Comprehensive Epilepsy Center
- **Mild hypomagnesemia was noted in two subjects at day 1 post-vector; administration should be considered if it occurs**
Magnesium levels are assessed and will be treated as relevant



IRB/DSMB Issues (2)

- **Try to localize seizures if they occur**
Continuous EEG monitoring pre-therapy and at 24 hr post-vector and day 14 will be carried out in all subjects; if seizures occur, the EEGs will be used to localize anatomic sites and attempt to correlate the sites of activity with sites of vector administration
- **What was the genotype of subject 4?**
IVS5-1G>C homozygous

IRB/DSMB Issues (3)

- **For the future, track genotype vs adverse events to determine if a correlation exists**
This will be done
- **It was noted that the continuous EEG monitoring was added**
- **It was clarified that the “school” that subject 4 attended is a term used by the parents to refer to the day facility for care/physical therapy**

Consent Issues (3)

Consent post-death subject 4

- **Page 5, Paragraph 5**

“On November 2004, a research subject enrolled in this protocol died after having episodes of multiple seizures (status epilepticus). We are unable to determine whether the cause of the child’s seizures was due to the natural history of the disease, the surgical procedure and/or drug administration in the setting of the disease, the research study drug (biologic vector), or some combination thereof. Two MRI scans of this subject’s brain showed no areas of inflammation, in the areas of vector administration or elsewhere and assessment of the fluid surrounding the brain also showed no evidence of inflammation. None of the other subjects enrolled in the study have experienced seizures following vector administration. In signing this consent you acknowledge an understanding of the risks of induction of continuous seizures (status epilepticus) and death.”

Subsequent Recruitment – How Were Potential Subjects Contacted? What Role Did the Foundation Play? How Many of the Subject’s Families Were Personally Known to the Investigators Prior to Enrollment?

- The death of subject 4 is explicitly stated in the informed consent
- The Foundation plays no role in the clinical study
- The PI met some of the families (not the subjects) at various meetings, but is divorced from decisions regarding enrollment

Should the Informed Consent Address Financial Risks in the Case of Serious Adverse Events?

- **It does** (page 8, paragraph 3)

“In accordance with Federal regulations, we are obliged to inform you about the Medical Center's policy, in the event physical injury occurs. If, as a result of your participation, your child experiences physical injury from known or unknown risks of the research procedures as described, immediate medical care and treatment, including hospitalization if necessary, will be available. No monetary compensation, however, is available and you will be responsible for the costs of such medical treatment either directly or through your medical insurance and/or other forms of medical coverage.”

- At the time of consent, the implications of this policy are fully explained

VII. Preclinical Animal Studies

- Is there a need for additional animal studies, perhaps including studies of the AAV vector in CLN2 knockout mice (these mice became available only recently)?
- Would it be possible to evaluate the expression of the transgene in the knockout model and assess the levels of TPP-1 produced? Given that 6-10% of normal TPP-1 levels are needed to prevent dysfunction, could the knockout model be used to determine if this level can be achieved?

VII. Preclinical Animal Studies (2)

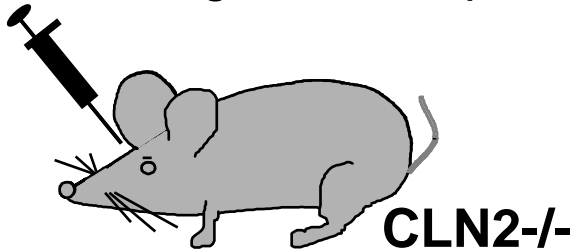
- What preclinical research was most decisive in the decision to move to clinical trials? What alternative treatments exist for treatment or amelioration of disease?
- The original protocol stated that non-human primate studies of delivery of the vector to the CNS were ongoing; what are the updated data from these studies?

VII. Preclinical Animal Studies (3)

- What is the current data on the delivery of AAV2 vectors to the CNS in the two other applications of this delivery system for Canavan's disease and Parkinson's? Have there been any AEs? Is there evidence that the transgenes persist?

Studies with CLN2^{-/-} Mice with the Clinical Vector

- AAV2_{CU}hCLN2
1.8x10⁹ genome copies



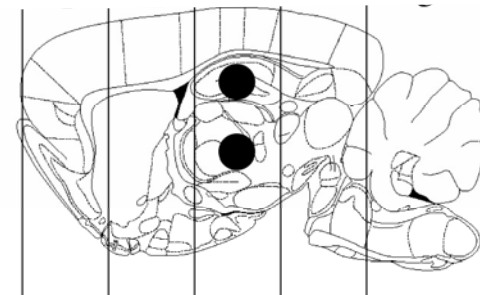
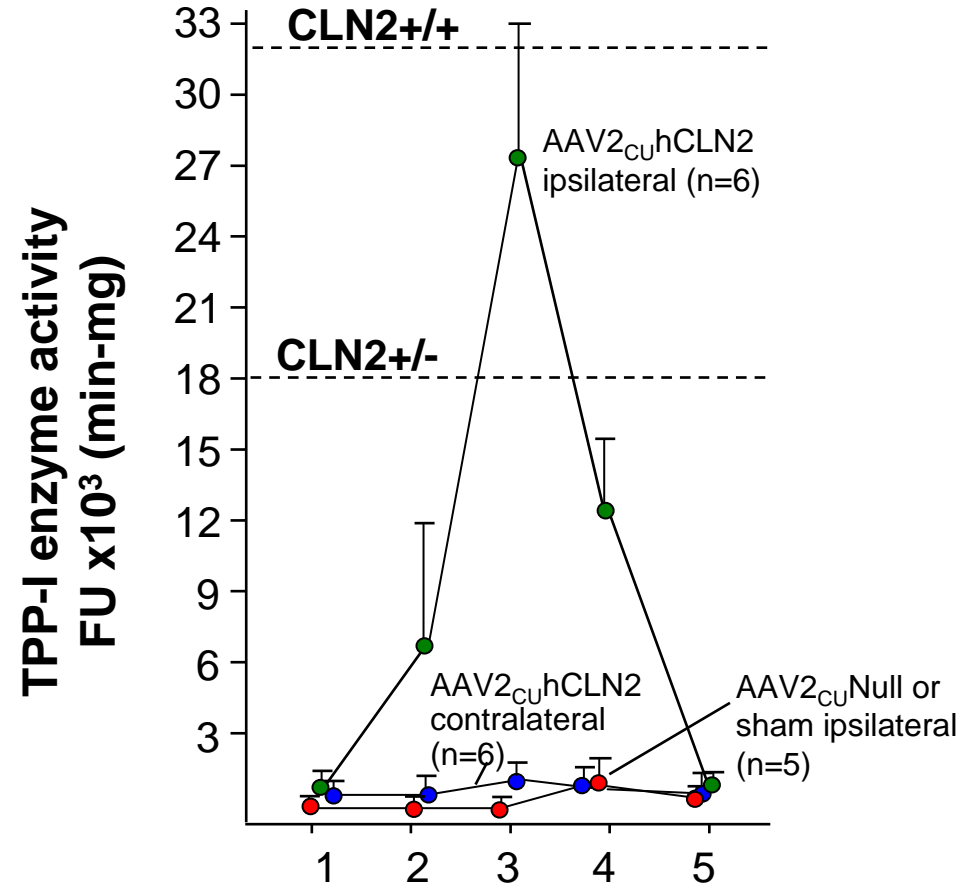
6 wk

Evaluate

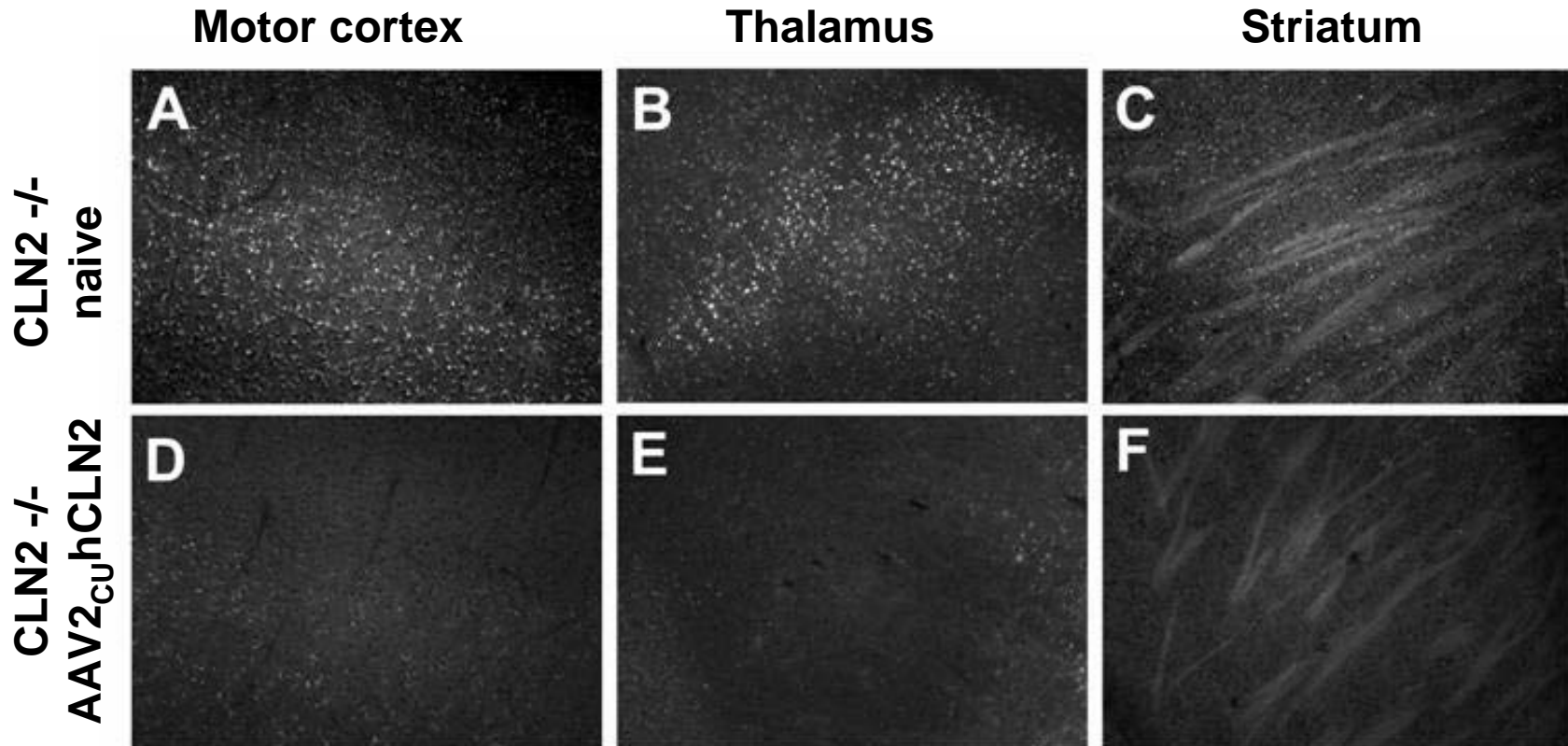
- TPP-I expression by enzymatic assay

Collaborators

- M. Passini, Genzyme
- P. Lobel, D. Sleat, UMDNJ
- B. Davidson, U Iowa



Studies with CLN2^{-/-} Mice with the Clinical Vector (13 wk post vector)



Reduction of autofluorescent storage material

Summary of Studies with CLN2-/- Mice with the Clinical Vector

- No safety issues
- TPP-I levels are much greater than the 5-10% needed for correction
- Storage granules are significantly reduced (30-70%) in motor cortex, striatum, hippocampus, thalamus, cerebellum
- Rotorod functional improvement 8 to 10 wk post-administration

Decisive and Follow-up Pre-clinical Data

- LINCL is a fatal neurodegenerative disease, with onset ages 2-4, and death ages 8-12
- There are no alternative treatments

Decisive and Follow-up Pre-clinical Data (2)

- Studies of AAV2 mediated gene transfer in rats indicated that TPP-I could be expressed in various parts of the brain
- TPP-I expression levels in the CNS persisted for at least 18 months after gene transfer using the clinical vector
- TPP-I levels in the CNS of treated rats and non-human primates exceeded the normal endogenous levels at >10%, levels consistent with therapeutic efficacy

Decisive and Follow-up Pre-clinical Data (3)

- Local cross-correction and axonal transport result in vector-derived TPP-I in CNS sites distant from the injection site
- Formal safety/toxicology studies in rats over 12 months and non-human primates over 12 months showed no significant concerns

Decision to Move to the Clinic

- LINCL is a fatal disorder
- Pre-clinical data showed the therapy was safe and is consistent with therapeutic efficacy

VII. Preclinical Animal Studies (3)

- What is the current data on the delivery of AAV2 vectors to the CNS in the two other applications of this delivery system for Canavan's disease and Parkinson's? Have there been any AEs? Is there evidence that the transgenes persist?
 - The only information available to us is public information; the RAC should have information directly from the investigators

Questions From Dr. Martha Bohn

3-14-05

- Has there been any testing of the vector in the immature non-human primate brain?
 - No
- Was the same vector stock used for all subjects as well as non-human primates?
Could we provide the QA test results for the vectors stocks used?
 - Multiple stocks were used; all passed the same rigorous lot release agreed to by the FDA

Lot Release for AAV2_{CU}hCLN2

Weill Medical College Of Cornell University: Gene Therapy Core Facility
1300 York Avenue, Box 228, New York, NY 10021

Quality Control Document			
Title: Lot Release Clinical Grade Adeno Associated Virus AAV2_{CU}hCLN2			
GTCF Lot Number: 4060216-RAAV			
Originator Devika Dhanraj	Rev. Date/Rev. No. 03/02/04 / 1	Effective Date: 5/27/04	Part No. 80013

Approvals

Name	Title/Department	Signature	Date
Devika Zachariah	QA/QC Coordinator	<i>D Zachariah</i>	6/21/04
Stephen Kaminsky	Manager/GMP	<i>Steve Kaminsky</i>	6/21/04

Date of Manufacture: 05/18/04

Expiration Date: N/A

Test	Method Reference	Specification	Test Results
Sterility	Contract lab	Negative	Negative
Mycoplasma	Contract lab	Negative	Negative
IN VITRO assay for the presence of viral contaminants	Contract lab	Negative	Negative
Limulus Amebocyte Lysate (LAL) - endotoxin assay	Contract lab	<20 EU/mL	0.5EU/mL
Adenovirus Virus DNA Detection	Contract lab	Negative	Negative
Residual host cell DNA	Contract lab	< 100 ng per dose	60 ng per dose
rcAAV	PCR and Taqman	For Information Only	< 1 in 10 ⁷ pu
Expression cassette	PCR	Detected	Detected

Weill Medical College of Cornell University
Gene Therapy Core Facility

QA/QC Signature *D Zachariah* 7/1/04

Test	Method Reference	Specification	Test Results
Transgene product	Western	Detected	Detected
Transgene function	TPP-1 Assay	> 200 fluorescent units/min	734 fluorescent units/min
Viral Capsid	Western	Detection of VP1,2,3. > 90% band density	Detection of VP1,2,3. > 90% band density
Genomic structural integrity	PCR and Sequencing	Predicted pattern observed	Predicted pattern observed
AAV titer	Infectious titer/PCR of product amplified on C12 cells	For information Only	> 8 x 10 ⁹ and < 8 x 10 ¹⁰
Viral DNA copies	Taqman	For information only	7.05 x 10 ¹⁰ gc/mL
Viral particle count	ELISA	≥ 1.0 x 10 ¹² pu/mL	1.5 x 10 ¹² pu/mL
PU/IU	Calculation	≤ 200	187
Lot yield and packaging: 12 vials at 400 µl, 14 vials at 20 µl			

Approval For Lot Release

Check appropriate box:
PASS FAIL

Name	Title	Signature	Date
Devika Zachariah	QA/QC Coordinator	<i>D Zachariah</i>	6/21/04
Stephen Kaminsky	Manager/ GMP	<i>Steve Kaminsky</i>	6/21/04
Ronald G. Crystal	Research & Development	<i>RGC</i>	6/21/04

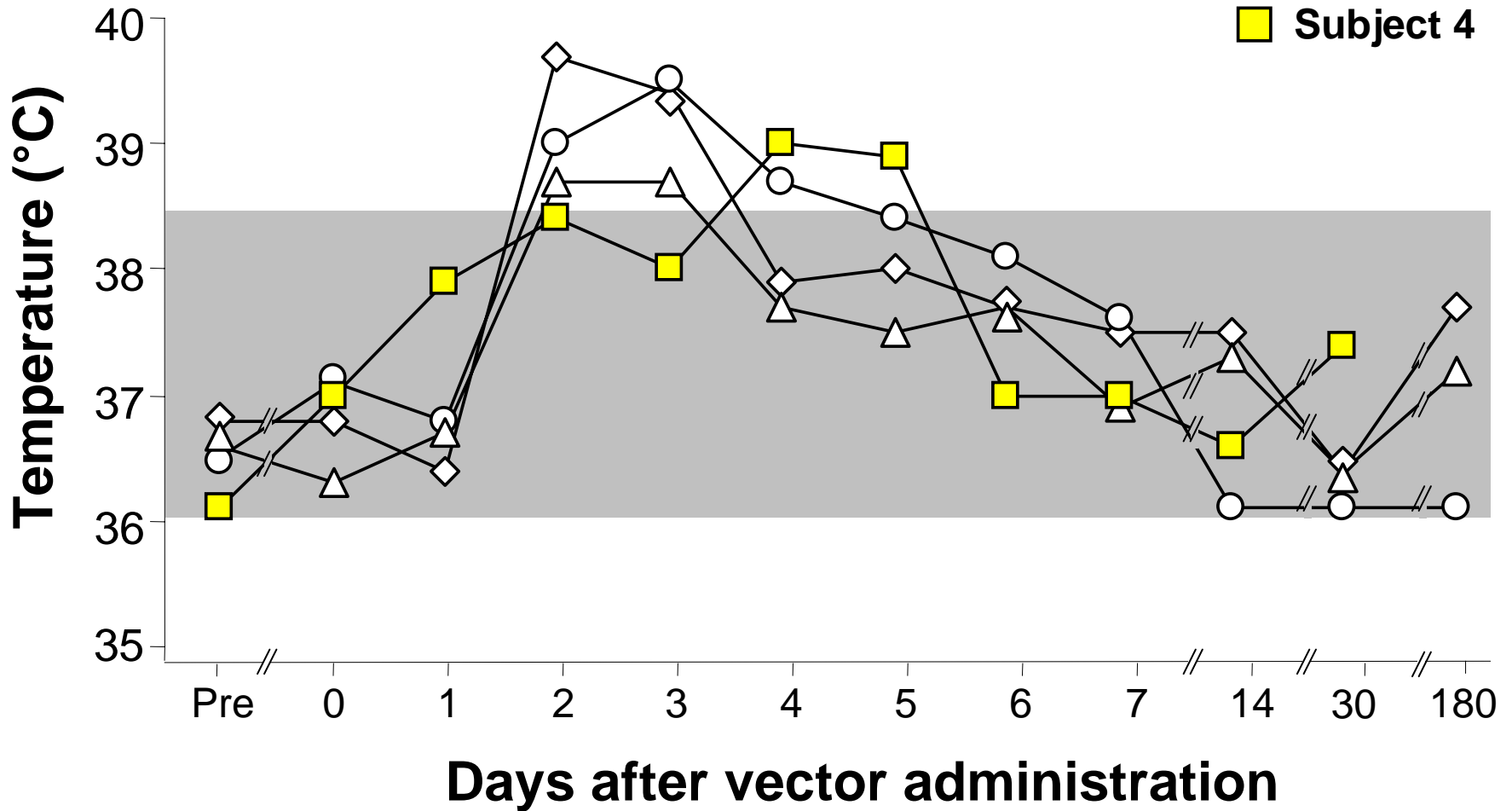
Definitions: pu = particle units; IU = infectious units

Questions From Dr. Martha Bohn

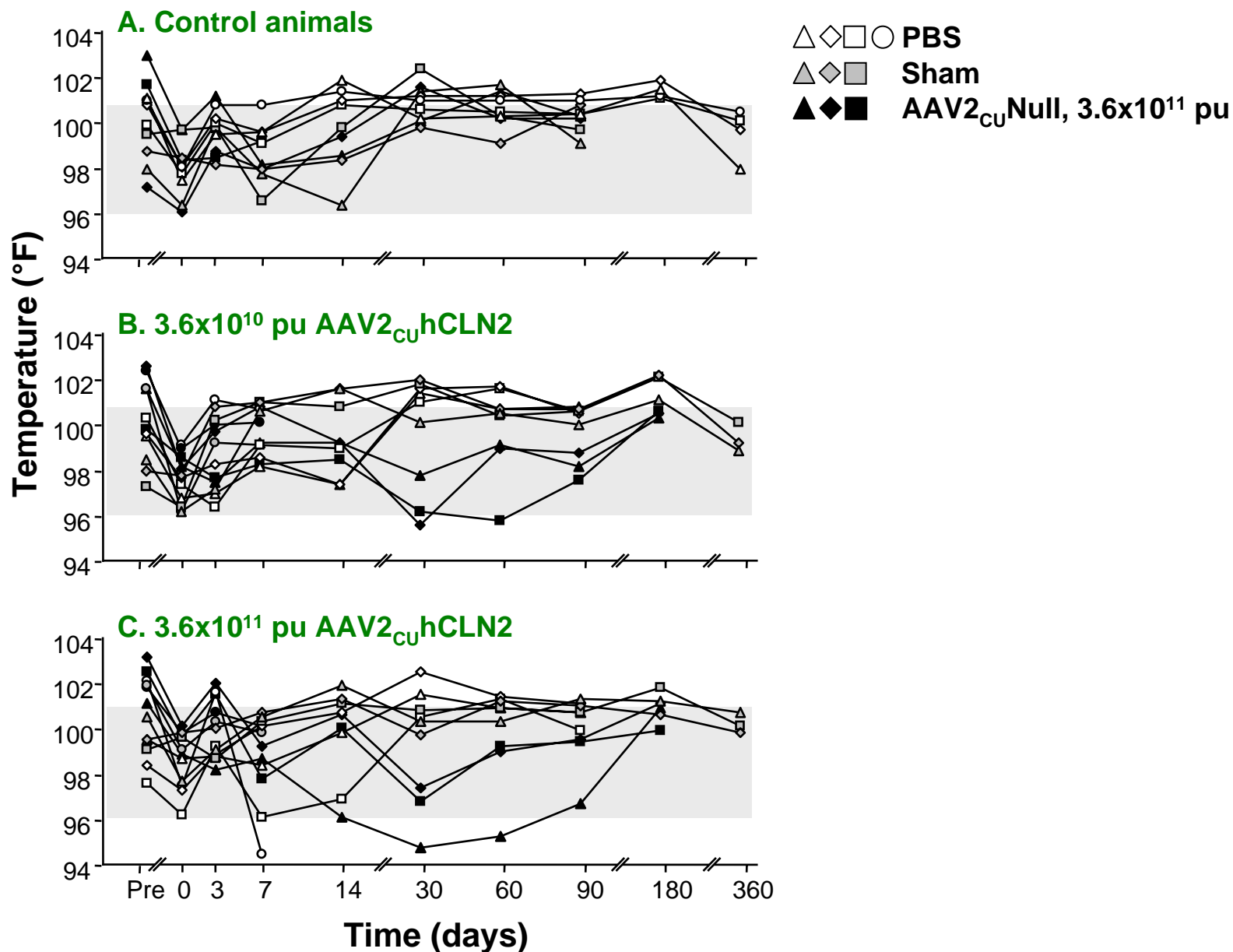
3-14-05

- Did the monkeys spike fevers or show any systemic changes that reflect those observed in children?
 - No

Temperature Curves in Batten Disease Gene Transfer Subjects



Long term Toxicology Evaluation of AAV2_{CU}hCLN2 Injection into the Brain of Non-human Primates: Temperature



Questions From Dr. Martha Bohn 3-14-05

“It is unfortunate that no neuropathologic evaluation will be possible due to lack of autopsy material. While it is important to respect the wishes of patients/participants, it might be helpful to include information in the informed consent that would outline what would be done with the autopsy material and why the evaluation of this material is important”

- **Statement in the consent (page 8, paragraph 5)**

“Consent for autopsy. Your child's physicians do not believe that the gene transfer or the tests your child will undergo will lead to adverse effects that could cause his/her death. However, as the parent/ legal guardian of a participant in a new research study, it is important that you understand that should death of your child occur while in this research study, the doctors need to be able to find out the exact cause of death. The doctors will ask you to give permission for a full autopsy of your child. We cannot force you to agree to this, but if you check “Yes”, you are indicating your agreement that an autopsy be carried out. Please check “Yes” or “No” below, to indicate your wishes for an autopsy to be performed on your child.

_____ Yes, it is my wish that an autopsy be performed on my child.

_____ (Initials of parent/legal guardian)

_____ No, it is not my wish to have an autopsy be performed on my child.

_____ (Initials of parent/legal guardian)

NIH guidelines for research (Appendix M-III-B-2-c) involving recombinant DNA molecules, states as ‘To obtain vital information about the safety and efficacy of gene transfer, subjects should be informed that at the time of death, no matter what the cause, permission for an autopsy will be requested of their families. Subjects should be asked to advise their families of the request and of its scientific and medical importance’.”

- This was previously reviewed by the RAC (submitted 12-23-03, Letter of Exemption 1-20-04)
- If the RAC has a general statement that is recommended for all gene transfer protocols relevant to autopsies, we would be pleased to incorporate it

VIII. Role of the Nathan's Battle Foundation

- **Did the foundation have an independent scientific review of this protocol? Does it have a scientific advisory board or committee? If so, how is it composed?**
 - We have no involvement or knowledge of the internal workings of the Foundation
- **Are any of the other physicians participating in this study, for example, the neurosurgeons, pediatrician and neurologist who evaluate prospective subjects, involved with the Foundation?**
 - No
- **Were there any subjects enrolled whose families were not involved with the Foundation? If not, in principle, would lack of involvement present an obstacle to enrollment?**
 - Subject enrollment was done (if eligible) on sequence of assessment in the screening study
 - Other than 2 of the subjects of the 11 that have been screened, we have no knowledge of the relationship of any of the subject's families with the Foundation
 - Relationship to the Foundation plays no role in decisions regarding enrollment
 - The Foundation plays no role and has no impact on any decisions regarding the clinical study

VIII. Role of the Nathan's Battle Foundation (2)

- **Was the preclinical research supporting this trial funded by the same Foundation?**
 - The pre-clinical research was funded, in part, by Nathan's Battle Foundation, funds from the Department of Genetic Medicine, and more recently, NINDS U01 NS047458
- **If so, how much of the cost was borne by the Foundation?**
 - Approximately 50% of the preclinical research

VIII. Role of the Nathan's Battle Foundation (3)

Was preclinical research evaluated by an scientific body prior to FDA submission?

- **Yes – the NIH OBA-RAC reviewed it**
 - Submitted December 23, 2003
 - Letter of Exemption January 20, 2004

VIII. Role of the Nathan's Battle Foundation (4)

Peer Review – Presentations at National Meetings

Abstracts

- Sondhi D, et al. A Corrective Gene Delivery Strategy for the Neurodegenerative Lysosomal Storage Disease Late Infantile Neuronal Ceroid Lipofuscinosis. *Molecular Therapy*, 2002, Vol. 5, No. 5, Part 2 of 2 Parts, S247, # 754
- Giannaris EL, et al. Comparison of AAV Serotypes 2 and 5 Based Vectors for Corrective Gene Therapy for Late Infantile Neuronal Ceroid Lipofuscinosis. *Molecular Therapy*, 2003, Vol. 5, No. 5, Part 2 of 2 Parts, S89, # 224
- Sanders CT, et al. Corrective Gene Therapy for Late Infantile Neuronal Ceroid Lipofuscinosis by AAV Serotypes 2 and 5 Based Vectors. Society for Neuroscience. Annual meeting in New Orleans, 2003
- Sondhi D, et al. Long Term Expression of TPP-I in Rat and Non-human Primate Brain following Administration of AAV2_{CU}hCLN2, a candidate Gene Therapy Treatment for Late Infantile Neuronal Ceroid Lipofuscinosis. *Molecular Therapy*, Volume 9, 2004, Supplement 1, S406, # 1060, **oral presentation**
- Hackett NR, et al. Safety of Administration of AAV2CUhCLN2, a candidate treatment for Late Infantile Neuronal Ceroid Lipofuscinosis to the Brain of Rats and Non-human Primates, *Molecular Therapy*, 2004, Volume 9, Supplement 1, S165, # 432

VIII. Role of the Nathan's Battle Foundation (5)

Peer Reviewed Publications

- Sondhi D, Hackett NR, Apblett RL, Kaminsky SM, Pergolizzi RG, Crystal RG. Feasibility of gene therapy for late neuronal ceroid lipofuscinosis. Arch Neurology 2001; 58: 1793-1798.
- Crystal RG, Sondhi D, Hackett NR, Kaminsky SM, Worgall S, Steig P, Souweidane M, Hosain S, Heier L, Ballon M, Dinner M, Wisniewski K, Kaplitt M, Greenwald BM, Howell JD, Strybing K, Dyke J, Voss H. Administration of a replication deficient adeno-associated virus gene transfer vector expressing the human CLN2 cDNA to the brain of children with late infantile neuronal ceroid lipofuscinosis. Hum Gene Ther 2004; 15:1131-1154.
- Sondhi D, Peterson DA, Giannaris EL, Sanders CT, Mendez BS, De B, Rostkowski A, Blanchard B, Bjugstad K, Sladek JR Jr, Redmond DE, Leopold PL, Kaminsky SM, Hackett NR, Crystal. AAV2-mediated CLN2 gene transfer to rodent and non-human primate brain results in long term TPP-I expression compatible with therapy for LINCL. Gene Ther 2005; 12: (in press)

VIII. Role of the Nathan's Battle Foundation (6)

- **How much are the clinical studies estimated to cost? How much of the cost is borne by the Foundation?**
 - Total cost approximately \$4 million over 3 yr
 - Approximately 3% by Weill Cornell NIH GCRC
 - Approximately 40% by the Foundation
 - Approximately 57% by the Department of Genetic Medicine

VIII. Role of the Nathan's Battle Foundation (6)

- **Are there plans to submit either preclinical or clinical study proposals to NIH as an investigator-initiated research proposal?**
 - Yes; submitted and funded; future preclinical studies funded by NINDS U01 NS047458
 - Plans for NIH proposals for future clinical studies will be determined by outcome of the current clinical study and ongoing preclinical studies

IX. Next Steps in Continuation of the Protocol

- **Are there plans to continue enrollment? If so, would the next child enrolled be from group A (severely ill) or group B (less severely ill)?**
 - Yes; 1 more from group A, then move to group B (n=6)
- **If new subjects will be enrolled, how will they be selected from among the potentially eligible children?**
 - In the same, unbiased fashion in which other subjects have been chosen
- **Are there other changes to the protocol under consideration?**
 - No; all changes are in amendments 12, 13 which are included in the annual report