

Lorenzo's oil in Adrenoleukodystrophy

What is known now and any
lessons we can take from the
history of its study

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Adrenoleukodystrophy (ALD)

X-linked disorder - Xq28

incidence 1:17,000, all races affected

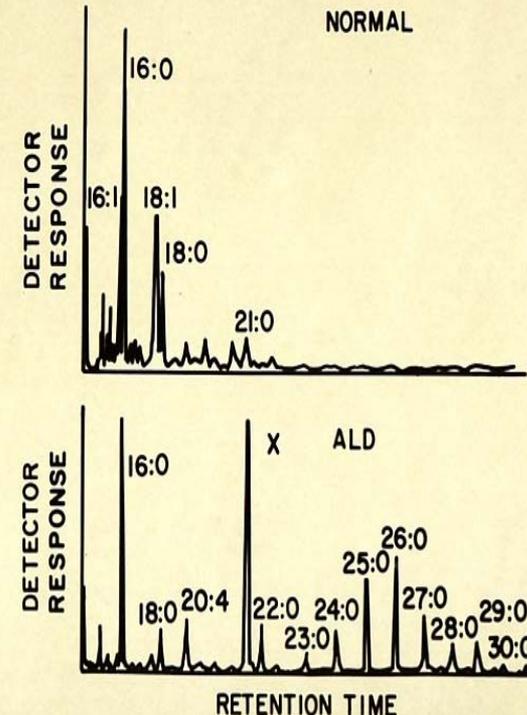
Peroxisomal ATPase Binding Cassette Protein (ABCD1)

Defect in peroxisomal beta oxidation

Accumulation of very long chain fatty acids (VLCFA)

Affects myelin, adrenal cortex, Leydig cells of the testes

FATTY ACID METHYL ESTERS OF BRAIN CHOLESTEROL ESTERS (GAS CHROMATOGRAPHY)

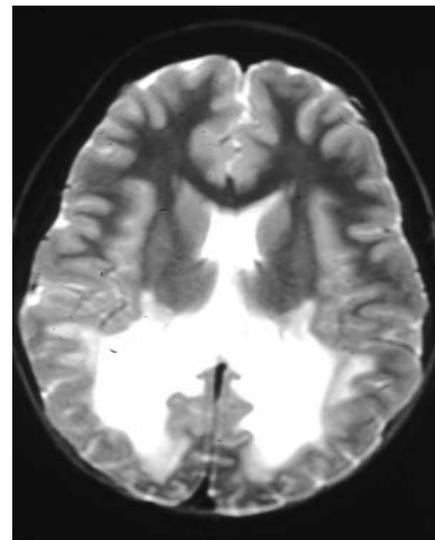


Igarashi et al.
JNeurochem
26:851-860, 1976

Variable Manifestations (phenotypes) of ALD and Relative Frequency

- **Cerebral (35%)**
 - Diffuse inflammatory demyelination, rapid progression.
 - Childhood form (onset 4-8 years) most common
- **Adrenomyeloneuropathy (AMN) (40-65%)**
 - Distal axonopathy mainly in spinal cord.
 - Paraparesis in young adults, progress over decades
- **Addison Disease only (20-30% at onset)**
 - Most develop AMN later
- **Asymptomatic**
- **>50% of heterozygous women develop AMN in middle age or later**

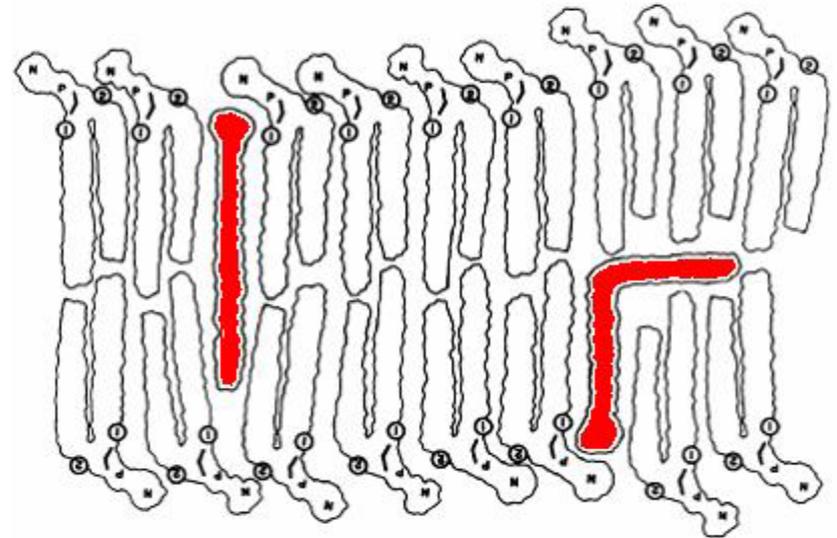
Neither the gene defect nor the biochemical abnormality predicts the phenotype. A genetic modifier has been postulated



Role of VLCFA in pathogenesis

- Extremely insoluble in water and alters properties of membranes
- Viscosity of red cell membranes is increased
- Cultured adrenocortical cells—added VLCFA increased microviscosity of membranes and decreased cortisol release
- Inclusion in adrenocortical cells
- Binding with albumin is altered
- Inclusion of C26:0 in model membrane perturbs structure and stability
- Impairs stability of axonal or myelin membranes?
- Role as a trigger of immune response?

Schematic of C26:0 in PC Bilayers

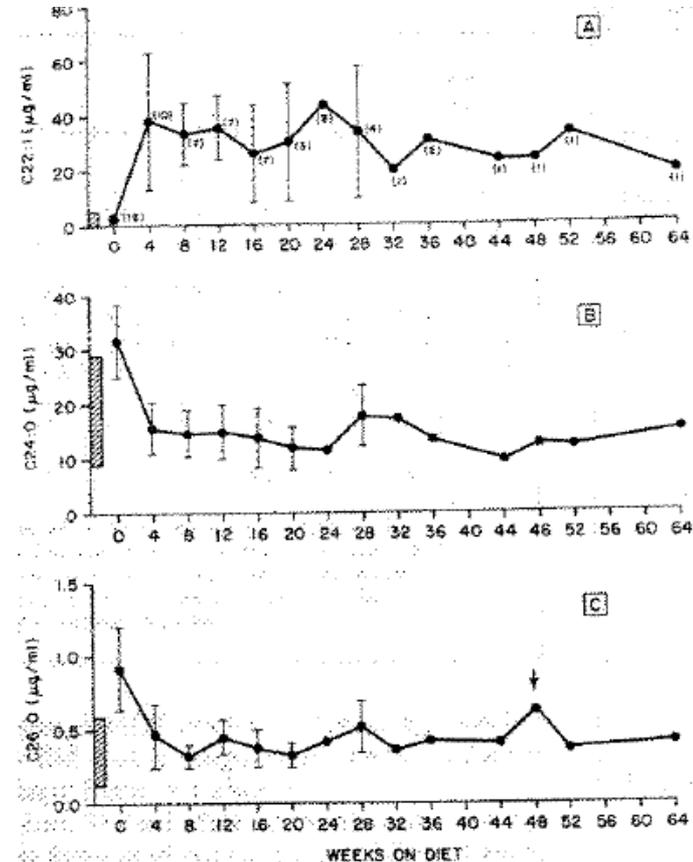


Early dietary experience

- Adrenoleukodystrophy: Evidence that abnormal very long chain fatty acids of brain cholesterol esters are of exogenous origin (Kishimoto et al 1980)
- Adrenoleukodystrophy: Effects of Dietary Restriction of Very Long Chain Fatty Acids and of Administration of Carnitine and Clofibrate on Clinical Status and Plasma Fatty Acids (Brown et al 1982)
- Adrenoleukodystrophy: Oleic acid lowers fibroblast saturated C22-C26 (Rizzo et al 1986)
- A new dietary therapy for adrenoleukodystrophy: biochemical and preliminary clinical results in 36 patients. (Moser et al 1987)
- Dietary erucic acid therapy for X-linked adrenoleukodystrophy (Rizzo et al 1989)

Dietary erucic acid therapy for X-linked adrenoleukodystrophy (Rizzo et al 1989)

- 4:1 volume of oleic to erucic acid (Lorenzo's oil)
- Crossover study between GTO and GTE/GTO for 3 week periods
 - 5 patients
 - Plasma C26:0 decreased on GTE/GTO
- 12 newly diagnosed cerebral individuals for 2-19 months.
 - C26:0 declined in 4-8 weeks
 - 8/12 on tx > 6 months
 - 5/8 showed deterioration
- Discussion
 - Did lower VLCFA in plasma
 - Clinical response was less than encouraging
 - Was it getting into the brain and were VLCFA being lowered there?
 - Could require months; myelin composition may not have changed
 - Rapid, progressive demyelination may not be affected
 - Detrimental effects could have offset any benefits

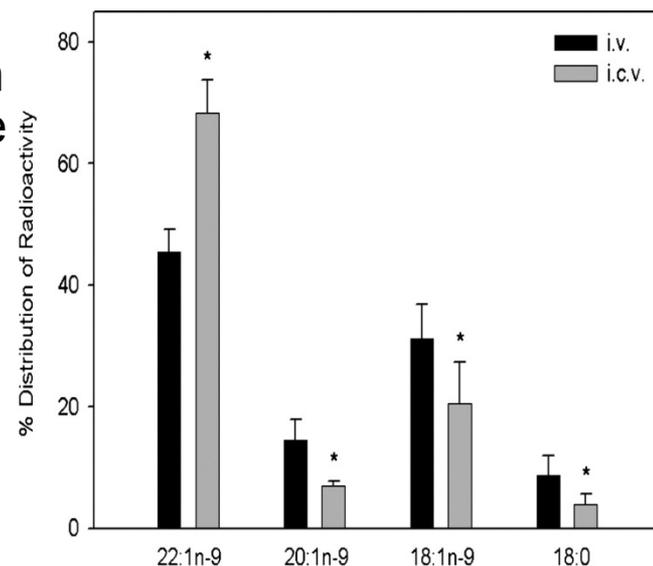


Effect on manifestations of ALD

- No effect on childhood cerebral disease
- Adrenomyeloneuropathy – no definitive answer
 - Cappa et al (1990)– cerebral demyelination in only 2/11 treated individuals
 - Kaplan et al (1993)– VEP did not improve despite therapy
 - Aubourg et al (1993) (n=24); varying phenotypes including cerebral disease, boys, and heterozygotes; 9/14 men worsened
 - Van Geel et al (1999) (n=22); varying phenotypes including heterozygotes; generally progress
- All of these studies were uncontrolled
- Small number of individuals studied with a wide range of ages, disability, and phenotype
- Limited information on compliance and effective reduction of VLCFA
- In spite of the poor design of the clinical evaluation, the lack of clear improvement led to the presumption that oil was ineffective in all forms of ALD.

Erucic acid entry into brain

- Poulos et al (1994)
 - Unable to detect any changes in the brain indicating that little erucic acid crossed the blood brain barrier
 - Limited value in correcting the accumulation of saturated very long chain fatty acids in the brain
- Rasmussen et al (1994)
 - 4 treated, 7 untreated
 - 1 out of 4 patients had decr VLCFA in brain
 - Erucic acid was not detected in brain
- Golovko and Murphy (2006)
 - Showed that it did cross the blood brain barrier in rodents and was rapidly metabolized



Distribution of infused [14-14C]22:1n-9 (i.v. or i.c.v.) among different n-9 family fatty acids, demonstrating the metabolism of the tracer via chain shortening

X-ALD Lorenzo's Oil Prevention Study in Boys: Rationale

- Saturated VLCFA (C26:0) excess
 - Principal biochemical abnormality
 - Contributes to pathogenesis
 - LO normalizes plasma VLCFA without serious adverse events
- Open trial
 - Placebo-controlled study not feasible
 - Disease severity
 - Concern about equipoise due to biochemical effect

X-ALD Lorenzo's Oil Prevention Study

Association between development of abnormality and change in plasma VLCFA

- Asymptomatic boys with normal MRI at baseline (n=89)
- Treated with Lorenzo's oil and reduced fat diet
- Followed by clinical exam, MRI, and neuropsych testing
- Mean C26:0 reduction in treated group: 0.49 $\mu\text{g/ml}$
- Serial plasma C26:0 curve in each of the boys was plotted against time (months of follow-up). Length adjusted area under the C26:0 curve was calculated to assess mean C26:0 levels over time.

Thus LAUC ($\mu\text{g/ml}$) is another representation of mean C26:0 over time.

Overall Clinical Outcome: 89-Member Study Group

Table 2. Overall Clinical Outcome: 89-Member Study Group

Characteristic	No. (%)
Living	81 (91)
Deceased	8 (9)
Neurologically normal and normal MRI results	66 (74)
MRI abnormalities and neurologically normal	13 (15)
MRI and neurological abnormalities*	8 (9)

Abbreviation: MRI, magnetic resonance image.

*Two patients had missing MRI results and had developed neurological abnormalities.

Moser, H. W. et al. Arch Neurol 2005;62:1073-1080.

Preventative Study Results

- The association between the LAUC and the development of MRI abnormalities in asymptomatic patients with ALD suggests that long-term reduction of C26:0 levels reduces the risk of developing brain MRI abnormalities in asymptomatic boys with ALD
- The most recent year of C26:0 observations did not show this significant association
- Time weighted estimate of average plasma C26:0 over study period (LAUC) was significantly associated with risk reduction
 - **0.1 µg/ml reduction of plasma C26:0 LAUC reduces risk of cerebral X-ALD by 36%**
 - **Two-fold or greater reduction of risk feasible**
- Long-term elevation of the plasma C26:0 level is more deleterious, and hence long-term lowering of plasma VLCFA levels should be more beneficial
- **Substantial and prolonged lowering of C26:0 levels may be required to achieve significant reduction in risk of developing MRI abnormality**

Limitations of interpretation

- Follow-up period was relatively short
- Limited understanding of the factors that cause the profound differences between the inflammatory cerebral phenotype and the noninflammatory AMN phenotype
 - Over half of them never develop childhood cerebral disease and thus for unknown reasons appear resistant to this phenotype

Preventative therapy in AMN and Carriers

- **Placebo-controlled Study**
 - Diet and either GTO-GTE or a placebo (soybean oil)
- **Planned enrollment 120 men with “pure” AMN and 120 symptomatic carriers**
- **4 year duration**
- **Yearly evaluation**
 - **Neurologic exam**
 - **MRI of brain and cervical spinal cord**
 - **Nutrition**
 - **Quantitative Motion analysis**
- **Outcome measures**
 - **Clinical status – Kurtzke FSS, EDSS, AADS**
 - Correlated with reduction in C26:0 levels
 - **Use of Quantitative functional measures and MRI markers**
 - **Development of cerebral disease in men**

Study Interruption

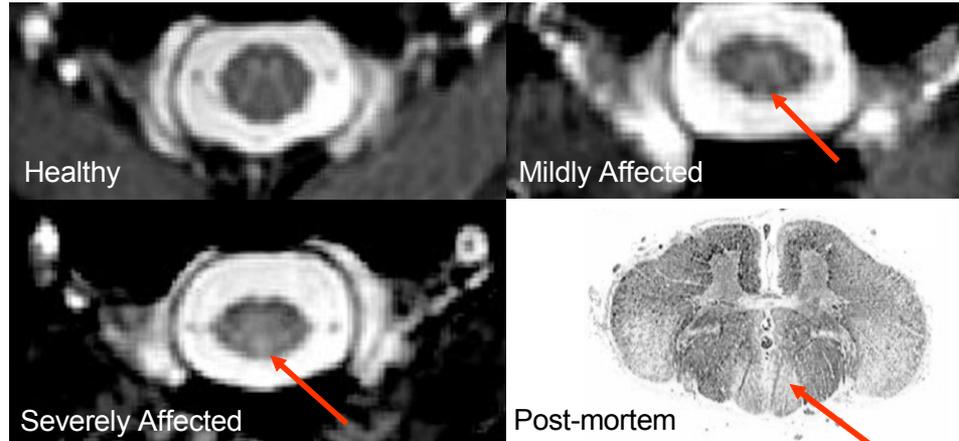
- DSMB on October 31, 2008 delivered a formal recommendation to terminate the study
- Decision made to halt new enrollment and treatment and consult with sponsors
- Issues
 - (1) inadequate study recruitment and retention
 - (2) efficacy/ futility
 - (3) safety concerns of the soybean oil placebo
- Ultimately accepted recommendation

Interim Analysis

- Clinical benefit to the patient remains the primary outcome of the study
- Use of associated measures as outlined in the proposal
 - Spinal cord imaging
 - Physical measures gathered by the Motion Analysis Laboratory
- Division of treated individuals into groups based on C26:0 levels

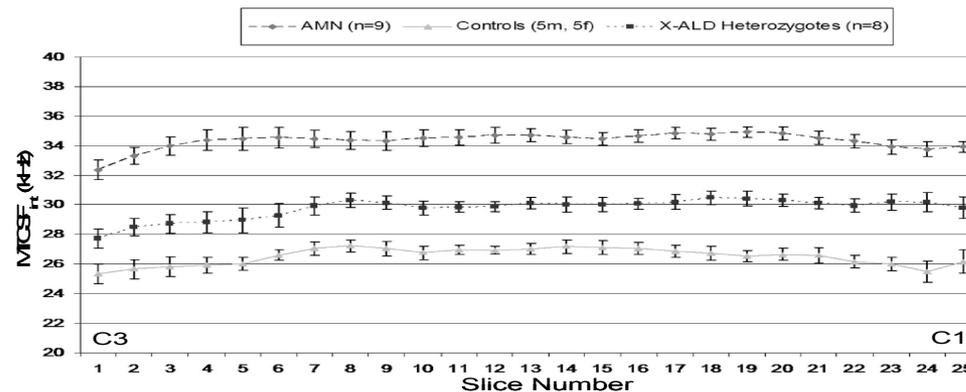
Interim Analysis

Magnetization Transfer Imaging



MT weighted Images

Post-mortem Reprinted with permission of Powers J, et al.

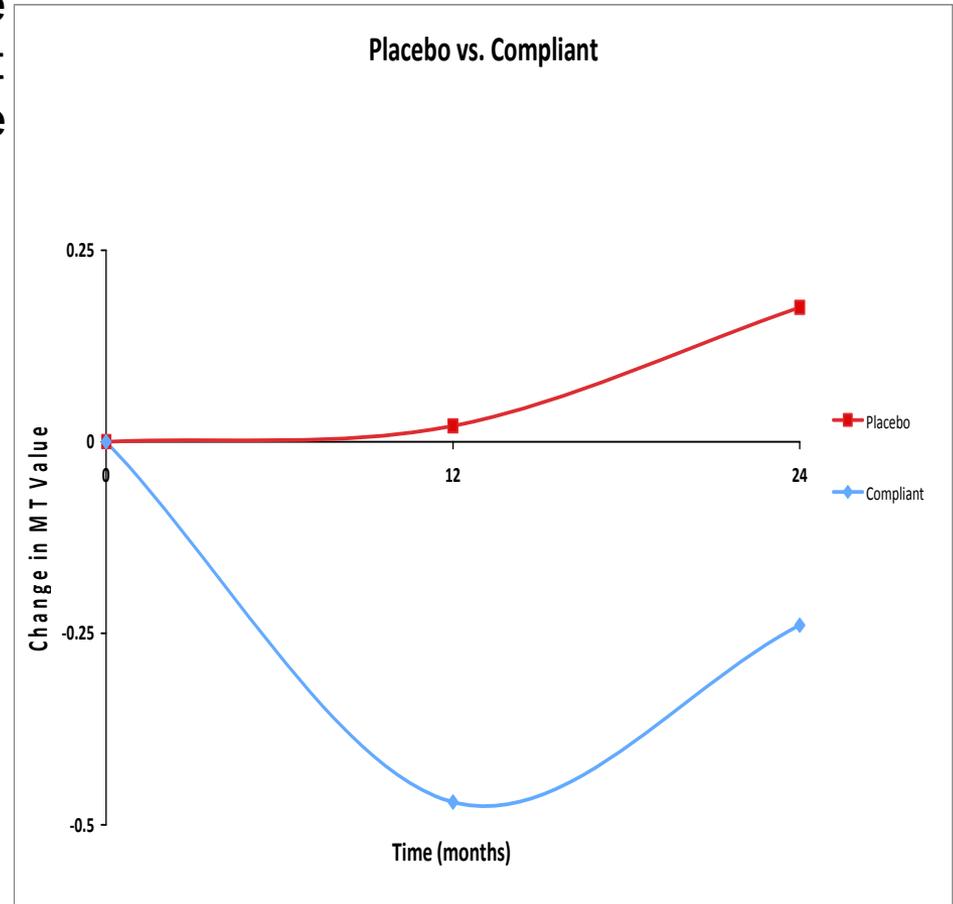


Diagnosis (mean \pm SD)	AMN (34.34 \pm 1.03)	Heterozygotes (29.76 \pm 1.03)
Heterozygotes (29.76 \pm 1.03)	p < 0.001	
Controls (26.82 \pm 1.38)	p < 0.001	p < 0.001

Interim Analysis

Magnetization Transfer Imaging

- MT MRI data were obtained in the dorsal column for each participant at baseline, 12 and 24 month time points.
- Defined Δ MT as the difference in mean MT value between baseline/12 months and baseline/24 months
- Positive Δ MT indicates greater abnormality at the 2nd time point [reflective of worsening]
- Negative Δ MT indicates less abnormality at the 2nd time point [reflective of normalization]



Interim Analysis

Functional Measures

- Clinical Neurologic Measures
 - Kurtzke FSS and EDSS – Developed primarily for MS
 - Adult Adrenoleukodystrophy Disability Scale (AADS)
- Quantitative Motion Analysis
 - Multiple measures of strength, balance, and sensation
 - Functional measures of walking
 - “Get up and Go”
 - Requires subject to stand up from a chair, walk a short distance, return, and sit down again.
 - Reliable and valid test for quantifying functional mobility

Get up and Go

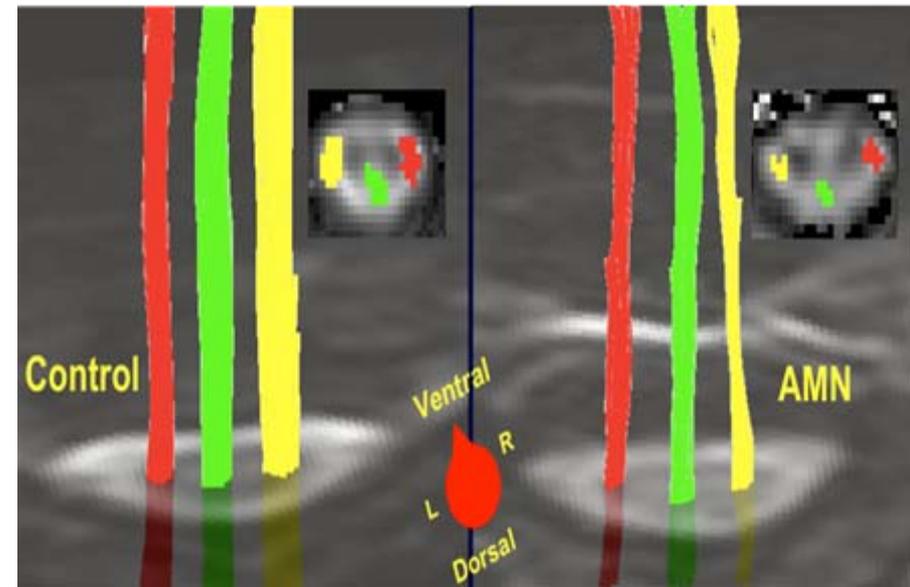
- The odds of functional walking improving 6 times more likely in the compliant group than in the placebo group.
- The relative risk of improvement was 1.7 with 95% confidence intervals of 0.58-61.85
- N for compliant was 7 and placebo 16

Conclusions of analysis

- The proportion showing improvements in MT imaging and functional status were greater in compliant v. placebo at year 1 and 2, but not statistically significant
- Should be sufficient power at year 2, but not at year 1.
- Plenty of caveats
 - Tendency for estimates of conditional power to increase as result of imputing the test statistic for a larger proportion of the study population
 - Unable to be certain that observed trends will continue
 - No corrections were made for multiple comparisons
 - They had issues with compliant versus noncompliant
 - **Intent to treat certainly washes out any trends**

Future Directions

- Presently completing analysis
- Plan to redesign a study around this analysis
 - Improved functional measures
 - Quantifiable MRI parameters
- Lowering of C26:0 will be a primary outcome



Observations – Lorenzo's Oil

- After ~ 20 years – still don't know for certain what role VLCFA or Lorenzo's oil has in ALD
- However, if past experience is any indication, Lorenzo's oil will not be going away anytime soon
- Relatively low risk, inexpensive, and technologically simple therapy
- With newborn screening, numbers of individuals who are asymptomatic will be increasing

Observations – Clinical Trials

- Controlled studies in well-defined populations are worth the effort
- Natural history comparisons may be useful
- Carefully developed functional measures are extraordinarily important
 - Walking measures in AMN
- Surrogate or Biomarkers need to be respected
- New MR imaging modalities may allow shorter trials

Acknowledgements

- Patients and their families

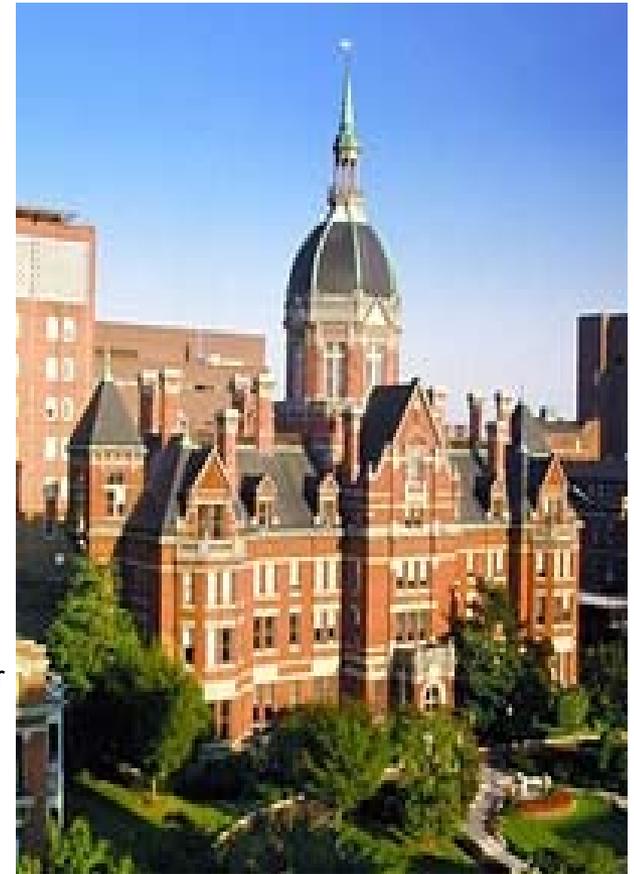
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In Memory of Hugo Moser
(1924-2007)



Efficacy of Lorenzo's oil

Safety concerns prompted interim analysis

- Efficacy
 - Clinical measures at year 1 and 2 were not significantly different between treated and untreated
- Futility
 - No strong argument to support continuing the trial based on the chance that it might show the LO group to be superior to the placebo.
- The DSMB also states that they examined the “C:26” levels and found a decrease in the majority of participants.
- DSMB would not examine the correlation with lowering VLCFA stating it was not a designated outcome.

Initial Response to Efficacy concerns

- Multiple studies of Lorenzo's oil that show that it does not affect the course of AMN in one year's time.
- No correlation made with effective reduction of C26:0 levels
- Confirmation of these facts was not what we set out to do

Proposed actions and issues presented to sponsor

- Safety
 - Not really a concern, but would monitor
- Efficacy
 - Evidence that reduction of C26:0 was beneficial
- Recruitment and Retention
 - Reasons were clearly known and were being addressed
- Issues
 - Retention of present participants while study is suspended
 - Concern for additional interruptions
 - Dealing with interruption in treatment analysis