

Summary of the Workshop on Translational Research Priorities for Infantile (CLN1) and Late Infantile (CLN2) Forms of Batten Disease

A workshop was held on November 11-12, 2010 on the topic of research priorities in Batten Disease. The primary focus of this workshop was the CLN1 and CLN2 forms of the disease. Leaders in the field were invited to participate together with scientists from other fields whose technology might be brought to bear on translational research opportunities in Batten Disease. During the course of their presentations, scientists expressed palpable enthusiasm for collaborative interactions and potential research opportunities in the field. Extensive discussion took place in three sessions, during which time gaps in current scientific knowledge and strategies to fill these gaps were identified.

A survey of conferees was conducted at the end of the workshop. Twenty-four respondents answered each of four questions (list top three areas for further research; list three areas of cooperation among different fields; list three observations learned during workshop; and should this workshop be repeated at a specified time period). Remarkable consistency and themes emerged regarding research priorities, serving as a basis for recommendations that are summarized below. All 24 respondents felt that the workshop should be repeated. Eighteen respondents felt that the workshop should be repeated in two years, four respondents in one year and two respondents in three years.

1. Clinical registry, tissue bank, sensitivity testing, unified clinical rating and biomarkers.

Today, registries of cases of Batten Disease are maintained in several EU countries and the US. Essential data elements of all current registries should be shared. A single, complete and accurate worldwide patient registry is urgently needed. In the absence of such a registry, a US registry should be developed as soon as possible.

Impressive progress has been made by the BDSRA in identifying and cataloguing incident cases of Batten Disease in the US. However, tracking of clinical course of disease is currently incomplete. A clinical registry should include not only demographic and clinical data but also a tissue bank and the means to perform and resolve logistical issues regarding high-throughput in vitro sensitivity testing of already developed drugs. A complete and accurate registry should include patient contact information, updated clinical information and regular clinical assessment at one site or a limited number (2 or 3) of sites. Biological samples are currently collected and stored at several laboratories located throughout the US. Data elements maintained at tissue collection sites should be shared, operations and procedures should be standardized, and information should be centralized at the BDSRA.

Although clinical phenotypes have been identified, the natural history of Batten Disease requires better definition. Unfortunately, multiple scales are used to gauge clinical disease activity, while rigorous studies and reports in the literature have been limited regarding the natural history of INCL and LINCL. Development of a unified clinical rating scale will be critical to assurance of comparative, high quality data across efficacy studies.

Biomarkers for CNS toxicity should be identified and validated as signals that predict outcome. Chemical markers of neuronal health, imaging markers of the brain (such as ventricular size, grey matter volume, etc) and whole brain spectroanalysis are needed. Biochemical markers may

be used to improve the power of clinical studies, gain knowledge about mechanism of disease and optimize drug development. Examples of biomarkers that have been useful in other neurological disorders include T-gangliosides, GFAP, P-Tau and NFL. Age-specific levels of such biomarkers should be applied to individuals with Batten Disease. Elucidation of primary substrates and characterization of lipofuscin are required for the development of markers that assess tissue load of substrates whose presence triggers apoptosis.

2. Biology and pathophysiology of Batten Disease.

A better understanding of the molecular biology of Batten Disease is required. Proteins have been identified for only three of the nine types of Batten Disease. Primary defects caused by Batten Disease must be distinguished from secondary consequences. For example, inflammation is a commonly observed pathological finding but whether it is a cause or effect of clinical sequel is unknown. A better understanding of types of aberrant splicing and identification of targets of splicing are required to advance the field. Functions of proteins involved in the pathogenesis of Batten Disease must be better defined. Antisense mediated correction of aberrant splicing is potentially useful in treatment of Batten Disease. Recently, small oligomers (5-mer oligos) have been found to stimulate the correction of an exon-skipping mutation. Unlike larger oligomers, the 5-mer may cross the blood brain barrier. Issues regarding this approach include the need to create a customized oligo for each patient and the need to perform safety studies on each oligo.

3. Efficient delivery systems for CNS therapy.

Successful management of Batten Disease requires treatment of both peripheral tissues and the central nervous system. Delivery of genes, proteins, small molecules and stem cells of varying potentiality to the CNS requires optimization for the treatment of neurological manifestations of the disease. Parenteral infusion of enzyme (i.e., enzyme replacement therapy or ERT) results in short-lived detection of enzyme in the CNS of mice. Nevertheless, a clinical benefit in vivo persisted for an extended period of time (i.e., 6-7 month) in these animals. A sensitive bioassay for enzyme should be developed to better understand the pharmacology of ERT.

Although strategies for molecular targeting of brain endothelial cells and ependymal cells have been developed, biological effects require definition. In vivo studies of vectors constructed with epitopes for these CNS cells should be conducted in canines, swine, sheep or non-human primates. Delivery systems such as minipumps may be useful in delivering PPT1 to the CNS. Recently, nanotechnological strategies have been applied to the treatment of fatal genetic diseases, including the lysosomal storage disorders. Polymers ranging in size from 100 to 300 nanometers in diameter may be designed to carry hydrophilic or hydrophobic cargo to the CNS. Advantages of this technology include the potential for controlled release of pharmaceuticals. Application of nanomedicine strategies to the treatment of INCL and LINCL, using molecular targets may provide a potentially powerful strategy to treat these diseases.

4. Clinical trials using gene therapy or stem cell therapy.

Clinical trials using gene therapy or stem cell therapy. Gene therapy is used today in a second phase 1 clinical trial of children with LINCL. This trial uses direct intracortical injection of vector constructed with the AAVrh.10 serotype, a vector/serotype that in non-human primates has increased spread throughout the cortex at 90 days after injection compared to prior vector/serotypes. Neurologists conduct blinded clinical evaluations to objectively assess outcomes, using each patient as his/her own control. In addition, clinical markers are used,

including level of n-acetyl-aspartate, MRI and whole brain spectroscopy analysis, in an attempt to assess response to therapy.

New vectors are needed in order to achieve even higher levels of global CNS delivery. Results of studies of AAVs suggest that an adequate amount of virus may be obtained by parenteral (10^{13} particle units), intracisternal (10^{12} particle units) or intrathecal (10^{11} particle units) injection, and that self-complementary AAV may be advantageous. Distribution throughout the CNS in primates was demonstrated. Additional research is needed to identify optimal vectors and determine the most appropriate delivery system for genes.

Results of a phase 1 trial using neural stem cell therapy were presented, and outcomes were reviewed. Phase 1b studies are now underway for children of age 6 months to 6 years who have INCL or LINCL. According to literature from Stem Cells, Inc, preclinical studies in a mouse model of INCL has shown that purified human neural stem cells can be directly transplanted into the central nervous system (CNS), after which they engraft, migrate, and differentiate into neurons, astrocytes, and oligodendrocytes. Questions to be addressed include proof of in vivo differentiation, growth and migration of neural stem cells that are placed in the CNS of humans. Results are still pending on the initial study regarding success of these measures.

5. Novel approaches to treatment.

Data were presented on several potential therapeutic approaches to INCL and LINCL, including nonsense suppression, using compounds that permit exon skipping and/or stop codon readthrough. Reagents such as gentamycin/G418, amikacin and ptc 124 may promote readthrough by up to 10% by inhibiting the termination reaction. Poly-l-aspartic acid reduces toxicity from gentamycin and enhances readthrough. Other unique approaches to treatment may include the use of statin therapy (as means of inducing NOS), administration of corticosteroids (to modulate the immune reaction) and administration of glutamate receptor targeting compounds (to modulate apoptosis) and treatment with macrolides or lithium. Testing for efficacy of these pharmaceuticals requires adequately powered studies of matched test and control groups, and assessment of objective outcomes measures. In addition, mechanistic studies of antiepileptic and anesthetic agents are needed to better understand the full potential of clinical therapies in current use.

6. Combination therapy

Results of preliminary studies suggest that combination therapy may be more effective than individual therapy in animals treated in vivo. In addition to improved motor function, animals treated with gene therapy, ERT and Cystagon had a greater increase in enzyme level, decreased storage material and improved motor function and overall survival, compared to animals treated

with each of these therapies alone. In other preliminary studies, the combination of gene therapy with bone marrow transplant resulted in a synergistic increase in survival. The impact of combination therapy (i.e., gene therapy, ERT, substrate reduction and/or stem cell therapy) should be investigated in large animal models.

Summary of Recommendations.

Exciting results were presented at this Workshop that may be applied to new treatments of INCL and LINCL. During the meeting, several spontaneous collaborations were initiated among the conferees. In particular, interactions emerged among researchers in the field with scientists in other fields (including nanotechnology, AAV vector development and molecular biology). These encouraging signs suggest that now is an appropriate time to issue a **request for applications** for funding new and unique proposals in order to:

- ◆ Develop an accurate and complete patient registry that includes demographic and clinical data, a tissue repository, capability of high throughput drug sensitivity testing, and robust assays to assess biological activity.
- ◆ Derive a unified clinical rating scale for use in clinical trials;
- ◆ Identify appropriate biomarkers of progression of disease;
- ◆ Characterize storage material (i.e., lipofuscin);
- ◆ Optimize non-invasive delivery of proteins, genes, small molecules and stem cells to the CNS;
- ◆ Improve gene expression after in vivo treatment;
- ◆ Determine biological effects of transplanted stem cells in vivo and examine other stem cell populations such as pluripotential stem cells;
- ◆ Evaluate novel approaches to therapy, including nonsense suppression, glutamate receptor targeting compounds, statin therapy, steroid therapy and antisense mediated correction of aberrant splicing, macrolides, lithium and molecular targets in the NO pathway;
- ◆ Perform mechanistic studies of pharmaceutical agents in current use that ameliorate symptoms, including antiepileptic and anesthetic agents;
- ◆ Conduct in vivo studies in large animals to assess molecular targeting and to determine whether combination therapy is more effective than single modality therapy.

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