

Scientific Meeting on
Metachromatic Leukodystrophy
Soho Hotel, London
December 2005

The Scientific Meeting on Metachromatic Leukodystrophy was held at the Soho Hotel in London on December 17th, 2005, and was jointly sponsored by the United Leukodystrophy Foundation, the Myelin Project and the Kemp Family. This meeting was a follow-up to the MLD Initiative Meeting in May of 2005 that had been sponsored by the Myelin Project. Dr. Hugo Moser thanked the Kemp family for their generous support and imparted the sad news of the death of Dr. Krivit. Dr. Krivit had been a pioneer in the field of bone marrow transplantation and an inspiration in developing new therapeutic strategies for diseases such as MLD.

At the prior meeting in May it was evident that the advances toward new therapies were proceeding more rapidly than the clinical community had been aware of. The purpose of the current meeting was therefore to focus on the clinician's response to the impending clinical trials. The goals of the meeting were to review the clinical research on MLD and discuss possible clinical trials. This document is a summary of the presentations (A), discussions (B), and recommendations (C) of the meeting.

A. Presentations

Dr. Hugo Moser from the Kennedy Krieger Institute, Baltimore, presented a review of the natural history of all published MLD patients (approximately 200 cases). Plots of the age of onset showed the striking preponderance of the late infantile form of the disease. Further peaks of onset were concentrated in the juvenile period and adult period. (One adult patient with MLD developed his first symptoms at the age of 60.) The Kaplan- Meier survival curves showed a striking difference between the early and late onset of the disease. There is a rapid progression and early death in the late infantile form of the disease. In some adolescents and adults the duration of the illness can last up to 40 years. Further, among the later onset cases there were four in whom peripheral neuropathy developed without CNS involvement and four with severe CNS manifestations lacking peripheral signs. Hence marked variability in the peripheral nerve findings is found in the adult patient. Dr. Moser emphasized the wealth of material available in the literature for the study of the natural history of MLD.

Dr. Arvan Fluharty from the UCLA Mental Retardation Research Center, Los Angeles, presented a compilation of the mutations of the arylsulfatase A gene. He listed more than 100 mutations but emphasized that this compilation is only a limited sample. It is very Eurocentric with no data from Africa and little from South America and Asia. Overall, only 50-60% of all known cases have defined mutations. It is of great importance to pursue mutation analysis, since (1) some mutations predict severity of phenotype, (2) misdiagnosis due to pseudodeficiency can be avoided, and (3) with more data a clearer genotype-phenotype correlation might emerge.

Dr. Fluharty has established a system that involves sequencing 6 sections of the gene in one sequencing run, each one of these covering approximately 400-500 base pairs (at least one exon

and all the introns). There is one report that it can be done in a single segment but this is prone to error. Quality control is partly ensured by the fact that the gene is sequenced in both directions. Overall, due to the short length of the gene sequencing does not pose great technical difficulties.

A major limitation, however, appears to be the cost of sequencing the gene. In his experience Dr. Fluharty has found that families are not able to pay more than \$ US 500 for mutation analysis. He calculated that the minimum cost for sequencing, not including the cost of a technician, is \$ US 350. [Dr. Kolodny pointed out that currently the entire set of analyses (testing of the entire family as well as prenatal diagnosis) costs \$ US 1200 at his institution.] Perhaps European centers that provide the analysis free of charge can assist the families in need. Concurrently, it is important to convince insurance companies of the necessity to perform this analysis, in particular within the United States.

Dr. Ingeborg Kraegeloh-Mann from the University Children's Hospital, Tuebingen, gave a presentation on the current techniques for assessing the general clinical status of MLD patients. The goal of the clinical assessment is to delineate the natural history, as well as the clinical course, following initiation of therapy.

For the infantile form of the disease developmental motor milestones appear most appropriate. Parents often reliably recall motor milestones and describe them with ease. Both gross and fine motor milestones have been standardized in the Gross Motor Function Classification System (GMFCS) and the Bimanual Fine Motor Function (BFMF) system. For cognitive function language milestones are the most reliable. The onset of language regression or loss of communication through language can easily be recorded. In less severely involved children the first occurrence of problems with attention and concentration and behavioral problems can be used. For patients with more severe involvement loss of visual fixation and communication can be used. Beyond the recording of function neurological signs can also be documented. This is more difficult but can be accomplished by careful consensus and definition (spasticity, dyskinesia, etc.). Ultimately, illustration of these symptoms by means of video appears to be the best way to build reliable consensus and collect comparable data.

Dr. Nicole Baumann from the Hopital de la Salpetriere, Paris, reported on the techniques for evaluating the cognitive status in adult MLD patients. She outlined a series of tests that could be easily performed in most countries and help overcome the difficulties in obtaining quantitative measures of cognition. These tests include the Mini Mental Status (MMS), memory tests for encoding and retrieval as well as the frontal assessment battery. Further it would be useful to implement the depression scale (Montgomery and Asberg) and search for common psychiatric pattern according to criteria of the DSM4 (diagnostic and statistical manual of mental disorders) for schizophrenia.

Dr. Sakkubai Naidu from the Kennedy Krieger Institute, Baltimore, presented current neuroimaging techniques in the study of MLD. She commented on similarities and differences to adrenoleukodystrophy and Krabbe. She pointed out that in MLD we lack a reliable scoring system for MRI abnormalities, as it exists for ALD (Loes scoring system). In the juvenile onset MLD cases MRI reveals frontal lobe disease and thinning of the anterior corpus callosum. The adult onset cases also show cerebellar atrophy but there is little clinical evidence for ataxia. A

fact that has often been neglected in the past is that there is prominent spinal cord involvement that today can be quantified using techniques such as magnetization transfer. Diffusion tensor imaging also allows for quantitation of abnormalities within specific tracts of the spinal cord. Little data on proton MR spectroscopy is available but this technique may be potentially important.

Dr. Maria Sessa from the San Raffaele Scientific Institute, Milan, reported on current techniques for evaluating peripheral nerve function in MLD patients. Data comes from the evaluation of a cohort of 24 patients with different forms of MLD. She outlined the conduction velocity methodology that allows differentiation of demyelination versus axonopathy. Peripheral neuropathy is observed in all late infantile patients; involvement of PNS is more variable in later onset patients. When present peripheral neuropathy is a demyelinating neuropathy. All late infantile patients have a severe polyneuropathy, while in later onset patients, if present, polyneuropathy is mild. Peripheral neuropathy remains quite stable a long time. All patients were sequenced and it has been observed that peripheral neuropathy is always associated with at least one severe mutation. It has been proposed that this observation might be useful for prognostic indication and also for the selection of patients for innovative therapies.

Dr. Arvan Fluharty presented biochemical and genetic procedures for the diagnosis of MLD. In the usual situation clinical evidence leads to a MRI indicating a leukodystrophy. Subsequent enzymatic assays suggest a possible deficiency of arylsulfatase A (ARSA). Due to the interference of ARSB in the most commonly used ARSA assays an ARSA enzyme activity in the 5-20 % range cannot establish or eliminate the diagnosis of MLD; therefore, one or more of the following additional tests are necessary: urinary sulfatide excretion, other evidence of sulfatide storage, such as metachromasia in a nerve biopsy, or molecular genetic testing of the ARSA gene.

The ARSA assay is usually performed in leukocytes but can also be done in cultured cells. It is also possible to perform on urine but it is not nearly as reliable and reproducible. A relatively cheap technique that is available in most clinical labs is the colorimetric assay. Differentiation of arylsulfatases can be performed by various methods of selective inhibition, differential thermal inactivation or separation of enzymes. Lastly, there is a wide range of arylsulfatase activity in fibroblasts within the normal population. MLD patients are easily differentiated but carrier states are not. A great overlap exists between carrier states and low enzyme individuals which today we term pseudodeficiency. For the practical purposes of a clinical trial the disease should be defined as arylsulfatase A deficiency. For diagnostic purposes it is necessary to demonstrate enzyme deficiency in leukocytes, show sulfatides in urine and also exclude pseudodeficiency by mutation analysis.

Dr. Charles Peters from the Children's Mercy Hospital, Kansas City, gave an overview of the results of hematopoietic stem cell transplantation (HSCT) in MLD. He emphasized that one of the main problems of bone marrow transplant in MLD is the relatively slow turnover of microglia in the setting of a rapidly progressive disease. Newer techniques such as transplantation of umbilical cord blood have become available but show a limited quality of enzyme activity and have not shown a benefit over bone marrow transplantation. Dr. Peters presented the bone marrow transplantation data for MLD from the University of Minnesota from 1984 – 2004. A

total of 37 patients with MLD had been transplanted: 3 with late infantile onset (age range 0.2-0.7 years), 19 with juvenile onset (age range 1.2-11.4 years) and 15 adult patients (age range 16.1-46.8 years). All patients had received matching donor stem cells and had undergone a preparative regimen. In late onset-MLD, cognitive function declined during the 1 to 2 years after stem cell transplantation but remained stable as long as 20 years thereafter. He concluded that HSCT arrests the course of the central demyelinating process in late onset MLD if treated early and that HSCT does not help the demyelinating peripheral neuropathy present in MLD.

Dr. Alfred Kohlschuetter from the University of Hamburg, Germany, gave a presentation on possible structures and problems of a central patient registry. He discussed the various known registries that are in use in Germany and France. These include the Leukonet (the German leukodystrophy network), GeNeMove (a German network for hereditary movement disorders), and the French HC Forum that currently already covers several genetic disorders. In these registries data is entered anonymously online and encoded in the process but visible to all authorized members of the registry. An international registry would pose challenges beyond those known on a national level. A central database has to be decided on to which each cooperating country and its medical centers contribute. On an international level the nature of the data, the intervals and strategies of evaluation have to be decided upon. Further, the participating physicians have to agree upon procedures of patient consent and the routes of establishing contact with primary care physicians and patient families. To address all these issues a MLD database task force will have to be created. This task force should also facilitate communication with local regulatory agencies.

Patrick Aubourg from the Hopital Saint-Vincent de Paul, Paris, gave a brief overview of new human therapies that will become available over the next five years. He presented proof of concept of various therapies in the ARSA deficient mice, in particular of enzyme replacement therapy (ERT), hematopoietic stem cell gene therapy and intracerebral gene therapy. ERT reduces sulfatide storage in PNS and improves PNS function. Unexpectedly, ERT also reduces sulfatide storage to a certain extent in the CNS – suggesting the possibility that ERT may also be of benefit to patients with CNS disease. Post- and cotranslational modifications as well as pharmacokinetics and pharmacodynamics will determine the optimal enzyme parameters.

Allogeneic stem cell transplantation has limited effect in the juvenile form of MLD and no effect in the infantile form of MLD. Bone-marrow derived macrophages/microglia overexpressing ARSA are more effective than wild-type cells at preventing neurodegeneration in ARSA deficient mice. Evaluation of HSC gene therapy is being undertaken in symptomatic ARSA deficient mice, including analysis of a dose-effect relationship. Safety issues remain related to overexpression of ARSA and insertional mutagenesis (lentivector). Intracerebral delivery of ARSA might be quicker and more effective in arresting the demyelinating process. In contrast to other lysosomal storage disorders, the site of vector injection can be guided by lesions seen at brain MRI. In ARSA deficient mice it has been shown that intracerebral injection of lentiviral or AAV vector can ameliorate CNS disease. Dr. Aubourg mentioned encouraging results with AAV in animal models of other lysosomal storage disorders.

In summary, the questions remain whether ERT will have long-term benefit for the CNS; one may envisage that ERT could be used in combination with other approaches. HSC gene therapy

shows a lag time between the transplantation of genetically corrected HSC and the replacement of microglia expressing ARSA in brain. Given the differential effect of the proposed therapies upon the central and peripheral nervous system, combining gene therapy with enzyme replacement therapy would be propitious.

B. Discussion

Natural history data on MLD

The meeting discussed the published lifespan history and questioned whether the literature was more pessimistic than real life experience. Furthermore, the severity of the phenotype can, to a limited extent, be predicted by mutation analysis. Several members of the meeting agreed that cases with longer survival rarely get published. The meeting emphasized the importance of continued collection of natural history. For the purpose of clinical trials the published natural history data alone may not be sufficient.

Issues regarding Gene Sequencing in MLD.

The consensus was reached that mutation analysis can and should be pursued as essential inclusion criterion for any clinical trial. Mutation analysis will be used for prenatal testing and early detection, vital information in light of possible future treatments. Occasionally patients with low arylsulfatase A activity get misdiagnosed as MLD. Urine sulfatides and mutation analysis are then necessary to establish the diagnosis. Further, severity of phenotype can be predicted by mutation analysis, an added reason to include mutation analysis in any future studies.

Strategies for sequencing the gene were discussed. The reliability of gene sequencing is enhanced by performing it in two segments and quality control is partly ensured by the fact that the gene is sequenced in both directions. Another possibility would be a modified skip reaction that allows exclusion of 10 of the most frequent mutations in a single reaction. The meeting decided that mutation analysis of all patients is feasible, if the funding can be arranged. Members of the meeting from Italy and France mentioned that the analysis is free in their countries. This analysis, however, only includes the patient and not the rest of the family. It was suggested that if the costs of sequencing cannot be covered by a family in the U.S., two or three centers in Europe can be set up to which the sample can be sent for analysis. An international listing of centers that can perform mutation analysis will be compiled. This will be of particular interest for physicians in the U.S. who are looking for a center that they can refer to, in case insurance coverage is denied and families cannot come up with the finances.

Neurologic symptoms and clinical parameters in MLD.

For the purpose of clinical trials the battery of tests will differ by age group and therapeutic trial. A proper developmental assessment for each age group will be necessary. Simple tests such as swallowing or a walking test may be of greatest utility in the setting of a clinical trial (simple functional markers). These measures should be simple enough that they are widely available and can be assessed within a short time period. The parameters gathered should take into account pertinent measures of the nervous system. Cognitive symptoms to be assessed in MLD will also differ by age group and the therapeutic trial envisioned. Simple functional markers of speech and

behavior may be of greatest utility for clinical trials. Newly developed instruments, such as the saccadometer, may be beneficial but have yet to be validated.

Neurophysiological parameters

Nerve conduction velocities are very useful both in the diagnostic phase and in the follow up. Brainstem auditory evoked responses (BAER) are altered in the early phase of the disease. The group from San Raffaele performed BAER in all their patients. Even if results seem variable, further analyses are necessary to evaluate the usefulness of BAER as surrogate end point in clinical trials. Somatosensory and motor evoked potentials may be helpful for spinal cord evaluation.

MR imaging parameters in MLD.

Given the prominent CNS involvement in MLD, imaging studies will be an essential component of any clinical trial. MR imaging studies, particularly for volumetric data, should be performed. This can easily be accomplished with conventional MR imaging techniques available in most academic centers. More advanced imaging techniques (MR spectroscopy, magnetization transfer and diffusion tensor imaging) will not be used as primary markers but may become surrogate markers at a later point. Improved surrogate markers may enable trials of shorter duration in the future. Unlike ALD, contrast enhancement will less likely yield helpful information.

Biochemical parameters.

In the setting of a clinical trial levels of enzyme activity can be assessed in leukocytes. Urinary sulfatides can also be quantified but may reflect renal function more than anything else. Perhaps measurement of arylsulfatase A in the cerebrospinal fluid is a marker that can be followed during the trial of intracerebral gene therapy. If a biochemical marker is used, then its relationship to clinical endpoints should be demonstrated.

Central Patient Registry.

An international registry is necessary. National responsibilities shall be assigned and several members of this meeting have volunteered to be representatives. Already established national databases (e.g. cerebral palsy and Fabry database) can provide contact information and possibly be used as role model. These databases should collect disease specific information and be open to extension/addition. A task force will be directed by Dr. Kohlschuetter. Initial demographic and socioeconomic data will be gathered. Interaction with regulatory agencies in each individual country will be guided through this task force. Problems with data acquisition and storing will be addressed. To this end a questionnaire will be generated and goals will be addressed in a stepwise fashion and communicated regularly with members of this meeting. In order to accomplish this funding will be necessary. Through a physician committee similar to the German model requests for funding may be generated. The myelin project and pharmaceutical partnership will be of assistance.

Ethical issues

Patient anonymity will be guaranteed within the MLD registry and information will be encoded and not contain patient identifying information. Issues arise regarding inclusion of healthy siblings in the registry. How can this information be included, since healthy siblings frequently

do not seek out physicians? Further, how can this information be kept anonymous? Perhaps the patients themselves can be the source of the information. Together with the ULF and the Myelin Project, the task force will address this issue. Anonymous data acquisition will not require registration. Coded access can be given to individual physicians to look up their patient information. Further, the meeting decided that given the progressive course of the disease it would not be ethical to include a placebo arm in the clinical trials.

Selection of patients for new human therapies

Enzyme Replacement Therapy:

- Infantile patients who have been recently diagnosed (within 3 months), not patients who are vegetative or tetraparetic.
- Juvenile patients who are not candidates for bone marrow transplantation (with or without peripheral nerve involvement), but who have been recently diagnosed.
- Transplanted juvenile patients
- Asymptomatic patients who are not eligible for gene therapy (e.g. patients whose parents refuse HSCT / intracerebral gene therapy)

Given the high variability in the clinical presentation, the adult patient is a less suitable candidate for initial assessment of therapeutic efficacy. If enough patients can be included within the next two years, then at that point adult patients could be included as well. (The compassionate use program may apply to adults not currently included in this study.)

Gene Therapy (hematopoietic stem cell therapy and intracerebral):

Asymptomatic late infantile patients will be studied.

Asymptomatic or nearly asymptomatic patients will be included. It has been suggested that the patients should not be older than 6 months for the HSCT and not older than 12 months for the intracerebral gene therapy. It could also be a juvenile patient diagnosed at an early stage who is a BMT candidate without a donor or a patient whose parents refuse BMT. In either case the patient should not have overt clinical symptoms. Performance of the intracerebral gene therapy would likely entail immunosuppression followed by local injections after creation of burr holes.

Given the differential effect of the proposed therapies upon the central and peripheral nervous system it seems ideal to combine gene therapy with enzyme replacement therapy.

Safety issues of new human therapies.

In the animal model enzyme replacement at a dose of 20mg/kg for 4 weeks has been well tolerated. The dose in humans will be much lower and the only known risk is that of anaphylaxis. This is currently being evaluated in animal models.

Regarding HSC gene therapy, with vector production having been started, it is anticipated that the first patient will be treated by the end of 2007. According to the Italian regulation the observation period after treatment is anticipated to be 8 years. Safety concerns for the lentivirus vector are currently being addressed. In France regulations are in place that three patients have to be treated for an 18-month period each before other patients can be treated. The use of the adenovirus vector has already been approved but issues will arise regarding the intracerebral injections. This is currently being evaluated in the monkey model.

Safety concerns for the lentivirus vector are also currently being addressed. The use of the adeno-associated virus vector has already been approved but issues will arise regarding the intracerebral injections. This is currently being evaluated in the monkey model. With vector production having been started it is anticipated that for hematopoietic stem cell therapy, the first patient will be treated by 2007. The observation period after treatment is anticipated to be 8 years, in fact for life in hematopoietic stem cell therapy in which an HIV1-derived lentivector is used

Endpoints of Trials.

Primary endpoint of the ERT study would be safety. Secondary endpoints would encompass biochemical and clinical measures. Both peripheral and central nervous system function could be assessed. Endpoints of the gene therapy study would be the same as those for the enzyme replacement study. Primary endpoints would be safety and secondary endpoints would be biochemical and clinical. Perhaps measurement of arylsulfatase A in the cerebrospinal fluid could be a marker that would be followed.

The meeting decided that the specific clinical, imaging and biochemical parameters had to now be decided upon for each of the proposed clinical trials.

International Communication and Collaboration.

An international task force will continue the collaborative initiative. Further, the meeting decided that this information should be disseminated to various parent organizations. This should include the United Leukodystrophy Foundation in the U.S., The Myelin Project, the French Lysosomal disease association (Vaincre les Maladies Lysosomales, VML) and the lysosomal organization Global Organisation for Lysosomal Disorders (GOLD) based in London. In Italy, parents of leukodystrophy patients are starting to be organized and could also be a point of contact. The meeting felt that in disseminating information there was the risk of creating unrealistic expectations. It was decided that undue expectation could be met through responsible regular reporting. The immediately applicable shall be differentiated from the long-term goals and expectations.

C. Recommendations

1. The participants recognized that basic research has advanced to the point that new therapies for MLD will become available within the next few years. Members of the meeting realize the urgency in addressing implementation of these therapies and recommend continued regular meetings to provide the clinician's response to the impending clinical trials.
2. The participants advocated newborn screening for MLD based on the novel therapies available. It will likely take 2-3 years to validate newborn screening, and hopefully by that time therapies will have evolved further to justify such a screening programme.
3. The participants agreed that for any clinical trial protocol it would be necessary to have data regarding the mutation analysis. Mutation analysis not only provides more accurate diagnosis but also enhances our understanding of the genotype phenotype relationship in MLD. The meeting recommends that mutation analysis be an inclusion criterion for any future clinical trial.

4. An international task force under the direction of Dr. Kohlschütter has been established. The goals of this task force are to establish an international MLD registry and gather demographic and socioeconomic data. The task force will regularly communicate with members of the meeting. The meeting recommends that the task force meet within the next three months.
5. Select patient populations will be identified for the initial clinical trials of enzyme replacement therapy and gene therapy. Primary endpoints of these trials will assess safety issues. Secondary endpoints will assess the effect upon clinical, biochemical and imaging parameters. The meeting recommends that the task force achieve closer definition of the secondary outcome measures based on the information provided at this meeting.
6. Pursuit of new therapies for MLD will require additional funds. Funding will be needed to support mutation analysis and for the establishment of an international MLD registry. The meeting recommends that the task force address these financial issues and report back to members of the meeting.
7. Participants placed strong emphasis on dissemination and transparency of information. This information will not only be exchanged with the international members of the meeting but also with parent organizations throughout the world. They recommend responsible regular reporting of progress in the field. This is intended to create realistic expectations regarding the prospects of therapy in MLD.
8. It should be noted that these are recommendations from one panel of experts. In order to avoid misunderstandings, it has to be clearly stated that scientists must also meet the requirements of the regulatory authorities from their own countries and from the scientific committees evaluating the proposals, particularly in regard to issues such as inclusion criteria, safety and efficacy parameters for clinical trials.