



# 2012 ELA Families / Scientists meeting

March 31 & April 1, 2012  
Paris, France

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During the 2012 ELA Families / Scientists meeting, patients and families of the association met and exchanged with 41 international experts invited to present in lay language their research work on leukodystrophies and myelin repair. This year, 8 workshops on diseases, 3 discussion groups and a forum were organized. In this report, you will find the abstracts of the diseases' workshops.



## Summary

### Diseases' workshops

- Workshop on ALD/AMN\*
- Workshop on Refsum disease
- Workshop on MLD\*
- Workshop on Krabbe disease
- Workshop on CACH/VWM\* syndrome, Alexander disease, MLC\* and Canavan disease
- Workshop on PMD\* and other hypomyelinating leukodystrophies
- Workshop on undetermined leukodystrophies
- Workshop on Aicardi-Goutières syndrome

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\*ALD : AdrenoLeukoDystrophy ; AMN : AdrenoMyeloNeuropathy ; MLD : Metachromatic Leukodystrophy ; CACH/VWM : Childhood Ataxia with Central nervous system Hypomyelination / Vanishing White Matter ; MLC : Megalencephalic Leukoencephalopathy with subcortical Cysts ; PMD : Pelizaeus-Merzbacher Disease.

## Diseases' workshops

### Workshop on ALD/AMN

#### *Clinical trials*

#### **Gene therapy for adrenoleukodystrophy: results from the clinical trial**

**Dr. Nathalie Cartier-Lacave (France)**

Hematopoietic stem cell transplant from a donor is the only effective treatment for cerebral forms of adrenoleukodystrophy when performed at an early stage of the disease and when a compatible donor (of bone marrow or umbilical cord) is available. As soon as the gene was cloned, our objective was to correct the patient's own hematopoietic stem cells using a viral vector in order to offer an autologous transplant of the corrected cells. The HIV virus, the only virus able to effectively correct bone marrow cells, was used. Our preclinical results allowed us to obtain the authorization from the French Medicine Agency (ANSM) to treat 4 children suffering from cerebral ALD using gene therapy. Their CD34+ cells were collected and corrected using an HIV-ALD vector and then re injected when all the safety tests were validated. The treatment was perfectly tolerated. We found in the children's blood a stable percentage of leukocytes (white blood cells) expressing the normal ALD protein. All the safety tests conducted to date are satisfactory. In the first 2 children treated, the clinical and radiological courses observed over the 5 years' follow-up was similar to that observed after transplantation of normal donor cells. The results are encouraging and show that gene therapy can stabilize cerebral ALD. Our objective is to follow up this study by conducting a second study including a larger number of patients with cerebral ALD. Production of sufficient amounts of clinical-grade vector currently represents the major issue in terms of availability, quality and cost. It is our priority.



#### **Bone marrow transplantation for AMN patients with cerebral involvement**

**Pr. Wolfgang Köhler (Germany)**

Adrenomyeloneuropathy (AMN) as the most frequent adulthood phenotype of X-linked Adrenoleukodystrophy (X-ALD) is clinically defined by a chronic progressive spastic paraparesis, sensory disturbances and bladder dysfunction. The disease is characterized by an inborn error of metabolism of very long chain fatty acids (VLCFA). The development of neurological symptoms is caused by the toxic influence of elevated VLCFA and oxidative stress leading to axonal nerve damage and myelin degeneration. About 5-10% of adulthood X-ALD patients primarily present with cerebral inflammatory brain involvement right from the beginning of their disease. Another 20 (to 50%) of patients with the originally non-inflammatory AMN variant develop inflammatory disease later in their clinical course. Cerebral inflammatory brain involvement always is associated with severe deterioration of clinical symptoms and prognosis. Experiences from hematopoietic stem cell transplantation (HSCT) in childhood inflammatory disease demonstrates progression-free survival rates of up to 80% in HSCT treated boys. Early treatment results in adult patients failed to demonstrate similar good results, partly because neurological symptoms and extensive brain involvement already was present at treatment



initiation. In our current pilot project we therefore re-defined adult patients who should be eligible for HSCT by clinical and MRI criteria. First results from this treatment strategy show that general risks and survival rates, similar to those in childhood ALD are possible. HSCT therefore appears to be a new and promising treatment option for patients at early stages of adulthood cerebral disease.

### **Clinical trial in AMN patients: Validation of oxidative stress biomarkers, efficiency and safety of a cocktail of antioxidants**

**Dr. Stéphane Fourcade (Spain)**

X-linked adrenoleukodystrophy is a neurodegenerative disease due to the loss of function in the peroxisomal protein ABCD1. The most frequent phenotype is adrenomyeloneuropathy (AMN) which is characterized by axonal degeneration in the spinal cord. This gives rise to spastic paresis and peripheral neuropathy. Currently, there is no effective treatment for AMN.

Over the 5 past years, we demonstrated the fundamental role played by oxidative stress in the neurodegenerative process leading to the disease. Then we discovered that a cocktail of antioxidants decreased and normalized the majority of oxidative stress markers in a murine model of the disease. Moreover, this cocktail is also able to alleviate the clinical symptoms and axonal degeneration.

On the basis of those results, we are currently conducting a clinical trial to test the safety of the antioxidant cocktail and its efficacy to correct impaired oxidative stress markers in AMN patients. The clinical trial began in September 2011 with 13 Spanish patients who received dose A of the antioxidant cocktail for 2 months. During the 2-month washout period, the oxidative stress markers were quantified and their levels compared to those obtained prior to the treatment. In only 1 patient did the oxidative markers fall. Since February 2012, the patient is treated with dose A for 1 year and has 2 scheduled visits, one after 6 months and the other at the end of treatment. For the other 12 patients, antioxidant dose A was not sufficient to reduce these parameters. For that reason, these patients are currently receiving a higher dose B for a period of 3 months (start of the treatment: February 2012). A 2-month 'washout' period will follow in order to quantify the markers. If the parameters have improved, the patients will be treated with dose B for 1 year (same protocol as for dosage A (2 visits)). If not, treatment will be discontinued. In addition to the oxidative stress marker analysis, clinical evaluation has been or will be conducted at the beginning and end of the clinical trial.



### **Improvement of motor function in patients with AMN**

**Pr. Wolfgang Köhler (Germany)**

Leg stiffness and walking disabilities are leading symptoms in patients with Adrenomyeloneuropathy (AMN). The reasons for this spasticity are spinal cord long nerve fiber and myelin damage. The nerve fibers of the spinal cord carry the electrical impulses that transport information from and to the brain to support the complex control system of the human body. Each microscopically thin nerve fiber is insulated by a regular pattern of myelin sheaths along its length that allow for the efficient conduction of electrical impulses. In AMN some of these myelin sheaths can be damaged leading to an impairment or even disruption of neural conduction of movement information from brain to the muscle. Nerve impulses "short circuit" in demyelinated axons, much like electricity in a wire whose insulation is stripped. Thus, even though a demyelinated axon is alive, it is unable transmit motor or sensory impulses.

Treatment with 4-Aminopyridine (4-AP) permits the axon to transmit impulses again, improve nerve conduction thus leading to reduced spasticity and improved walking abilities in patients with multiple

sclerosis. Since in AMN similar clinical symptoms are present, we treated 18 AMN patients with 4-AP using a standardized study protocol. 13 out of 18 patients improved significantly regarding a timed-25 foot walking distance. 15 out of 18 patients reported significant reduction of spasticity and pain, 9 out of 18 improved with their bladder function. In summary treatment with 4-AP may significantly improve walking disabilities caused by spastic paraparesis in most patients with AMN.

## Workshop on Refsum disease

### Care

#### Eating for Health – Managing Adult Refsums Disease by Diet Eleanor Baldwin (United Kingdom)



Adult Refsum's Disease is a condition where the body has a severely limited ability to break down a fatty acid called phytanic acid (3,7,11,15 tetra-methyl-hexadecanoic acid). This fatty acid comes from the diet. If the intake of the fatty acid is limited, then the phytanic acid levels in the body gradually decrease. In some cases it has been possible to achieve very low phytanic acid levels through careful food choice and weight management. Good control of plasma phytanic acid levels improves health and prevents the development of some of the serious complications of Adult Refsum's Disease.

There are four main principles of healthy eating for people with Adult Refsum's Disease:

1. Avoid rapid weight loss or fasting. Rapid weight loss can cause large rises in the phytanic acid levels in the blood because any stored phytanic acid in the liver is quickly released during fasting. Nutritional supplements are available that can help support good nutrition during illness.
2. Base your food choices on starchy vegetables and grains (bread, pasta, rice, potatoes), fruit, vegetables, moderate portions of chicken, pork, soya and fat free dairy products.
3. Avoid foods rich in phytanic acid such as dairy products containing fat, beef products, lamb, sheep and fish.
4. Read food labels and avoid foods containing butter, fish oil, cheese, milk fat, cream.

Fat soluble vitamin supplementation (D, E, K) and essential fatty acid supplements (as Omacor) may be necessary.

**NOTE:** *The diet sheet for Refsum disease is available upon request.*

### Perspectives

#### Clinical, biochemical, genetic and therapeutic aspects of Refsum disease Pr. Ronald Wanders (The Netherlands)

##### 1. Clinical aspects

##### Introduction

In the 1940s, the Norwegian Sigwald Refsum identified a new disease that he named 'heredopathia atactica polyneuriformis' and which, according to him, was characterized by 4 symptoms:

- retinitis pigmentosa;
- cerebellar ataxia;



- chronic polyneuropathy and
- persistent elevation of protein concentration in cerebrospinal fluid without cell increase.

A major advance in the elucidation of the pathophysiology of what is now known as Refsum's disease was the discovery, by Klenk and Kahlke, in the early 1960s, of an accumulation of phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) in the plasma and tissues of patients presenting with the symptoms described above. Refsum's disease was thus found to be a disorder of lipid metabolism. Phytanic acid assay now enables diagnosis in patients only presenting with some of the symptoms described by Refsum, thus redefining the semiology of the disease. Retinitis pigmentosa of early onset and anosmia (*i.e.* the loss of the sense of smell) are found in almost all patients, while polyneuropathy, deafness, cerebellar ataxia, ichthyosis and cardiac arrhythmia are less frequent. The initial signs of the disease may emerge at any age: in young children but also in adults aged over 50 years.

#### *Ophthalmologic impairment*

The earliest symptom of Refsum's disease is generally hemeralopia (*i.e.* a progressive reduction in night vision or progressive night blindness). Secondly (often after a few years), retinitis pigmentosa may give rise to a narrowing of the visual field and to blindness. An electroretinogram (ERG) may contribute to the diagnosis of hemeralopia, which is otherwise difficult to diagnose, particularly in young children.

#### *Anosmia*

Anosmia (*i.e.* the loss of the sense of smell) is found in almost all patients presenting with Refsum's disease when they undergo olfactory tests.

#### *Polyneuropathy*

Patients with Refsum's disease may present with chronic sensorimotor polyneuropathy, which is asymmetric and progressive in nature if it is not (sufficiently) treated. The polyneuropathy is not always obvious when Refsum's disease is diagnosed due to its course characterized by episodes of progression and remission. The polyneuropathy may, in the long term, induce muscular atrophy and motor deficit not only of the legs but also the trunk. Most patients also show sensory disorders.

#### *Deafness*

Deafness is perceptive, bilateral and symmetrical and impacts high frequencies and conversational frequencies. Deafness may be moderate or severe.

If necessary, the diagnosis may be confirmed by auditory evoked potentials.

#### *Cerebellar ataxia*

Cerebellar ataxia is generally considered one of the major clinical symptoms of Refsum's disease despite the fact that its clinical onset occurs later than that of retinitis pigmentosa and polyneuropathy. Patients with cerebellar ataxia suffer, in particular, from gait disorders.

#### *Ichthyosis*

Ichthyosis is characterized by the accumulation of scales on the skin giving it a rough appearance. It only affects a minority of patients with Refsum's disease and the patients generally show the initial signs during adolescence. Onset during infancy is less common.

#### *Cardiac impairment*

The complications of a cardiomyopathy such as arrhythmia or heart failure are frequently the cause of death in patients with Refsum's disease.

## 2. Biochemical and genetic aspects

### *Diagnosis*

Suspected Refsum's disease may be confirmed by plasma phytanic acid measurement performed by a certified laboratory. An assay result greater than 100  $\mu\text{mol/L}$  strongly suggests Refsum's disease since the normal level is less than 10  $\mu\text{mol/L}$ . Diagnosis may subsequently be confirmed by sequencing the gene encoding phytanoyl-CoA hydroxylase showing 2 mutations: one per chromosome inherited from the parents.

## 3. Therapeutic aspects

Currently, there is no curative treatment for Refsum's disease. Treatment focuses on reducing plasma phytanic acid levels in order to slow disease progression.

Plasmapheresis is used to treat cardiac arrhythmia, ichthyosis and neurological complications such as motor deficit in the context of polyneuropathy and pronounced ataxia. The symptoms generally respond very well to marked reduction in plasma phytanic acid levels. Retinitis pigmentosa, anosmia and deafness, on the other hand, do not seem reversible.

### *Long-term management*

- Low-phytanic acid diet (in contrast to what is widely believed, it is not necessary to avoid green vegetables; however, dairy products, ruminant meat and fish are to be avoided).
- Regular and sufficient calorie intake to prevent catabolic lipolysis, which would lead to mobilization of the phytanic acid stored in liver and adipose tissue.
- Supplementary precautions (such as parenteral nutrition) are to be taken in the event of a surgical procedure.
- Follow-up by a cardiologist is recommended in order to prevent cardiomyopathy complications such as arrhythmia and heart failure.
- Symptomatic treatment using moisturizing creams to alleviate ichthyosis.

### *A mouse model of Refsum's disease facilitates research on new treatments.*

Refsum disease treatment is currently based on the reduction of plasma phytanic acid levels through a diet recommended by Eleanor Baldwin. Such diet is, however, difficult to observe. Plasmapheresis treatment is effective but associated with certain health risks. A drug enabling the decrease of phytanic acid levels would thus considerably improve the quality of life of patients suffering from the disease. We previously studied omega-oxidation of phytanic acid, a way of degradation that may constitute an alternative to alpha-oxidation of phytanic acid, which is deficient in patients with Refsum disease. We have identified drugs prescribed for other diseases, such as bezafibrate and fenofibrate, which may induce omega-oxidation of phytanic acid. We now wish to determine whether those drugs are effective *in vivo*. Accordingly, we have developed a mouse model of Refsum disease. These mice are asymptomatic when their diet contains very little phytanic acid. However, the mice show ataxia and polyneuropathy when the diet is supplemented with phytol or phytanic acid (phytol is a precursor of phytanic acid). This closely reflects what is observed in Refsum disease in human. We will present the results obtained using the mouse model. We are also continuing our commitment to designing new therapeutic strategies in the future.

## Workshop on MLD

### *Clinical trials*

#### **Phase I/II clinical trial of hematopoietic stem cell gene therapy for the treatment of Metachromatic Leukodystrophy**

**Dr. Maria Sessa (Italy)**



Metachromatic Leukodystrophy (MLD), consequent to the deficiency of Arylsulfatase A (ARSA), is characterized by severe dysmyelination of central and peripheral nervous systems, for which no effective treatments exist.

Based on the preclinical efficacy and safety data we obtained in the MLD murine model and in relevant cells from healthy donors and MLD patients, a gene therapy clinical trial based on transplantation of Hematopoietic Stem Cell transduced with a lentiviral vector (LV) containing the human ARSA cDNA was implemented.

The study, approved by the Italian Superior Institute of Health in March 2010 and by the Ethical Committee of our Institute, is a monocentric (conducted exclusively at San Raffaele Telethon Institute for Gene Therapy, in Milan), open, not randomized, prospective study, comparative with a non-contemporary population of controls studied within a natural history study of the disease performed in our Institute. As expected from the criteria for experimentation in pediatric patients, the study is of Phase I/II, therefore evaluating not only safety, but also effectiveness of the treatment. To demonstrate efficacy, candidate patients should have an expected disease progression and survival time, which would allow evaluation of potential clinical benefits and safety of the proposed therapy. Therefore, inclusion criteria foresee either late infantile patients in pre-symptomatic phase, or early juvenile in pre- or early- symptomatic phase. Together with safety end-points related to the myeloablative conditioning regimen based on IV Busulfan and to the use of LV, efficacy will be assessed as reduction in the progression of the clinical motor impairment in treated patients as compared to control patients, and as a significant increase of residual ARSA activity as compared to pre-treatment values.

In the first year of enrolment, three patients affected by the late infantile variant of the disease have been treated in the pre-symptomatic phase. Good hematopoietic reconstitution occurred in all treated patients and no severe adverse events related to the treatment were reported, confirming a good safety profile for this short-term observation. For the evaluation of efficacy, all patients have been followed with clinical evaluation inclusive of Gross Motor Function Measure (GMFM), and instrumental tests inclusive of ElectroNeuroGraphy (ENG) and Brain Magnetic Resonance (MR).

Up to now, only patient 1, who was treated at 15 months, has a follow-up long enough to allow us to make some comments on efficacy. According to disease onset in his two older late infantile-MLD siblings, he was expected to develop the first symptoms by 18 months of age. Both siblings by age 30 months were wheelchair-bound. On the contrary, patient 1 at 28 months was able to stand independently and required only single aid for walking and running. Furthermore, ENG and brain MR remained quite stable at follow-up at 12 months post-treatment.

Even if these data are extremely positive and support us in continuing enrollment as planned, only the scheduled follow-up at two years of all the treated patients will allow us to eventually demonstrate the efficacy of the treatment in slowing/halting disease progression.

## **Presentation of the phase I/II clinical trial based on enzyme replacement therapy using HGT-1110 enzyme**

**Dr. Eric Crombez (Shire)**

Shire Human Genetic Therapies is sponsoring a clinical trial to evaluate the safety of an experimental intrathecal enzyme replacement therapy for patients with metachromatic leukodystrophy (MLD). The study, HGT-MLD-070, has a planned duration of 40 weeks for each enrolled patient with the primary goal to evaluate safety of ascending doses of the investigational drug in patients using a surgically implanted device. All enrolled patients will be randomized to treatment with investigational enzyme replacement therapy.



Further details about the study, including complete eligibility criteria and open clinical trial sites, will be available at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (keyword metachromatic leukodystrophy; NCT01510028). With questions about eligibility, please direct interested patients and families to their physician.

### *Perspectives*

## **Gene therapy for metachromatic leukodystrophy by intracerebral ARSA gene transfer**

**Dr. Caroline Sevin (France)**



Metachromatic leukodystrophy (MLD) is a severe demyelinating disease due to a deficiency in the enzyme arylsulfatase A (ARSA). We have demonstrated the safety and efficacy of intracerebral injection of an adeno-associated vector (AAV of serotype 5) expressing the deficient enzyme in the MLD mouse and primate.

More recently, we evaluated the potential of using a new treatment vector (AAV10), which diffuses in the brain better than AAV5. We were able to show the marked superiority of the new vector in the MLD mouse. We also observed the presence of the therapeutic protein ARSA in oligodendrocytes (cells that produce myelin) and the long-term

efficacy (up to 1 year) of the procedure.

In view of setting up a clinical trial, we optimized the neurosurgical procedure to allow simultaneous injection of the therapeutic vector into 12 different brain sites in less than 2.5 hours. The procedure has been validated in the non-human primate showing that injection of the AAV10-ARSA vector induces significant over-expression of ARSA throughout the brain with no adverse effect. The toxicological studies required have now been completed and the clinical protocol is under review by the French Medicine Agency (ANSM). The phase I/II study (safety and efficacy study) should begin before the end of 2012. Five patients with a rapidly progressive form of MLD but at an early stage of the disease will be recruited. The therapeutic vector will be injected into the brain under cerebral imaging guidance. The safety and efficacy parameters will be evaluated over 2 years, a sufficient follow-up period to assess the potential therapeutic efficacy of the intracerebral gene therapy.

## **Modification of the ARSA enzyme for a better crossing of the blood-brain barrier**

**Dr. Ulrich Matzner (Germany)**

Metachromatic leukodystrophy is a lysosomal storage disease which is caused by a functional deficiency of the lysosomal enzyme Arylsulfatase A (ARSA). Using mice with a deficiency of ARSA as an animal model of the human disease, we are working on enzyme replacement therapy for MLD. This therapy approach is based on repeated intravenous infusion of active ARSA, produced by bioengineered cells under bioreactor conditions. Enzyme replacement therapy is approved and clinically used for several lysosomal storage diseases which have in common that they do not affect

the brain. Lysosomal storage diseases like MLD which mainly affect the brain are believed to be untreatable by enzyme replacement therapy because of the blood-brain barrier which prevents entry of intravenously injected enzyme from the circulation to the brain tissue. As we could show for MLD-mice in previous studies, the blood-brain barrier is not absolutely tight for ARSA, but allows transfer of approximately 0.07% of the total amount of intravenously injected enzyme. By using very high injection doses ( $\geq 20$  mg ARSA per kg body weight), the small fraction reaching the brain accumulates to concentrations being sufficient to reduce brain storage and improve central nervous system functions. Since minor differences in the residual ARSA activity of MLD patients have a strong impact on the severity and course of the disease, also moderate increases of the transfer rate are expected to significantly improve therapeutic efficacy of enzyme replacement therapy.



The blood-brain barrier has evolved to protect the brain from infections, toxins and metabolites which may disturb signal conduction in the central nervous system. For nutrients and compounds which are needed by brain cells, specific transporters and carriers exist. They bind specific cargo molecules (*e.g.* glucose) on the blood side, migrate through the blood-brain barrier to the brain side and deliver the cargo molecule to brain cells. Because healthy individuals express active ARSA in all cells of the brain, there is no need to transport ARSA across the blood-brain barrier and no specific ARSA-transporter at the blood-brain barrier exists.

In order to get more ARSA into the brain tissue, we try to modify ARSA in a way that it can be bound and transferred by existing transporter systems of the blood-brain barrier. Based on published data, we focus on a transporter called LRP (for low density lipoprotein receptor-related protein) which is very active in transferring specific cargo molecules (called lipoproteins) from the circulation to the brain tissue. The specificity of the lipoprotein-LRP interaction is due to a certain portion of the lipoprotein which, like a key into a lock, precisely fits into the binding pocket of LRP. Our idea is to transfer the key motif from the lipoproteins to the ARSA enzyme so that the modified enzyme is erroneously recognized and transported by LRP. Several key motifs have been selected from literature data and fused to the ARSA enzyme. The resulting ARSA-fusion proteins have then been produced on a laboratory scale and submitted to a battery of biochemical tests which allow conclusions about their enzymatic activity, stability, cellular uptake and other features. Binding to LRP was tested under cell culture conditions using cells which overexpress and which are deficient for LRP, respectively. Transport across the blood-brain barrier was analysed in a cell culture system of the barrier which consists of brain endothelial cells isolated from pig brain. Subsequently, MLD-mice were injected with the ARSA-fusion proteins and the transfer to the brain tissue was measured. Thus far, two of the constructed ARSA-fusion proteins successfully passed all steps of the quality control process. Their transfer rates across the cell culture blood-brain barrier were approximately doubled compared to unmodified ARSA. Also in MLD-mice, brain delivery was significantly increased. Consequently, the two ARSA-fusion proteins might increase the therapeutic efficacy of enzyme replacement trials. The fusion proteins are presently produced in larger quantities allowing preclinical studies in MLD-mice. Various biochemical, histological and behavioral parameters will be analysed to examine the therapeutic potency of this approach.

## **Workshop on Krabbe disease**

### **Current issues with diagnosing and treating patients with Krabbe disease**

**Pr. David Wenger (USA)**

Krabbe disease or globoid cell leukodystrophy (GLD) is a genetic leukodystrophy caused by mutations in the galactocerebrosidase (GALC) gene. Both healthy parents of an affected individual have one copy a disease-causing mutation in the *GALC* gene. When an individual inherits two copies of the mutated gene, he or she will have very low GALC activity. This causes a buildup of some important



galactose-containing lipids that are found primarily in the white matter or myelin of the central and peripheral nervous systems. While most patients present initially as infants, later-onset forms are also recognized. Usually there is a delay in the diagnosis of most patients until certain symptoms initiate genetic testing. This delay may limit the success of any treatment aimed at preventing or repairing damage to the nervous system. In order to obtain a diagnosis earlier, newborn screening for Krabbe disease was instituted in 2006 in New York State. Using an automated enzyme-based test on dried blood spots the laboratory in Albany, New York can test about 1000 samples per workday (about 260,000 per year). At this time about 1.4 million babies have been screened. Newborns with a GALC value below a certain daily cut-off value are retested, and if the value is still low, a more conventional test (in use for

about 40 years) is performed in Dr. Wenger's laboratory and mutation analysis of the *GALC* gene is also done. However this testing has resulted in serious medical and ethical issues that are still being addressed. All individuals who screen positive (low GALC activity) do have mutations in the *GALC* gene; some are clearly disease-causing (they have been found in confirmed patients or result in a greatly altered or unstable GALC enzyme), some have been found only in late-onset (non-infantile) patients, some are mutations never seen before and some changes are polymorphisms (normal variations in the gene that can lower the measured activity but do not cause disease). The finding of either previously unseen mutations or mutations found in late-onset patients causes serious uncertainties for both the family and the physician. The determination of when and if the individual might develop Krabbe disease is critically important to the success of the program.

When therapy should be started requires careful clinical evaluation and neurodiagnostic studies. It is not known what environmental or additional genetic factors may precipitate the onset of disease in the older individuals. While the clinical course in most late-onset patients progresses slowly, some have a rapid downhill course. Treatment options are limited at this time. Those newborns with very low GALC activity measured in this laboratory and two mutations in the *GALC* gene that have been found in patients with an infantile presentation are offered hematopoietic (blood) stem cell transplantation (HSCT) within one month of life. At this time stem cells from the umbilical cords of unrelated individuals usually can provide a suitable donor. When HSCT is performed in pre-symptomatic or very mildly affected infants it can extend the lives of these individuals. However, these patients can have significant motor and cognitive, especially expressive language, deficits. Better treatments are needed. Animal models of Krabbe disease provide us with systems for testing different therapies to be sure they work and are safe before being tried in human patients. There have been many studies in the mouse model. Some treatments only resulted in a small increase in lifespan and some were found to be not well tolerated by the mice. Since the mouse model, like the human patients, are missing GALC activity, attempts have been made to supply the missing enzyme by gene therapy by inserting the gene in a virus that is not pathogenic, and injecting this vector into the brains of mice with Krabbe disease. While there was some extension of life with previous viral vectors, the gains were moderate. We are currently testing a new viral vector, called AAVrh10 containing the *GALC* gene. Injecting this vector into the brain and into a blood vessel has shown promise in significantly prolonging the lives of treated mice (from about 40 days to 150+ days). These mice are fertile and show few signs of the disease until very late in their lives. The latest data will be presented. However, it appears that more than one approach may be needed to prevent and correct the pathology seen in both the central and peripheral nervous systems in this disease. Hopefully new approaches will lead to improved therapies for human patients.

## Workshop on CACH/VWM syndrome – Alexander disease - MLC – Canavan disease and other vacuolating leukodystrophies

### CACH/VWM syndrome

#### Insights gained from the mouse model for CACH/VWM disease

Pr. Orna Elroy-Stein (Israel)

eIF2B-related leukodystrophy, also called Vanishing White Matter disease (VWM), or Childhood-Ataxia and Central nervous system Hypomyelination (CACH), is caused by recessive mutations in any of the five genes encoding eukaryotic translation initiation factor 2B (eIF2B). eIF2B has a major role in governing the rate of global protein synthesis under normal and stress conditions. We have developed a mouse model for the disease by introducing a mutation into the gene encoding the catalytic subunit of eIF2B. We specifically chose a mutation that in the homozygous state leads to a classical form of the disease in humans. Time-course magnetic resonance imaging (MRI) and electron microscopy revealed for the first time delayed post-natal development of the brain white matter. This anomaly was confirmed by abnormal abundance of oligodendrocytes and astrocytes and abnormal level of major myelin proteins in young animals. Genome-wide analysis revealed delayed waves of gene expression and pointed at down-regulation of many oligodendrocytic-specific genes during the peak of myelin formation. Moreover, the abnormally expressed stress-related genes underline the need of the mutant brain to cope with its inborn hypersensitivity to cellular stress. Most importantly, mutant mice failed to recover from experimentally-induced demyelination, reflecting their increased sensitivity to brain insults. The abnormal astrogliosis observed in the mutant mice in response to insults is in agreement with the atypical astrogliosis reported for human patients. The mouse model provided important new information related to the impaired ability of the mutant astrocytes and microglial cells to produce and secrete a wide range of proteins which are important for brain repair in response to acute demand. Among these proteins are cytokines and chemokines which are normally produced and secreted in the brain as part of an inflammatory response following physiological stress. All together, the mouse model provided evidence that the mutation in eIF2B is responsible for abnormal myelin formation in young animals, abnormal myelin maintenance in mature animals, and impaired ability to recover from stress-induced brain damage. It also provided lists of abnormally expressed genes which will enable the discovery of the molecular circuits involved in this pathology and may provide the basis for rational drug design in the future.



### Alexander disease

#### Update on research in Alexander Disease

Dr. Albee Messing (USA)



Alexander disease is a generally fatal disorder that results from mutations in a single gene, *GFAP*. Previous studies have stated that in two-thirds of patients the disease becomes apparent within the first two years of life, and half of these patients die before the age of 6. Three important studies have appeared within the past year that provide the first estimate of prevalence, propose a new classification system, describe longer survival periods than previously known, and increase our understanding of variability even within families with multiple affected individuals carrying the same mutation.

Yoshida *et al.* (2011) conducted a survey of neurology clinics throughout Japan to identify all Alexander disease patients in this defined population.

They arrived at an overall estimate of 1 in 2.7 million. Interestingly, approximately half of these patients were considered adult-onset, which is very different from the previous notions that adult-onset patients accounted for only 10% of the cases.

Prust *et al.* (2011) conducted a review 215 patients that includes nearly all published as well as 30 previously unpublished individuals. In contrast to the most common classification system that groups patients by age of onset - infantile (before the age of 2), juvenile (between 2-13), and adult (>13) - this study instead proposes a different grouping that is based on location of lesions within the nervous system. In this revised classification system Alexander disease is considered to manifest as either type I (all early onset) or type II (onset throughout the lifespan). Patients with type I disease have forebrain predominance of lesions, whereas patients with type II disease have hindbrain predominance. Survival is also much longer than previously appreciated. Type I patients have a median survival of 14 years from diagnosis, and type II patients have a median survival of 25 years for diagnosis.

Finally, Messing *et al.* (2012) reported genetic studies on two of the most historically important families in Alexander disease research. Although most patients with Alexander disease have no family history of disease, and arise from newly occurring dominant mutations in GFAP, families with multiple affected individuals have been known for some time. One such family, with two affected children and unaffected parents, contributed to speculation about recessive modes of inheritance or germ line mosaicism. Our studies instead show that both of these families have dominant mutations in GFAP, are not examples of germline mosaicism, and reveal the marked variability in severity found among family members with the same mutation. We propose for the purpose of genetic counseling that the risk of germ line mosaicism be presented as less than 1%, and possibly much less.

## MLC

### **New insights into the molecular pathophysiology of MLC disease**

**Pr. Raul Estevez (Spain)**

MLC is a rare type of leukodystrophy characterized by chronic brain white matter edema. Until very recently, *MLC1* was the only gene associated with MLC. In collaboration with the group of van der Knaap, we have identified *GLIALCAM* as the second gene associated with MLC. GlialCAM protein is necessary as an escort protein needed for MLC1 targeting to cell junctions and MLC-causing mutations abolish this targeting. In addition, GlialCAM serves as an auxiliary subunit of the CIC-2 chloride channel regulating its targeting and its function. Strikingly, the CIC-2 and the MLC1 knockout mice exhibit vacuolation of the brain that resembles what is found in MLC patients. Astrocytes with reduced expression of MLC1 or GlialCAM have altered chloride currents. These results suggest that MLC is caused by a disturbance in the function of astrocytic chloride channels, leading to defects in water homeostasis. We will present these discoveries and discuss with families how these new results have suggested new therapeutical strategies.



## Canavan disease

### **Clinical trial testing the effects of high dose of triacetin in Canavan disease**

**Dr. Gheona W. Altarescu (Israel)**

Canavan disease (CD; MIM#271900) is a rare autosomal recessive neurodegenerative disorder, presenting in early infancy. The course of the disease is variable, but it is invariably fatal, and death



usually occurs during the first and second decade of life. CD is caused by mutations in the *ASPA* gene, which codes for the enzyme aspartoacylase (ASPA). ASPA normally breaks down N-acetylaspartate (NAA) to acetate and aspartic acid. The lack of NAA-degrading enzyme activity, leads to excess accumulation of NAA in the brain, and deficiency of acetate, which is necessary for myelin lipid synthesis.

Glyceryltriacetate (GTA) is a short-chain triglyceride with three acetate moieties on a glycerol backbone and is a proven effective acetate precursor. Intra-gastric administration of GTA to tremour mice results in greatly increased brain acetate levels, and improved motor functions. GTA given to infants with CD at a low dose showed no improvement in their clinical status, but also no detectable toxicity was noted. We present

for the first time the safety profile of high dose GTA in 2 patients with CD.

We treated 2 infants with CD at ages 8 months and 1 year with high dose GTA, for 4.5 and 6 months respectively. We started at 0.5g/kg body weight daily, doubling the dose every 3 days until a maximum of 4.5 g/kg body weight /day was reached.

No significant side effects were observed except for possible increased gastric acidity at the highest dose of 5 g/Kg body weight /day, for which Omeprazole was administered. No toxicity was noted. Although the treatment resulted in no motor improvement, it was well tolerated. The lack of clinical improvement might be explained mainly by the late onset of treatment, when already significant brain damage was present. We hope that better results may be achieved if therapeutic intervention starts at an earlier stage of CNS development, prior to 3 months of age. Further larger studies of CD patients below age 3 months are required in order to test the long-term efficacy of this drug.

## Workshop on PMD and other hypomyelinating leukodystrophies

### Phase I Study of the Safety and Preliminary Efficacy of Intracerebral Transplantation of HuCNS-SC® Cells for Connatal Pelizaeus-Merzbacher disease (PMD)

Dr. David Rowitch (USA)

Patients with Pelizaeus-Merzbacher Disease (PMD) are born with a gene mutation that results in insufficient myelination of nerve fibers in the brain. The connatal form of PMD manifests in early life and leads to neurological impairment and eventually death. Currently, there are no disease-modifying approved treatments for PMD.



Myelin, which is produced by special cells called oligodendrocytes, insulates nerve fibers to allow electrical signals to be conducted normally. In addition to other leukodystrophies, more common diseases of myelination include cerebral palsy, transverse myelitis and multiple sclerosis. Preclinical studies performed by StemCells, Inc (SCI) demonstrated that, when transplanted into an animal model of hypomyelination (the shiverer mouse), purified human central nervous system stem cells (HuCNS-SC) engraft and differentiate into mature oligodendrocytes and form myelin sheaths around host nerve fibers. This animal model data formed the rationale to proceed with human testing in PMD.

This Phase I clinical study, sponsored by SCI and authorized by the U.S. Food and Drug Administration (FDA), was designed to evaluate the safety and preliminary efficacy of HuCNS-SC cells as a treatment for PMD. The study was approved by and conducted at the University of California, San Francisco, California. Four patients with conatal PMD received HuCNS-SC transplants and underwent intensive follow-up assessments between February 2010 and February 2012.

The study carried out was done to assess safety regarding surgery, immunosuppression and HuCNS-SC cells and also to look for signs of myelin formation by MRI. Four boys with early-severe (conatal) PMD received transplants with HuCNS-SC into white matter.

The following are preliminary findings.

- From a safety perspective: The neurosurgical procedure, immunosuppression and the HuCNS-SC cells did not generate safety concerns a one year after transplant.
- There was no neurological deterioration in any subject. This non-controlled trial was not designed to determine clinical efficacy.
- Preliminary MRI Findings: We detected MRI changes only in the regions of brain that received cell transplant. The pattern of findings suggest physical properties of myelin. We conclude that HuCNS-SC can engraft in human brain and suggest the capability to form myelin. Further work is needed to confirm this.
- Our conclusion: This is the first demonstration of human neural stem cell engraftment with evidence of myelin formation in living subjects.  
What are the next steps? The findings, once confirmed in peer-review, support carrying out a well-designed larger clinical study for PMD to show efficacy. We expect that this later study would involve a European partner to enroll PMD patients.

Further research is needed to understand the precise biological causes of leukodystrophies to design new and logical therapies including cell-based approaches. We would like to thank the families for their support and willingness to participate in cutting edge research.

### **Olesoxime and morpholinos: new therapeutic approaches for the treatment of PMD**

**Dr. Méline Bégou (France)**



Pelizaeus-Merzbacher disease (PMD) is due to mutations in the gene that codes for myelin proteolipid protein (PLP1). Proteolipid proteins (PLP, DM20) constitute about 50% of the total myelin proteins of the central nervous system. In the brain, myelin is synthesized by glial cells, oligodendrocytes, to form an insulating sheath around the axons of neurons and thus enable fast conduction of the nerve influx. In the majority of cases, PMD results from the acquisition of supplementary copies of the *PLP* gene (duplication) and, in a smaller proportion of cases, there are abnormalities in the sequence of the gene, which are responsible for the production of an abnormal protein (mutation). In the case of certain mutations leading to the absence of protein, patients show a more moderate form of the disease called spastic paraplegia

type 2 (SPG2). Currently, there are no appropriate therapies for these diseases.

Transgenic mouse models of PMD expressing supplementary copies of *PLP1* and *SPG2* genes are being used in our work. In the mouse, the mutations induce neurological disorders and abnormalities of the central nervous system similar to those observed in man.

For several years now, our objective has been to develop new therapeutic strategies in those mouse models with the aim of:

- 1) reducing the over-expression of normal PLP using the morpholinos<sup>®</sup> antisense oligonucleotide technology;
- 2) slowing or even correcting the axonal degeneration occurring in spastic paraplegia type 2.

The encouraging preliminary results show the efficacy of morpholinos<sup>®</sup> oligonucleotides in decreasing protein expression in oligodendrocytes *in vitro* and *in vivo*. A new drug, Olesoxime,

developed by the Trophos® company, is under evaluation in our animal models. Prior preclinical studies have shown that the drug promotes the function and survival of neurons and other types of cells placed under pathological stress conditions through its interaction on the mitochondrial membrane transition pore.

### **Clinical aspects of leukodystrophies related to RNA Pol III**

**Dr. Nicole Wolf (The Netherlands)**

Hypomyelination is a heterogeneous group of inherited white matter disorders. The best-known disorder with hypomyelination is Pelizaeus-Merzbacher disease. In 2005, we described a novel hypomyelinating disorder in which another symptom, hypodontia (lack of teeth and delayed tooth eruption) was a prominent finding in young children. Later it was discovered that in adolescents, puberty development did not start as usual. The disease was therefore named 4H syndrome, 4H standing for hypomyelination, hypodontia and hypogonadotropic hypogonadism. In 2011, Canadian and Japanese groups identified two genes causing 4H syndrome, *POLR3A* and *POLR3B*. In the meantime, it became clear that not all patients always had all 4 symptoms. There were patients with only hypomyelination, other patients had hypomyelination and delayed or absent puberty, and others showed all symptoms including abnormal tooth development. Other important symptoms in many patients are deterioration with minor infections, growth problems and myopia. Natal teeth (presence of teeth at birth) affect 20% of all children with 4H syndrome. The problems with tooth eruption sometimes necessitate dental operations.



Neurological symptoms are usually evident early, and many children are evaluated round the age of 2 to 3 years because of balance problems when walking and mildly delayed development. Some children never learn to walk without support, but this is the exception. Fine motor skills are better than gross motor skills. Speech problems are also common, and many children have difficulties to pronounce certain sounds. Most children need special education as they have mild to moderate learning difficulties. In most, the neurological symptoms deteriorate in the second decade. Walking becomes more difficult, and they need a rolator or wheelchair. Many become completely wheelchair dependent in adolescence. Also talking and swallowing become more difficult, and vision deteriorates. Spasticity is often not very prominent. Gradually, all motor functions are lost, and patients communicate with eye or head movements. Life expectancy is reduced in many patients. Other patients have a much more stable disease course and only show mild or even no deterioration. At the moment, we cannot predict disease course in young children. If there is deterioration, it usually continues at a slow pace.

### **Mutations of *POLR3A* and *POLR3B* genes cause a hypomyelinating leukodystrophy by dysfunction of Pol III**

**Pr. Bernard Brais (Canada)**

The leukodystrophies are a heterogeneous group of degenerative diseases characterized by abnormal white matter in the central nervous system. Our research group has identified the first mutations in *POLR3A* and *POLR3B* genes responsible for the majority of cases of hypomyelinating leukodystrophies fully or partially fitting the characteristics of the 4H syndrome (hypomyelination, hypogonadotropic hypogonadism and hypodontia). These 2 genes encode for the catalytic subunits of RNA-polymerase III (Pol III). For that reason, we have named the diseases 'Pol III-linked leukodystrophies'.



We demonstrated by studying patients' cell lines and the brain from one patient that there was a significant reduction in POLR3A and POLR3B at the protein level. We have developed a biochip that enables quantification of all the known RNA (or transcripts) sequences that are produced by Pol III. The preliminary results in the patients' lines show changes in the level of expression of some Pol III transcripts. Some of the decreases are likely to affect protein synthesis in the central nervous system and this may result in neuronal dysfunction observed in this subtype of hypomyelinating leukodystrophy.

## Workshop on undetermined leukodystrophies

### Leukodystrophies of the adult

#### Pr. Pierre Labauge (France)

Leukodystrophies, defined by myelin impairment of genetic etiology, are most frequently diagnosed in children. Adult forms are rare and poorly known.

The diagnostic orientation toward a genetic mechanism underlying impairment of the white matter in adults is based on a number of arguments:

- 1) Clinical argument: progressive onset of clinical symptoms such as gait disorders or cognitive impairment, rarely epileptic seizures;
- 2) Neuroradiological arguments: symmetrical impairment of the white matter on the first MRI. The etiologic diagnosis is based on the mechanism of white matter impairment: either a vascular mechanism or direct impairment of the myelin, most frequently of the demyelination type.



Hypomyelination is in fact very rare in adults. Some neuroradiological aspects help the diagnosis of vascular diseases, particularly impairment of the basal ganglia, brainstem and the presence of microbleeds in echo-gradient sequences. The two most frequent genetic vascular diseases involving vascular lesions are CADASIL disease and those due to a mutation of collagen IV (Col IV).

Other rarer diseases may be encountered, in particular connective tissue diseases such as pseudoxanthoma elasticum and certain mitochondrial cytopathies. When a vascular mechanism is not confirmed, an investigation for an enzymatic etiology has to be conducted. An initial standardized analysis must be performed.

The most frequent causes in adults are adrenomyeloneuropathy, metachromatic leukodystrophy and cerebrotendinous xanthomatosis. A large number of patients do not receive a precise diagnosis.

### Undetermined leukodystrophies, clinical and molecular approach

#### Drs. Imen Dorboz et Samia Oumlil (France)



Undetermined leukodystrophies account for about 40% of all leukodystrophies. Their great heterogeneity makes their study particularly difficult despite the technical progress in imaging and molecular biology. Despite the limited therapeutic resources currently available, the identification of new causal genes is essential in order to further elucidate the disease and give families a genetic counseling. In order to do so, it is necessary to identify homogeneous patient subgroups to guide the molecular genetics. Accordingly, we have set up a computer database in order to pool all the clinical and non-clinical data of our patients as well as the samples available so as to investigate for a cause of the

disease. The perfectly anonymous database will also allow a better follow-up of the patients via an interface accessible to the patient himself or his attending physician. Its European extension in the frame of the 'Leukotreat' project currently permits the registration of large number of patients suffering from leukodystrophy of known etiology and available for clinical trials. With this database, families were selected to accelerate the research on new causal genes in leukodystrophies of undetermined cause.



**Identification of a new leukodystrophy of the adult  
Pr. Frédéric Sedel (France)**



While leukodystrophies are usually considered childhood diseases, there are adult-onset forms. Onset may occur at very advanced age. Thanks to the ELA Push project, designed to identify new leukodystrophies of undetermined cause, we have identified the gene responsible for a new adult leukodystrophy in 3 women of North African origin not belonging to the same family and aged 59, 64 and 51 years, respectively. The initial symptoms of the disease appeared at age 57 years in the first patient (dizziness, tinnitus), 44 years in the second (isolated tremor) and at about 35 years for the third (visual and psychiatric disorders). Subsequently, the symptoms progressed little. Brain MRI, conducted fortuitously in some patients, showed very characteristic abnormalities enabling the comparison of these patients and the identification of the causal gene by exome sequencing.

**A new leukoencephalopathy and a new gene: leukoencephalopathy with thalamus and brainstem involvement and high lactate, LTBL**

**Pr. Marjo van der Knaap (The Netherlands)**

Despite the progress made in the last decade, unclassified leukoencephalopathies still constitute a challenging problem. A considerable proportion of the patients with significant white matter abnormalities on MRI remain without a specific diagnosis. Our approach is to determine novel leukoencephalopathies among them by MRI pattern recognition.

With our MRI database of approximately 3000 leukoencephalopathies of unknown origin, we identified 7 patients with the same distinct MRI pattern. MRI showed signal abnormalities of the deep cerebral white matter (sparing a periventricular zone), thalamus, basal ganglia, brain stem and cerebellar white matter between the ages of nine months and two years. Especially the signal abnormalities in the cerebral white matter and thalamus were striking. MRS showed elevated lactate in the areas of abnormal signal. On follow-up, abnormalities gradually improved with limited remaining MRI abnormalities. Lactate in MRS disappeared. The clinical picture was similar for all patients. After a normal or almost normal initial development, regression occurred in the second half year of life with spasticity and loss of milestones. From the second year on, clinical improvement occurred. The remaining permanent deficits were variable in severity. No second episode of regression occurred until now. Lactate in blood and CSF was elevated during the period of clinical regression and normalized after that. The gene defect in this group of patients was determined by exome sequencing.



After identification of the gene defect in the above group of patients, the gene was analyzed in an additional group of patients with a similar but more severe phenotype. The patients presented soon after birth with a devastating encephalopathy with increasing spasticity and absence of all

development. MRI showed diffuse abnormality of the cerebral white matter (sparing a thin periventricular rim only), thalamus, basal ganglia, brain stem and cerebellar white matter. Additionally, agenesis of the posterior part of the corpus callosum was seen. On follow-up, no improvement, but atrophy of the affected structures occurred. Clinically, the patients stabilized but did not improve. Lactate remained high. These patients were found to have mutations in the same gene.

The disease has an autosomal recessive mode of inheritance. It is called leukoencephalopathy with thalamus and brainstem involvement and high lactate, LTBL.

## Workshop on Aicardi-Goutières syndrome

### An update on Aicardi-Goutières syndrome

Dr. Yanick Crow (United Kingdom)



Aicardi-Goutières syndrome (AGS) is an inherited inflammatory disease, which is characterized by inappropriate activation of the immune system leading to destruction of the white matter of the brain and consequent mental and physical disability. We have previously identified five genes from *AGS1* to 5: *TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C* and *SAMHD1* when mutated can cause AGS. All five of these genes have been demonstrated to play a role in nucleic acid metabolism.

Our research work focuses on the *AGS5* gene and its encoded protein SAMHD1. We, and others, have made significant advances towards better understanding SAMHD1 function. Of particular note, we have demonstrated that SAMHD1 has a role in DNA precursor metabolism (Goldstone *et al.* Nature 2011). This work, undertaken in collaboration with two other groups, has defined the crystal structure and biological function of SAMHD1, which is now known to be a potent so-called dGTP-stimulated triphosphohydrolase. The derived protein structure will help to better inform future functional studies of SAMHD1 as well as already providing important insights into how AGS associated variants affect protein function.

In addition to this groundbreaking work, we have focused on developing tools/methodologies which will enable us to study the function of SAMHD1 in more detail. Specifically, in collaboration with a company called MRC-Holland, we have piloted a test, which allows rapid screening of AGS patients for *SAMHD1* gene deletions. This assay has now been incorporated into our diagnostic protocol used to test new cases of AGS. Furthermore, we have optimised assays to facilitate the study of SAMHD1 protein expression and localization in normal and patient cells. Finally, in order to gain additional insight into the cellular pathways and processes in which SAMHD1 participates, we have initiated a number of studies aimed at identifying other proteins that interact with SAMHD1.



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