LSHM-CT-2006-018692

HUE-MAN

TOWARDS THE DEVELOPMENT OF AN EFFECTIVE ENZYME REPLACEMENT THERAPY FOR HUMAN ALPHA-MANNOSIDOSIS

Instrument: STREP
Thematic Priority: Life Sciences, Genomics and Biotechnology for Health

Publishable final activity report

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Project web-site: http://www.biochem.uni-kiel.de/hue-man/

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Section 1 – Project execution

Introduction

The aim of the HUE-MAN project was to set the ground for the introduction of the effective treatment of the rare disorder human alpha Mannosidosis using enzyme replacement therapy (ERT) as the generally accepted and most promising therapy for lysosomal storage disorders. Based on our initial observation that correction of storage in many tissues of a mouse model for this disease including brain was found after administration of lysosomal acid α-Mannosidase (LAMAN) from bovine kidney, and human and mouse recombinant LAMAN our major goals were to investigate and establish clinical parameters in the mouse model and a natural history study of the human disease in order to define clinical endpoints for the future clinical trials in α-Mannosidosis. Furthermore, in parallel, HUE-MAN wanted to establish conditions for the production of rhLAMAN that can pave the way for a First Clinical Trial in Man.

HUE-MAN brought together cell and molecular biologists, clinicians with regular patient contact, neuropharmacologists with expertise in behavioural analysis, epidemiologists and biochemists with experience in large scale enzyme production and toxicology testing to set up the conditions and knowledge for eventually introducing the reLAMAN drug into first clinical trials. The combination of top level European scientists and clinicians was aimed to facilitate the integration of research capacities across Europe, increasing coherence and providing critical mass of investigators. The integrated multidisciplinary research enabled direct interactions between technology and biology and will provide the knowledge base essential for the rational design of therapeutic interventions. The unique composition and collection of expertises of this consortium was of major importance to pave the way for a successful introduction of ERT for human patients suffering from Alpha-Mannosidosis. The ultimate aim was to push forward the development a high quality, safe and effective drug.

Background

Within FP5 the collaborative research project EURAMAN successfully established an enzyme replacement therapy for a mouse model of α-Mannosidosis. EURAMAN also provided important informations about fundamental scientific questions dealing with the biology, structure-function relationship and carbohydrate metabolism of the lysosomal α-Mannosidase enzyme. The main objective of the HUE-MAN project was to transfer and expand the knowledge obtained from the EURAMAN project to investigate and establish clinical parameters in the mouse model and a natural history study of the human disease in order to define clinical endpoints for the future clinical trials in α-Mannosidosis.

α-Mannosidosis is a lysosomal storage disorder that is caused by the deficiency of lysosomal α-Mannosidase (LAMAN) and is known to occur in man, cattle and cat. The deficiency of LAMAN causes the intralysosomal accumulation of oligosaccharides

carrying α 1,2-, α 1,3- and α 1,6-mannosyl residues at their non-reducing termini. These oligosaccharides mainly originate from the intralysosomal degradation of glycoproteins with N-linked oligosaccharides. The lysosomal storage is observed in a wide range of cell types and tissues, including neurons in all regions of the brain. The clinical phenotype of α-Mannosidosis is heterogenous, ranging from severe infantile forms to mild juvenile forms with moderate mental retardation, dysostosis multiplex, coarsening of the face, impaired hearing, recurrent infections and mild hepatosplenomegaly. Multiple mutations are found in human α-Mannosidosis, but a genotype–phenotype correlation is not apparent. The prevalence of α-Mannosidase disease has been estimated to be 1 in 500.000. Up to 2004 at least 112 cases have appeared in the literature. The disease is not specific to any ethnic group or α-Mannosidosis mutation.

Enzyme replacement and bone marrow transplantation (BMT) are the major therapeutic options in lysosomal storage disorders. Enzyme replacement therapy (ERT) is an effective means to improve the clinical manifestations in type I Gaucher disease and has meanwhile been approved for several lysosomal storage disorders including some involvingthe brain. Reports on ERT in α-Mannosidosis were so far lacking. The few attempts of BMT in human α-Mannosidosis have had variable outcomes, but have indicated that successful engraftment can increase language, social and motor skills. To obtain a more accessible model for the study of therapeutic modalities in α-Mannosidosis we have generated a mouse model for α-Mannosidosis by disrupting the gene for LAMAN. The morphological and biochemical alterations in mouse α-Mannosidosis closely resemble those reported in human α-Mannosidosis and the phenotype corresponds to a mild form of the human disease. The generation of a mouse model for α-Mannosidosis and the production of the recombinant LAMAN have made it possible to study the efficacy of ERT in this lysosomal storage disorder.

Project objectives

The project was divided into five objectives:

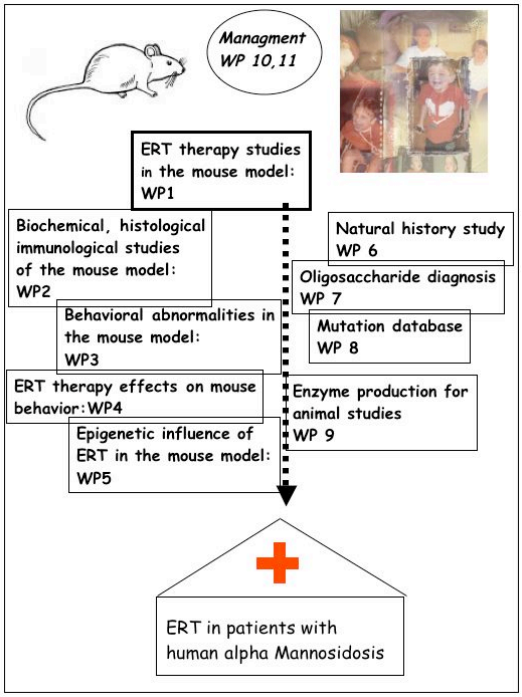
(1) To explore the efficacy of enzyme replacement therapy in the mouse model of alpha-Mannosidosis. Treated mice were analysed for lysosomal storage, enzyme activity, immunological response, behavior and gene expression (WP1,WP2, WP3, WP4, WP5).

(2) To systematically determine the natural history of patients suffering from alpha Mannosidosis. Clinical phenotypes of selected European patients were determined and important medical data were collected (WP6; WP10).

(3) To find new diagnostic parameters which are suitable for analysis of a possible benefit of LAMAN-treatment. Mass spectrometry analysis technology was developed and used to determine the quantities of oligomannosides in mouse and patient samples (WP7).

(4) To systematically define genotype/phenotype relationships and to collect patient data. Functional and structural characterization of disease causing mutations and the establishment of an alpha Mannosidosis mutation database were performed (WP5,8).

(5) To establish the production of human recombinant LAMAN to provide the enzyme needed for the studies using the mouse model. Efficient fermentation and purification protocols were developed and sufficient enzyme was produced for the preclinical animal studies (WP9).



Outline of HUE-MAN project

Work performed

WP1 Enzyme replacement therapy in the mouse model P1,P2a,P4	A working system for enzyme replacement therapy (ERT) of the preclinical mouse model including breeding of α-Mannosidosis mice, intravenous injections, taking blood from the retroorbital plexus after injection, enzyme activity measurements in serum and tissue, perfusion of mice for sugar and histological analysis, sugar extraction and TLC analyis was established. Various short term experiments with injection of different doses of rhLAMAN (25-500U/kg) to determine the most effective dose of LAMAN treatment in the mouse model were performed. Also long term studies in the classical mouse model were performed. The generation of a new immunotolerant α-mannosidosis mouse model was initiated.
WP2 Characterization of alpha Mannosidase knockout mice P1,P2a,P4,P9	Various biochemical and histological experiments were performed to further characterize the lysosomal phenotype of α-Mannosidois mice before and after ERT. Experiments were also established and performed in which the antibody response to injected rhLAMAN in alpha Mannosidase deficient mice was monitored over time.
WP3 Behavioral abnormalities in the mouse alpha Mannosidosis P3, P1,,P2a	It was shown that Mannosidosis mice differ and can be discriminated from their wildtype littermates in tests of emotionality and cognitive performance, which is likely related to the emotional and cognitive alterations in Mannosidosis patients. A detailed analysis of the behavioural and other neurofunctional characteristics of alpha mannosidase deficient mice was performed. A battery of well-established tests for different behavioural & afferent neuronal functions was applied. We also examined the possible age-dependency of the behavioural

	observations. In addition, electro-physiological recordings on hippocampal slices from Mannosidosis mice were performed.
WP4 Effect on CNS pathology and behavior after treatment of human LAMAN **P3, P1,,P2a**	The effects of treatment with human LAMAN was tested using the multimetric battery that was fined tuned during WP3. The animals received intravenous injections of human LAMAN, and were tested to assess the therapeutic efficacy of these injections. Apart from the therapeutic efficacy, the multimetric assessment was used to investigate the possible adverse effects of the experimental procedure on behavioural functions.
WP5 Epigenetic influence of ERT in the mouse model **P4, P1,P2a**	The impact of ERT on gene expression in the model organ liver was described. The goal was to understand the molecular effect of neutral sugar storage and ERT-mediated clearance. A screening for possible effects was performed using microarrays. The candidate genes and pathways obtained from the transcription profiling were analysed in more depth in functional follow-up experiments.
WP6 Natural history of human patients with alpha-Mannosidosis **P2b, P5,,P6,P7,P8a,P9,P10**	Data on the natural course of the disease in a larger patient population should be collected and evaluated for the clinical management of the disease and to form a baseline for efficacy assessment of potentially therapeutics. The study design was a prospective**,** longitudinal, multi-center, multinational of the patient cohort registered in Norway, United Kingdom, Czech Republic, Germany, and USA. The patients will be assessed clinically, biochemically and with other assessments relevant for the disease and defined in the protocol for the study. The study plan include at least 2 visits over a 24 months period.
WP7 Development of oligosaccharide analysis of tissues originating from mouse models	The development and application of sensitive methods mostly based on mass spectrometry analysis (MS) for quantification and identification of the

P9, P8a,P2b,P1	precise structures of the oligosaccharides (oligomannosides) present in the urine and serum of patients was performed. Efficiency of the ERT treatment was correlated with the disapearance of the storage material (high mannose-type oligosaccharides) in tissues and biological fluids (serum, urine). present in plasma, urine, and tissues - To apply the optimized procedures for the analysis of samples (mouse urine, serum and tissues before and after ERT)
WP8 Mutation database, genetic epidemiology of human alpha-Mannosidosis **P8b, P2b,,P8a,P10**	A relational mutation database was constructed that will aid the understanding of the natural history of α-Mannosidosis. A description of allelic variants (mutations) was included and form the core of the database. These data were related to annotations of genotypes – to ethnic-, population- and geographic associations - to a detailed and structured set of clinical information - to properties of the mutant enzymes such as residual activity (if any) - intracellular transport and processing (missense mutations) and enzyme 3D-stucture. The alpha-Mannosidosis mutation database was further developed by creating and implementing new applications which allow the user to retrieve data and information across datasets. Molecular and clinical data were subjected to thorough quality assurance and subsequently bulk imported. The database has been adapted to www deployment.
WP9 Production of human recombinant LAMAN **P2a**	The human recombinant LAMAN enzyme was cloned and expressed. In order to provide sufficient amounts of the enzyme for the animal studies it was necessary to develop the process and produce 0.5 grams of enzyme. The enzyme was produced in CHO mammalian cells. The procedure of enzyme purification was optimized and adapted for large scale production for toxicology studies in animals. Analytical

	methods, for the analysis and characterization of the rhLAMAN final product, including posttranslational modifications, were also developed.
WP10 Documentation and result management of the natural history study **P1, P2b, P5,,P6,P7,P8a,P8b**	Case Record Form's (CRF) were created, CRF's (monitoring) were collected, statistical analyses and medical writing was performed. We were able to collect data in a structured and manageable way in order to secure validity of data and evaluate results statistically and report the results in a structured way, usable for regulatory purposes.
WP11 Management **P1**	The project management has organised seven project meetings, one in Kiel, Brussels, Perugia, Copenhagen, Prague, Bodoe/Tromsoe, and the final meeting in Kiel, Germany. In addition phone, skype conferences and small meetings with selected participants from the clinical part and the basic science part of HUE-MAN were organized. The project website was maintained. A request for the extension of the HUE-MAN network for 6 months was submitted to the EU. A second amendmend request is currently under preparation reflecting the change of the legal status of participant 6 from January 1st 2009. The participants of the HUE-MAN project have given presentations in international congresses and published many articles. The HUE-MAN project has been active in research training. The periodic and final reports and corresponding cost statements have been prepared.

Major achievements:

The present project is of great value for the introduction of recombinant human lysosomal alpha-mannosidase into the first clinical trials of ERT in patients. In line with our plans we have been able to evaluate the benefit of alpha mannosidase ERT in the mouse model and we have made significant advances to get a suitable protocol for treatment of this lysosomal storage disorder. Importantly, the correction of storage has been followed over time in visceral organs but also in the central nervous system. A possible improvement of the brain associated symptoms has been analysed by sophisticated behavioral monitoring of treated and non treated mice, respectively. We have initiated a study in the mouse model of the hitherto unrecognized role of epigenetic factors contributing to the manifestation of the disease. On the other hand a systematic clinical and diagnostic natural history study of the disease in human patients was successfully started. This study enables us to define the critical parameters and hallmarks of the disease which will eventually be used in phase I and phase II clinical trials to evaluate the efficacy of the ERT in patients. Apart from providing sufficient enzyme for the preclinical studies the optimisation of the production of the human recombinant LAMAN enzyme has been pushed forward and will be of major importance for the successful introduction of this enzyme into clinical phase trials.

We could successfully develop the enzyme production and the replacement therapy in the mouse model. We have obtained important information about dose and interval of ERT treatment, about the mode of correction in peripheral neurons and neurons of the CNS, about behavioral profiles of the mouse model with and without ERT, about differentially expressed genes in treated and untreated mice. We could further analyse in detail the oligosaccharide storage by newly developed mass spectrometry analysis and started to establish this methodology for the analysis for clinical use.

The major achievements of the project are summarized below:

(1) A direct prerequisite for an effective enzyme replacement therapy in patients is **the development of a suitable therapeutic protocol** aiming to reduce the storage burden in peripheral organs and the central nervous system of the mouse model. Injection experiments using different doses of recombinant human LAMAN and administration of the drug at different intervals have revealed the best and successful therapeutic strategy. The biochemical and histological analysis of storage material in the brain and other tissues has been accompanied by a detailed behavioral analysis of alpha mannosidase knockout mice which have undergone ERT treatment. These examinations revealed **significant improvements after enzyme replacement therapy** in all parameters tested. Preliminary long term experiments with an injection schedule of 16 weeks (injection every other week) revealed that the chosen conditions reduced the storage in visceral (e.g. spleen) tissues but not in the central nervous system. However, the mice developed a severe immune response against the injected enzyme followed by a high mortality, therefore serious long term studies were precluded. To circumvent the

immunological reaction, we have **generated an immune-tolerant alpha-Mannosidosis mouse model** which will allow chronic long-term ERT-studies.

(2) **Therapeutic correction of storage lesions** after LAMAN enzyme replacement in the preclinical mouse model in the central nervous system could be demonstrated. The **correction of lysosomal storage in the CNS** worked when higher doses of enzyme (more than 250 U/g b.w. or 40 mg/g b.w.) were injected. An uptake of the recombinant enzyme could be demonstrated in neurons of the CNS and PNS. The morphological correction in brain was accompanied by **an improvement in behavioural performance** after repeated enzyme injections. These analyses of alpha-Mannosidosis mice revealed a distinct and specific neuropathology (e.g. regional astrogliosis, loss of Purkinje cells, sphinolipid storage) in the cerebellum of alpha-Mannosidosis mice that has not been described before. With the results of these studies and the use of the newly generated immuntolerant mouse model which allows long term treatment we finally are able to monitor the reduction of the oligosaccharide storage but also to diminish the storage linked neuropathology that can then be used as a "clinical" endpoint to study the effectiveness of ERT in brain.

(3) Furthermore, it was a major achievement that a **sensitive neuromotor performance test** was developed during this project - the use of an adaptation of this method enabled us to reveal subtle neuromotor impairment in mannosidosis mice. The detailed behavioural assessment also provided further support to the definition of alpha-mannosidase-deficient mice as a **valid preclinical model** of human mannosidosis. The behavioural defects described in the mannosidosis mice mimic the emotional, neuromotor and cognitive alterations of mannosidosis patients. The prominent storage-related alterations in hippocampal CA3-CA1 cells and pathways could at least partly underlie the functional changes in the mouse model, and could have important pathophysiological consequences.

(4) By performing a genome wide transcriptome analysis we **identified 33 differentially expressed genes**. Eleven genes were selected due to their high fold change in expression level. We confirmed the differential expression of eight genes by RealTime PCR. By the microarray analyses it became obvious that particularly older α-mannosidase mice show genetic alterations particularly in those genes encoding inflammatory proteins. The ERT treatment of α-mannosidosis mice partially **corrected the differential gene expression** and we were able to show that one group of differentially expressed genes were sensitive for ERT while a second group of genes were resistant to the ERT treatment. In-depth analyses of the lysosomal protease cathepsin B revealed an altered processing of the cathepsin B with a simultaneous upregulation of the cathepsin B enzyme activity. The altered processing of cathepsin B and other lysosomal proteins in α-mannosidosis mice was analysed. We were able to demonstrate that this is due to **an impaired trimming of N-glycans** on the native lysosomal proteins. We showed for the first time that the lysosomal α-mannosidase also possesses exoglycosidase activity towards N-glycans of native lysosomal proteins in vitro as well as in vivo since in ERT-treated α-mannosidase mice the molecular shift of lysosomal proteins were partially normalized.

(5) The **natural history study of patients** suffering from human alpha Mannosidosis has been performed. **Approval of Ethics Commitees** in all countries of our clinical assessment centers were obtained. A **GCP protocol has been established** and approved. To verify a possible benefit of alpha mannosidase enzyme replacement therapy in patients the **clinical hallmarks** are being defined and crucial parameters, which are suspected to change after treatment will be determined. **45 patients were included in the study**. The four clinical sites included all national and eligible patients, that consented to participate, but only 29 eligible patients were identified in the 4 involved countries. **Recruitment of patients from the rest of Europe** was therefore necessary. At the German site patients were further recruited from Spain, Denmark, France and Italy. At the Norwegian site patients were further recruited from Sweden and Finland and in the Czech Republic, attempts were made to recruit a patient from Bulgaria. The investigational sites had to overcome many obstacles in order to include patients from abroad. Interpreter assistance, travel and accommodation had to be arranged. The last patient was included 29APR2008. Two patients withdrew from the study due to severe worsening of concomitant illness and 9 patients missed the 24 months assessment, due to late recruitment and the end of the reporting period. The objective of the protocol was to recruit 38 patients and 45 patients were included. Out of these, 34 had the final visit after 24 months. The vast majority of patients were followed for a minimum of 18 months. This is in itself a great achievement and the data that has been collected (see appendix 2) will be used to pinpoint **potential endpoints for the forthcoming clinical trials**.

(6) The clinical phenotypes for the majority of European patients have been determined, and are being compared with the genotypes. Environmental and other factors that affect clinical phenotype are being assessed. An **improved diagnostic service** has been offered to European alpha-Mannosidosis families. Patient information (personal and clinical data) had been collected through a questionnaire and DNA samples had been obtained from most of the patients. Patients DNA samples have been subjected to **comprehensive mutation analysis** of the gene encoding lysosomal alpha-mannosidase (*MAN2B1*). By the end of the project **135 mannosidosis mutations have been identified** and altogether **322 independent disease alleles have been detected in 164 unrelated families**. Missense mutations have underwent further characterisation in cell culture systems. For the construction of a **relational mutation database** an industry-standard database system (Oracle) was chosen as the software-platform. The web-application for the consortium members has been completed and the consortium version of the database is now accessible from the internet. The public version, which is a restricted version of the consortium version of the database, will be deployed on the internet as soon as all data have been published in scientific journals (see below). This interactive and contextual database harbors all relevant data on alpha-Mannosidosis patients - their genotypes and molecular and clinical phenotypes. The database (http://amamutdb.no) will enable the significance of mutations to be understood and, furthermore, it will allow the **analysis of genotype/phenotype relationships**.

(7) The understanding of the pathophysiology of alpha-Mannosidosis can lead to the development of new clinical markers that are related to the clinical severity and that can be used to predict the progress and outcome of the disease. The present markers, enzyme assays and determination of accumulating oligomannoses in the urine, relate to neither the clinical severity nor the progress and outcome. We therefore have **successfully developed mass spectrometry and HPLC sensitive analysis methods** to screen and **quantify high-mannose type oligosaccharides present in plasma, urine, and tissues** of α-Mannosidosis knock-out mouse. These procedures have been applied before and after enzyme replacement therapy to various tissues and urine originating from animals. The results lead to a **quantitative and qualitative determination of individual oligosaccharide species** and a clear **assessment of the ERT efficiency** in the preclinical model.

(8) The human recombinant **LAMAN** enzyme has been cloned and expressed by Zymenex. The **production process has been developed** and **sufficient quantities enzyme were produced** to allow the studies using the mouse model to be performed. A **large scale production of rhLAMAN** for toxicology studies in animals was developed, which includes a 250L fed-batch bioreactor followed by a 4-step chromatographic purification with additional TFF, virus inactivation, virus filtration and formulation steps. A number of analytical methods (RP-HPLC, Enzyme Activity, Protein determination, SDS-PAGE, IEF, Sialic Acids, Carbohydrate composition, Western Blot for HCP and rhLAMAN, SEC and HCP ELISA) used for the analysis, **characterization and posttranslational modifications** of rhLAMAN have also been developed.

(9) We have organised **effective research training**, meetings and communication events between laboratories and clinical centers. This has greatly stimulated the collaborative network and will facilitate the achievement of the research goals of the HUE-MAN project.

(10) The dissemination of knowledge was most prominently expressed by a number of **press releases, publications, communications and presentations at international conferences and approvals** by different authorities.

Potential applications:

The ultimate impact of the proposal is in providing the basis for therapeutic strategy to treat the rare disorder alpha-Mannosidosis. The principle feasibility of ERT in the mouse model of alpha-Mannosidosis was shown. In the treated mice corrections of lysosomal storage in viceral organs but also -most importantly- brain was demonstrated making the introduction of ERT for human alpha Mannosidosis into clinical trials a very promising project. The HUE-MAN project was of substantial importance to establish preclinical and clinical therapy protocols, to set out the conditions for a large scale and toxicity monitored drug production, to better understand and define the typical hallmarks of this disease and to evaluate epigenetic factors influencing the disease progression. All these goals of HUE-MAN have added new important informations which will pave the way to realise a causative cure of this still untreatable disease.
The major technological prospect of this research has been the achievement of a medicament for curing alpha-Mannosidosis. This research project was intended to decrease the economical burden, and to improve the prospects of a new medicament, to the point where there is an incentive among biotechnological companies to produce this drug.

Section 2 – Dissemination

Public Dissemination

Meetings and Collaboration Network

HUE-MAN project started by a Kick-off meeting in Kiel in May 2006, followed by meetings in Venice (June 2006; Natural History Study Coordination Meeting), Brussels (January 2007), Copenhagen (May, 2007, Blood-Brain-Barrier Discussion Workshop), Perugia (September 2007), Copenhagen, Denmark taking place from May 9th until May 10th 2008; October 2008, Prague (Chech Republic); Meeting, May 2009, Bodoe/Tromsoe, Norway and the final Meeting, September 2009, Kiel, Germany.

All researchers and clinicians of the HUE-MAN consortium met. The responsible scientists/clincicians/SME-leaders of each workpackage presented the work status and young scientists gave presentations on their ongoing projects.

An extensive collaboration network has been set and this has included several visits of scientists and clinicians between the laboratories and clinical centers.

Websites

A HUE-MAN website was established in the end of June 2006. The website:

http://www.biochem.uni-kiel.de/hue-man/

describes the HUE-MAN project and the knowledge generated in the project are displayed on these web sites that are open to the public and scientific with links to the web sites of the PIs. Additionally, the website contains an intranet accessible to HUE-MAN scientists. The intranet contains all meeting agendas, minutes and progress reports. Additionally, the intranet provides databases of different reagents freely distributed amongst the NCL-models scientists. The website was maintained through the coordination office and updated on a regular base. A number of patient requests came in through this intranet platform.

The HUE-MAN project has presented numerous scientific presentations, posters, public presentations, research training and other dissemination activities, also listed below.

An alpha-Mannosidosis mutation database (http://amamutdb.no) was devoloped and deployed on the interenet on September 2009, to be accessible to HUE-MAN consortium members. The public version, which is a restricted version of the consortium version of the database, will be deployed on the internet as soon as all data have been published in scientific journals. The website is fully interactive with a user-friendly graphical interface. A choice of search tools is available. Description of allelic variants (mutations) constitutes the core of the database. These data are related to patients and annotations of genotypes –

to ethnic-, population- and geographic associations - to a detailed and structured set of clinical information - to properties of the mutant enzymes such as residual activity (if any) - intracellular transport and processing (missense mutations) and enzyme 3D-stucture. The database will enable the significance of mutations to be understood and, furthermore, it will allow the analysis of genotype/phenotype relationships.

Parent organizations

HUE-MAN and in particular the clinical participants of HUE-MAN are in regular contact to various parent organizations such as the ISMRD (International Advocate for Glycoprotein Storage Diseases) and the Society for Mucopolysaccharide Disease and the German Gesellschaft für Mukopolysaccharidosen to discuss recent developments and to keep the contact to local clinicians, parents and patients.
The details of the HUE-MAN project have been presented to the UK MPS Society, a parent group that provides advocacy support within the UK for patients affected by MPS and related disorders including alpha-Mannosidosis.

University level teaching

Many of the PIs are involved in teaching in their local Universities and use the case of Mannosidosis and the project related to the new therapy of Mannosidosis where possible.
Examples of the University teaching include:
University Kiel: Annual Course on Biochemistry for Medical Students; BSc and Master courses of Biochemistry
University Göttingen: Bi-annual seminar course on Biochemistry for Medical students; specialized courses for students of "Molekulare Medizin" as well as for BSc and Master students for Biology.
Charles University in Prague: Inherited metabolic disorders as a regular seminar in the course of pediatrics for Medical Students
University of Manchester: Two annual courses on metabolic diseases (including LSDs). One to non-specialist paediatric colleagues within the North of England and a second one aimed specifically at clinical trainees inherited metaolic disease. New treatments including the work towards ceveloping a therapy for mannosidosis are always discussed during these meetings.
University of Mainz: Annual Course on Lysosomal Storage Disorders (Nierstein, June 7-12, 2002-2009

Other dissemination

TV and newspaper apperances are also an important way to effectively target information to multiple key audiences including othe scientists, the families of affected children, healthcare professionals who support them, and the general public. The coordinator but also some of the participants appeared in national and regional discussions and publications about the HUE-MAN project and the goals to get a new therapy for this rare disorder:

Press releases/media contact (see also: http://www.uni-kiel.de/Biochemie/hue-man/media.htm for full details):

- Saftig, P. Forscher entwickeln Medikament gegen Alpha-Mannosidose Die Naturheilkunde, 10/2006, 11
- Saftig, P. Gen-Defekt ausgleichen Life Science Nord, 07/2006, 7
- D´Hooge, R. Europese steun voor onderzoek zeldzame ziekten Campuskrant, 06/2006, 7
- Saftig, P. EU-Projekt am 01. April gestartet Eurobrief, 06/2006, 4
- Saftig, P. Dem Gen-Defekt gegensteuern BIOforum 4/2006, 6
- Saftig, P. Dem Gen-Defekt gegensteuern Kieler Nachrichten, Nr. 125, 05/2006
- Saftig, P.Overcoming a Genetic Defect Press Release CAU Kiel, 43, 05/2006
- Saftig, P.Gemeinsam gegen den Gendefekt Schleswig-Holsteinische Landeszeitung, 186, 08/2005
- Beck, M. Auch Arzneien für seltene Erkrankungen können lukrativ sein: Frankfurter Allgemeine Zeitung, 8.6.2007, page N2
- Dr E.Wraith, Partner 5, and prof. Zeman Partner 7 had an interview about enzyme replacement therapy in Czech TV, May 17th, 2007.
- Novel Natural History Study to answer questions regarding Rare Disease, July 4, 2007 (www.zymenex.com)
- Dr E Wraith had an exhitbition at Manchester Museum of Science on new therapies for Genetic Disease that centred on the introduction of ERT as a therapy.

Official documents /approvals related to HUE-MAN activities:

- The study is registered on www.ClinicalTrials.gov ID: NCT00498420 in order to eligible for publishing in scientific journals.
- The drug (recombinant human alpha-mannosidase) has been approved Orphan Designation 02FEB2006 by US Food and Drug Administration: http://www.fda.gov/orphan/designat/alldes.rtf
- The drug (recombinant human alpha-mannosidase) has been approved Orphan Medicinal Product Designation in the European Union 26JAN2005 by the European Commission: http://ec.europa.eu/enterprise/pharmaceuticals/register/alforphreg.htm
- Construction of a mutation database, for alpha-Mannosidosis: Renewal of the approval from The Norwegian Data Inspectorate, April 10th, 2007.
- The natural history of alpha-Mannosidosis: Approval from the Regional Committee for Research Ethics, Northern-Norway (Tromsø, 9th of June/ 2007 / QLTR-2000-02458) and The Norwegian Data Inspectorate (May, 2007).

Scientific Dissemination

Publishing research papers, review articles and book chapters:
Participants of HUE-MAN have published original articles and reviews in leading peer-reviewed research journals. There are a number of papers which are currently in the planning stage.

• A number of papers directly linked to HUE-MAN activities: 35 (see the list below)

• A number of papers which are linked to the activities of HUE-MAN: 115 (see the list in the periodic activity reports)

Presentations at scientific meetings/congress contributions:

33 talks and presentations (poster) at scientific meetings (see the list in the periodic activity reports)

Grant support:
Much of the data generated will form the basis of grant applications,many of which are on a collaborative basis with participants in this project. In particular the promising data on the enzyme replacement therapy in the preclinical mouse model stimulated an application to the EU:FP 7 framework call (HEALTH.2010.2.4.4-1: Clicical Development of Enzyme Replacement Therapy in Alpha-Mannosidosis using recombinant human enzyme; ALPHA-MAN). The application involving 12 partners from University and industry was submitted November 17th 2009.

Clinical Apects:
In addition to the scientific dissemination, certain clinical aspects exist:

- Teaching clincians at the Villa Metabolica, Mainz
- Nierstein Course on lysosomal storage disorders
- Course on lysosomal storage disease as a part of postgraduate curriculum in pediatrics in Czech republic
- Teaching clinicians and trainees at Manchester University

Publications which resulted from HUE-MAN projects from 2006 until 2009:

Beck M, Widmer U. Assessing multiorgan disease severity: scoring systems and applicability in LSDs. Acta Paediatr Suppl. 2006; 95: 41-42.

Beck M. New therapeutic options for lysosomal storage disorders: enzyme replacement, small molecules and gene therapy. Hum Genet. 2007 Mar;121(1):1-22.

Blanz J. Stroobants S, Lüllmann-Rauch R, Morelle W, Lüdemann M, D´Hooge R, Reuterwall H, Michalski J.C, Fogh J, Andersson C, Saftig P. (2008) Reversal of peripheral and central nervous storage and ataxia after recombinant enzyme replacement therapy in alpha-mannosidosis mice. Hum. Mol. Genet. , in press

Blanz, J., Saftig, P. Enzymersatztherapie bei der lysosomalen Speichererkrankung alpha-Mannosidose Deutsche Zeitung für klinische Forschung 11/12-2006, 42-49

Brantova O. Asfaw B, Sladkova J, Poupetova H, Zivny J, Magner M, Krusek J, Hansikova H, Ledvinova J, Tesarova M , Zeman, J. (2009) Ultrastructural and functional abnormalities of mitochondria in cultivated fibroblasts from α-mannosidosis patients. Biologia 64/2: 394-401

Caeyenberghs K, Balschun D, Roces DP, Schwake M, Saftig P, D'Hooge R.Multivariate neurocognitive and emotional profile of a Mannosidosis murine model for therapy assessment. Neurobiol Dis. 2006 Aug;23(2):422-32.

Castelnovo G, Levade T, Riise Stensland HM, Nonnon MJ, Berges MA, Tollersrud OK, Labauge P. 2007 [Adult leukoencephalopathy caused by alpha-Mannosidosis deficiency] Rev Neurol (Paris). 163(3):359-61.

D'Hooge R, Lullmann-Rauch R, Beckers T, Balschun D, Schwake M, Reiss K, von Figura K, Saftig P. Neurocognitive and psychotiform behavioral alterations and enhanced hippocampal long-term potentiation in transgenic mice displaying neuropathological features of human alpha-Mannosidosis. J Neurosci. 2005 Jul 13;25(28):6539-49.

Damme M, Morelle W, Schmidt B, Andersson C, Fogh J, Michalski JC, Lübke T Impaired lysosomal trimming of N-linked oligosaccharides leads to hyperglycosylation of native lysosomal proteins in mannosidosis mice. Mol. Biol. Cell, in press

de la Salle H, Mariotti S, Angenieux C, Gilleron M, Garcia-Alles LF, Malm D, Berg T, Paoletti S, Maitre B, Mourey L, Salamero J, Cazenave JP, Hanau D, Mori L, Puzo G, De Libero G. Assistance of microbial glycolipid antigen processing by CD1e. Science. 2005 Nov 25;310(5752):1321-4.

Faid V, Evjen G, Tollersrud OK, Michalski JC, Morelle W. Site-specific glycosylation analysis of the bovine lysosomal alpha-mannosidase. Glycobiology. 2006 May;16(5):440-61.

Faid V, Michalski J.C, Morelle W. A mass spectrometric strategy for profiling glycoproteinoses, Pompe disease, and sialic acid storage diseases. Proteomics - Clinical Applications, 2008, 2, 528-543

Kollmann K, Damme M, Deuschl F, Kahle J, D'Hooge R, Lüllmann-Rauch R, Lübke T (2009) Molecular characterization and gene disruption of Scpep1 in mice. FEBS J. 276(5):1356-1369

Kollmann K, Mutenda KE, Balleininger M, Eckermann E, von Figura K, Schmidt B, Lübke, T (2005) Identification of novel lysosomal matrix proteins by proteome analysis. Proteomics. 5(15):3966-78

Leroy, T., Silva,M., D'Hooge, R., Aerts, J.M., Berckmans, D. (2009) Automated gait analysis in the open-field test for laboratory mice. Behavior Research Methods 41: 148-153.

Lübke T, Lobel P, Sleat D. (2009) Proteomics of the lysosome. Biochim Biophys Acta 1793(4):625-35

Lyons, M.J., Espinoza, L., Stensland, H., Holden, K. (2007) Early onset alpha mannosidosis with slow progression in three Hispanic males. Dev. Med. & Child Neurol. 49, 854-857

Magner M, Buganová M, Asfaw B, Poupatová H, Ledvinová J, Brantová O, Stesland H.M. a Zeman J. Klinické projevy a výsledky laboratorních vyšetření u čtyř pacientů s alfa-manosidózou. Čes.-slov. Pediat., 2008, roč. 63, č.12, s.677-682

Malm D *"Alfa-mannosidose"* NEL (Norsk Elektronisk Legehåndbok) 2006 http://www.legehandboka.no/

Malm D, Nilssen O. Alpha-Mannosidosis (October 2005 through 2007) In: GeneReviews: Clinical GeneticInformation Resource .Copyright, University of Washington, Seattle.Available at http://www.geneclinics.org

Malm D, Pantel J, Linaker OM. Psychiatric symptoms in alpha-Mannosidosis. J Intellect Disabil Res. 2005 Nov;49(Pt 11):865-71.

Malm, D. and Nilssen, O. (2008) Alpha-mannosidosis. Orphanet J. of Rare Diseases, 3, 21

Morelle W, Canis K, Chirat F, Faid V, Michalski JC. The use of mass spectrometry for the proteomic analysis of glycosylation. Proteomics. 2006 Jul;6(14):3993-4015.

Morelle W, Faid V, Chirat F, Michalski J.C. Analysis of N- and O-linked glycans from glycoproteins using maldi-tof mass spectrometry. Methods Mol Biol., 2009, 534, 5-21

Morelle W, Michalski J.C. Analysis of protein glycosylation by mass spectrometry. Nature Protoc., 2007, 2, 1585-1602

Riise Stensland HMF, Persichetti E, Sorriso C, Hansen GM, Bibi L, Paciotti S, Balducci C, Beccari T (2008) Identification of two novel beta-mannosidosis associated sequence variants. Biochemical analysis of beta-mannosidase (MANBA) missense mutations. Mol Genet Metab 94:476-480

Sabourdy P, Labauge P, Riise Stensland HMF, Nieto M, Garces VL, Renard D, Castelnovo G, de Champfleur N, Levade T (2009) A MANBA mutation resulting in residual beta-mannosidase activity associated with severe leukoencephalopathy: a possible pseudodeficeincy variant. BMC Med Genet 10:84 (online Sept 3rd - doi 10.1186/1471-2350-10-84)

Saftig P. and Klumperman J. (2009) Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nat. Rev. Moll. Cell Biol., in press

Saftig, P. (2006) Lysosomes (ed. Paul Saftig), Springer, New York

Schieweck O, Damme M, Schröder B, Hasilik A, Schmidt B, Lübke T (2009) NCU-G1 is a highly glycosylated integral membrane protein of the lysosome. Biochem J. 422(1):83-90

Stroobants, S., Leroy, T., Eckhardt, M., Aerts, J.M.; Berckmans, D., D´Hooge, R. (2008) Early signes of neurolipidosis-related behavioral alterations in a murine model of metachromatic leucodystrophy. Beh. Brain Res. 189, 306-316

Wraith JE (2006) Limitations of enzyme replacement therapy: Current and future. J Inherit Metab Dis; 29(2-3):422-447.

Wraith JE. Metabolic Disorders in Endocrine and Metabolic Disorders, in Illustrated Textbook of Paediatrics, 3rd edition, eds Lissauer T, Clayden G, Mosby, Edindurgh, 2007.

Wraith JE. Mucopolysaccharidoses and Oligosaccharidoses. In Inborn Metabolic Diseases, 4th edition, eds Fernandes J, Saudubray J-M, van den Berghe G, Walter J H, Springer-Verlag, Berlin, 2006.

Wynn RF, Stubbs M, Ozyilmaz N, Wraith JE, Bigger B. (2009) Cellular therapy of lysosomal storage disorders: Current status and future prospects. Current Paediatric Reviews 5: (in press)