PROJECT FINAL REPORT

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[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.
[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm ; logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

4.1 FINAL PUBLISHABLE SUMMARY REPORT

This section must be of suitable quality to enable direct publication by the Commission and should preferably not exceed 40 pages. This report should address a wide audience, including the general public.

The publishable summary has to include **5 distinct parts** described below:

- An executive summary (not exceeding 1 page).
- A summary description of project context and objectives (not exceeding 4 pages).
- A description of the main S&T results/foregrounds (not exceeding 25 pages),
- The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results (not exceeding 10 pages).
- The address of the project public website, if applicable as well as relevant contact details.

Furthermore, project logo, diagrams or photographs illustrating and promoting the work of the project (including videos, etc…), as well as the list of all beneficiaries with the corresponding contact names can be submitted without any restriction.

Executive summary (not exceeding 1 page)

The EUCLYD consortium has established a unique research programme to study lysosomal storage diseases by developing a scientific work plan that has promoted collaborative scientific interactions between outstanding communities of basic and clinical investigators. EUCLYD has studied the mechanisms underlying the symptoms of lysosomal storage disorders to then progress towards the testing of novel therapeutic approaches with the goal of setting the scenario to directly reach patient's "bedside."

Lysosomal Storage Diseases (LSDs) are caused by genetic defects that affect the synthesis or processing of lysosomal hydrolases. Therefore, a lysosomal disorder can be due to a defect in a specific hydrolase, by deficiencies in activator proteins, in the receptors or in the trafficking of enzymes. This leads to an intracellular accumulation of a variety of undegraded cellular substrates, including sphigolipids, glycosaminoglycan and glycogen. Forty to fifty lysosomal storage diseases are presently known. As a group they occur with an estimated frequency of 1/2000, but each of them is rare.
The four specific LSDs chosen in this research programme (Pompe disease, Gaucher disease, MPS VI, MSD) have each unique characteristics, they all differ for the type of stored material and for the involvement of different tissues. This results in different challenges in the treatment of patients and has therefore implications for the choice of specific therapeutic strategies.

The overall goal of the project has been *i)* to study specific lysosomal diseases, *ii)* to understand the pathophysiology, *iii)* to determine the natural history of these rare diseases in order to develop tools to measure therapy efficacy, and *iv)* to test and develop novel therapeutic approaches utilizing appropriate animal models of human disorders. The final objective of the EUCLYD consortium has been to shed light on the course and pathophysiology of lysosomal storage diseases and to develop proof-of principle tests on novel therapeutic approaches.

According to the experimental plan, the consortium has studied the pathophysiology of MSD, Gaucher Disease, PD and MPS VI (WP1). These studies have unveiled the basic mechanisms to explain disease phenotype. This knowledge has also allowed developing therapies to contrast the devastating effects of accumulation, and to understand whether and when therapies should be devised. In addition, research has been focused on the natural history of Gaucher Disease, Pompe Disease and MPS VI (WP2). The study of the natural history of these disorders is essential to evaluate the effectiveness of therapy. Novel therapeutic strategies for LSDs consist of the application of small molecules that act as chaperones to increase the residual activity of the lysosomal enzymes, known as enzyme enhancement therapy (WP3) or small molecules that inhibit substrate synthesis, known as substrate reduction (WP4). Finally, the consortium has tested and developed new protocols utilizing Adeno-Associated Virus (AAV) vectors to directly administer the wild-type gene into a factory organ taking advantage of normal lysosomal enzyme trafficking and a phenomenon known as "cross-correction" (WP5).

Individuals with lysosomal disorders, which largely affect children in the first decade of life, will greatly benefit from continual refinement and optimization of the current therapy, as well as from the development of new treatment modalities that offer improvements in efficacy, cost, safety and availability. By developing an experimental plan to understand the basis, the natural history and to test potential therapies, the EUCLYD consortium has provided an enormous impact on the health of children who are being devastated by this type of progressive, debilitating and often lethal group of disorders.

Summary description of project context and objectives (not exceeding 4 pages)

Lysosomes are membrane-enclosed compartments, filled with hydrolytic enzymes that are used for the degradation of macromolecules. Proteins and other substrates are delivered to the lysosomes by various pathways including endocytosis, and autophagy, a pathway utilized by the cell for the disposal of obsolete parts. Many steps are necessary for the correct synthesis and processing of lysosomal enzymes.
Lysosomal Storage Diseases (LSDs) are caused by genetic defects that affect the synthesis or processing of lysosomal hydrolases. Therefore, a lysosomal disorder can be due to a defect in a specific hydrolase, by deficiencies in activator proteins, in the receptors or in the trafficking of enzymes. This leads to an intracellular accumulation of a variety of undegraded cellular substrates, including sphigolipids, glycosaminoglycan and glycogen. Forty to fifty lysosomal storage diseases are presently known. As a group they occur with an estimated frequency of 1/2000, but each of them is rare.

The EUCLYD consortium has established a unique research programme to study LSDs by promoting collaborative scientific interactions between outstanding communities of basic and clinical investigators. To improve human health, scientific discoveries must be translated into practical applications. EUCLYD has focused on the study of the mechanisms underlying the symptoms of LSDs to then progress towards clinical applications through the development of novel therapeutic approaches with the final goal of moving from “bench to bedside”.

Among the LSDs presently known, EUCLYD has focused on: **Gaucher disease**, **Pompe disease**, **Mucopolysaccharidosis VI** (MPS VI) and **Multiple Sulfatase Deficiency** (MSD). The four specific LSDs chosen in this research programme have each unique characteristics, they all differ for the type of stored material and for the involvement of different tissues. This results in different challenges in the treatment of patients and has therefore implications for the choice of specific therapeutic strategies.

The overall goal of the project has been *i)* to study specific lysosomal diseases, *ii)* to understand the pathophysiology, *iii)* to determine the natural history of these rare diseases in order to develop tools to measure therapy efficacy, and *iv)* to test and develop novel therapeutic approaches utilizing appropriate animal models of human disorders.

The **specific objectives** have been to:

1. Characterize the pathophysiology of MSD, MPS VI and PD to identify disease mechanisms and markers. This characterization has been done utilizing specific markers and assays that have allowed to quantify and verify the mechanisms studied.

2. Determine factors that modify clinical diversity between PD patients bearing the same GAA haplotype. This has been determined by using specific assays.

3. Identify tissue-pathology markers as determinants of PD stage. The success of therapeutic intervention seems largely determined by the stage of disease at start of treatment. Furthermore, these markers could help in predicting efficacy of therapy.

4. Characterize the natural history of a subset of LSDs in order to have the correct tools to evaluate treatment efficacy and to obtain information to develop standardized European protocols for the different LSDs, starting with Pompe disease and MPS VI. This has allowed collecting homogeneous information on a large number of patients and, ultimately, has been useful to further validate the information available until now on the therapeutic outcome.

5. Explore the use of enzyme enhancement by pharmacological chaperones, for the treatment of Pompe disease.

6. Determine the effect of miglustat and other substrate inhibitory analogues, such as the morpholino compounds, on *de novo* biosynthesis of glucosylceramide and determine whether their action serves as an inhibitor of cytokine release that accompanies Gaucher disease.

7. Develop novel gene therapy approaches using state-of-the-art gene transfer technologies based on AAV vectors for therapy of systemic signs of lysosomal storage diseases (LSD).

The final objective of the EUCLYD consortium has thus been to shed light on the course and pathophysiology of lysosomal storage diseases and to develop proof-of principle tests on novel therapeutic approaches. Therapeutic approaches can only be tested if disease mechanisms are understood to identify pathogenetic markers and natural history to evaluate therapeutic outcome and long-term therapy efficacy.

A formal evaluation of therapy efficacy in lysosomal diseases is difficult due to the marked variability of clinical phenotypes. Enzyme replacement therapy and enhancement therapies have been developed to treat these disorders, albeit with variable results. New treatment strategies have been considered for patients with LSDs including gene therapy, substrate reduction therapy and chaperone therapy. Individuals with lysosomal disorders will greatly benefit from continual refinement and optimization of the current therapy, as well as from the development of new treatment modalities that offer improvements in efficacy, cost, safety and availability.

Finally, it is important to stress that LSDs are characterized by a progressive, highly debilitating or lethal course, causing severe handicap in patients resulting in significant social burden. Moreover, many of these diseases affect children in the first decade of life. By developing an experimental plan to understand the basis, the natural history and to test potential therapies, the EUCLYD consortium has provided an enormous impact on the health of children who are being devastated by this type of progressive, debilitating and often lethal group of disorders.

Description of the main S&T results/foregrounds (not exceeding 25 pages)

The interest of the EUCLYD consortium is the study of Lysosomal Storage Diseases (LSDs), a heterogeneous group of disorders that encompass around 50 distinct metabolic diseases. The incidence of LSDs as a group is 1/2000, but, taken individually, each disease is very rare and many have an incidence of less than 1:100,000. However, one important peculiarity of LSDs is that data indicate that the basis of the diseases and pathogenetic mechanisms of the symptoms may have common grounds; therefore, the study of a few more relevant examples, such as Gaucher Disease, Pompe Disease, Mucopolysaccharidosis VI (MPS VI) and Multiple Sulfatase Deficiency (MSD), may have huge implications for the entire group of disorders.

According to the experimental plan, the consortium has studied the pathophysiology of MSD, Gaucher Disease, PD and MPS VI (**WP1**). These studies have unveiled the basic mechanisms to explain disease phenotype. This knowledge has also allowed developing therapies to contrast the devastating effects of accumulation, and to understand whether and when therapies should be devised. In addition, research has been focused on the natural history of Gaucher Disease, Pompe Disease and MPS VI (**WP2**). The study of the natural history of these disorders is essential to evaluate the effectiveness of therapy. Novel therapeutic strategies for LSDs consist of the application of small molecules that act as chaperones to increase the residual activity of the lysosomal enzymes, known as enzyme enhancement therapy (**WP3**) or small molecules that inhibit substrate synthesis, known as substrate reduction (**WP4**). Finally, the consortium has tested and developed new protocols utilizing Adeno-Associated Virus (AAV) vectors to directly administer the wild-type gene into a factory organ taking advantage of normal lysosomal enzyme trafficking and a phenomenon known as “cross-correction” (**WP5**).

The main S&T results obtained by the EUCLYD consortium are outlined below and in the following pages.

LSD: pathophysiology

In eukaryotes, most of the cellular clearing processes occur in a specialized organelle, the lysosome, which receives and degrades macromolecules from the secretory, endocytic, autophagic and phagocytic membrane-trafficking pathways. Lysosomes contain proteins with different functions, including hydrolases, transmembrane proteins involved in membrane fusion and transport, and a complex proton pump that is required for the acidification of the organelle. Importantly, the lysosome is involved in numerous diseases including lysosomal storage diseases (LSDs), characterized by lysosomal dysfunction and defects in intracellular trafficking. This leads to intracellular accumulation of a variety of undegraded cellular substrates, including sphingolipids, glycosaminoglycans and glycogen. In most cases these disorders are characterized by a progressive, highly debilitating or lethal course, associated with neurodegeneration, causing severe handicap in patients and resulting in a significant social burden.

As the degradative requirements of the cell vary depending upon tissue type, age and environmental conditions, it is reasonable to expect the existence of systems that allow regulation of lysosomal function. Genetic programs that control organelle biogenesis and function may mediate such coordination and facilitate the coordination of complex functions, such as cellular clearance. We discovered a gene regulatory network (CLEAR: Coordinated Lysosomal Enhancement And Regulation) (Sardiello et al., 2009) that controls lysosomal biogenesis and function. The transcription factor TFEB acts as a modulator of the CLEAR network and is physiologically activated by lysosomal storage. Upon activation, TFEB translocates into the nucleus, binds to CLEAR target sites in the promoter of lysosomal genes, and induces lysosomal biogenesis (Sardiello et al., 2009). We then demonstrated that TFEB controls lysosomal exocytosis (Medina et al., in press). During lysosomal exocytosis, a Ca^{2+}-regulated process, lysosomes are docked to the cell surface and fuse

with the plasma membrane (PM), emptying their content outside the cell. This process has an important role in secretion and PM repair. We showed that TFEB regulates lysosomal exocytosis. TFEB increases the pool of lysosomes in the proximity of the PM and promotes their fusion by raising intracellular Ca^{2+} levels through the activation of the lysosomal Ca^{2+} channel called MCOLN1. Induction of lysosomal exocytosis by TFEB overexpression rescued pathologic storage and restored normal cellular morphology both *in vitro* and *in vivo* in lysosomal storage diseases (LSDs, MSD). Our data indicate that lysosomal exocytosis may directly modulate cellular clearance and suggest a novel therapeutic strategy for disorders associated to intracellular storage.

Pompe disease: pathophysiology and natural history

With an estimated incidence of 1 in 40.000 Pompe disease is indeed a rare disease. The pathogenesis is well understood in the sense that the disease is caused by sequence variations in the gene coding for acid α-glucosidase. Some of these variations are harmless but others lead to loss of function. Acid α-glucosidase is one of the estimated 50 enzymes that together secure lysosomal functioning. Proper function of acid α-glucosidase is essential for the degradation of glycogen that has entered the lysosomes by autophagy, a process by which cells and tissues renew themselves. Shortage or complete lack of acid α-glucosidase leads to storage of glycogen and cellular damage. As muscle cells are most vulnerable to glycogen storage, acid α-glucosidase deficiency leads to muscle weakness and wasting, predominantly affecting the proximal muscles and the pulmonary function of affected individuals. Infants without any acid α-glucosidase activity succumb in the first year of life due to cardio-respiratory failure.

The European collaborative effort of EUCLYD has enabled to address the many aspects of Pompe disease in parallel: i.e. the underlying mutations and their effect, renewal of diagnostic procedures and introduction of methods to estimate the "CRIM status" and the antibody titer evoked by enzyme therapy, the role of autophagy, the role of muscle fibre type involvement, the natural course including survival, muscle function, pulmonary function, hearing and cardiac function in children, the impact of disease modifying factors, the effect of enzyme replacement therapy, the corrective effect of chaperones on pathogenic forms of acid α-glucosidase and the additive effect of chaperones when administered in combination with enzyme replacement therapy.

The EUCLYD consortium has successfully characterized multiple cellular abnormalities in Pompe disease (PD) cultured fibroblasts (including intralysosomal storage of glycogen, increased number of multivesicular bodies, activation of autophagy, expansion of the Golgi apparatus), and identified abnormalities in the trafficking and recycling of the cation-independent mannose-6-phosphate receptor (CI-MPR). All these abnormalities appeared to be more prominent in severe and intermediate PD fibroblasts, apparently correlating with disease severity.

During the three years of the EUCLYD programme the number of patients cared for by the clinicians of the consortium (EMC, UMC) has substantially increased, mainly as a result of the approval and market introduction of enzyme replacement therapy (Myozyme) in 2006. Managing the increasing number of patients has required the design of a study protocol and a package of clinical assessments to secure the systematic follow-up of all patients. All protocols are now in place to diagnose the patients at an early stage of disease with improved clinical and laboratory procedures. EMC, in particular, has established an assay to measure acid α-glucosidase activity in white blood cells, and other methods to measure the activity in dried blood spots.
The making of blood smears and staining of the lymphocytes with Periodic Acid Schiff reagent - to demonstrate glycogen storage - was shown to assist in the diagnosis of Pompe disease patients with both very early onset (< 1 month of age) as well as very late onset (> 70 years of age) of symptoms. This finding was unexpected since patients with late onset of symptoms do not store glycogen in their cultured skin cells while patients with early onset of symptoms do. The difference between glycogen storage in lymphocytes *versus* skin cells might lay in the differential expression of the acid α-glucosidase gene in these different cell types although work is still in progress to fill this

knowledge gap (the planned experiments will be completed with financial support of EMC within the end of 2011). Hopefully, the new generation sequencing technology that has meanwhile become available will also shed light on the genetic factors that substantially modify the clinical course of Pompe disease. The latter relates to the observation in our continuously growing patient population that patients with the "same" acid α-glucosidase genotype can have a widely differing course of disease with regard to age of onset. Skin cells of 12 patients with very early onset and 12 patients with very late onset of symptoms have been collected and are presently being analyzed for acid α-glucosidase activity, glycogen storage and mRNA expression level of all expressed genes. These latter activities enabled by EUCLYD hopefully will guide to new ways of therapeutic intervention.

The systematic follow-up of large numbers of patients with Pompe disease over the past three years has enabled us to accurately document the natural course of Pompe disease and the effects of enzyme replacement therapy.

By mutation analyses of the *GAA* gene we characterized the genotype in all enrolled patients (UMC, N=48) and identified some genotype-phenotype correlations (Herzog et al., in preparation). Furthermore our studies contributed to the description of dilated arteriopathies in Pompe disease, an unprecedented disease complication. Additionally, we recognized, by interim analysis, high prevalence of respiratory insufficiency and abnormal breathing during sleep due to diaphragmal weakness years prior to diagnosis in new diagnosed adult Pompe patients. To increase awareness and to study the natural course of diaphragmal weakness in adult Pompe patients a cooperative study in the 3 largest German Pompe centres has begun. Timely diagnosis will improve the long-term outcome by optimizing respiratory management and by early initiation of enzyme replacement therapy.

Study results in the infantile Pompe cohort have raised the issue of antibody formation against rhGAA. As a consequence, we studied the impact of antibody formation on safety and efficacy of rhGAA in infantile Pompe patients. We also developed new methods to monitor antibody formation against the therapeutic enzyme and to establish the "CRIM status" of severely affected infants. "CRIM" stands for any small amount of acid α-glucosidase that is detectable in the patient's tissues. CRIM-negative patients are more prone to develop high antibody titers during enzyme therapy than CRIM-positive patients. We investigated the role of CRIM status in 11 of our infantile patients and confirmed previous findings that a CRIM-negative status is a poor prognostic factor for successful clinical outcome, but the correlation between the height of the antibody titer (the height of the immune response) and the clinical outcome is not very strict. An incidental finding of a very high antibody response in an adult patient receiving enzyme replacement therapy has led to in-depth analysis of the effect of antibody formation as a result of enzyme replacement therapy. It has resulted in an understanding of what antibody formation and low *versus* high antibody titers actually means for the successful treatment of patients.

The newly developed methods for determining the CRIM status and the height of antibody titers are now routinely applied as diagnostic tool and for the follow-up of patients during treatment. EMC offers diagnostic services for Pompe disease at the level of enzyme assay and DNA analysis to many European and non-European countries; CRIM tests, antibody testing and analysis of the functional effect of newly discovered acid α-glucosidase mutations are included.

As to the natural course of Pompe disease, in the context of EUCLYD and related programmes, a study was performed to gather information that is yet lacking on the survival of adults with Pompe disease. This study, which was carried out in a large sample of individuals (268 adults), was completed and showed for the first time that untreated patients have a higher mortality than that observed in the general population. The levels of disability and handicap/participation are the most important factors associated with mortality.

The condition of 94 adult patients who did not receive enzyme replacement therapy was monitored during their regular visits to hospital (EMC). At study entry, the mean age of these patients was 50

years old (range 25-75 yrs). The skeletal muscles of the proximal lower extremities and the trunk were most affected, and the muscle weakness increased by 1.3% to 2.6 points/year. The forced vital capacity in sitting position deteriorated by 1% point per year (p=0.06), and in supine position by 1,3% points per year (p=0.02). A substantial number of patients had less well-known features of Pompe disease such as ptosis (23%), bulbar weakness (28%), or scapular winging (33%). Hearing loss was studied in 58 affected adults using tympanometry and pure-tone audiometry and appeared to be no feature of Pompe disease in adults. Fatique was a frequent finding. The bone mineral density (BMD) was measured in 36 adults and 10 children with Pompe disease and was found to be significantly lower than in the reference healthy population.

Pompe disease: enzyme replacement (ERT) and enzyme enhancement (EET) therapy

The characterization of the CI-MPR pathway in PD cells has important clinical implications. For PD, like for some of the most prevalent lysosomal storage diseases, enzyme replacement therapy (ERT) with recombinant human alpha-glucosidase (rhGAA) is the only approved treatment. ERT is based on the concept that recombinant lysosomal hydrolases can be internalized by cells and tissues through the mannose or mannose-6-phosphate receptor (MPR) pathways and are ultimately delivered to lysosomes, where they replace the function of the defective hydrolases. Thus, the integrity of the mannose-6-phosphate pathway is a requisite for the efficacy of ERT. In PD, ERT efficacy is variable in different patients and it is known that some tissues (like the skeletal muscle) are refractory to this treatment. The finding of a deranged CI-MPR function in PD cells may provide an explanation for the variable response to ERT (and indicate possible therapeutic strategies directed toward the correction of these abnormalities).

Sixty-nine patients were monitored before and after the start of enzyme replacement therapy (EMC). In this group of patients with a treatment duration of 2-4 yrs the muscle strength improved by 1.4 to 4.0 points per year. The pulmonary function (FVC) in sitting position (N=62) stabilized, but the FVC in supine position ($p<0.05$) (N=54) declined by 1% per year. The best responding patients were those of younger age, with shorter disease duration and with the least disease severity. It is certain that the occurrence of high antibody titers hampers enzyme therapy, but it is not fully understood why some patient generate high antibody titers while others don't.

In addition, we have provided the proof-of-principle for the use of pharmacological chaperones as a potential therapy of PD. Enzyme enhancement therapy (EET) is based on the concept that loss-of-function diseases are often due to missense mutations causing misfolding and degradation of catalytically competent enzyme proteins. A partial rescue of enzyme activity may be obtained by active site-directed competitive inhibitors, that can improve folding and stability of mutated proteins with altered conformations by acting as folding templates.
We demonstrated that two imino sugars, 1-deoxynojirimycin (DNJ) and its alkylated derivative N-butyl deoxynojirimycin (NB-DNJ, Miglustat) are effective in enhancing GAA residual activity in fibroblasts from PD patient carrying specific mutations of the *GAA* gene and act as pharmacological chaperones. This approach, however, has important limitations as it may be applicable only to patients with specific *GAA* gene mutations. We estimated that about 10-20% of PD patients may be amenable to EET.

This limitation could be apparently overcome according to the results obtained in another study, in which we showed that chaperones are not only effective in enhancing mutated GAA activity, but can also potentiate the efficacy of ERT. Co-administration of rhGAA and NB-DNJ to PD fibroblasts resulted in improved trafficking of the recombinant enzyme to lysosomes, improved maturation and increased intracellular GAA activity. Thus, these results indicate a synergistic effect between pharmacological chaperones and ERT. The synergistic effect between EET and ERT was confirmed in a certain number (>10) of PD cell lines. In addition, preliminary and unpublished results obtained by the partners of the EUCLYD consortium have demonstrated that this synergy between ERT and EET is also observed in another lysosomal storage disease, Fabry disease.

The implications of the results obtained in our studies are:
- the use of pharmacological chaperones should not be restricted only to patients with responsive mutations, but may be extended to any PD patient on ERT, with obvious advantages for the cure of patients.
- our data provide the rationale for the use of combination therapeutic protocols in the treatment of PD (and possibly other lysosomal storage diseases). PD, like most lysosomal diseases, is a complex disorder, characterized by generalized glycogen storage and multi-organ involvement. It is reasonable to think that approaches based on the combination of different therapies may be more effective in correcting all the aspects of the disease.

These hypotheses are now being translated into an Italian multicentre clinical trial, beyond the scope of the EUCLYD programme.

Gaucher disease: pathophysiology and natural history

Gaucher disease is an autosomal recessive LSD due to the deficiency of β-glucocerebrosidase. Gaucher disease is characterized by the storage of sphingolipids (glucosylceramide, GlcCer) primarily within cells of phagocyte origin ('Gaucher cells'). Gaucher disease is the less rare LSD, with an estimated incidence ranging between 1:57,000 and 1.16:100,000 (although it is significantly higher in specific populations as Ashkenazi Jewish descent). The clinical phenotype of the disease includes visceral (hepatosplenomegaly), hematologic (cytopenia), and skeletal (osteolytic lesions, bone crises, femoral Erlenmeyer flask deformity) manifestations.

Gaucher patients under the care of clinics supervised by the EUCLYD partners are now registered in independent databases, including patients receiving substrate inhibitors. The consortium has assembled a clinical database of Gaucher patients, which can be interrogated for further evaluation of licensed substrate inhibitor treatment with miglustat. Plasma samples are available for analysis from several groups, including those receiving no treatment, enzyme replacement therapy and substrate reduction therapy with miglustat. Their clinical behaviour and effects of miglustat have been recorded and several toxic unwanted effects have been experienced, which are currently being annotated for documentation and reporting in the literature (see also below).

Concerning patient-reported outcome measures, the EuroQol 5D (EQ5D) quality of life summary measure is significantly lower (median 0.679) in those patients with a history of osteonecrosis, compared with those with no such history, (median 0.796, $p<0.01$). The health-state score was also found to be significantly lower (median 0.626) in those who had suffered a fragility fracture, representing osteoporosis, than in those who had not (median 0.796, $p<0.001$).

The consortium has completed studies to investigate the relationship between circulating chemokines and cytokines and the most disabling complication affecting life quality of patients with Gaucher disease – osteonecrosis. The concentration of the chemokine biomarker, PARC/CCL18, appears to be significantly greater in patients with a history of osteonecrosis ($p<0.01$) and there is a significant correlation between the number of anatomical sites and the elevation of this chemokine. Subsequent studies have been completed to quantify cytokines/chemokines and many of these molecules have been found to be elevated in Gaucher disease before treatment.

We have completed multiplex assays in samples obtained from a cohort of 100 adults with Gaucher disease attending 3 referral centres who have been independently evaluated for quality of life and disease-related complications as part of a structured assessment compounding an independent clinical bone registry.

Gaucher disease: enzyme replacement (ERT), gene therapy, substrate reduction

The consortium has completed a study of the osseous manifestations of adult Gaucher disease in the mature era of enzyme therapy. While this treatment has the capacity to prevent many of the emergent manifestations of Gaucher disease, and may reverse the haematological and visceral effects, its benefit on the skeleton has yet to be determined and has been the subject of the work supported by EUCLYD. In 9 adults with Gaucher disease who had received enzyme replacement therapy from childhood out of 100 surveyed, one patient presented with established bone disease (osteonecrosis) and was treated. A second patient with an episode of osteonecrosis aged 5, was treated from 9 years. Of the remaining 7, none had osteonecrosis either before or after treatment, and of the total number of 9, none experienced osteonecrosis after the start of treatment. These findings strongly suggest that early introduction of therapy is critical and all these patients were free of active disease in the skeleton and had excellent quality of life as mature adults.

Concerning the development of gene therapy for Gaucher disease, hematopoietic stem cell-based gene therapy offers the possibility of permanent correction for genetic disorders of the hematopoietic system. However, optimization of present protocols is required before gene therapy can be safely applied as general treatment of genetic diseases. The EUCLYD consortium used a mouse model of type 1 Gaucher disease (GD) to demonstrate the feasibility of a low-risk conditioning regimen instead of standard radiation, which is associated with severe adverse effects. We first wanted to establish what level of engraftment and glucosylceramidase (GCase) activity is required to correct the pathology of the type 1 GD mouse. Our results demonstrate that a median wild-type (WT) cell engraftment of 7%, corresponding to GCase activity levels above 10 nmoles/hour and mg protein, was sufficient to reverse pathology in the bone marrow and spleen in the GD mouse. Moreover, we applied nonmyeloablative doses of busulfan as a pretransplant conditioning regimen and showed that even WT cell engraftment in the range of 1%–10% can confer a beneficial therapeutical outcome in this disease model. Taken together, our data provide encouraging evidence for the possibility of developing safe and efficient conditioning protocols for diseases that require only a low level of normal or gene-corrected cells for a permanent and beneficial therapeutic outcome.

To generate safer gene therapeutic approaches, we have generated several self-inactivating lentiviral vectors that are less likely to cause serious insertional mutagenesis effects by upregulating cancer-promoting genes. These vectors contain the glucocerebrosidase gene driven by the SFFV promoter (strong promoter control vector) and vectors containing either the CD11b promoter (tissue-specific for myeloid cells) or the PGK promoter (a relatively weak promoter in hematopoietic cells. All vectors contain the *GFP* gene, which is expressed through an IRES between the two genes. These vectors have been constructed and can transduce primary murine hematopoietic cells from type 1 Gaucher mice. All vectors increase the expression of the glucocerebrosidase enzyme to levels high enough to correct the enzyme deficiency when the transduced cells are grown *in vitro*. Due to difficulties in inducing the phenotype, the *in vivo* gene therapy studies have been delayed by one year. They are all in progress now and we expect to have findings one year from now that show enzyme correction in Gaucher mice *in vivo*. Similarly, studies to analyze insertional mutagenesis will be finished at the same time.

Several substrate inhibitors have been investigated by members of the consortium for Gaucher disease, including Rhodamine B, to restrict the biosynthesis of the macromolecular substrates stored in lysosomes. Imino sugars (miglustat) are in current licensed use in Gaucher disease; an unrelated inhibitor, GENZ112638, Eliglustat tartrate, is in clinical trial for Gaucher disease and is a ceramide analogue. Unfortunately, the potency of Rhodamine and other related compounds under exploration as inhibitors of the biosynthesis of glycosaminoglycans was insufficient for further investigation clinically with inhibitory actions in the high micromolar or low minimolar range.

Full clinical laboratory tests have been conducted in patients receiving miglustat in both centres (UCAM, UMC). The effects of the treatment on disease behaviour as reflected by chemokines haematopoietic indices and visceral size have been studied. In several instances miglustat proved

unsatisfactory and a full report on the role of this agent in both stable and unstable patients is shortly to be submitted.

The trial of GENZ-112638 in the authentic model of Gaucher disease in genetically modified mice induced with the interferon analogue, poly[I:C] has been completed. Preliminary analysis has shown marked reduction of plasmaglucoceramide at all 3 doses of the substrate inhibitor in the test, compared with the matched controlled diet. Further studies of the effect of the inhibitor are awaiting mass spectroscopic analysis but all relevant tissues from a large number of experimental animals have been collected.

Further collaborations among members of the EUCLYD consortium are under way to improve the expression of the Gaucher phenotype for further study and a supply of second-generation inhibitors has been arranged with the Genzyme Corporation for further detailed characterization and study. Unfortunately, the full-blown Gaucher phenotype was not evident in the inducible model and a programme of further collaborative breeding with scientists in ULUND is in progress to ensure more profound glucocylceramidase sufficiency in the study animals.

However, a unique phenotype in the long-term survivors harbouring the Cre gene recombinase gene (potential Gaucher) has been compared with wild-type, mutant heterozygotes and Cre negative animals, irrespective of the inducing agent. Several animals show massive splenomegaly and lymphoid aggregates indicative of spontaneous tumours. These animals are actively under investigation as a model of B-cell malignancy, which complicates adult Gaucher disease in humans. Within a few weeks preliminary data on whether substrate inhibitors suppress this phenotype will be available.

MPS VI: pathophysiology and natural history

Mucopolysaccharidosis VI (MPS VI) is caused by deficient arylsulfatase B (ARSB) activity resulting in lysosomal storage of glycosaminoglycans (GAGs). MPS VI is characterized by dysostosis multiplex, organomegaly, corneal clouding, and heart valve thickening. The incidence of MPS VI is even lower than that of Pompe disease.

Our studies on the pathogenesis of MPS VI have indicated that non-lysosomal degradation pathways are impaired both in MPS VI cell lines and in MPS VI rat tissues as a result of dermatan sulfate accumulation. These pathways can be both targets of new experimental therapies and biomarkers for follow-up of existing treatments.

The clinicians of the EUCLYD consortium have developed protocols for the diagnosis and clinical follow-up of patients with this disease as has been done for patients affected by Pompe disease. The systematic collection of data by EMC has led thus far to the description of the cardiac problems, the collection of genotypes, the production of antibodies for CRIM testing (as in Pompe disease), the development of an ELISA assay to measure the height of the immune response against enzyme replacement therapy, the assessment of antibody titers in 9 patients receiving enzyme therapy, and the documentation of the clinical effects of therapy over a period of 1.5 years. With the help of a newly designed questionnaire (MPS Survey) we have also assessed the quality of life of patients with MPS VI, like we did for Pompe disease, in a collaborative effort with the Dutch patient association (VKS). All these activities performed at EMC and by its partners in the context of EUCLYD have led to an upgrading of the quality of care in and outside Europe for patients with these rare lysosomal storage disorders.

MPSVI: enzyme replacement (ERT), gene therapy, substrate reduction

The first years with enzyme replacement therapy in MPS VI have demonstrated that patients benefit from ERT regarding quality of life, lung function and endurance. However, we described disease complications in relation to the phenotype, which did not respond to enzyme replacement

therapy. Early diagnosis and timely intervention of these complications, such as craniocervical stenosis or heart valve abnormalities, are essential for optimal management. Our studies have focused on a diagnostic algorithm to detect craniocervical stenosis timely before irreversible changes occur.
In addition, we found that onset of craniocervical stenosis as well as heart valve findings vary substantially between patients with classical and attenuated/slowly progressive phenotype. Additional to mutations in the *ARSB*-gene we identified severity of growth retardation and GAG excretion as clinical predictive factors for the phenotype.

In addition, we have set-up a gene therapy protocol in animal models of MPS VI based on the systemic delivery of adeno-associated viral (AAV) vectors 8 and on the conversion of the liver in a factory for long-term sustained release of arylsulfatase B to deficient tissues. We have shown that this strategy results in significant improvement of the biochemical, pathological, skeletal and motor function anomalies in MPS VI rats and cats. In MPS VI rats, as results of the null ARSB mutation causing the disease, upon AAV administration animals develop an immune response to the recombinant ARSB which limits therapeutic efficacy and can be prevented by co-administration of immune-suppressive drugs. This does not occur in MPS VI cats bearing missense ARSB mutations and expressing an inactive ARSB enzyme. Indeed, in MPS VI cats a single intravascular administration of AAV8 results in ARSB expression up to 3 years after vector delivery, the last time point of the analysis. This bodes well towards the clinical development of gene therapy for MPS VI.

Finally, we have provided preliminary evidence supporting a novel therapeutic approach based on substrate reduction for the treatment of mucopolysaccharidoses (MPS). This approach is also based on the use of small molecule drugs (like pharmacological chaperones) and has the potential to overcome some of the limitations of ERT, such as the insufficient distribution of recombinant enzymes in tissues and organs and the need for frequent intravenous infusions.

As substrate-reducing agent, we tested a member of beta-D-xylosides, a class of variably substituted compounds that in earlier studies have been shown to prime GAG polymerization independently of core proteins, by serving as acceptors in the first galactosylation and the subsequent elongation steps. We showed that this drug can reduce glycosaminoglycan (GAG) and proteoglycan (PG) synthesis in cultured fibroblasts from patients with different MPS (MPS IIIA, II and VI).

We also tested the effects of beta-D-xylosides on the synthesis of PGs in the mouse model of MPSIIIA that recapitulates the phenotype of the disease. In a preliminary short-term test 1 month-old mice were treated with 50 mg/day for 2 months intraperitoneally. During treatment with the beta-D-xyloside the mice did not show clinically overt signs of toxicity. At the age of 3 months the animals were examined, sacrificed, and GAG content of different tissues was assayed and compared to those obtained in 3 month-old MPSIIIA untreated mice. In xyloside-treated mice GAG content was decreased in the liver and brain.

MSD: gene therapy

The landmark of MSD deficiency is the severe or lack of activity of the entire repertoire of endogenous sulfatases. This is due to genetic defects affecting the sulfatase modifying factor 1 (*SUMF1*) gene. *SUMF1* is a so-called modifier gene whose key activity is to activate sulfatases through an enzymatic activity. As a consequence, important metabolic functions of cells are impaired, eventually leading to the abnormal storage of toxic compounds (glycosaminoglycans; GAGs) within the sub cellular organelles called lysosomes. Gene therapy holds promise to correct this pathological lysosomal storage based on the correction of the underlying DNA defects affecting SUMF1. One promising system relies on gene transfer with modified viral vectors of the correct copy of the gene in affected cells. In addition, since *SUMF1* is expressed in any tissue of the body one major challenge to cure the disease is to transfer the correct copy of *SUMF1* efficiently in the

whole body. Previous studies have indicated the so-called viral vector based on Adeno-associated virus (AAV) type 9 as a very efficient delivery vehicle. Based on this premise we generated AAV9 vectors containing the *SUMF1* gene and administered them into neonatal *Sumf1-/-* mice, early during disease progression. As a result, we showed that visceral organs exposed to the vector were efficiently infected by AAV9-CMV-SUMF1 vector, and this, in turn, resulted in the global visceral activation of sulfatases. As shown in **Fig. 1**, the activation of sulfatases led to clearance of GAGs accumulation within distinct organs.

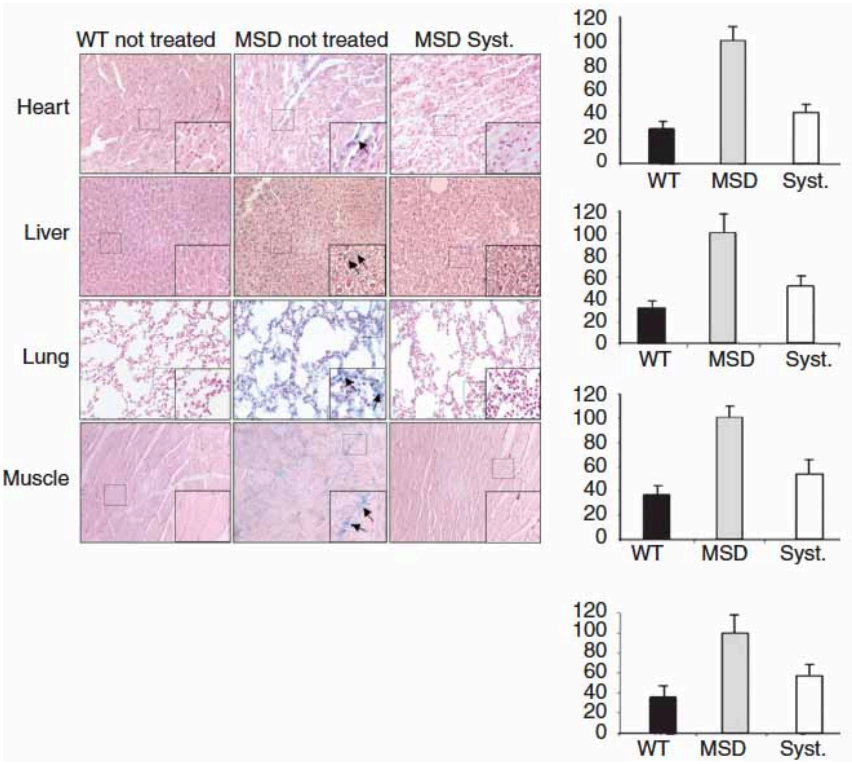

Figure 1. Right panel, alcian blue stained tissue sections of the heart, liver, lung, and muscle of wild-type mice (WT), MSD mice, MSD administered intravenously through the temporal vein with AAV vector (MSD Syst.). Squares represent zoom-in portion of tissues (dotted squares). Black arrows show glycosaminoglycan (GAG) accumulation. 20x magnification.
Left panel, quantitative analysis of GAG accumulation in treated MSD mice. GAG content in the tissue homogenates (tissues as indicated) from the control (WT), untreated MSD, MSD rAAV9 vector-CMV-SUMF1-injected through the temporal vein (Syst.). The GAG content is indicated as percentage and all of the tissues from treated mice reached normal levels, as seen in the wild-type animals. The error bars indicate the SEM.

In addition, we showed that administration of AAV9-CMV-SUMF1 in neonatal MSD mice resulted in significant amelioration of the inflammatory status of visceral organs including the liver (**Fig. 2**) and rescue of behavioural motor defects affecting bone-joints, as showed in **Fig. 3**.

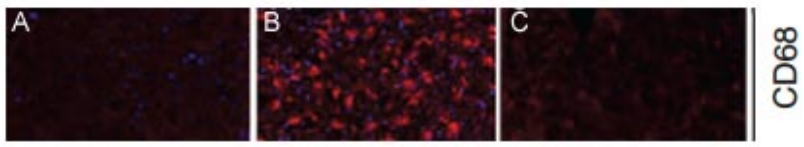

Figure 2. CD68 immunofluorescence revealed a massive activation of macrophages in the liver (**B**) of untreated MSD mice, as opposed to (**A**) wild-type mice. Treatment with rAAV9 vector-CMV-SUMF1 resulted in a reduction of macrophage activation in the liver of MSD mice administered intravenously with rAAV vector (**C**).

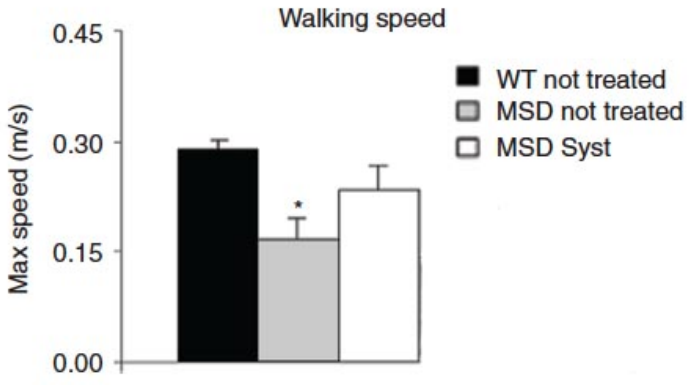

Figure 3. Maximal walking speed in the open field (5 minutes recording) was dramatically impaired in 3–4 months old MSD mice. Systemic injection of rAAV9 vector rescued this behavioural defect.

In summary, this proof-of-concept study provides converging evidence at different levels of analysis that rAAV9 vector mediated SUMF1 injection can significantly ameliorate the visceral phenotype of a mouse model of MSD, which is perhaps the most severe lysosomal storage disorder affecting the whole body.

References cited in text

Herzog A, Hartung R, **Reuser A**, Hermanns P, Runz H, Gökce S, Pohlenz J, **Beck M**, **Mengel E**. Pompe disease in 42 German patients: Molecular analysis of the *GAA* gene, natural course and genotype-phenotype correlations. *In preparation*.

Medina DL, Fraldi A, Bouché V, Annunziata F, Mansueto G, Spampanato C, Puri C, Pignata A, Martina JA, Sardiello M, Polishchuk R, Puertollano R, **Ballabio A**. Transcriptional activation of lysosomal exocytosis promotes cellular clearance. *Dev Cell*, *in press*.

Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, Di Malta C, Donaudy F, Embrione V, Polishchuk RS, Banfi S, **Parenti G**, Cattaneo E, **Ballabio A** (2009). A gene network regulating lysosomal biogenesis and function. *Science* 325: 473-477.

Potential impact and main dissemination activities and exploitation of results (not exceeding 10 pages)

Potential impact

The EUCLYD consortium has aimed at establishing a unique research programme to tackle lysosomal storage diseases by putting together a consortium that has promoted collaborative scientific interactions between the outstanding communities of basic and clinical investigators. The consortium and scientific programme was thought to allow the interaction between basic scientists and clinicians: basic science observations to provide clinicians developing therapeutic approaches with new tools for use in patients and for assessment of their impact, and clinical researchers to make novel observations regarding natural history, mutant-phenotype correlations on the nature and progression of disease that often stimulate basic investigations.

The potential impact of the work obtained by EUCLYD on the understanding and potential treatment of rare diseases due to metabolic disorders such as lysosomal storage dysfunction is described below:

- The characterization of the pathophysiology of MSD, MPSVI and PD has helped to identify disease mechanisms and markers and determine whether the treatment of secondary effects due to substrate accumulation can help/revert patient symptoms.

- The identification of tissue-pathology markers as determinants of PD stage have been crucial to determine the success of therapeutic intervention. These studies have potentially led to the identification of factors that modify the onset and clinical course of Pompe disease in patients with residual acid α-glucosidase activity as well as to the identification of biomarkers that can be used to monitor the stage of disease and the effect of therapy. The findings are expected to be very relevant for the design of measures that can be taken to improve the natural course of Pompe disease and the outcome of therapeutic interventions, such as enzyme replacement therapy, chaperone based therapy and gene therapy.

- The characterization of the natural history of a subset of LSDs will lead to a better insight in the natural course of rare LSDs and the response to therapy of patients with different ages and levels of disease severity. This information is important for the optimal timing and dosing of expensive new therapies like enzyme replacement therapy and for the development of treatment start- and stop criteria. Furthermore, in-depth knowledge has been obtained on the therapeutic effects on specific tissues, such as skeletal muscle and bone, the use of biomarkers in monitoring the effects of treatment, and the role of antibody formation and inflammatory parameters. Finally, the follow-up protocols and disease severity scales for Pompe disease and the MPSes can serve as a framework for studying other, very rare LSDs, such as fucosidosis, Farber disease and Niemann-Pick disease type A/B.

- The experimental results obtained have added information on the use of enzyme enhancement as an alternative therapy for Pompe disease and on the possible combined use of pharmacological chaperones and ERT.

- Determining the safety and specificity of the putative therapeutic effects of iminosugars and the morpholino compounds as substrate-reducing drugs for the treatment of glycosphingolipidoses are of key importance in the long-term safety and benefit of this therapeutic modality; although work is still in progress towards the obtainment of an authentic viable model of Gaucher disease in the experimental mouse, the experimental results obtained thus far have been critical to a deeper understanding of the mechanism of action of these agents. The results have been informative on the specificity of the substrate-reducing drugs and their long-term therapeutic effect at their presumed biochemical site of action and have given confidence as to their safety

and applicability in the other target lysosomal diseases for which their use has been proposed e.g. Fabry disease, Niemann Pick disease type C affecting the liver, GM1 gangliosidosis and the secondary glycosphingolipid storage disorders in MPSIII and related glycosaminoglycan storage diseases.

- The experimental results obtained on gene therapy have provided proof-of-principle that gene therapy may represent a new therapy for MSD or overcome some of the limitations of ERT for GD, PD and MPS VI.

Main dissemination activities and exploitation of results

The partners of the EUCLYD consortium have given particular attention towards the dissemination of the results of the project throughout these three years by presenting their data in international conferences, workshops and meetings and by publishing their results in high impact, peer-reviewed international journals (see Templates A1 and A2 for more details).

The EUCLYD website has been developed and is online (www.euclyd.eu), as planned, which has favoured the dissemination of the project to the scientific community, patient groups, industry, biotechnology, and training institutions, and has facilitated the inter-communication among partners as well as the acceleration in work progression.

To this end, the site consists of a public area and a private area that is password protected (**username**: partners; **password**: europa3).
In the public area, which is easily accessible for any internet user, a first section (What is EUCLYD?) provides general information on the focus of the project, the diseases of interest to the consortium, and the aims and impact of the project in terms of EU scientific and societal objectives.
A more specific description of the project work packages and their objectives is also provided together with a general overview of each partner making up the consortium, the role in the project, the key personnel and publications acknowledging EUCLYD. Links to other websites, relevant events and participation to meetings and symposia is also available. Finally, a separate section (Internal Use) with sensitive data (such as the TA, the contract and terms, the reports submitted to the EU, download to single publications etc.) is restricted to the partners of the consortium and is password protected.

The EUCLYD management team has also given particular attention to keep the website up to date with the consortium annual meetings, including the PPTs presented by each partner and Minutes of each meeting, and with the publications acknowledging EUCLYD and EU funding. The PPTs, Minutes of the meetings and full articles of EUCLYD publications are again restricted to the partners and the EU and therefore are password protected.

As planned, the partners have been able to exploit the results of the project in their local research programmes, and have been successful in seeking additional major funding from national research agencies, foundations and industries to further extend the work carried out in the project towards the experimental characterization of the most interesting findings.

Concerning the exploitation of results and contacts with industry, the consortium has continued to strengthen its contacts with the two industries (Actelion and Amicus) manufacturing Miglustat and similar compounds to develop new therapies using these drugs and to identify new indications for drugs already available in the treatment of LSDs. The partners involved in the treatment of patients (Prof. Andria, Prof. Beck, Prof. Cox, Prof. Reuser) have continued to involve the industries cited above in discussing preliminary data and implications of results in order to optimize the transfer of results from "bench to bedside". In particular, UMC, LUND and UCAM now have a secure collaboration and we are confident that several further publications acknowledging EUCLYD will result within the next year at the latest (see for example, Herzog et al., in preparation in section 1.9 of periodic report Month 19-36).
Unfortunately, work has been complicated by the worldwide total cessation of imiglucerase manufacture from June 2009. Since October 2010, supplies have been restored partially, but many patients have required adjustment to different enzyme preparations (VPRIV and taliglucerase) and additional patients have been started on miglustat (see WP4, M4.7 in periodic report Month 19-36 for major details).

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4.2 USE AND DISSEMINATION OF FOREGROUND

A plan for use and dissemination of foreground (including socio-economic impact and target groups for the results of the research) shall be established at the end of the project. It should, where appropriate, be an update of the initial plan in Annex I for use and dissemination of foreground and be consistent with the report on societal implications on the use and dissemination of foreground (section 4.3 – H).

The plan should consist of:

- Section A

  This section should describe the dissemination measures, including any scientific publications relating to foreground. **Its content will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

- Section B

  This section should specify the exploitable foreground and provide the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential **will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

Section A (public)

This section includes two templates

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES*

* Papers in preparation have been excluded from the list below (please refer to periodic report Month 19-36 for publications in prep)

NO.	Title	Main author (EUCLYD partner)	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[3] (if available)	Is/Will open access[4] provided to this publication?
1	A randomized study of alglucosidase alfa in late-onset Pompe's disease	van der Ploeg	N Engl J Med	362			2010	1396-1406	See EUCLYD publications pdf	YES
2	Pompe's disease	van der Ploeg, Reuser	Lancet	372			2008	1342-1353	See EUCLYD publications pdf	YES
3	TFEB Links Autophagy to Lysosomal Biogenesis	Ballabio	Science	332			2011	1429-1433	See EUCLYD publications pdf	YES
4	A gene network regulating lysosomal	Ballabio	Science	325			2009	473-477	See EUCLYD	YES

[3] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[4] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

	biogenesis and function								publications pdf	
5	Transcriptional activation of lysosomal exocytosis promotes cellular clearance	Ballabio	Dev Cell	In press			In press	In press		ND
6	Lentiviral gene therapy of murine hematopoietic stem cells ameliorates the Pompe disease phenotype	van der Ploeg, Reuser	Blood	115			2010	5329-5337	See EUCLYD publications pdf	YES
7	Treating lysosomal storage diseases with pharmacological chaperones. From concept to clinics	Parenti	EMBO Mol Med	1			2009	268-279	See EUCLYD publications pdf	YES
8	Altered expression and distribution of cathepsins in neuronopathic forms of Gaucher disease and in other sphingolipidoses	Karlsson	Hum Mol Genet	19			2010	3583-3590	See EUCLYD publications pdf	YES
9	No evidence for activation of the unfolded protein response in neuronopathic models of Gaucher disease	Karlsson	Hum Mol Genet	18			2009	1482-1488	See EUCLYD publications pdf	YES
10	First experience with enzyme replacement therapy during pregnancy and lactation in Pompe disease	Reuser, van der Ploeg	Neurology	Submitted			Submitted	Submitted		ND
11	Successful low-risk hematopoietic cell therapy in a mouse model of type 1 Gaucher disease	Karlsson	Stem Cells	27			2009	744-752	See EUCLYD publications pdf	YES
12	Long-term amelioration of feline Mucopolysaccharidosis VI after AAV-mediated liver gene transfer	Auricchio	Mol Ther	19			2011	461-469	See EUCLYD publications pdf	NO
13	Efficacy of a combined intracerebral and systemic gene delivery approach for the treatment of a severe lysosomal storage disorder	Auricchio, Surace, Ballabio	Mol Ther	19			2011	860-869	See EUCLYD publications pdf	NO
14	The pharmacological chaperone N-butyldeoxynojirimycin enhances enzyme replacement therapy in Pompe disease fibroblasts	Andria, Parenti	Mol Ther	17			2009	964-971	See EUCLYD publications pdf	YES
15	Fatigue in neuromuscular disorders: focus on Guillain-Barré syndrome and	van der Ploeg, van Doorn	Cell Mol Life Sci	67			2010	701-713	See EUCLYD publications	YES

	Pompe disease								pdf	
16	Update of the Pompe Disease Mutation Database with functional effects of 22 previously known and 46 new Sequence Variants in *GAA*	Reuser	Hum Mutat	Submitted			Submitted	Submitted		ND
17	Design and validation of a metabolic disorder resequencing microarray (BRUM1)	Reuser	Hum Mutat	31			2010	858-865	See EUCLYD publications pdf	YES
18	The pharmacological chaperone 1-deoxynojirimycin increases the activity and lysosomal trafficking of multiple mutant forms of acid alpha-glucosidase	Andria, Parenti	Hum Mutat	30			2009	1683-1692	See EUCLYD publications pdf	YES
19	Cardiac involvement in adults with Pompe disease	van der Ploeg	J Intern Med	264			2008	333-339	See EUCLYD publications pdf	YES
20	Survival and associated factors in 268 adults with Pompe disease prior to treatment with enzyme replacement therapy	Reuser, van Doorn, van der Ploeg	Orphanet J Rare Dis	6			2011	34	See EUCLYD publications pdf	YES
21	Noninvasive repetitive imaging of Somatostatin Receptor 2 gene transfer with positron emission tomography	Auricchio	Hum Gene Ther	22			2011	189-196	See EUCLYD publications pdf	YES
22	Different serum enzyme levels are required for the rescue of the various systemic features in Mucopolysaccharidoses	Auricchio	Hum Gene Ther	21			2010	555-569	See EUCLYD publications pdf	NO
23	Reply to the letter to the editor by Papadimas et al.: "Bone mineral density in adult patients with Pompe disease"	Reuser, van der Ploeg	Bone	48			2011	418-419	See EUCLYD publications pdf	ND
24	Low bone mass in Pompe disease; muscular strength as a predictor of bone mineral density	Reuser, van der Ploeg	Bone	47			2010	643-649	See EUCLYD publications pdf	YES
25	Osseous manifestations of adult Gaucher disease in the era of enzyme replacement therapy	Cox	Medicine	90			2011	52-60	See EUCLYD publications pdf	NO
26	Enzyme replacement therapy with	Mengel	J Neurol	257			2010	91-97	See EUCLYD	YES

	alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial								publications pdf	
27	Burden of illness of Pompe disease in patients only receiving supportive care	van der Ploeg	J Inherit Metab Dis	Apr 16 [Epub ahead of print]			2011	Apr 16 [Epub ahead of print]	See EUCLYD publications pdf	YES
28	Dietary modifications in patients receiving miglustat	Cox	J Inherit Metab Dis	Sep 16 [Epub ahead of print]			2010	Sep 16 [Epub ahead of print]	See EUCLYD publications pdf	YES
29	PAS-positive lymphocyte vacuoles can be used as diagnostic screening test for Pompe disease	Reuser, van der Ploeg	J Inherit Metab Dis	33			2010	133-139	See EUCLYD publications pdf	YES
30	Hearing loss in Pompe disease revisited: results from a study of 24 children	Reuser, van der Ploeg	J Inherit Metab Dis	33			2010	597-602	See EUCLYD publications pdf	YES
31	Enzyme analysis for Pompe disease in leukocytes; superior results with natural substrate compared with artificial substrates	Reuser	J Inherit Metab Dis	32			2009	416-423	See EUCLYD publications pdf	YES
32	Rate of progression and predictive factors for pulmonary outcome in children and adults with Pompe disease	van Doorn, van der Ploeg	Mol Genet Metab	In press			In press	In press		ND
33	Expanding the clinical spectrum of late-onset Pompe disease: Dilated arteriopathy involving the thoracic aorta, a novel vascular phenotype uncovered	Mengel	Mol Genet Metab	May 5 [Epub ahead of print]			2011	May 5 [Epub ahead of print]	See EUCLYD publications pdf	YES
34	Hemoglobin precipitation greatly improves 4-methylumbelliferone-based diagnostic assays for lysosomal storage diseases in dried blood spots	van der Ploeg, Reuser	Mol Genet Metab	102			2011	44-48	See EUCLYD publications pdf	YES
35	Improved assay for differential diagnosis between Pompe disease and acid α-glucosidase pseudodeficiency on dried blood spots	Reuser	Mol Genet Metab	103			2011	12-17	See EUCLYD publications pdf	YES

36	Home treatment with intravenous enzyme replacement therapy with idursulfase for mucopolysaccharidosis type II - data from the Hunter Outcome Survey	van der Ploeg	Mol Genet Metab	101			2010	123-129	See EUCLYD publications pdf	YES
37	High antibody titer in an adult with Pompe disease affects treatment with alglucoisidase alfa	van Doorn, van der Ploeg, Reuser	Mol Genet Metab	101			2010	338-345	See EUCLYD publications pdf	YES
38	High frequency of acid α-glucosidase pseudodeficiency complicates newborn screening for glycogen storage disease type II in the Japanese Population	Reuser	Mol Genet Metab	97			2009	190-195	See EUCLYD publications pdf	YES
39	Expensive drugs for rare disorders: to treat or not to treat? The case of enzyme replacement therapy for mucopolysaccharidosis VI	Beck	Curr Med Res Opin	25			2009	1285-1293	See EUCLYD publications pdf	YES
40	Development of novel therapies in murine models for Gaucher disease	Karlsson	Clin Ther	31			2009	Suppl C	See EUCLYD publications pdf	YES
41	Cardiac evaluation in children and adults with Pompe disease sharing the common c.-32-13T > G genotype rarely reveals abnormalities	Reuser, van der Ploeg	J Neurol Sci	275			2008	46-50	See EUCLYD publications pdf	YES
42	A case of adult-onset Pompe disease presenting with severe fatigue and selective involvement of type 1 muscle fibers	van Doorn, Reuser, van der Ploeg	Neuromuscul Disord	21			2011	232-234	See EUCLYD publications pdf	YES
43	Effect of enzyme therapy in juvenile patients with Pompe disease: A three-year open-label study	Reuser, van der Ploeg	Neuromuscul Disord	20			2010	775-782	See EUCLYD publications pdf	YES
44	Where do we stand in enzyme replacement therapy in Pompe's disease?	van der Ploeg	Neuromuscul Disord	20			2010	773-774	See EUCLYD publications pdf	YES
45	Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease	Reuser, van der Ploeg	Neuromuscul Disord	19			2009	113-117	See EUCLYD publications pdf	YES
46	Abnormal autophagy, ubiquitination,	Auricchio	Pathogenetics	2			2009	4	See EUCLYD	YES

	inflammation and apoptosis are dependent upon lysosomal storage and are useful biomarkers of mucopolysaccharidosis VI								publications pdf	
47	Abnormal mannose-6-phosphate receptor trafficking impairs recombinant alpha-glucosidase uptake in Pompe disease fibroblasts	Andria, Parenti	Pathogenetics	1			2008	6	See EUCLYD publications pdf	YES
48	Biomarkers for osteonecrosis in Gaucher disease	Cox	Exp Opin Med Diag	Submitted			Submitted	Submitted		ND
49	Enzymatic and molecular strategies to diagnose Pompe disease	Reuser	Exp Opin Med Diag	4			2010	79-89	See EUCLYD publications pdf	ND
50	Bones, joints and teeth development in Mucopolysaccharidoses: Relevance to therapeutic options	van der Ploeg, Reuser	Biochem Biophys Acta	Submitted			Submitted	Submitted		ND
51	Understanding and Treatment of Rare Metabolic Disorders	Andria	Projects				2011	72-73	See EUCLYD publications pdf	ND
52	Potential biomarkers of osteonecrosis in Gaucher disease	Cox	Blood Cell Mol Dis	46			2011	27-33	See EUCLYD publications pdf	YES
53	Ptosis, extra ocular motility disorder, and myopia as features of Pompe disease	van der Ploeg	Orbit	30			2011	111-113	See EUCLYD publications pdf	YES
54	Evaluation of disease severity in mucopolysaccharidosis	Beck	J Ped Rehab Med	3			2010	39-46	See EUCLYD publications pdf	ND
55	Eliglustat tartrate, an orally active glucocerebroside synthase inhibitor for the potential treatment of Gaucher disease and other lysosomal storage diseases	Cox	Curr Opin Investig Drugs	11			2010	1169-1181	See EUCLYD publications pdf	NO
56	Gaucher disease: clinical profile and therapeutic developments	Cox	Biologics	4			2010	299-313	See EUCLYD publications pdf	YES
57	Alglucosidase alfa: Long term use in	Beck	Ther Clin Risk	5			2009	767-772	See EUCLYD	YES

	the treatment of patients with Pompe disease		Manag						publications pdf	
58	Does enzyme replacement therapy influence the ocular changes in type VI mucopolysaccharidosis?	Beck	Graefes Arch Clin Exp Ophthalmol	247			2009	975-980	See EUCLYD publications pdf	YES
59	Mucopolysaccharidose type II en type VI: de ziekten van Hunter en van Maroteaux-Lamy	van der Ploeg	Tijdschr Kindergeneeskd	78			2010	62-69	See EUCLYD publications pdf	ND
60	The Pompe Disease Mutation Database at www.pompecenter.nl	Reuser	Mutation Database	Last update, December 2, 2010			Last update, December 2, 2010	Last update, December 2, 2010		YES

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

NO.	Type of activities[5]	Main leader	Title	Date	Place	Type of audience[6]	Size of audience[7]	Countries addressed
1	Conference	Mengel	German Society of Pediatrics	**2011**: September 18	Potsdam, Germany	Scientific Community	M	EU/DE
2	Conference	Mengel	SSIEM 2011	August	Geneva, Switzerland	Scientific Community	L	EU
3	Conference	Andria	Europaediatrics 2011	June 24	Vienna, Austria	Scientific Community	L	EU
4	Conference	Surace	American Society of Gene and Cellular Therapy (ASGCT) Conference	May 18-21	Seattle, WA, USA	Scientific Community	L	Worldwide
5	Workshop	de Vries	Diagnosis and management of Pompe disease	May 8	Thessaloniki, Greece	Scientific Community	S	EU
6	Meeting	van der Ploeg	PRG Meeting	April 16	Rotterdam, The Netherlands	Scientific Community	S	NL
7	Conference	van der Ploeg	ESN (Inherited Diseases in the Netherlands) Conference	April 12-13	Amsterdam, The Netherlands	Scientific Community	M	EU/NL
8	Conference	van der Ploeg	SPEDM, Portughese neuromuscular society	March 26	Coimbra, Portugal	Scientific Community	M	EU/PT
9	Conference	Parenti Auricchio	Telethon Convention 2011	March 7-9	Riva del Garda, Italy	Scientific Community	L	EU/IT
10	Workshop	Lampe	Workshop on Craniocervical stenosis in MPS VI	March	Vancouver, Canada	Scientific Community	M	EU/USA
11	Symposium	van der Ploeg	Symposium on Lysosomal Storage	February 2	Alkmaar, The Netherlands	Scientific	S	NL

[5] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[6] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias ('multiple choices' is possible.

[7] **For size of audience the EUCLYD management team created 3 categories: small (S) for meetings and seminars (20-70 people), medium (M) for workshops (≤100), and large (L) for conferences or congresses (≥300).**

			Disorders			Community		
12	Workshop	Mengel	German Workshop on infantile Pompe disease	January 16	Frankfurt, Germany	Scientific Community	S	DE
13	Workshop	Reuser	Diagnostic DNA analysis and functional assays	**2010**: December 6	Rotterdam, The Netherlands	Scientific Community	S	NL
14	Conference	van der Ploeg	Dutch patient association for metabolic diseases (VKS) Conference	November 26	Amersfoort, The Netherlands	Scientific Community	M	NL
15	Workshop	van der Ploeg Herzog, Mengel	Genzyme: Steps Forward in Pompe Disease	November 19-21	London, UK	Scientific Community	M	Worldwide
16	Workshop	de Vries	Portuguese metabolic disease society	November	Faro, Portugal	Scientific Community	S	EU/PT
17	Meeting	Reuser, van der Ploeg	8th Pompe disease Expert Day	October 27-28	Rotterdam, The Netherlands	Scientific Community	S	NL
18	Symposium	van der Ploeg	MPS and the heart international Symposium	October 13-15	Rio de Janeiro, Brasil	Scientific Community	M	Worldwide
19	Meeting	Karlsson	The Second Gaucher Leadership Forum: Keeping an Eye on the Future	October 1-2	London, UK	Scientific Community	M	EU/UK
20	Workshop	Reuser, van der Ploeg	177th ENMC International Workshop on Pompe disease	September 10-12	Naarden, The Netherlands	Scientific Community	M	Worldwide
21	Workshop	van der Ploeg	Workshop in Neonatal Screening for Pompe disease and other Lysosomal Storage Disorders	August 24	Amsterdam, The Netherlands	Scientific Community	M	EU/NL
22	Meeting	Reuser	Sophia Children's Hospital Reunion	June 29	Rotterdam, The Netherlands	Scientific Community	S	NL
23	Course	Reuser	9th Postdoctoral Course on Lysosomal Storage Diseases	June 7-8	Nierstein, Germany	Scientific Community	S	DE
24	Meeting	Parenti	6th European metabolic group Meeting	June 4	Lisbon, Portugal	Scientific Community	M	EU
25	Conference	van der Ploeg	VSN, Dutch Patient Association Neuromuscular Diseases	May 29	Zoetermeer, The Netherlands	Scientific Community	M	EU/NL
26	Conference	van der Ploeg	ESN, Inherited Diseases in the Netherlands	May 19-21	Leuven, Belgium	Scientific Community	M	NL/BE
27	Meeting	Auricchio	The American Society of Gene Therapy, 13th Annual Meeting	May 17-22	Washington DC, WA, USA	Scientific Community	L	Worldwide
28	Meeting	Reuser, van der Ploeg	7th Pompe disease Expert Day	May 4-5	Rotterdam, The Netherlands	Scientific Community	S	NL
29	Conference	Karlsson	The Swedish Gaucher Foundation	April 24	Malmö, Sweden	Scientific	M	SE

						Community		
30	Conference	Karlsson	7th Annual Conference of The British Society for Gene Therapy	March 29-31	London, UK	Scientific Community	M	UK
31	Course	Reuser, van der Ploeg	Orphan Europe Academy. Lysosomal Storage Disorders Course	March 18-19	Manchester, UK	Scientific Community	S	UK
32	Meeting	Ballabio	The American Society for Cell Biology, 49th Annual Meeting	**2009**: December 5-9	San Diego, CA, USA	Scientific Community	L	Worldwide
33	Conference	van der Ploeg	Dutch patient association for metabolic diseases (VKS) Conference	November 28	Amersfoort, The Netherlands	Scientific Community	M	EU/NL
34	Meeting	Parenti	Chaperone Therapy Meeting	November 21-22	Munich, Germany	Scientific Community	S	DE
35	Workshop	van den Hout, van der Ploeg, de Vries, Reuser Beck	Genzyme: Steps Forward in Pompe Disease	November 19-21	München, Germany	Scientific Community	M	Worldwide
36	Workshop	Parenti	Workshop su malattia di Pompe	November 19	Florence, Italy	Scientific Community	S	IT
37	Workshop	Reuser	Dutch Neuromuscular Research Center	November 11-12	Beekbergen, The Netherlands	Scientific Community	S	NL
38	Symposium	Beck	Symposium on Pompe disease	November 7	Manheim, Germany	Scientific Community	S	DE
39	Workshop	Reuser	Shire. 9th International Workshop on Lysosomal Storage Diseases	November 5-7	Barcelona, Spain	Scientific Community	M	Worldwide
40	Meeting	Beck	Satellite Meeting: Bone Disease in Patients with Mucopolysaccharidoses	October 31	Curia, Portugal	Scientific Community	M	Worldwide
41	Meeting	Ballabio	The American Society of Human Genetics, 59th Annual Meeting	October 20-24	Honolulu, Hawaii	Scientific Community	L	Worldwide
42	Conference	Andria, Parenti	Italian Society for Metabolic Diseases and Neonatal Screenings	October 12-13	Cagliari, Italy	Scientific Community	M	EU/IT
43	Symposium	Auricchio Beck	Symposium: MPS and the Eye	October 7-9	Venice, Italy	Scientific Community	M	EU/IT
44	Conference	Auricchio	International Centre for Genetic Engineering and Biotecnology (ICGEB)	October 6	Trieste, Italy	Scientific Community	L	Worldwide
45	Conference	Andria	XXII Convegno Nazionale AIMPS	October 2-4	Florence, Italy	Scientific Community	M	IT
46	Conference	van der Ploeg	International Society Inborn Errors of Metabolism Conference (ICIEM)	August 31	San Diego, CA, USA	Scientific Community	L	Worldwide

47	Conference	Andria	3° Congresso Nazionale FIMP	September 30-October 3	Rome, Italy	Scientific Community	M	IT
48	Meeting	Auricchio	*In vivo* application of AAV Meeting	September 3–4	Nantes, France	Scientific Community	S	EU/FR
49	Conference	Parenti	Convegno UNIAMO: "Malattie Rare tra Presente e Futuro. Innovazione, Assistenza, Ricerca, Società"	July 3-5	Venice, Italy	Scientific Community	M	EU/IT
50	Workshop	Reuser	Workshop on diagnostic bloodspot analysis in Pompe disease	June 23-24	Amsterdam, The Netherlands	Scientific Community	S	NL
51	Conference	Reuser, van der Ploeg	VSN, Dutch Patient Association Neuromuscular Diseases	June 20	Velthoven, The Netherlands	Scientific Community	M	EU/NL
52	Meeting	Reuser, van der Ploeg	6th Pompe disease Expert Day	June 10-11	Rotterdam, The Netherlands	Scientific Community	S	NL
53	Course	Reuser	8th Postdoctoral Course on Lysosomal Storage Diseases	June 8	Nierstein, Germany	Scientific Community	S	DE
54	Meeting	Auricchio	The American Society of Gene Therapy, 12th Annual Meeting	May 27–30	San Diego, CA, USA	Scientific Community	L	Worldwide
55	Meeting	Karlsson	First European Gaucher Leadership Forum	May 14-15	Milan, Italy	Scientific Community	S	IT
56	Symposium	Reuser	Shire. 9th International Symposium on Lysosomal Storage Diseases	April 2-4	Frankfurt, Germany	Scientific Community	M	Worldwide
57	Course	Andria	XXI Corso di aggiornamento - Malattie da accumulo Lisosomiale: la diagnosi precoce e le nuove possibilità di trattamento	March 17-19	Parma, Italy	Scientific Community	S	IT
58	Conference	Auricchio	Telethon Convention 2009	March 9-11	Riva Del Garda, Italy	Scientific Community	L	EU/IT
59	Workshop	Andria	3rd European Brains for brain workshop	March 6-8	Frankfurt, Germany	Scientific Community	S	EU/DE
60	Meeting	Reuser, van der Ploeg	5th Pompe disease Expert Day	**2008** November 26-27	Rotterdam, The Netherlands	Scientific Community	S	NL
61	Conference	Andria	Congresso Milano Pediatria	November 20-22	Milan, Italy	Scientific Community	M	IT
62	Meeting	Auricchio	The American Society of Gene Therapy, 11th Annual Meeting	November 13-16	Brugge, Belgium	Scientific Community	L	Worldwide
63	Workshop	Reuser	International Workshop on Lysosomal Storage Disorders	November 7-8	Lisbon, Portugal	Scientific Community	L	Worldwide
64	Meeting	Karlsson	The German Society of Gene Therapy, 15th	October 8-10	Berlin, Germany	Scientific	M	EU/DE

			Annual Meeting			Community		
65	Course	Reuser	7th Postdoctoral Course on Lysosomal Storage Diseases	June 8-11	Nierstein, Germany	Scientific Community	S	DE
66	Workshop	Cox Karlsson	Proceedings of the 8th European Working Group on Gaucher Disease (EWGGD)	June 4-7	Budapest, Hungary	Scientific Community	M	EU
67	Meeting	Ballabio, Auricchio, Surace	The American Society of Gene Therapy, 10th Annual Meeting	May 28- June 1	Boston, MS, USA	Scientific Community	L	Worldwide

Section B (Confidential[8] or public: confidential information to be marked clearly)
Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

No patents, trademarks, registered designs or the like have been produced by the EUCLYD consortium. Templates B1 and B2 have thus been omitted from the Final Report of the project.

[8] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

4.3 REPORT ON SOCIETAL IMPLICATIONS

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	Health-F2-2008-201678
Title of Project:	A European Consortium for Lysosomal Storage Diseases
Name and Title of Coordinator:	Generoso Andria, MD

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***0Yes X No***
2. Please indicate whether your project involved any of the following issues (tick box):	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	YES
• Did the project involve patients?	YES
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human genetic material?	
• Did the project involve Human biological samples?	YES
• Did the project involve Human data collection?	YES
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	YES

• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	YES
• Were those animals transgenic small laboratory animals?	YES
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	0 Yes X No
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator		1
Work package leaders		4
Experienced researchers (i.e. PhD holders)	5	12
PhD Students	5	0
Other	7	3

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	3
Of which, indicate the number of men:	2

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

X Yes
○ No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
X	Design and implement an equal opportunity policy	○	○	X	○	○
X	Set targets to achieve a gender balance in the workforce	○	○	X	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

○ Yes- please specify

X No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

X Yes- please specify EU PUBLIC WEBSITE

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

○ Main discipline[9]: 3

○ Associated discipline[9]: 3.1 ○ Associated discipline[9]: 3.2

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*

○ Yes
X No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No
○ Yes- in determining what research should be performed
○ Yes - in implementing the research
○ Yes, in communicating /disseminating / using the results of the project

[9] Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ○ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	60
To how many of these is open access[10] provided?	42
How many of these are published in open access journals?	
How many of these are published in open repositories?	
To how many of these is open access not provided?	5
Please check all applicable reasons for not providing open access:	
X publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available X no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[11]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		N/A
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	N/A
	Registered design	N/A
	Other	N/A
17. How many spin-off companies were created / are planned as a direct result of the project?		N/A
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑ Increase in employment, or	❑ In small & medium-sized enterprises
❑ Safeguard employment, or	❑ In large companies
❑ Decrease in employment,	❑ None of the above / not relevant to the project
X Difficult to estimate / not possible to quantify	

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*

[10] Open Access is defined as free of charge access for anyone via Internet.
[11] For instance: classification for security project.

Difficult to estimate / not possible to quantify	X

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes X No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes X No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

❑	Press Release	X	Coverage in specialist press
❑	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	❑	Coverage in national press
❑	Radio coverage / report	X	Coverage in international press
X	Brochures /posters / flyers	X	Website for the general public / internet
❑	DVD /Film /Multimedia	X	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	X	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as

geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]