Clinical trial of gene therapy for MPS VI - a severe lysosomal storage disorder

Final Report Summary - MEUSIX (Clinical trial of gene therapy for MPS VI - a severe lysosomal storage disorder)

Executive Summary:
As a preliminary step of the project, the Partners planned the production of AAV2/8.TBG.hARSB clinical vector under GMP conditions for use as an Investigational Medicinal Product (IMP) and provide a safety report for regulatory authorities. To discuss the project, Partners met with the Italian Regulatory Authority (ISS), which gave them advice related to chemistry, manufacturing, and controls (CMCs) issues. Partners evaluated and selected contract research organizations (CROs) and contract manufacturing organizations (CMOs) to produce and test the IMP; they also chose the company providing the qualified person (QP) for IMP approval. The company conducted an audit of the selected CMOs and CROs: the audit identified issues with the CMO responsible for manufacturing the IMP. Partner 2 and the CMO have implemented corrective actions to address the audit findings; the process has been established, and Partner 2 completed the IMP manufacture.

Main achievements of the project:
- The IMP was successfully produced, with the final fill/finish taking place in June 2015 and product delivered to the study sponsor’s designated storage facility in September 2016.
Non-clinical studies evaluating the safety of AAV2/8.TBG.hARSB administration were performed according to Good Laboratory Practice (GLP) principles, using a GMP-like vector lot indicated as “Tox lot” for use in non-clinical studies. All the results have been reported in the IMPD.

- The IMPD has been submitted to the Competent Italian Authorities (AIFA and ISS) and to the Ethics Committee, obtaining approval on February 22, 2017.

- Partners have established an Ethics Advisory Group (EAG), overseeing the development of guidelines for the consent forms and ensuring uniformity of operations in all participating centers.

- Study consent forms and parental information sheets have been developed with a uniform set of information. Forms have been adapted to the local participating centers.

- Partners have also established the independent Data and Drug Safety Monitoring Board, to assess the progress, safety data and critical efficacy endpoints of the study.

- The authorization of the clinical site for the use of vector has been obtained. Moreover, as in Italy the Clinical Units have to be compliant to the Determina AIFA 19.06.2015 we also prepared the request for this authorization and got it on February 25, 2017.

- The pre-submission meeting at EMA (European Medicine Agency) has also been completed.

- The clinical protocol has been prepared with the contribution of Partners 1, 4, 5, 6 and 7 and submitted to ISS/AIFA and Ethics Committees.

- The clinical study was approved in Italy on February 22, 2017.

- On May 3 2017, the CRO conducted the site initiation visit, so the primary site in Naples was authorized to screen patients and during that visit was also tested the eCRF with the contribution of P8.

- As the study protocol states that the trial should start with adult patients, we commenced to individuate possible eligible patients in order to start the first infusion contacting the other two referring sites in Turkey and The Netherlands. The first adult patient resulted to be a Turkish one.

- A protocol amendment (concerning the primary safety endpoints, exclusion criteria, risks associated with gene therapy infusion of the IMP) was submitted to Competent Authority on June 14, 2017 and was approved on July 17, 2017.

- Assays realized on patients samples have been validated according to the Good Clinical Practices by P3 with the contribution of P1.

- The first patient received the gene therapy IMP in October 11, 2017. No any particular adverse events occurred. He is now conducting follow up visits at the referring site in Ankara.

- A second patient (from Italy) was screened in November 6, 2017. He was treated in January 2018.

- Three patients have been treated until June 2018, the trial is going on, as well as the follow-up activities.

Project Context and Objectives:

The Partners identified five main milestones, which are the overall objectives of MeuSIX proposal:

1. to produce AAV2/8.TBG.hARSB vector for a gene therapy human clinical trial, according to Good Manufacturing Practice (GMP) (clinical-grade);
2. to perform pre-clinical pharmacological and toxicological studies using the AAV2/8.TBG.hARSB vector;
3. to design a Phase I/II clinical trial, in which the GMP AAV2/8.TBG.hARSB vector is tested in MPS VI patients, to generate data related to pharmacokinetics, pharmacodynamics, safety, and efficacy;
4. to produce and file the documents required to obtain authorization from Italian, Turkish and Dutch regulatory agencies to execute a Phase I/II clinical trial;
5. to perform a multicenter Phase I/II clinical trial to investigate the safety and efficacy of AAV2/8.TBG.hARSB gene therapy for MPS VI.

1) To produce Good Manufacturing Practice (GMP) (clinical-grade) AAV2/8-TBG-hARSB vector for a gene therapy human clinical trial, according to Good Manufacturing Practice (GMP) (clinical-grade).
As for any clinical trial involving in vivo gene delivery of viral vectors, as a first step we needed to generate AAV2/8.TBG.hARSB under good manufacturing practice (GMP) conditions. Partner 2 had a long-standing expertise on AAV vector use and production, since they originally cloned AAV2/8 and demonstrated its greater liver transduction efficiency compared to other AAV serotype. Partner 2 engaged a qualified Contract Manufacturing Organization (CMO) to perform vector manufacturing in compliance with current Good Manufacturing Practices (GMP) regulations and guidelines. AAV2/8.TBG.hARSB will be produced by triple transfection of HEK 293 cells and purified from culture medium by a physical method. The vector was tested in compliance with GMPs (according to the Italian and European regulations) for identity, adventitious agents, purity, and potency before was released to the Sponsor for further pre-clinical testing.

2) To perform pre-clinical pharmacological (pharmacodynamics and pharmacokinetics), immunological, and toxicological studies using the GMP AAV2/8.TBG.hARSB vector required to perform a human clinical trial.

To obtain a safety evaluation of the investigational medicinal product (IMP) AAV2/8.TBG.hARSB GMP vector, in compliance with Good Laboratory Practice (GLP), testing was carried out in mice. Specifically, we completed pre-clinical toxicity and safety studies in MPS VI transgenic mice and wild type mice. In addition, part of the pre-clinical toxicity studies were complemented with data obtained in MPS VI cats through non-GLP studies.

The studies determined potential risks associated with AAV2/8.TBG.hARSB vector administration including vector quality, according to EMA and Italian regulatory authorities.

3) To design a Phase I/II clinical trial testing the GMP AAV2/8.TBG.hARSB vector in MPS VI patients to generate data in terms of pharmokinetics, pharmacodynamics, safety, and efficacy.

The design of the trial took into account safety endpoints including those required to evaluate toxicity, immune reactions to both vector and transgene, and biodistribution.

Although safety is the primary objective of the trial, efficacy will also be evaluated. This study is powered at 80% to detect as statistically significant a decrease in urinary GAGs of at least 50 μg/mg creatinine in favour of gene-therapy, as compared to historical reference levels of MPS VI untreated patients. A clinical study protocol including the multicenter trial inclusion/exclusion criteria, the primary and secondary endpoints of safety as well as of efficacy has been drafted by the clinical members of the consortium. The power analysis of this study was evaluated by a team of expert statisticians (Partner 7), led by Prof. Maria Grazia Valsecchi at the University of Milano-Bicocca who have long-standing experience in rare disease clinical trials.

4) To produce and file the documents required to obtain authorizations to perform a Phase I/II clinical trial from the Italian, Turkish and Dutch regulatory agencies.

The strategy based on liver-directed gene therapy for the treatment of MPS VI patients has already received the ODD status from the FDA (ODD 11-350) and from the EMA (EU/3/11/864). This part of the project focused on the production and filing by the Sponsor Partner 1 of the required regulatory documents, including: the request for protocol assistance to the EMA; the request for authorization of the clinical sites to the clinical use of genetically modified organisms (GMO); the preparation of the Investigational Medicinal Product Dossier (IMPD); the Investigator’s Brochure (IB); the Clinical Trial Application. This included the submission of all necessary documentation to the regulatory authorities as well as all the regulatory supports for the duration of the trial.

5) To perform a multicenter Phase I/II clinical trial to investigate the safety and efficacy of AAV2/8.TBG.hARSB gene therapy for MPS VI.
The pre-clinical data generated by Partner 1 have shown that liver-directed gene therapy results in systemic secretion and widespread uptake of the vector-derived ARSB, which ultimately leads to long-term biochemical and functional correction of the disease. The major goal of this part of the project is to translate these encouraging pre-clinical results into the clinic and to demonstrate that gene therapy for MPS VI is safe and at least as effective as ERT. Building on the deliverables from the previous objectives, we plan to perform an investigator-driven, multicentre Phase I/II clinical trial designed as an open label dose escalation study to test the safety and efficacy of intravascular administrations of AAV2/8.TBG.hARSB to MPS VI patients of 4 years of age and older. The trial involves the Partner 4 as the primary clinical site and Partners 5 and 6 as secondary sites.

Project Results:
As a preliminary step of the project, the Partners planned the production of AAV2/8.TBG.hARSB clinical vector under GMP conditions for use as an Investigational Medicinal Product (IMP) and provide a safety report for regulatory authorities.

To discuss the project, Partners met with the Italian Regulatory Authority (ISS), which gave them advice related to chemistry, manufacturing, and controls (CMCs) issues. Partner 2 evaluated and selected contract research organizations (CROs) and contract manufacturing organizations (CMOs) to produce and test the IMP. Partner 2 successfully delivered well-established manufacturing processes and product-specific test methods to the selected CMO and CROs, and worked to develop and optimize the ARSB gene therapy vector production process.

Partners 1 and 2 have chosen a company that will provide the qualified person (QP), which will give approval for the IMP. Partner 1 engaged the company, which conducted an audit of the selected CMOs and CROs. The audit identified issues with the CMO, which is responsible for manufacturing the IMP; Partner 2 and the CMO have implemented corrective actions to address the audit findings; the process has been established, and Partner 2 completed the IMP manufacture.

The ISS has stated that GMP-like material could be used in non-clinical studies, and thus Partner 2 has provided vector for use in non-clinical work during tech transfer. The Partner has also provided data on vector stability in the medium used for infusion in animals during the non-clinical studies. Since long-term stability data are requested as part of the Investigational Medicinal Product Dossier (IMPD), additional material was also produced for a long-term stability study.

Partners have established an independent Data and Drug Safety Monitoring Board, to assess the progress, safety data and critical efficacy endpoints of the study. The Board includes qualified clinicians, pharmacologists and ethics experts.

Partners have also established an Ethics Advisory Group (EAG), composed of medical ethicists and members of patient organizations that give guidance on the ethical aspects of patient treatment and care. The EAG oversees the development of guidelines for the consent forms and ensure uniformity of operations in all participating centers.

The project also oversees the production and filing of the required regulatory documents; the following actions have been taken in order to fulfil this process: preparation of the pre-submission meeting with ISS, request for authorization of the clinical site for the use of vector, and preparation of the IMPD. A pre-submission meeting at EMA (European Medicine Agency) has also been completed. Scientific advice has been asked on clinical aspects of the proposed trial design, including age inclusion criteria, enrolment of patients based on genotype, vector doses and dose-escalation design, endpoints, and patients return to enzyme replacement therapy.
In particular,

REGENXBIO selected contract manufacturing organizations (CMO’s) to produce and analyze the Investigational Medicinal Product (IMP) within the timeline specified in the grant. The subcontractors selected to produce the vector meeting EU standards are the following:

- Belfer Gene Therapy Core Facility, Weill Cornell Medical College, NY, USA responsible for manufacturing, testing, validation (referred to as “Cornell” in report);
- WuXi AppTec Inc., Philadelphia, PA, responsible for QC testing not performed by Cornell;
- Aldevron, Fargo, North Dakota, USA responsible for supplying plasmids as raw materials for vector production;
- SAFC, Carlsbad, CA, responsible for aseptic fill and finish activity.

The IMP was successfully produced, with the final fill/finish taking place in June, 2015 and product delivered to the study sponsor’s designated storage facility in September, 2016.

The Partners decided that it was necessary a stability study in the infusion medium. The objective of this study was to test the stability of the AAV2/8.TBG.hARSB vector in the infusion medium at room temperature. The stability study was performed by Partners 1, 3, and 4. It was performed for each dose cohort, i.e 2E+11, 6E+11 and 2E+12 gc/kg. After dilution, vector was incubated at RT up to 8 hours. For the 3 experiments, no reduction in both physical and infectious titers was observed after incubation at room temperature in the infusion medium.

Non-clinical studies aimed at evaluating the safety of AAV2/8.TBG.hARSB administration were performed according to Good Laboratory Practice (GLP) principles, using a GMP-like vector lot indicated as “Tox lot” for use in non-clinical studies. The “Tox lot” is representative of the process used for the drug product (DP) production, which is consistent with the recommendations in the Note for Guidance on Quality, Pre-clinical and Clinical Aspects of Gene Therapy Medicinal Products (CPMP/BWP/3088/99). This lot was labelled for research use only and shipped, under standard procedures for importation of research materials, to the test facility - Centre de Recherches Biologiques (CERB, France) - where animals were injected.

Locations: Analyses were conducted at both the test facility and at additional test sites
- Centre de Recherches Biologiques (CERB) , Chemin de Montifault, 18800 Baugy – France; Study: Vector administration, General health and clinical sign evaluation Haematology, Clinical Chemistry, Necropsy and samples collection for other studies.
- Belfer Gene Therapy Core facility; Department of Genetic Medicine, Weill Cornell Medical College; 515 East 71st Street, Room S-1000, New York, NY 10021- United States; Study: Formulation analysis
- Propath UK Limited; Willow Court, Netherwood Road; Hereford, HR2 6JU - United Kingdom; Study: Histotechnology
- JPF Consultancy; PO Box 27, Woodbridge; IP13 9WX Suffol - United Kingdom; Study: Histopathology
- GENOSAFE; 1 rue de l’Internationale; BP 40064; 91002 Evry Cedex- France Study: Biodistribution analysis

GLP non-clinical studies were mainly performed in heterozygous C57/BL6-TgARSBC91S mice, an immune-tolerant to hARSB mouse model that was developed in collaboration with Taconis Artemis (Germany) and expanded later by Taconis Europe. The model is a wild-type C57/BL6 mouse that harbor a hARSB ORF carrying the C91S point mutation (Brooks et al., 1995) in the ROSA26 locus (Zambrowicz et al., 1997). This mutation inactivates the ARSB enzymatic activity, preserving the protein conformation and structure.
thus making this mouse immune tolerant to wt hARSB. We generated this model to easily control over the copy number of the C91S ARSB human allele. Unlike in the transgenic MPS VI mouse model we used in our previous studies and in the dose response study, where the copy number is variable, here the C91S ARSB transgene is inserted in a site-specific manner at the ROSA 26 locus and is thus present in one copy in heterozygous animals and in two copies in homozygous ones. Therefore the levels of immune-tolerance to the hARSB transgene product of the C57/BL6-TgARSBC91S mice should be consistent inter- and intra-littermates. In addition, a wild type mouse model, i.e. C57/BL6-TgARSBC91S mice, is easier to manage and more affordable compared to a disease model such as the transgenic MPS VI mouse model. Additionally, adult (sexually mature) male rabbits were used to in-depth investigate the risk of germline transmission. As recommended by EMA guidelines (EMEA/273954/2005), if the vector is detected in gonads, more detailed information is needed to elucidate whether stem cells are transduced. This is possible by investigating sperm in male animals at different time points taking the duration of spermatogenesis into account. Since external sperm collection is not possible in mice, we decided to use New Zealand male rabbits, which are a suitable model for germline transmission studies (Favaro et al., 2009; Favaro et al., 2011). A dose-dependent response to the test item, i.e. AAV2/8.TBG.hARSB in its final formulation, was observed in MPS VI transgenic mice. The only exception was represented by GAGs in visceral organs that were strongly reduced in all animals, regardless of the dose administered and the serum and tissue ARSB levels achieved. These findings support what previously found in our studies performed in MPS VI cats, rats and mice, that very low ARSB tissue levels are sufficient to achieve lysosomal storage clearance (Cotugno et al., 2011; Cotugno et al., 2010; Ferla et al., 2014). The maximal therapeutic efficacy was achieved at the dose of 2x1012 gc/kg with normalization of GAGs in urine, visceral tissue and myocardium and marked to complete clearance of GAG storage also in heart valves. Similar but slightly variable results were obtained in mice receiving the intermediate dose of test item. Our findings also suggested that the dose of 2x1011 gc/kg could be considered as the minimal effective dose. Indeed, circulating ARSB was consistently detectable only in 2 out 4 mice of this dose cohort. Nevertheless, urinary GAGs were on average reduced by 30% and such reduction was observed even in those mice with undetectable serum ARSB levels. As above-mentioned, GAGs storage in liver, kidney and spleen was significantly reduced in all mice receiving this dose of vector. Conversely, GAGs clearance in myocardium was variable and related to serum ARSB, whereas minimal to absent reduction was observed in heart valves. Therefore, these data confirm what observed in MPS VI cats receiving 2x1011 gc/kg of vector. Indeed, despite serum ARSB levels similar to those in untreated MPS VI cats, they showed phenotype improvement, such as reduction of urinary, tissue GAGs and MVT, and also amelioration of femur length and motor activity were observed (Cotugno et al., 2011; Ferla et al., 2013). These findings suggested that the doses of AAV2/8.TBG.hARSB vector to be tested in a clinical trial for MPS VI should range between 2x1011 and 2x1012 gc/kg. Results show that AAV2/8.TBG.hARSB vector is expressed in all organs investigated, most importantly in liver, gall bladder and stomach but not in tissues of the digestive system (salivary gland, jejunum, caecum, colon, pancreas). The vector was not expressed in organs from urogenital system in males and females suggesting that no germline transmission should occur. In addition, in spite of a low amount of RNA tested from blood and bone marrow, no expression was found in these two fluids. At last, as expected a decrease of RNA vector copy number is observed along time (Day 180) in males and females. All the results have been reported in the IMPD (submitted as deliverable 3.8). The IMPD has been submitted to the Competent Italian Authorities (AIFA and ISS) and to the Ethics Committee, obtaining approval on February 22, 2017. A comprehensive description of the main findings of the non-clinical studies of gene therapy for MPS VI has been reported in: “Non-clinical Safety

In Italy, the request for authorization is a two-step process: 1) a document named “Notifica di Impianto” needs to be filed to request authorization for the spaces (rooms) in which the clinical trial will be conducted and the vector will be used; 2) after obtaining the authorization of “Impianto”, and after the positive opinion from ISS on the quality of vector and safety of non-clinical studies in animals, a second document needs to be filed to request the “Notifica di Impiego” which is the authorization to the use of vector.

The vector administration will occur at the Department of Translational Medicine (former Department of Pediatrics) of the Università degli Studi di Napoli “Federico II” Medical School (Partner 4). The site in which the clinical trial will take place has obtained the authorization for the clinical use of genetically modified organisms, so the application named “Notifica di Impianto” was obtained from Ministero della Salute on May 9, 2014. Finally, we obtained the “Notifica di Impiego” (to the clinical use of genetically modified organism) on February 3, 2017. In the documentation provided for this authorization we were asked to give plenty of information concerning the viral vector and his production. In addition, we were requested to give information about the personnel who had the skills to participate to the clinical trial. We also gave information concerning the vector infusion and the design of the trial (partner 4 and 7). For this purpose, we created the Standard Operating Procedures (SOPs) for dose calculation, infusion set and trial conduction. Moreover, in Italy, in order to operate as structure for Phase I/II studies, the Clinical Units have to be compliant to the Determina AIFA 19.06.2015. We got this authorization on February 25, 2017.

The clinical protocol has been prepared with the contribution of Partners 1, 4 and 7 and submitted to ISS/AIFA and Ethics Committee. The study was approved in Italy on February 22, 2017. On May 3 2017, the CRO conduced the SIV (Site initiation visit) so the primary site in Naples was authorized to screen patients and during that visit was also tested the eCRF.

As the study protocol states that the trial should start with adult patients, we commenced to individuate possible eligible patients in order to start the first infusion contacting the other two referring sites in Turkey and The Netherlands. The first adult patient results to be a Turkish one. The screening visit for this patient was scheduled on May 14, 2017, but a few day before his arrival in Naples, the visit was postponed to prepare a protocol amendment as explained in WP5.

We made an amendment to the protocol concerning the following points:
1) Primary safety endpoints
Safety of the administration. This will be determined through clinical evaluations recording the body temperature, heart rate, blood pressure, respiratory rate, complete physical exam, and monitoring of adverse reactions (e.g. hives, breathing difficulties, or death). Overall safety and tolerability will be also determined through laboratory investigations and imaging studies (i.e. liver ultrasound). Inflammation of the liver, as shown by an elevation in transaminases, and thyroids hormones will be also included in the safety evaluation. Evaluations of kidney function will be performed by serial evaluations of the following parameters in blood and urine: creatinine albumin, total protein and BUN and C3 and C4 blood levels.
2) Exclusion criteria
Presence of serum antibodies anti-ARSB above the limit of detection of the assay (antibodies anti-ARSB level >31250 or positive to the value of dilution 1.10 according to the performed assay) at Screening
3) Risks associated with gene therapy

Renal disease is a rare complication of ERT and it occurs as a consequence of extensive deposition of immune complexes made of anti-ARSB antibodies and the enzyme delivered intravenously by ERT. In the context of gene therapy, sustained production and blood release of ARSB from transduced hepatocytes might result in higher risk of immune complex formation. Although this is a theoretical risk, we will carefully evaluate renal function and complement levels in the enrolled subjects.

4) Each urine sample will be divided in two aliquots, one for uGAG analysis and the other for determination of creatinine concentration.

5) Blood collection will be performed for standard tests described above, as well as esoteric tests including ARSB activity, anti-AAV8, NAB and antibodies for ARSB protein. PBMCs will be collected for ELISPOT assay, a research laboratory study that measures cell mediated immune response. This test will be performed on blood, except for the samples from Children’s Hospital, Hacettepe University, Ankara, Turkey. In this case, the test will be performed on serum.

- The kidney function will be evaluated by the following parameters in blood (as part of the serum chemistry test for renal function) and urine: creatinine, albumin, total protein and BUN
- C3 and C4 will be also evaluated in blood.

- Urinary GAG
- Peripheral blood, saliva, urine, stool and semen (if applicable) PCR to detect vector shedding will be included in each subject evaluations.

For the following test a back sample will be collected and stored at clinic site:
- Serum collection for ARSB protein level, serum collection for PBMC test (only for samples from Hacettepe University site, Ankara, Turkey), serum collection for antibodies anti-AAV8, anti-ARSB and for neutralizing antibodies, serum collection for vector shedding,
- Urine collection for uGAG test, urine for vector shedding,
- Leukocytes for ARSB enzyme activity.

In any case the total blood volume collected is within the limiting range of 1% - 5% of total blood volume collected over 24 hours and up to 10% of total blood volume over 8 weeks (Howie 2011).

6) Tertiary efficacy endpoints will include:
- Height and Weight
- Health Assessment Questionnaire and Childhood Health Assessment Questionnaire (HAQ, CHAQ) scores,
- Visual acuity and ocular abnormalities by full ocular examination.
- Cardiac function through ECG and echocardiography to monitoring cardiac parameters

7) Starting from week 2 prednisolone will be administrated to the subject, as soon as we detect the increase of ALT and AST.

8) All subjects returning to ERT after gene therapy will remain in the study and will continue to be follow up...
Amendment was submitted to Competent Authority on June 14, 2017 and approved on July 17, 2017. The screening visit for the first patient, was planned on May 2017 but it was postponed to proceed to the protocol amendment. In order to minimize the discomfort for the patient, since he is Turkish, it was decided to conduct screening and baseline assessments in a single session on July 2017. Based on the results, he was eligible and he came in Naples again to receive the treatment on October 11, 2017 and now he is conducting follow up visits at the referring site in Ankara.

According to the clinical protocol, as transaminase levels were above 1.5 1.5-fold compared to baseline 2 (-5 days) and the subject started the glucocorticoid therapy (oral prednisolone of 1 mg/kg/die in children and 60 mg/die in adults). The patient is still being treated the glucocorticoid therapy. No any particular adverse event occurred.

On November 6, 2017 we screened the second patient and his results are compliant with the inclusion criteria. He will be treated in January 2018.

The Erasmus MC participated, together with HU, UNIMIB and INFORMA to work package 4 (WP4), which focused on the design and performance of the gene therapy clinical trial in MPS VI patients. The task of the Erasmus MC in this work package was to report on molecular and clinical characteristics of MPS VI patients, to screen and identify MPS VI patients as candidate to the gene therapy clinical trial bases on inclusion and exclusion criteria and to report on the safety and efficacy data. We present the clinical and molecular characteristics of the Dutch MPS VI patients, in addition we describe research projects related to the deliverables of the MeuSix project. The study protocol was approved by the Medical Ethical Committee at Erasmus MC University Medical Center. In the Netherlands, treatment and standardized clinical follow-up of MPS VI patients take place at a single national expertise center, which was officially appointed by the Ministry of Health in June 2015 (Erasmus MC, Center for Lysosomal and Metabolic disease). All patients were treated with 1 mg/kg recombinant human arylsulfatase B (galsulfase, Naglazyme®, Biomarin Corporation). In line with the study protocol all patients were assessed every 2-4 times per years. Follow up of these patients started at baseline, just before of start ERT. At every assessment serum and urine samples were obtained and patients were seen by a pediatrician specialized in metabolic disorders, a child neurologist, and a physiotherapist (2 times per year). Standard assessment that were performed include the assessment of pulmonary function (FVC and polysomnography), a cardiac evaluation, size of liver and spleen, joint mobility (goniometry), hearing function, radiologic examinations, and urinary glycosaminoglycan (GAG) level. The physiotherapist performed a 6 Minute Walk Test (6MWT), BRUCE and a 4 meter stair climb. Additional to a clinical follow-up patients underwent a standardized cognitive assessment. The details are described in Ebbink et al. Quality of life was assessed with the PedsQol, the HAQ and the SF-36. Currently both care and research has focused on the effects of ERT on hip and joint problems. These problems lead to severe disability for patients with MPS VI. The development of hip problems over time and underlying pathophysiology are described in Oussoren et al, 2017.

Assays realized on patients samples have to be validated according to the Good Clinical Practices by Partner 3 Genosafe. Prior to validation, tests have to be 1) transferred from Partner 1 to Partner 3, 2) qualified.
During the Period, Genosafe qualified 3 assays: ARSB activity assessment, ECL assay for the detection of circulating antibodies against hARSB in human sera, Assay for the titration of neutralizing antibodies against rhARSB binding activities in human activities. The qualification consisted in determining the different parameters that will be applied during the validation process. This process consists in locking out these parameters, and demonstrating that the method is robust, specific, and sensitive enough.

Electronic Case Report Forms (eCRFs). The design and implementation of the eCRFs has been strictly intertwined with the elaboration of the Study Protocol. The Protocol itself has been reviewed several times, in accordance with the indications of the clinicians and of the experts: after the first Protocol drafts released in the previous project period, two different versions of the Protocol have been delivered: in September 2016 and in May 2017. Accordingly, several versions of the Case Report Forms (CRFs) design (paper based) and of the eCRFs version have been realised. An updated version of the CRFs design was released and evaluated in June 2016, with the aim to check any possible problem in the implementation of the several conditions given by the Protocol, before the implementation of the web based eCRFs. At the beginning of September 2016 a meeting was held in Rome, in the Telethon offices, to close the design phase of the CRFs. In November 2016 complete and tested eCRFs were released. After the release of the second version of the Study Protocol, a second version of the eCRFs was released in May 2017.

Ethics and clinical safety are of primary importance in the MeuSIX study particularly as it includes vulnerable populations as children. The main task of this essential Work package is the supervision of ethics and safety of the clinical trial. This has been carried out through the establishment of an ethics advisory group (EAG) including medical ethicists and members of patient organizations including the Italian, French, Spanish, Dutch, Turkish and American MPS Societies. The EAG has helped us in advising on ethical aspects of patient treatment and care on the development of guidelines for the MeuSIX study consent forms and also on parent information sheets, to ensure a uniform approach in all participating centres (in conjunction with WP3). The EAG has given also important contribution in preparing an amendment request concerning the introduction of a new exclusion criterion and periodic monitoring of renal function. It has been observed that MPS VI patients undergoing enzymatic replacement therapy, developed immunocomplexes that alter renal function. It was therefore considered appropriate to include, as a safety measure, a new exclusion criterion to exclude those MPS VI patients who have a high titer of ARSB antibodies, which may be the cause of the development of immunocomplexes and to monitor renal function through periodic creatinine, total proteins, albumin and BUN tests and complement (C3 and C4). Moreover, it was decided to evaluate the effectiveness of the therapy also on cardiac function, which is generally compromised in MPSVI patients, through ECG and echocardiogram.

The Amendment has been approved by AIFA/ISS and by the Ethics Committee.

The first patient received the gene therapy IMP in October 11, 2017. No any particular adverse events occurred. He is now conducting follow up visits at the referring site in Ankara. A second patient (from Italy) was screened in November 6, 2017. He was treated in January 2018. Three patients have been treated until June 2018, the trial is going on, as well as the follow-up activities.
has been reported to the study coordinator and sponsor. The CTA has been submitted to Ethics Committee of Federico II University that approved it (please find attached the authorization from CE and the list of Members belonging to the CE, along this report).

Study consent forms and parental information sheets have been developed with a uniform set of information. Forms have been adapted to the local participating centres. Please find attached (at the end of this report) the final versions of consent forms in English, Italian and Turkish languages. The forms in Dutch language are waiting for the approval from the Dutch Ethics Committee, which is expected to be obtained in few weeks.

EAG group continuously work in ensuring that the study complies with the appropriate European laws.

Communication within the consortium has been always open and consistent. Participants were very frequently in contact with each other via teleconferences, email or Skype. They exchanged ideas, discuss progress of research and project objectives, and make strategic decisions regarding scientific planning and financial aspects of the programme.

Potential Impact:
The MeuSIX program responds to the call HEALTH.2012.2.4.4-1: Pre-clinical and/or clinical development of substances with a clear potential as orphan drugs. FP7-HEALTH-2012-INNOVATION-1. This call is aimed at the development of “pre-clinical studies of EU designated orphan medicinal products”. MeuSIX has put together a consortium of leading basic scientists, expert clinicians and SME’s to develop a unique program to specifically respond to this call to develop a novel gene therapy trial for a rare genetic disorder, namely MPS VI. The 2011 work programme aims at developing a new improved therapy for patients affected with rare diseases “to alleviate the negative impact of the disease on the quality of life of these patients and their families”. MeuSIX directly addresses this objective by developing a one-in-a-life-time treatment for MPS VI, that will lead to a positive impact on the life of patients and their families and lift the significant economic burden current treatments have on national health care systems. This will aid the commission and the International Rare Disease Research Consortium (IRDiRC), of which Partner 1 is a member, to meet the goal “to deliver 200 new therapies for patients affected by rare diseases by 2020”. The director of TIGEM (Telethon Institute of Genetics and Medicine – Partner 1) Dr. Andrea Ballabio is on the IRDiRC scientific advisory board. The consortium plans to attend the IRDiRC Working Groups in order to interact with the group members and update them on the progress of the project.

The major expected impacts of MeuSIX are:
1) To overcome the limitations of ERT for MPS VI:
ERT with recombinant rhARSB (Naglazyme) has been approved by the FDA and the EMA and is currently recommended as a therapy for MPS VI. However, ERT has several limitations. First, rhARSB has a short half-life (26, 27) requiring weekly intravenous infusions that carry a risk of allergic reaction and often require a central venous access, which in turn carries risk of sepsis. Second, some organs and tissues like bone, cartilage and cornea are not corrected likely because of limited biodistribution. Third, the cost of ERT is extremely high thus preventing access to therapy to some patients especially in underdeveloped countries.

Our pre-clinical data in MPS VI animal models suggest that gene therapy based on a single intravascular injection of AAV2/8rhARSB can lead to a significant reduction of ARSBI gene expression and would lead to an improvement in clinical symptoms.
administration of AAV2/8 expressing ARSB has the potential to overcome each of the ERT limitations:

- we have shown that a single intravascular administration of AAV2/8 provides normal circulating levels of ARSB up to 3 years, the last time point of the observation. Data from the ongoing clinical trial with AAV2/8 in hemophilia B patients show long-term expression of FIX. Thus AAV2/8-mediated ARSB liver gene transfer may avoid the weekly infusions associated with ERT.

- we have shown significant improvement of MPS VI cats cartilage and bone storage as well as mitral valve thickness following AAV2/8 intravascular administration. It is thus possible to hypothesize that the stable levels of ARSB expression provided by gene therapy are advantageous over the peak-and-drop levels associated with rhARSB infusions.

- following the positive results observed in the AAV2/8 hemophilia B trial, it has been hypothesized that the single gene therapy administration may reduce the costs of treatment/patient from the current $400,000 (FIX prophylaxis/therapy) to $30,000 (AAV2/8 administration). For MPS VI the drop in costs would be even higher, considering that ERT costs 150,000-450,000 euro/patient/year. Thus, overall gene therapy may provide a significant improvement in the treatment of MPS VI.

The clinical trial we propose to develop within the timeframe of this project has the goal of investigating this hypothesis in line with the IRDiRC goals.

2) To exploit the data for future marketing authorization requests to develop a drug accessible to MPS VI patients

The clinical trial we plan to perform is a collaboration between academic sites, which have provided the pre-clinical evidence of the efficacy of gene therapy for MPS VI (Partner 1) or have expertise in diagnosis and management of MPS VI patients (Partners 4-6), and industry (Partners 2 and 3). Partner 2 is an US-based SME, which owns the intellectual property on AAV2/8 and has recently engaged in collaborations with several academic Partners (including the: University of Pennsylvania, University of North Carolina, University College London, TIGEM) who are leaders in pre-clinical research involving AAV2/8. These collaborations have the goal of moving AAV2/8-mediated gene transfer into the first phases of clinical development. In addition, Partner 2 has access to state-of-the-art GMP-AAV2/8 production, as well as in-house regulatory, toxicology and clinical immunology expertise, all very relevant for the clinical development of AAV2/8. Partner 3 is an EU-based SME leader in the evaluation under GLP conditions of the efficacy and safety of gene therapy products. The collaboration with these industrial partners as well as with expert CROs (for the pre-clinical toxicology studies and to monitor the clinical multicenter study) will ensure that the quality of the data produced within this trial will comply with the standards required to obtain market authorization. In addition, taking advantage of the ODD status that has been granted to Partner 1 for the development of AAV2/8-mediated gene therapy for MPS VI, the consortium is determined to seek advice from the EMA on the design of both the pre-clinical and clinical studies. This will additionally assure that the data produced in the context of this project can be further exploited to obtain market authorization for the treatment, thus potentially limiting the number and size of additional pivotal trials needed to support product approval.

3) To establish a platform for stable secretion of systemic proteins for other rare diseases:

If proven safe and effective the approach we are proposing in MPS VI patients could be extended to other LSDs without CNS involvement, which rely on ERT as the current therapeutic option, and thus suffer from the same limitations of this treatment: these include Fabry, Pompe and Gaucher diseases. More generally, AAV2/8-mediated gene transfer to liver may be applicable to several other rare diseases, including
conditions which like LSDs rely on sustained release of therapeutic proteins (i.e. alpha1-antitrypsin deficiency or hemophilias) or liver-specific conditions like urea cycle disorders, organic acidemias, familial hypercholesterolemia or inherited hyperbilirubinemias. This is also relevant to the IRDiRC goals to develop over 200 new drugs for rare diseases in the near future.

Issues to be solved for the achievement of the expected impact of the MeuSIX program

The consortium will need to contend with a number of issues to achieve its objectives. We are optimistic that with our resources and expertise we will effectively address each issue:

1) MPS VI is a rare condition and therefore patient recruitment may be challenging. Combining patient registries from three international referral centers will allow the recruitment of an appropriate number of patients for the trial.

2) As in Italy and The Netherlands (but not necessarily in Turkey), patients are treated with ERT after being diagnosed with MPS VI, we anticipate that the majority of the patients will be under ERT at the time of the enrollment in the trial. Thus, to evaluate the efficacy of gene therapy we will need to discontinue ERT, which poses ethical challenges. However, we believe that a 4-month ERT interruption should be a period long enough to determine whether the gene therapy is effective and at the same time not too long to have a significant impact on disease progression. Should gene therapy appear less effective than ERT, four months after vector infusions patients will return to ERT.

3) To our knowledge this will be only the second clinical trial using AAV2/8 in humans and will be the first for a metabolic disease. However, a group at University College London and St. Jude Children Hospital in Memphis has recently administered successfully and safely AAV2/8 vectors systemically in hemophilia B subjects.

4) A European consortium with a broader perspective of regulatory issues will be more effective in its dialogue with EMA and in delivering product to patients following market authorization.

In summary, the proposed collaborative project will provide added value for Europe because it will enable us to establish a European network for the development of gene therapies for the treatment of MPS VI and other LSDs. Only tertiary centers have the necessary resources to carry out clinical trials for rare diseases. Cross-training, technology exchange and sharing of clinical data, will allow the establishment in Europe of three clinical centers where liver-directed gene therapy can be performed. The three centers will also begin to disseminate their know-how to other European centers with the ultimate goal of making gene therapy for MPS VI and more generally other LSDs available across the European Union. European funding will be critical to develop this unique research scaffold aimed at developing novel efficient therapies for rare diseases.

List of Websites:

http://meusix.tigem.it/

Project Office Address

MeuSIX Project
Telethon Institute of Genetics and Medicine (TIGEM)

Via Campi Flegrei, 34
80078, Pozzuoli, Naples
Italy

Last update: 3 September 2018
Record number: 239227