Major Publications

None reported yet.

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A Clinical Trial of Gene Therapy for Mucopolysaccharidosis VI, a Severe Lysosomal Storage Disorder (MeuSIX)

Contract No.: 304999; EU contribution: € 5,995,041;
Total costs: € 7,877,621.40; Starting date: 01/12/2012;
Duration: 60 months

Background and Objectives

Mucopolysaccharidosis VI (MPS VI) is a rare lysosomal storage disease caused by deficient arylsulfatase B (ARSB) activity. Clinical manifestations include hydrocephalus, spinal cord compression, corneal clouding, hearing loss, coarse facial features, macroglossia, heart valve disease, cardiomyopathy, respiratory insufficiency, hepatosplenomegaly, inguinal and abdominal hernias, dwarfism/growth retardation, skeletal dysplasia, and joint stiffness. Cognitive functions are usually normal, although physical and visual impairment may limit psychomotor performance.

The Food and Drug Administration (FDA) and European Medicines Agency (EMA) have approved enzyme replacement therapy (ERT) with recombinant ARSB (galsulphase; Naglazyme; BioMarin Pharmaceutical Inc.) for the treatment of MPS VI. However, current clinical evidence shows that ERT has limitations: (1) efficacy is very limited in some tissue types, (2) weekly intravenous infusions are needed to achieve disease amelioration, and (3) elevated costs make treatment inaccessible for many patients.

Gene therapy has the potential to provide a more effective, long-term solution for MPS VI: a single administration of adeno-associated viral (AAV) vectors targeting the liver can provide a lifelong source of ARSB.

Nonclinical studies in rodent and feline models of MPS VI showed that a single administration of AAV vector serotype 8 (AAV2/8) encoding ARSB converts the liver into a factory organ, which provides enzyme for systemic distribution, and results in biochemical, pathological, skeletal, and functional improvements.[1–6] Treated MPS VI cats showed stable, within the normal range, levels of circulating ARSB activity, up to 6 years postinjection without immune responses (Ferla and Auricchio, unpublished data).

Encouraged by these ground-breaking nonclinical results, the MeuSIX consortium will conduct a multicenter phase I/II clinical trial to investigate the safety and efficacy of AAV-mediated gene therapy in patients with MPS VI. Orphan drug designation for the use of MPS VI therapeutic AAV vector has been obtained from both the EMA and the U.S. FDA.

To achieve this goal, MeuSIX has the following objectives:

1. to produce AAV2/8.TBG.hARSB vector for a gene therapy human clinical trial, according to Good Manufacturing Practice (GMP);
2. to perform nonclinical pharmacological and toxicological studies using the AAV2/8.TBG.hARSB vector;
3. to design a phase I/II clinical trial, in which the GMP 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 regulatory authorities to execute a phase I/II clinical trial; and
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.

In conclusion, MeuSIX has been designed to investigate the safety and efficacy of intravascular administrations of AAV2/8 in MPS VI patients. Positive results from this phase I/II study will support an eventual licensure of AAV2/8.TBG.hARSB for the treatment of MPS VI.

Approach and Methodology

The approach initially focuses on the production of GMP AAV2/8.TBG.hARSB vector and on investigation of the safety and efficacy of the vector in animals. In parallel, the consortium will generate all the necessary paperwork for the production and filing of the required regulatory documents for authorization to perform the trial, including request for protocol assistance to the EMA to appropriately develop strategies regarding the clinical aspects of the study; request for the authorization of the clinical sites to the

clinical use of genetically modified organisms; preparation of the Investigational Medicinal Product Dossier, Investigator's Brochure, and Clinical Trial Application.

Once approval from the competent authorities is received, the consortium will proceed with the multicenter phase I/II MPS VI clinical trial. All patients included in the trial will be appropriately characterized at the molecular and clinical levels before entering the trial. We aim at enrolling MPS VI patients from three different sites: Federico II University Hospital (Italy), Hacettepe University (Turkey), and Erasmus MC (The Netherlands). The vector will be administered at Federico II University, where also the baseline and key follow-up visits to the patients will be performed; the other two sites will perform additional visits on the patients they will contribute.

An ethics committee, the Ethics Advisory Group, will advise on all ethics aspects of patient treatment and care. The project has also developed appropriate plans to engage with patient organizations, including the Italian, French, Dutch, Turkish, Spanish, British, and American MPS societies, including a program through the project website to disseminate results and to update on the progress of the project.

In addition, an independent *ad hoc* Data and Drug Safety Monitoring Board has been established; it includes qualified clinicians, biostatisticians, and ethics experts assessing the progress, safety data, and critical efficacy endpoints of the study.

Main Findings

The main findings of MeuSIX to date are presented below:

1. Preliminary meeting with the Italian Regulatory Authority, which resulted in advice from the agency related to chemistry, manufacturing, and controls issues.
2. The partners evaluated and selected contract research organizations (CROs) and contract manufacturing organizations (CMOs) to produce and test the investigational medicinal product (IMP); they successfully delivered 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. The manufacturing process has now been established, and the IMP production is in process.
3. While the manufacturing of clinical vector proceeds, nonclinical studies, under good laboratory practice (GLP) conditions, are being performed to test toxicity, biodistribution, and expression of the vector in animals. The studies in animals are performed with material produced with a GMP-comparable process. Partners developed the related analytical methods and assays, established the standard operating procedures, and evaluated power analysis of this study. At the present time, GLP vector administration in animals has been completed, and later phases of the investigation are underway.
4. The consortium's clinical members have drafted a study protocol including the inclusion/exclusion criteria and the primary and secondary endpoints of safety and efficacy.
5. The Ethics Advisory Group is overseeing the development of guidelines for the consent forms and ensuring uniformity of operations in all participating centers.
6. The MeuSIX consortium is performing molecular and clinical characterizations of MPS VI patients that will lead to the identification of potential candidates to be included on the trial from the Erasmus MC, Hacettepe University, and Federico II University Hospitals.

Expected Outcome

The MPS VI gene therapy trial will be the first gene therapy clinical trial in which AAV2/8 is used to treat a metabolic disease. Given its innovative approach, it may serve as a pioneering framework for clinical trials for other diseases caused by lysosomal enzyme deficiency, and in general for inborn errors of liver metabolism. The results from this study may indicate that the liver, converted into a factory organ via administration of viral vectors, supports production and secretion of therapeutic proteins, in an efficient and safe manner.

Major Publications

Brands M, Güngör D, van den Hout H, et al. Pain: a prevalent feature in patients with mucopolysaccharidosis. Results of a cross-sectional national survey. J Inherit Metab Dis 2015;38: 323–331.

Brands M, Roelants J, de Krijger R, et al. Macrophage involvement in mitral valve pathology in mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome). Am J Med Genet A 2013;161:2550–2553.

Ferla R, Claudiani P, Cotugno G, et al. Similar therapeutic efficacy between a single administration of gene therapy and multiple administrations of recombinant enzyme in a mouse model of lysosomal storage disease. Hum Gene Ther 2014; 25:609–618.

Ferla R, Claudiani P, Savarese M, et al. Prevalence of anti-adeno-associated virus serotype 8 neutralizing antibodies and arylsulfatase B cross-reactive immunologic material in mucopolysaccharidosis VI patient candidates for a gene therapy trial. Hum Gene Ther 2015;26:145–152.

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Website: meusix.tigem.it

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Gene Therapy for X-Linked Chronic Granulomatous Disease (Net4CGD)

Contract No.: 305011; EU contribution: € 5,999,607;
Total costs: € 8,302,977.60; Starting date: 01/12/2012;
Duration: 48 months

Background and Objectives

The Net4CGD European consortium is focused on the clinical development of gene therapy for patients with the X-linked form of chronic granulomatous disease (X-CGD). CGD is a debilitating primary immunodeficiency affecting children and young adults.[1] This rare inherited disorder of the phagocytes is caused by absence of NADPH oxidase activity and characterized by the inability of monocytes and neutrophils to produce reactive oxygen species in response to stimuli. The X-linked recessive form is the most frequent (65% of cases) and is caused by mutations in the *CYBB* gene (encoding gp91phox). Affected patients present an elevated susceptibility to bacterial and fungal infections, as well as an excessive inflammatory response leading to granuloma formation. Invasive aspergillosis is the leading cause of death in patients with CGD. Conventional treatment of CGD consists of lifelong prophylaxis with antibiotics, antimycotics, and/or interferon gamma. To date, hematopoietic stem cell transplantation (HSCT) with a suitable donor allows permanent cure of CGD, and well-tolerated reduced intensity conditioning regimens have been established.[2] However, at least one-third of patients do not have an HLA-matched compatible donor, and HSCT in these patients is associated with high morbidity and mortality. Gene therapy based on autologous HSC correction may represent a definitive cure for CGD patients for whom allogeneic HSCT is not possible.[3]

Several members of the Net4CGD consortium have already attempted hematopoietic gene correction of X-CGD using gp91 gammaretroviral gene transfer vectors. While functional correction and clinical benefit was initially achieved, problems arose, linked to insertional mutagenesis, vector silencing, and lack of long-term engraftment.[1] A new lentiviral vector (LV) was developed to express gp91phox in myeloid cells.[4] Encouraging results obtained in preclinical studies and through the compassionate treatment of a patient in London have prompted us to test the LV in a multicenter study in several European centers expert in CGD. The trial sponsor is Genethon, a nonprofit organization dedicated to the development of gene therapy in rare diseases. An orphan drug designation was obtained for autologous hematopoietic cells genetically modified with an LV containing the human *gp91* (phox) gene (EU/3/12/957—EMA/OD/118/11).

The Net4CGD project is focused on the clinical development of this new orphan drug, which could rapidly become a new treatment option for patients with X-CGD. This will be achieved through the following objectives:

- Conducting a phase I/II trial in eligible X-CGD patients, with LV gene-modified autologous HSC to test the safety and efficacy of the technology
- Collecting high-quality data by conducting trials in expert institutions, testing the same LV product with harmonized procedures and protocols to facilitate product registration
- Assessing functional innate immune restoration obtained by hematopoietic gene therapy
- Obtaining large-scale ''omics'' and vector genome stability analysis, including bioinformatic data mining, dynamic sequence data storage, and whole integrome sequencing, to evaluate vector safety in man

Approach and Methodology

The Net4CGD consortium includes seven partner laboratories with scientific and clinical expertise, three small and medium enterprises in the biotechnological and service sector, and one partner fully dedicated to the management of the consortium. The main tasks for the consortium over the next 4 years include (1) manufacturing clinical-grade vector to support clinical studies, (2) conducting a multicenter phase I/II trial in eligible X-CGD patients, with lentiviral gene-modified autologous hematopoietic stem cells to evaluate the safety and efficacy of the product, (3) ensuring high-quality and harmonization of products and procedure to facilitate future product registration, and (4) obtaining state-of-the-art information on biological efficacy and safety in patients by assessing immune restoration and large-scale integrome data. In addition, the consortium aims to communicate effectively the results of its efforts toward the scientific community, patients, families, and the general public.

Main Findings

A European multicenter gene therapy trial for X-CGD has been successfully initiated since the start of the project, and three centers are currently open to recruit adult or pediatric patients.

A unique Investigational Medicinal Product Dossier was submitted by the sponsor to the different competent national regulatory authorities in the United Kingdom, Switzerland, and Germany. The clinical trial is registered at the European