Gene Therapy for X-linked Chronic Granulomatous Disease (CGD)

Reporting

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Final Report Summary - NET4CGD (Gene Therapy for X-linked Chronic Granulomatous Disease (CGD))

Executive Summary:
The Net4CGD project is focused on the clinical development of a new orphan drug that can rapidly become a new treatment option for patients with the X-linked form of chronic granulomatous disease (X-CGD). This severe disease without satisfactory treatment is a rare monogenic disorder of the phagocytes causing immunodeficiency with intractable infections, granulomas and hyper-inflammation. Gene therapy had already been attempted in this disease but without success. The Net4CGD consortium aimed to develop a new, safe and effective gene therapy approach using a lentiviral vector that strongly expresses the transgene in phagocytes. An orphan drug designation was obtained in the perspective of bringing a new product to patients.

Overall, the main objective of Net4CGD was to rapidly develop an effective and safe new treatment for X-CGD by demonstrating its safety and efficacy in phase I/II clinical trials conducted in several centers. The challenges for the consortium were to manufacture sufficient amounts of a clinical-grade vector, to design and conduct a safe clinical protocol, to treat X-CGD patients with this vector successfully and to generate high quality clinical and biological data that could facilitate future product registration.

The Net4CGD consortium included 7 scientific and clinical partners, 3 small and medium enterprises (SMEs) in the biotechnological and service sector and 1 partner fully dedicated to the management of the consortium.

The consortium successfully manufactured 4 lots of clinical-grade G1XCGD vector in compliance with Good Manufacturing Practice (GMP). The partners of the consortium worked together to harmonize the preparation of the investigational medicinal product (IMP) (patient's autologous cells transduced with the vector) under GMP conditions. A clinical protocol was designed for a single administration of the IMP following myeloablative conditioning of patients who do not have a compatible hematopoietic stem cell (HSC) donor.
The initial plan was to conduct a single European multi-centric gene therapy trial for X-CGD in 4 European centers. Approval was obtained in 3 European countries (UK, Germany and Switzerland) and a separate supportive study was approved in France. Yet, the accrual of X-CGD patients in the trials was slow or delayed. It was necessary to obtain an extension of the project period from 48 to 66 months to meet the clinical goals.

At the end of the project, 7 X-CGD patients have been treated including 4 patients followed for 12 months and 1 patient for 2 years. An interim analysis shows the engraftment of gene-marked cells with polyclonal and functional correction. Variable levels of gene-corrected granulocytes were obtained among patients but 3 patients show high levels of prolonged correction suggesting engraftment of corrected HSC. Chronic inflammation, which has negative effects on HSC and their transduction, could be a possible cause of the variable quality of the infused product. Gene therapy seems well tolerated. Clinical ameliorations of patients were noted after gene therapy although severe adverse events were also reported. Based on the safety data collected and advice from an independent ethics expert and Data Safety Monitoring Board (DSMB), the risk-benefit balance of the product remains favourable at this time. More information is needed and will be obtained by the continuation of clinical trials. To communicate on the project advances, a website was established (www.net4CGD.eu). The Consortium was represented at several international meetings and generated several peer-reviewed scientific publications.

Altogether, the Net4CGD consortium has reached its goals. Results have advanced our knowledge on gene therapy, for CGD and similar diseases for which myeloid correction is required. Results have also strengthened the expertise of industrial partners and service providers in the field. Importantly, Net4CGD provides a perspective for a new treatment to patients. A company, Orchard Therapeutics, will develop the product in the perspective of its registration. This would be the first potentially curative option for X-CGD patients without access to allogeneic HSC transplants, and could become one of the first Advanced Therapy Medicinal Products (ATMPs) registered in Europe.

Project Context and Objectives:
The Net4CGD project has been focused on the clinical development of a new orphan drug that can rapidly become a new treatment option for patients with the X-linked form of chronic granulomatous disease (X-CGD). This rare immunodeficiency is a severe monogenic disorder of the phagocytes causing intractable infections, granulomas and hyper-inflammation. It is the most common genetic form of CGD and is caused by mutations in the CYBB (gp91Phox) gene. There is no cure for X-CGD patients unable to receive an allogeneic hematopoietic stem cell (HSC) transplant. Several members of the consortium have already attempted gene correction of X-GCD using gammaretroviral vectors but with unsatisfactory results. In these first trials, engraftment of gene-modified autologous HSC was achieved but clinical success was compromised by gammaretroviral vector inactivation and insertion mutagenesis. The Net4CGD consortium was established to develop an advanced vector and advanced clinical protocol to treat CGD patients. Prior to the project, encouraging preclinical efficacy and safety data were obtained with a third generation lentiviral vector expressing a codon-optimized CYBB cDNA under control of a chimeric promoter strongly expressed in myeloid cells. The lentiviral vector was capable of efficient transduction of hematopoietic cells and of strong expression of the transgene in phagocytes, supporting its clinical application. An orphan drug designation was obtained from the European Medicines Agency (EMA) in the perspective of bringing this product to patients in case of successful results in clinical studies.

The main goal of the Net4CGD project was to develop the first safe and effective clinical protocol to successfully treat X-CGD patients and to generate high quality clinical and biological data that could facilitate the product registration and bring it to patients. The project aimed i) to conduct a multi-center phase I/II trial in eligible X-CGD patients, with lentiviral gene-modified autologous HSC to evaluate the safety and efficacy of the procedure, ii) to obtain state-of the art information on biological efficacy and safety in patients by assessing immune restoration and large-scale integrome data, iii) to ensure high-quality and harmonization of products and procedure to facilitate registration and to develop a commercialization plan capable of bringing a potential product to patients, and iv) In addition, the consortium aimed to communicate effectively the results of its efforts towards the scientific community, patients, families and the general public.

The Net4CGD consortium included 7 scientific partners all experts in gene therapy, including 4 partners who are clinical references on the disease; 3 small and medium enterprises (SMEs) in the biotechnological and service sector and 1 partner fully dedicated to the management of the consortium.

The total duration of the project, initially set at 4 years, was extended to 5 ½ years (66 months (M)).

The main work packages of the project (and their deliverable periods) were:
WP1. Manufacturing clinical grade of the new lentiviral vector to support clinical studies (M6-M54)
WP2. Manufacturing the cell therapy product for the trial (M12)
WP3. To conduct the first gene therapy studies in man if a rHIV vector for X-CGD (M3-M66)
WP4. Immune reconstitution and clonality analyses after gene therapy for X-CGD (M54-M66)
WP5. Dissemination and ethics (M3-M66)
WP6. Coordination (M1-M66)

The quantification of objectives included measurable deliverables and tasks such as:
1) Approvals of clinical trials at national levels: Approval for at least 1 Centre was scheduled at M12
2) Vector manufacture, quality control (QC) and release of lots for clinical use: Release of the first clinical lot was scheduled at M3 and completion of the manufacture of all needed lots was expected at M54
3) Number of patients treated in the study: 5 patients were expected to be included, treated and analysed at M66

Description of the work performed from the beginning of the project and key results achieved.

Since the beginning of the project, the consortium has successfully developed a new vector for clinical use. The G1XCGD vector is a latest-generation rHIV-derived lentiviral vector allowing strong expression of the transgene in myeloid cells and capable of correcting the GCD defect in phagocytes. The process for the large-scale manufacture of clinical-grade G1XCGD lentiviral vector has been implemented in compliance with Good Manufacturing Practice (GMP). Four lots of clinical-grade G1XCGD lentiviral vector have been manufactured for the clinical trial providing sufficient material to conduct the study considering the enrolment. Analytical procedures for the vector testing were developed and were improved, in particular to optimize vector usage and to reduce the costs of testing.

The partners of the consortium have worked together to harmonize the preparation of the investigational medicinal product (IMP) in GMP. The IMP is the patient's autologous cells transduced with the vector. Specification for cell dose, cell viability and transduction levels were established. A process was developed for fresh and cryopreserved products. A careful analysis of XCGD patient hematopoietic stem and progenitor cells showed abnormal contents and suggested that hyperinflammation observed in patients could be responsible for a relative reduction in primitive HSC content and for a relative expansion of the short-term myeloid engrafting cell population. As a consequence, a phenotypic analysis was performed on the IMP during development.

A clinical protocol was designed for a single administration of the IMP following myeloablative conditioning of patients who do not have a compatible HSC donor. An initial assessment of the risk benefit of the clinical study was made at the beginning of the project and approved by an independent ethics expert. This initial assessment concluded that there is a clear benefit to conduct the clinical trial using the G1XCGD lentiviral viral to treat patients suffering from CGD and who have no treatment option. All ethical and regulatory aspects of such a project have been taken into consideration and are included in the management of the project. An ethics specialist was included in the scientific board. Regular ethical monitoring updates have been submitted to the European Commission. A Data Safety Monitoring Board (DSMB) has been assembled by the sponsor of the trial and consulted.

As patients are very rare, coordinated action was taken to ensure maximum recruitment and information on patients. The initial plan was to conduct a European multi-centric gene therapy trial for X-CGD in 4 European centers. A unique Clinical Trial Application (CTA) was therefore prepared and submitted to the different competent national regulatory authorities. The clinical trial (registered at the EMA under EudraCT: 2012-000242-35) was first approved in the UK in January 2013. Subsequent approval was obtained in Germany and Switzerland, respectively in July 2014 and September 2014, but lack of recruitment in these sites, and necessity to focus efforts in budgetary and time constraints, led to their closing in 2017. The French site participation was delayed due to changes in local manufacturing regulation, providing an opportunity to conduct a separate supportive study. The French study was approved in December 2015 (EudraCT: 2014-002222-12).

The first X-CGD patient was treated with the G1XCGD vector in July 2013 in London. Although the patient died for reasons unrelated to the drug product, the valuable information gained together with the high transduction efficiency achieved (70%) set a note of optimism for the treatment of forthcoming patients. However, patient recruitment proved to be slower than initially planned in spite of the consortium efforts. Thus, to allow the treatment of patients in Europe with a meaningful duration of observation, we requested an extension of the project. The project was initially planned for 4 years and we were granted an extension of 18 months to be able to treat at least 5 patients with a follow-up of at least 1 year on 4 patients and a follow-up of 2 years on 1 patient.

At the end of the project (M66) 7 X-CGD patients have been treated with lentiviral gene therapy and 3 patients have been followed-up for at least 12 months. Shortly after the end of the project, in September 2018, 4 patients will have been followed for 12 months and
one patient for 2 years. An interim analysis of the study shows the engraftment of gene-marked cells with polyclonal populations of gene corrected cells and functional correction. Some variability was observed among patients, in the level of recovery of gene marked granulocytes and oxidase functional correction. A negative effect of inflammation on HSC maintenance in CGD patients was identified by several partners in the consortium (Weisser et al. 2016) and could be a possible cause of this variation. Yet persistence of gene marked cells in the longest follow up suggests that successful engraftment of gene corrected long-term-repopulating HSC can be achieved. From the preliminary results obtained so far, it appears that the treatment is well tolerated. Clinical ameliorations have been noted although severe adverse events were reported. Based on the safety data collected, the risk-benefit balance of the product remains favourable. To conclude, more information is needed and will be obtained by the continuation of patient monitoring and inclusion of additional patients in clinical trials after the end of the project.

A collaboration with teams in the US in Boston, Bethesda and Los Angeles, enabled a parallel gene therapy study with the same G1XCGD vector manufactured also by Genethon. Encouraging results obtained on the first US X-CGD patients were communicated to the Net4CGD consortium and are coherent with each other. The Europe and US clinical trials are ongoing.

Overall, such encouraging results warrant the pursuit of developing lentiviral gene therapy for X-CGD towards registration. A company, Orchard Therapeutics, expressed interest in taking over the development of the product and an agreement was reached in the perspective of registration of the product.

To communicate on the project, a website has been established and is regularly updated (www.net4CGD.eu). Public newsletters were issued. The Consortium has been represented at several international meetings in particular through the attendance of several partners at important international meetings in Gene Therapy (ESGCT, ASGCT) or Rare Diseases (IRDIRC). The Consortium has published several articles on CGD.

In conclusion, as of M66 at the end of the project, the Net4CGD consortium has achieved all of its objectives. It has developed a promising new orphan drug to treat and potentially cure X-CGD patients. This promising treatment has a concrete path to commercialization.

The first results that are emerging from the treatment of X-CGD patients by lentiviral gene therapy are encouraging in terms of feasibility, safety and efficacy. More information is needed and will be obtained by the continuation of patient monitoring and inclusion of additional patients in clinical trials. A company, Orchard Therapeutics, will enable the pursuit of a clinical study in the perspective of registration.

If positive results are confirmed in the future, a product could soon become accessible to X-CGD patients. This will be the first potentially curative option for this severe disease. The Net4CGD consortium will therefore have contributed a major advancement in the treatment of this severe condition by providing clinical and biological results, and by establishing the essential expertise in manufacturing and quality controls which is necessary for the development of advanced therapeutic medicinal products (ATMPs). The utility of this approach and the qualities of the vector provide useful information to extend towards the treatment of other forms of CGD or to other disorders for which high-level myeloid gene expression is desirable.

Project Results:

WP1 WORK PACKAGE 01: TO MANUFACTURE CLINICAL LOTS OF THE pCCL chimGP91 LV TO SUPPORT THE CLINICAL STUDIES FOR X-CGD GENE THERAPY

Introduction: An advanced lentiviral vector, called G1XCGD, was developed to improve the treatment of X-CGD patients. The G1XCGD vector is a third generation replication-defective, self-inactivating, hybrid viral particle made by core proteins derived from HIV-1 and the envelope glycoprotein of the unrelated Vescicular Stomatitis Virus (VSV). The transfer vector encodes the GP91 transgene of 1712 nucleotides corresponding to a codon-optimized cDNA sequence of the human CYBB gene also known as GP91-PHOX or NOX2 gene. The GP91 transgene is expressed under control of a heterologous synthetic promoter named chimeric promoter which was described by Partner 2 (UCL) (Santilli et al. 2011). The chimeric promoter was obtained by the fusion of two previously described promoter regions, the Cathepsin G and c-fes gene flanking regions. The GP91 transgene expression cassette also contains a post-transcriptional regulatory element called PRE4, which is a safety-enhanced version of the woodchuck post-transcriptional regulatory element (WPRE). The G1XCGD lentiviral vector leads to the integration of a 5.5 kb proviral sequence into the cellular genome.

Preclinical testing of the G1XCGD vector was conducted prior to the project by Partners 2, 3 and 1 together with other collaborators. Standardized pharmacodynamics, biosafety and biodistribution testing was conducted in mice and in human cells. High levels of
transduction and therapeutically-relevant levels of NADPH activity could be achieved in myeloid cells. Hematopoietic cells expressing functional g91 phox protein levels engrafted showing the expected biodistribution in mice. Extensive in vitro and in vivo genotoxicity studies found no evidence for adverse events related to the G1XCDG transduced cells. These results were eventually published (Brendel et al. 2018) and provided the bases for developing the G1XCGD vector in clinical trials.

In the clinical trial, the G1XCGD vector is a component used to transduce patient-autologous CD34+ cells ex vivo. The transduced cells constitute the investigational medicinal drug product (IMP) which is expected to restore hematopoiesis and therapeutic levels of gene-corrected cells in the patients.

Objectives: The objective of WP1 was to provide clinical-grade vector to all centers for the conduct of the trial.

Partners involved: WP1 was led by the R&D organization Genethon (GNT-Partner 1) with the participation of the SME Genosafe (GNS-Partner 7). The following tasks were implemented.

Tasks:

Task 1.1: Large-scale GMP adaptation of vector manufacturing processes (M1-M3)
Partners involved: GNT

Partner 1 (GNT) had extensive prior experience with the manufacture of clinical-grade lentiviral vectors (Merten et al. 2011). Based on this experience GNT was able to rapidly develop a process adapted to the G1XCGD vector. The G1XCGD vector was produced using transient transfection of four separate plasmids into 293T cells: A transfer plasmid and three separate accessory plasmids encoding respectively HIV gag pol, VSV-G and HIV Rev. The production system is based on 4 non-overlapping expression constructs and the 3' LTR of the transfer vector has been deleted in the U3 region (SIN design) in order to strongly reduce the risk of replication and the generation of Replication Competent Lentiviruses (RCLs).

The manufacturing process of G1XCGD lentiviral vectors is composed of three principal steps:

i. Quadri-transfection of 293T producer cells with transfer and accessory plasmids
ii. Harvests and purification: The culture medium in which particles are released is treated to digest residual plasmid DNA, and collected. The crude vector batch is concentrated and purified by sequential operations based on membrane and chromatography steps to remove DNA and proteins from the medium. The vector is formulated in X-Vivo20 medium, a culture medium compatible with transduction of patients' cells.
iii. Sterile 0.2µm filtration and aseptic filling of 1 mL vials is performed in a monitored biosafety cabinet and the vector is then frozen at <-70°C.

The G1XCGD vector is supplied frozen in single-use vials as a sterile, preservative-free concentrate at a minimum concentration of 2E+08 infectious genomes/milliliter (IG/mL) in X-Vivo 20 medium.

All steps of the production process are performed under Good Manufacturing Practices in an accredited pharmaceutical establishment. All steps are monitored by the quality assurance (QA) specialists. The vector is certified and released for use, by the establishment's Qualified Person.

Several engineering runs of G1XCGD vector were generated by GNT prior to GMP production. Such engineering runs enable practice and also establish the manufacturing operation procedures and the specifications of the product for its release for clinical use.

Task 1.1 was fully achieved in time. The process was fully developped to manufacture the G1XCGD lentiviral vector within 3 months of starting Net4CGD. As planned we achieved Milestone MS11 at Month 3 by releasing a first G1XCGD vector batch certificate.

Task 1.2: Development of a simplified and sensitive RCL assay (M1-M6)
Partners involved: GNS

The safety of the G1XCGD vector must be tested to comply with European Pharmacopeia EP 5.14 "Gene transfer medicinal products for human use" to demonstrate the absence of replicative particles in the preparation, prior to its use in the clinic.
The current assay for the detection of replication-competent lentiviruses (RCL) is a P24 decrease assay based on a publication by Escarpe et al. (Escarpe et al. 2003). This assay has already been used to control several batches of clinical grade rHIV vector that have been authorized for clinical trials. However, the G1XCGD vector has a high titer and the established procedures was based on a low multiplicity of infection (MOI) making this assay very labour intensive and impractical. To facilitate the implementation of an RCL assay and to reduce its cost, Partner 7 (GNS) collaborated with Partner 1 (GNT) to develop an equally-sensitive assay at reduced scale.

The approach consisted in reducing the number of indicator cells to transduce by infecting these cells at a higher MOI, all the while avoiding a cytotoxic effect or inhibition of the infection efficacy or inhibition of cell growth. Based on a statistical analysis, a pooling strategy was implemented to reduce the number of flasks to passage during the duration of the test.

Several experiments were performed to develop this new RCL pooling assay:
1. Definition of the highest MOI for the test during a RCL testing experiment,
2. Determination of the cytotoxic effect of the vector, its ability of cell growth inhibition,
3. Determination of the ability of the vector of replication inhibition
4. Setting up a « pooling » protocol: culture flasks would be pooled 2 by 2 from the 2nd cell passage, reducing this way the number of flasks to manipulate in the case of testing a batch of lentiviral vector with a very high titer. We used a pre-GMP lot of vector to test the pooling protocol showing that this batch batch was free of RCL.
5. Confirmation that the sensitivity of the initial and the modified protocol were equivalent.

Deliverable D1.12 (RCL test development) was done at Month 6. Moreover, an even more complete validation of the test (step 5) was accomplished and finalized at Month 24.

This modified RCL test has been reported in a publication (Corre et al. 2016) and has been implemented to test the G1XCGD batches used in the clinic. This RCL pooling assay is reducing by more than half the cost of the initial RCL assay while retaining the same sensitivity. Thus, Partner GNS will complete its service portfolio with a cost-effective new RCL testing option.

Task 1.3: Manufacture, QC and release of vector for clinical use (M3-M54)
Partners involved: GNT & GNS

In Task 1.1 GNT developed the large-scale processes to manufacture and control the G1XCGD vector in GMP. Then, GNT manufactured and released four lots of G1XCGD vector for clinical use.

The quality control operations included 3 types of testing.

i. Environmental testing to verify the air quality and procedures according to the GMP.

ii. Product release testing which includes microbial and viral safety, detection of replication competent lentiviruses (RCL), identity and integrity, residual impurities and biopotency testing. Some of the quality control testing is performed directly at GNT, and tests that GNT cannot perform are subcontracted to GMP-compliant and audited operators. RCL testing is performed by GNS using an improved method (Task 1.2).

iii. Stability testing. A stability plan is established by the manufacturer to determine the expiration date of the vector. The stability testing is based on infectious titer (measured as infectious genome/mL (IG/mL) and physical titer measured by P24 concentration. The container integrity is also verified. This stability study gathers data at 3, 6, 9, 12, 18, 24, 36, 48 and 60 months as specified in the stability program. The stability testing is performed by GNS. The stability of each vector lot is determined based on the results obtained in the stability plan time points and also based on results obtained with prior lots of G1XCGD vector.

GNT has manufactured 4 lots of G1XCGD vector for the Net4CGD trials. The results on these lots show that the manufacture of the vector in GMP is conform to all specifications established. All lots have been certified and released for clinical use. The manufacturing and control plans were approved by all regulatory authorities approving the clinical trials in the UK, Germany, Switzerland and France.

Based on physical and infectious titers and biopotency activity of the 4 lots which are coherent with the preceding engineering runs, it can be concluded that the production process is reproducible and robust. The stability testing plan results on the clinical units showed stability up to Month 60.

Approximately 250 mL of final product vector is manufactured for each batch of G1XCGD vector. After subtraction of product for quality control testing and archival, approximately 150-160 units of 1mL remain for clinical use and are dispatched to the centers using
GMP-compliant transport and storage at \(-70^\circ\text{C}\).

As planned initially, GNT delivered the first vector manufacture and controls by month 36 (D1.13). Considering the slow inclusion rate in the trial and perishable nature of the vector and the extension of the project period, GNT postponed the manufacture of the 4th lot by a few months and this was reported at M54. In total, four lots of G1XCGD vector were provided to conduct the clinical studies in Europe and this represents altogether, 660 vials of available clinical vector. This has been sufficient to conduct the planned studies and will support treatment of a few additional patients post project.

Future developments in vector manufacture.
As of November 2, 2016, the pharmaceutical establishment of Genethon, formerly Genethon BioProd, in which the G1XCGD vectors were produced, has become a commercial company called Yposkesi. The transition from Genethon Bioprod to Yposkesi has had no impact on the Net4CGD project deliverables and tasks, since all vector lots had already been released. Remaining stocks of vector will be kept at Yposkesi until the end of the clinical studies. In the future, the production of additional G1XCGD vector could be subcontracted to Yposkesi for additional trials in the perspective of product registration and commercialization.

In conclusion for WP1:
The consortium has successfully developed the new G1XCGD vector which is a latest-generation lentiviral vector allowing strong expression of the transgene in myeloid cells and corrects the CGD defect in phagocytes. The consortium has developed a robust process for the large-scale manufacture of the G1XCGD vector in compliance with good manufacturing practices (GMP). Analytical procedures were developed and improved. Four clinical-grade lots of G1XCGD vector were manufactured in a pharmaceutical establishment and were released conform to specifications. The cryopreserved vector is stable up to 60 months post-production. Four lots of G1XCGD vector were provided to the clinical centers and were used to treat CGD patients with encouraging results. All objectives, tasks, deliverables and milestones of WP1 were met and timelines respected.

WORK PACKAGE 02: TO MANUFACTURE THE CELL THERAPY PRODUCT FOR THE TRIAL (M1-M66)

Introduction:
The IMP for the treatment of X-CGD is an ATMPs) which will provide extended treatment options for patients with this disease. Furthermore, it will inform the utility of this approach and vector configuration for treatment of other disorders for which high-level myeloid gene expression is desirable. The vector proposed for use in this study is novel (Orphan drug designation obtained Dec 7, 2011, EMA/OD/118/11), and generation of patient-specific ATMPs with this agent is therefore innovative.

Objectives: The objective of WP2 was to harmonize cell culture and transduction processes across participating centers, to optimize the transduction of cells using GMP-grade vector and to produce ATMPs for the treatment of patients.

Partners involved: WP2 was led by UCL (Partner 2). Three manufacturing sites for ATMPs for X-CGD included: UCL (Partner 2), APHP (Partner 6) and the SME EUFETS (Partner 8) with the participation of GNT (Partner 1) providing vector and media, GSH (Partner 3) for functional assays and the SME GNS (Partner 7) for quality controls. The following tasks were implemented.

Tasks:

Task 2.1: Harmonisation of cell culture and transduction (M1-M6)
Partners involved: APHP, UCL, GSH, EUFETS & GNT

Partners APHP and UCL have established experience in preparation of GMP-grade lentiviral vector transduced haematopoietic stem cells (HSC) for trials in WAS, ALD, ADA and Beta-thalassemia. Prior to the project, Partner UCL had also treated a patient with X-CGD using the LV specified in this proposal under a ‘specials’ exemption of current ATMP legislation. This exemption allows the treatment of individual patients outside a clinical trial format and is justified by clinical need. For the Net4CGD study, we planned to recruit patients from across Europe and to manufacture transduced cells at several sites located in different countries. Three manufacturing sites are therefore established at the initiation of the project (UCL in London, APHP in Paris and EUFETS in Germany). With geographically distant manufacturing sites, harmonisation of procedures to manufacture transduced cell products is essential to the success of this multi-centre trial. Harmonisation of HSC culture conditions and transduction protocols may lead to improvements in efficiency and safety of ATMP manufacture.
The objective of Task 2.1 was to establish and to harmonize the procedures for selection, culture and transduction of CD34+ cells at the participating manufacture sites under GMP conditions.

Representatives from the manufacturing sites have met to discuss their experience with transduction of HSC and current preferred method for transduction. The protocol for CD34+ selection is equivalent at all sites. To harmonize the key steps of transduction across the sites, the most important parameters were identified such as cell density, vector concentration, timing of transduction culture periods, source of raw materials, culture containers and final product excipient. Experiments were performed at various sites to optimise these various parameters and also to evaluate closed systems (culture bags) as an alternative to open (flask) tissue culture containers. Established SOPs for selection of CD34+ cells from each site were shared with the partners and equivalency checked.

The vector, a critical component for the manufacture of the ATMP, is manufactured by GNT and is supplied to all sites. GNT also supplies the XVIVO20 medium which is used for the G1XCGD vector formulation and for the transduction of patients cells. A consortium purchasing arrangement has been set up for cytokines. The agreement to purchase and use raw materials from the same suppliers has significantly enhanced the harmonisation across sites. The use of the same time schedule i.e. selection and transduction on consecutive afternoons is also a big step in harmonisation. In fact all the key parameters of manufacture have been aligned at all sites.

An SOP was generated and is meeting all the pre-release criteria. UCL and Genethon have worked together to write and standardise a template Certificate of Analysis (CoA) that includes the release criteria. This form can be used by all the manufacturing sites to report the data to Genethon for each batch of GMP ATMP produced. This CoA will therefore aid comparison of ATMPs manufactured at different sites as the study progresses. The partners have agreed on a common production SOP and agreed common release criteria, both of which have been included in the Investigational Medicinal Product Dossier (IMPD). The harmonized protocol was used to apply for authorisation for the clinical trial. This harmonised protocol is therefore the base template for manufacturing at any site. Any further optimisation of the protocol can be employed if it is validated to GMP standard.

Comparative experiments were performed to harmonize the type of vessels used in transduction procedures and validated both bags and flasks.

The critical results of this comparison are the vector copy number (VCN) achieved per condition. The cell counts, viability and purity (CD34 %) are also measured to compare the quality of the transduced cell product achieved by the two methods.

The method for the detection of RCL in CD34+ patient cells transduced with the G1XCGD vector, before and after cryopreservation, was developed by GNS, based on previous experience.

The first ATMP was made using this harmonised method and it was of satisfactory quality. The SOP has been reviewed by the qualified person used by the UCL site in the process of releasing the ATMP made in 2013. The release criteria set were included in the IMPD submitted to the Medicines and Healthcare products Regulatory Agency (MHRA), UK and were passed.

Task 2.2: Optimisation of transduction using GMP-grade lentiviral preparations (M1-M12)

Partners involved: UCL, APHP, GNS, EUFETS & GNT

Following establishment of harmonised methodology, each of the manufacturing sites (UCL, APHP and EUFETS) have conducted trial-run transductions using clinical grade lentiviral preparations, and under GMP conditions. Whenever possible X-CGD cells were used but most of the time, mobilised CD34+ cells from normal individuals were used as cells from CGD patients are not widely available. Several types of improvements were made in the transduction protocol. Step-wise improvements were conducted around the initial protocol. In parallel, additional changes such as cryopreservation or addition of transduction additives were made to reflect the scientific and clinical knowledge acquired during the project.

Step-wise improvements were tested to consolidate the initial protocol. Each change improved the quality of the transduced cell product compared to previous protocols and included: new media formulation (the replacement of human transferrin by optiferrin a plant-derived transferrin for improved viral safety), switch to tissue culture treated flasks and the validation of protamine sulphate in comparison to retronectin which is used in other protocols. Cell yields, viability, and transduction efficiency (qPCR) were analysed for each transduction. Transduced cells were also tested for microbial contamination and for vector preparation contaminants. The final resultant procedure was used to transduce a small number of CGD patient cells and was shown to be effective in transducing these
cells and achieving good cell viability, purity, transduction efficiency and microbiological sterility. A small number of CGD patient cells were also engrafted into immunodeficient NSG-mice for similar analyses. These results were used to determine minimum specifications for each parameter (for example, >50% cell viability determined by 7AAD flow cytometric staining and/or trypan blue, and vector copy number of >0.3 VCN). These specifications are used to ‘release’ the cell product for use in patients. There was admittedly some variation in the scale-up data e.g. viability from 76-99%. This variability is likely due to different factors such as the reduced starting viability of frozen starting cells compared to other experiments using fresh cells, and variations between cells from different donors. These variations led us to set generous specifications for release of the product as we would expect to see such variability in patient cells.

Several formulation excipients were compared to determine the stability of the IMP. EUFETS following the agreed SOP, measured the transduction and stability of the product. Results showed that transduced cells formulated in 0.9 % NaCl + 2 % HSA (human serum albumin) were stable for at least 5 hours in terms of viability and hematopoietic function.

Cryopreserved cells. The APHP Paris has developed a modified protocol to improve the transduction of primitive HSC and for the cryopreservation of the product. Phenotypic and functional studies on HSC in CGD patients and in the murine model of the disease have shown that inflammation, and in particular IL-1b increased myeloid differentiation and cell cycle entry, is reducing the primitive HSC content in CGD bone marrow (Weisser et al. 2016). These observations prompted the APHP group to design a separate protocol for the collect, transduction and cryopreservation of cells in X-CGD patients, aiming to preserve the maximal amount of HSC.

Cryopreservation of the cells was found to be necessary to enable several collects of HSC to reach sufficient HSC numbers and to control the product before infusion. As the initial protocol was based on fresh cells, it was necessary to validate the change. The method to detect RCL in cryopreserved transduced CD34+ cells was validated by GNS. Comparisons between fresh and cryopreserved cell products was performed by UCL and by GNT for the amendment of the clinical protocol in the UK. The APHP team also worked to improve the quality of the mobilized HSC and their transduction as described below.

Task 2.3: Production of GMP-grade ATMPs for patients (M12-M66)

Partners involved: UCL, APHP, EUFETS & GNT

First ATMP. The UCL site was the first to recruit and to enrol a suitable patient into the trial and so was the first to manufacture an ATMP for a patient at Month 8 of the project (ahead of schedule). The establishment of SOP, optimisation and harmonisation of the procedure over the preceding months as described in deliverables D2.21 and D2.22 served to both improve both the quality of the ATMP and to train new staff in the GMP procedure for this first, and for future manufacturing runs. The ATMP was released against criteria described in the certificate of analysis (CoA) of deliverable D2.23. The SOP and CoA were validated by the QP at UCL and the release criteria which were included in the IMPD submitted to the MHRA were passed. The batch folder is the legal record of the ATMP manufacture which the UCL site is required to store for 30 years.

Cell dose is a critical parameter for a good engraftment following a CD34+ cell transplant whether it is gene therapy or traditional bone marrow transplant. The recognised minimum cell dose for success in such procedures is 1 x 106 CD34+/kg which was used in the initial CoA as a minimum cell dose specification. The cell dose achieved in the first ATMP manufactured was 15.6 x 106 CD34+/kg, which is much above this threshold. The cells were 86% viable which was also very good compared to previous data. The functional read-outs of the product were also excellent. The haematopoietic activity was tested by plating of cells in a medium that encourages growth of haematopoietic cell colonies. The product performed very well with 885 of 1000 cells plated going on to form colonies. In our other lentiviral vector trials we typically see approximately 300-800 cells forming colonies per 1000 plated thus the results for this ATMP were at the excellent end of the spectrum. The ideal vector copy number per cell is 1-2 VCN and this ATMP fell exactly in that range with a VCN of 1.46. Finally, when a small sample of the ATMP was used to test the phagocytic capacity of neutrophils derived from the ATMP the results showed that 27 ± 2 % of the cells had active function of the corrected gp91phox gene.

The CoA of the ATMP manufactured for UK01 was provided to the Program Officer during periodic reporting.

Subsequent manufacture of ATMP. Subsequently, and since the beginning of the study, a total of 9 GMP-grade ATMPs were manufactured in London and in Paris altogether using slightly different manufacturing processes from the first ATMP. Transduction protocols were improved and some products were prepared fresh and others cryopreserved according to the evolution of the clinical protocol and according to the patient conditions. All changes were communicated regularly to the Partners throughout the Net4CGD consortia meetings.
Since the beginning of the study a total of 6 GMP-grade ATMPs were manufactured at partner UCL for 4 patients treated in London. Four of these products were then infused and the patients range between 5-22 months of follow-up at the conclusion of the Net4CGD project. Two products that were manufactured but not infused contained less than the minimum 3 x 10^6/kg CD34+ cell dose which was chosen for the amended protocol using cryopreserved cells. This was due to insufficient CD34+ cells mobilisation from the patients in the starting material. The patients were recalled for a second mobilisation at which time an improved mobilisation was achieved and an ATMP with greater CD34+ cell dose was created and infused.

A total of 3 products were made at APHP in Paris for 2 patients. All three products were infused. Two products were manufactured for patient FR01 as the initial ATMP batch had insufficient CD34+ cells/kg. The first patient FR01 has engrafted and reached 18 months follow up whilst the second patient also engrafted and is at 6 months follow up. The team in Paris worked to optimize the transduction of CGD cells. They used a new additive with effects described on CXCR4 homing, expression of survivin blocking of caspase 3 and enhanced survival and anti-inflammatory effects. Whereas the inflammatory cytokine IFN-γ decreased transduction, the transduction additive compound which has anti-inflammatoryary effects, was found instead to increase transduction of X-CGD CD34+ cells significantly. This additive was used to increase transduction levels on the second French patient who was treated off-protocol. After observing > 3 month hematopoietic recovery and stable marking, the French protocol was amended to include this modification in the manufacture of the IMP. The APHP team has prepared a patent application for this modification.

EUFETS participated in the optimisation and harmonisation of the ATMP manufacturing process but due to lack of patients at the Swiss/German recruitment sites, and following a strategic decision made by the sponsor of trial, EUFETS was not required to manufacture any ATMPs for patients.

GNT supplied the G1XCGD lentiviral vector that was used for all ATMP manufactures at all sites. GNT contributed to the change control for the cryopreserved protocol in collaboration with UCL, APHP and EUFETS. GNT also performed side investigations to confirm the absence of DNA methylation induced by G1XCGD on CD34+ cells, in collaboration with DKFZ and GATC.

**WORK PACKAGE 03: TO CONDUCT THE FIRST GENE THERAPY STUDIES IN MAN OF A rHIV VECTOR FOR X-CGD (M1-M66)**

**Introduction:** X-CGD is a severe disease and there is no cure for X-CGD patients without HLA-compatible allogeneic HSC donors. Prior to the start of the project, several centers attempted gene correction of X-GCD using gammaretroviral vectors but with unsatisfactory results. In these first trials, engraftment of gene-modified autologous HSC was achieved but clinical success was compromised by gammaretroviral vector inactivation and insertion mutagenesis. The Net4CGD consortium was established to develop an advanced lentiviral vector and advanced clinical protocol to treat CGD patients.

**Objectives:**

- The objectives of WP03 are to perform a phase I/II, non randomized, multicenter, open-label study of a LV transduced CD34+ cells in Patients with X-Linked CGD in four European centers of reference for CGD expert in gene therapy, and to demonstrate the safety and the efficacy of this new vector.

**Partners involved:** GNT, UCL, GSH, UHF, UZH, APHP

**Tasks:**
Task 3.1: Regulatory and clinical preparation of approvals for a multicenter Phase I/II trial in London/Frankfurt/Zurich/Paris (M1-M66)
Partners involved: GNT, UCL, GSH, UHF, UZH, APHP

A multicentric European gene therapy trial for the X-linked form of CGD was planned in the perspective of product registration. The product tested is an advanced therapy medicinal product (ATMP) consisting of autologous CD34+ cells transduced with the G1XCGD LV. The sponsor of the trial is GNT.

The initial clinical study was planned in 4 countries, including United Kingdom (UCL), Switzerland (UZH), Germany (UHF) and France (APHP). An IMPD was prepared and submitted to the different competent national regulatory authorities and Ethics Committees of each of the consortium clinical partners. A change in the French national legislation delayed the submission in France. During this delay, a slightly different clinical study was designed in France to address key questions on product quality with however the same patients’ population (inclusion/exclusion criteria), study visit schedule and assessments/endpoints.

Therefore, two parallel clinical studies were initiated: one study in United Kingdom, Switzerland and Germany (G1XCGD.01) and one study in France (G1XCGD.02).

After the initial clinical trial approvals (CTA), several amendments were submitted in the UK and in France. These amendments were made to add investigation sites, to modify the manufacture or administration of product, to include newly identified risks.

A major change was made in the G1XCGD.01 study protocol and was approved by the MHRA in Nov 2016 (M48) to allow the cryopreservation of the IMP. This change facilitates the clinical management of the patient. This cryopreserved product now allows to confirm the entire sterility of the IMP in order to guarantee the safety profile of the administered IMP. It also allows measures of biopotency of the administered IMP and maximizes the chance of successful engraftment for the patient. In addition, the potentially toxic myeloablative conditioning with chemotherapeutic agents will only be initiated when IMP is released. This cryopreserved product allows the completion of the crucial IMP preparation, conditioning period and administration with more flexibility in the clinical management of the patient, and offering the patient highest chance of success of this therapeutic approach.

All the submissions to competent authorities in UK, France, Germany and Switzerland since the beginning of the project are listed below.

G1XCGD.01 Protocol – UK - Submissions to MHRA. Initial CTA and 7 amendments.
G1XCGD.01 Protocol – UK - Submissions to GTAC. Initial CTA and 10 amendments or updates.
G1XCGD.01 Protocol – UK - Submissions to R&D. Initial CTA and 9 amendments or updates.
G1XCGD.02 Protocol – France - Submissions to ANSM. Initial CTA and 5 amendments or notifications.
G1XCGD.02 Protocol – France - Submissions to ethical committee. as above
G1XCGD.01 Protocol – CH - Submissions to SwissMedic and ethical committee. 6 procedures.
G1XCGD.01 Protocol – Ger - Submissions to CA and EC. 4 procedures.

Task 3.2: Opening centers, Conduct of the trial and monitoring (M12-M66)
Partners involved: GNT, UCL, UHF, UZH, APHP

Since the beginning of the project, 6 clinical centers have been opened to participate in the clinical studies. The first site opened for enrolment was GOSH in London (March 2013), then Frankfurt Hospital (November 2014), Zurich Hospital (January 2015) and APHP Necker Hospital in March 2016. In the UK, in addition to UCL, the RFH and UCLH sites were opened in July 2016 to increase participation and to enable the treatment respectively of adult and teenage patients.

Since the beginning of the project, 7 X-CGD patients have been treated by gene therapy with the G1XCGD vector:
Five patients have been treated in the protocol in London and Paris between 26-Jul-2013 and 02-July-2018.
Two patients have been treated as special license / compassionate use (out of study protocol) in London and Paris respectively on 18-Jul-2016 and 23-Nov-2017.

The initial accrual of the patients in the trials has been slow. Inclusions of the first patients are spaced from one another for safety reasons but 3 years took place between the treatment of the first and of the second patient. This long interruption has 2 possible
The first reason is that this is a very rare disease requiring referrals of patients from multiple centers and countries. After opening the first center in the UK, it took between 18-24 months to open 2 additional centers that could increase recruitment in the G1XCGD protocol. The second reason is an interruption following the death of the first patient treated in London. This patient deceased about 3 months post gene therapy of a severe respiratory distress. This death was found unlikely related to the study drug and possibly related to the study procedure. The case was reviewed by the study Data Safety Monitoring Board (DSMB) committee which did not modify or interrupt the protocol. To recruit patients, the consortium made additional efforts to publicize the trial and to contact patients associations.

No patients have been treated in Switzerland and Germany. In Germany, a severely sick patient was identified and after evaluation by the DSMB, the patient was screened for the study in 2014. However, this patient could not be treated and deceased. Since then, no other patient was screened in Frankfurt. Recruitment of adult patients was hampered by common chronic organ-dysfunctions in this cohort that did not allow the application of the toxic conditioning regimen defined in the protocol. In Switzerland in Zurich, several eligible patients had been screened. However, no patients were finally included into the study, following the sponsor's decision due to a lack of resources and time constraints. Ultimately, more than 2 years after opening the sites and almost 5 years since the start of the project, both the Frankfurt and Zurich sites were closed on September 2017.

Task 3.3: Interim study report (M62-M66)
Partners involved: GNT, UCL, UHF, UZH, APHP

The scope of an interim study report is to describe the treatment of the first X-CGD patients who received lentiviral gene therapy in the Net4CGD project.

At the initiation of the Net4CGD project, a European multicentric clinical study for the gene therapy of X-CGD was planned in 4 countries: UK, Germany, Switzerland and France. The principal investigator (PI) of the European multicentric study is A. Thrasher (G1XCGD.01 protocol). However, a change in the French national legislation delayed the submission in France and provided an opportunity to assess if the quality of transduced stem cells could be optimized using a protocol supportive of the multicentric European study. The PI of the French study is S. Blanche (G1XCGD.02 protocol). Both studies are sponsored by Genethon.

The primary objectives of the two studies are to evaluate:
- (a) the safety and efficacy of biochemical, and
- (b) the functional reconstitution in progeny of engrafted cells and stability at 12 months.

The primary endpoints are:
- (a) the measure of safety by the incidence of adverse events,
- (b) the restoration and stability over time of the NADPH functioning granulocytes assessed by a DHR test (≥ 5% of expressing cells at 12 months).

The secondary objectives are to provide evidence of clinical efficacy in terms of augmented immunity, transduction levels and by engraftment kinetics and stability.

The European studies were initially planned in 2012 to include 5 patients per site. A revised objective was established in 2016 for the European Commission Net4CGD project extension, to achieve 5 treated patients before the end of the Net4CGD EC funding period (May 2018).

At the time of reporting (M66), 7 patients have been treated with the lentiviral vector G1XCGD. Five patients have been treated in the two clinical trials in London or Paris and two patients have been treated out of protocol according to special license / compassionate use regulations.

The key dates and status of treated patients are summarized below:

Five patients have been treated in the protocol:
UK1  Treated 26-Jul-2013. Patient deceased due to severe respiratory illness, unlikely related to the study drug and possibly related to the study procedure.
FR01  Treated 19-Sep-2016. Patient doing well at last follow-up visit.
UK1-05  Treated 10-Apr-2017. Patient doing well except for new liver lesions observed in March 2018 but no safety concern related to gene therapy.
UK3-01  Treated 08-Sep-2017. Patient doing well at last follow-up visit.
FR02  Treated 02-July-2018. Too soon to evaluate at time of report.

Two patients have been treated as special license / compassionate use (out of study protocol):
The patients have been followed-up in terms of efficacy and safety according to the study protocols approved by the competent authorities.

Several patients in Europe show sustained marking in myeloid cells for periods greater than 6 months, suggesting that primitive HSC were transduced, allowing long-term engraftment of gene corrected cells. Based on the review of the safety data collected during the covered period and cumulatively the risk-benefit balance of the product remains favourable.

Details on the clinical status of patients prior to, and after gene therapy, as well as results on transgene expression, vector copy number, functional restoration and safety evaluations are provided in D3.33.

In addition to the European studies, Genethon and UCL have collaborated with a US consortium to test the same lentiviral vector in X-CGD patients in a multicentric US study. So far, five X-CGD patients have been treated in the US and showed similar biological and clinical results as in Europe. The US results have been presented to the Net4CGD consortium on two occasions.

Our results show for the first time the feasibility and sustained efficacy of gene therapy in X-CGD. These results were achieved with a lentiviral vector providing superior transduction efficacy in HSC and myeloid cells than the gammaretroviral vector which was used in prior studies. This long-term engraftment without safety concerns had not been demonstrated in previous clinical trials.

Gene therapy seems to provide a rapid clinical benefit in patients. These results confirm what was observed earlier in 2011 in London in a CGD patient treated in compassionate use with the same vector in an off-study special license situation prior to the start of Net4CGD. From the preliminary results obtained so far, it appears that the treatment is well tolerated in most patients but severe adverse events were observed that were linked to the treatment procedure. Variability was observed in the level of recovery of functionally-corrected granulocytes. The origin of this variability is currently unknown. Thus, more information will be obtained by the continuation of patient monitoring and inclusion of additional patients in clinical trials.

While the clinical results are encouraging, the development of an ex-vivo gene therapy product in the context of a severe ultra-rare disease is complex. During this European project, the recruitment of patients was difficult. Before the start of the clinical trial and periodically during the 5 years of the consortium, the number of eligible patients has been reviewed and validated with the clinical sites. Additional sites were opened in the UK to try to increase recruitment rates into the studies. However, 2 participating European sites were not able to recruit eligible patients. Eventually, these two sites have been closed (in Germany and in Switzerland). Through a collaboration with the US we were able to gain information on five patients treated with the same vector. The European and US clinical trials are still ongoing.

In conclusion, the objective of this interim report was to obtain preliminary results from the first 5 treated patients and it has been successfully achieved as 7 patients were finally treated.

Task 3.4: Regulatory follow-up towards registration (M1-M66)
Partners involved: GNT

The underlying strategy of the Net4CGD project is to develop the product towards registration by conducting a two-year clinical study that would facilitate a rapid registration of the product in case of positive results.

In that strategic perspective, the following steps were taken:

- At the European level, to take advantage of the EU incentives on orphan drug development, the sponsor obtained an orphan drug designation, which ensures protocol assistance at the EMA and marketing exclusivity rights. The orphan drug application is updated regularly.

- To support the authorisation of a medicine for children by the EMA, a pediatric investigation plan was submitted in 2012 to ensure that the necessary data are obtained through studies in children.

- Separate but comparable studies were conducted in multiple centers to increase patient accrual. The list of centers opened, and all regulatory and ethical approvals are described above (Task 3.1).
The two clinical studies comply with National and European legislation and ethics (*). European guidelines for gene therapy in humans are strictly followed. All necessary official related approvals have been obtained prior to the start of the work and made available to the Governing board and European Commission (through the periodic reports). The ethical officer reviewed the periodic ethical reports submitted to the European Commission as well as this report (A2_ statement letter 2018).

(*) Study conducted in accordance with:
- The updated Declaration of Helsinki adopted by the World Medical Association,
- The ethical principles in the Charter of Fundamental Rights of the European Union, in particular Article 24 concerning the Rights of the Child,
- The directive 2001/20/EC of the European Parliament and the Council (April 2001) relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use,
- The ICH (International Conference on Harmonization) Good Clinical Practice (GCP) guidelines, and
- The national and local requirements for ethics as they apply in each of the participating countries for the approval of studies on humans.

Pharmacovigilance and study results are carefully monitored with the support of an independent and expert Data Safety Monitoring Board (DSMB). An annual Development Safety Update Report (DSUR) and Investigator Brochure (IB) were updated annually according to the regulation. Scientific advice is provided by regular discussions with investigators in Europe and in the US where a collaborative trial is also conducted, using the same vector.

An independent ethical evaluation has been provided to the consortium to review the risk benefit of the clinical study. Dr. Fuchs, an independent expert from Bonn University, Germany, has reviewed the initial assessment of the risk benefit of the clinical study and has provided a regular evaluation of the trial in relation with the study sponsor representative, Dr. Honnet. Regular Ethical reports have been provided to the Program Officer during the period of Net4CGD.

Since the beginning of the Net4CGD study, 7 patients were treated and 3 patients will be treated after the end of the consortium. All patients engrafted with gene-modified cells and ≥ 5% of marking and transgene-expressing cells are found at 6 months in all cases (with exception of a patient who died before 6 months).

The first XCGD patient treated by gene therapy in the study (UK1.01) experienced a Serious Adverse Event (SAE) which led to the patient's death on 05 November 2013 (3 months post-gene therapy). The fatal event of acute respiratory distress syndrome was not considered as related to the gene therapy and did not alter the safety profile of the Investigational Medicinal Product (IMP). The study investigator reported that the event is indicative of an idiopathic pneumonia syndrome with potential contributors including pre-existing disease, infection, late effects of busulfan conditioning and possibly delayed T cell response against pre-existing antigens so possibly related to the study procedure. An exceptional DSMB meeting was held on 09 January 2014 to discuss the case report of the deceased patient. After reviewing the safety data of the patient, the DSMB agreed with the investigator's conclusion and recommended continuation of the study with no modification (this was reported in detail in D3.35 at Month 18 of the project).

After this case, 8 cases corresponding to 8 suspected expected serious adverse reactions and 1 suspected unexpected serious adverse reaction (SUSAR), have been reported in 4 patients.

One patient experienced an immune reconstitution inflammatory syndrome (IRIS) in May 2017, assessed as unexpected and related to the IMP, and resolved at the time of the report. This same patient also experienced increased creatinine in July 2017, persistent thrombocytopenia and anemia, both in August 2017, all resolved at the time of the report and new liver lesions appeared in March 2018, still ongoing at the time of the report.

A second patient experienced granulomatous colitis in October 2017, now resolved.

A third patient was reported with symptomatic anemia in January 2017 and catheter site removal bleeding in March 2017, both events being resolved at the time of the report. To be noted that these events occurred before gene therapy administration.

A fourth patient experienced febrile neutropenia in November 2017 and recovered within a couple of days.

Based on the review of the safety data collected during the covered period and cumulatively the risk-benefit balance of the product remains favourable.

To facilitate the development of the product, the sponsor has explored the possibility for partnership with a commercial company. Since the beginning of 2018, Orchard Therapeutics has licensed the rights to the study results in the perspective of further clinical
In conclusion for WP3: The objective of WP3 have been fully achieved. Two phase I/II gene therapy studies have been initiated in UK and in France to evaluate the efficacy and safety of using LV transduced CD34+ cells to treat patients with X-Linked CGD.

In spite of the difficulty in recruiting patients, 7 patients were treated. Preliminary clinical results confirm the feasibility of the study and the safety of the lentiviral vector. Preliminary results show sustained engraftment of gene-marked cells at different levels in patients. Phagocytes have restored oxidase activity and all patients experience clinical amelioration in spite of remaining manifestations of the disease.

During the project, the manufacturing of the product was improved. A cryopreserved product can now be used to facilitate clinical management of the patient, and offering the patient highest chance of success of this therapeutic approach.

In the future, the product will be further developed by Orchard Therapeutics in the perspective of registering the product and making it available to patients with X-CGD.

All of the deliverables of WP3 have been completed fully and returned in time: D3.31 (IMPD submission); D3.32 (First patient included); D3.33 (Interim report); D3.34 (Assessment of risk/benefit of the study for ethical evaluation); D3.35 (Periodic Ethical Report-M18); D3.36 (Periodic Ethical Report-M30); D3.37 (Periodic Ethical Report -M48) and D3.38 (Periodic Ethical Report -M66).

The WP3 Milestone MS31 (All sites opened) was finalized with a slight delay at M24 instead of M15 for the first protocol, but 6 sites were eventually opened for enrollment.

WORK PACKAGE 04: IMMUNORECONSTITUTION AND CLONALITY ANALYSIS AFTER GENE THERAPY FOR X-CGD (M3-M66)

Partners involved: GNT, UCL, GSH, UHF, UZH, GNS, DKFZ, GATC, APHP

Introduction: The project aimed to conduct a multi-center phase I/II trial in eligible X-CGD patients, with lentiviral gene-modified autologous HSC to obtain state-of the art information on biological efficacy and safety in patients by assessing immune restoration and large-scale integrome data.

Work package objectives:

- To assess reconstitution of immune competence after gene therapy for CGD
- To assess superoxide production and NET formation in gene transduced cells and their derivatives
- To assess gene marking levels
- To define vector integration sites
- To analyze changes in clonal distribution with time
- To assess gene modification of hematopoietic stem cells

Partners involved: Partners GNT, UCL, GSH, UHF, UZH, GNS, DKFZ, GATC have developed testing or are involved in monitoring of patients.

Tasks:

Task 4.1: Immunological tests and related data analysis (M3-M66)
Partners involved: GSH, UCL, UZH, UHF, APHP

Neutrophils of X-CGD patients are impaired in ROS (reactive oxygen species) production leading to a defect in microbial killing. Any attempt to cure CGD by gene therapy has to reconstitute the ability of neutrophils to produce ROS and improve the killing of microorganisms. In addition, the refinement of methodologies to determine reconstitution of functional activity is also a matter of continued development within the field for an accurate assessment of the efficiency of the treatment. Two functional assays are routinely used to diagnose neutrophil phagocyte defects such as that in CGD and to monitor neutrophil function. Both tests measure the ability of neutrophils to release reactive oxygen species (ROS) via NADPH oxidase. The nitro-blue tetrazolium (NBT) assay, is a microscopic evaluation test which measures the reduction of NBT to formazan in the presence of superoxide. Formazan forms blue deposits which are visible by light microscopy. The more quantitative Dihydro-Rhodamine-123 (DHR) assay is a flow cytometry based
test. Activation of neutrophils from normal individual will result in oxidation of DHR to a fluorescent compound rhodamine-123 which can be detected by flow cytometry. This task describes the study of both immune and haematopoietic functions in CGD patients treated by gene therapy (GT).

A total of 7 patients have received myeloablative chemotherapy followed by infusion of 1-2 ATMP per patient. Only 5 have been monitored for periods greater than 3 months. The follow up period ranges from 5 to 18 months.

DHR and NBT tests are used for the diagnosis of neutrophil function. X-CGD patients have little or no superoxide production when stimulated in vitro. Increased neutrophil superoxide production following GT treatment as measured by DHR and NBT assays.

Data from DHR assays clearly shows initial recovery of neutrophil superoxide production in all patients, due to the introduction of the correct copy of the gp91phox gene. However, the kinetics of this functionality vary greatly in the patient group. Patients UK 1.03 UK 3.01 and FR04 have sustained neutrophil function >30% whereas DHR response in the other 2 patients that were followed up for > 3 months appears to decline over time.

The NBT assay can be difficult to interpret and often the clinical diagnostics lab reports a result of ‘unable to analyse’, hence the development of other neutrophil function tests was included in the WP4. Assessing fungal killing by phagocytes. Partner UZH has more recently set up an alternative method of assessing fungal killing by granulocytes (Bianchi et al. 2009). This assay focusses upon the antimicrobial activity of granulocytes by the formation of Neutrophil Extracellular Traps (NETs). These NETs consist of extracellular granulocytic DNA decorated with cellular proteins and were shown to possess antibacterial as well as antimicrobial activity. NET formation (NETosis) is triggered by ROS production and therefore absent in CGD. In previous clinical trials of GT for X-CGD, Partner UZH has shown that reconstitution of NET formation by gene therapy in a patient with CGD restored neutrophil elimination of A nidulans conidia and hyphae and correlated with rapid cure of preexisting therapy refractory invasive pulmonary aspergillosis (Bianchi et al. 2009).

By co-incubation of granulocytes with a NET stimulus e.g. PMA or the fungal spores of candida albicans and SYTOX Green reagent only extracellular DNA is stained. The SYTOX Green-derived fluorescent signal indicating NET formation can be followed by measurement of the kinetics, by fluorescence endpoint measurement or the fluorescence can be visualized by fluorescence microscopy.

Peripheral blood derived granulocytes from patient FR02 before gene therapy (GT) were stimulated with candida and analysed for NET formation. Analysis of the kinetics of NET formation, endpoint measurement as well as fluorescent microscopy analysis clearly indicated the absence of NET formation in granulocytes of the patient FR02 before GT. The absence of NET formation is most likely due to the absence of the triggering ROS signal. Correspondingly, ROS formation by glucose oxidase enzyme activity restored NETosis in patient FR02 cells prior to GT.

The killing of aspergillus fumigatus hyphes by granulocyte was tested in an in vitro assay. After 70 minutes of co-incubation of hyphae with granulocytes, granulocytes were lyzed and remaining fungal mitochondrial activity was quantified in a MTT assay. In the assay high mitochondrial activity and thereby viability results in a high absorbance at 570nm; fungal killing results in decrease in 570nm absorbance signal intensity.

As expected co-incubation of aspergillus hyphes with healthy control granulocytes resulted in a decrease fungal viability within 70 minutes whilst no signal reduction could be observed for granulocytes of patient FR02 before GT, which is in line with absent ROS production and with absent NETosis before GT.

For patient UK03-01 prior to GT, results were comparable to patient FR02. NET formation was not detectable as was true for Aspergillus killing activity.

One month after GT, patient FR01 clearly showed reconstitution of NET formation as well as of Aspergillus killing activity. PMA
stimulation of patient neutrophils induced an increase in DNA release in the NET assay indicating restoration of NET formation. Co-incubation of Aspergillus hyphae with granulocytes for 70 minutes reduced viability of remaining hyphae as revealed by a drop in OD (570nm) in the MTT assay of remaining hyphae after co-incubation.

An overall result, i.e. restoration of NET formation and restoration of Aspergillus killing directly comparable to patient FR01, was observed for patient FR04 3 months after GT.

Quantification of IL-1β release by blood monocytes. CGD is known to be associated with a hyperinflammatory phenotype. CGD monocytes of patients in non-infectious inflammatory conditions were reported to show an increased caspase I activity resulting in an increased IL-1β release (Meissner et al. 2010). Therefore partner UZH analyzed IL-1β release of blood monocytes of GT patient samples.

The cells were isolated from heparinized blood at the day of arrival to the test site, and cultured at usual cell culture conditions overnight. The next day, patient monocytes and healthy traveling control monocytes were stimulated with/without 500 ng/ml lipopolysaccharides (LPS; from E.coli; Sigma-Aldrich) for 5 hours. The level of IL-1β in cell culture supernatants was measured using an appropriate human IL-1β ELISA detection kit (Sigma-Aldrich) in full accordance to the manufacturer's instructions. This way blood monocytes of Paris patients FR01 (15 months after GT), FR02 (before GT), FR03 (before GT), FR04 (3 months after GT) and corresponding travel-control samples (HD) were analyzed.

In the absence of LPS priming, blood monocytes isolated from patients FR02 (prior to GT) and FR04 (3 months after GT) had accordingly 22 and 2.5 fold-increased levels of IL-1β release in comparison to healthy traveling controls. The release of IL-1β by unstimulated monocytes of patients FR01 and FR03 were comparable to the values of control subjects.

In all healthy control and all patient samples LPS treatment stimulated IL-1β release. In patients FR02 and FR03, not yet treated by GT, the levels of IL-1β release upon pro-inflammatory LPS stimulation were accordingly 6.5 and 4.5 fold-higher than corresponding values of healthy traveling controls exceeding levels in their corresponding healthy controls. In contrast, in patients FR01 and FR04 who underwent GT, upon LPS stimulation the levels of IL-1β were not increased over the values of healthy traveling controls in similar conditions.

Metagenome analysis to try to identify pathogens or infections in patients.
In partnership with GSH and GATC, the metagenome analysis of infections in plasma samples from 2 patients at 2 timepoints were analysed by GATC using cell free DNA (cfDNA). Although the results were not informative for the course of infections for these patients, valuable information about the analysis method was used to refine the analysis parameters.

Task 4.2: Standard molecular follow up (M16-M66)
Partners involved: UCL, GSH, UZH, APHP, GNT, GNS

Analysis of the vector copy number (VCN) confirms insertion of the correct copy of the gp91phox cDNA in the HSC cell products. As the gp91phox cDNA is inserted under the control of a myeloid cell specific chimeric promoter it is particularly important to analyse VCN in the different leukocyte subsets found in peripheral blood.

The molecular follow-up also comprises the recombinant competent lentiviral (RCL) assay of samples of both the ATMP and patient monitoring samples during the follow up period. The RCL assay is an important safety test designed to detect any opportunistic recombination with wild-type HIV elements. The RCL assays are carried out by partner GNS. All samples collected during the reportable period have been assayed and no detectable RCL has been reported in this assay.

Partner GATC has provided promoter methylation analysis based on Next Generation Sequencing of patient samples. GATC will analyse the 7 samples recently shipped at time of final report and results will be available after the end of the project.

Task 4.3: Genomics testing and related data analysis (M16-M66)
Partners involved: GNT, DKFZ, GATC

The analysis of vector integration sites is an essential component in the follow-up studies in gene therapy trials, since it allows the definition of clonal heterogeneity and contribution to gene marked hematopoiesis of individual clones during hematopoietic
reconstitution and homeostasis. In addition, clonality analyses based on linear amplification mediated (LAM) PCR assays became an integral part of vector biosafety assessment, capable of uncovering vector-mediated clonal distortions and outgrowth of individual gene-corrected cells. Coupling insertion analyses with next generation sequencing, either nr/LAM-PCR or enrichment of vector sequences followed by direct sequencing, it becomes possible to integrate in vivo clonality monitoring studies with downstream functional analyses. The identified insertion sites can be subjected to bioinformatical analyses and characterized with regard to their exact localization on chromosomes and other genomic structures.

In WP4, analysis of vector insertion sites in blood cells i.e. 6, 12 and 24 months post transplantation will provide a description of the integrome, vector genome stability and genetic disease-related integration characteristics (Gabriel et al. 2009; Giordano et al. 2007; Paruzynski et al. 2010). Partner 9 DFKZ has developed bioinformatical tools (Afzal et al. 2017) capable of managing and dynamically storing vast amount of sequence information, enabling straightforward safety assessment and functional analyses. We now know that the distribution of vector integration is not only influenced by the vector type, but also the type of target cells and tissue as well as genetic disease background may play a more determinant role than formerly anticipated. Thus, large-scale integrome analyses will provide insight whether a CGD specific landscape of LV integrations exist.

The deliverable D4.44 objective was to collect samples from the first patients receiving gene therapy at 6, 12 and 24 months following infusion and to send them to partner DFKZ for analysis. Partner DFKZ should store the samples until all time points have been collected for each patient and then perform the integration sites analysis.

DFKZ is capable of applying a variety of methods for insertion site analysis. PCR based methods LAM and nrLAM (Paruzynski et al. 2010); PCR based with Shearing Extension Primer Tag Selection linker Mediated PCR (S-EPTS/LM; adapted from (Schmidt et al. 2001)) and non-PCR based with shearing Target Enrichment Sequencing (TES; not published, method described in the WP4 at M54 interim report).

The choice of method is dependent on various factors: DNA amount available, vector copy number and project related questions. Nr/LAM-PCR and S-EPTS/LM-PCR are pure dedicated integration site analysis methods, while non-PCR based TES allows in addition to retrieve information on vector genome integrity over time and vector copy number of the same sample in parallel. Because of the low VCN (<0.1) of the first patient samples retrieved, we decided to use a PCR-based method: S-EPTS/LM-PCR which in principle is quantitatively more accurate than nr/LAM-PCR due to the lack of restriction enzyme usage.

As patients have now received treatment in the study post-infusion samples have been generated for integration site analysis, samples were shipped, for the three UK and two French CGD patients.

Three samples have been collected and shipped to partner DFKZ from patient UK 1.03. There are 3 months of remaining follow up to reach the 24m time point. Two samples from patient UK 1.05 and one from UK 3.01 have also been shipped together with the UK 1.03 samples. All remaining samples will be collected by end of September 2019. Patient UK 1.01 unfortunately did not reach the first time point thus no samples were collected.

Now that two sites have treated patients, we can see that the collection of samples for analysis is occurring as planned at the patient sites. Further time points are to be collected throughout 2018 and 2019. The existing samples were successfully transported from partner UCL, UK and partner APHP, France to the partner DFKZ in Germany. Co-ordination across international borders in this way is complex. We need to have appropriate patient consent and a system for anonymization to ensure patient identifiable information is kept private and to keep good records to maintain traceability and show evidence that all relevant laws governing the use of samples of blood & tissue from human research subjects are respected. These records must be maintained for 30 years after the completion of the clinical study. We need to utilise experienced shipping contractors to ensure integrity of the sample and timely passing through customs. The systems for transfer of samples from UK and France to the analysis partner in Germany are now in place and working well.

The inclusion of an additional time point at month 18 is a beneficial amendment that will increase the data available for analysis. Insertional mutagenesis can progress very rapidly and the course of treatment if mutagenesis were to occur would nullify the option to collect the next time point. Therefore, it is useful to have a time point between the 12 and 24 months visits in case of this potential adverse event.

The first samples from patient FR01 have been analysed. Insertion sites were analysed in unsorted peripheral blood mononuclear cells...
(mixed leukocytes), granulocytes (neutrophils, eosinophils, basophils, mast cells) and sorted leukocyte subsets CD14 (monocytes) CD15 (neutrophils) CD34 (stem cells). The analysis of the first patient’s blood samples (FR01) has generated quality data, although one has to note that the limited amount of DNA available combined with the low vector copy number did exclude large scale integration profiling to dissect disease specific characteristics of vector integration and persistence in the patients. Nevertheless, integration site information is collected and can serve for Meta-Analysis in future. Multiple sites were found. The 10 strongest clones changes between the 6 and 12 month time points. Although some strong clones were seen e.g. CD2AP accounts for 50% of sequence reads in PBMCs at 12 months, no single clone exceeded 20% frequency at more than one time point. No clones increased in frequency from 6 month to 12 months. Importantly, no frequent integrations were found in or nearby to the MECOM or LMO2 gene which are known oncogenes where the gamma retroviral vectors inserted contributing to insertional mutagenesis.

In conclusion for WP4: The monitoring results are very encouraging for the use of G1XCGD in treatment of X-CGD patients who do not have a suitable matched donor available for haematopoietic stem cell allo-transplantation (HSCT). The haematopoietic recovery is functional and sustained >18 months. The inserted gene is also detectable in several leukocyte subsets and again, the copy numbers are sustained over time, even though some variability is observed in the levels of reconstitution among patients. Three of the patients show high levels of sustained marking. The safety profile so far is good with no detected RCL or insertional site mutagenesis. The standard therapy for X-CGD, HSCT, carries considerable risks, and a high failure rate in this patient group. If the current study continues to show proof of concept for treatment of X-CGD with lentiviral gene therapy it might be possible to propose gene therapy also for patients with available donors for allo-HSCT.

All deliverables of WP4 were completed in full and returned between M54 and M66 thanks to the extension of the project: D4.41 (Hematopoietic reconstitution of the first patients); D4.42 (Reconstitution of immune functions of the first patients); D4.43 (Gene marking levels of the first patients) and D4.44 (Clonality analysis and D4.45 (Promoter methylation analysis). Milestones MS41 (reconstitution of superoxide production), MS42 (killing of Aspergillus by the gene modified cells) MS43 (Analysis of retroviral integration sites) MS 44 (Transduction of hematopoietic cells) and MS45 (long-term engraftment) have all been achieved as they generated experimental data.

WORK PACKAGE 05: DISSEMINATION & ETHICS (M1-M66)
Partners involved: GNT, UCL, GSH, APHP, DKFZ, GATC, FINOVATIS

Work package objectives:

• To forward all ethical approval documentation to the European Commission (in relation with Task 3.1)
• To manage, communicate and disseminate the knowledge, 
• To optimise the knowledge exploitation

Task 5.1: Follow-up of ethical issues (M1-M66)
Partners involved: GNT and all partners

The aim of the Net4CGD program is to develop gene therapy for children and adult with X-CGD. For this experimental treatment, it is essential to ensure that the ethical aspects of the clinical study are properly addressed. An initial assessment of the risk benefit of the clinical study was made at the beginning of the project, concluding that there is a clear benefit to conduct the clinical trial using the G1XCGD lentiviral viral to treat patients suffering from CGD and who have no treatment option. All ethical and regulatory aspects of such a project have been taken into consideration and are included in the management of the project. Ethical review and approval of the protocol by competent authorities was conducted prior to start the study. Ethical aspects are also regularly evaluated by the sponsor, investigators and advisors throughout the study. Periodic ethical reports included:

- Update on the regulatory activities of the study: submissions and approvals of the clinical trial
- Update of the clinical activities: patient’s recruitment, safety results including Data Safety Monitoring activities (Data Safety Monitoring Board= DSMB)
- Risk/benefit assessment update.
- Review of the external ethics expert.

The Periodic ethical report at M66 (D3.38) concluded that the sponsor of the trial and coordinator of the WP strictly followed the regulation and collected all the approval related to this project. The independent ethics expert concluded that there was no debate
about the quality of the product.

Task 5.2: Knowledge management (M1-M66)
Partners involved: GNT and all partners

The www.Net4cgd.eu website has been and is updated regularly. This public website provides an update on the publications issued from the consortium. Practical information on the laboratories is also provided (cell therapy facility at UCL for instance). This public website also contains an internal webspace restricted to members of the consortium.

Task 5.3: Knowledge dissemination and communication (M1-M66)
Partners involved: GNT and all partners

The knowledge dissemination and communication plan was established to communicate within the project and also to a broader interested community. This plan includes the following items.

a) A Web portal (www.net4CGD.eu) which is regularly updated by partner FINOVATIS.

b) A generic poster describing Net4CGD consortium has been published by Cordis (http://cordis.europa.eu/result/rcn/165015_en.html).

c) CGD study newsletters have been prepared for the consortium clinical teams to inform them on the advance of the project. These restricted-access CGD study newsletter were sent in March 2014 (M15), in August 2014 (M20), in January 2015 (M25) and in January 2016 (M36).

d) A public newsletter of the consortium to inform the public and patient of the trials. It contains links to a description of the project in several languages, as well as a question-and-answer document for patients and families. The Net4CGD public-access newsletters have been advertised on the Net4CGD website (www.net4CGD.eu) (latest newsletter June 2018 – see update D5.54). There were also distributed to the consortium partners.

e) Annual workshops have been organized (see update D6.62). After the first kick-off meeting in Evry in 2013, five annual meetings occurred (Stresa, Frankfurt, London, Paris, Evry). The annual workshops were used to present results, prepare for the progress review and reporting, take decisions.

f) Participation of Net4CGD partners at international conferences on their travel funds is encouraged to indirectly sponsor the event and to disseminate our activities. Several consortium members (Thrasher, Grez, Galy, Kuehlcke, Schmidt, Cavazzana) have presented at international meetings (European Society of Cell and Gene Therapy, American Society of cell and Gene Therapy, or the IrDIRC meetings) and reached broad scientific audiences.

g) Contacts have been established with CGD/PID patients’ organizations (UK CGD Association and the International PID Association). In collaboration with these associations a Questions/Answers document has been elaborated explaining the objectives and details of the clinical trial in a language accessible to patients. This document has been translated in several European languages (English, French, German) and provided to the PIs in each clinical site in order to facilitate their communication with the patients and parents and disseminated through the patients organization networks including Facebook, websites, twitter. In addition, a clinical study summary explaining the project in a few lines has been disseminated through the same channels and also through CORDIS channels in several European languages (English, French, German, Italian, Spanish, Polish).

h) Peer-reviewed publications. Several publications were published since the beginning of the project.

Task 5.4: Development of exploitation and commercialisation plans (M1-M66)
Partners involved: GNT and all partners

The Net4CGD project was initiated to facilitate the development of an orphan drug into a commercial product. Several X-CGD patients have now been treated by lentiviral gene therapy in London and Paris. The first results are encouraging, showing a good tolerance to the treatment, with rapid hematopoietic reconstitution, stable expression of the transgene and restoration of superoxide activity in
greater than 5% of cells. The longest follow-up in patients is close to 2 years. The safety profile of the vector is favourable. Thus, further development of the G1XCGD product in the perspective of registration and commercialisation seems warranted.

Registration of an ATMP is a centralized procedure at the EMA. This process is usually conducted by SMEs and involves the evaluation of clinical criteria, pharmacovigilance, risk assessment, scientific evaluation of quality data and of non-clinical safety data, as well as marketing plans. If achieved, the registration and commercialization of a G1XCGD gene therapy product would be the first potentially curative option for patients without HLA-compatible HSC donor. Such product would be very innovative as it could be potentially the first lentiviral and HSC-based gene therapy on the market.

As a strategy towards registration, Partner 1 (GNT) registered the G1XCGD gene therapy product as an orphan drug by the EMA, to benefit from the incentives such as protocol assistance at reduced charge and 10 years of market exclusivity.

Agreements were secured between partners involved in the preclinical development of the product.

During the course of the Net4CGD project, the sponsor of the clinical studies, Partner 1 (GNT), searched for partnerships able to support the efforts of registering the G1XCGD product. Genethon engaged discussions with Orchard Therapeutics, a new biotechnology SME issued from Partner 2 (UCL) and which is interested in developing the G1XCGD drug towards commercialization. Consortium partners were informed of these discussions and have all agreed to let the due diligence process take place.

In December 2017, an agreement was signed between GNT and Orchard Therapeutics to option the license the X-CGD program in a perspective of further developing a product towards registration. This was announced by a press release (annex). In February 2018, Orchard Therapeutics exercised its option and has licensed the clinical trial data from Genethon. The involvement of Orchard Therapeutics in the project is now enabling the pursuit of the clinical trial after the end of the funding from Net4CGD. This provides a very concrete perspective for the future commercialization of a product for patients.

In conclusion for WP5: The Net4CGD project has successfully achieved its objectives to disseminate its activities in professional and public circles. Successful contacts and agreements were made with Orchard Therapeutics, a dedicated SME that plans to develop the product towards a marketed gene therapy drug.

All deliverables D5.51 (collected ethical approvals), D5.52 (Net4CGD website for scientific and public communities), D5.53 (Net4CGD dissemination plan), D5.54 (annual newsletters) and D5.55 (commercial development plan) have been fully completed. With the extension of the project to M66 enabled the completion of a substantial interim report since it describes clinical results on 7 patients including 4 patients with long term (>6M) follow up.

WORK PACKAGE 06: COORDINATION (M1-M66)
Partners involved: GNT & FINOVATIS

Introduction: A workpackage has been dedicated to the administrative, scientific and financial management of the consortium as well as coordination of communication and reporting.

Work package objectives:

- To communicate all necessary information to the consortium and to the European Commission.
- To coordinate the Work Packages as outlined in the work program, e.g. defining interfaces and ensuring the hand-over of results within the project.
- To monitor and manage the quality control of deliverables and milestones.
- To assess results and progress against milestones and deliverables.
- To coordinate and supervise all legal and contractual aspects including the preparation and update of the Consortium Agreement.
- To monitor budget and expenditures.
- To ensure quality control and submission of progress reports to the European Commission.
- To submit financial statements to the European Commission.
- To coordinate exploitation and dissemination related activities (see WP5).
- To provide detailed work plan updates for the forthcoming activity period.
Partners involved: FINOVATIS

Tasks:

Task 6.1: Project coordination, Execution of the General Assembly’s decisions (M1-M66)
Partners involved: GNT, FINOVATIS and all partners

In-person (face-to-face) annual consortium meetings:

During the course of the project, 6 NET4CGD Annual Meetings were held. These meetings included all of the Partner institutions PIs as well as key personnels and collaborators of the project. The kick off meeting of the Consortium was a first scientific workshop of the consortium and was organized on 30 April 2013. This event was partly open to the public and was attended by about 100 persons. Pr Seger gave an inaugural presentation.

The first Annual consortium meeting was organized in Stresa, Italy 25-26 February 2014 and was a joint meeting with the FP7 project Cell-PID, as proposed in our DoW. The scientific program of this first annual Workshop was provided in the M18 reporting.

The second Annual consortium meeting was organized in Frankfurt, Germany on March 3, 2015. It was attended by our ethics advisor (Dr. Fuchs) and by a new member of the scientific advisory committee (Dr. Candotti). The scientific program of this second annual Workshop was provided in the M36 reporting.

The third Annual consortium meeting was organized in London at the Institute of Child Health April 18, 2016. The scientific program of this third annual Workshop is provided in the M54 reporting.

The fourth Annual workshop meeting was organized in Paris at the Imagine Institute on 21 November 2016. A guest speaker, Dr. Harry Malech from the NIH was invited. Dr. Malech is a world renowned expert in CGD and in gene therapy. He is also an investigator of the US trial and presented the first results on the US patients to the consortium. The scientific program of this fourth annual Workshop is provided in M54 reporting.

The fifth Annual consortium meeting was held in Evry at Genethon on 11-12 April 2018. As a guest speaker, we invited Dr Donald Kohn, PI of the multicentric US study which is using the same vector and is done in collaboration with Genethon and UCL. Dr. Kohn presented an update of the US trial. The UK and FR trials were also presented. In addition, the meeting included scientific presentations. The scientific program of this fith annual Workshop is provided in the M66 reporting.

At all meetings, an agenda was prepared including time for scientific presentations and time for project discussions in the form of chaired plenary sessions.

During these face-to-face meetings, the NET4CGD consortium had the opportunity to discuss the status of the work programme achievements, including a review of the current scientific and clinical findings as well as a discussion of clinical trial design and up-to-date advances.

The objectives of the annual meetings were to discuss in particular:

- Update on ongoing tasks and deliverable achievements of the project
- Discuss the status of the trials, ethics, regulatory approvals, patient recruitment, treatment and monitoring studies
- Review of CGD gene therapy activities elsewhere including to CGD gene therapy in the US.
- General coordination and management discussion with a focus on:
  - Reporting activities (periodic and final reporting) and next deadlines
  - Financial considerations throughout the project and more intensively in the context of the project extension and project closure.

During these annual meetings, General Assemblies (GENA) were scheduled as part of the “general session on management”. All institutions involved in the NET4CGD project were represented by their respective PI (or substitute) here serving as decision-making body in the project. Financial considerations were discussed with the partners. Partners were also reminded about reporting activities timelines and deadlines.
Minutes were always prepared from each of these annual meetings. All decisions of the GENA (clinical, technical and financial management) have been carried out. Minutes of the GENA meetings have been documented and circulated to all partners in due course before being finalized and reported to the Program Officer at each periodic report in the form of deliverable reports to be uploaded onto the EC portal.

Consortium conference calls:

Several conference calls were organized during the course of the project, providing interactions between partners in-between annual meetings. These conference calls served to keep updates on progress made on the project with a focus on the clinical activities of the consortium, as well as to discuss more strategical issues e.g. the preparation and submission of a budgetary amendment to the EC. These calls also served to remind partners on the dissemination activities to be performed in the context of the project with a focus on the NET4CGD website and to plan for the next NET4CGD annual meetings.

At these conference calls, all partnering institutions were represented, therefore allowing project-related information to be shared and decisions to be made by the consortium as a whole.

Task 6.2: Progress reporting – Work plan monitoring and Periodic reporting (M1-M66)
Partners involved: GNT, FINOVATIS and all partners

Over the course of the 4 contractual periods of the project, the management team at GNT and FINOVATIS dedicated much of its time to setting up all actions, tools and systems necessary to ensure the good implementation of the project. This was done in order to ease partners’ work and avoid unnecessary administrative burden.

- Preparation for the contractual reporting (financial and technical) by generating and providing templates and instructions
- Preparation for the final reporting by generating and providing templates and instructions
- Creation and distribution of the deliverable report templates
- Preparation and submission of the contractual reporting (technical and financial) including deliverable reports, list of dissemination activities and publications for each period
- Preparation for the annual meetings including preparing agendas, logistics, etc.
- Presentation of management issues (reporting, deadlines, budget, etc.) at the consortium meeting
- Preparation and distribution of management tools to partners (including templates, information on certificate on the methodology, reporting guidelines, reporting action plan, reporting timeline, second internal management report, etc.)
- Support to the coordinating institution on budget follow-up and interim payment redistribution
- Coordination of the funding reallocation strategy incl. GENA approval - Modification of the budget distribution following the extension of the duration of the project
- Coordination of the NET4CGD amendment request to the EC (no formal amendment necessary as indicated by the EC)
- Interface with the EC requesting advice, and recommendations on behalf of partners
- Support for annual reporting on administrative and financial issues
- Technical support to partners on the participant portal
- Collection and recording of dissemination activities and NET4CGD related publications and subsequent uploading onto the participant portal
- Discussions with partners regarding budget issues.

Regular telephone conferences (TC) have been implemented with the coordination team and regular telephone and email updates are circulated to partners to remind them of deliverables/milestones due date. FINOVATIS is offering support to all partners in order to facilitate communication both externally and internally, streamline reporting activities and act as link with the EC services.

Task 6.3: Work package coordination (M1-M66)
Partners involved: GNT and all WP leaders

Routine monitoring of individual (WP and partner specific) tasks is in place via WP leaders and the coordination team through the regular TC updates. A monthly check is carried out to ensure there are no significant deviation for the submission of prospective deliverables and milestones. Although the project has progressed very rapidly and very well in the initial phase (M1-M18), deviation to the original work programme, deliverables and milestones plans were anticipated because of the slow recruitment of patients which
delayed the expected analysis. TCs were then organised with the PIs and key personnel involved in the clinical trials in order to address this issue and actively work on finding solutions to better disseminate information on the trial and to increase recruitment. This has been brought to the attention of the European Commission via the scientific officer.

Over the 3rd and now 4th period, significant efforts were deployed to increase patients’ enrolment and treatment, which was successful as more patients have been recruited and treated in the UK and in France over the last period of the project. Altogether, the patient inclusion has allowed the NET4CGD consortium to meet the EC extension objectives, as detailed in the revised DoW.

Task 6.4: Annual GENA meetings (M1-M66)
Partners involved: GNT, FINOVATIS and all partners

In line with the work programme, GENA workshops have been organised either during the annual face-to-face consortium meeting or via teleconference when needed. Minutes of the GENA workshops were circulated to the partners via email shortly after each meeting / teleconferences.

In conclusion for WP6, the thorough and regular management of Net4CGD has enabled the documentation of activities and decisions taken during the project; has facilitated the adjustments made to the project to achieve the objectives; and has enabled an efficient management of funds allocated to the consortium by the EC.

All deliverables of WP6 (D6.61 “Procedures preparation and implementation coordinator’s everyday management” and D6.62 “Annual GENA workshops”) have been completed and returned on time. To be noted, the project was extended to 66 months instead of the 54 months originally planned. Additional management activities and enhanced focus of the scientific and clinical activities were necessary for this extension and were managed in WP6. The scientific officer was approached on that subject. No official amendment was to be requested as no change to the work programme was to be reported. However, a reorganisation of the internal budget was implemented within the Partners to achieve the objectives.

Potential Impact:
Net4CGD has been a successful European initiative to implement advanced gene therapy in patients, with encouraging clinical results. It has built an established European excellence in research and medicine to achieve a world-leading research programme across multiple European centres. It will have a long-lasting contribution to healthcare and tangible benefits for patients. An effective gene therapy drug that is effective in a single treatment provides benefits, both in terms of quality of life and also in terms of the socio-economic burden of genetic disease. The impacts arising from Net4CGD range from fundamental studies on advanced vector technology and haematopoietic stem cell biology, rigorous pre-clinical validation of advanced gene therapies, and application of multicentre European clinical studies. Furthermore, the knowledge gained forms a sustainable platform on which to build novel treatments based on similar technologies in a wider collection of inherited and acquired disease.

Development of new therapeutic approaches for rare disorders

The first clinical results that are emerging from the Net4CGD studies in X-CGD patients suggest that lentiviral gene therapy is well-tolerated and can provide sustained gene correction in granulocytes helping to control infections and auto-immunity. These results provide the first demonstration that GT can provide sustained clinical efficacy in this disease. In the past, several unsuccessful attempts were made at GT in X-CGD. Patients either failed to engraft or, when engrafting, they developed complications linked to insertional mutagenesis or transgene silencing, requiring very complex clinical management (Grez et al. 2011; Siler et al. 2015). Thus, lentiviral GT provides a real advance in the therapeutic management of CGD.

X-CGD is demonstrably a very rare disease (RD). The European Society for Immunodeficiencies (ESID) developed, since 1993, a main patient and research registry concerning all PID including X-CGD (http://www.esid.org/registry) lists about 1200 living patients per 100 000 EU inhabitants with phagocytic disorders in 2011 database. This represents about 8% of all PIDs. Based on several published studies we estimate the prevalence of all-type-CGD in Europe to be 0.018:10,000 inhabitants. Related to the European Community (751 070 367 inhabitants in 2018; source: https://www.populationdata.net/continents/europe/) this represents a total number of 1352 potential patients. Comparatively, the prevalence of CGD in the USA is estimated to be about 0.013:10,000 inhabitants (Winkelstein, Marino et al. 2000). As X-CGD accounts for two thirds of the cases (Roos, van Bruggen et al. 2003), thus prevalence of X-CGD can be estimated to 0.012:10,000 inhabitants in Europe which represents in total around 900 patients with this specific form of disease A survey made in 2009 discloses clinical data from 429 European patients. From these, a total of 69 lived in Germany and 84 in France with 78-89% of the patients having the X-linked form of CGD. A similar survey conducted in UK has revealed 69 patients with X-CGD.
Modern therapy for CGD includes aggressive and prolonged administration of antibiotics and prednisone (Lanini et al. 2017). Conventional treatment often consists of lifelong anti-infectious prophylaxis and/or gamma IFN to improve neutrophil and monocyte function and prevent recurring infections. IFN-gamma is an approved orphan drug for CGD. However, controversy remains about its routine administration in CGD. IFN gamma prophylaxis is offered only in selected CGD cases by most European physicians, while it is rather universally prescribed in the USA. Long-term oral prophylaxis with trimethoprim-sulfamethoxazole is an accepted practice in the management of patients with CGD. Patients have long periods of infection-free periods, which result from the prevention of infections caused by *Staphylococcus aureus*, without increasing the incidence of fungal infections. Use of ketoconazole has not been found to provide any protection against *Aspergillus* infections, while itraconazole may prove to be efficacious in this regard. Granulocyte transfusions from granulocyte colony-stimulating factor (and dexamethasone-stimulated donors) are sometimes performed in patients with disseminated invasive aspergillosis. However, granulocyte transfusions can cause numerous side effects, and it is not known whether the success of the therapy outweighs the health risks.

Hematopoietic stem cell transplantation (HSCT) may be considered as an early treatment option for CGD. Similar outcomes can be obtained with stem cells from matched related donors and matched unrelated donors. Bone marrow transplantation is only used as a last resort as this treatment has been only partially successful. Transplantations with non-perfectly matched donors/receivers are presently discouraged.

Mutations in CGD that result in 5 to 10% of normal functioning amounts of NADPH have a mild phenotype and better clinical prognosis than that of patients with complete absence of any NADPH oxidase activity. Similarly, female carriers of X-linked CGD who have only 3 to 5% oxidase-normal neutrophils rarely get serious infections suggestive of the CGD clinical phenotype. Thus, even low levels or partial correction by gene therapy of CGD was expected to provide significant clinical benefits for patients. The overarching aim of the Net4CGD project was to develop a treatment for X-linked CGD by autologous ex vivo gene therapy using a novel G1XCGD-modified HSC vector, which has demonstrated a clear potential against CGD disorders and has been granted Orphan Drug Designation (ODD) (EMA/OD/118/11; decision Dec 7, 2011) prior to the start of the project. Hence, the project focused on conducting the first gene therapy studies of a lentiviral vector for X-CGD. The results achieved so far in the interim study report suggest the feasibility, safety and efficacy of X-CGD lentiviral gene in humans which could bring significant clinical benefit over existing treatments.

More clinical information is needed and will be obtained by the continuation of patient monitoring and inclusion of additional patients in clinical trials. A company, Orchard Therapeutics, will enable the pursuit of trials in the perspective of registration.

Impact of Net4CGD on patient's quality of life and the socio-economic burden of CGD

With the potential to provide new, cost-effective and clinically effective treatment of CGD, Net4CGD is expected to enhance the social conditions of patients and their families. The financial and emotional burden of CGD patients is extremely high. CGD patients suffer from severe recurrent bacterial and fungal infections of body surfaces, e.g. the skin, the airways and the gut, as well as the draining lymph nodes. Following contiguous and haematogenously spread, a wide range of internal organs can be affected, e.g. the liver and the bones. The major clinical manifestations of CGD are therefore pyoderma, pneumonia, inflammation of the gastrointestinal tract, lymphadenitis, liver abscess and osteomyelitis. A high level of vigilance is necessary in searching for these infections. Patients with CGD routinely undergo roentgenograms of the chest and skeleton and CT scans of internal organs due to the frequency and severity of complications. Because marrow transplantation is the only known cure for CGD, vigorous supportive care along with the use of gamma IFN continues to form the foundation of treatment. With early intervention, many lesions can be managed by conservative medical means; but more serious events require hospitalization and a diagnostic/therapeutic approach applicable to any patient with severe infection. In general, antibiotic therapy for the offending organisms is indicated and purulent masses should be drained. The cause of fever and prostration cannot always be established, and empiric treatment with broad-spectrum parenteral antibiotics is often required for prolonged periods until the initial sedimentation rate approaches normal values. *Aspergillus* spp. Infection requires treatment with amphotericin B or, in refractory cases, with granulocyte transfusions. Glucocorticoids also may be useful in the treatment of patients
with antral and urethral obstruction. Despite this, management of CGD patients with antimicrobial prophylaxis, early and aggressive treatment of infections, and interferon gamma which are the cornerstones of current therapy for CGD remains fraught with significant morbidity and a finite risk of death.

The average annual treatment cost of a CGD patient who is not eligible for HSCT is 70,000 – 100,000 € per year (up to 6 weeks hospitalization, constant antibiotic treatment, interferon therapy, supportive care). The average cost of allogeneic HSCT is about 140,000 €. The treatment of X-CGD by autologous gene-modified cells developed within Net4CGD has the potential to solve the problem of donor availability and lead to a fully curative treatment for CGD. This would represent an unprecedented step towards the eradication of CGD and could lead to a significant reduction in the socio-economic burden of CGD disorders by decreasing the morbidity normally associated with allogeneic HSCT and reducing the hospitalization periods and associated costs required/treatment (e.g. for expensive antibiotics / antimycotics). Additional clinical data and socio-economic studies will be needed to further explore the impact of X-CGD gene therapy.

Critical mass of expertise of Net4CGD towards novel gene therapy technologies and clinical trials and integration with other EU projects

Net4CGD’s ambitious and innovative clinical programme required a multidisciplinary and complementary approach combining expertise in molecular biology, biochemistry, immunology, clinical trials (trial organisation, protocols, conduct, etc.) ethics and regulatory issues (implementation of consent forms, clinical authorisations, clinical dossiers, etc.) together with specific clinical expertise of CGD disorders. The Net4CGD consortium has brought together recognised partners from internationally established institutions under the guidance of Genethon which has a proven track record of managing international research projects on rare genetic disorders. This allowed the direct communication of the project’s outcomes to major patient organisations internationally and the rapid implementation of new, improved treatments for patients. Indeed, several Partners of the Net4CGD consortium are closely linked patients organisations such as the CGD Research Trust (a UK-based parent organization dedicated to the dissemination of information and treatment options for CGD patients) and the French Muscular Dystrophy Association (AFM), GIS-Maladies Rares and RIS association in France.

The Net4CGD project included, through a relevant and high quality multidisciplinary approach, top world-class experts in all above mentioned fields with a significant and adapted overall critical mass of 794.10 person months.

New research avenues and clinical standards

Lentiviral vectors have shown the unique ability of permanently engineering mammalian cells and altering their genetic characters. Lentiviruses as virus vectors present several advantages such as high effectiveness in transfection when compared to other vectors (plasmids, onco-retroviral vectors). Besides playing a central role in cellular engineering mechanisms, LV transfected cell systems offer improved yields, smaller bioreactors, and enhanced economics for biogenerics manufacturers. Lentiviral vectors are being increasingly exploited for delivering genes in a variety of anticancer strategies, including stem cell therapy. Study findings pinpoint the fact that the rapidly emerging lentiviral vector technology platform, as developed within Net4CGD, offer tremendous technological and economic advantages in the emerging gene therapy market. In addition, the progress made in CGD within Net4CGD are expected to impact other diseases that can be treated with similar approaches such as other hematopoietic or immune diseases and stem cell-based therapies. This project is expected to increase competitiveness in scientific and clinical expertise in gene therapy and manufacturing, serving to maintain excellence and contribute to employment in the biotechnology and health sector.

The phase I/II clinical trials of the Net4CGD project is performed as a collaborative multicentric effort sponsored by Genethon and with Adrian Thrasher (UCL) as coordinating investigator of the European multicentric clinical study (G1XCGD.01 protocol) and Stéphane Blanche for the French study (G1XCGD.02 protocol). Details of the trial were posted on dedicated clinical trial registries (e.g. clinicaltrials.gov”) and on specialized patient websites. The scientific advances generated during this project (natural history, study design and results, recommendations on harmonization of standards of care, etc.) are or will be published in peer-reviewed scientific journals and presented at relevant scientific conferences. Open discussions occurred within the consortium and also with national and international Health Authorities (eg EMA) on study procedures and clinically meaningful outcome measures and endpoints during the lifetime of the project. It is envisaged the clinical trial protocols to be developed during Net4CGD will become an accepted standard for future clinical trials in CGD and related immunodeficiency disorders. The various study sites across the EU should thus contribute to the harmonization of standards of care in multiple geographical regions representing different ethnicities. The harmonization of such standards of care will benefit patients regardless the outcome of the intervention trials.
The project has also helped to advance the understanding of the CGD physiopathology. Several members of the consortium have published on the negative effects of inflammation on the maintenance of primitive HSC in CGD mice (Weisser et al. 2016). Measures of HSC phenotype, functional activity and transduction were included in the clinical study to investigate these aspects in the patients. In France, a modification was made to the transduction protocol to counteract possible effects of inflammation on transduction and maintenance of HSC during IMP manufacture.

Facilitate risk assessment in gene therapy

Partner 9 and 10 delineated a CGD-specific landscape of LV integrations, and the gathered clonality and integration site data are stored in a dynamic database, allowing comprehensive downstream functional analyses for vector biosafety and risk assessment in gene therapy approaches. According to current knowledge, all serious adverse events were related to insertional mutagenesis as a root cause of initially benign, then premalignant clonal dominance, initiated by vector related gene activation. However, during the first 2000 decade, improvements and efficient technologies to identify and sequence these integration sites emerged. Thereof, linear amplification-mediated (LAM) PCR shows the most convincing approach for highly sensitive comprehensive integration site analyses to assess gene therapy agents and gene modified cells. With a combinatorial approach of LAM-PCR/mrLAM-PCR and next generation sequencing, time- and cost-efficiency of integration site sequence retrieval has been improved by several logs compared to standard Sanger sequencing technologies.

The ability to probe the integrome, to study insertion sites and to decipher the role of methylation is vital to fully elucidate the safety profile of both the lentiviral vector platform utilised in Net4CGD, and other methods of gene therapy. These techniques will assist in development of more informative predictions of insertional mutagenesis potential which may eventually replace the traditional employment of animal models for pre-clinical toxicology for ATMPs and could be further developed and exploited in other gene therapy clinical trials going forwards. This continuity of the research, and uniquely the immediate dissemination of such technologies to other partners has been enabled by the cooperation brought about by the European Commission Framework Programme.

Developing a European translational research capability in gene therapy

Europe has been world-leading in the application of cell and gene therapy and this is particularly the case for CGD. Much of this effort has been lead by individual major Centres expert in the disease, facilitated by wider collaborative efforts particularly on disease mechanisms and a fundamental knowledge (for example CONSORT, EU 6th framework, CELL-PID FP7). The adoption of a European regulatory framework for advanced medicines, and the rapidity of technological advancement demands greater dissemination of information, skills, and translational activity, to ensure maximisation of potential to patients with otherwise untreatable conditions, has facilitated the adoption of new technologies, and harmonisation of study protocols and regulatory requirements. Net4CGD has therefore contributed to the implementation of these strategies and helped to strengthen Europe's lead on gene therapy.

Inclusion of the European Biotechnology Industry

Net4CGD mobilized critical resources from the EU biotechnology industry to validate the new proposed gene transfer technology against CGD together with the implementation of manufacturing processes and scale-up capability according to the requirements of GMP. Net4CGD included among its partners the leading and most active European experts and biotechnology companies currently engaged in advanced gene and cell therapies:

- EUFETS: GMP cell transduction process
- GATC: Bioinformatics & Sequencing
- Genosafe: Quality control tests (GMP vectors) and patients follow-up

Net4CGD facilitated exploitation of their know-how to address the specific requirements and technical challenges raised by the different technological platforms, specific genes and cell types. The critical materials production and quality control (excluding specific viral testing performed by GNS) required for the vector manufacture and cell product have be subcontracted to certified companies (such as Plasmid Factory, Lonza, etc.) thus contributing to develop their competitiveness. Moreover, the lentiviral production was ensured by Genethon, a world leading expert in this field. The production capacity of Genethon has recently been transferred to an affiliate company, YposKesi which is a commercial entity.

The project has generated some important know how and industrial property which globally will advance the industrial sector of gene therapy. The vector manufacturing operations, the IMP manufacturing SOPs, manufacturing data and technology, quality control tests...
and clinical trial data all constitute very specific and highly valuable know how. This may be needed in support of a medicines licence application for the manufacture of ATMP by the same or comparable method as developed by the Net4CGD partners. Globally, this know how will strengthen the field of SMEs and industries in gene therapy, contributing to value and employment of this sector.

Market perspectives and impact of Net4CGD on SME development and competitiveness

Biotechnologies are creating a revolution in health care. Companies are moving from drug discovery and development based on medicinal chemistry to designing and developing biological drugs using information provided by genomics. In particular, gene therapy is expected to change the treatment of disease by actually curing diseases rather than treating symptoms. Incidences of congenital diseases and other genetic disorders are increasing among people worldwide. Besides, the cases of diseases, such as cancer, eye disorders, and hearing disorders are increasing in the developed countries. Pharmaceutical drugs have proved ineffective for several disorders, such as Parkinson's disease, cancer, bubble boy disease, and many more. Due to this, the researchers and companies across the globe are investing heavily on the R&D of gene therapies. As a result of efforts made by the researchers and technological innovations in the healthcare industry, gene therapy has emerged as the fastest growing market. The global gene therapy market is difficult to forecast and very divergent numbers have been suggested. This is due to the still emerging nature of this technology. However, one of the most recent evaluation estimates the global market for gene therapy to reach US$9.500 billion by the year 2018 (Report “Gene Therapy: Technologies and Markets” by Prof. K. K. Jain. Jain PharmaBiotech. Basel Switzerland. May 2014). Key factors driving market growth include not only demand for novel and efficient therapies to treat cancers and other indications with high unmet needs, but also a still unprecedented master of the manufacturing process for these new and highly complex biomedicines.

In less than a couple of decades, gene therapy has witnessed significant advances. From conceptual stage, gene therapy progressed to the current clinical trials stage in various disease conditions. Gene therapy has witnessed many clinical successes in the area of treating inherited conditions such as neurological diseases (adrenoleukodystrophy, metachromatic leukodystrophy, spinal muscular atrophy...) immune deficiencies and blood disorders (SCIDs, Wiskott Aldrich syndrome, thalassemia, Fanconi Anemia A), hemophilies, retinal diseases, but also in several acquired diseases among which mainly cancers. In 2016, the first autologous ex vivo gene therapy, Strimvelis®, was approved by the EMA. In 2018, two CAR T cell products were approved. Relieving the concerns about gene therapy efficacy and safety through iterative successes and applications in well-controlled studies, will create a paradigm shift in risk/reward perception and is expected to further open up market opportunities.

Demonstrating the improved efficacy and safety of gene therapy have been the top priorities for researchers in gene therapy and one of the primary aims of Net4CGD.

Recent market surveys showed that, of the total gene therapy trials conducted and ongoing in worldwide cancer trials account for about 65% of the total. These trials are vastly dominated (95%) by Phase-I and Phase-II, which is to reconnect with the still emerging nature of this therapeutic approach. Europe stands in a good position in this highly innovative therapeutic field; in 2017, on 2600 clinical trials conducted or still active in the world, Europe accounts for almost 600 of these. The U.K. France, Switzerland and Germany stand out as the premier European research centres, accounting for more 70% of the total number of European gene therapy trials. At present, 1380 gene therapy trials are registered at www.clinicaltrials.gov (keywords: gene therapy or gene transfer, Active studies) and 481 open gene therapy studies are mentioned to be funded by Industry.

Gene therapy has a recognized potential to effectively treat devastating inherited diseases for which there is little hope of finding a conventional cure, cancers and infectious conditions. Eventually, along with the development of personalized medicine, gene therapy will become a fundamental part of modern medicine, although the timescale for this is difficult to foresee.

The Net4CGD consortium is in an ideal position to exploit the project’s innovations benefiting from strong market pull and low barriers to entry. Orchard Therapeutics, a new biotechnology SME issued from Partner 2 (UCL) is interested in developing the G1XCGD drug towards commercialization. During the course of the project, the sponsor of the clinical studies, Partner 1 (GNT), searched for partnerships able to support the efforts of registering the G1XCGD product. Genethon engaged discussions with Orchard Therapeutics, and in December 2017, an agreement was signed between GNT and Orchard Therapeutics to option the license the X-CGD program in a perspective of further developing a product towards registration. In February 2018, Orchard Therapeutics exercised its option and has licensed the clinical trial data from Genethon. The involvement of Orchard Therapeutics in the project is now enabling the pursuit of the clinical trial after the end of the funding from Net4CGD. This provides a very concrete perspective for the future commercialization of a product for patients.

The main dissemination activities are described in section 4.2 and include peer-reviewed original publications and reviews, scientific
presentations at international professional meetings and press releases. The advancement of the project could be followed on the Net4CGD website.
List of Websites:
http://www.net4cgd.eu/