

Final Publishable Summary Report

GENEGRAFT
(Contract N° HEALTH-F2-2011-261392)



Executive Summary

Recessive Dystrophic Epidermolysis Bullosa (RDEB) is one of the most severe rare genetic skin diseases of children and adults characterized by skin blistering resulting from lack of expression of type VII collagen (C7). There is no treatment for this life-threatening disease. In March 2009, we obtained the orphan drug designation for the Medicinal product: "Skin equivalent graft genetically corrected with a *COL7A1*-encoding SIN retroviral vector", which was developed during the THERAPEUSKIN project (FP6 GA N° 511974) to treat RDEB patients by *ex vivo* gene therapy. This approach uses autologous skin grafts made of primary keratinocytes and fibroblasts genetically corrected with a safe (SIN) retroviral vector expressing C7 under the control of the EF1alpha promoter. We have demonstrated the feasibility of the approach in pre-clinical studies in mice and the absence of tumorigenicity. We now aim at preparing a first clinical trial in 3 selected RDEB patients.

This project has involved the transfer and the adaptation from the research laboratory to clinic, of the entire experimental procedure for genetic correction of RDEB skin equivalents suitable for transplantation in patients. A GMP grade viral batch with high transduction efficiencies was produced and validated. Standard operating procedures (SOPs) were established, the best Good Manufacturing Practice (GMP) culture system were selected, the graft preparation was scale-up and validated by the production center which was certified by the Spanish regulatory authorities for this gene therapy protocol. We have identified 3 adult RDEB patients from France and the UK highly suitable for the clinical trial, who show high proliferative capacities, produce full length C7 and are predicted to be tolerant to C7. Safety assessment of gene-corrected skin equivalents included determination of provirus integrity, vector copy number, integration site analysis, bio-distribution and tumorigenicity assays which were all satisfactory and showed a high safety profile. The regulatory and safety issues of these procedures related to the preparation of clinical grade genetically modified cells suitable for human transplantation have been fully addressed.

The regulatory dossier for Clinical Trial authorization has been submitted to the French agency (Agence National de Sécurité du Médicament, ANSM). Favorable opinion from the ANSM will allow to treat these three patients. This "bench to bedside" project will serve as a proof of principle of safe gene therapy for RDEB. It has the potential to bring clinical improvement to EB sufferers and to represent a significant progress in the treatment of this devastating skin disease.

I. Project context and objectives

The concept of the project is that **transplantation of autologous skin equivalents made of genetically corrected epidermal stem cells and dermal fibroblasts using a safe SIN retroviral vector has a therapeutic potential** for a severe genetic skin disease like recessive dystrophic epidermolysis bullosa (RDEB).

The objectives of the project were to complete all the steps required to achieve a **phase I/II clinical trial for gene therapy for RDEB using transplantation of the investigational medicinal product “Skin equivalent graft genetically corrected with a COL7A1-encoding SIN retroviral vector”** for which we have obtained the orphan drug designation (EU/3/09/630) in April 2009.

a. Concepts underlying the project

RDEB is one of the most severe genetic skin diseases and is caused by recessive mutations in *COL7A1* encoding type VII collagen (C7), the constituent of anchoring fibres which form essential structures for dermal-epidermal adherence (Varki et al., 2007). As a recessive disease, heterozygote carriers are not affected, and **long-term expression of a normal copy of COL7A1 in the target cells has the potential to revert the disease phenotype**. There is no treatment for RDEB, and *ex vivo* gene therapy using genetic correction of epidermal cells and fibroblasts has the potential to bring clinical benefit to RDEB patients.

Type VII collagen is produced and secreted locally by basal keratinocytes and dermal fibroblasts, and both cell types contribute to anchoring fibril formation. Therefore, stable genetic correction of keratinocytes and fibroblasts by integrating retroviral or lentiviral vectors should restore type VII collagen function. It should be emphasized that the epidermis is a constantly renewing tissue that is completely regenerated every 21 days in humans. This occurs through a differentiation process from the deep (basal) layers of the epidermis to the superficial layers (granular) and the stratum corneum leading to desquamation. Epidermal self-renewal is in fact due to epidermal stem cells which correspond only to a minority of basal cells of the interfollicular epidermis and which originate mainly from the hair bulge. Therefore, **efficient genetic correction** of epidermal cells leading to permanent expression of the transgene requires that a sufficient number of **epidermal stem cells** are genetically corrected to prevent loss of genetically corrected cell through the desquamation process, but also that the transgene is **integrated** into the genome of the target cell to insure transmission of the transgene to daughter cells. There is currently no specific cell marker for epidermal stem cells which could be reliably used to select or enrich epidermal stem cells in culture. Maintenance of these stem cells in culture requires the use of specific culture conditions involving an irradiated 3T3 feeder layer described by Rheinwald and Green (Rheinwald and Green, 1975). Culture of fibroblasts is easier to achieve and these cells are not subjected to a high loss rate *in vivo*.

Skin transplantation using autologous epithelial sheets is a robust and efficient technique which has been used for more than twenty years to treat severely burned patients (De Luca et al., 1992; Ronfard et al. 2000; Rue et al. 1993). The high proliferative capacity of epidermal stem cells is maintained under the culture conditions described above, allowing for the generation of large surfaces of autologous epithelial sheets which can be efficiently grafted onto the patient after 2-3 weeks and which provide permanent and definitive correction of the skin defect.

It has been shown that **epidermal stem cells genetically corrected with a classical retroviral vector expressing the LAMB3 cDNA under the control of the viral LTR promoter can restore dermal epidermal adherence in 3 patients with junctional EB due to LAMB3 mutations** (Mavilio et al., 2006, Bauer et al. 2017, Hirsch et al. 2017). This proof of concept for a form of EB which is clinically and

genetically distinct from RDEB, led us to develop a similar approach for genetic correction of epidermal stem cells and dermal fibroblasts to treat patients with RDEB.

However, *COL7A1*, unlike *LAMB3*, is encoded by a large 8.9 kb cDNA and the addition of a human promoter results in a transgene around 10.5 kb, which is at the upper limit of the insert capacity of retroviral vectors. In fact, we were successful in designing such a vector in the context of a previous European project (FP6 THERAPEUSKIN) which efficiently expresses type VII collagen (see below). This result supported the concept that such a large cDNA could indeed be efficiently packaged into a retroviral particle and remain functional.

Recently, genetically corrected autologous epidermal grafts were shown to be safe and effective to treat chronic wounds in 4 patients with RDEB (Siprashvili *et al.* 2016). This trial used epithelial sheets.

A skin equivalent model made of a fibrin matrix in which embedded fibroblasts support the growth of an epithelial sheet has been successfully used to treat burned patients (Llames *et al.*, 2004). We favoured this model which allows genetic correction of the epidermal and dermal component of the skin which is highly relevant in RDEB since both cell types produce C7 and the dermis contributes to severe disease complications such as fibrosis, contractures and the development of skin cancer.

An important concept on which the project relies on is **the safety** of the approach. The use of an integrating viral vector is required to provide permanent expression of the transgene, but this type of vector has been shown to expose to the risk of insertional mutagenesis. Therefore, we used a Self Inactivating (SIN) retroviral vector in which the deletion of the enhancer in the viral 3'LTR prevents the activation of proto-oncogenes located at the vicinity of the insertion site. In these vectors, a heterologous promoter should be added to drive the expression of the transgene. We used the human Elongating factor 1 alpha (EF1 α) promoter to drive *COL7A1* expression. Both the SIN nature and the human promoter contribute to the safety of the viral vector.

Another aspect of safety is the risk for the patient treated by gene therapy to develop an **immune response towards the therapeutic product**, in this case type VII collagen. Indeed, RDEB patients who do not produce any type VII collagen molecule are at high risk of developing an adverse immune reaction against the therapeutic molecule, which would lead to the loss of the graft. This concept of immune intolerance to a protein which is not recognized as being a self protein, prompted us to carefully select the first patients to be treated among patients who do express detectable full length (but mutated) type VII collagen. We thus developed specific immunological tests (ELISA and ELISPOT) assays to detect the immune tolerance of the patients towards type VII collagen (Pendaries and *et al.* *Gene.Ther.in press* 2009)

The coordinator team has developed a method to predict and monitor possible humoral and/or cellular immune response towards type VII collagen, we have developed two *in vitro* tests that measure i) the frequency of circulating antibodies raised against type VII collagen in the blood stream; ii) the frequency of reactive T-cells against type VII collagen in the blood stream (Pendaries *et al.*, 2010). These tests display high sensitivity (68%) and specificity (96%) as determined by ROC test (Pendaries *et al.*, 2010). Should the ELISA test be positive, direct immunofluorescence analysis on skin biopsy will be performed to detect binding of type VII collagen antibodies to the basement membrane zone.

In the light of these considerations, it is clear that the concept of transplantation of skin equivalents made of genetically corrected keratinocytes and fibroblasts represents a promising therapeutic option, providing that the specific issues related with the safety and the efficacy of the approach are properly addressed. We have completed all the required pre-clinical steps in a previous European project (FP6 THERAPEUSKIN) and could demonstrate the proof of principle of this approach in mice (Titeux *et al.* *Mol Ther* 2009).

b. Objectives

The subject of the project is to find a **durable and efficient** treatment for Recessive Dystrophic Epidermolysis Bullosa (RDEB), one of the most severe **orphan diseases** of the skin which affects children and adults.

The objectives of the GENEGRIFT project are to develop a **safe and efficient *ex vivo*** gene therapy approach for a permanent treatment of RDEB. The proof of principle of this approach has been demonstrated by the coordinator's team (Titeux *et al.* 2010) in a pre-clinical model in the context of a previous European project (FP6 THERAPEUSKIN). The approach uses skin equivalents made of genetically corrected keratinocytes and fibroblasts which will be grafted on a limited surface of the skin in up to 3 patients with RDEB.

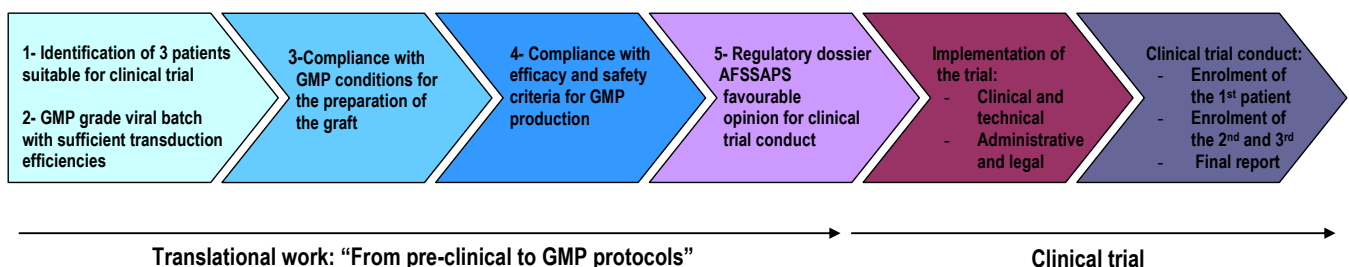
The coordinator's team obtained the **orphan drug designation** for this Medicinal product "Skin equivalent graft genetically corrected with a COL7A1-encoding SIN retroviral vector" (EU/3/09/630) in March 2009. The GENEGRIFT project aims at using this new Investigational medicinal product **to translate these pre-clinical results into a phase I/II clinical trial** to treat a small number of RDEB patients on a limited area.

Throughout this project, a specific focus has been made on the **safety** aspect of the approach, as reflected by the choice of a SIN retroviral vector and a human promoter to drive *COL7A1* expression. These features represent key assets for safety for this gene therapy trial.

THIS "BENCH TO BEDSIDE" PROJECT WHICH WILL SERVE AS A PROOF OF PRINCIPLE OF SAFE GENE THERAPY FOR RDEB. IT HAS THE POTENTIAL TO BRING CLINICAL IMPROVEMENT TO THESE YOUNG PATIENTS AND TO REPRESENT A SIGNIFICANT PROGRESS IN THE TREATMENT OF THIS DEVASTATING SKIN DISEASE. INDEED, THE SKIN LESIONS OFTEN PREDOMINATE ON THE EXTREMITIES (HANDS, FEET, ARMS, LEGS), AND RESTORING DERMAL-EPIDERMAL ADHESION IN THESE AREAS WOULD REPRESENT A MAJOR IMPROVEMENT IN THEIR SKIN CONDITION.

GENEGRIFT proposes an innovative solution by exploiting partners' high scientific and technical development in *ex vivo* gene therapy **to establish a clinical trial model for RDEB treatment (fig 1).**

Figure 1. GENEGRIFT: A new therapeutic option through gene therapy technology to improve the condition of patients suffering from RDEB



This is a **translational project**, which aims to achieve a **first phase I/II clinical trial for *ex vivo* gene therapy** in 3 RDEB patients. The therapeutic principle is the transplantation of skin equivalents genetically corrected with a safe SIN retroviral vector. This trial will address the safety of the technique and its potential to lead to clinical benefit.

This pilot clinical trial involves a limited number of patients since it is a phase 1/2 clinical trial for a rare condition which aims to demonstrate first the safety of the procedure and to a lesser extent the efficacy of the treatment. While only a small area of skin will be initially engrafted (300 cm²), the biopsy performed on the selected patient will allow the cell-banking of keratinocytes and fibroblasts. These cells can subsequently be used to produce large areas of skin equivalent. A single epidermal stem cell has enough proliferative and self-renewing capabilities to generate several square-meter of epidermis (Claudinot et al., 2005). Therefore, a 5 mm punch biopsy will be sufficient for the treatment of a patient.

This innovative strategy relies on the completion of several steps which belong to two chronological parts: the **translational work from pre-clinical to GMP protocols and the preparation of the clinical trial**.

Partners of this project have a long-standing experience in pre-clinical and clinical studies in RDEB or in gene therapy trials. Their interaction in GENEGRAFT combines specific and complementary expertise required for the **translational nature of the project**. This project fosters progress towards a safe medicinal product for RDEB and it is expected that it could be extended to other genetic skin diseases.

Genegrafft consortium

N°	Principal investigators	Beneficiary name	Acronym	Country	Expertise
1	Alain Hovnanian Matthias Titeux Sonia Gaucher	Institut National de la Santé et de la Recherche Médicale	INSERM	France	Genetics, gene and cell therapy, genetic skin diseases, clinical trials
2	Christine Bodemer	Assistance Publique – Hôpitaux de Paris	NEM-APHP	France	Paediatric dermatology
3	John McGrath Su Lwin	Kings's College London	KCL	United Kingdom	Dermatology, genetic skin diseases
4	Klaus Kuhlcke Rainer Low	EUFETS GMBH	EUFETS	Germany	GMP manufacturing of gene therapy products
5	Didier Caizergues Hélène Lafolly	GENETHON	Genethon	France	Health/Pharmaceutical Legislation
6	Patricia Joseph-Mathieu Dahlia Tsakirooulos	INSERM - TRANSFERT SA	IT	France	Project management and Technology transfer
7	John Dart Clare Robinson	DEBRA International	Debra	Austria	Dermatology, molecular Dermatology
9	Marcela Del Rio Fernando Larcher	Universidad Carlos III de Madrid	UC3M	Spain	Cell therapy, Gene therapy, Clinical trial
10	Gustavo Melen Manuel Ramirez Orellana	Fundación para la Investigación Biomédica del Hospital Niño Jesús	FIBHNI	Spain	GMP manufacturing for cell and gene therapy

II. A description of the main S&T results

We proposed the following innovative approach comprising 7 synergistic work packages:

WP1: PATIENT RECRUITMENT AND SELECTION

This WP aims at identifying 3 adult patients with RDEB from two large international cohorts of patients, with optimal clinical and biological features for a first clinical trial **EBGEN STUDY** ([clinical.trial.gov](https://clinicaltrials.gov))

The criteria **for patient pre-selection** have been refined to optimize the safety and feasibility of the phase I/II clinical trial. They include clinical, molecular, biochemical, immunological and general criteria and also take into account the nature of the anesthetic and surgical procedures. From a pre-selection of 70 patients from the UK and from France presenting with moderate or severe RDEB, 30 adult patients have been **enrolled in the EBGen study**. The consortium has agreed that adult patients only should be proposed for the EBgraft trial, given the total surface of chronic wounds to be treated (6 grafts of 50 cm² each, *i.e.* 300 cm² per patient) and the anesthetic procedure chosen (loco-regional or neuroleptanalgesia). These 30 patients have been short-listed according to their suitability for the clinical trial taking into account their age, clinical presentation, the location, number and extend of their chronic wounds and blistered areas, as well as their immune-reactivity towards wild-type type VII collagen. Of these patients, 6 patients with optimal clinical and biological features were identified as good to very good candidates. Keratinocyte proliferative capacities of the 3 best candidates were high confirming that they were highly suitable for the gene therapy trial. In conclusion, we have identified **3 patients who are the best candidates for the gene-therapy trial** and additional 3 patients who are also very good candidates and will serve as a back-up to the “best candidate” group.

Assessment of the immune response towards type VII collagen: To predict a possible immune reaction towards type VII collagen, we had previously set up highly sensitive and specific ELISA and ELISPOT assays (Pendaries *et al.*, 2010). These assays have now been optimized and we have modified the purification process to enhance the yield of type VII collagen recovery and to shorten the procedure. The yield has been improved by 20 fold and the duration of the process has been reduced from 2 days to 8 hours. These substantial progresses made in type VII collagen production and purification have been essential to ensure a better availability and lower costs for ELISA and ELISPOT testing. They have allowed us to perform these assays for the majority of the 30 RDEB patients enrolled in EBGen, including the 6 best candidates. These assays will also be essential during the one-year follow up period post-grafting to assess B and T-cell reactivity towards wild-type type VII collagen in the 3 patients who will be grafted.

WP2: CLINICAL GRADE VIRAL BATCHES AND VALIDATION

Development of an optimized γ -retroviral SIN-vector expressing type VII collagen (*COL7A1*) cDNA (transgene) and allowing efficient transduction and expression of type VII collagen in RDEB patient fibroblasts and keratinocytes was essential for the feasibility of the project. The therapeutic vector had to be produced in a packaging cell able to pseudotype the vector with an amphotropic envelope. A suitable SIN-vector was characterized by EUFETS, termed pCMS-EF1.COL7A1.SIN1 (E890) that was able to transfer *COL7A1* cDNA with very high efficiency into primary target cells. This therapeutic vector was integrated into a plasmid-based exchange construct that was used to target the viral genome into a retroviral packaging cell. This packaging cell was used to generate test batches of the therapeutic vector and to optimize the production process.

In parallel, we have developed a **new functional titration method** based on FACS detection of type VII collagen expression that allows for rapid and accurate quantification of transduced cells. This protocol was used to perform functional titration of the viral supernatants (pilot runs approx 1.5 liter) and to precisely measure the level of transduction on primary keratinocytes and fibroblasts. We then tested the effect of different sequence modifications in the pCMS backbone to improve the viral titres of the supernatant.

Next, a new packaging cell line was developed which achieved the production of the SIN *COL7A1* vectors with high titres ($2 \cdot 10^6$ ip/ml up to $4 \cdot 10^6$ ip/ml) allowing for the use of non-concentrated and raw supernatants for further clinical use.

Two batches of raw (non-purified) viral supernatants were used to transduce primary RDEB keratinocytes and fibroblasts with a high level of efficiency, which validated the producer clone. PSB-cells were expanded to a **master cell bank** (MCB) that was tested for safety (advantageous virus, RCR, etc.) and was **fully characterized and certified**.

The **certified master cell bank** was used to produce a **clinical grade retroviral vector batch**, which has subsequently been tested, **certified and released in March 2015 and is ready for use in the clinical trial**.

WP3. TRANSFER AND ADAPTATION OF THE KNOWHOW AND PROTOCOLS FROM RESEARCH TO GMP STANDARDS

The aim of this WP was to establish Standard Operating Procedures for GMP protocols for cell culture, efficient transduction of primary RDEB keratinocytes and fibroblasts and large scale skin equivalent production.

Beneficiary 10 (FUNDACION PARA LA INVESTIGACION BIOMEDICA HOSPITAL INFANTIL UNIVERSITARIO NINO JESUS – FIBHNI) joined the consortium in June 2015 and is an academic GMP certified gene and cell therapy laboratory based in Madrid. Its role to produce the Advanced Therapy Medicine under GMP conditions for the clinical trial to be performed in Paris was crucial. FIBHNI has selected the best GMP-grade reagents for keratinocyte and fibroblast culture from skin biopsies, for their genetic correction and for their use to generate skin equivalents. All these GMP grade raw material have been approved by the Spanish Agency for GMP-manufacturing processes. FIBHNI has also elaborated Standard Operating Procedures (SOPS) from biopsy processing and establishment of primary cultures to skin equivalent (sheet) assembly, conditioning and packaging. As part of the manufacturing process validation, three transfer batches were successfully generated under GMP conditions from three different RDEB patients. A cell bank of transduced and non-transduced keratinocytes and fibroblasts from each these three RDEB patients was made under GMP conditions in GMP facilities. Genetically corrected skin equivalents were generated. The process of the preparation of genetically corrected skin equivalents has been agreed by the Spanish Agency.

WP4. TOXICOLOGY AND SAFETY STUDIES

This WP aimed at demonstrating the safety of the approach. Safety assessment included demonstration of absence of tumorigenicity of the approach during preclinical development and the validation of quality control tests. These tests included the demonstration of the provirus integrity and the integration site analysis. Further tests to monitor the graft and the patient performed during the clinical trial will include the quality control of the graft prior to the grafting (provirus integrity, detection of RCR and other infectious agents), the surveillance of the immune response towards type VII collagen after the grafting and the establishment of the integration site pattern prior to grafting and after 6 months of follow-up.

Assessment of provirus integrity: The study of *COL7A1* rearrangements was required to demonstrate the safety of the approach and for the quality control of transduced cells prior to grafting the skin equivalent onto patients. Preliminary observations led to the conclusion that rearrangements occurred during the reverse transcription step as a result of template switching activity of the viral reverse transcriptase. Southern-blot and Western-blot analyses were used to characterize integrated *COL7A1* provirus rearrangements. The presence of *COL7A1* rearrangements could be detected at the genomic and/or protein levels, although abnormal bands at the protein level were difficult to distinguish from physiological proteolytic degradation products. For this reason, we concluded that provirus rearrangements had to be investigated at the DNA level. In addition, to estimate the frequency of these events and to try to get insights into the mechanism involved, we have set up a large scale experiment to isolate a significant number of rearrangement events from molecular analysis of isolated transduced clones. Our results confirmed that provirus rearrangements occurred after the infection of target cells. Analyses of the breakpoint sequences failed to reveal any rearrangement hotspot. We could estimate the frequency of genomic rearrangement of the transgene after transduction and showed that truncated proteins were detectable in a minority of clones. Our results were consistent with a mechanism involving the reverse-transcription step. They have led us to propose a detection method based on Southern-blot analysis of transduced bulked cell populations.

Bridging studies: In order to document the safety with the new vector that will be used in the clinic, we have designed protocols for bridging studies in mice including tumorigenicity, monitoring of the graft and biodistribution studies. The protocols and the strategy cover the gap between the first generation vector and the vector optimized during the first two periods of the project by Eufets and INSERM.

Following the ANSM (Agence Nationale de Sécurité du Médicament) scientific advice in December 2015, we have designed and carried out two bridging toxicology studies, and have organized proviral integration site analysis on two validation runs. Studies were carried out following the principles of GLP with defined SOPs to ensure sound scientific data.

The subcutaneous tumorigenicity study showed that nude mice developed no tumour, were healthy with normal weight curves 80 days after subcutaneous injection of 2.10⁶ of genetically engineered RDEB keratinocytes and fibroblasts.

The monitoring of mice grafted with skin equivalents made of transduced cells showed that they did not present with a higher rate of morbidity compared to control groups, showed no sign of illness, no weight loss and no tumor formation. Biodistribution study based on genomic DNA analysis extracted from collected organs is ongoing, but preliminary data show no dissemination of transduced cells. Proviral integration site analysis was performed on transfer batches of transduced cells as advised by the ANSM. A subcontractor has performed integration site analysis with linear amplification mediated PCR (LAM-PCR) with two enzymes and non-restrictive LAM-PCR (nrLAM-PCR) in duplicate. NGS and bioinformatics analysis of the samples were performed on cultured fibroblasts and keratinocytes from two RDEB patients and from the same cells following transduction with the SIN *COL7A1* retroviral vector. Overall, the analyses of SIN-RV transduced fibroblasts and keratinocytes obtained from two subjects showed a highly polyclonal vector integration profile with no preferred integration in/nearby genes previously involved in serious adverse event in gene therapy.

WP5: REGULATORY AND ETHICAL ISSUES

This WP aimed to fulfil all the regulatory and ethical requirements to obtain clinical trial authorisation. The clinical and non-clinical regulatory documents have been completed and clinical trial authorization has been requested from the ANSM. Beneficiary 5 (GENETHON) has organized and implemented the preparation of the dossier for the Clinical Trial Authorization (CTA) including the Investigational Medicinal Product Dossier (IMPD).

Chemical and pharmaceutical quality documentation of the IMPD:

The documentation regarding the viral vector part has been finalized. The documentation on pharmacological development including the manufacturing process, the production process and validation, the container closure system and the stability of the IMP have been finalized. The quality control has been completed.

Non-clinical evaluation (pre-clinical studies) of the IMPD included *in vitro* and *in vivo* proof of concept studies which have been successfully completed, and safety studies for which two complementary bridging toxicology studies have been performed and analysed (see WP5). Integration site analysis has been achieved and showed good safety profiles (see WP5).

All clinical documents of the IMPD (Clinical protocol, informed consent forms, the Case report form and the Investigator's Brochure) have been finalized (see WP6) and the Clinical Trial Application was submitted to the ANSM on November 24th 2017.

Additional requirements have been fulfilled, including updating of the annual report of the Orphan Drug Designation Dossier; a scientific advice with the ANSM was held in December 2015; the GMOs agreements have been obtained from the Haut Comité des Biotechnologies; FIBHNJ was GMP certified by the Spanish Agency; authorization for skin biopsy exportation was obtained from the French Ministry of Health.

WP6: IMPLEMENTATION OF THE CLINICAL TRIAL

This WP aimed at obtaining the accreditation of the Gene Therapy Centre to conduct the trial and to implement the clinical trial according to the authorized protocol leading to the opening of the site.

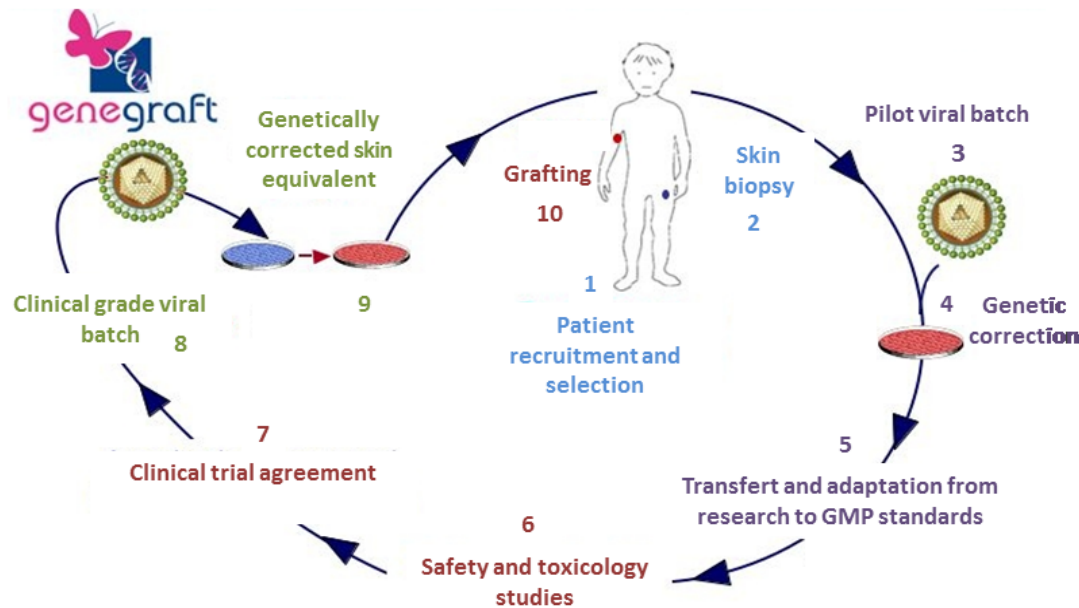
FIBHNJ obtained a GMP compliance certificate from the Spanish Regulatory Agency (AEMPS) on September 12th 2017. The design and the logistics of the clinical trial have been thoroughly discussed and defined by the consortium. Specifically, the primary and secondary objectives have been defined as well as the primary and secondary endpoints. The anesthetic and surgical procedures have been delineated. The number and size of the grafts, the nature, number and size of the skin lesions to be grafted, the monitoring and the follow-up period have been outlined. With regards to the logistics, the procedures for certification of the clinical department, for the research laboratory and the shipment of the biological material have been identified, and contacts with the relevant administrations have been made.

WP7: CLINICAL TRIAL CONDUCT

This WP aimed at implementing the treatment of 3 adult patients using clinical grade skin equivalents grafts genetically corrected with a COL7A1 encoding retroviral vector (orphan drug designation EU/3/09/630) and monitoring the tolerance and the effectiveness of this graft.

This WP has not been completed yet due to pending authorisation from ANSM (expected in June 2018).

GENEGRAFT: Phase I/II ex vivo gene therapy clinical trial for Recessive dystrophic Epidermolysis Bullosa



Overview of the major steps of the GENEGRAFT clinical trial

III. Impact and main dissemination activities

The objective of the project was to achieve the **first clinical trial for ex vivo gene therapy of Recessive Dystrophic Epidermolysis Bullosa (RDEB)** one of the most severe rare inherited skin disorders, using autologous skin equivalents genetically corrected with a secured SIN *COL7A1* retroviral vector. The programme has successfully **translated current scientific knowledge and expertise into novel, specific and preventive therapy** while actual medical approach is limited to palliatives procedures.

This project required highly qualified and experienced scientists and clinicians in *ex vivo* gene therapy for human genetic diseases and also specific structure for gene therapy in humans. GENEGRAFT gathered the critical mass of expertise in rare inherited diseases, gene therapy in human, viral vector production and clinical trials to perform basic and clinical research on Epidermolysis Bullosa. The relationships between the GENEGRAFT scientists and Debra International and other patient associations allowed strategic exchanges for the recruitment of patients for survey and dissemination of the results.

This **new therapeutic strategy** addresses the clinical and molecular diversity of RDEB patients. Transplantation of genetically corrected skin opens new fields in DEB therapeutic research and has a **strong potential to bring clinical improvement to RDEB patients and to represent a major progress in the treatment of this devastating disease.**

Transplantation of genetically corrected skin will provide an essential and permanent treatment of chronic and large skin wounds because gene-corrected skin equivalents have the potential to definitely cure the treated area. Indeed, transduced epidermal stem cells and fibroblasts contained in the gene-corrected skin equivalents will maintain their division and proliferation potential, allowing for long term expression and deposition of type VII collagen at the dermal-epidermal junction. This will allow the formation of functional anchoring fibrils. Therefore, treated areas will

locally reverse the disease phenotype. They will prevent the formation of blisters, skin inflammation, retraction and skin cancer.

By performing this research and development at the European level and in direct link with the European policy on rare diseases, the results of the GENEGRIFT project will serve as a model for the treatment of RDEB and in other forms of severe epidermolysis bullosa. It is also expected that this model could be extended to other genetic skin diseases, as well as non- dermatological disorders. The results of the project will benefit the entire scientific community and will have positive impacts on the health of thousands of European citizens.

- **Impact on patients and their families**

Epidermolysis Bullosa is a very severe disease causing disability, deformity and in most severe cases early death which affects day-to-day patients' and their families' activities. Parents or helpers involvement in taking care of the children is required every day and lifelong. Patients are often unable to live a normal life.

Effective treatment of RDEB will have strong, positive knock-on effects on the quality of life of patients and their families. Restoring skin cohesion over the most vulnerable areas such as the hands, forearms, feet and legs to prevent the recurrence of skin lesions and skin cancers should result in a significant reduction in the functional and systemic complications of RDEB, including aggressive and recurrent skin cancers which represent the first cause of death of these young patients. Consequently, vital prognosis would improve, and the frequency and duration of hospitalisation would be reduced. Further anticipated benefits include a reduction in pain and the prevention of deformity, with a positive effect as regards to the ability to carry out normal everyday activities. It is hoped, then, that physical improvements will result in lesser social handicap and better mental health. In addition, by cutting down the workload and stress involved in the care of the young patients, the positive impact of the therapy on their families would be considerable.

- **Impact on the public health system**

In Europe, Dystrophic Epidermolysis Bullosa (DEB) affects approximately **30,000 individuals** (prevalence of about 60/million) and approximately **400-500,000 people worldwide and most patients are children and young adults**. The patients suffer since birth from skin blistering, and from severe local and systemic complications resulting in poor prognosis. Current medical care protocols for RDEB patients are limited to palliative procedures to treat bullous lesions and severe complications of the disease (joint contractures, malnutrition, infections, sepsis and aggressive skin cancer). However, these treatments cannot prevent recurrent blistering arising from defective type VII collagen expression. This creates a tremendous medical, economic and psychological burden for patients through recurrent medical care, hospitalizations and surgical interventions.

It is expected that the clinical benefits of a novel and effective therapy for RDEB would prevent the occurrence of the most severe complications of the disease, thus improving the functional and vital prognosis of patients. Among the most severe complications are aggressive and recurrent skin cancers (squamous cell carcinomas) which occur at very high frequency on chronic wounds of RDEB patients and represent the first cause of death in these young patients.

In France, the cost of hospital care for RDEB has been estimated at over 900€ per day excluding expensive consumables for the treatment of skin lesions. In the long term, the costs of gene therapy will be offset by saving the costs of palliative health care. This should encourage local and national authorities to give access to this treatment to the patients.

▪ Dissemination of Genegraft results

Dissemination of the project results was accomplished via the usual channel of scientific information - publications in peer-reviewed journals, seminars, teaching, oral and poster presentations at professional meetings and the construction and maintenance of a **project website**.

DEBRA international actively relayed information on the research and therapy prospects to patients and their families. Debra reported on GENEGRIFT research progress on its own website (www.debra-international.org) and facilitated dissemination to policy makers, patient/parent organizations, regional/national/international societies via EB workshops, health manifestations and forums. Moreover, the work of Debra raises the profile of RDEB among the wider public and uncovers societal implications of the research work. These aspects are expected to continue after the project.

Such a close collaboration between researchers and Debra International led to a better understanding of the problems linked to the quality of life of EB patients and to a more efficient translation of Genegraft scientific results into the clinical practice.

Project scientific publications

Hovnanian A and GENEGRIFT consortium. Phase I/II ex vivo gene therapy clinical trial for recessive dystrophic epidermolysis bullosa using skin equivalent grafts genetically corrected with a COL7A1-encoding SIN retroviral c-vector (GENEGRIFT). *Hum Mol Ther*, 2014, 65-66 (DOI 10.1089/humc.2014.2508)

Hennig K, Raasch L, Kolbe C, Weidner S, Leisegang M, Uckert W, Titeux M, Hovnanian A, Kuehlcke K, Loew R. HEK293-based production platform for γ -retroviral (self-inactivating) vectors: application for safe and efficient transfer of COL7A1 cDNA. *Hum Gene Ther Clin Dev*. 2014 Dec;25(4):218-28. doi: 10.1089/humc.2014.083.

Project website address: www.genegraft.eu

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