RECOMB Project Final Report

STEM-CELL BASED GENE THERAPY FOR RECOMBINATION DEFICIENT SEVERE COMBINED IMMUNODEFICIENCY

www.recomb.eu

Recomb
gene therapy

SUMMARY

Recomb is a multi-stakeholder research consortium aiming to advance a novel treatment for severe combined immunodeficiency (SCID) by conducting a clinical trial using gene therapy for one of the most common types of SCID: RAG1-SCID.

The consortium, started in 2018, brings together clinical and research professionals from 16 European and 1 Israeli institutes with expertise in the management of primary immunodeficiencies, such as SCID. The project received funding from the European Union Horizon 2020 programme from January 2018 to December 2024.

SCID comprises a group of rare diseases in which cells in the adaptive immune system fail to develop properly. These infants are born without a functional adaptive immune system, thus typically experience a wide range of serious, eventually life-threatening infections, and die within the first year of life unless effective treatment is given.

Recomb aimed to progress the development of autologous haematopoietic stem cell-based gene therapy for RAG1-SCID. This means that the genetic mutation causing RAG1-SCID would be corrected in the patient's stem cells before those are transplanted back to the patient.

To do so, the patients' own blood-forming stem cells are collected and sent to the transduction site at Leiden University Medical Center (LUMC) in the Netherlands. Then, the genetically modified stem cells are returned to the participating clinical centres and transplanted to the patients. This implies that families will be able to avoid travelling across borders with their newborn child and having to stay several months at a foreign treatment centre, away from their daily social life and duties.

Before starting the clinical trial to treat patients, the safety, efficacy and stability of the technology was demonstrated in preclinical models. Once confirmed, the trial opened in the Netherlands, Spain, Poland, the United Kingdom and Italy. In addition to these Recomb clinical centres, two non-Recomb collaborating centres were added from Turkey and Australia to empower recruitment of study patients. To date, five patients have been enrolled and infused in this ongoing study, all of whom are clinically stable.

Preliminary safety and efficacy data are promising. Long-term effects of gene therapy will continue to be monitored at both cellular and molecular levels using standardised protocols. This first-in-human real-world experience provides proof of concept that lentiviral RAG1-SCID gene therapy is effective and safe, and can be successfully conducted in a multinational trial design.

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Abbreviations

EMA	European Medicines Agency
GMP	Good Manufacturing Practice
GvHD	Graft-versus-host disease
HSCT	Haematopoietic stem cell transplant
IPOPI	International Patient Organisation for Primary Immunodeficiencies
LV	Lentiviral
LUMC	Leiden University Medical Center
PID	Primary Immunodeficiency
RAG1	Recombination Activating Gene-1
SCID	Severe combined immunodeficiency
SIN	Self-Inactivating

RECOMB project final report (1st edition).

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Context and Objectives

Gene therapy for rare inherited immune disorders has become a clinical reality. Especially for SCID, two major types of SCID (ADA-SCID, X-SCID) have been successfully treated by autologous stem cell-based gene therapy. However, for one of the most common groups of SCID, the SCID underlying recombination defects, this has not yet occurred due to the higher complexities of the affected genes.

The Recomb project aimed to fill the unmet medical need for one of the most common categories of SCID, recombination activating gene-1 (RAG-1) deficient SCID, by performing a Phase I/II clinical trial using autologous hematopoietic stem cell-based gene therapy.

To this end, within the consortium, investigators successfully produced lentiviral vector batches, proved their safety and efficacy in preclinical models, and a multicentre, multinational clinical trial has commenced with input from regulatory authorities such as the European Medicines Agency (EMA), as well as the International Patient Organisation for Primary Immunodeficiencies (IPOPI) patient advocacy group.

This ongoing, prospective, non-randomised, open-label, multi-centre phase I/II intervention study is designed to treat children up to 24 months of age with RAG1-deficient SCID with an indication for allogeneic hematopoietic stem cell transplantation but lacking an HLA-matched donor.

Although the EU-funding has ended for the project, the trial is still currently recruiting in the following Recomb clinical centres: Leiden University Medical Center (LUMC) in Leiden, the Netherlands; Fundació Hospital Universitari Vall d'Hebron - Institut de Recerca (VHIR) in Barcelona, Spain; Wroclaw Medical University in Wroclaw, Poland; University College London Great Ormond Street Institute of Child Health (UCL GOSH) in London, United Kingdom; and Bambino Gesù Children's Hospital in Rome, Italy, as well as in two non-Recomb centres Erciyes University in Kayseri, Turkey and Murdoch Children's Research Institute in Melbourne, Australia. Clinical centres outside the trial may refer their patients to LUMC in Leiden.

Recomb objectives:

1. To provide a curative treatment for RAG1-SCID, filling a currently unmet medical need.
2. To prove the feasibility of the concept of *"Cells travel to the transduction site, not patients".*
3. To build upon and develop harmonised and standardised immune-monitoring protocols based on the EuroFlow approach.
4. To provide a health technology assessment.
5. To provide preclinical and clinical data to initiate a commercial trajectory.
6. To significantly increase Europe's expertise in gene therapy strategies.

Introduction

Severe combined immunodeficiency (SCID)

Severe combined immunodeficiency is a rare, life-threatening genetic disease in which the cells of the adaptive immune system fail to develop. To date, more than 20 genes have been identified to cause SCID phenotypes, of which one of the most common types is the recombination-deficient SCID (RAG-SCID). SCID affects 1:58,000 infants who may often seem healthy at birth but will typically experience a wide range of serious, life-threatening infections early in life and die within the first year without effective treatment.[1]

When it is diagnosed, the first aim is to treat active infections and prevent further infections. These treatments, however, are only temporary solutions, often partly effective, and they do not treat the underlying condition. Mutations in RAG1, RAG2 and Artemis genes cause the most common recombination defects. Currently, the standard of care is allogeneic haematopoietic stem cell transplantation (HSCT). In allogeneic HSCT, the deficient immune system is corrected by replacing the patient's bone marrow with healthy, unmodified allogeneic donor stem cells from which all immune cells can properly develop.

Despite improvements to this technique, the transplant outcome and overall survival vary for patients lacking matched family donors. Moreover, allogeneic HSCT is intrinsically associated with the risk of graft-versus-host disease (GvHD), an immune reaction of donor T-cells directed against the recipient's organs and tissues. As allogeneic HSCT still has limitations, there is an urgent need for new therapies based on the genetic correction of autologous stem cells, where the patient's own cells are modified and transplanted back.

GENE THERAPY

Gene therapy is a novel type of treatment for genetic conditions that involves correcting the faulty gene in the patient's stem cells. In gene therapy, working copies of the missing gene are inserted into the patient's DNA using a vector. Vectors are "vehicles" for delivering genetic material, such as DNA, into a cell. A vector is often a bacterium or a virus that has been inactivated, so that it no longer causes a disease.

Currently, the only treatment for RAG1-SCID is HSCT, and it has limitations. In gene therapy, a stem cell donor is not necessary because the patient's own stem cells are used and modified.

THE RECOMB PROJECT

Recomb is a research consortium furthering the development of stem cell-based gene therapy as a life-saving alternative for RAG1-SCID patients. Recomb started in 2018 and is coordinated by the LUMC, in Leiden, the Netherlands. It brings together world-renowned clinical and research professionals from 17 stakeholders who have expertise in primary immunodeficiencies, and some have previously conducted the first successful clinical trials using autologous stem cell-based gene therapy for X-SCID and ADA-SCID in patients lacking a matched donor.

Recomb's aim was to perform a first-in-human clinical study and provide treatment for RAG1-SCID patients. We aimed to correct the deficiency by delivering the therapeutic gene into the target cells using a vector. After successful transduction, the introduced gene is passed to all newly formed immune cells, thus restoring the immune function and curing the patient for life after a single treatment. Using the patient's own stem cells will exclude GvHD risk and increase survival.

A unique aspect of the protocol is that the patient's cells, not the patient, are transported to the transduction site at LUMC. After the cells are genetically modified, they are returned to the local centres for transplantation. Thus, the therapy is more comfortable and less expensive for patients.

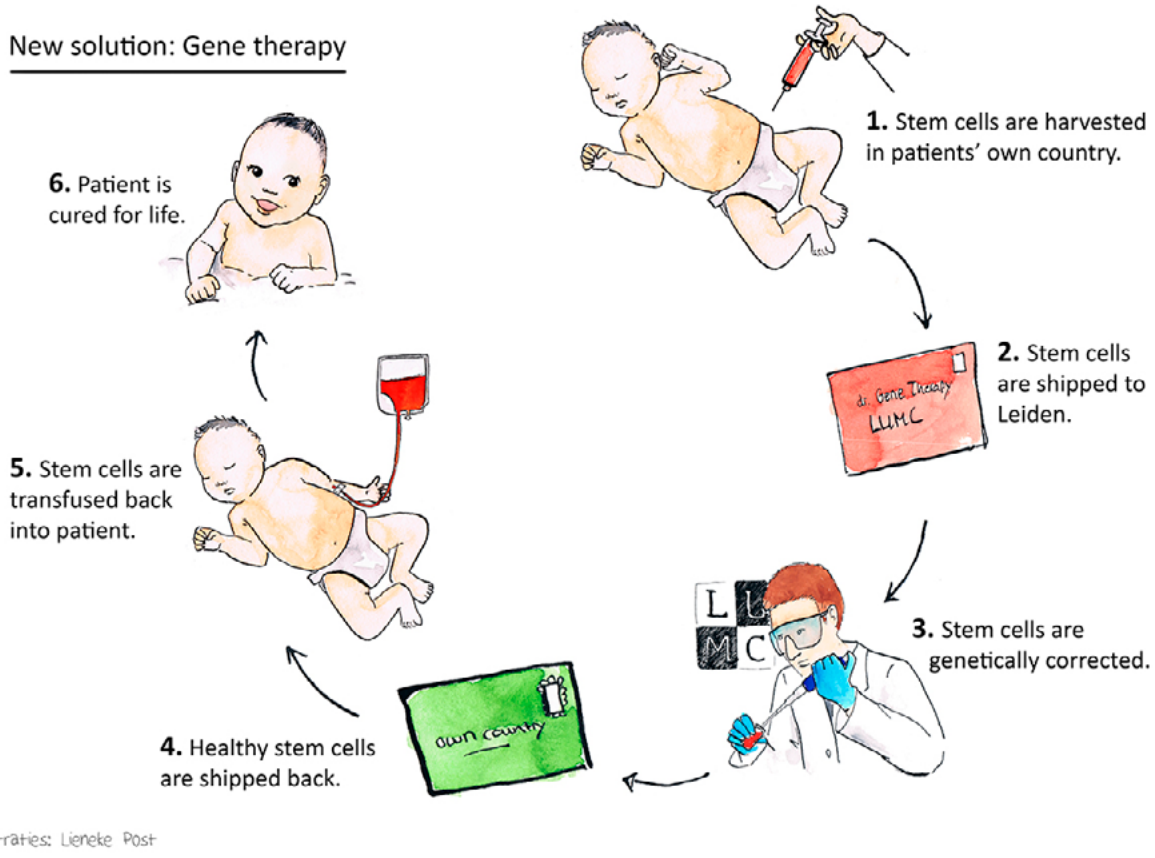

Figure 1 - The Recomb concept of gene therapy for RAG1-SCID.

The efficacy and feasibility of using gene therapy to treat RAG1-SCID were studied at five levels:

- **Produce:** Production of the RAG1 lentiviral (LV) vector.
- **Develop:** Efficacy and safety of the GMP-grade vector in preclinical models with mouse cells and patient cells.
- **Treat:** Conduct a clinical trial.
- **Evaluate:** Assess phenotypic and functional immune reconstitution as well as molecular reconstitution. Obtain new insights into TCR and BCR repertoire formation and development in humans.
- **Impact:** Cost-effectiveness and ethical issues were also studied. We created awareness of the study results among all stakeholders through dissemination and communication to reach clinical practice and deliver a business plan to capitalise on the study results.

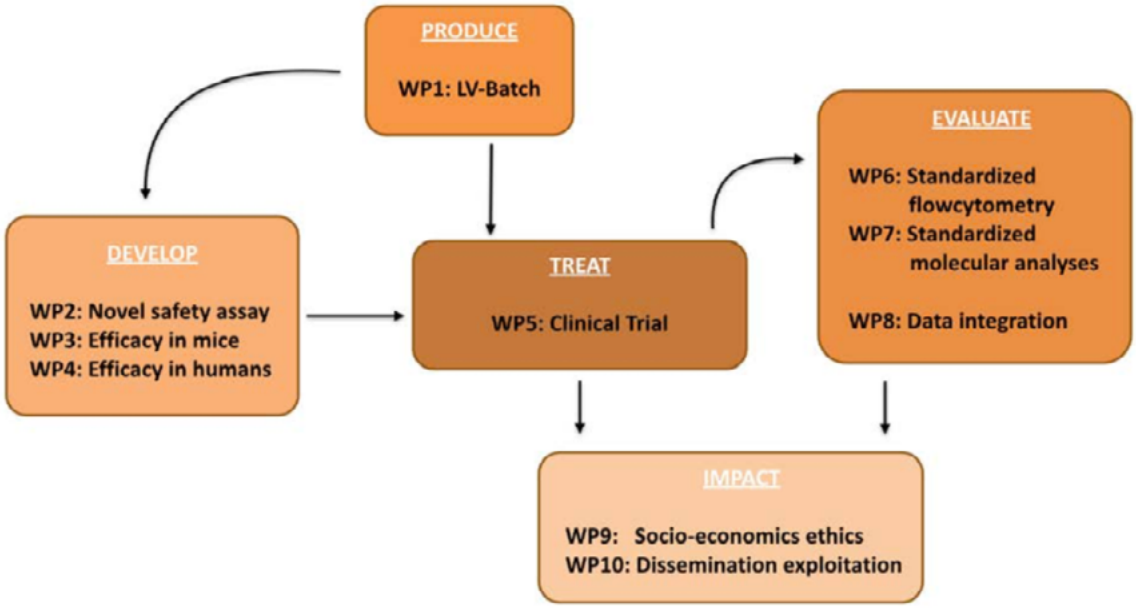

Figure 2 - Project chart showing the activities in Recomb and their dependencies.

PART 1
Producing preclinical and clinical lentiviral vector batches

A vector or vehicle is needed to deliver the correct gene to patient cells with the RAG1-SCID mutation so that patients can be treated. Therefore, one of the first steps of the project was to produce self-inactivating (SIN) vectors, which have demonstrated in recent trials to be safe in patients with Primary Immunodeficiency (PID).

As such, SIN lentiviral vectors encoding codon-optimised RAG1 were successfully produced for preclinical tests (pre-GMP batch) and for clinical use (GMP batch) by Batavia Biosciences under direction of and with extensive help from the Staal lab, LUMC.

These batches were tested for quality and stability before use with additional tests described in the next part.

PART 2
Preclinical models proving efficacy and safety of the produced LV vectors

In gene therapy trials for SCID and other immune disorders, oncogenic events have occurred due to insertional activation of proto-oncogenes by integration of the therapeutic vector. However, it is possible to predict such insertional mutagenic events.

During Recomb, the partners developed improved in vitro immortalisation assays as the new standard for evaluating the safety of gene therapy vectors. This allowed them to better detect vector-associated proto-oncogene activation of the lymphoid compartment and to investigate the effect of disease background on the risk of insertional transformation [2,3].

Once these new cellular assays were developed, the efficacy, safety, toxicity, stability and bio-distribution of pre-GMP and GMP LV vector batches were evaluated in mouse stem cells and human patient-specific stem cells by the Staal lab, LUMC, with support from the Schambach' lab, Hannover Medical School.

These vector batches were fully characterised and deemed safe in preclinical evaluations. They demonstrated successful transduction of bone-marrow-derived hematopoietic stem and progenitor cells while the cells maintained differentiation capacity without toxicity, making these vectors compatible with a gene therapy clinical approach.

Due to the need to know more about the long-term effects of gene therapy and to optimise tools to evaluate its safety, the consortium is still developing new techniques and reviewing the existing literature in order to provide a detailed protocol for the research community [4,5].

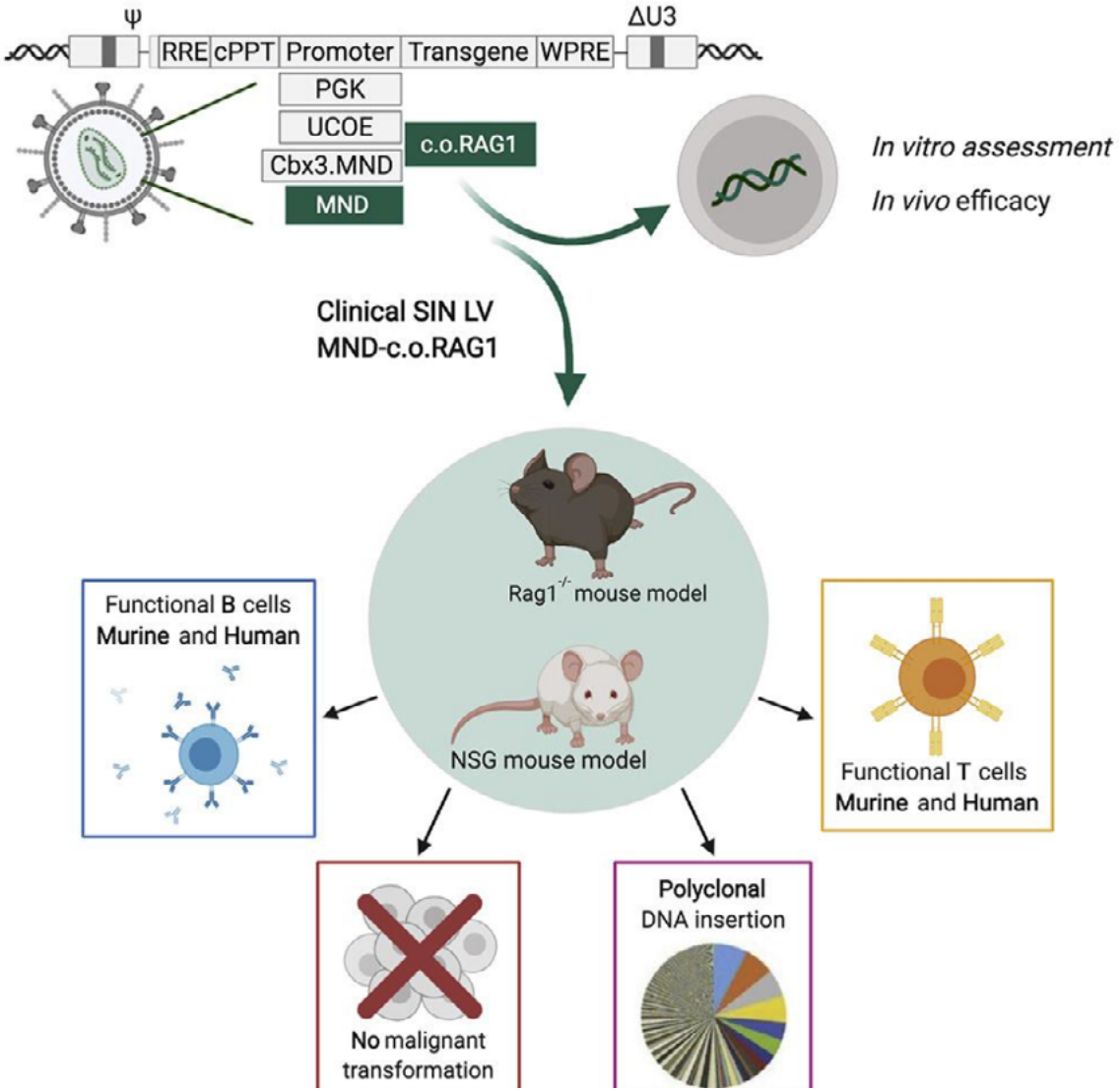

Figure 3 - Design of murine model to test efficacy of the SIN LV vector.[3]

PART 3
Conducting a clinical trial to treat patients with RAG1-SCID

Designing and opening the clinical trial

Once the LV vector carrying a codon-optimised RAG1 gene was developed and proven safe and efficacious in preclinical validation studies, the next step was to initiate a clinical trial. The Recomb clinical team designed a phase I/II clinical trial with the objective to evaluate the safety and efficacy of this gene therapy in infants with RAG1-deficient SCID.

The clinical study was ethically approved and opened first in the Netherlands in 2021, and it is currently open for recruitment in the following Recomb centres:

• **The Netherlands** - Leiden University Medical Centre (LUMC), Department of Pediatrics/ Willem-Alexander Children's Hospital in Leiden.

• **Spain** - Fundació Hospital Universitari Vall d'Hebron- Institut de Recerca (VHIR) in Barcelona.

• **Poland** - Wroclaw Medical University, Department of Pediatric Hematology/Oncology and BMT Supraregional Center of Pediatric Oncology "Cape of Hope" in Wroclaw.

• **United Kingdom** - University College London Great Ormond Street Institute of Child Health (UCL GOSH) in London.

• **Italy** - Bambino Gesù Children's Hospital in Rome.

In addition to these Recomb clinical centres, two non-Recomb collaborating centres were added to empower recruitment of study patients:

• **Turkey** - Erciyes Üniversitesi TIP Fakültesi Pediatrik Hemtoloji Onkoloji BD Erciyes Pediatrik Kemik iligi Nakli Merkezi in Kayseri.

• **Australia** - The Royal Children's Hospital in Melbourne.

The study is a prospective, non-randomised, open-label, multi-centre phase I/II intervention study designed to treat **children up to 24 months of age with RAG1-deficient SCID with an indication for allogeneic hematopoietic stem cell transplantation but lacking an HLA-matched donor.**

One of the innovative aspects of this study is that autologous cells are harvested from the patients at their host centre (the clinical sites) and then transported to a central facility (LUMC) for stem cell enrichment of the graft and LV transduction.

Gene-modified cells are cryopreserved using GMP-established protocols and analysed for defined release criteria, including CD34+ cell number, sterility, vector transduction efficiency (VCN/cell; RAG1 expression), and other quality tests required by the regulatory agencies. Cells that meet these criteria are shipped to the host centre for re-infusion into the patients.

Using this approach, the need to transport vulnerable, critically ill patients from their host site is circumvented. This study may therefore serve as a basis for other gene therapy studies in patients with immunohaematological disorders.

Clinical trial results

During the overall inclusion period, the study coordinator (LUMC) was consulted on 15 potential candidate patients from seven different countries/centres. Patients were selected based as per protocol in-/exclusion criteria (Table 1).

Table 1: Protocol in-/exclusion criteria.

CRITERIA	
INCLUSION	**EXCLUSION**
Genetically confirmed RAG1-SCID	HLA-matched donor available
Age below 2 years	Peripheral T cells <300/µl and/or naïve T cells < 1/µl
No HLA-matched donor available	Omenn Syndrome
Peripheral T cells <300/µl and/or naïve T cells < 1/µl	Previous stem cell transplantation
No Omenn Syndrome	Significant organ dysfunction

So far, the trial has recruited **five patients** who have successfully been treated with the investigational medical product (IMP) (genetically modified cells of the patient). The collection of blood stem cells has been successfully conducted in all study subjects. International transportation of the stem cell product was uneventful, and an IMP was generated for all patients in the GMP facility of the central manufacturing site at LUMC.

The follow-up of the five patients ranges from 8-47 months. While the follow-up of all study patients is ongoing as defined in the protocol, several interim conclusions can be drawn.

All patients are alive and well at the last follow-up (August 2025). Immune recovery is encouraging in all patients, leading to discontinuation of immunoglobulin supplementation in the first three patients with sufficient follow-up. No substantial toxicities or side effects related to the treatment have been observed.

No signs of insertional mutagenesis have been reported during follow-up in any of the patients. Therefore, the interim results unequivocally support continuation of the current trial and recruitment of additional study patients.

This first-in-human real-world experience provides proof of concept that lentiviral RAG1-SCID gene therapy is effective and safe, and can be successfully conducted in a multinational trial design.

The Recomb clinical trial is set to continue until December 31, 2029. Clinical centres outside Recomb may refer their patients to LUMC.

For more details, contact the LUMC team (Prof. Staal and Prof. Lankester) via rag1trial@lumc.nl.

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PART 4
Phenotypic, functional and molecular evaluation of the treatment

As is customary for conventional HSCT, as well as haematopoietic stem cell–based gene therapy approaches, data on transplant-specific parameters (e.g. cell dose, type of conditioning, general outcome of the transplant) are collected. Interpretation of the immune development of treated patients in comparison with normal immune system build-up occurring during the first years of life, as well as of the response to challenges such as vaccines, provides an in-depth insight into the degree of immune reconstitution and, therefore, of the efficacy of the treatment.

The analysis of these data allows for the study of reconstitution kinetics of adaptive immunity at the cellular level in direct relation to repertoire reconstitution at the molecular level.

This allows for the outcome of the treatment to be registered and used for comparison across centres, which in turn provides more information on the safety and efficacy of gene therapy and allows specialists to optimise the treatment for future patients.

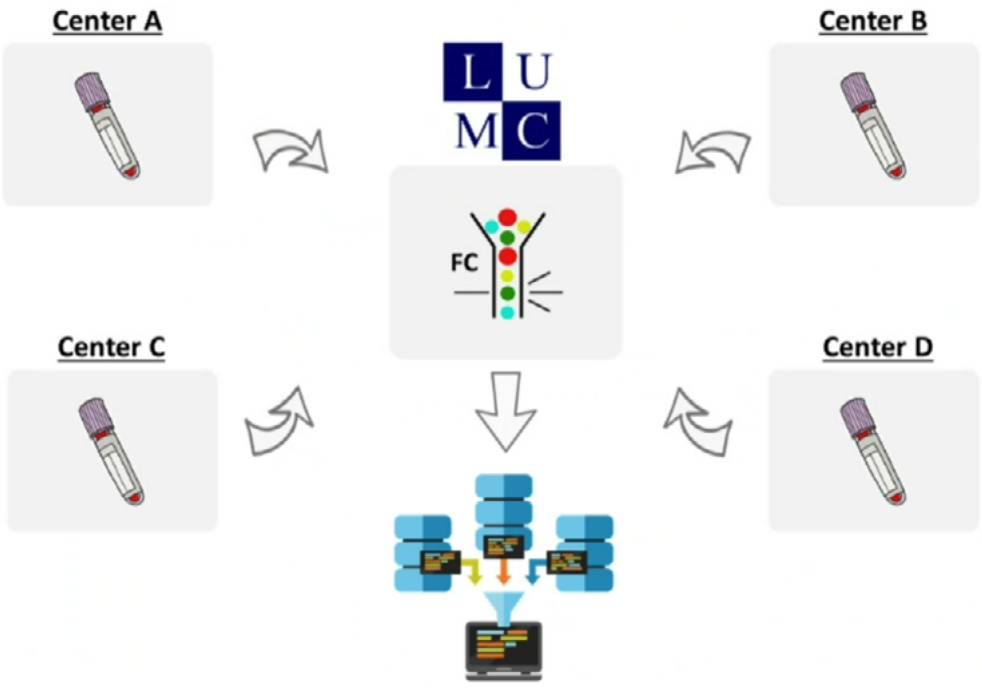


Figure 4: Centralised sample processing (FC - flow cytometry).

To accomplish this, highly standardised protocols and tools, such as those already developed by EuroFlow (from which LUMC is a member) for flow cytometric PID diagnosis and immune monitoring, were optimised and validated. Cells from these patients were also evaluated in parallel to confirm the safety and efficacy of LV vectors. Next-generation sequencing and state-of-the-art bioinformatics were used to identify the integration site of the new genes in the modified cells.

The flow cytometry panels that were developed for peripheral blood allowed us to identify a multitude of immune cell subsets in both peripheral blood and bone marrow, reflecting the maturation process and the regeneration of T, B, and NK-cell functional compartments.[6] Even though the analyses were done in very small volumes of blood, small populations were still detectable, showing the sensitivity of the panels. Additionally, patients showed restoration of the ability to create highly diverse populations of B and T cells.

Importantly, these essays demonstrated full immune reconstitution of the first clinical trial patients, hinting at a potentially safe and efficacious cure for RAG1-SCID.

PART 5
Recomb's Impact

Socio-economic and ethical evaluation of the trial

Recomb's achievements, as outlined so far, underline that a first-in-human academic stem cell gene therapy trial can be successfully implemented by a multinational consortium of dedicated expert centres for rare diseases.

Gene therapy has the potential to combine effective and curative treatment for RAG1-SCID with acceptable healthcare costs. However, the experimental character of the approach may cause a dilemma in clinical decision-making for parents of candidate patients.

To strengthen the arguments for a curative and innovative treatment like Recomb suggests, multiple aspects are essential, such as ethical concerns and cost-effectiveness compared to current approved treatments. This evaluation is important, both from an individual's parents' and a societal perspective. To do this, Recomb committed to:

- Evaluating the 'ethical' and social acceptability, both community and individual/parent perceived, of the proposed gene therapy trial; and

- Performing an economic evaluation of gene therapy as compared to conventional allogeneic HSCT, which is the current best available treatment.

To evaluate the social acceptability of gene therapy, the proposal was to inquire patients about their quality of life, 'acceptability and distress' and 'anxiety and depression'. Due to the small pool of patients enrolled in the Recomb clinical trial, investigators extended their interviews to other non-RAG1 SCID, severe inborn errors of immunity for which allogeneic HSCT is currently the standard of care and gene therapy may become available as well. This work is ongoing and will provide a much better chance to give a reliable perspective on a) satisfaction with the information provided on the gene therapy trial and with the consent process, b) confidence in the proposed gene therapy and c) the concept of shared decision making.

Briefly, inquired patients reported a relatively high use of physiotherapy, psychologists, emergency visits, and outpatient and inpatient hospital care.

A detailed economic evaluation, including the comparison of allogeneic HSCT and gene therapy, is currently in progress.[7-9]

The consortium was interested in the concept of unmet needs; whether First-In-Human trials can be therapeutic; the concepts of risks and benefits; and group relatedness.

Dissemination and exploitation of project results

A big component of Recomb's impact can be attributed to the dissemination and exploitation of results. This is crucial to ensure that a range of stakeholders in the scientific community, patient groups, the public, policymakers, and industry representatives are aware of the project's existence, progress and achievements. It is also important to facilitate the exploitation of the results of Recomb in the commercial and industrial sector. Additionally, maintaining the patient community informed is essential to ensure that the right patients and caregivers are aware of the clinical trial and can seek information if they wish to be included.

To achieve this, the IPOPI, in collaboration with all Recomb partners, prepared a Recomb website and social media to share informative videos and leaflets. These materials feature key information about SCID, the available treatments and the Recomb clinical trial, and were disseminated extensively to reach a wide audience.

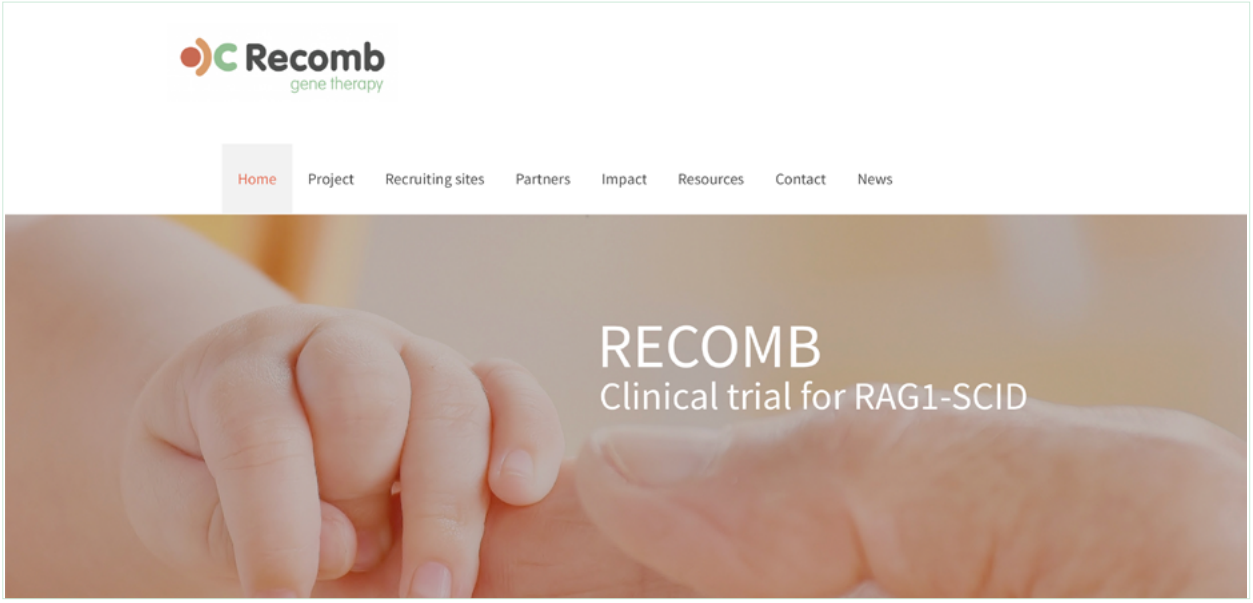


*Figure 5: Recomb website (*www.recomb.eu*).*

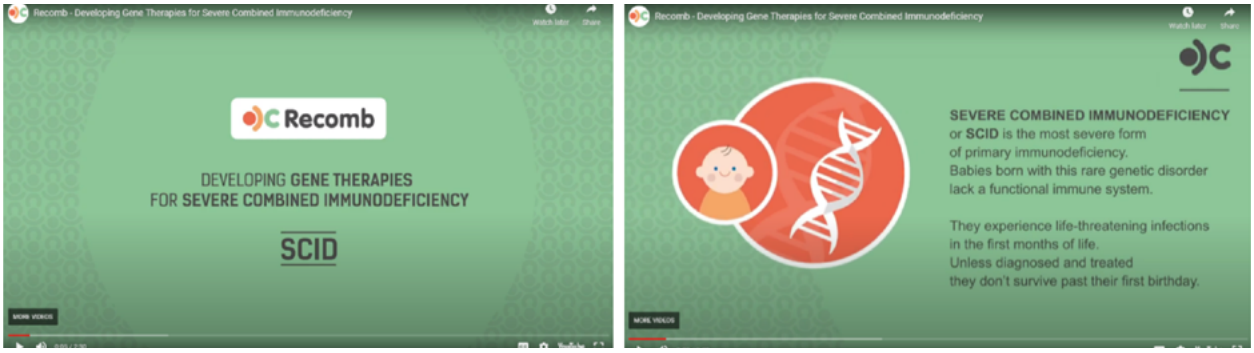


Figure 6: Recomb videos Recomb - Developing Gene Therapies for Severe Combined Immunodeficiency.

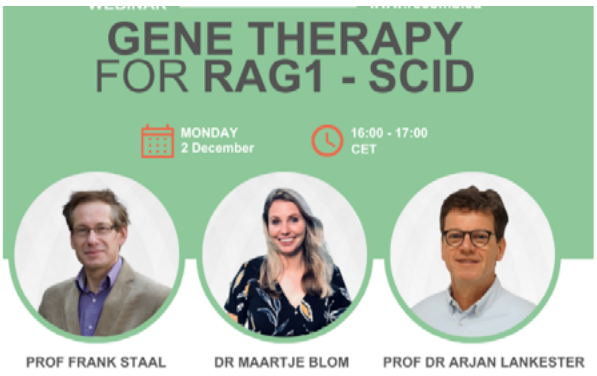
Additionally, a flyer concerning the recruitment process was published in English and recently translated into Dutch, German, Polish, Turkish, Italian and Spanish. This flyer is designed to reach and connect with patients across Europe and help them learn more about the trial and the inclusion criteria.

Figure 7: Advertisement about the flyers shared on social media.

The consortium has produced several publications:

- 31 articles in prestigious journals (26 peer-reviewed)
- 4 publications in conference proceedings/workshop
- 7 articles in IPOPI newsletters (4123 subscribers)

Additionally, to facilitate the digital dissemination of results to relevant stakeholders, two webinars were held:



1st Webinar on January 2021

- Overview of gene therapy and how it works for RAG1-SCID and detailed the status and prospects of RECOMB
- 117 registrations (34 countries)

2nd Webinar on December 2024

- Share the findings of RECOMB after treating 5 patients and preliminary data on the cost-benefit of gene therapy.
- 64 registrations (17 countries)

Through activities such as conferences and social media, the main audiences, like the scientific community, the general public and PID patients, were reached and informed about Recomb's milestones.

SOCIETY AND ECONOMY

CURRENT STATUS

1) The only currently available treatment for RAG-SCID is allogeneic stem cell transplantation (HSCT) with less favorable survival in mismatched HSCT recipients; Graft vs. host disease regularly affects HSCT recipients, especially with mismatched donors.

2) The current treatment is suboptimal and can therefore be expensive.

3) Significant increase in the number of RAG-SCID patients on the horizon with newborn screening for SCID.

4) Current healthcare for SCID and other severe forms of PID is orientated around HSCT as the definitive treatment option.

EXPECTED IMPACT

1) Provide a curative treatment with gene therapy using autologous HSCs, which will: Increase survival; Eliminate risk of graft vs. host disease. High level of safety and efficacy.

2) Gene therapy has the potential towill significantly reduce healthcare costs for the treatment of (RAG) SCID in the long term, and potentially many others.

3) The Recomb program will contribute to make gene therapy a realistic option for the majority of SCID patients.

4) A new model including highly specialized centres with the expertise to receive, transduce and return cells to remote clinical units in the EU, will likely become a global standard.

PATIENTS AND FAMILY

CURRENT STATUS

1) Current gene therapy treatment requires families to travel and find accommodation at the location of the treatment centre.

2) Insufficient engagement of patients in research.

EXPECTED IMPACT

1) In the Recomb trial, cells - and not patients - will travel to the coordinating study centersite, and genetically modified stem cells will be returned to the participating and expert clinical centres and transplanted to the patients.

2) Patients are actively involved due to the participation of The International Patient Organisation for Primary Immunodeficiencies (IPOPI)

SCIENCE AND CLINICAL PRACTICE

CURRENT STATUS

1) Europe is the global leader in developing stem cell-based gene therapy due to SCID programmes granted by EU, but still over 50% of SCID patients do not have access to gene therapy as a treatment option.
2) Industry investments in gene therapy to treat rare diseases (orphan diseases) such as SCID are sparse and there have been notable setbacks in recent years.
3) Current knowledge base regarding lymphoid development in humans is incomplete.
4) Lack of EU-wide guidelines in assessing efficacy of treatments.
5) Many other diseases lack safe and efficient (curative) therapies.

EXPECTED IMPACT

1) Recomb building on these projects will reinforce this leading role by delivering gene therapy as treatment for more patients.

2) Recomb will engage with industry towards further research and development of orphan medicines and may stimulate public-private partnerships.

3) Recomb will provide new scientific insights regarding haematopoiesis and lymphocyte development in humans.

4) Recomb will develop harmonised and highly standardised protocols and tools for monitoring the short and long-term effects of gene therapy.

5) The knowledge and expertise obtained during the Recomb program can also be applied to other diseases that can be treated with autologous HSC gene therapy,
i.e. other SCIDs, immune disorders, lysosomal storage diseases and hemoglobinopathies, such as β-thalassemia and sickle cell anaemia

With the success of this project, many advantageous impacts are envisaged on patients' lives, society and economy, as well as on scientific and clinical practice. Most importantly, it is anticipated to provide treatment for RAG1-SCID patients with higher efficacy and safety. Moreover, it is expected to be more comfortable and less expensive for the patients. Additionally, due to cryopreservation of the product, its distribution is facilitated to several centres, with the possibility to reach any affected child in the EU.

This new model, including highly specialised centres with the expertise to receive, transduce and return cells to remote clinical units in the EU, will likely become a global standard. Gene therapy may be cost-effective for the treatment of RAG1-SCID in the long term and potentially many others. Given the success with X-SCID and ADA-SCID, gene therapy will likely become the treatment of choice for the majority of SCID patients, offering an effective treatment option for over 70% of all genetically defined SCID patients in Europe and beyond. It is becoming more important to provide such therapy as the number of diagnosed RAG1-SCID patients is expected to increase due to newborn screening studies.

Along with these ambitions, the consortium expects that the obtained knowledge can also be applied to other diseases that can be treated with autologous stem cell-based gene therapy, i.e. SCIDs, immune and lysosomal storage diseases and haemoglobinopathies. The successful treatment of the first patients provides clinical proof of concept for Recomb's approach to treating RAG1-SCID and a promising perspective towards the above-mentioned expectations.

Conclusions

A lentiviral-based approach was developed for transferring the therapeutic RAG1 gene into CD34+ HSCs to correct this mutation. Proof of principle was obtained in preclinical mouse studies. It supported the feasibility of using gene-corrected stem cells to restore immune function by restoring B- and T-cell production. As a result of the Recomb project, the disease-modifying effect of these CD34+-RAG1 cells is now being tested in human patients.

The vector batches for use in human cells were successfully produced and tested. When evaluating in vitro safety, assays showed that the vectors were not genotoxic and deemed safe for the trial. Moreover, the development of a novel, combined assay for preclinical risk assessment to provide more supportive data for regulatory evaluations is underway.

In vitro and in vivo preclinical evaluations were performed in Rag1-Omenn syndrome models, Rag1-deficient mouse cells, and RAG1-SCID patient cells to help determine the full clinical spectrum of patients that can benefit from the therapy. Efficacy testing in hypomorphic Rag1 mouse models showed significant amelioration of the disease phenotype. The long-term efficacy and safety assessed in Rag1-deficient mice detected no signs of toxicity and normal survival. The vector also efficiently transduced RAG1-SCID patient cells and restored stable RAG1 expression with no toxicity. Altogether, these assessments confirmed the efficacy and safety of gene therapy.

After vector efficacy, safety and stability were confirmed, the trial opened in the Netherlands, Spain, Poland, the UK, , Turkey and Australia. So far, five patients have been enrolled and infused in this ongoing study, all of whom are clinically stable. Preliminary safety and efficacy data are promising. Long-term effects of gene therapy will continue to be monitored at both cellular and molecular levels using standardised protocols. This first-in-human real-world experience provides proof of concept that lentiviral RAG1-SCID gene therapy is effective and safe, and can be successfully conducted in a multinational trial design.

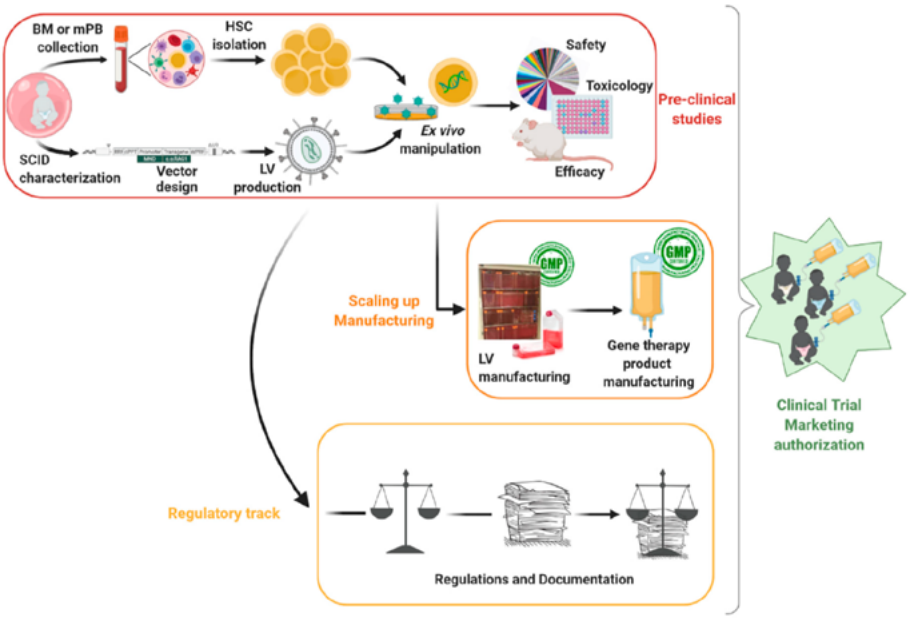


Figure 8: Overview of the pre-clinical assessments of gene therapy treatment: From disease modelling to clinical application (Bone Marrow (BM); mobilised Peripheral Blood (mPB); Hematopoietic Stem Cell (HSC); Lentiviral Vector (LV); Severe Combined Immunodeficiency (SCID)). Figure reproduced from [10]

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