

**MagSelectoFection .EU**



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magselectofection

Combined isolation and stable nonviral transfection of hematopoietic cells – a novel platform technology for ex vivo hematopoietic stem cell gene therapy.

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**Publishable Executive Summary – Second Reporting Period**

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**Project coordinator name:** Christian Plank, Ph.D.

**Project coordinator organisation name:**

Klinikum rechts der Isar der Technischen Universität München

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## **Publishable Executive Summary**

The feasibility of ex vivo gene therapy in humans has been demonstrated with retrovirally transduced hematopoietic stem cells. At the same time, risks associated with the use of retroviral vectors have become apparent. Therefore, this consortium intends to develop a novel nonviral integrated *ex-vivo* cell separation/transfection platform, suitable for site-specific genomic integration of transfected nucleic acids into non-coding regions of the host genome. This concept is intended to be applied to hematopoietic stem cells (HSC) and to be validated with established preclinical models of SCID-X1, a rare hereditary immuno deficiency. The *Magselectofection* platform technology is based on a clinically approved magnetic cell separation technique (MACS Technology) combined with magnetically enhanced transfection (Magnetofection) and on nucleic acid constructs that provide site-specific genomic integration, either the phage phiC31 integrase system or, alternatively, a drug-inducible AAV-derived replicase/integrase system.

The work programme comprises 3 major objectives: Establishing, Validating, and, Disseminating and Exploiting *Magselectofection*. Continued efforts of the consortium in transfecting HSC with nonviral vectors have not succeeded so far. Therefore, the *Magselectofection* platform was first established with hematopoietic cell lines, furthermore with peripheral blood lymphocytes and was then extended to other cell types including mesenchymal stroma cells from umbilical cord. Furthermore, the technology was extended to the use with lentiviral gene vectors. Establishing the technology has comprised the development and characterization of novel magnetic vector formulations, demonstrating proof of principle on Miltenyi MACS cell separation columns and process development towards using *Magselectofection* technology with clinically approved CliniMACS instruments.

In parallel, the phiC31 integrase system and the AAV-derived replicase/integrase system were further refined, optimized and characterized. A protein transduction reagent was developed and launched on the reagent market that can also be used to transport phiC31 integrase protein into cells. In order to exploit the high transduction efficiency of lentiviral vectors while avoiding the biological risks associated with their non-specific integration into the host genomes, non-integrating lentiviral vectors are developed that are able to direct site-specific integration via the phiC31 integrase.

The work programme also includes characterizing the migration behaviour of transfected / transduced cells as well as their homing and engraftment in animal models. Members of this consortium have shown that a magnetic transfection reagent efficient in *Magselectofection* of hematopoietic cell lines compromises to some extent the proliferation, the motility and the clonogenic function of CD34+ hematopoietic progenitor cells, an important finding with respect to developing nonviral transfection reagents compatible with the biological functions of HSCs. Characterizing homing and engraftment within this project also comprises magnetic cell labeling for cell tracking by MRI. To this end, a collection of iron magnetic nanoparticles was screened and particles for efficient cell labeling, generating strong contrast in MRI were identified.

In summary, the *Magselectofection* platform technology will be available in the near future as a standardized technique for transfecting / transducing a variety of cell types relevant in current biomedical research and applications. However, progress within this project also highlights and identifies the technical and biological challenges associated with genetically modifying HSCs, challenges the extent of which has not been foreseen when starting this project.

## **Background**

The clinical success in curing patients suffering from X-linked severe combined immuno deficiency (SCID-X) has demonstrated the great potential of gene therapy. In the same study, the shortcomings and serious biological risks associated with the use of current viral gene vector technology became evident. Several patients treated with retrovirally transduced hematopoietic stem cells developed a lymphoproliferative disease due to insertional mutagenesis caused by the uncontrolled integration properties of the vector. Hence, safe alternatives are required to realize gene therapeutic concepts involving the stable genetic modification of cells.

Members of this consortium have previously developed independent technologies for magnetic cell separation, for magnetic field assisted nucleic acid delivery to cells (Magnetofection), nucleic acid constructs that are suitable for stable integration into eukaryotic genomes, disease-relevant transgenic mouse models to evaluate the biological characteristics and therapeutic potential of transgenic cells under pre-clinical settings as well as tools for expression profiling. There are strong personal and scientific links to the EU-funded CONSERT project. The basic objective of the Magselectofection project is to combine the named independent technologies and skills in order to generate a novel integrated platform technology for the genetic modification of cells in clinical and research applications that is simple, efficient and safe.

## **Objectives**

### **Establishing Magselectofection**

- Combining magnetic cell sorting and transfection based on Miltenyi's clinically approved MACS Technology and Magnetofection (magnetically guided nucleic acid delivery) for manipulation of hematopoietic cells.
- Achieving stable and regulatable transfected gene expression in hematopoietic *stem* cells by site-specific genomic integration of delivered nucleic acids upon Magselectofection with plasmid constructs harboring the phiC31 integrase system and, alternatively, a drug-inducible AAV-derived replicase/integrase system.

### **Validating Magselectofection**

- Characterizing this technology: Analysis of genomic integration sites, transcriptome profiling, characterization of stable and inducible transfected gene expression.
- Validating this technology in transgenic SCID-X mouse models: Evaluation of homing, engraftment and persistence in transgenic animal models using molecular biological tools and magnetic resonance imaging.
- Demonstrating the therapeutic efficacy and assessing associated risks of the technology in transgenic mouse models.

### **Disseminating and exploiting Magselectofection**

- Transfer into research and clinics through the participating companies.

## Results:

Magnetic cell separation by MACS technology from Miltenyi Biotec and Magnetofection gene transfer technology were successfully integrated. To this end, proof of concept was provided with hematopoietic cell lines (Jurkat and K562), with primary peripheral blood lymphocytes and with mesenchymal stromal cells (umbilical cord stromal cells able for differentiation into several tissues). It was demonstrated that Magselectofection can be practiced with nonviral as well with lentiviral vectors. To this end, detailed studies of vector association with a collection of magnetic nanoparticles was carried out including a detailed biophysical characterization of magnetic vector constructs. In this manner, magnetic compositions optimized for Magselectofection were identified. It was shown that Magselectofection can be scaled to the use with various formats of Miltenyi MACS technology.

As so far the use of nonviral vectors did not yield transfection of HSCs, ongoing efforts within the consortium are dedicated to developing non-integrating lentiviral vectors that make use of the phiC31 integrase system for achieving more site-specific genomic integration. Non integrating codon-optimized phiC31 integrase lentiviral vectors have been generated and are currently further refined. Important progress in site-specific genomic integration has been achieved with a plasmid construct for the integration of large genes that contains the integration cassette based on the cis-acting elements of the adeno-associated virus type 2 and an expression cassette for the AAV replicase-recombinase Rep78. A relatively high efficiency of Rep78-mediated integration at a specific site on human chromosome 19 has been obtained in cell lines and in transgenic mice. Major progress was also made with the phiC31 integrase system. A protein transduction reagent developed within this consortium was successfully used to transport the recombinant phiC31 protein into target cells, which constitutes an important step in realizing genomic integration with nonviral vector technology. The protein was produced by a consortium member after tedious cloning and purification work.

Important progress has been made with the collection, in vitro expansion and banking of umbilical cord stromal cells residing in Wharton's jelly of cord tissue. This was a prerequisite for demonstrating that Magselectofection can be carried out with this cell type. In fact, nonviral Magselectofection is effective in genetically modifying this cell type without compromising its differentiation potential. In contrast, as already mentioned, HSCs so far were not amenable to nonviral magnetofection. It turned out that HSCs treated with a nonviral magnetic vector become compromised with respect to their the proliferation, motility and clonogenic functions. Interestingly, the extent of vector impact was different among cells from two different donors. So far, there has been hardly any knowledge on the impact of nonviral vectors on the biological function of HSCs. Hence, these preliminary findings constitute an important progress in the development of tools for the genetic modification of this cell type.

For establishing cell tracking by MRI we have screened a collection of magnetic nanoparticles for magnetic cell labelling in conjunction with magnetofection. We have found that magnetofection is a suitable procedure for a one-process transfection and magnetic cell labelling. Magnetic particles and vector formulations were identified that yield high transfection efficiency and generate strong contrast of magnetically labelled cells in MRI. As little as a few thousand cells in a fibrin phantom can be localized in this manner.

So far, this project has generated new knowledge in a broad spectrum of scientific disciplines. It can be envisaged that this interdisciplinary collaboration will generate important progress with novel tools for nucleic acid, gene and cell therapies.

**Potential applications:**

Multiple nucleic acid, gene and cell therapy applications that can be envisaged such as:

- Using the method to induce RNA interference (RNAi) in hematopoietic stem cells to fight HIV infections.
- Using the method for the transfection of lymphocytes for adoptive immunotherapy of cancer.
- Transfection of neuronal stem cells for ex vivo gene therapy of central nervous system disorders.
- Ex vivo transfection of stem cells or progenitor cells for tissue engineering (bone, cartilage reconstruction, tendon and wound healing etc.).
- Cell tracking, molecular imaging. The magnetic nanoparticles used in Magselectofection are suitable contrast agents for magnetic resonance imaging. Thus, the method can be used to introduce these particles in any cell of interest, optionally along with other agents for molecular imaging (e.g. fluorescent dyes).

**Project web-site:** <http://www.magselectofection.eu>

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**Coordinator**

Dr. Christian Plank  
Klinikum rechts der Isar der TU München  
Institute of Experimental Oncology  
Ismaninger Str. 22, D-81675 Munich, Germany  
T: (+49) 89 4140 4453; F: (+49) 89 4140 4476  
Email: plank@lrz.tum.de

## Partners

Principle Investigator(s)	Participant organisation name, contact details
Christian Plank	Klinikum rechts der Isar der Technischen Universität München Ismaningerstr. 22 81675 München Germany Tel. +49-89-4140-4453, Fax +49-89-4140-4476 <a href="mailto:plank@lrz.tum.de">plank@lrz.tum.de</a>
Gerard Wagemaker	Erasmus University Medical Center Faculty Building, Department of Hematology Dr. Molewaterplein 50 3015 GE Rotterdam The Netherlands Tel. +31(0)10-4088302 / 408 7766 /secretariat 4087756 Fax +31(0)10-408 9470 <a href="mailto:g.wagemaker@erasmusmc.nl">g.wagemaker@erasmusmc.nl</a>
Fulvio Mavilio	Fondazione Centro San Raffaele del Monte Tabor via Olgettina 58 20132 Milano Italia Tel. +39-02-2643 4701 <a href="mailto:f.mavilio@hsr.it">f.mavilio@hsr.it</a>
Tsvee Lapidot Orit Kollet	Weizmann Institute of Science Wolfson Building Rehovot 76100 Israel Tel. +972-8-934 2481,4262, Fax +972-8-934 4141 <a href="mailto:tsvee.lapidot@weizmann.ac.il">tsvee.lapidot@weizmann.ac.il</a> <a href="mailto:orit.kollet@weizmann.ac.il">orit.kollet@weizmann.ac.il</a>
Zygmunt Pojda	M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology Department of Experimental Hematology and Cord Blood Bank 02-781 Warszawa Poland <a href="mailto:zpojda@coi.waw.pl">zpojda@coi.waw.pl</a>
Ian Johnston Michael Apel	Miltenyi Biotec GmbH Friedrich-Ebert-Straße 68 51429 Bergisch Gladbach Germany Tel. +49(0)2204-8306-0, Fax +49(0)2204-85197 <a href="mailto:MichaelA@miltenyibiotec.de">MichaelA@miltenyibiotec.de</a> <a href="mailto:ianJ@miltenyibiotec.de">ianJ@miltenyibiotec.de</a>
Peter Steinlein	Research Institute of Molecular Pathology Dr. Bohr-Gasse 7 1030 Vienna Austria Tel. +43(1)-797 30, Fax +43(1)-798 7153 <a href="mailto:steinlein@imp.univie.ac.at">steinlein@imp.univie.ac.at</a>
Melania Babincova Peter Babinec	Comenius University Department of Nuclear Physics and Biophysics Mlynská dolina F1 842 48 Bratislava Slovakia Tel. +386-421-760295674 <a href="mailto:babinec@fmph.uniba.sk">babinec@fmph.uniba.sk</a> , <a href="mailto:babincova@fmph.uniba.sk">babincova@fmph.uniba.sk</a>
Olivier Zelphati	OZ Biosciences Parc Scientifique et Technologique de Marseille-Luminy BP 13 Marseille 13273 Cedex 9 France Tel. +33(0)4-9182 8174, Fax +33(0)4-9182 8170 <a href="mailto:ozelphati@ozbiosciences.com">ozelphati@ozbiosciences.com</a>
Joseph Rosenecker <u>Subcontractors:</u> Carsten Rudolph Florian Hoffmann	Ludwig-Maximilians-Universität Klinikum der Universität München, Dr. von Haunersches Kinderspital Kubus Rückgebäude Lindwurmstr. 2a 80337 München Germany Tel. +49(0)89-5160 7711, Fax +49(0)89-5160 4421 <a href="mailto:joseph.rosenecker@med.uni-muenchen.de">joseph.rosenecker@med.uni-muenchen.de</a>