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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

Instrument: STREP

Thematic Priority: [FP6-2004-LIFESCIHEALTH-5] [Life sciences, genomics and biotechnology for health]

Final Activity Report

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Project coordinator name: Christian Plank, PhD.

Project coordinator organisation name:

Klinikum rechts der Isar der Technischen Universität München

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PUBLISHABLE FINAL ACTIVITY REPORT

1. Project Execution

This project was conceived in early 2004 and was submitted for review in November of that year. By that time, the feasibility of ex vivo gene therapy in humans had been demonstrated with retrovirally transduced hematopoietic stem cells. At the same time, risks associated with the use of retroviral vectors had become apparent. Therefore, this consortium had intended 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 was 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 was to be based on a clinically approved magnetic cell separation technique (MACS Technology from project Partner Miltenyi Biotec, Germany) combined with magnetically enhanced transfection (Magnetofection[1]) and on nucleic acid constructs that were to provide site-specific genomic integration. These constructs were to be either based on nucleic acid sequences derived from the bacterial phage phiC31 or, alternatively, on nucleic acid sequences derived from adeno-associated virus. At the time when the project was conceived, site-specific genomic integration upon using such constructs had been reported in the literature[2, 3]. Hence, the very ambitious overall goal of this project was establishing a new technology that in the long run might make the use of viruses for gene delivery obsolete. In this manner, the biological risks associated with the use of viruses would be avoided. The ambitious overall goal has not been reached. Also now, in the year 2010, no single nonviral system for gene delivery exists that amounts to the efficiency of viral systems. Truly site-specific and risk-free genomic integration is still a long-term goal that poses numerous technical and biological challenges. However, during the Magselectofection project and in parallel, in international research, very substantial progress has been made towards cell therapies that involve the use of genetically engineered cells.

From a 2010 perspective, the feasibility of using *ex vivo* genetically engineered cells for therapy in humans has been demonstrated using various cell types including tumor cells[4-6], lymphocytes and dendritic cells[7, 8], fibroblasts[9,10], hematopoietic stem cells (HSCs)[11, 12] and mesenchymal stem cells (MSCs)[13,14]. Actual or potential applications are as diverse as immuno gene therapy for the treatment of cancer[4-6], cancer therapy with T-cells expressing chimeric T-cell receptors[15, 16], the treatment of hereditary diseases[11, 12, 17-19], and a plethora of applications in regenerative medicine[20]. With the emerging field of induced pluripotent stem cells (iPS), research in genetically engineered cell therapies has reached yet another level of pace and dimension. Future clinical applications will require efficient, reliable, standardized methods for cell manipulation. For optimized reproducibility and wide practicality in a decentralized manner, such methods should comprise a minimum number of handling steps[21], be cost-effective and amenable to automation in a closed system. Such methods have been lacking so far. Upon concluding the Magselectofection project, the project partners state that the now existing Magselectofection technology platform has the potential to close the tremendous gap between clinical research applications of genetically engineered cells and the requirements for future widespread clinical use.

The project consortium has succeeded in combining nanomagnetic cell separation in one procedure with the genetic modification of cells, in particular of stem cells. Magselectofection, if carried out according to an optimized protocol, produces a high yield of selected target cells at high purity of which a high percentage is genetically modified. In particular, thus selected and modified cells display high viability and excellent biological functionality. Hematopoietic and mesenchymal stem cells that have been engineered by Magselectofection retain their pluripotent characteristics and can differentiate into multiple lineages. Magselectofected hematopoietic stem cells can reconstitute the hematopoietic compartment in mouse models. Magselectofection is a platform that comprises a minimum number of handling steps, it is simple to execute, it is efficient and it is amenable to automation. First commercial products for research use will be launched in the year 2010. A product for clinical use will be developed depending on demand.

1.1 Project Objectives

The original project objectives as detailed in the grant proposal were as follows:

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, transcriptom 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.

1.2 Project Partners

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The project partners were chosen according to their respective expertise which they could contribute to the project. At the coordinator's institution, Klinikum rechts der Isar, Technische Universität München, Christian Plank and coworkers had previously developed the Magnetofection technology. That is gene delivery enhanced and guided by gradient magnetic fields that act on gene vectors which are associated with magnetic nanoparticles. Magnetofection has been one of the two technological pillars of Magselectofection.

Prof. Gerard Wagmaker, PI at Erasmus University, has been one of the pioneers in cell therapies. He has been the coordinator of the CONSERT project which joined many of the most distinguished European researchers in gene therapy, and in particular in gene therapy with hematopoietic stem cells. Wagemaker's group has contributed to this project their protocols and experience with hematopoietic stem cell isolation, cultivation and genetic modification. Furthermore, Wagemaker's group had previously established transgenic mouse models which could be used for the validation of Magselectofection by demonstrating the functionality of magselectofected cells.

Prof. Fulvio Mavilio also counts among the European pioneers in gene therapy and has also been a member of the CONSERT consortium. Apart from his profound experience and great diversity of research expertise, a system for site-specific genomic integration copied from adeno-associated virus has been one of the biological pillars of this project.

Orit Kollet working with Prof. Tsvee Lapidot at the Weizmann Institute of Science in Israel, in this project, count among the worldwide leading cell biologists with respect to their expertise in defining stem cell biology, stem cell homing and function. Based on this expertise, they have been, together with Prof. Wagemaker and coworkers, the essential participants in this project for validating Magselectofection.

Prof. Zygmunt Pojda from the M. Sklodowska-Curie Memorial Cancer Center in Poland, affiliated with the Polish national cord blood bank, has contributed his expertise with the standardized isolation and cultivation of stem cells. He has provided some of the technology-oriented partners with stem cells has also contributed to validating Magselectofection with mesenchymal stromal cells isolated from human umbilical cord.

Ian Johnston and Michael Apel work with the worldwide market leader in nanomagnetic cell separation, Miltenyi Biotec. The MACS® cell separation technology of Miltenyi was the second technological pillars of this project. MACS® is an essential ingredient of the now established Magselectofection technology. Ian Johnston and coworkers at Miltenyi were instrumental in initially establishing Magselectofection and in subsequently upgrading the method to their automated instrument CliniMACS which is approved for clinical use.

Dr. Peter Steinlein from the well-known Research Institute of Molecular Pathology in Vienna, was supposed to contribute his expertise in gene expression profiling, once the stable integration of nucleic acids upon nonviral gene delivery in hematopoietic stem cells was achieved. As this goal could not be reached within this project, he has served as an advisor but could unfortunately not contribute experimental support.

Profs. Melania Babincova and Peter Babinec from the Physics Department of the Comenius University in Bratislava have been among the pioneers in Magnetic Drug Targeting. Their intended role in the project was establishing tracking of magselectofected hematopoietic stem cells by magnetic resonance imaging (MRI). As will be detailed below, nonviral transfection of hematopoietic stem cells turned out to be an unexpectedly difficult challenge to be partially overcome only towards the termination of the project. Hence after initial MRI studies and a shift in some objectives of the project, the major contribution from Comenius University was the design and construction of novel magnetic devices for Magselectofection configurations and a prototype of a combined magnetic cell separation and electroporation apparatus. The full potential of these developments can only be exploited in follow-up projects.

Olivier Zelphati, CEO of OZ Biosciences, contributed to this project their well standardized technologies for nonviral gene delivery from which several partners have profited during this project. Together with Miltenyi Biotec, an important role of OZ Biosciences as an SME is in the dissemination and exploitation of technology thas has emerged from this project. In fact, during this project OZB has launched several new products that at least in part have emerged from R&D at OZB and partner

laboratories during the project. In this manner, the international scientific community is able to take advantage from the Magselectofection research results. Technology emerging from this project will be made available to researchers worldwide in a well standardized manner with robust user protocols.

Finally, Joseph Rosenecker and subcontractors from Ludwig-Maximilians University in Munich count among the leading experts in Europe in gene delivery to the airways. In this context, they had a major interest in the stable genetic modification of target cells in order to achieve sustained expression of therapeutic proteins. As such, they have focused on the bacteriophage phiC31 integrase which in fact could be used for a stable genetic modification of some cell lines. They contributed to this project various constructs and knowledge on genomic integration using such constructs. In particular, they were able to produce the phiC31 integrase as a recombinant protein which they could introduce into cells using a protein transduction reagent of Partner OZ Biosciences. This research has contributed to the successful launch of OZ Bioscience's protein transduction reagent Pro-DeliverIN™.

Originally, there have been two further partners. One was Prof. Michele Calos from Stanford University who previously had done the pioneering work with the phiC31 integrase system. However, Stanford University, intended to join as a third party could not join because of incompatibilities between established Stanford policies and EU legislation. So Prof. Calos contributed her advice and nucleic acid constructs without joining the project. And in the first year, there was the legal department of the Fraunhofer Institute in Munich who together with the coordinating institution has negotiated the consortium agreement with the partners. After having completed this task, Fraunhofer desired to quit, in part due to an internal re-organisation of departments. The management duties attributed to Fraunhofer were further on taken over by the coordinator.

1.3 Evolvement of the project, achievements and backlashes

Although the grant proposal had been recommended by the reviewers for funding in early 2005, it took until May 2006 to officially commence the project. The reason for this delay was a complicated negotiation phase which was compromised by the rotation of project officers in Brussels.

The work programme has been structured in 7 workpackages and a management workpackage:

WP1: Development of Magnetoselectofection

WP2: Standardized hematopoietic stem cell isolation and nuclear targeting of nucleic acids and proteins in hematopoietic stem cells

WP3: Design and synthesis of plasmids and application of integrase technology

WP4: Evaluation of transplanted stem cells using transplantation assays

WP5: Transplantation and improved engraftment in animal models

WP6: Analysis of genomic integration sites and transcription profiling

WP7: Towards clinical studies: preclinical evaluation of efficacy and side effects

It is evident that the consortium expected to rapidly overcome technical hurdles and that it would then immediately move forward to animal models and finally to preparation for clinical studies. The whole consortium has started their work with great enthusiasm. During the first reporting period, the basic principle of Magselectofection (Figures 1, 2, 4) was established with cell lines and proof of principle was provided (WP1). New magnetic nanoparticles and magnetic and nonmagnetic transfection reagents were developed during this period. A novel protein transduction reagent was developed and launched on the reagents market. Given the vast experience of several project partners with hematopoietic stem cells, their standardized isolation and cultivation (WP2) did not represent any problem. Progress with the two integrase technologies was immediate and substantial (WP3). A screening of a whole collection of magnetic nanoparticles for magnetic resonance imaging rapidly has indentified particles suitable for this purpose. But already in the first reporting period, it turned out that nonviral gene delivery to hematopoietic stem cells is a tremendous challenge that could not be simply overcome within a short period of time. Hence, work packages 4 to 7 had to

change focus. Partners working in these areas continued their basic research with stem cells that were not genetically modified or that were engineered with lentiviral gene vectors. This resulted in important "collateral benefit" in the stem cell field in general. Also, it turned out early on that the phiC31 integrase system was not that site-specific and universally applicable as reported earlier.[22, 23] In particular it turned out that this system worked very poorly in hematopoietic cell lines.[24] Therefore, a shift in focus was envisaged by the consortium and was implemented starting in the second reporting period. Instead of only focusing on hematopoietic stem cells, the new strategy was to develop Magselectofection as a general tool for gene delivery, viral and and nonviral and for multiple cell types. The rationale for this was that many forms of cell therapies with genetically engineered cells were emerging and the notion that viral vectors, in particular lentiviral ones, would remain the most efficient vehicles for nucleic acid delivery for years to come. And as simple, standardized and at the same time economic methods for gene delivery were lacking, there was a good reason for extending the scope of this project. Therefore, the coordinator proposed a new strategy for the technology-oriented groups in the consortium on how to proceed further which is shown in the flow chart, Figure 3. From then on, the project has proceeded highly successfully.

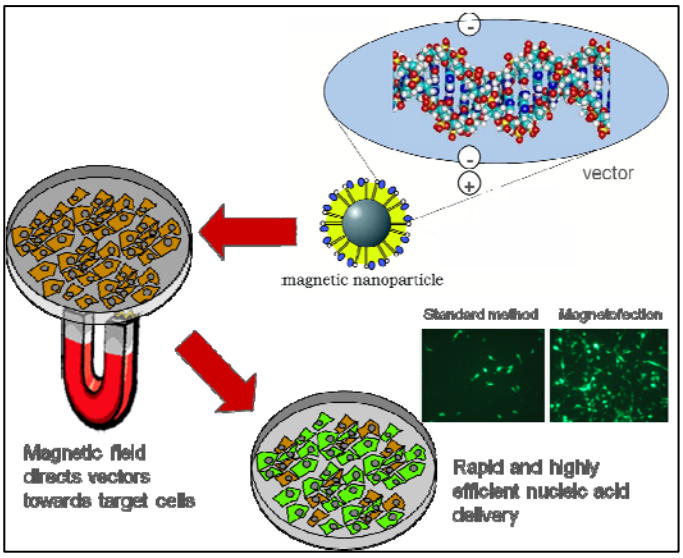


Figure 1. The principle of Magnetofection. Magnetofection has been one of the technological pillars of the Magselectofection project. Gene vectors such as naked plasmid DNA, lipoplexes or polyplexes or viral gene vectors are associated with magnetic nanoparticles. After addition to cell culture supernatants, a gradient magnetic field is applied to sediment the full vector dose on the target cells. In standard gene delivery, vectors will encounter their target cells only by the exceedingly slow process of diffusion. This very limitation is overcome by Magnetofection. Therefore, Magnetofection results in a strongly improved dose-response profile, rapid kinetics of gene delivery and a dramatic enhancement of gene transfer efficiency.

Figure 2. The principle of Magselectofection. Magselectofection combines the procedures of magnetic cell separation and Magnetofection in one integrated technology. In a mixed cell population, target cells are magnetically labeled by mixing with magnetic nanoparticles that are modified on their surface with a target cell-specific antibody (MicroBeads). In the meantime, viral or nonviral vectors are associated with a different type of magnetic nanoparticles and are loaded on a magnetic cell separation column (A). Subsequently, the column is placed in a magnetic separator and the mixed cell population is added (B). Non target cells elute through the column and only target cells are retained. During this time, only the target cells become genetically modified. In the last step (C), the column is removed from the magnet, target cells are eluted and cultivated until further use.

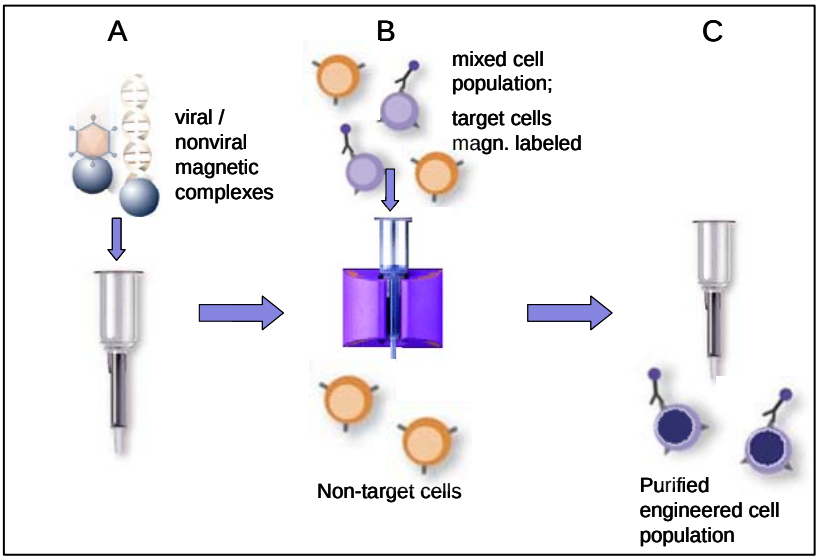
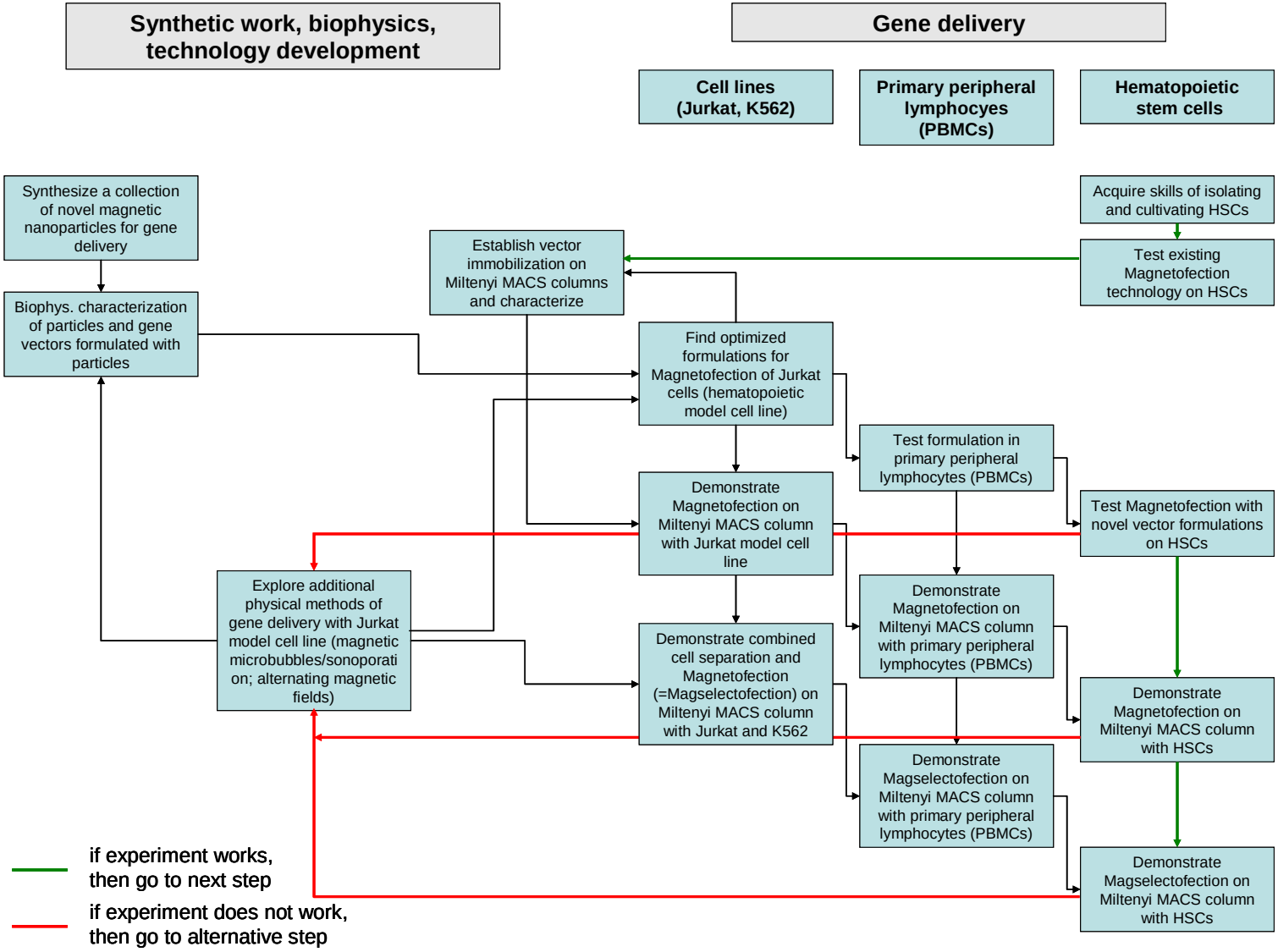


Figure 3. Experimental Strategy for Magselectofection after the first reporting period.



As mentioned, Magnetofection of cell lines on MACS® cell separation columns was well established by the end of the first reporting period. The strategy was to proceed in a stepwise manner with coordinated parallel research efforts. Magselectofection was to be firmly established with a model cell mixture. The hematopoietic cell lines Jurkat T-cells and K562 erythroleukemic were used for this purpose. In parallel, there should be a continuous effort for synthesizing improved magnetic nanoparticles and for characterizing their complexes with viral and nonviral vectors. At the same time, alternative physical methods of gene delivery for implementing Magselectofection were to be explored. This part has been taken over by Parnters 1 and 9 in the consortium. Novel magnet configurations for Magselectofection and a novel prototype for combined electroporation and magnetic cell separation have resulted from this work. And numerous improved magnetic nanoparticle types, especially for viral gene delivery were developed. At the same time, Partner 10 in the consortium, OZ Biosciences, developed novel lipid formulations for both delivering nucleic acids and proteins. From this resulted new products that were launched on the market. But in particular, one of the lipids delivered a final breakthrough with nonviral Magselectofection of HSCs. According to Figure 3, when Magselectofection was firmly established with the Jurkat/K562 model cell mixture, one would proceed with Magselectofection of primary lymphocytes. If successful, the final step would be Magselectofection of hematopoietic stem cells. Not included in the chart shown in Figure 3 were the activities of Partners 1 and 5 in applying Magselectofection to mesenchymal-like stem cells isolated from human umbilical cords (hUC-MSC). This work turned out highly successful.

Figure 4 shows results obtained with nonviral Magselectofection of the Jurkat/K562 model cell mixture. Cell recovery and cell viability were excellent (not shown). The purity of magselectofected cells was high and so was reporter gene expression. About 30% of the target cell population were transfected while the non-target cell population remained essentially unaffected. Studies with radioactive labeled DNA showed that more than 80% of the applied DNA dose became associated with the target cell population and were recovered with these cells.

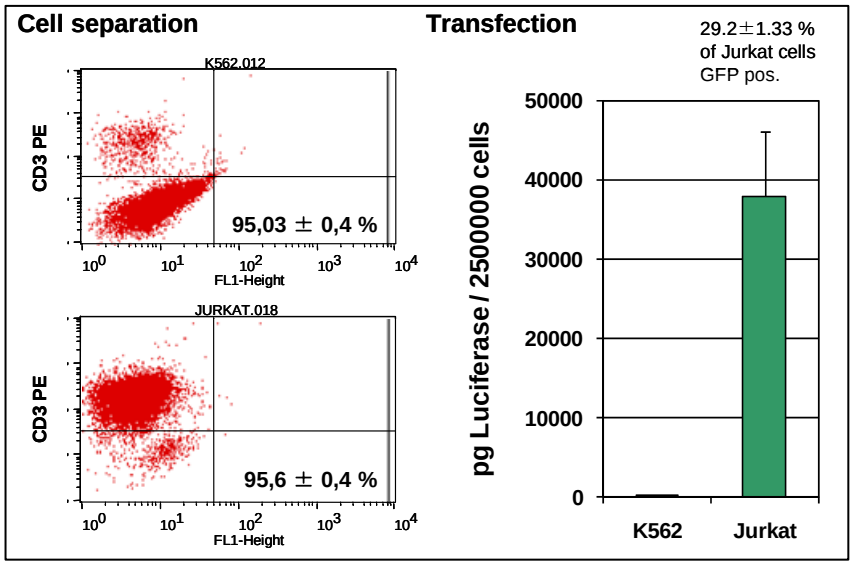


Figure 4. Magselectofection with a Jurkat/K562 model cell mixture. A mixture of 2.5 x 10^6 Jurkat T cells and 2.5 x 10^6 K562 cells was treated with CD2 MicroBeads and passed through a MACS® column which was loaded with PEI-Mag2/DF-Gold/pBLuc magnetic lipoplexes. PEI-Mag2 are magnetic nanoparticles from Partner 1 and DF-Gold is Dreamfect Gold, a cationic lipid transfection reagent from Partner 10. The $CD2^-$ cell fraction in the effluent (K562 cells) and the $CD2^+$ cells positively selected in the column (Jurkat T cells) were treated with a CD3-PE antibody and analyzed for the percentage of CD3-PE-positive cells using FACS analysis. Luciferase expression in the effluent ($CD3^-$/$CD2^-$ cells) and in the cell fraction that was magnetically selected with CD2 beads ($CD3^+$/$CD2^+$ cells) was determined 48h later. The same procedure was carried out using the green fluorescent protein reporter gene.

In parallel, Partner 6, Miltenyi Biotec, carried out Magselectofection studies with the model Jurkat and mixtures of suspension (1881) and adherent (HEK 293) cell lines and found that transfection efficiency wasacceptable but that the cell separation efficiency was disappointing. The cell separation efficiency depended strongly on the relative frequency of the target cell population in the model cell mixture. Ifthe relative frequency was high, the cell separation efficiency was acceptable. If it was low, the cellseparation efficiency was poor (Figure 5).

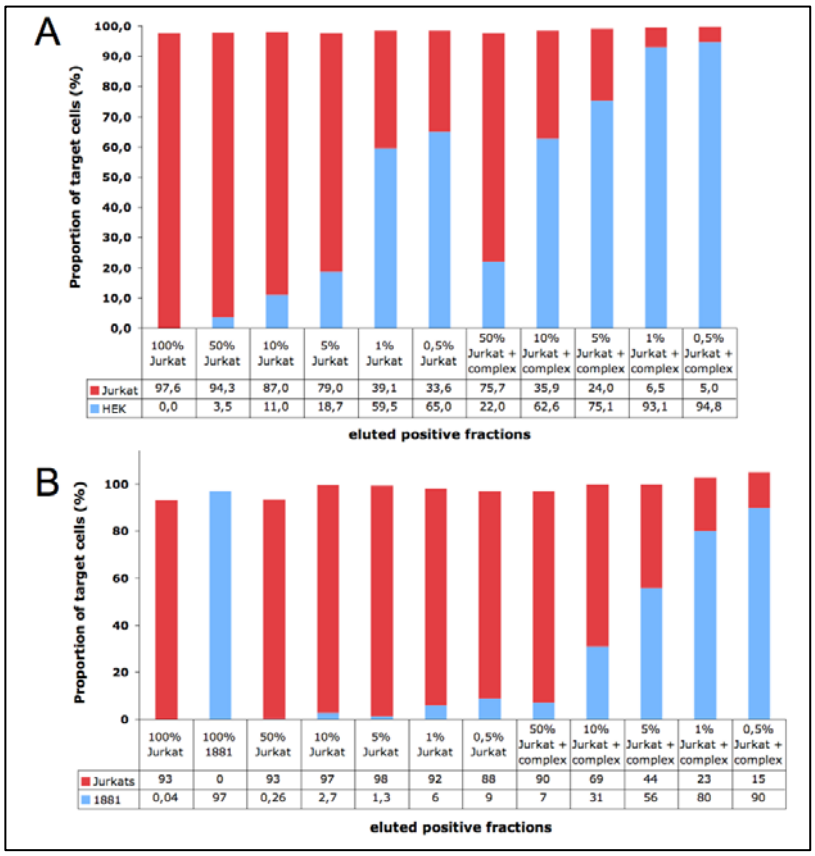
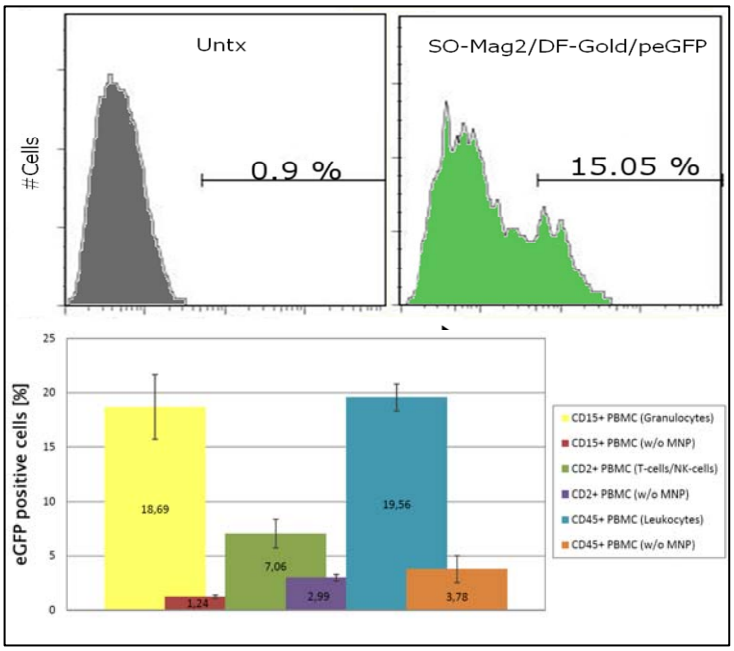


Figure 5. The presence of magnetic transfection complex on MACS columns drastically impairs cell separation performance. LS columns were either pretreated with magnetic transfection complex (20 µg pmaxGFP plasmid, 20 µg S35 particles and 80 µL Dreamfect Gold) or left untreated. Jurkat cells were labelled with CD45 MicroBeads and mixed with different proportions of CD271-expressing HEK 293 cells (A) or mouse 1881 pre-B cells (B). $1x10^6$ total cells were then loaded onto the complex-treated columns (+ complex) or untreated columns, washed with 0.5 mL RPMI and incubated for 30 minutes at room temperature. The cells were then eluted and the proportions of each cell population quantified immediately by flow cytometry after staining with CD3-PE (Jurkat cells) and CD271-APC (HEK 293 or 1881 cells) using the MACSQuant Analyzer (Miltenyi Biotec GmbH). For example in B from a mixture of 1% Jurkat/99% 1881-CD271 cells, the Jurkat cells are enriched to 92%.

Based on these results, it was concluded that a final Magselectofection procedure should comprise at least two cell separation steps. One or more pre-selection steps on unmodified cell separation columns followed by a final step on a vector-modified column. This procedure turned out highly efficient for Jurkat/K562 model cell mixtures upon nonviral and lentiviral Magselectofection, but also for mouse and human hematopoietic stem cells. But before looking at that, the applicability of nonviral and lentiviral Magselectofection to primary human peripheral blood mononuclear cells (PBMC) was examined. It turned out that a high percentage of $CD15^+$ cells could be transfected upon nonviral Magselectofection. Lentiviral Magnetofection on a magnetic cell separation column was highly efficient for $CD15^+$ cells (granulocytes) and $CD45^+$ cells (leukocytes) (Figure 6).

Figure 6. Nonviral Magselectofection and lentiviral Magnetofection of PBMCs on a MACS® column. Above: PBMCs were labeled with anti-CD15 MicroBeads. $CD15^+$cells were pre-selected on an unmodified cell separation column followed by passing through a vector-loaded column (Dreamfect Gold magnetic complex). The transfection efficiency was unexpectedly high.
Below: PBMCs extracted from whole blood were induced via the T Cell Activation/Expansion Kit from Miltenyi, mimicking antigen-presenting cells and activating resting T cells from PBMCs. After activation, PBMCs were labelled either with CD2 MicroBeads (T and NK cells), CD15 MicroBeads (Granulocytes) and CD45 MicroBeads (Leukocytes). After transduction on a lentiviral vector-loaded MACS® column, the positively selected cell fraction were collected and reactivated 24h post-transduction with the T Cell Activation/Expansion Kit. Activated and transduced cells were then incubated at 37°C; 5% CO2 in a 48-well plate and eGFP expression analysed after 48h–96h by FACS.



While the shown results do not highlight the time-consuming optimization procedures that were required for obtaining them, they clearly demonstrate that Magselectofection had become an efficient procedure for transfecting and transducing hematopoietic target cell populations already during the second reporting period. Before proceeding to hematopoietic stem cells, Partners 1 and 5 have focused on Magselectofection of hUC-MSC. This cell type is of major interest in ongoing research with respect to cell therapy applications in regenerative medicine. Again, the same procedures were carried out as described above. Pre-selected cells were passed over nonviral or lentiviral vector-loaded MACS® columns. We were interested in the transfection/transduction efficiency and in whether Magselectofection would compromise the differentiation potential of this cell type. It turned out that the transfection efficiency was high, the transduction efficiency was around 100%, the dose-response profile and cell viability were excellent and that the differentiation potential of the cells was maintained (Figures 7 and 8).

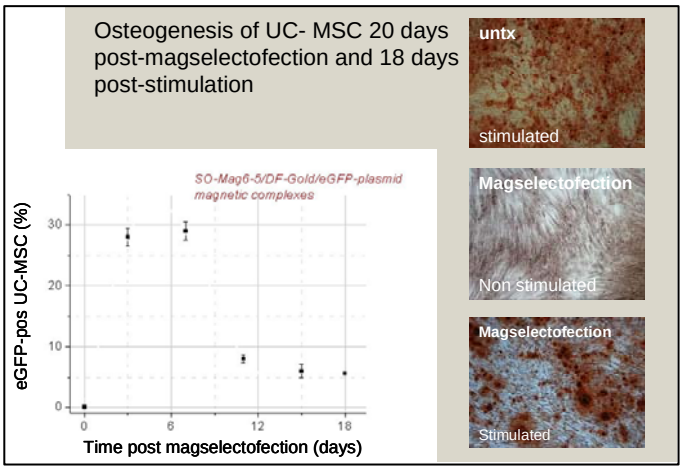
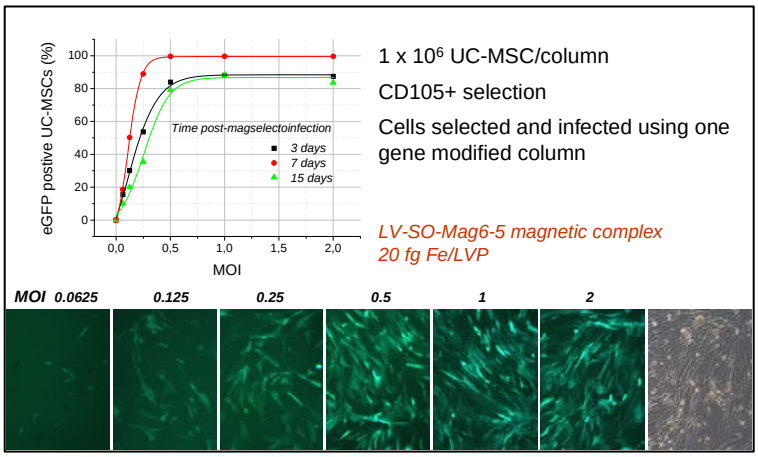


Figure 7. Nonviral Magselectofection of hUC-MSC. hUC-MSCs were labeled using CD105 MicroBeads and were magselectofected with the magnetic lipoplexes SO-Mag2/DF-Gold/eGFP. Two days after magselectofection, the cells were stimulated using an osteogenic medium, and 18 days post-stimulation, the cells were analyzed using alizarin red staining. Left: Time course of eGFP expression determined by FACS analysis. Right: Microscopy images of untreated (untx) stimulated hUC-MSCs and the non-stimulated and stimulated hUC-MSCs days after magselectofection; the bar represents 200 µm.

Figure 8. Lentiviral Magselectofection of hUC-MSC. The cells were labeled with CD105 MicroBeads and magselectofected with the lentiviral magnetic complexes SO-Mag2/LV.eGFP. Very high transduction efficiency is achieved at very low MOI. Reporter gene expression persists at a high level. Cell differentiation potential is not compromised (data not shown).



While this work was carried out, the partners at Weizmann Institute focused on the transfection of mobilized hematopoietic stem cells from human donors. For this purpose they first used nonmagnetic DNA complexes with the cationic lipid SM4-31 which was developed by OZ Biosciences during the project. Initial results reported in the second reporting period indicated that these cells got transfected at surprisingly high frequency. But propidium iodide staining indicated that the GFP positive signal originated from dead cells. Only more detailed analysis demonstrated that the cells were viable, although compromised in cell migration and proliferation. Subsequent experiments demonstrated SM4-31 transfected cells displayed normal clonogenic capacity 2 weeks after transfection, and their homing to bone marrow in a mouse model was normal. This was an essential achievement with respect to the initial objectives of the Magselectofection project and has spurred intensive efforts with Magselectofection of HSC during the last reporting period. Erasmus University and the partner from Warsaw together with Partner 1 have focused on lentiviral Magselectofection while the colleagues at Weizmann Institute continued their work with nonviral Magselectofection.

It was consistently shown that lentiviral Magselectofection is highly efficient, especially when the target cells are present at low concentration, a situation one is typically confronted with with clinical isolates. Wagemaker's group could show that under these settings, standard lentiviral transduction yields poor efficiency, while Magselectofetion is highly efficient. Furthermore, the transduced cells formed colonies in a colony forming assay (Figure 9). Similar observations were made with Sca-1$^+$ mouse hematopoietic stem cells (Figure 10).

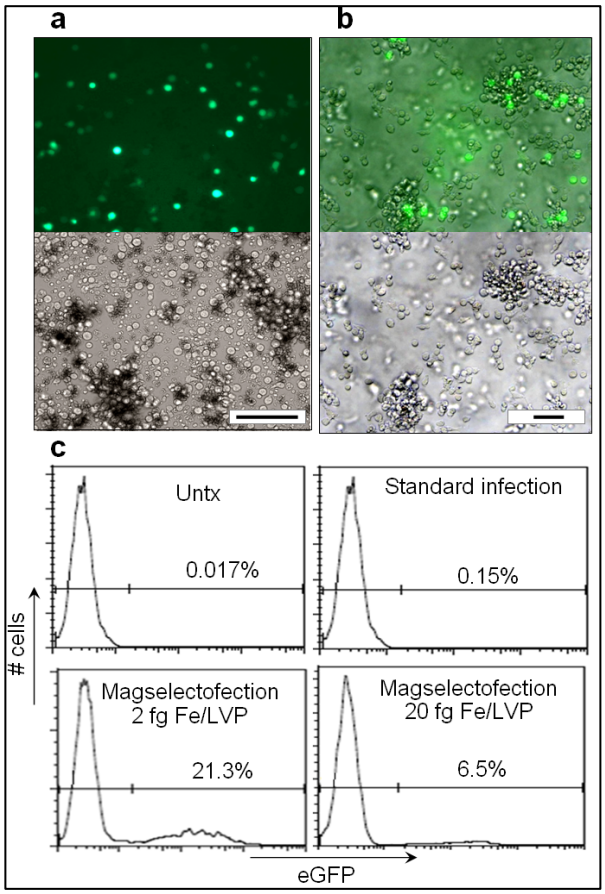


Figure 9. Lentiviral Magselectofection of human hematopoietic stem cells isolated from cord blood. hCB-HSCs were transduced at a low cell density of 1.5 x 10^5 cells/ml without cell stimulation before magselectofection. (a) Fluorescence and bright field microscopy images of the hCB-HSCs taken on day 3 after magselectofection with the magnetic complexes formulated at 2 fg Fe/VP. (b) (top) Overlay of the bright field and fluorescence microscopy images and (bottom) the bright field microscopy image of hCB-HSCs differentiated using a colony-forming assay taken 6 days after magselectofection with the magnetic complexes formulated at 20 fg Fe/VP. The bars represent 100 µm. (c) Histogram plots of the untreated hCB-HSCs (untx), cells transduced using the standard infection protocol or viral magselectofection with the complexes formulated at 2 or 20 fg Fe/VP.

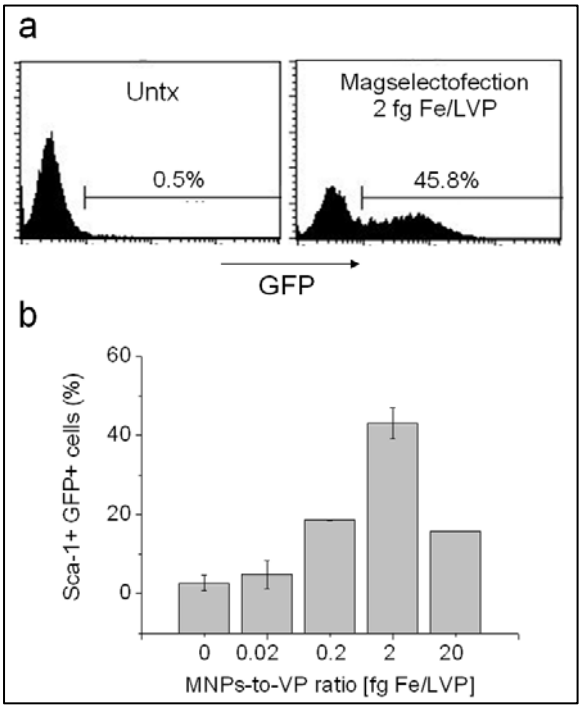


Figure 10. Magselectofection of Sca-1$^+$ mouse hematopoietic stem cells. Sca-1$^+$ cells were enriched over unmodified LS columns according to the manufacturer's protocol (Miltenyi). Subsequently, 2x10^6 Sca-1$^+$ cells were magselectofected within an MS column modified with SO-Mag2/LV-PGK-eGFP magnetic complexes formulated at different MNPs-to-VP ratios and an MOI of 3. (a) FACS data on the percentage of GFP-positive cells 6 days after magselectofection. (b) percentage of GFP-positive cells 6 days after magselectofection versus MNP-to-VP ratio.

After having optimized the lentiviral Magselectofection setup, Wagemaker's group at Erasmus could finally show that magselectofected Sca-1$^+$ mouse hematopoietic stem cells were functional in engrafting and repopulating the hematpoietic compartment in irradiated *Il2rg-/-* mice (Figure 11). At the same time, Orit Kollet and Tsvee Lapidot at Weizmann were able to demonstrate nonviral Magselectofection of mobilized bone marrow HSCs from human donors and to show their full functionality in colony formation and homing in bone marrow in NOD/SCID mice in vivo (Figures 12-14). Taken together, these achievements can be considered a final breakthrough with demonstrating the functionality of Magselectofection with hematopoietic stem cells.

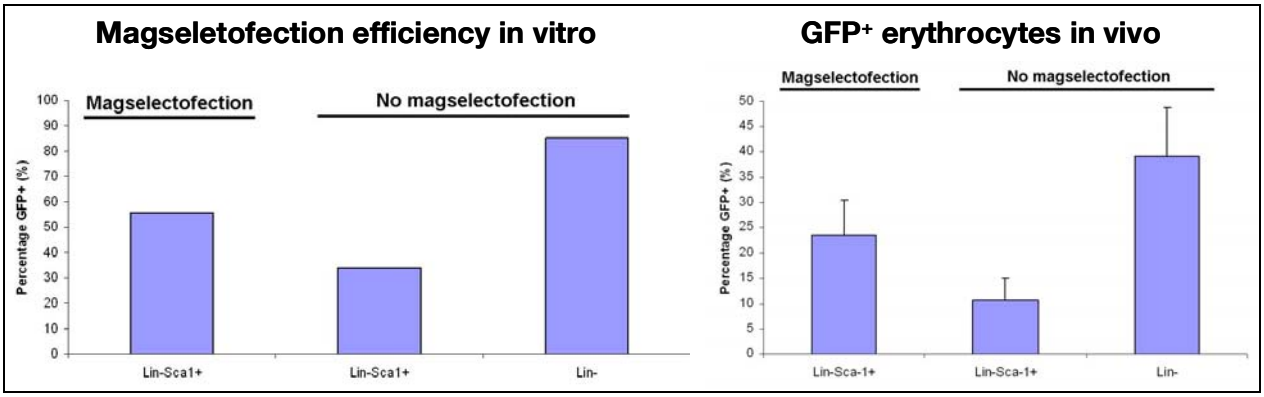


Figure 11. Magselectofection results in significant transduction as compared to standard overnight transduction (left) and yields GFP positive erythrocytes 1 month after transplantation in *Il2rg-/-* mice. Lineage depletion was performed followed by Sca-1$^+$ enrichment on a unmodified MACS® column. For Magselectofection, the Lin-Sca-1$^+$ cells were transduced in a lentiviral vector-modified column (MOI 3), released from the column and cultured overnight. For the cells that were not subjected to Magselectofection, these were run through a second unmodified column and transduced overnight during culture. The cells were subsequently transplanted into 6Gy total body irradiated *Il2rg-/-* mice. Magselectofection resulted in significant transduction and reconstitution of transplanted cells based on GFP expression in erythrocytes, which was improved compared to overnight transduction on Lin-Sca-1$^+$ cells. Transduction efficiencies are compared to Lin- cells, which were transduced overnight.

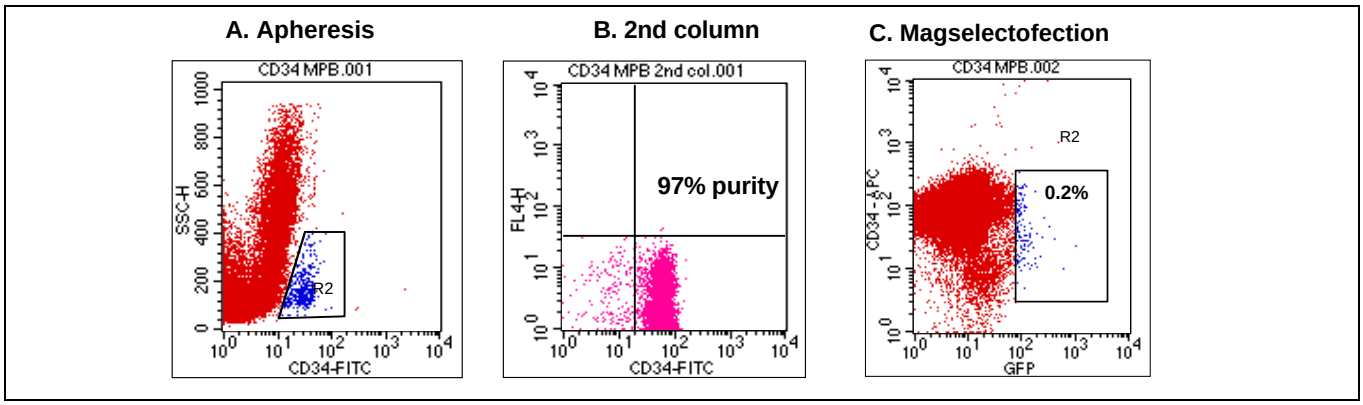


Figure 12. Isolation and nonviral Magselectofection of G-CSF mobilized CD34$^+$ progenitor cells from healthy human donors. CD34$^+$ progenitors were isolated from peripheral blood samples obtained from healthy donors that were pre-treated with standard clinic protocols with 5 daily injections of G-CSF for mobilization of hematopoietic stem cells. G-CSF-induced mobilized peripheral blood (MPB) CD34$^+$ cells were labeled with anti human CD34 Abs conjugated to magnetic beads (Miltenyi) according to the manufacturer's instructions, and subjected to magnetic separation using 2 sequential columns. When the labeled cells were loaded to the second column, transfection mix was added. The transfection mix was prepared with GFP plasmid, Dreamfect Gold transfection reagent from OZ Biosciences and magnetic nanoparticles from the Plank lab according to protocol developed by Plank Lab. The cells were left on the column for 30 minutes.
Eluted MPB CD34+ cells were seeded in 24w plate, in RPMI+10% FCS in the presence of the cytokine combinations: SCF and IL-6 (50 ng/ml) with and without Norepinephrine (1 uM). Cells were cultured for 48h and 96h.

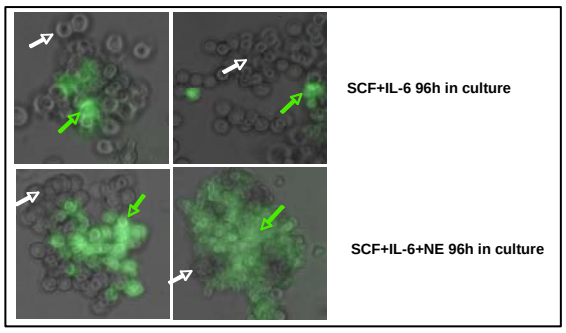


Figure 13. Colony formation by GFP-transfected MPB CD34+ enriched cells. Colonies were scored by using fluorescent microscope, that allows merging of images taken in bright field (to document all the colony cells) and dark field (to document fluorescent GFP cells). Colonies developed from cells cultured for 48h and 96h, demonstrated similar phenotype in which most of the colonies did not express any GFP signal, some were all green, and some demonstrated bi-phenotypical expression of GFP. GFP-positive cells are marked by green arrows, GFP-negative cells are marked by white arrows. In both time periods, adding NE to the cultures led to increased number and size of the GFP+ colonies. Representative images of 96h cultures are presented.

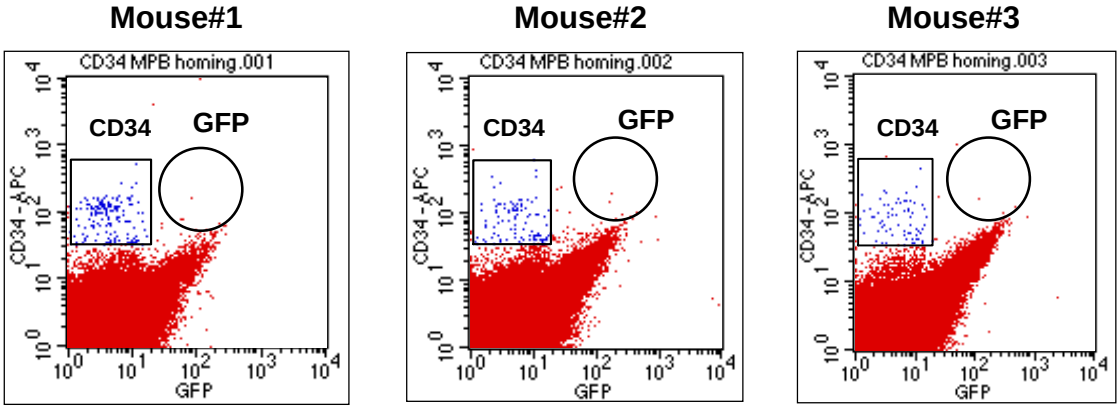


Figure 14. In vivo functional homing of enriched MPB CD34⁺ magselectofected cells to the bone marrow of immune deficient mice. Magselectofected cells were harvested and transplanted to sublethally irradiated immune deficient mice ($1x10^6$ cells/mouse), to allow functional cell homing to the murine bone marrow. 16 hours later, murine bone marrow was harvested, bones were flushed and the presence of human CD34⁺ cells and GFP signals were evaluated by flow cytometry. Three representative mice are presented. While human CD34⁺ progenitor cells could easily identified in the murine bone marrow (squares), cells positive for both CD34 and GFP were hardly observed (circles), indicating successful and functional homing of the transplant but a low expression of GFP by transfected cells in the murine bone marrow.

The above results show only the most important findings with respect to demonstrating the feasibility and functional potency of Magselectofection. The substantial collateral benefit of this project should not be neglected. Many person months have been invested in the integrase part of this project and important knowledge has been generated. For example, one task was the design and construction of plasmid vectors for site-specific integration of therapeutic genes into the human genome, based on the integrase properties of the AAV Rep78 protein. Partner FCSR has developed a two-plasmid system where, upon transfection, Rep is transiently expressed under the control of a PGK promoter and mediates integration of a transgene expression cassette flanked by AAV ITRs. The system can mediate site-specific integration at the AAVS1 locus on chromosome 19 of cassettes up to 12 kb in human cells at an overall efficiency of 5 to 15%, depending on the target cell and the size of the transgene. 50% of the integrations were site-specific. To improve the efficiency of the system, a 350-bp sequence encompassing the p5 promoter and the 5' portion of the *rep* open reading frame was added at the 5' ITR. This element contains binding sites for transcription factors such as YY1, and an additional Rep-binding element, which increases the number of copies integrated site-specifically into the human genome. To overcome the apparently rate-limiting efficiency of Rep expression in hematopoietic cells, Partner FCSR developed an alternative technology based on integration-defective lentiviral vectors (IDLV). These vectors are packaged with an integrase mutant that prevents integration of the viral genome while leaving unaffected the capacity of the virions to transduce at high efficiency human primary cells. Expression of Rep78, driven by a PGK promoter, was detected up to 10 days in infected cells, allowing a consistent increase in the efficiency of site-specific integration.

Major activities were also dedicated to developing the phiC31 integrase system for the purposes of Magselectofection. Although still not sufficiently functional in this context, several publications have resulted from this work.[24-26] As it was necessary for Magselectofection to develop novel and optimize existing gene transfer technology, this project has also contributed to progress with viral and nonviral vector systems in general. This has been published.[4, 24-64] Also, it should be noted that this project contributed via Partner FCSR to the development of a high-throughput technology based on LM-PCR and pyrosequencing was developed to map genome-wide the non specific, Rep-mediated integrations in the human genome. And the project has also contributed via the Wagemaker group to the development of a software that links integration sites to functionality of neighboring genes, expression levels and other parameters characterizing integration sites. The methodology of the analysis of gene expression levels to be implemented is currently under study. This program will be made available to the project partners as a tool in conjunction with high throughput sequencing of integration sites.

1.4 Future perspectives

Miltenyi Biotec in collaboration with the Plank group, Partner 1, has started upgrading Magselectofection to the CliniMACS instrument. Both nonviral and lentiviral Magselectofection was performed successfully in this setting. However, transfection/transduction efficiency and target cell recovery still need to be optimized in this large scale system. Figure 15 shows a semi-automated setup for Magselectofection on CliniMACS and Figure 16 shows preliminary results that have been obtained with lentiviral transduction of hUC-MSC on this instrument in comparison with results obtained upon manual Magselectofection on Miltenyi LS cell separation columns.

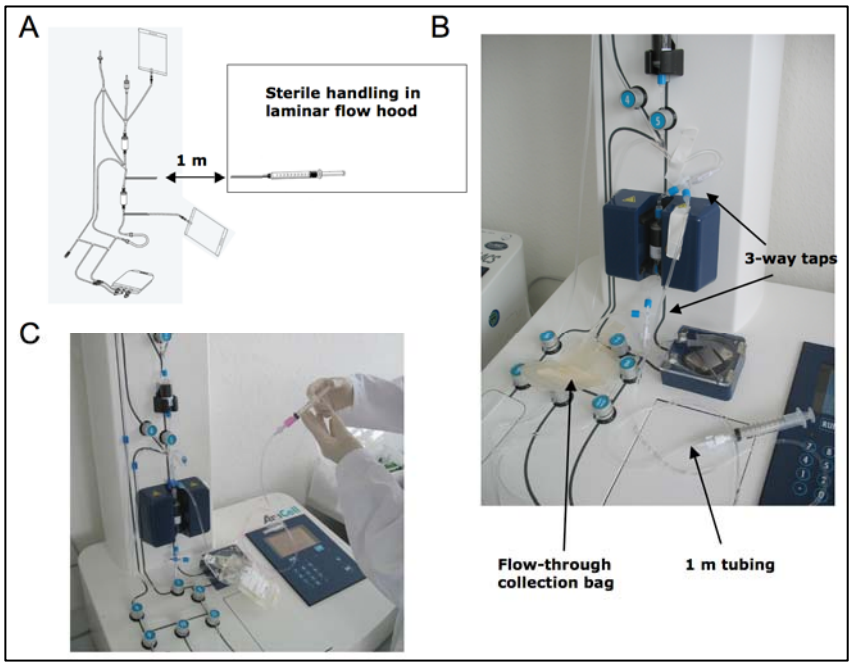


Figure 15: CliniMACS Tubing Sets for Magselectofection: prototype 2. (A, B) CliniMACS Tubing Set modified with 3-way tap, collection bag and 1 m of tubing to allow sterile, in-process application of magnetic transfection complex. (C) Application of pCMV-K^k, S35 magnetic particle, DreamFect Gold transfection complexes to 2.5x10^7 CD45 MicroBead-immobilised Jurkat cells.

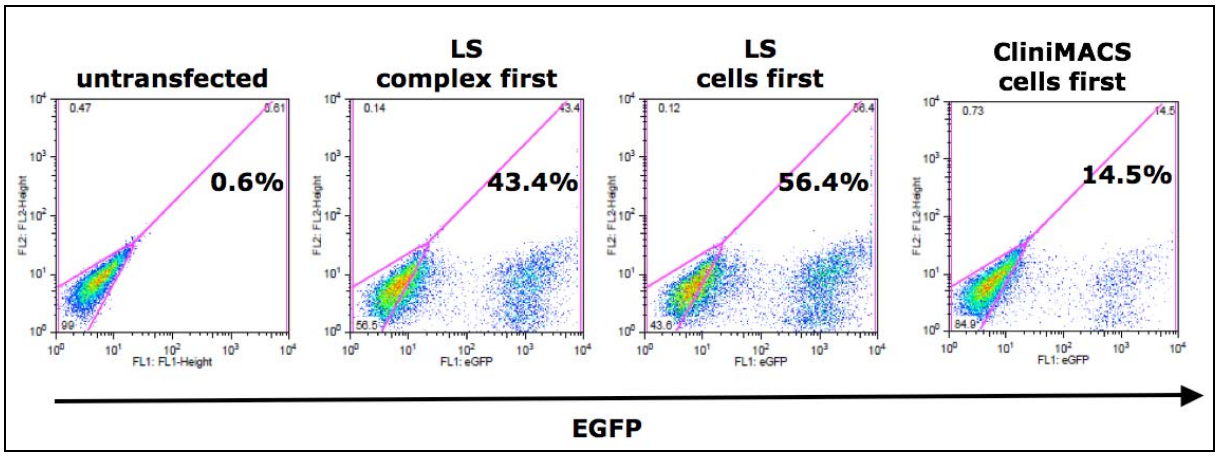


Figure 16: Lentiviral magselectofection of primary UC-MSCs in the CliniMACS. 2.5x10^6 UC-MSCs were labelled with CD105 MicroBeads and magselectofected on LS columns using the standard protocol (complex added first) or with the reverse protocol (cells added first). 1x10^7 UC-MSCs, labelled with CD105 MicroBeads were used for the CliniMACS magselectofection on prototype 2 CliniMACS Tubing Sets. The magnetic lentiviral transduction complexes were generated using S35 PEI beads at a ratio of 20 fg Fe per infectious lentiviral particle and 1 infectious virion was applied for each target cell (moi=1). 2 days post-magselectofection, the cells were analysed for GFP expression by flow cytometry.

Magselectofection on the smaller scale manual cell separation columns is fully functional. The novel technology will yield products in the near future for research applications which will greatly facilitate the daily work of researchers working in cellular engineering. There is a realistic perspective that modules of the Magselectofection technology will enter clinical use as an essential tool in the production of genetically engineered effector cells. The therapeutic use of such cells may be as widespread as cancer therapy, gene therapy of hereditary diseases, therapy of metabolic diseases, tissue engineering and regenerative medice.

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2. Dissemination and use

Section 1 – Exploitable Knowledge and its use

This has been a publicly funded project. Thus the highest ranking objective of the dissemination plan has been making results from this project accessible to the scientific community and the interested public. This has been done by publication of the results in scientific journals, presentation at scientific meetings, posting on the project web page and on the individual partners' home pages, exchange of results with members of the CONSERT project (coordinated by Gerard Wagemaker from Partner 2). Press releases by the individual partners are encouraged. Every partner has their own existing collaboration networks, which is another basis for the rapid dissemination of knowledge.

This project has a main focus on technology development. In this respect, the participation of commercial partners has been instrumental. Miltenyi Biotec is the market leader in magnetic cell separation and is interested in Magselectofection as a novel product and so is OZ Biosciences. The participation of these partners will greatly facilitate and expedite market access and penetration for the novel technology.

Commercially exploitable knowledge and its use

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
1. How to combine magnetic cell separation and nucleic acid delivery	Magselectofection technology	1. Life sciences 2. Biomedical research 3. Clinical research 4. Medical	Probably 2011	Patent on Magnetofection IPR from Miltenyi on mag. cell sep.	Partic. 1, 6, 10
2. Knowledge on how to prepare specific magn. nanoparticles	Magnetic nanoparticles	1. Life sciences research (cell separation, gene delivery) 2. Medical (MRI contrast agents) 3. Medical (drug carrier)		Trade secret	Partic. 1, 6, 10
3. Transfection technology	DreamFect Gold Research reagent Lipid SM4-31	Life sciences research	2007 2008	No patent is planned for this product Patent was filed on December, 19th 2007. FR N° 07/08861	Partic. 10
4. Magnetofection	ViroMag R/L Research reagent for viral application	Life sciences research and medical	2006	Magnetofection patent	Plank & Bergemann Partic. 10
5. Protein delivery system:	Pro-DeliverIN Research reagent Ab-DeliverIN Research reagent	Life sciences research	2007	Patent was filed on December, 19th 2007. FR N° 07/0886107	Partic. 10

Explanations

Partners involved in the (potential) exploitation are identical with the ones listed in the overview table. Together with the knowledge about manufacturing suitable magnetic nanoparticles, the knowledge of how to combine magnetic cell separation and nucleic acid delivery is the key knowledge for performing Magselectofection. This knowledge will be published and will also be part of the application notes of commercial Magselectofection kits. The knowledge is protected by patents or patent applications as listed in the table. However, a more essential protection is trade secrets on the manufacturing of magnetic nanoparticles which are in the possession of OZ Biosciences and independently of Miltenyi Biotec. Non-commercial exploitation will be by the scientific community

who will use this published knowledge for their research purposes. Commercial exploitation will be by the project partners Miltenyi Biotec and OZ Biosciences. The first product to be launched will be magnetic nanoparticles for Magselectofection together with a protocol for performing Magselectofection. OZ Biosciences and Miltenyi will negotiate an agreement for commercialization of a complete Magselectofection kit, comprising magnetic cell separation columns and a separator magnet from Miltenyi and magnetic nanoparticles and optionally a nucleic acid from OZ Biosciences. All of this may be suitable for licencing, for example under OEM contracts, if economically meaningful.

Market considerations are too early for Magselectofection technology. However, in regenerative medicine alone, Magselectofection meets a billion dollar market. As a truly enabling technology, Magselectofection may be worth several hundred million Euros over a product cycle. The listed OZ Biosciences products have been launched already (Exploitable Knowledge 3 – 5). Such products can bring revenues of several hundred thousand Euros per year within a few years of market presence if successful in the hands of customers. Substantial additional work is required to exploit the full potential scope of Magselectofection products, in part as outlined in this report.

Section 2 – Dissemination of Knowledge

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Online since May 2006	Project webpage www.magselectofection.eu	Project partners, public	worlwide	?	Partner 1
19.6.05	Press release	Press, public	Germany, worldwide		Partner 1
18. or 25.9.05	Newspaper article (Verdens Gang)	Public	Norway		Partner 1
2.8.05	Newspaper article (Münchner Merkur)	Public	Germany	thousands	Partner 1
17.8.05	Newspaper article (Süddeutsche Zeitung)	Public	Germany	Thousands	Partner 1
25. 08.05	TV Broadcast (ARD Mittagsmagazin)	Public	Germany	thousands	Partner 1
13.6.06	Magazine article (Apotheken Umschau)	Public	Germany	Millions (accoring to publisher)	Partner 1
Nov. 2006	Clinigene satellite meeting at ESGT congress Athens	Research	Europe	50	All partners, repres. by Partner 1
May. 2006	6th international conference: scientific and clinical applications of magnetic carriers	Research	World wide	500	Partner 10
December 2006	46th American Society of Cell Biology Meeting	Research	World wide	7000	Partner 10
April 2006	Experimental Biology 2007 annual conference	Research	World wide	15000	Partner 10
Feb. – April 2007	Press Release	General and research	Worldwide		Partner 10
Feb. 2007	Flyers DreamFect Gold	Research	Worldwide		Partner 10

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
April 2007	Flyers Pro-DeliverIN	Research	Worldwide		Partner 10
April-May 2007	Experimental Biology 2007 annual conference	Research	World wide	15000	Partner 10
May 2007	Sc Clin App Mag Nanocarr	Nanotechnology	Worldwide	600	Partner 1
May-June 2007	ASGT 2007	Research / Pharmaceutical	Worldwide	2000	Partner 1, 2, 3, 11
November 2007	ESGT 2007	Research / Pharmaceutical	Worldwide	1000	Partner 1, 2, 3, 11
January–June 2008	Junior Citizen Panel on Nanomedicine	Juveniles 16 to 25 years old	Germany	100	Partner 1
May-June 2008	ASGT 2008	Research / Pharmaceutical	Worldwide	2000	Partner 2, 3, 10, 11
October 2008	ESGT 2008	Research / Pharmaceutical	Worldwide	1000	Partner 1, 2, 3, 11
April 2009	AACR annual conference	Research / Pharmaceutical	World wide	16000	Partner 10
May 2009	ASGT annual conference 2009	Research / Pharmaceutical	Worldwide	4000	Partner 10
Novemerb 2009	ESGT 2009	Research / Pharmaceutical	Worldwide	1000	Partner 1, 2, 3, 11

Commercial presentations of OZ Biosciences at the following conferences

- 6th international conference: scientific and clinical applications of magnetic carriers, May17-20, Krems (Austria). Presentation of the Magnetofection technology
- 46th American Society of Cell Biology 2006. December 10-13 San Diego (USA). Presentation & exhibits of the Magnetofection and lipid-based transfection reagents
- Experimental Biology 2007, annual meeting, April 28-30, Washington DC (USA). Presentation & exhibits of DreamFect Gold & Pro-DeliverIN reagents
- February to April 2007, press release for the launching of DreamFect Gold (promotion in Sciences, Nature, Biotechniques, GEN, gazette du laboratoire and Journal of Biological Chemistry).
- American Society of Gene Therapy 11th Annual Meeting May-June 2008, Presentation & exhibits of Magnetofection technology especially ViroMag (Partner 10). Abstracts, other partners.
- ESGT: Annual meeting of the European Society of Gene and Cell Therapy, October 27-30, 2007, organized by Partner 2. Abstracts.
- 6th international conference: scientific and clinical applications of magnetic carriers, May17-20, Krems (Austria). Presentation of the Magnetofection technology
- The 100th Annual Meeting of the American Association for Cancer Research (AACR), 18-22 April 2009, Denver (USA). Presentation & exhibits of DreamFect Gold, ViroMag R/L & Pro-DeliverIN reagents
- American Society of Gene Therapy (ASGT) 12th Annual Meeting 27-30 May 2009, San Diego (USA). Presentation & exhibits of Magnetofection technology especially ViroMag, DreamFect Gold, & Pro-DeliverIN reagents.

Scientific presentations at conferences

- Niek P. van Til, Carla J.L. Oerlemans-Bergs, Fatima S.F. Aerts Kaya, Trudi P. Visser, Gerard Wagemaker, Monique M.A. Verstegen. Cell-density and viral vector load are determinant factors for ex vivo lentiviral transduction efficiency of hematopoietic stem cells. Am. Soc. Gene Therapy, Seattle WA, USA, May 30-June 3, 2007.
- Gerard Wagemaker. Hematopoietic stem cells as targets for gene therapy of inherited diseases. Hemopoietic Stem Cells Transplantation. International Meeting dedicated to the memory of Raisa Gorbacheva. St-Petersburg, Russia, September 21-22, 2007

- Huston M, Horsman S, Brugman MH, Stubbs A, van Til NP, de Ridder D, van der Spek P, Wagemaker G. MAVRIC: a web based tool for retroviral vector integration site analyses. XVth Annual Congress Eur. Soc. Gene and Cell Therapy, Rotterdam, October 27-30, 2007.
- Huston M, van Til NP, Visser TP, Wagemaker G, MMA Verstegen. Lentiviral transfer of the human IL2Rγ gene into lineage depleted hematopoietic cells results in efficient phenotypic correction of IL2Rγ-/- mice. XVth Annual Congress Eur. Soc. Gene and Cell Therapy, Rotterdam, October 27-30, 2007.
- Gerard Wagemaker. Hematopoietic stem cell gene therapy in inherited disorders. Seminar given at the Hadassah Medical center, Jerusalem, Feb. 5, 2008; at Schneider Children's Hospital, Tel Aviv, February 27, 2008; at The Chaim Sheba Medical Center, Tel Hashomer, March 3, 2008; at the Weizmann Institute of Science, Rohovot, Israel, April 10, 2008.
- Gerard Wagemaker. Gammaretroviral vector integrations (RVIS) relate to hematopoietic gene expression patterns. Seminar at HSR-TIGET, Milan, Italy, April 7, 2008.
- Huston M, van Til NP, Visser TP, MMA Verstegen, Wagemaker G. Lentiviral transfer of the human IL2Rγ gene into lineage depleted hematopoietic cells results in efficient phenotypic correction of IL2Rγ-/- mice. ASGT 11th Annual Meeting, May 28 - June 1, 2008, Boston, Massachusetts, USA
- Huston M, Horsman S, Brugman MH, Stubbs A, van Til NP, de Ridder D, van der Spek P, Wagemaker G. MAVRIC: a web based tool for viral vector integration site analyses. ASGT 11th Annual Meeting, May 28 - June 1, 2008, Boston, Massachusetts, USA
- Van Til NP, Sarwari R, Aerts Kaya F, Visser T, Verstegen MMA, Wagemaker G. Safety assessment in murine recipients of hematopoietic cells transduced with gammaretroviral or lentiviral vectors: evidence for significantly reduced genotoxicity of SIN lentiviral vectors. ASGT 11th Annual Meeting, May 28 - June 1, 2008, Boston, Massachusetts, USA
- Gerard Wagemaker. Hematopoietic stem cells as targets of gene therapy for inherited diseases - the next generation of applications. Second Raisa Corbacheva Memorial International Meeting on Hemopoietic Stem Cell Transplantation. St Petersburg, Russia, September 2008
- Gerard Wagemaker. Hematopoietic stem cell gene therapy. Keynote Lecture Magselectofection Contractor's Meeting, Rehovoth, Israel, September 2008.
- Gerard Wagemaker. Risks and Risk Perception. International Symposium "The Ethics of Gene Therapy in Inherited Diseases", Brugge, Belgium, November 2008
- Gerard Wagemaker. Hematopoietic stem cell gene therapy. VIth National Symposium Biological Basis Of Treatment Of Oncological And Hematological Diseases. Moscow, January 2009
- Gerard Wagemaker. Hematopoietic stem cell gene therapy – an update. Plenary Lecture, Hacettepe Children's Hospital, Ankara, Turkey, February 2009.
- Niek van Til. Lentiviral gene therapy of murine hematopoietic stem cells using a native *IL2RG* promoter region corrects the SCID-X1 phenotype. Voorjaarsvergadering NVGT 2009. Nijmegen, May 2009
- Huston M, van Til NP, Visser TP, Sawari R, Verstegen MMA, Wagemaker G. Lentiviral gene therapy of murine hematopoietic stem cells using a native *IL2RG* promoter region corrects the SCID-X1 phenotype. 12th Annual Meeting of the American Society of Gene Therapy, May 27 - 30, 2009, San Diego, California
- Gerard Wagemaker. A Next Generation Of Therapeutic Approaches To Inherited Disorders: Gene Therapy Of Hematopoietic Stem Cells. Biotechnology 2009, Stem Cell Conference. Tel Aviv, Israel, June 2009.
- Vlaskou, O. Mykhaylyk, R. Giunta, I. Neshkova, N. Hellwig, F. Kroetz, C. Bergemann, and C. Plank. Magnetic Microbubbles: New Carriers for Localized Gene and Drug Delivery. Abstract to the Ninth Annual Meeting of the American Society of Gene Therapy, Baltimore, Maryland, May 31-June 4, 2006**.** Molecular Therapy. 13:S290 (2006).
- C. Plank, "Combined isolation and stable nonviral transfection of hematopoetic stem cells – a novel platform technology for ex vivo hematopoetic stem cell gene therapy". Clinigene Satellite Meeting ESGT 2006, November 9-10, 2006, Athens, Greece.

- Y.S. Antequera, O. Mykhaylyk, E. Hammerschmid, and C. Plank. Magselectofection - Combined magnetic cell separation and magnetofection. Abstract to the XVth Annual Congress of the European Society of Gene and Cell Therapy. 27–30 October, ***2007***, Rotterdam. Human Gene Therapy. 18:1048-1048 (2007).
- Plank, O. Mykhaylyk, H. Perea, J. Aigner, A. Steingoetter, and R. Botnar. Nucleic acid delivery to magnetically labeled cells and its potential to modify cell engraftments. Abstract to the XVth Annual Congress of the European Society of Gene and Cell Therapy. 27–30 October, ***2007***, Rotterdam. Human Gene Therapy. 18:1059-1059 (2007).
- C. Plank, "Localized Nucleic Acid Delivery Using Magnetic Nanoparticles and Magnetic Force". NanoBionics IV - from Molecules to Applications. Marburg (Germany), September 17-21, 2007.
- Sauer, A.; de Bruin, K.; Plank, C.; Braeuchle, C. Internalization dynamics and localization of magnetic transfection complexes.Abstract to the XVIth Annual Congress of the European Society of Gene and Cell Therapy, Brussels, Belgium, November 12-16. 2008. Human Gene Therapy 2008, 19, (10), 1151-1151.
- Mykhaylyk, O.; Vlaskou, D.; Hammerschmid, E.; Zelphati, O.; Resenecker, J.; Plank, C. siRNA Magnetofection in vitro. Abstract to the XVIth Annual Congress of the European Society of Gene and Cell Therapy, Brussels, Belgium, November 12-16. 2008. Human Gene Therapy 2008, 19, (10), 1170-1170.
- Antequera, Y. S.; Cengizeroglu, A.; Hammerschmid, E.; Mykhaylyk, O.; Plank, C. Magselectofection - combined magnetic cell separation and magnetofection: A novel non-viral and viral technology for ex-vivo gene therapy. Abstract to the XVIth Annual Congress of the European Society of Gene and Cell Therapy, Brussels, Belgium, November 12-16. 2008. Human Gene Therapy 2008, 19, (10), 1145-1146.
- C. Plank, "Localized nucleic acid delivery using magnetic nanoparticles and magnetic force." UK Magnetics Society - 22nd Ewing Event and Afternoon Seminar - 9 December 2008.
- C. Plank, "Combined isolation and stable nonviral transfection of hematopoietic stem cells – a novel platform technology for ex vivo hematopoetic stem cell gene therapy."Symposium on Recombinase-based non-viral gene transfer. Satellite meeting of the XVIth Annual Congress of the European Society of Cell and Gene Therapy, Bruges, Belgium, Nov. 13-16, 2008.
- C. Plank, "Localized nucleic acid delivery using magnetic nanoparticles and magnetic force".Nanosens 2008, 29.-30.9.2008 Tech Gate Vienna. Organizer: Austrian Research Centers.
- C. Plank, "Localized nucleic acid delivery using magnetic nanoparticles and magnetic force". Colloidal Nanoparticles - From Synthesis to Biological Applications. University of Marburg, 15.9.-17.9. 2008.
- C. Plank, "Localized nucleic acid delivery using magnetic nanoparticles and magnetic force".ICONSAT 2008 - International Conference on Nano Science and Technology. February 27-29, 2008, Chennai, India. Convenor: Prof. Baldev Raj,Distinguished Scientist & Director Indira Gandhi Centre for Atomic Research.
- C. Plank, "Nanomagnetic Delivery of Innovative Therapeutics". 3rd Annual Global Symposium on Nanobiotechnology. California NanoSystems Institute (CNSI), University of California, Los Angeles, November 19-29, 2009.
- C. Plank, "Recent advances with magnetically guided nucleic acid delivery (Magnetofection)". Fifth National Nanoscience and Nanotechnology Symposium, Anadolu University, June 8 - 12, 2009 in Eskisehir, Turkey.
- C. Plank, "Nanomagnetic Delivery of Innovative Therapeutics". ICONSAT 2010 - International Conference on Nano Science and Technology. February 17-20, 2010, Mumbai, India. Keynote lecture.
- Schajnovitz A, Itkin T, Ludin A, Kalinkovich A, Lapid K, Seger R, Lapidot T. Functional SDF-1 Secretion from BM Stromal Cells Is a Cell Contact-Dependent Event Mediated by Cx43 and Cx45 Gap-Junctions. Abstract of this study was presented in the annual meeting of the American Society of Hematology (ASH, San Francisco, USA, 08) as oral presentation, and in the coming

annual meeting of the Israel Stem Cell Society (ISCS, Tel Aviv, June 09), also selected for oral presentation.

- A.KRAFČÍK, M. BABINCOVÁ, AND P. BABINEC, Analýza trajektórií magnetických nanočastíc v magentických poliach pre magentickú separáciu buniek, cielenie liečiv a génovú terapiu. Presented as a poster in PREVEDA VIRTUAL CONFERENCE, May 2009, Bratislava, Slovakia, http://konferencia.preveda.sk/forum/downloads/abstrakty/Krafcik.pdf.
- A.KRAFČÍK, M. BABINCOVÁ, AND P. BABINEC, Analysis of trajectory of magnetic particles in magnetic fields for cell separation and transfection, p. 58, CECE 2009, 6th International Interdisciplinary Meeting on Bioanalysis, November 5 – 8, 2009, PÉCS, HUNGARY.
- Carsten Rudolph: "Targeting gene delivery for lung cancer treatment", 8th International Conference and Workshop on Biological Barriers - in vitro Tools, Nanotoxicology, and Nanomedicine, Saarbrücken, 22.03.2010
- Carsten Rudolph: „Nichtvirale Integration von Genen zur Behandlung von Lungenerkrankungen, BioFuture Tagung, Berlin, 27.01.2010
- Carsten Rudolph: "Therapie des angeborenen Surfactant Protein B-Mangels durch Inhalation von Nukleinsäuren", Seminar im Hämatologikum des Helmholtz-Zentrums München, 26.11.2009
- Carsten Rudolph: "PhiC31 vector - Cell type-dependent activity of the Streptomyces bacteriophage phiC31integrase", Symposium on Recombinase-based nonviral gene transfer, November 12, 2008, Brugge, Belgium
- Carsten Rudolph: "Nucleic acid treatment for functional correction of Surfactant Protein B deficiency and Cystic Fibrosis", Joint of German Society for Gene Therapy (DGGT) and the European Society of Gene and Cell Therapy (ESGCT), Hannover, 24.11.2009
- Carsten Rudolph: "Physicochemical methods for gene delivery, part II", Joint of German Society for Gene Therapy (DGGT) and the European Society of Gene and Cell Therapy (ESGCT), Hannover, 21.11.2009
- Carsten Rudolph: "Nucleic Acid Delivery and Targeting to the Lung", Knowledge-Forum Merck KGaA, Darmstadt, 05.08.2009
- Carsten Rudolph: "Genomic integration for long-term gene expression using nonviral gene delivery systems", SMi Quantitative Polymerase Chain Reaction and Transcriptional Profiling, London, UK, 15th & 16th June 2009
- Carsten Rudolph:"Therapie des angeborenen Surfactant Protein B-Mangels durch Inhalation von Nukleinsäuren", Pneumologie Symposium, München, 25.04.2009
- Carsten Rudolph: "Targeted delivery of nonviral gene vectors to the lung", Drug Delivery Summit, London, UK, 02.09.2009
- Carsten Rudolph: „Targeting of nucleic acids to the lung", Seminar in Pharmaceutics, Universität des Saarlandes, Saarbrücken, 13.01.2009
- Carsten Rudolph: „Perspektiven der Nanomedizin in der Pneumologie", XV. Deutsches Aerosol Therapie Seminar, 08.11.2008, Marburg
- Carsten Rudolph: „Targeting of gene vectors to the lungs", Meeting of the Cluster of Excellence "Nanosystems Initiative Munich", Münsing am Starnberger See, 06.11.2009
- Carsten Rudolph: "Targeted Gene Delivery to the Lung", Annual Meeting of the British Society of Gene Therapy, Edinburgh, 08.04.2008
- Carsten Rudolph: "Strategies to improve gene delivery to the lung using biological and physical targeting mechanisms", Oxford Gene Therapy Seminar Series, Oxford, 27.03.2008
- Carsten Rudolph: „Untersuchungen zur kontrollierten genomischen Integration des humanen Surfactant Protein B- (hSP-B) Gens mittels nichtviraler Gentransfersysteme im Mausmodell", BioFuture Tagung, Berlin, 29.01.2008
- Carsten Rudolph: „Optimierungsstrategien für die Aerosolapplikation DNS-haltiger Nanopartikel", Friedrich Löffler Institut, Tübingen, 01.08.2007

- Carsten Rudolph: „Gentherapie bei Surfactantprotein-B-Defizienz - eine realistische Vision?“ Jahrestagung der Gesellschaft für Neonatologie und pädiatrische Intensivmedizin, 15.06.2007 Hamburg
- Carsten Rudolph: „SP-B Defizienz: Mutationen, Klinik – kann Gentherapie hier wirklich helfen?“, Jahrestagung der Gesellschaft für pädiatrische Pneumologie, München, 22.03.2007
- Carsten Rudolph: “PhiC31 integrase mediated long-term gene expression in lung cells in vitro and *in vivo*”, The 14th Annual Congress of the ESGCT, Athens, Greece, 9-12 November 2006
- Carsten Rudolph: Berliner Gentherapie-Seminar der Charité und Max-Planck-Gesellschaft, "Langanhaltende Genenexpression in der Lunge mittels nichtviraler Gentransfersysteme" 01.11.2006
- Carsten Rudolph: Seminarreihe der Klinischen Chemie und Klinischen Biochemie der Medizinischen Fakultät der Ludwig Maximilians Universität, "Aerosolapplikation synthetischer Gentransfercarrier im Mausmodell", 19.09.2006
- Carsten Rudolph: 4th JOINT WORKSHOP “Transplantation and stem cell research”, OPBG (Roma) & Dr. von Haunersches Kinderspital (Munich), May 12th, 2006, „ Aerosol gene delivery – targeting of broncho-alveolar stem cells”
- Carsten Rudolph: 4. Jahrestagung des Tierschutz-Informations-Zentrums für die Biomedizinische Forschung der Medizinischen Fakultät München - Stammzellforschung - Onkologie - TiermodelleTransgene Tiere und Versuchstierbelastung, München: „Gentransfer in Stammzellen - Eine Strategie zur Therapie von Erbkrankheiten“

Project-related published abstracts and papers of the consortium

1. Cédric Sapet; Nicolas Laurent ; Loïc Le Gourrierec ; Séverine Augier and Olivier Zelphati. *In vitro* and *in vivo* Magnetofection: a move towards gene therapy. Annales de Biologie Clinique 2010 In press
2. Guilbaud M., Chadeuf G., Avolio F., Francoise A., Moullier P., Recchia A., Salvetti A. (2008). Relative influence of the adeno-associated virus (AAV) type 2 p5 element for recombinant AAV vector site-specific integration. J Virol. 82:2590-2593.
3. Pike-Overzet K, Burg M van der, Wagemaker G, Dongen JJ van, Staal FJ. New Insights and Unresolved Issues Regarding Insertional Mutagenesis in X-linked SCID Gene Therapy. *Mol Ther 2007;15:1910-1916*
4. Pike-Overzet K, Ridder D de, Weerkamp F, Baert MR, Verstegen MM, Brugman MH, Howe SJ, Reinders MJ, Thrasher AJ, Wagemaker G, Dongen JJ van, Staal FJ. Ectopic retroviral expression of LMO2, but not IL2Rgamma, blocks human T-cell development from CD34+ cells: implications for leukemogenesis in gene therapy. *Leukemia. 2007;21:754-63*
5. Inverse Regulation of Hematopoietic Progenitor Cell Egress by MT1-MMP and RECK. This study applies the modified protocol for transfection of CD34 cells with siRNA. Abstracts submitted recently to the annual meetings of the International Society of Stem Cell Research (ISSCR, Sidney, Australia, June 07), and the European Hematology Association (EHA, Vienna, Austria, June 07, oral presentation) and The European Group for Blood and Marrow Transplantation (EBMT, Florence, Italy, April 08. Abstract was presented in the plenary session and received the basic science prize). A manuscript is submitted.
6. The CD45 phosphatase regulates migration, proliferation and retention of progenitor cells, involving osteoclast-mediated remodeling of metaphysial bone trabecules. Abstract was submitted to the annual meeting of the Israel Stem Cell Society (ISCS, Tel Aviv, Israel, June 07) and selected for poster presentation. Revised manuscript is under consideration.
7. Deichmann A, Hacein-Bey-Abina S, Schmidt M, Garrigue A, Brugman MH, Hu J, Glimm H, Gyapay G, Prum B, Fraser CC, Fischer N, Schwarzwaelder K, Siegler ML, Ridder D de, Pike-Overzet K, Howe SJ, Thrasher AJ, Wagemaker G, Abel U, Staal FJ, Delabesse E, Villeval JL, Aronow B, Hue C, Prinz C, Wissler M, Klanke C, Weissenbach J, Alexander I, Fischer A, Kalle C von, Cavazzana-Calvo M. Vector integration is nonrandom and clustered and influences the fate of lymphopoiesis in SCID-X1 gene therapy. *J Clin Invest. 2007;117:2225-32*

8. Kustikova OS, Geiger H, Li Z, Brugman MH, Chambers SM, Shaw CA, Pike-Overzet K, Ridder D de, Staal FJT, Keudell G von, Cornils K, Jekumar Nattamai K, Modlich U, Wagemaker G, Goodell MA, Fehse B, Baum Chr. Retroviral vector insertion sites associated with dominant hematopoietic clones mark "stemness" pathways. *Blood 2007;109:1897-907*

9. Schwarzwaelder K, Howe SJ, Schmidt M, Brugman MH, Deichmann A, Glimm H, Schmidt S, Prinz C, Wissler M, King DJ, Zhang F, Parsley KL, Gilmour KC, Sinclair J, Bayford J, Peraj R, Pike-Overzet K, Staal FJ, Ridder D de, Kinnon C, Abel U, Wagemaker G, Gaspar HB, Thrasher AJ, Kalle C von. Gammaretrovirus-mediated correction of SCID-X1 is associated with skewed vector integration site distribution in vivo. *J Clin Invest. 2007;117:2241-9*

10. Board of the European Society of Gene and Cell Therapy, Executive Committee of the Clinigene Network of Excellence, and *Executive of the Consert Integrated Project*. Case of Leukaemia Associated with X-Linked Severe Combined Immunodeficiency Gene Therapy Trial in London. Commentary. Human Gene Ther. 19:3–4 (January 2008)

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Section 3 – Publishable results

Everything listed in Sections 1 and 2 is publishable.