"Persisting Transgenesis"

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Name of the scientific representative of the project's co-ordinator[1], Title and Organisation:
Prof. Luigi NALDINI, MD, PhD, Università Vita San Raffaele (USR), Milano, Italy

Tel: +39 02-2643-4681

Fax: +39 02-2643-4621/4668

E-mail: naldini.luigi@hsr.it

Project website address: http://www.persist-project.eu/

[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.

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1 Executive summary

PERSIST stands for “Persisting Transgenesis”. It was a Large Scale Integrated Project (IP) in the FP7 Health theme that addressed the topic HEALTH-2007-1.4-4 concerning the “Development of emerging gene therapy tools and technologies for clinical application”. The PERSIST consortium brought together 27 partners from 9 European countries and was coordinated by Prof. Luigi Naldini of the San Raffaele University (USR) in Milano, Italy.

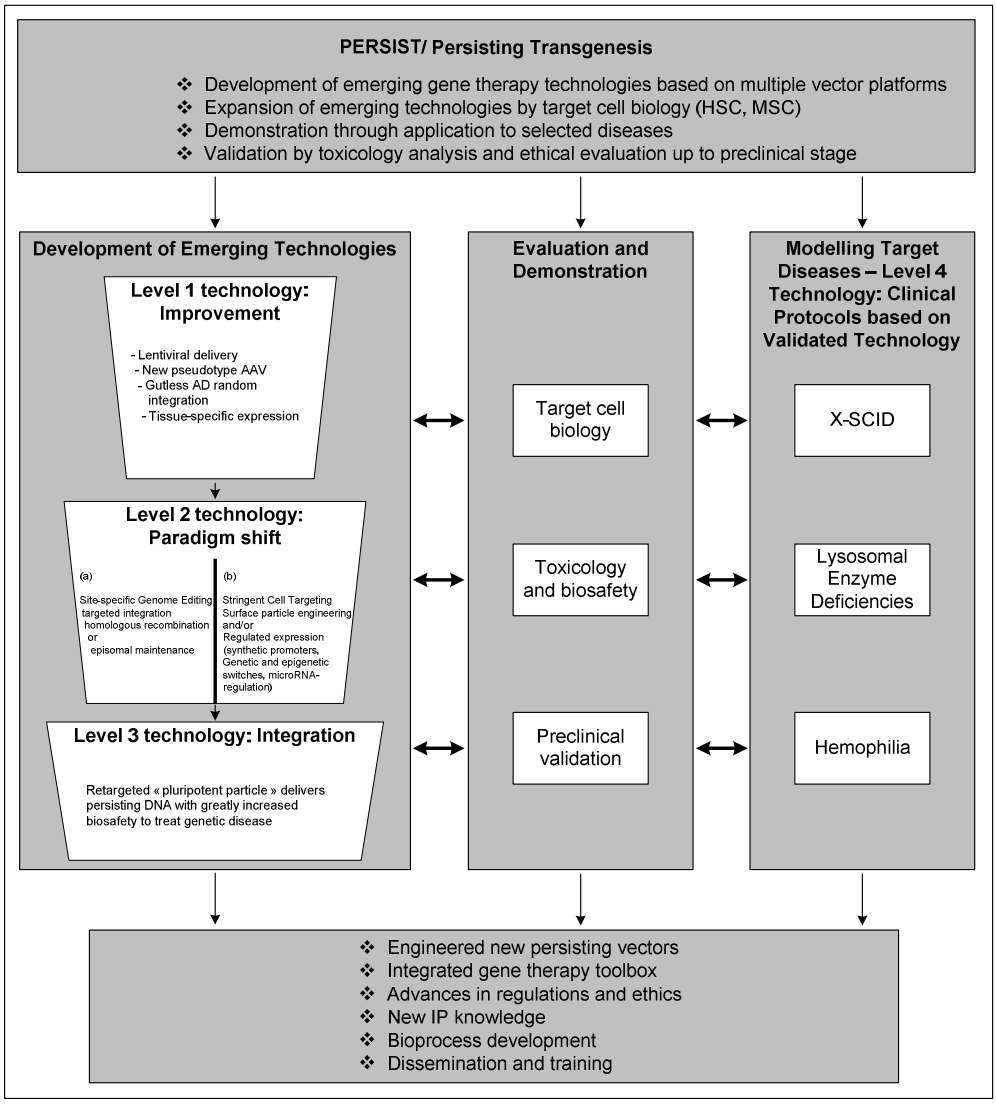
The project aimed to achieve the following main objectives strategic for advancing the field of genetic engineering and persisting gene expression:

- Long-term gene transfer without adverse effect on the host chromatin, including stable non-integrative gene transfer and targeted integration without impact on endogenous gene transcription,
- Site-specific genome modification, allowing a range of approaches such as in situ gene correction, somatic gene disruption and site-specific gene addition,
- Tight control on target cell selection or immune recognition by engineering the vector particle,
- Tight control on transgene expression exploiting recent discoveries on gene expression control, such as microRNA, on chromatin structure and organization, and designer transcriptional switches.

Partners in the consortium represented outstanding experts in the field of genetic engineering and genomic analysis. They pioneered the use of Zinc Finger Nucleases, engineered recombinases and transposases for gene targeting, synthetic promoters, epigenetic switches and micro-RNA for regulating gene expression, developed state-of-the-art gene delivery platforms based on lentiviral, AAV and gutless adenoviral vectors, conducted several first-in-man clinical trials and productively collaborated in previous FP projects.

The PERSIST project developed highly innovative gene targeting, editing and delivery technologies by capitalizing on the latest discoveries in gene expression control. These new technologies offer a radical solution to the problem of precisely controlling the fate and expression of exogenous genetic information in gene therapy. They were validated for application to severe diseases such as inherited immunodeficiencies, storage disorders, haemophilias, as well as some type of cancer. These diseases represent crucial paradigms for testing novel therapeutic options based on genetic modification of hematopoietic stem cells (HSCs), lymphocytes, mesenchymal stem cells (MSCs), hepatocytes, and induced Pluripotent stem Cells (iPSCs).

The figure hereafter represents the overall scientific approach adopted in the PERSIST project.
PERSIST consisted of 16 work packages of which 6 focused on vector innovations, 6 on applications and evaluation, 1 on bioprocess development and manufacturing, 1 on ethics and regulatory issues, and 1 on training and dissemination activities including IPR aspects. The last one (WP16), dedicated to project management, coordinated all other work packages together.



2 Description of project context and objectives

2.1 Vector innovation

The overall objective of the first WP was to develop tools for effective gene delivery to cells without toxicity arising as a result of semi-random genome integration. Considering that the high efficiency of lentivirus-mediated gene transfer, coupled with specific modifications to limit or re-direct integration may have widespread utility, the consortium planned to explore the mechanisms of action, the effectiveness of gene transfer and expression, and the potential for toxicity of integrase-defective lentiviral vectors. Then the reagents would be utilised for correction of models of disease outlined in WP10-12 according to technologies developed in WP3-4.

New methods to deliver DNA-modifying enzymes, mainly via transfer of mRNA or proteins, as required for the target cell populations of PERSIST's disease-related work program, were explored in WP2. Partners used episomal and non-integrating lentivirus-based modes of DNA delivery to express the enzymes of interest. Efficiency and biosafety of mRNA and protein delivery could be determined using novel technologies and experimental systems introduced by the investigators of PERSIST, including both physicochemical and viral methods. The goal was to define novel and potentially clinically useful means of transient delivery of DNA-

modifying enzymes into somatic (stem) cells, and to explore basic mechanisms of mRNA and protein delivery into somatic cells.

The objective of WP3 was the development of integrase- and transposase-based technology for efficient and safe site-specific integration of therapeutic transgenes into the human genome. This included the design and testing of Rep and phiC31 integrases, Sleeping Beauty-derived transposases, and group-II introns with increased targeting efficiency and reduced genotoxicity. New hybrid vector platforms for the combined delivery of site-specific recombinase/transposase and donor DNA for site-specific integration were developed and tested in relevant target cells. Genome-wide analysis of the full spectrum of integration properties of integrases and transposases was crucial to assess their potential genotoxicity for clinical applications. In addition, the WP aimed at developing a platform based on large-capacity HD-Ad vectors to deliver integrase- and transposase-based integration systems to specific cell and tissues in vitro and in vivo, and at validating the new site-specific integration technology in cells (hematopoietic stem cells, epidermal stem cells) and organs (liver, muscle) of clinical relevance.

The main activities of the WP4 “Homologous recombination-based gene targeting” aimed to (i) improve ZFN performance in terms of on target efficacy, specificity and limit their toxicity, (ii) develop effective delivery strategies for ZFN and donor DNA templates into target cell types relevant for gene therapy application, such as lymphocytes and hematopoietic stem cells (HSC), (iii) identify genomic target sites tolerant to insertion and allowing robust expression (safe genomic harbours”); (iv) generate ZFN-based vector systems for efficient gene addition to a selected “safe genomic harbour” and validate them in the above mentioned relevant cell types. A new design for autonomous Zinc Finger Nucleases was developed and allowed simultaneous delivery of multiple ZFN pairs targeting different loci. Moreover, a safe genomic acceptor site for transgenes was validated by showing robust transgene expression upon targeted integration without any detectable impact on endogenous transcription at the targeted and flanking loci. This strategy allowed defining a novel modality of “sustainable” gene transfer with minimal to undetectable impact on the acceptor genome. In addition, a new strategy to assess ZFN specificity genome wide in vivo was developed and used to validate the amazing specificity of late-generation designer ZFN action on the human genome.

The work plan described in the WP5 related to “Surface targeting” aimed at i) exploring the resources of envelope glycoproteins and cellular molecules that can naturally interact with specific target receptors ii) engineering these components to optimise their incorporation on LV surface iii) developing methodologies to display on the LV surface ligands that specifically bind receptors expressed on target cells iv) evaluating these strategies to target cells of interest such as HSC and hepatocytes v) optimizing and testing the developed LV to target these cells in vivo. During the last months of the project, the activities mainly focused on testing the novel BAEVgp pseudotyped lentivectors for transduction of human and macaque HSCs and comparison to RDTR and VSVG pseudotyped vectors. Partners involved in this WP also investigated whether HSCs ex vivo transduced by CD133-LV could repopulate in immunodeficient mice and whether CD133-LV was superior to commonly used VSV-G LV in transducing long-term repopulating CD133+/CD34+ HSC.

The activities carried out in WP6 involved (i) the definition of microRNA activity in different hematopoietic lineages, (ii) the identification of hematopoietic stem cell (HSC) and progenitor cell-specific microRNAs, (iii) the combination of epigenetics and RNA interference to control transgene expression, (iv) the design of novel synthetic transcription factors which control therapeutic transgenes in response to physiologic/pathologic metabolites, (v) the *in vivo* validation of hepatocyte-specific promoters developed using novel bio-informatics algorithms and (vi) the evaluation of immune responses to microRNA-regulated gene transfer vectors.

2.2 Applications and evaluations

Providing a complete Application Pharmacokinetics and Safety platform environment for all WP programs and their participants was the main objective of the work package 7. Specifically, this work aimed to perform high-throughput sequencing and distribution analyses of integration sites in order to assess the biosafety of viral vectors for clinical gene therapy. Toward this objective, we investigated non integrating vector systems based on integration-deficient lentiviral vectors as a potential safer alternative by reducing the risk of insertional mutagenesis. We optimized the performance of comprehensive genome-wide integration site (IS) analysis based on genomic accessibility models. An integration site sequence analysis and data management platform

to optimize and 'being prepared' for increasing numbers of raw sequence reads and applicability of non-bioinformatics scientists was set up.

The first objective of the WP 8 was to develop *in vitro* methods designed to expand and/or select cord blood-derived HSCs that have been genetically modified by the new technologies introduced throughout the activity of the Consortium (WP1 to WP6). Secondly, we planned to develop *in vivo* approaches aimed to specifically expand HSCs carrying the desired genetic modification, and assess efficacy and safety of the new gene therapy technologies. During the first period of the project the main work consisted of investigating *in vitro* expansion of human cord blood and bone marrow derived HSC with the objective of establishing methods for HSC expansion. A novel regulator, Cripto, could be discovered and evaluated for use in HSC expansion protocols.

The workpackage 9 had the objective to set the stage for therapy development using gene modified Mesenchymal Stem Cell (MSC) and to use MSC as a most versatile cell type to evaluate genomic and functional efficacy and safety of the technologies developed in WPs 2-4 and 6. MSC were procured and expanded from normal mouse and human BM and from BM of mice and individuals suffering from target diseases of WP10 and WP11. Pilot experiments from selected expanded cells, either or not transduced with LV-GFP, to establish genome wide gene expression profiles by Affymetrix arrays were tested. Pompe's disease specific MSCs were successfully used to establish titers of therapeutic vectors with direct assessment of functional transgene expression levels by fluorescence microscopy.

Within the "Disease Models" cluster, the new technologies for targeted gene transfer were successfully applied to develop novel treatments for severe diseases in relevant animal models. The development of tools for effective gene therapy of SCID-X1 without toxicity arising as a result of semi-random genome integration and constitutive transgene expression was the main goal of WP 10. This involved the implementation of a new protocol for efficient targeted integration of a corrective IL2RG transgene downstream its endogenous promoter into human HSC using a combination of ZFNs transiently expressed by mRNA transfection and donor template for homologous recombination delivered by integrase-defective lentiviral vectors. This strategy achieved efficient correction of human and mouse HSC and phenotypically corrected a mouse model of SCID-X1 disease, which is caused by inherited mutations of the IL2RG gene.

The two main objectives of WP 11 *Gene therapy for Lysosomal Enzyme Deficiencies* were the implementation of novel, microRNA-regulated lentiviral vectors for the HSC-based gene therapy of two model diseases, i.e globoid cell leukodystrophy (GLD, Krabbe's disease) and a glucosidase deficiency (Pompe's disease).

WP12 focused on the development of improved gene therapy strategies for hemophilia A and B. This involved the determination of a persistent phenotypic correction of the bleeding disorders by using the newly developed vector designs based on either stealth AAV or microRNA-regulated lentiviral vectors.

2.3 Industrialization

WP13 aimed at translating the upstream research projects towards bioprocess development for the manufacturing of clinical products.
The main work items that were addressed in this workpackage were the optimisation of large scale production processes for lentiviral vectors and the optimisation of *ex vivo* transduction protocols for human target cells.

2.4 Other activities

Two work packages were designed to cover all the aspects related to IPR management, ethics and societal issues, training and dissemination.

WP14 monitored and supervised all the ethical aspects that occurred during the course of the project. Indeed, achieving a successful research in the gene therapy field requires common protocols, standards, normalisation. WP14 focused on a survey of the state of the ethical discussion on human gene therapy, as well as to the assessment of risks and individual benefits. The aim was to ensure that all experiments performed in the project and all clinical protocols developed by PERSIST complied with the national and European rules.

The dissemination and training activities, as well as the management of IPR aspects, were addressed in WP15. Dissemination of knowledge was an essential concern of PERSIST. The projects aimed at generating new biological, bioinformatics and technical skills that need to be promoted to the scientific community and to citizens groups. Training programmes were developed for PhD students, post-doctoral fellows, internal and external personnel.

3 Description of the main S&T results/foregrounds

3.1 WP1 – Non integrating vector technology and persisting episomes

Characterisation of integrase mutants with respect to efficiency of gene expression and residual integration events

The use of semi-randomly integrating vectors may raise concerns regarding the potential risk of insertional mutagenesis. We tested several HIV integrase mutants and attachment site mutants for efficiency of integration. Essentially the D64V integrase mutant reduced activity to background levels which could not be further enhanced through combination with other mutations. We therefore investigated liver gene transfer by integrase-defective lentiviral vectors (IDLV) containing an inactivating mutation in the integrase (D64V). Hepatocyte-targeted expression using micro-RNA regulated IDLV resulted in sustained and robust induction of immune tolerance to both intracellular and secreted proteins despite the reduced transgene expression levels compared to their integrase-competent vector counterparts. IDLV-mediated and hepatocyte-targeted coagulation factor IX (FIX) expression prevented induction of neutralizing antibodies to FIX even after antigen re-challenge in hemophilia B mice and accounted for relatively prolonged therapeutic FIX expression levels. Upon delivery of intracellular model antigens we showed that hepatocyte-targeted IDLV induced transgene-specific regulatory T cells that contributed to the observed immune tolerance. Deep sequencing of IDLV-transduced livers showed only rare genomic integrations that had no preference for gene coding regions and occurred mostly by a mechanism inconsistent with residual integrase activity. IDLV thus provide an attractive platform for tolerogenic expression of intracellular or secreted proteins in the liver with substantially reduced risk of insertional mutagenesis. In other studies we have shown that hydrodynamic injection of IDLV increases the transduction efficiency of both IDLV and integrase-competent LV. As part of ongoing work, we have recently shown that codon optimisation of the FVIII cDNA results in much superior gene expression from liver transduced by LV.

Development of integrase-defective lentiviral vectors for targeted genome integration in specific models of human disease

We have developed a gene targeting system to correct two models of human immunodeficiency. The first utilizes the classical mouse SCID mutation, a model of the very rare human DNA-dependent protein kinase catalytic subunit (DNA-PKcs, PRKDC) deficiency. The system relies on the use of integration-deficient lentiviral vectors (IDLVs) for delivery of the targeting cassette and the zinc-finger nuclease (ZFN) monomer genes. We previously reported the development of assays to demonstrate ZFN activity and gene repair of mouse Prkdc, the production of zinc-finger nuclease and the required lentiviral constructs, and Prkdc gene targeting in both Tert-immortalised mouse SCID fibroblasts and lin-SCID haematopietic stem cells (HSCs). Upon primary transplantation of gene-corrected SCID HSCs into sublethally irradiated SCID mice, we have observed double-positive CD4/CD8 cells in the thymus, and single-positive CD3, CD4 and CD8 cells in peripheral blood. Correction of the SCID mutation and concurrent incorporation of the diagnostic restriction site have been confirmed by deep sequencing of thymic DNA. Upon secondary transplantation (currently a year from the beginning of the primary transplant) we have observed single-positive CD3, CD4 and CD8 cells in blood. Our observations suggest that we have been able to correct the T-cell deficiency of Prkdc SCID mice by transplantation of ex vivo gene corrected SCID HSCs, indicating that gene repair-based rescue of SCID disease is a feasible approach.

The second model exploits IDLV-mediated ZFNs specific for the IL2RG gene which is mutated in the commonest genetic form of human SCID (SCID-X1). We corrected the mutation of the human IL2RG gene in HSPC from a SCID-X1 mouse model (INDY mouse; for its description see WP10). Efficacy of gene-correction was tested by monitoring immune reconstitution of recipient INDY or RAG2 -/- γC-/- mice transplanted with the treated HSPC. Over time we observed increasing levels of T and B cells expressing the human IL2RG gene, indicating that gene-correction allows full restoration of the lymphocyte lineages and immune system. In summary, we demonstrated that targeted gene-correction can be successfully performed in primary murine HSPC, and that this strategy leads to immune reconstitution of this disease model.

These studies are state of the art examples of how genome engineering can be used to provide corrective gene therapy in models of inherited immunodeficiencies. The background of immunodeficiency provides an

added advantage because there are profound selection advantages to corrected cells, but progress is such that clinical trials in this area can be anticipated within the next 5 years.

3.2 WP2 - mRNA and protein transduction of DNA-modifying enzymes

Novel approaches to deliver mRNA or proteins into cells.

Existing methods for the transient and dose-controlled delivery of DNA-modifying enzymes mostly rely on physicochemical transfection systems. Cell death encountered during transfection may be substantial, can be problematic especially when targeting rare cell sources such as primitive hematopoietic cells or stem cells in which toxic stress may induce severe functional alterations. Within WP2, we developed and further improved two novel approaches to deliver mRNA or protein into cells, using retrovirus-like particles which do not elicit major cytotoxicity. The first approach, termed RMT, delivers mRNA, which offers a highly flexible platform as structural limitations are minor. Importantly, this approach does not induce high levels of expression of the protein of interest, which can be a major advantage if the enzyme encoded by the mRNA has a dose-limiting toxicity. The second approach, termed RPT, delivers proteins in the framework of a retroviral polyprotein that undergoes the essential steps of the retroviral life cycle (assembly, budding from producer cells in the form of retroviral particles, proteolytic maturation of the polyprotein to release its subunits, uptake into cells in the form of mature particles containing the protein of interest as Trojan horses, and release inside target cells following particle disassembly). RPT may induce high levels of protein transduction, and can be dose-controlled simply by varying the number of particles per cell. Both, RMT and RPT, allow cell-specific delivery of their cargo, because cell uptake is controlled by a receptor which may be cell-specific, depending on the nature of the envelope protein used for particle production. For both, RMT and RPT, disadvantages of retroviral technologies, such as the co-transfer of DNA with untargeted integration properties, can be avoided. The expected use of RMT and RPT is for the genetic modification of mammalian cells in biological research, biotechnology and cell&gene therapy. We demonstrated the efficiency of these new methods and elucidated their mechanism of action. The expected use of these novel platform technologies covers the delivery of various DNA-modifying enzymes (transposases, nucleases, recombinases, integrases).

Reducing toxicity of DNA-modifying enzymes

We took two complementary approaches to reduce the toxicity of DNA-modifying enzymes: (i) through engineering of the protein of interest, (ii) via modification of the delivery platform and expression levels. The PERSIST partners demonstrated the suitability of the first approach by modifying three major classes of DNA-modifying enzymes: integrases, nucleases and transposases. To reduce toxic effects, we developed novel experimental systems that record off-target effects with high sensitivity. Furthermore, we recorded cytotoxicity in careful comparative studies, and developed novel mutants or hybrid proteins with DNA-modifying properties. With regard to the second approach, our network performed careful dose-escalation studies using different delivery platforms, again using sensitive readout systems to score off-target effects with high sensitivity. We discovered novel mechanisms of cytotoxicity induced by the over-expression of DNA modifying enzymes, and demonstrated that dose-controlled delivery allows efficient genetic modification of target cells while circumventing overt cell damage. The expected use of these discoveries includes the design of novel, retargeted DNA-modifying enzymes that are well tolerated by the target cells, thus establishing a strong basis especially for the putative therapeutic use of DNA-modifying enzymes in gene therapy.

3.3 WP3 - Targeted integration

Development of phiC31-based site-specific integration technology

The bacteriophage derived PhiC31 integrase system is an attractive tool for efficient somatic integration of transgenes into the human genome. Although integration specificities of up to 15% were demonstrated for the PhiC31 integrase system in mammalian cells, optimization of activity and enhanced recombination at specific DNA target sites are still required for the usage of this system for therapeutic applications. We generated functional PhiC31 fusion proteins in which the PhiC31 integrase is linked to a DNA-binding domain (DBD) to direct binding of PhiC31 integrase to specific DNA sequences and to increase recombination activities at the zinc finger DNA-binding site (DBS). We explored established DBDs such as the synthetic polydactyl zinc finger E2C to target a specific sequence on chromosome 17 and the AAV Rep78 protein targeting the AAVS1 site on chromosome 19. Increased recombination efficiencies were observed for all groups which received the

PhiC31-E2C fusion protein independent of the plasmid ratio applied. These results represent the first evidence that a PhiC31 fusion protein can be redirected to a specific DNA sequence, and open the way to its utilization in gene therapy applications.

Development of a gene targeting strategy based on the Sleeping Beauty transposon/transposase system

The Sleeping Beauty (SB) transposon is a nonviral, integrating vector system with proven efficacy in preclinical animal models, holding promise for future clinical applications. We successfully tested transposase-based integration systems in different cells in vitro and in vivo, showing the potential of this system for human gene therapy. The aim of this research was to improve gene transfer efficiency, reduce off-target effects and increase biosafety by incorporating naturally occurring as well as synthetic DNA binding domains into the framework of SB transposition. A series of fusion constructs consisting of the N-terminal DNA-binding domain of AAV Rep or E2C and the transposases or the N57 domain of SB were generated. A plasmid-based transposition assay showed that Rep/SB yielded a 15-fold enrichment of transposition at a particular site near a Rep-targeted site. Genome-wide insertion site analysis indicated that an approach based on interactions between the SB transposase and Rep/N57 enriched transgene insertions at Rep-targeted sites. This study provides a comparative insight into target site selection properties of transposons, as well as proof-of-principle for targeted chromosomal transposition by composite protein-protein and protein-DNA interactions.

Site-specific integration in human epithelial stem cells

Epithelial stem cells (EpSCs) are an important target in a gene therapy approach to inherited skin adhesion defects. We designed a gene targeting approach aimed at site-specific insertion of a gene into a identified “safe harbour” location, the adeno-associated virus integration site (AAVS1) locus on chromosome 19, in the genome of human EpSCs. The strategy is based on the use of AAVS1-specific zinc-finger nucleases (ZFNs) to induce a targeted double-strand break (DSB) and stimulate a specialized form of homologous recombination (HR) known as homology-directed DNA repair. Simultaneous provision of a suitably designed donor DNA cassette, in which the gene of interest is flanked by sequences homologous to the target site, results in the site-specific addition of the corrective DNA to the targeted site. The AAVS1 locus allows for robust transgene expression without perturbation of the neighbouring gene expression. ZFNs were delivered via integrase-defective lentiviral vectors (IDLVs) or adenoviral (Ad) vectors, which do not persist in actively replicating cells. We provided proof of principle that ZFN-mediated, targeted gene addition can be achieved in human keratinocytes and in long-term repopulating EpSCs in a validated pre-clinical model of xenotransplantation of human skin equivalents on immunodeficient mice. Deep sequencing of the AAVS1 locus showed that ZFN-induced double-strand breaks are mostly repaired by non-homologous end joining in primary cells, indicating that poor induction of the HR-dependent DNA repair pathway, and not the efficiency of gene targeting, is the most significant limitation for targeted gene integration in human stem cells. This study has important implication on the application of ZFN-based technology to EpSC-based therapy.

Development of a clinical-grade gene delivery technology platform based on HD-Ad vectors

Efficient delivery of integrase- and transposase-based gene transfer system to specific cell targets is a crucial aspect in a translational approach towards clinical gene therapy. The use of high-capacity HD-Ad vectors to deliver integrase- and transposase-based cassettes has been pioneered by our project. The goal of this part of the WP3 was the development of optimized HD-Ad vector backbones, helper plasmids encoding capsids with different target specificity, and cell lines for the production of clinical grade vectors. We have investigated the capacity of the adenoviral E2A- and bacteriophage P1 Cre-expressing packaging cell line PEC 3.30 (PER.E2A.Cre clone 3.30) to support the production of various “gutless”/helper-dependent AdV (HD-AdV) batches. This cell line was generated, isolated and characterized under Good Laboratory Practice guidelines and was derived from PER.C6 cells, which constitute an established platform for the clinical-grade production of RCA-free (Replication-Competent Adenovirus) AdV batches. The task was successfully completed, with the development of SOPs that can be immediately transferred into production.

3.4 WP4 - Homologous recombination-based gene targeting

Improvements in designer endonucleases platforms

A novel development in the genome engineering arena was the use of ZFNs to introduce targeted deletions in a chromosome. To this end, two pairs of ZFNs (1L/1R and 2L/2R) had to be expressed in a cell to target two adjacent sites on the same chromosome. The first experiments showed that protein engineering was

successfully employed to generate two autonomously acting ZFN pairs that maintained high cleavage activity but showed reduced ZFN-associated toxicity. By identifying autonomous ZFN pairs that induced gene targeting at the same efficacy as previously published obligate heterodimeric ZFNs but with significantly less toxicity, we demonstrated that autonomous ZFNs proved useful in targeted genome engineering approaches wherever an application requires the expression of two distinct ZFN pairs.

We also established a novel designer nuclease platform based on transcription activator-like effectors (TALENs).

Development of optimized protocols for targeted integration and gene editing in relevant cell types for gene therapy

An adenoviral-based (AdV 5/35 serotype) platform was established as the best performing delivery approach for transient and robust ZFN expression in human primary T cells and fibroblasts, followed by donor template delivery by integrase-defective lentiviral vectors (IDLV) for homology-driven transgene insertion. This protocol allowed robust and reproducible gene targeting, editing and targeted integration in human primary lymphocytes.

Another protocol was optimized for the efficient delivery of the molecular machinery required to induce homology-driven transgene insertion into selected genomic target sites in human hematopoietic/progenitor cells (HSPC), which is based on mRNA transfection to transiently express ZFNs and IDLV infection for donor template delivery. This protocol was optimized for targeted integration of several types of transgene expression cassettes into the AAVS1 “safe harbour” site or into a mutational hotspot of the IL2RG gene of long-term repopulating HSC with high efficiency and reproducibility. This advance fulfills a key goal of the project and represents a major step toward the clinical exploitation of the benefits of the new technologies

Development of a new unbiased method for genome-wide assessment of designer endonuclease specificity

We developed a novel strategy to comprehensively map ZFN off-target activity genome-wide and showed that: i) the intended ZFN target site was by far the most frequently hit genomic locus, ii) the ZFN off-target activity was limited to a small fraction of genomic sites with sequence homology to the intended target site; iii) a consensus sequence for the actual cleavage could be established *in vivo*; iv) the degree of sequence homology alone was not sufficient to predict the actual ZFN off-target activity; v) ZFN engineering - such as using the obligate heterodimeric FokI - substantially decreases off-target activity. This method has wide applicability to virtually all platforms of designer endonucleases.

Identification and validation of genomic safe harbour sites for transgene insertion

Even if we previously described the use of Zinc Finger Nucleases (ZFN) and Integrase Defective Lentiviral Vectors to insert transgenes into preselected human genomic sites, little was known of suitable safe harbour loci, and about the impact of transgene insertion on the targeted locus and vice versa. The studies conducted by the consortium highlighted a strategy to achieve site-specific integration and predictable transgene expression without perturbing endogenous transcription in human T lymphocytes, fibroblasts, NSC and iPS cells. By investigating the transcriptional and epigenetic impact of a panel of different GFP expression cassettes targeted by the ZFN technology into two genomic sites, the CCR5 gene and AAVS1 locus, in different cell types, including primary T lymphocytes, we showed that insertion into AAVS1 was conducive to robust transgene expression without interfering with the transcription of the endogenous genes at and near the insertion sites, thus validating AAVS1 as a safe harbour for transgene insertion in lymphocytes, iPSC and Neural Stem Cells. Based on these results AAVS1-specific TALENs were also generated and shown to cleave the target site as effectively as ZFNs. Moreover, TALEN-mediated integration of a reporter cassette into AAVS1 has been demonstrated in human cell lines and human iPS cells.

A new strategy for genetic editing of T-cell specificity

The use of Zinc Finger Nucleases (ZFN) designed to permanently disrupt a predefined gene, coupled to lentiviral-mediated gene transfer, enables the precise substitution of a biological function in primary cells. This technology was used to completely and permanently redirect T cell specificities toward tumor antigens through editing of the TCR in primary human T cells. Zinc-finger nucleases (ZFNs) promoting the disruption of endogenous TCR β and α chain genes (TCR complete editing) permitted the elimination of the risk of TCR mispairing thus increasing efficacy and limiting toxicity of TCR gene transfer (shown in vitro and in humanized

murine models). While the complete editing procedure currently requires multiple manipulation steps, 'single TCR editing', based on the ZFN-mediated knock-down of a single endogenous TCR chain (α or β) followed by the introduction of the tumor-specific TCR, enables the generation of redirected T cells devoid of their natural TCR repertoire during a single round of T cell activation, improving the feasibility of the clinical translation of this approach.

Targeted integration and gene correction in iPSC.

We demonstrated for the first time gene correction of primary fibroblasts from Fanconi anemia A patients by the insertion of the FANCA gene in a safe harbour locus, and of fibroblasts from SCID-X1 by insertion of a functional transgene downstream its endogenous promoter. As a first step fibroblasts were corrected by directed homologous repair (DHR) in the targeted locus using an adenoviral vector expressing ZFN specifically designed for the target site and a donor integrase defective lentiviral vector carrying the corrective gene. As a next step corrected fibroblasts were reprogrammed using an excisable lentiviral vector and several clones of iPS cells were selected, showing different pluripotency markers. It was demonstrated that at least 6 out of the twelve clones had the transgene cassette integrated in the targeted locus. Pursuing the objective to differentiate iPS cell clones into HSCs with the final goal of correcting the disease phenotype of FA and SCID mice, several protocols were developed based on the sequential differentiation of embryoid bodies and subsequent co-culture of pre-differentiated cells to hemangioblast in different stromas. The efficiency of two hematopoietic stromas to differentiate iPSC lines into CD41 + cells with hematopoietic graft potential was evaluated. It was shown that (i) the specific insertion of FANCA in the AAVS1 safe harbor facilitated the generation of disease-free iPSCs from FA-A patients; (ii) telomerase facilitated both the gene editing and the generation of disease-free iPSCs from FA-A patients; (iii) hematopoietic progenitors could be efficiently generated from gene-edited hFANCA-/- iPSCs. Concerning SCID-X1, we differentiated lymphoid and myeloid progenitors from iPSCs derived from gene corrected SCID-X1 fibroblasts and showed functional correction of the original mutation by expression of the in situ corrected gamma chain gene.

3.5 WP5 - Surface targeting

Newly engineered envelope glycoproteins for efficient incorporation into vectors/study of vector entry and assembly

In WP5 we developed for the first time to our knowledge LVs which allow efficient transduction of human quiescent T and B cells and DCs and this without changing their phenotype. We were also able to decipher the entry mechanism used by these new pseudotypes for entry into quiescent lymphocytes and engineered mutant measles virus (MV)-LVs fit for in vivo use, which resist to MV-specific neutralizing antibodies. Additionally, by engineering of the measles virus glycoprotein H, we showed targeting with an unprecedented specificity and efficiency in mixed cultures of primary cells, e.g. for endothelial cells via CD105, upon local intracerebral injection to glutamate receptor positive neurons and also upon systemic injection to tumor cells (Anliker et al., 2010). This was possible by the display of carefully designed single chain antibodies or DARPINs on the measles virus gp H. Furthermore, we demonstrated for three different targeting vectors (CD133-LV, CD105-LV, CD20-LV) that they are all partially protected against MV neutralizing antibodies in human plasma. In addition a novel technology was explored through the design and production of inorganic nanoparticles interacting with viral envelopes thereby modifying their cell tropism.
These vectors carrying the glycoproteins of measles virus at their surface provide a significant advance in the field of gene therapy and immunotherapy since they may overcome current limitations of classical lentiviral vectors for genetic modification ex vivo and in vivo of hematopoietic cells.

Engineering of lentiviral vectors for in vivo targeting to hematopoietic stem cells (HSCs): ex vivo evaluation

By display of an early cytokine such as SCF on the surface of a lentiviral vector, we achieved a strong selectivity for HSC gene transfer ex vivo and this vector allowed correction of Fanconi anemia patient cells with success. Using the MV gp engineered LVs, additionally a HSC targeting vector was developed with high specificity for CD133 positive human HSC cells, validated ex vivo. Using a competitive setup, in which the two different vectors were distinguished via green or blue fluorescent protein expression, we found that CD133-LVs are superior to VSV-G LVs in transducing HSCs with an increased and extended proliferative capacity ex vivo. In addition, we engineered for the first time a LV pseudotyped with a BaEVgp derived from a baboon endogenous retrovirus. These vectors stably transduced up to 80-90 % of human CD34+ cells and macaque CD34+ cells ex vivo.

HSC-targeted vectors: in vivo evaluation

We showed that the 'RDTR/SCF' HSC-targeted vectors allowed efficient transduction of hCD34+ lineage negative cells in the BM of human hematochimeric mice upon intrafemural injection. The transduced hCD34+ immature progenitors were maintained for 2 months in the BM and showed that upon isolation of these in vivo transduced hCD34+ cells they could still differentiate into myeloid progenitors. This strategy was transferred to SIV vectors that allowed efficient ex vivo transduction of macaque BM CD34+ cells and when reinfusion of these transduced CD34+ cells was combined with a reduced intensity conditioning regimen, cell marking persisted for more than 3 years in macaques. In vivo intra-bone marrow injection of the SCF-displaying SIV vector combined with a positive selection marker allowed an enrichment of gene marked cells in macaques. First reconstitution experiments in immunodeficient mice indicated that the CD133+/CD34+ cells that were ex vivo transduced by CD133-LVs or BAEV-LVs efficiently reconstitute mice in all hematopoietic lineages in bone marrow, thymus, spleen and blood demonstrating that CD133-LV and BAEV-LVs transduced HSCs. Competitive reconstitution experiments with equivalent amounts of VSVG-LVs and CD133-LVs transduced hCD34+ cells showed that the CD133-LV transduced progeny cells dominated in 80% of the mice. RDTR/SCF-LVs and CD133-LVs might in the future allow bypassing ex vivo handling and simplify gene therapy for many hematopoietic diseases by direct in vivo inoculation into the patient.

3.6 WP6 - Regulated gene expression (miRNA or promoters)

We followed two main objectives that were:

- Establishing miRNA-controlled tissue- or lineage-specific transgene expression systems with particular emphasis on compartment- and lineage-specific expression in the hematopoietic system
- Defining microRNA activity in different hematopoietic lineages

Based on available profiling data, we rationally chose a panel of 10 microRNAs and characterized their activity across the whole hematopoietic system making use of novel, bidirectional miRNA reporter vector specific for the queried miRNAs. Multiple hematopoietic cell populations were isolated from the peripheral blood, bone marrow, spleen and thymus of the reconstituted mice and immunophenotyped by multicolor flow cytometry. Concomitant assessment of reporter expression in each of these subpopulations allowed for a massively parallel characterization of the activity of the queried miRNAs across the whole hematopoietic system. The following significant results were obtained: we identified miRNAs with activity in the myeloid lineage (miR-223), in the lymphoid lineage (miR-150, miR-181a) and in hematopoietic stem and progenitor cells (including miR-126, miR-130a). This knowledge was then exploited to build lentiviral vectors (LVs) with miRNA-regulated transgene cassettes, which lacked or showed reduced expression in the above-mentioned compartments. As a proof-of-concept, we showed that HSPC-detargeted expression of galactocerebrosidase (GALC) overcame the engraftment block of HSPC transduced with GALC LVs and provided therapeutic GALC expression in the myeloid progeny resulting in prolonged survival in a mouse model for Krabbe disease. Next, miRNA-based HSPC de-targeting was optimized for human hematopoietic cells from cord blood and bone marrow, and combined with tissue-specific promoters. We achieved unprecedented myeloid-specific transgene expression, entirely sparing the human CD34+ HSC compartment while providing high absolute levels of transgene expression in mature myeloid cells. These new vectors are now entering preclinical development for autologous HSPC gene therapy of globoid leukodystrophy and chronic granulomatous disease.

Self-inactivating lentiviral vectors harboring internal myeloid specific promoters were evaluated for their potential use in a clinical gene-therapy trial X-linked Chronic Granulomatous Disease. The results from this work provided the basis for the selection of a suitable candidate vector for extensive preclinical testing. In order to find a suitable candidate which fulfills the requirements, five candidate therapeutic vectors harboring different regulatory elements were selected based on results from pilot experiments. The regulatory elements used to drive expression of codon optimized gp91phox were the microRNA223 promoter (223), a chimeric promoter consisting of a fusion between the cathepsin G and c-Fes promoters (chim), the myeloid related protein 8 promoter (MRP8), and two vectors in which an ubiquitous-acting chromatin element (UCOE) was placed upstream of the MRP8-promoter. In addition, a vector containing a myeloid-specific promoter (MSP) and a posttranscriptional control element (miRNA 126) was tested in parallel. Based on promising data from *ex vivo* differentiated primary hematopoietic stem and progenitor cells of human and murine origin, two vectors, one carrying the chimeric promoter and the other the MSP promoter and the 126 miRNA were selected for *in vivo* studies. In summary, both vectors mediated high and copy number dependent expression

of the transgene with high fidelity in myeloid cells. Myeloid specificity was shown by the lack of transgene expression in lymphoid lineages and very low expression levels in hematopoietic stem and progenitor cells. A strong upregulation of gp91phox expression during the differentiation of hematopoietic stem and progenitor cells into granulocytes could be shown *in vitro* and *in vivo* for murine and human gp91phox-deficient cells, leading to an efficient reconstitution of NADPH-oxidase activity. The expression was stable over an extended period of time *in vivo* with no signs of epigenetic silencing or any signs of hematologic abnormalities. In conclusion, when comparing the newly generated lentiviral vectors with the γ-retroviral construct used in clinical trials for X-CGD, major improvements in vector safety and transgene expression have been achieved. This will likely be reflected by better therapeutic results and no severe adverse events in a future gene therapy trial.

We have established the cartography of KRAB-ZFP gene expression in the hematopoietic system, and analyzed the phenotypic and molecular consequences of its inactivation in hematopoietic stem cells.
We have determined that the stage-specific induction of two KRAB-ZFPs in erythroblasts is necessary for the suppression of mitophagy genes-targeting miRNAs, hence for allowing eythropoietic differentiation. We have also determined that inactivating KRAB/KAP1-mediated regulation in long-term repopulating HSC abrogates their ability to undergo asymmetric cell division, apparently through the downregulation of genes involved in the recognition of the stem cell niche. These findings demonstrate that KRAB/KAP-mediated epigenetic regulation and RNA interference combine to control erythropoiesis, paving the way to designed approaches to regulate transgene expression in the hematopoietic system, notably in the red cell lineage.

We have designed novel synthetic transcription control modalities that respond to traceless non molecular cues such as electromagnetic waves, and allow optogenetic control of transcription. We have applied this novel promising strategy to a device that enhances blood-glucose homeostasis in mice.

3.7 WP7 - Pharmacodynamics and Biosafety

Provide a complete application pharmacokinetics and safety platform environment for all IP programs and their participants

The implementation of a complete and sophisticated application pharmacokinetics and biosafety platform enables the use of novel, most efficient and safe gene transfer vectors. With the developed platform new vectors can now be easily tested for the genotoxic risk. Furthermore their optimal application parameters towards highly effective gene therapy trials can be investigated. Our understanding of high and low frequent integrating viruses and their influence on the biology of the affected cells and potential clinical outcomes benefits enormously of novel high throughput next generation sequencing technologies. We have established LAM/nrLAM-PCR for a variety of vector systems used in preclinical and clinical gene therapy trials. Further modification and optimization using linker and vector specific primers fused with next generation sequencing primers enabled straight forward sequencing. Since the MiSeq sequencing technology provides up to 15 million read numbers compared to the established 454 pyrosequencing technology we have set up this new sequencing technology to enable a more profound landscape of the integration site repertoire. Therefore we have developed a specific boulde bar code strategy. The double barcoding strategy combined with MiSeq personal sequencer platform enables reliable and extensive but cost effective identification of IS which will be used to all further IS analysis studies in preclinical and clinical settings. Time- and cost-efficiency of integration site sequence retrieval has been improved by several logs compared to standard Sanger sequencing technologies.

Establish a high-throughput integration site sequencing technology platform that will allow a sophisticated and complete assessment of the clonal cellular contributions

Large scale integration site profiling to monitor the cell fate of the gene corrected cell population and to assess vector biosafety will decisively help to translate gene therapeutic approaches to the clinics. Clonality analysis of viral vector-transduced cell populations represents a convincing approach to dissect the physiology of tissue and organ regeneration, to monitor the fate of individual gene-corrected cells in vivo, and to assess vector biosafety. Gene marking of hematopoietic stem cells not only allows the correction of monogenetic diseases but also allows to follow the fate of individual clones within the hematopoietic compartment since the retrieved vector integration sites are unique for each transduced cell clone. Screening for (potentially) harmful clones can be achieved using LAM-PCR combined with next-generation sequencing techniques, whereas a special emphasis lies on directly quantifying individual clonal contributions using the sequence retrieval

frequencies ('read counts') of individual integration sites over time. With this combination we have set up a standardized strategy for large-scale clonal assessments in gene therapy studies. Using these methods we can tightly track and robustly quantify suspicious clones in current and future gene therapy studies.

Determine the clonal inventory of preclinical and clinical gene transfer studies and the vector induced influences on the host genome to reach a reliable and robust risk/benefit ratio (degree of genotoxicity) in the affected settings

Optimized vector constructs were tested for gene activation, suppression and interruption, resulting in clonal dominance, premalignant proliferation and malignant transformation in different settings. It was of great scientific interest to investigate the oncogenic potential of prototypical murine leukemia virus (MLV) –derived, γ-retroviral and lentiviral vectors.
The analysis of genomic distribution of retro-/lentiviral vectors is a powerful tool to monitor possible "vector-on-host" effects in gene therapy clinical trials but could also provide crucial information about "host-on-vector" influences based on target cell genetic and epigenetic state. On this issue we had the unique occasion together with our partners to compare the insertional profile of different viral vector in the context of genotoxicity of the used vectors. These results enabled us to generate the basis for technology development and thus to promote patient safety.

3.8 WP8 - Target Cell Biology: Hematopoietic Stem Cells (HSC)

The first objective was to develop methods for ex vivo expansion of human HSC.

Since Angiopoietin-like proteins (ANGPTLs) have been reported to function as novel factors, supporting expansion of human and murine hematopoietic stem cells *ex vivo,* we investigated whether these factors can reliably expand human $CD34^+$ scid-repopulating cells (SRCs) in a manner suitable for clinical translation. We confirmed published findings that Angptl5 can increase the engraftment of CD34+ cord blood cells in immuno-compromized mice and identified Angptl4 as a novel factor that can increase CD34+ engraftment. Our data suggest that ANGPTLs 4 and 5 may be useful for clinical purposes in the future, but further improvements of the protocol to reduce/eliminate variability are required. Better protein preparations may allow more reproducibility in the future.

The alternative strategy would be the use of Cripto. We identified Cripto as an important regulator of HSC quiescence and maintenance in the niche, and shown that it preserves stem cell properties *in vitro* for at least two weeks to allow significant engraftment of the HSC after transplantation. Although there is no evidence that Cripto can expand HSC *in vitro*, it is possible that Cripto can be used in future HSC expansion protocols since it preserves stemness of cultured HSC.

We also evaluated the influence of hypoxia on serum free hematopoietic expansion cultures stimulated with SCF, TPO, IGF2, Flt3L and Angptl3 (collectively termed STIFA3) for both mouse and human stem cells as tested in syngeneic mouse recipients and NOD/SCID mice, respectively. Competitive transplantation of the cultured equivalent of 120 or as few as 12 original LSK cells and secondary transplantation of bone marrow cells revealed an approximately 12-fold expansion of long-term repopulating HSCs. Consistent with the mouse studies, NOD/SCID repopulating cells in the CD34-positive population of human umbilical cord blood cells, stimulated with the equivalent human growth factors under hypoxic conditions, expanded within 7 days approximately 15-fold ((confidence limits 12- to18-fold). These findings provide an essential basis for clinical implementation of ex vivo stem cell expansion for both allogeneic cord blood and autologous gene modified stem cell transplantation.

The second objective consisted in determining whether stem cell expansion in vitro enabled HSC gene transfer by the leading new technologies developed in WP3 and WP4. A protocol for successful gene targeting and editing in human HSC has been developed by partner USR and is reported in WP4. This protocol exploits some strategies previously reported to support maintenance and limited expansion of human stem/early progenitors at least in short term culture.

3.9 WP9 - Target Cell Biology: Mesenchymal stem cells (MSC)

Mesenchymal stem cells (MSC) were procured, expanded and characterized from normal mouse and human BM and from BM of mice and individuals suffering from X-linked SCID, MPS I and Pompe disease. Genome wide gene expression profiles by Affymetrix arrays were generated for both untransduced and transduced MSC's and in collaboration with WP7, LV integration sites were determined using LAM-PCR and high throughput sequencing, related to the gene expression profiles of the cells and compared with similar data for mouse and human highly purified HSC available in our laboratories to detect possible stem cell type specific integration preferences.

3.10 WP10 - SCID-X1 target disease

Development of a clinically relevant murine model of SCID-X1 for gene correction

We have developed a mouse model (INDY) for gene targeting at the human IL2RG gene locus. This was achieved using conventional transgenic methodologies whereby the entire human locus replaced the murine locus. Furthermore, a common human mutation was introduced into exon 5 so that corrective strategies could later be addressed through analysis of restoration of immunity. The mouse was characterized by histological and immunological methods and was shown to display an identical phenotype to that of mice with a complete knockout of the murine il2rg gene (ie low numbers of B,T and NK cells). This validates the model as a platform for a wide variety of targeted gene correction strategies, including those utilized in this work package.

Reconstitution of immunity in transplant models of SCID-X1 using gene corrected human or mouse HSC

The development of ZFN technology enables targeted rather than random integration, and gene correction rather than replacement, as new paradigms for gene therapy. Whereas high levels of targeted gene modification has been reported in some clinically relevant primary cells, the demonstration of highly efficient targeted integration (TI) in human Hematopoietic Stem and Progenitor Cells (HSPC) remains challenging. Here, we combined Integrase Defective Lentiviral Vectors (IDLV) to deliver a donor template for homology-driven repair and mRNA transfection to drive a spike of ZFN expression in the treated HSPC. Optimization of this protocol facilitated targeted integration of a corrective cDNA into a mutational hotspot of the IL2RG gene of HSPCs with high efficiency and reproducibility. Upon xeno-transplantation in NSG mice we observed gene-targeted cells in both myeloid and lymphoid lineages as well as in the early progenitors' compartment of the bone marrow for several months post-transplant across multiple independent mice, indicating successful targeting of long-term repopulating stem cells. Importantly, HSPC targeted with the corrective IL2RG cDNA were capable of generating lymphoid cells that express the IL2RG protein and are dependent on its signaling. These lymphoid cells were endowed with a selective growth advantage over those carrying disruptive IL2RG mutations, proving that the corrective cDNA was able to functionally restore the gamma-chain coupled receptors. By exploiting a similar gene-targeting protocol developed for human HSPC, we also corrected the mutation of the human IL2RG gene in HSPC from INDY mice. By transplanting the treated HSPC into INDY or RAG2 -/- γc-/- mice, we observed increasing levels of T and B cells expressing the human IL2RG gene over time in the blood and lymphoid organs of the transplanted animals, indicating that the gene-corrected cells were endowed with a selective growth advantage over the un-corrected INDY cells. Tests for off-target effects or toxicities are ongoing.

3.11 WP11- Gene Therapy for Lysosomal Enzyme Deficiencies

Studies were carried out regarding the Pompe disease in order to develop lentiviral stem cell gene therapy for lysosomal enzyme deficiencies. Using lentiviral vectors and modules for construction of vectors with modified promoters and post-transcriptional regulatory elements, we tested the Phosphoglycerate kinase (PGK) and Ubiquitous chromatin opening element (UCOE) promoters against the Spleen Focus Forming Virus (SF) promoter used to obtain proof-of-principle. Results pointed to the PGK promoter as a very effective promoter for further clinical development in conjunction with the codon-optimized therapeutic transgene GAA. Complementary experiments established the minimum number of integrations/ cell, the minimum numbers of (selected/purified) cells transplanted, the optimal conditioning regimen as well as the optimal age for efficacy of the therapeutic intervention, i.e. reversal of the Pompe phenotype. The safety evaluation in Pompe mice

revealed neither general nor disease-specific genotoxicity. Promising results with stem cell gene therapy for Pompe disease were highlighted.

We developed a successful preclinical gene therapy model for Globoid Cell Leukodystrophy (GLD), or Krabbe disease, demonstrating the importance of precisely targeting expression only to the most appropriate cell types. Globoid leukodystrophy (GLD) is a fatal lysosomal storage disease (LSD) caused by deficiency of the enzyme galactocerebrosidase (GALC), which leads to the accumulation of substrates, particularly in the nervous system, resulting in neuroinflammation and demyelination. The aim of HSC transplantation in lysosomal disorders was to repopulate affected tissues with macrophages/microglia that expressed and secreted a functional hydrolase. Towards this goal, efficient transduction of HSPC was essential to ensure long term repopulation of gene corrected cells, and transgene over-expression in the progeny of transduced HSC was also required. Tests of transplantation of gene-corrected HSPC into newborn GLD mice showed positive results regarding the reduction of neuroinflammation in the brain and prevention of severe peripheral demyelination, as well as an increased survival of gene therapy-treated mice as compared to untreated affected controls. Progress on the proof of efficacy and safety has been achieved and opens opportunities for treating other metabolic diseases with HSC gene therapy.

We conducted a first-in-human trial of lentiviral vector mediated HSC gene therapy for Metachromatic Leukodystrophy (MLD) by using a state-of-the-art vector platform, with self-inactivating vector LTR and an internal moderately active promoter to express the therapeutic Arylsulfatase A cDNA into hematopoietic stem cells from three pre-symptomatic patients who showed genetic, biochemical and neurophysiological evidence of late infantile MLD. Results of the study revealed that extensive genetic engineering of human hematopoiesis can be achieved with lentiviral vectors and that this approach may offer therapeutic benefit for MLD patients.

3.12 WP12 - Haemophilia target disease

This WP focuses on the development of improved gene therapy strategies for hemophilia A and B. Significant progress was made to further validate the use of miR-regulated LV for FIX gene delivery in both FIX deficient mice and hemophilia B dogs demonstrating its ability to obtain sustained therapeutic FIX levels in both small and large preclinical models. The use of miR-regulation was necessary to prevent untoward extopic expression of clotting factors in antigen-presneting cells which is an important risk factor in the indication of inhibitory antibodies to FIX or FVIII. In hemo B dogs, therapeutic FIX levels were obtained and some transient self-limiting toxicities were apparent. Partial correction of the bleeding diathesis was achieved. We have improved the efficacy of these LV-based approaches using codon-optimized FIX (or FVIII) that boost clotting factor expression. Moreover, by incorporating a hyper-activating FIX mutation, much higher clotting activity could be obtained in hemophilic mice. To minimize the risk of insertional oncogenesis, we have explored the use of integration-deficient LV that showed reduced expression of FIX but sustained induction of immune tolerance to FIX. LV also prove particularly useful to express FVIII and achieve sustained FVIII expression in haemophilia A mice. To achieve this, LV were pseudotyped with GP64 to augment hepatocyte-specific transduction. Alternatively, LV tropism could be modified using CD105-specific scFv-mediated LV retargeting to LSECs in vivo. This resulted in unprecedented LSEC targeting specificity which opens new perspectives for the use of LSECs as target for expression of secretable proteins (FVIII, FIX). Proof-of-concept was established using Epo. LV targeting to CD34+ HSC was demonstrated using SCF as targeting modality. Muscle-directed gene transfer provides an alternative for expression of secretable (or membrane-bound) proteins though turns out to raise more important immunogenicity concerns that liver-directed gene therapy which was therefore prioritized. Based on the in silico design of liver-specific regulatory elements (designated as ‘hyperons’) we have generated robust liver-specific expression cassettes that enabled sustained expression of FIX or FVIII after gene therapy. In particular, AAV9 vectors were generated containing these hyperons that resulted in robust FIX and FVIII expression. In addition, translational studies in rhesus macaques resulted in robust human FIX expression (>30%) without any serious adverse events. This underscores the efficacy and safety of AAV-based approaches.
Finally, we have developed and validated a new gene transfer paradigm based on "immune stealth" AAV nanoparticles that could potentially prevent CTL-mediated elimination of AAV-transduced cells and thus enable long-term clotting factor expression without compromising the efficiency of AAV transduction. .

3.13 WP13 - Bioprocess development

Using a model producer cell line producing VSV-g pseudotyped LV vectors with GFP as transgene, obtained from B. Massie (CNRC, Montreal/Canada) we could show that such producer cell lines can be used at a reactor scale (stirred tank reactor) in serum-free medium allowing the consistent production of high titer lentiviral vectors after double induction using doxycycline/cumate. The principle of the double induction has been shown to be a very powerful tool avoiding the leaky expression of some of the more toxic viral proteins thus assuring the maintenance of a stable phenotype. The reactor cultures (2L) allowed the production of vector titers beyond 1-2 x 107 ip/ml. However no equivalent cell line could be obtained with a different transgene and efforts on stable lines were stopped

Optimised methods for manufacturing of lentiviral vectors
The GMP production of lentiviral vectors is a more complex process than used for other vectors employed in gene therapy. Due to the toxic nature of some of the packaging proteins, to date lentiviral vectors are mainly manufactured using transient transduction of 293T cells with packaging plasmid and subsequent harvest and purification of the vector particles. This limits the batch size of clinical batches because due to the transient nature of the process upscaling over a certain threshold is difficult and because a high percentage of the infectious particles are lost during purification. At the start of the PERSIST program the recovery of lentiviral vector particles was in the region between 10 and 20 %, resulting in batch sizes of ca. 5 x 10e10 inf. particles. This meant that one batch only was big enough to treat 2-4 patients in a typical clinical transduction scenario. Due to the optimisation of multiple steps in the manufacturing procedure (ratio of packaging constructs, transfection of 293 cells, chromatographic methods and media, concentration methods and media, filtration), a gradual increase in efficiency was accomplished by the partners. This resulted in recovery rates of nearly 50 % and in batch sizes of > 2 x 10e11 infectious particles, meaning that now 8 – 16 patients can be treated with one clinical batch. The optimised manufacturing processes for lentiviral vectors will be utilised by the three companies involved in this work package to produce lentiviral vectors for their own projects as well as for third party customers.

The use of lentiviral vectors pseudotyped with other env proteins than VSV-g will in principle allow specific targeting of selected cells or tissues in vivo which would be of very high interest. Therefore in view of the development of a scalable DSP protocol of lentiviral vectors pseudotyped by other env proteins than VSV-g, our efforts were directed towards the identification of critical conditions of different chromatographic principles and tangential flow filtration, the two main process steps in a DSP protocol, for preserving the infectivity/functionality of lentiviral vectors during purification. The use of these optimized conditions permitted vector (GaLV pseudotyped) recoveries of 40-45% (in a three step process consisting of one TFF, and two different chromatographic steps). The availability of a novel DSP protocol for the purification of lentiviral vectors pseudotyped with GaLV not only will be the base for setting-up purification protocols of clinical grade vectors which was not possible up to date due to the insufficient stability of the vectors. In addition, the knowledge may be applied to other env proteins other than VSV-g. Finally, the ability to purify LV with envelopes less fusogenic than VSVg should enable new perspectives on the (re) development of stable cell lines allowing protocols for the production of lentiviral vectors at reactor scale using inducible cell lines (“next generation producer cell lines”)

3.14 WP14 - Ethics and regulatory issues

PERSIST has developed a number of innovative technologies to improve gene transfer as treatment option for genetic diseases. Although translational activities were not in focus of PERSIST, it was important to consider any regulatory and ethical implications of the project as well as to integrate and harmonize the methodologies already at an early stage of development. WP14 of the PERSIST project was established to raise awareness for ethical and regulatory issues within the PERSIST partners and also beyond addressing especially the gene therapy community in Europe but also elsewhere.
At the beginning of the project a Project Ethic Committee (PEC) was set up composed of a representative from each participating country. With Dr. Fuchs (Germany), an external expert in ethical issues was selected as advisor, while Dr. Buchholz from partner PEI, which is the German federal authority for the authorization of biological medicinal products including gene therapy, took the lead in all regulatory issues. The PEC was responsible to identify regulatory and ethical problems coming up during the project. Moreover, it supervised that handling of human samples and all animal experimental work was performed according to European

legislation and ethical guidelines. Annual updates of all required documentations were collected from all PERSIST partners and were part of the regular Deliverables of this WP. As not being involved directly in clinical trials, education in ethical and regulatory issues was identified as the main requirement for PERSIST. Accordingly three ethical reflection and education units were organized by the PEC which took place during the annual PERSIST conference. In addition two international workshops on ethical and regulatory issues were organized by the PERSIST consortium as part of conferences of the European Society for Gene and Cell Therapy (ESGCT). Both workshops were well attended each time filling big lecture halls and resulted in very active discussions. Detailed reports about the topics covered by each workshop were part of the WP14 Deliverables.
Discussions with PERSIST partners at the annual meetings revealed the regulatory topics of main interest for the consortium. Lectures held by partner PEI covered especially amendments in the relevant legal framework and guidelines, such as the certification of quality and non-clinical data obtained during product development and the so called hospital exemption regulation (Regulation (EC) No 1394/2007 on advanced therapy medicinal products amending Directive 2001/83/EC). The latter turned out to be of special interest for many PERSIST partners, since it offers an additional option to apply gene therapy products in patients besides performing clinical trials. The specific requirements that must be fulfilled to apply a product under the hospital exemption regulation as well as the procedures for application and authorization were therefore discussed in most detail. Due to the broad interest, it was decided to present this in form of an article published by an international gene therapy journal to make the whole gene therapy field aware about this option.

Ethical and regulatory issues in gene therapy are directly linked to the welfare of European citizens, who can be participants in gene therapy clinical themselves or indirectly when relatives are included as patients. Although the number of genetic diseases in focus of gene therapy is still small and accordingly also the number of patients so far involved, it is expected that this will steadily increase in future. Moreover, advanced innovative therapies such as gene therapy are constantly in focus of the public press. Positive as well as negative outcomes of gene therapy studies therefore achieve immediate broad public attendance. Educating scientists and students in gene therapy regulatory and ethical issues as performed during the PERSIST project therefore is of key relevance to guarantee clinical trial protocols that will allow a maximum of safety and benefit for the participating patients. In addition, the WP14 activities will help to improve the translation of the innovative gene transfer technologies developed by the PERSIST partners into clinical trials, and thus to make medication available for EU citizens as fast as possible. Likewise, the discussion sessions organized by PERSIST will contribute to further harmonize the views between the EU member states on how to implement European ethical and regulatory guidelines and legislation.

3.15 WP15 - Training, dissemination and IPR

The PERSIST consortium has been very active in disseminating the results and more than 130 papers have so far been published in international peer-reviewed journals and several articles are in preparation or have been submitted for publication. Some of them have been published in the top tier international research journals with general readership (e.g. Science, JCI, Nature, Nature Biotech, Nature Genetics, Nature Medicine, Nature Methods, Cell Stem Cell) as well as a number in top tier speciality journals (e.g. the Gene Therapy journals, Blood, NAR, etc.).

Partners presented the project achievements at international conferences sponsored by FASEB and at the Annual ASGCT and ESCGT Meetings throughout the time of the project. The ESGCT annual congress is one of the most important and attended meetings in the field of Gene and Cell Therapy and gathers a large scientific community together that share their latest findings, outline future directions for innovative research, support basic and translational research and clinical applications of gene and cell therapy for inherited and acquired diseases. PERSIST partners have largely contributed papers and posters directly coming from the project activities. PERSIST has also sponsored these ESGCT Meetings and carried out actions such as the organisation of satellite public days (ESGCT Meeting 2009, 2010, 2011, 2012).

Each annual scientific meeting held by PERSIST in Leukerbad (CH- 2009-2011) and in Stresa (IT - February 2013) functions as a major platform for presentations and exchanges between the PhD students and Post-docs working in the project. Scientific sessions were programmed every year with more than two days were dedicated to scientific presentations by young researchers on the topics addressed in the project.

A mobility plan was drafted at the end of the first year involving already exchanges of researchers / visits of staff between eight partners. An international summer school for PhD students was organized by the German Society of Gene Therapy in 2009, with lecturers from the PERSIST consortium

The PERSIST website (www.persist-project.eu) was established during the first year of the project and has since then been continuously updated with information about PERSIST results and meeting and events with partners's participation, publications, and links to related organisations.

A database of Background Knowledge was created in the first year of the project and was updated regularly. In the last year of the project, a new project, SUPERSIST, was accepted by the EC for funding a follow-up experimental research based upon the innovative targeted integration technology developed in PERSIST. The objective of the new project is to pave the way towards clinical translation of selected new gene targeting technologies for correcting inherited mutations and empowering adoptive immunotherapy of cancer. It involves 4 partners from the present consortium for a duration of 3 years.

3.16 WP16 - Project Management

The role of the management team, composed by the project coordinator (USR) and the project management partner (NOVAMEN) was to ensure that consortium activities are performed in accordance to the EC expectations in terms of content and timescale.

The Management Manual of PERSIST was produced at Month 3 of the project and gave the main decision-making rules and the main procedures regarding the management of the project (reports, deliverables, communication, etc. More particularly, this deliverable D16.1 included: i) a description of the project management structure, organisation and procedures; ii) the composition, role and responsibilities of the different Project Committees as well as the responsibilities and duties of the Project Coordinator, Work Package Leaders and partners; iii) the general rules for meetings and communication; iv) the internal procedures for reporting and deliverables; v) the major milestones and project deliverables to be produced during the project.

The operational management organisation was installed since the beginning of the project. Strategic, management and workshops meetings were taking place every year to monitor the execution of the project and the work progress and to define the project strategies. They also hosted members of the Project Ethics Committee (PEC).
In addition to these Governing Board meetings, regularly meetings or conference calls were organised on the WP level to ensure effective communication and coordination of the scientific work.
Work progress and results were reported to the European Commission by the consortium in the Periodic Activity Reports that were submitted at Months 12, 24 and 36. The fourth scientific report covered the activities carried out during the last 18 months of the project.

A project mid-term review with the EC officer and two external reviewers was held in Brussels on July 6th, 2011. The project was reviewed in depth for technical achievements of the scientific WPs, for dissemination and publication of results, and for the overall project management. The reviewers pointed out that the project was progressing steadily towards achieving its objectives and suggested to strengthen 5 workpackages in particular.

The protection, management and transfer of IPR appeared to be an important and strategic issue for a successful implementation of PERSIST project, as well as for open opportunities for commercial exploitation. This element included the sharing of knowledge to comply with the rules of participation in FP7 programs,

4 Potential impact

PERSIST project has made a major contribution to strengthen the position of European research in the development and clinical translation of new technologies for genetic modification of somatic cells. This sets the path for innovation in a field whose success is largely dependent on the development of novel tools for "gene surgery" that operate with a defined mechanism of action and in the absence of major adverse effects. In the mid-term, these novel tools will become more and more important for applications in human gene therapy and biotechnology, thus improving human health and stimulating European economy.

4.1 Scientific perspectives

The "targeted integration" activities showed that the Sleeping Beauty (SB) transposon is a nonviral, integrating vector system with proven efficacy in preclinical animal models, and thus holds promise for future clinical applications. However, SB has a close-to-random insertion profile that could lead to genotoxic effects, thereby presenting a potential safety issue. Improvements in ZF technology and a careful choice of targeted genomic regions may improve the safety profile of SB for future clinical applications.

Surface engineering of lentiviral vectors yielded new types of vectors that allow targeted gene transfer into human hematopoietic stem cells. These tools might allow bypassing *ex vivo* handling and simplify gene therapy for many hematopoietic defects in the future by direct *in vivo* inoculation into the patient. Secondly, vectors carrying the glycoproteins of measles virus at their surface might overcome some limitations of classical lentiviral vectors for genetic modification *ex vivo* and *in vivo* of hematopoietic cells. Indeed, these new vectors perform efficiently for gene transfer into quiescent T, B and dendritic cells whereas classical vectors are inefficient. These vectors represent a versatile tool that can target lentiviral gene transfer to basically any cell type (immune cells, endothelial cells, cancer cells) with an unforeseen specificity: this opens the way to multiple gene therapy applications where specific *in vivo* gene transfer would be advantageous.

Several protocols have been developed and optimized for the efficient delivery of the molecular machinery required to induce homology-driven transgene insertion into selected genomic target sites in human lymphocytes and hematopoietic stem cells. These protocols allow targeted insertion of transgene cassettes into genomic "safe harbour" sites, which allow robust expression without any detectable detrimental effect on the recipient genome, as well as in situ correction of inherited mutant genes, with functional reconstitution both of function and physiological expression control. The scope and power of these new strategies have been validated in *in vivo* disease models and human hematochimeric mice. These are major advances that fulfill a key goal of the project and represent a major step toward the clinical exploitation of the benefits of the new technologies

Two novel regulators of Hematopoietic Stem Cells: Angiopoietin-like protein 4 (Angptl4) and Cripto have been discovered. They may provide the way to improve the culture conditions for HSC *ex vivo*.

The research performed within the work package 6 has resulted in the designing of next-generation gene therapy vectors which are characterized by regulated transgene expression, targeted only to the hematopoietic cell lineage and differentiation stage where a functional copy of the gene is actually needed for disease correction and thus avoiding off-target toxicity in the delicate stem- and progenitor cell compartment. This concept can be applied to a wide range of genetic and acquired diseases amenable to hematopoietic stem cell based gene therapy, and will improve the safety and long-term efficacy of the procedure. This will ultimately lead to highly innovate, curative treatments of genetic but also acquired diseases that may be more cost-effective than lifelong drug therapy.

One of the CGD (Chronic Granulomatous Disease) vectors developed by PERSIST has entered Phase I/II clinical trials. The successful treatment of CGD patients by gene therapy with the vectors developed in the WP6 will not only generate tangible benefits for the patients but will also provide a long-lasting contribution to healthcare. Furthermore the introduction of these vectors in clinical trials will generate a wealth of information on advanced vector technology, the treatment of immunodeficiencies and hematopoietic gene therapy.

No matter what their origin, strain and family, viruses have evolved exquisite strategies to reach and penetrate specific target cells where they hijack the cellular machinery to express viral genes and produce progeny particles. The ability to deliver and express genetic information to cells is the basis for exploiting viruses to

genetically modify the natural cell target or, upon manipulation of the viral receptor to retarget the virus, to genetically engineer different cell types. Specifically, retroviral vectors have been successfully used for the correction of inherited immune diseases in clinical trials. However, with increasing efficiency and success in clinical retroviral gene therapy, the occurrence of vector-induced severe side effects has also increased. In the workpackage 7 we have developed and performed qualitative and quantitative *in vitro* and *vivo* monitoring in gene marking studies, preclinical and clinical gene therapy settings. Towards safe clinical application of retroviral and lentiviral vectors uncovered target site preferences have proven to play substantial roles in the likelihood for insertional mutagenesis and cellular transformation. Knowledge gained from comprehensive integration site analysis of gene therapy patients and preclinical animal models to assess genotoxic safety of vectors are currently being actively transferred into the development of new and safer clinical protocols for the treatment of hematological and neurodegenerative diseases.
We have set up integration site landscapes of various viral vectors. Incorporation of new sophisticated technologies like next generation/deep sequencing combined with LAM/nrLAM-PCR helped to substantially improve in depth analyses and to monitor individual and global clone contribution in a hitherto unexpected resolution. Prospective monitoring of vector integration sites in clinical gene therapy studies is feasible, can detect possible side effects of gene therapy in real-time and thus has gained new insights in basic mechanisms leading to specific clonal behaviour *in vivo*. The safety of gene delivery systems represents an issue of major concern and thus significant efforts were directed towards this end.

We developed and optimized a protocol that allows achieving site-specific gene correction of the IL2RG gene in HSPC with efficiency compatible with clinical translation. We envisage prompt clinical translation of this approach, which may benefit SCID-X1 patients while avoiding the risks of allogeneic transplantation or HSC gene therapy with conventional integrating vectors. Additionally, the strategies that we are developing have potential application for gene correction of other primary immunodeficiences. The activities carried out in the workpackage 10 represent the first studies to demonstrate robust correction of a human disease using targeted integration at the disease gene locus in primary hematopoietic stem cells (human and murine). The platform technologies developed in this WP can be utilized to test other gene targeting strategies for SCID-X1, but more importantly will provide the basis for development of clinical protocols. It is anticipated that clinical studies will be implemented within five years after refinement of protocols and assessment of potential residual toxicities. The development of safe one-off curative autologous treatments for diseases such as SCID has attractive healthcare economic benefits when compared with current mis-matched allogeneic transplantation technologies as the survival rates without complications such as graft versus host disease, are likely to be much higher, and long term complications much lower.

By using the optimised methods resulting from WP13, the partners involved will be able to produce lentiviral vectors with much higher efficiency than was state of the art before the start of the PERSIST project. This will result in the possibility that more clinical trials using lentiviral vectors can be started which will benefit European translational science as well as patients suffering from disease that can be ameliorated by gene therapy with lentiviral vectors.
Due to the increased efficiency of the manufacturing process, the price of one patient dose will also be decreasing considerably, so that the cost burden for the conduct of clinical trials can be reduced.

4.2 Impact on health problems

PERSIST studies pave the way to clinical testing of innovative gene targeting technologies and establish a pipeline of solutions to outstanding hurdles currently limiting the exploitation of gene therapy for improving human health. The cutting-edge scientific knowledge gained by PERSIST project, is currently further developed by the academic centers of excellence involved, for translation into feasible and innovative progression of medical technology by the industry and SME partners.

Genetic diseases: The overall goal of PERSIST is the application of the new gene therapy strategies being developed to the definition of safe and effective gene therapy protocols for severe human disease. The advantage of gene therapies over currently available treatments is evident both for final users (patients) and for payers (National Health systems, health insurances....). Standard treatments rely on the life-long administration of animal derived or recombinant enzymes, which implies (a) For patients, potential immune reaction against such non-self proteins and continuous (life-saving) injections, (b) For payers, life-long

payments for each patient, therapies becoming increasingly expensive (dose and therefore price is related to the weight of the patient, increasing 20-30 times from babies to adults).

Leukemia: The incidence of acute leukemia (AL) is between 6 and 10 per 100,000 individuals per year. The usual treatment of AL is divided into two phases: induction of remission and post-remission therapy. The induction phase aims to decrease the number of leukemia cells to undetectable levels. Conventional treatment often result in complete remission in up to 60% (AML) - 80% (ALL) of adult patients and in up to 85% (AML) – 99% (ALL) of paediatric patients. However, such remissions are usually short-lived unless additional, post-remission therapy is given. Treatments for post-remission therapy such as allogeneic hematopoietic stem cell transplantation (HSCT) are of limited efficacy and can induce major serious complications, including infections, graft-versus-host disease (GvHD) and the development of new malignancies.
The TCR editing approach aims at exploiting leukemia-specific immunity while minimizing the risks of toxicity, including the risk of graft-versus-host disease (GvHD). This technology has the potential to become the standard of treatment for patients with high-risk acute leukaemia, reducing the risks of relapse and, ultimately, leading to increased survival and better quality of life.

Haemophilia is one of the most common hereditary diseases in man (frequency 1:5,000) and lead to uncontrollable bleeding episodes, primarily in joints leading to chronic crippling arthropathy or in internal organs and tissues which can have potential lethal consequences. The development of gene therapy for haemophilia constitutes a major research priority in its own right and serves as an ideal trailblazer for application of new gene therapy approaches for many different disease targets. Current treatment of haemophilia A and B consists of infusion of purified factor VIII (FVIII) or factor IX (FIX) concentrates, respectively. Although this treatment markedly improved the life expectancy of patients suffering from haemophilia, they are still at risk for life threatening bleeding episodes and chronic joint damage, especially since prophylactic treatment is restricted by the limited availability and high cost of purified clotting factors. Typically, the average cost per patient falls between 150,000€ and 350,000€/year, amounting to an estimated 15,000M€ - 35,000M€ in the EU member countries, excluding the costs of secondary complications. The World Federation of Haemophilia estimates that of the 400,000 patients with haemophilia worldwide, 300,000 patients receive either no, or very sporadic treatment by protein substitution therapy.
Thus, the innovation achieved in the context of PERSIST, mainly in WP12, may ultimately provide cost effective therapies/cures for all affected individuals. Moreover an important side effect of clotting factors substitution therapy is that some patients develop neutralizing antibodies against FIX or FVIII (clinically referred to as “inhibitors”) that render further substitution ineffective. The results achieved in the project indicate that gene therapy may provide a solution to this important side effect of conventional substitution therapy with purified clotting factors. Indeed, we have managed to induce immune tolerance using LV, IDLV, AAV and transposon platforms. We even achieved haemostatic correction and immune tolerance induction in haemophilia B mice with pre-existing inhibitory antibodies that were treated by gene therapy. The improvement of quality of life and life expectancy for patients in these inherited diseases does not require much argumentation, and neither does the economic benefit to the patients, their families and society as a whole when protracted invalidating disease courses can be corrected by a single medical intervention as ensured by the gene therapy approach, and as opposed to prolonged and eventually unsuccessful conventional symptomatic medical treatment, or a curative treatment such as allogeneic stem cell transplantation that carries high risks and is only accessible to a minority of patients.
The development of innovative gene therapy as outlined in this project for these four highly diverse example inherited diseases will not only have a profound impact for many inherited diseases and introduce an economically responsible therapeutic approach, but also set the stage for applications in many acquired diseases such as cancer and infectious diseases as well.

Rare diseases, of which 80% are genetic diseases, affect some 6% of the human population, amounting in Europe to around 30 million people. Since many rare diseases result in chronic disability and cost intensive care, the impact is disproportional and may well be over 20% of health care costs. Most of these diseases lack effective treatments, are managed by symptomatic treatment only and are amenable to gene therapy. The development of innovative gene therapy approaches as conducted in PERSIST for some severe inherited diseases will not only have a profound impact on these diseases but also on several other inherited diseases and introduce an economically responsible therapeutic approach. The project has investigated novel means of permanent genetic engineering of major target organs for advanced cell and gene therapy, and has delivered proof-of-principle that will lead to the development of novel clinical protocols.

Thus, PERSIST has contributed to overcome the uncertainty related to dose-limiting side effects of rather untargeted early generations of integrating vectors. Furthermore, our results are setting the stage for applications in acquired diseases such as cancer and infectious diseases as well. The outcome of PERSIST will result in faster discovery and development of better medicines to meet the great EU health challenges.

4.3 Dissemination of foreground

Publications during the first period (01/01/2009 – 31/12/2009)

Type [2]	References [3]
Article	Cartier-Lacave N., Hacein-Bey-Abina S., Bartholomae C. C., Veres G, Schmidt M., Kutschera I., Vidaud M., Abel U., Dal-Cortivo L., Caccavelli L., Mahlaoui N., Kiermer V., Mittelstaedt D., Bellesme C., Lahlou N., Lefrère F., Blanche S., Audit M., Payen E., Leboulch P., l'Homme B., Bougnères P., Von Kalle C., Fischer A., Cavazzana-Calvo M., Aubourg P. *Hematopoietic Stem Cell Gene Therapy With a Lentiviral Vector in X-linked Adrenoleukodystrophy*. **Science** 2009, Nov 6;326(5954):818-23.
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Article	Vink, C.A., Gaspar, H.B., Gabriel, R., Schmidt, M., McIvor, R.S., Thrasher, A.J., Qasim, W. *Sleeping beauty transposition from nonintegrating lentivirus.* **Molecular Therapy**. 17, 1197-1204, 2009.
Article	Maetzig, T., M. Galla, M. Brugman, R. Loew, C. Baum, A. Schambach. *Mechanisms controlling titer and expression of bidirectional lentiviral and γ-retroviral vectors.* **Gene Therapy** 2010 Mar;17(3):400-11, Epub 2009 Oct 22.
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Article	Modlich U, Navarro S, Zychlinski D, Maetzig T, Knoess S, Brugman MH, Schambach A, Charrier S, Galy A, Thrasher AJ, Bueren J, Baum C. *Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors*. **Molecular Therapy** 2009; 17:1919-28
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Publications during the second period (01/01/2010 – 31/12/2010)

Publications	Journal	Participants / Authors	Comments / Titles *
Feb 7, 2010	Nat Methods	MHH2	Reply to: Genome editing with modularly assembled zinc-finger nucleases.
July 1, 2011	Expert Opin Biol Ther.	MHH2	Generation and genetic modification of induced pluripotent stem cells.
Aug 16, 2010	Nucleic Acids Res.	MHH2	Autonomous zinc-finger nuclease pairs for targeted chromosomal deletion.
Feb 1, 2011	Curr Gene Ther.	MHH2	Zinc-Finger Nuclease Based Genome Surgery: It's all About Specificity.
April 2010	Mol. Ther. 18:1200-9.	MDC, VIB et al.	Comparative analysis of transposable element vector systems in human cells.
2010	Stem Cells 28:1760-71.	VIB/MDC	Novel hyperactive transposons for genetic modification of induced pluripotent and adult stem cells: A non-viral paradigm for coaxed differentiation.
14/01/2010	Nature	EPFL/Rowe	KAP1 controls endogenous retroviruses in embryonic stem cells
June 2010	Journal of Virology	EPFL/Maillard	The Specificity of TRIM5a-Mediated Restriction is Influenced by its Coiled-Coil Domain
March 2010	PloS Genetics	EPFL/Groner	KRAB-Zinc Finger Proteins and KAP1 Can Mediate Long-Range Transcriptional Repression through Heterochromatin Spreading
23/07/2010	Science	EPFL/Busskamp	Genetic Reactivation of Cone Photoreceptors Restores Visual Response in Retinitis Pigmentosa
09/07/2010	Science	EPFL/Trono	HIV Persistence and the Prosepect of Long-Term Drug-Free Remissions for HIV-Infected Individuals
16/07/2010	Stem Cells	EPFL/Laurenti	Inducible Gene and shRNA Expression in Resident Hematopoietic Stem Cells in Vivo
01/10/2010	Current Protocols in Neuroscience	EPFL/Barde	UNIT 4.21 Production and Titration of Lentiviral Vectors
02/11/2010	Journal of Virology	EPFL/Bulliard	Structure-Function Analyses Point to a Polynucleotide-Accommodating Groove Essential for APOBEC3A
Dec 2010	PLos One	EPFL/Friedli	A systematic enhancer screen using lentivector transgenesis identifies conserved and non-conserved functional elements at the olig1 and olig2 locus.
Dec 2010	The Journal of biological chemistry	EPFL/Maillard	Homology-based identification of capsid determinant that protect HIV1 from human TRIM5-a restriction
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Publications	Journal	Participants / Authors	Comments / Titles *
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Publications	Journal	Participants / Authors	Comments / Titles *
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23 Sep. 2010	Blood, 116:5507-5517, 2010	UNIMORE, USR	High-definition mapping of retroviral integration sites identifies active regulatory elements in human multipotent hematopoietic progenitors
22 Dec. 2010	PLoS One, 5:e15688, 2010	UNIMORE, USR, DKFZ	High-definition mapping of retroviral integration sites defines the fate of allogeneic T cells after donor lymphocyte infusion
2010	Nat. Methods 7, 929-935	PEI	Specific gene transfer to neurons, endothelial cells and hematopoietic progenitors with lentiviral vectors
22 July 2010	Blood;116(3):498-500	INSERM	Lévy C, Frecha C, Costa C, Rachinel N, Salles G, Cosset FL, Verhoeyen E. Lentiviral vectors and transduction of human cancer B cells
October 2010	Mol Ther.;18(10):1748-57. Epub 2010 Aug 24. Review	INSERM	Frecha C, Lévy C, Cosset FL, Verhoeyen E. Advances in the field of lentivector-based transduction of T and B lymphocytes for gene therapy
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2011	Journal of Controlled Release 150: 23-29	ETHZ	Kemmer C, Fluri DA, Witschi U, Passeraub A, Gutzwiller A and Fussenegger M. A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms
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2010	Current Opinion in Hematology 2010, 17:387–392	VIB	Janka Mátrai, Marinee K.L. Chuah and Thierry VandenDriessche. Preclinical and clinical progress in hemophilia gene therapy
2011	Hepatology 2011,	VIB, USR, DKFZ, VUB	J. Mátrai1, A. Cantore, C. Bartholomae, A. Annoni, W. Wang, A. Acosta-Sanchez, E. Samara-Kuko, L. De Waele, L. Ma, P. Genovese, M. Damo, A. Arens, K. Goudy, T. Nichols, C. von Kalle, M. Chuah, M. Grazia Roncarolo, M. Schmidt, T. VandenDriessche, L. Naldini Hepatocyte-targeted Expression by Integrase-Defective Lentiviral Vectors Induces Transgene-specific Immune Tolerance With Low Genotoxic Risk
August 2010	J Gene Med 2010; 12: doi: 10.1002/jgm.1500	VIB	Inge Petrus, Marinee Chuah, Thierry VandenDriessche Gene therapy strategies for hemophilia: benefits versus risks
January 2010	Molecular Therapy doi:10.1038/mt.2009.319	VIB	Janka Mátrai, Marinee K.L. Chuah and Thierry VandenDriessche. Recent Advances in Lentiviral Vector Development and Applications
1 July 2010	Blood, Volume 115, Number 26	ERASMUS	N. van Til, M. Stok, F. Aerts Kaya, M. de Waard, E. Farahbakhshian, T. Visser, M. Kroos, E. Jacobs, M. Willart, P. van der Wegen, B. Scholte, B. Lambrecht, D. Duncker, A. van der Ploeg, A. Reuser, M. Verstegen, G. Wagemaker Lentiviral gene therapy of murine hematopoietic stem cells ameliorates the Pompe disease phenotype
27 April 2010	PNAS, vol. 107, no. 17, 7805–7810	MHH1	C. Voelkel, M. Galla, T. Maetzig, E. Warlich, J. Kuehle, D. Zychlinski, J. Bode, T. Cantz, A. Schambach, C. Baum Protein transduction from retroviral Gag precursors

Publications during the third period (01/01/2011 – 31/12/2011)

Journal	Participants / authors	Title of publication
Virology, 2011. 411(2): p. 273-87	EPFL	Dynamic control of endogenous retroviruses during development
BMC genomics, 2011. 12(1): p. 378	EPFL	*S. Meylan, A. Groner, G. Ambrosini, N. Malani, S. Quenneveille, N. Zangger, A. Kapopoulou, A. Kauzlaric, J. Rougemont, A. Ciuffi, F.D. Bushman, P. Bucher and D. Trono* A gene-rich, transcriptionally active environment and the pre-deposition of repressive marks are predictive of susceptibility to KRAB/KAP1-mediated silencing
Chemistry & biology, 2011. 18(6): p. 805-15	EPFL	Measuring in vivo protein half-life
Curr Protoc Mouse Biol 1, 2011. 1: p. 169-184	EPFL	Lentiviral Vector Mediated Transgenesis
Gene Ther, 2011	EPFL	*Barde I., E. Laurenti, S. Verp, M. Wiznerowicz, S. Offner, A. Vironery, A. Galy, A. Trumpp and D. Trono* Lineage- and stage-restricted lentiviral vectors for the gene therapy of chronic granulomatous disease
Cell Host Microbe, 2011. 9(3): p. 170-2	EPFL	Profaning the ultimate sanctuary: HIV latency in hematopoietic stem cells
PLoS One, 2011. 6(3): p. e17634	EPFL	Chromosome conformation capture uncovers potential genome-wide interactions between human conserved non-coding sequences
Molecular cell, 2011. 44(3): p. 361-72	EPFL	In Embryonic Stem Cells, ZFP57/KAP1 Recognize a Methylated Hexanucleotide to Affect Chromatin and DNA Methylation of Imprinting Control Regions
Cell Death Differ, 2011. 18(5): p. 745-53	EPFL	Genomic instability in induced stem cells
Nucleic Acids Research, 2011, 1–14	MHH	*Gala M., A. Schambach, C. Falk, T. Maetzig, J. Kuehle, K. Lange, D. Zychlinski, N. Heinz, M. Brugman, G. Göhring, Z. Izsvak Z. Ivics and C. Baum* Avoiding cytotoxicity of transposases by dose-controlled mRNA delivery
Human Gene Therapy, 2011, 22:1	MHH	*Voelkel et al* Pseudotype-Independent Nonspecific Uptake of Gammaretroviral and Lentiviral Particles in Human Cells
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Nat Methods, 2011 Aug 21; 8(10):861-9	USR	*Lombardo et al* Site-specific integration and in situ tailoring of cassette design allow "sustainable" gene transfer.
Nat Biotechnol, 2011 Aug 7; 29(9):816-23	USR - DKFZ	*Gabriel R, Lombardo A, Arens A, Miller JC, Genovese P, Kaeppel C, Nowrouzi A, Bartholomae CC, Wang J, Friedman G, Holmes MC, Gregory PD, Glimm H, Schmidt M,Naldini L, von Kalle C.* An unbiased genome-wide analysis of zinc finger nuclease specificity
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Hum Gene Ther 22, 1249–1254	MRC, PEI	*Ageichik,A., et al* Lentiviral Vectors Targeted to MHC II Are Effective in Immunization
Mol Ther. 2011 Jul;19(7): 1193-8.	CIEMAT, USR, GENETHON, DKFZ, INSERM, UCL	*Tolar J, Adair JE, Antoniou M, Bartholomae CC, Becker PS, Blazar BR, Bueren J, Carroll T, Cavazzana-Calvo M, Clapp DW, Dalgleish R, Galy A, Gaspar HB, Hanenberg H, Von Kalle C, Kiem HP, Lindeman D, Naldini L, Navarro S, Renella R, Rio P, Sevilla J,*

		Schmidt M, Verhoeyen E, Wagner JE, Williams DA, Thrasher AJ Stem cell gene therapy for fanconi Anemia: report from the 1st international Fanconi anemia gene therapy working group meeting
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Nat Biotechnol. 2011 Aug 7; 29(9):816-23	USR, DKFZ	*Gabriel et al* An unbiased genome-wide analysis of zinc finger nuclease specificity
Nucleic Acids Res. 2011 Nov;39(21):9283-93	MHH	*Mussolino et al* A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity
PLoS One. 2011;6(12):e28911	MHH	*Osiak et al* Selection-independent generation of gene knockout mouse embryonic stem cells using zinc-finger nucleases
Biochemistry. 2011 Jun 7; 50(22):5033-41	MHH	*Shimizu et al* Adding fingers to an engineered zinc finger nuclease can reduce activity
Stem Cell Rev. 2011 Aug 27	MHH	*Höher et al* Highly Efficient Zinc-Finger Nuclease-Mediated Disruption of an eGFP Transgene in Keratinocyte Stem Cells without Impairment of Stem Cell Properties
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Science 2011, 332: 1565	ETHZ	*Ye H, Daoud-El Baba M, Peng R.W and Fussenegger M* A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice.
EMBO Mol Med. 2011 Feb;3(2) :89-101	USR	*Biasco al.* Integration profile of retroviral vector in gene therapy treated patients is cell-specific according to gene expression and chromatin conformation of target cell.
Mol Ther. 2011 Apr;19(4):703-10	USR	*Bartholomae et al* Lentiviral vector integration profiles differ in rodent postmitotic tissues
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Molecular Therapy vol. 19 no. 4, 723–730	USR	*Matsui H. et al*, A MicroRNA-regulated and GP64-pseudotyped Lentiviral Vector Mediates Stable Expression of FVIII in a Murine Model of Hemophilia A
Mol. Ther., Oct 19(10):1867-1877	ERASMUS, MHH, UNIMORE, UCL	*Huston M.W., van Til N.P., Visser T.P., Arshad S., Brugman M.H., Cattoglio C., Nowrouzi A., Li Y., Schambach A., Schmidt M., Baum C., von Kalle C., Mavilio F., Zhang F., Blundell M.P., Thrasher A., Verstegen M.M.A., Wagemaker G* Correction of murine SCID-X1 by lentiviral gene therapy using a codon optimized IL2RG gene and minimal pre-transplant conditioning
Mol. Ther., Nov 19(11):2031-2039	DKFZ, USR, UNIMORE, ERASMUS, MHH, UCL	*Deichmann A, Brugman MH, Bartholomae CC, Schwarzwaelder K, Verstegen MM, Howe SJ, Arens A, Ott MG, Hoelzer D, Seger R, Grez M, Hacein-Bey-Abina S, Cavazzana-Calvo M, Fischer A, Paruzynski A, Gabriel R, Glimm H, Abel U, Cattoglio C, Mavilio F, Cassani B, Aiuti A, Dunbar CE, Baum C, Gaspar HB, Thrasher AJ, von Kalle C, Schmidt M, Wagemaker G*

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Curr. Gene Ther., 11:399-405	UNIMORE	*Recchia A., Mavilio F* Site-specific integration by the Adeno-associated virus rep protein
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Human Gene Therapy 22:1043–1051	MDC, PEI	*Zoltan Ivics and Zsuzsanna Izsvak* Nonviral Gene Delivery with the Sleeping Beauty Transposon System
Expert Opin. Biol. Ther. (2012) 12(2):139-153	MDC, PEI	*Marta Swierczek, Zsuzsanna Izsvak and Zoltan Ivics* The Sleeping Beauty transposon system for clinical applications
Gene Ther. 2011 Oct 1;11(5): 363-74	LMU	*Martin Hausl, Wenli Zhang, Nadine Müther, Richard Voigtländer, Christina Rauschhuber, and Anja Ehrhardt* Development of adenovirus hybrid vectors for Sleeping Beauty transposition in large mammals

Publications during the fourth period (01/01/2012 – 30/06/2013)

Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Year of publication	Relevant pages	Permanent identifiers (if available)	Is/Will open access provided to this publication?
Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy	Biffi A	Science	July 2013		2013			No
Lentiviral Hematopoietic Stem Cell Gene Therapy in Patients with Wiskott-Aldrich Syndrome	Aiuti A	Science	July 2013		2013			no
A KRAB/KAP1-miRNA cascade regulates erythropoiesis through stage-specific control of mitophagy.	Barde I	Science	340(6130)	AAAS	2013	350-3	doi: 10.1126/science.1232398	no
A synthetic single-cell mammalian biocomputer performing programmable half-subtractor and half-adder calculations	Ausländer S	Nature	487(7405)	Nature publishing group	2012	123-7	doi: 10.1038/nature11149.	no
Angptl4 maintains in vivo repopulation capacity of CD34+ human cord blood cells	Blank U.	Eur J Heamatol	Sept., 89(3)	Blackwell publishing	2012	198-205	doi: 10.1111/j.1600-0609.2012.01812.x	no
Hematopoietic stem cells are regulated by Cripto as an intermediary of HIF-1α in the hypoxic bone marrow niche	Miharada	Annals of the New York Academy of Sciences	Aug, 1266	Blackwell publishing	2012	55-62	doi: 10.1111/j.1749-6632.2012.06564.x.	no
Escape of measles virus glycoprotein based lentiviral targeting vectors from neutralizing antibodies	Kneissl S	PLoS one	2012;7(10)		2012	e46667	doi: 10.1371/journal.pone.0046667	yes
Lentiviral vectors displaying	Levy C.	Molecular	20(9)	Nature	2012	1699-712	doi: 10.1038/mt.2012.96	no

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Stem cell factor-displaying SIV vectors together with a low conditioning regimen allow for long-term engraftment of gene marked autologous HSCs in macaques.	Verhoeyen E.	Hum. Gene Ther.	23(7)	Mary Ann Liebert, Inc	2012	754-68	doi: 10.1089/hum.2012.020	yes
Efficient transduction of healthy and malignant plasma cells by lentiviral vectors pseudotyped with measles virus glycoproteins	Schoenhals M	Leukemia	26(7)	Nature Publishing group	2012	1663-70	doi: 10.1038/leu.2012.36	no
Measles virus glycoproteins pseudotyped lentiviral vectors are highly superior over VSV-G pseudotypes for genetic modification of monocyte-derived DCs	Humbert J-M	J. Virol	86(9)	American society for Microbiology	2012	5192-203	doi: 10.1128/JVI.06283-11	yes
Glut1-mediated glucose transport regulates HIV infection	Loisel-Meyer S.	Proc Natl Acad Sci U S A	109(7)	National Academy of sciences	2012	2549-54	doi: 10.1073/pnas.1121427109	yes
A novel lentivector targets gene transfer into hHSC in marrow from patients with BM-failure-syndrome and in vivo in humanized mice.	Frecha C.	Blood	119(5)	American Society of Hematology	2012	1139-50	10.1182/blood-2011-04-346619	yes
The European hospital exemption clause-new option for gene therapy?	Buchholz C.	Hum. Gene Ther.	23(1)	Mary Ann Liebert, Inc	2012	7-12	doi: 10.1089/hum.2011.2529.	no
Corticospinal tract transduction: a comparison of seven adeno-associated viral vector serotypes and a non-	Hutson TH	Gene Ther.	19(1)	Nature Pub. Group	2012	49-60	doi: 10.1038/gt.2011.71	yes

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Highly potent delivery method of gp160 envelope vaccine combining lentivirus-like particles and DNA electrotransfer	Vandermeulen G.	J. Control release	159(3)	Elsevier	2012	376-83	doi: 10.1016/j.jconrel.2012.01.035	no
Lentiviral vectors encoding shRNAs efficiently transduce and knockdown LINGO-1 but induce an interferon response and cytotoxicity in CNS neurons	Hutson TH	J. Gene Med.	14(5)	John Wiley & Sons, Ltd	2012	299-315	doi: 10.1002/jgm.2626	no
AMP-activated protein kinase (AMPK) –dependent and –independent pathways regulate hypoxic inhibition of transepithelial Na+ transport across human airway epithelial cells	Tan CD	J. Pharmacol.	167(2)	British journal of pharmacology	2012	368-82	doi: 10.1111/j.1476-5381.2012.01993.x	no
Efficient gene expression from integration-deficient lentiviral vectors in the spinal cord	Peluffo H	Gene Ther.	20(6)	Nature publishing	2013	645-57	doi: 10.1038/gt.2012.78	no
Activation of EphA receptors mediates the recruitment of the adaptor protein Slap contributing to the downregulation of NMDA receptors	Semerdjieva, S	Mol. Cell Biol.	33(7)	American society for Microbiology	2013	1442-55	doi: 10.1128/MCB.01618-12	no
Retargeting Sleeping Beauty transposon insertions by engineered zinc finger DNA-binding domains	Voigt K	Mol. Ther	20(10)	Nature publishing	2012	1852-62	doi: 10.1038/mt.2012.126	no
Retargeting transposon insertions by the adeno-associated virus Rep protein	Ammar I	Nucleic Acids Res	40(14)	Oxford University Press	2012	6693-712	doi: 10.1093/nar/gks317	yes
Overexpression of the anti-apoptotic protein AVEN	Eißmann M	Oncogene	32(20)	Nature Publishing	2012	2586-91	doi: 10.1038/onc.2012.263	no

contributes to increased malignancy in hematopoietic neoplasms				group				
Integration of retroviral vectors	Gabriel R	Curr Opin Immunol	24(5)	Elsevier	2012	592-7	doi: 10.1016/j.coi.2012.08.006	no
Bioinformatic Clonality Analysis of Next-Generation Sequencing-Derived Viral Vector Integration Sites	Arens A	Hum Gene Ther Methods	23(2)	Mary Ann Liebert, Inc	2012	111-8	doi: 10.1089/hgtb.2011.219	No
Lentiviral vector-based insertional mutagenesis identifies genes associated with liver cancer	Ranzani M	Nath Methods	10(2)	Nature Publishing Group	2013	155-61	doi: 10.1038/nmeth.2331	yes
Preclinical safety and efficacy of human CD34(+) cells transduced with lentiviral vector for the treatment of Wiskott-Aldrich Syndrome	Scaramuzza S	Mol. Ther	21(1)	Nature Publishing Group	2013	175-84	doi: 10.1038/mt.2012.23	yes
The Sleeping Beauty transposon toolbox.	Ammar I	Methods Mol. Biol	859	Springer	2012	229-40	doi: 10.1007/978-1-61779-603-6_13	no
Retrovirus-based mRNA transfer for transient cell manipulation	Galla M.	Methods Mol. Biol	969	Springer	2013	139-161	doi: 10.1007/978-1-62703-260-5_10	no
Retroviral protein transfer: falling apart to make an impact	Maetzig T.	Curr Gene Ther	12(5)	Bentham science publishers	2013	389-409	10.2174/156652312802762581	no
Pseudotype-independent nonspecific uptake of gammaretroviral and lentiviral particles in human cells	Voelkel C.	Hum Gene Ther	23(3)	Mary Ann Liebert, Inc	2012	274-286	doi: 10.1089/hum.2011.011	no
Clinical progress in gene therapy: sustained partial correction of the bleeding disorder in patients suffering from severe hemophilia B	VandenDriessche T	Hum Gene Ther.	23(1)	Mary Ann Liebert, Inc	2012	4-6	doi: 10.1089/hum.2011.221	no
PiggyBac Toolbox	Di Matteo M.	Methods Mol. Biol	859	Spinger	2012	241-54	doi: 10.1007/978-1-61779-603-6_14.	no

Recent developments in transposon-mediated gene therapy	DI Matteo M	Expert Opin Biol Ther.	12(7)	Informa Plc	2012	841-58	doi: 10.1517/14712598.2012.684875	no
Recent progress in gene therapy for haemophilia.	Chuah MK	Hum Gene Ther	23(6)	Mary Ann Liebert, Inc	2012	557-65	doi: 10.1089/hum.2012.088	no
Hyper-functional coagulation factor IX improves the efficacy of gene therapy in hemophilic mice	Cantore A	Blood	120(23)	American Society of Hematology	2012	4517-20	doi: 10.1182/blood-2012-05-432591	no
Gene therapy for hemophilia	Chuah MK	J. Thromb. Hemost	Suppl. 1	John Wiley & Sons, Inc	2013	99-110	doi: 10.1111/jth.12215	yes
Specific gene delivery to liver sinusoidal and artery endothelial cells	Abel T.	Blood			2013			no
Prolonged gene expression in muscle is achieved without active immune tolerance using MicrorRNA 142.3p-regulated rAAV gene transfer.	Boisgerault F	Hum Gene Ther	24(4)	Mary Ann Liebert, Inc	2013	393-405	doi: 10.1089/hum.2012.208	no
Liver gene therapy by lentiviral vectors reverses anti-factor IX pre-existing immunity in hemophilic mice	Annoni, A.	EMBO Mol Med					In press	
Safer, Silencing-Resistant Lentiviral Vectors: Optimization of the Ubiquitous Chromatin-Opening Element through elimination of Aberrant Splicing	Knight S.	Journal of Virology	86(17)	American society for microbiology	2012	9088	10.1128/JVI.00485-12	yes
Physiological regulation of transgene expression by a lentiviral vector containing the A2UCOE linked to a myeloid promoter	Brendel C	Gene Ther.	19(10)	Nature publishing group	2012	1018-29	doi: 10.1038/gt.2011.167	no
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Chimeric TALE recombinases with programmable DNA sequence specificity	Mercer AC	Nucleic Acids Res	40(21)	Oxford University Press	2012	11163-72	doi: 10.1093/nar/gks875	yes
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IL-7 and IL-15 instruct the generation of human memory stem T cells from naïve precursors	Cieri N	Blood	121(4)	American Society of Hematology	2013	573-84	doi: 10.1182/blood-2012-05-431718	no
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Histone deacetylase inhibition activates transgene expression from integration-defective lentiviral vectors in dividing and non-dividing cells	Pelascini LP	Hum Gene Ther	24(1)	Mary Ann Liebert, Inc	2013	78-96	doi: 10.1089/hum.2012.069	no
The brown algae PI.LSU/2 group II intron-encoded protein has functional reverse transcriptase and maturase activities	Zerbato M	PLoS One	8(3)	PLoS	2013	e58263	doi: 10.1371/journal.pone.0058263	yes
Deletion of the LTR enhancer/promoter has no impact on the integration profile of MLV vectors in human hematopoietic progenitors.	Moiani A	PLoS One	8(1)	PLoS	2013	e55721	doi: 10.1371/journal.pone.0055721	yes
Targeted gene addition in human epithelial stem cells by zinc-finger nuclease-mediated homologous recombination.	Coluccio A	Mol Ther	21(9)	Nature publishing group	2013	1695-704	doi: 10.1038/mt.2013.143	No
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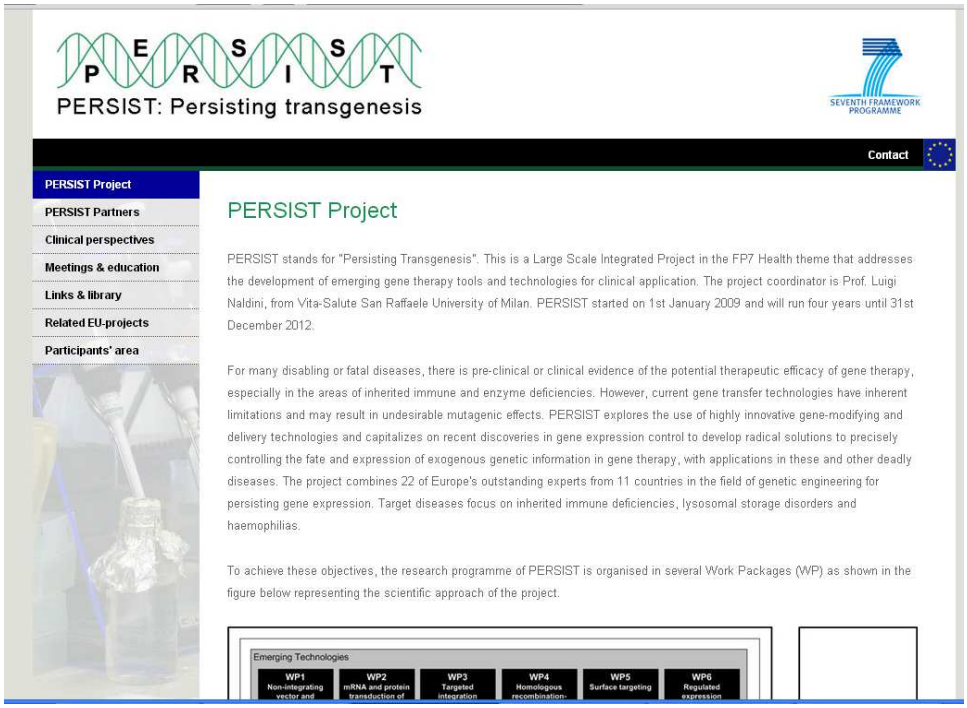
A double-switch vector system positively regulates transgene expression by endogenous microRNA expression (miR-ON vector)	Amendola M	Mol Ther	21(5)	Nature publishing group	2013	934-46	doi: 10.1038/mt.2013.12	no
Exploiting microRNA regulation for genetic engineering	Gentner B	Tissue Antigens	80(5)	John Wiley & Sons	2012	393-403		no
Attenuation of miR-126 activity expands HSC in vivo without exhaustion	Lechman ER	Cell Stem cell	11(6)	Elsevier	2012	799-811	doi: 10.1016/j.stem.2012.09.001	yes
A microRNA-based system for selecting and maintaining the pluripotent state in human induced pluripotent stem cells	Di Stefano B	Stem cells	29(11)	John Wiley & Sons	2011	1684-95	doi: 10.1002/stem.726	yes
Safer, silencing-resistant lentiviral vectors: Optimization of ubiquitous chromatin opening 3 element (UCOE) through elimination of aberrant splicing	Knight S	J. Virol.	86(17)	American Society for microbiology	2012	9088-95	doi: 10.1128/JVI.00485-12	yes
A designer circuit for the treatment of the metabolic syndrome	Haifeng Y	Proc. Natl. Acad. Sci. USA	110(1)	PNAS	2013	141-6	doi: 10.1073/pnas.1216801110	yes
A novel reporter system for bacterial and mammalian cells based on the non-ribosomal peptide indigoidine	Müller M	Metab Eng.	14(4)	Elsevier	2012	325-35	doi: 10.1016/j.ymben.2012.04.002	no
Increasing the dynamic control space of mammalian transcription devices by combinatorial assembly of homologous regulatory elements from different bacterial species	Bacchus W	Metab Eng	Jan;15	Elsevier	2013	144-50	doi: 10.1016/j.ymben.2012.11.003	no

Synthetic two-way communication between mammalian cells.	Bacchus W	Nat Biotechnol	30(10)	Nature publishing group	2012	991-6	doi: 10.1038/nbt.2351	no
The food additive vanillic acid controls transgene expression in mammalian cells and mice.	Gitzinger M	Nucleic Acids Res	40(5)	Oxford university Press	2012	e37	doi: 10.1093/nar/gkr1251	yes
Design and construction of synthetic gene networks in mammalian cells	Karlsson M	Methods Mol Biol	813	Springer	2012	359-76	doi: 10.1007/978-1-61779-412-4_22	no
Whole transcriptome characterization of aberrant splicing events induced by lentiviral vector integrations	Cesana D	J Clin Invest	122(5)	American society for clinical investigation	2012	1667-76	doi: 10.1172/JCI62189	yes
Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer	Provasi E	Nature Medicine	18(5)	Nature publishing group	2012	807-15	doi: 10.1038/nm.2700	no
The β-globin locus control region in combination with the EF1α short promoter allows enhanced lentiviral vector-mediated erythroid gene expression with conserved multilineage activity	Montiel-Equihua CA	Molecular Therapy	20(7)	Nature publishing group	2012	1400-9	doi: 10.1038/mt.2012.50	yes
Gene therapy on the move	Kaufmann K.	EMBO Mol Med	Sept 17	Wiley online library	2013		doi: 10.1002/emmm.201202287	yes

5 Contacts and addresses

5.1 Project website

http://www.persist-project.eu/



5.2 Project logo

5.3 Project participants

USR (Università Vita-Salute San Raffaele), Italy
Prof. L. NALDINI (project coordinator)

INSERM Lyon, France
Dr. F-L. COSSET

PEI (Paul-Ehrlich-Institut Langen), Germany

Prof. C.J. Buchholz, Dr. Z. Ivics

MHH (Hannover Medical School), Germany
Prof. C. Baum

EPFL (École Polytechnique Fédérale de Lausanne), Switzerland
Prof. D. Trono

ICH-UCL (Institute of Child Health London), UK
Prof. A. Trasher

LMU (Ludwig-Maximilians-Universität München), Germany
Dr. A. Ehrhardt

UNIMORE (University of Modena and Reggio Emilia), Italy
Prof. F. Mavilio

LUMC (Leids Universitair Medisch Centrum), The Netherlands
Dr. Manuel Gonçalves

ERASMUS (Erasmus University Medical Center), The Netherlands
Prof. G. Wagemaker

ULUND (Lunds Universitet), Sweden
Prof. S. Karlsson

DKFZ (Deutsches Krebsforschungszentrum NCT Heidelberg), Germany
Prof. C. von Kalle

ETHZ (ETH Zurich, Institute for Chemical and Bioengineering), Switzerland
Prof. M. Fussenegger

RHUL (Royal Holloway and Bedford New College), UK
Dr. R. Yáñez

EUFETS GmbH, Germany
Dr. K. Khülcke

GENETHON, France
Dr. A. Galy

GATC Biotech AG, Germany
Mr. T. Pohl

MOLMED S.p.A., Italy
Dr. F. Bellintani

NOVAMEN-ACIES, France
Dr. J-L. Roux

CIEMAT, Spain
Dr. J. A. Bueren

GSH (Georg-Speyer-Haus), Germany
Dr. M. Grez

VUB (Vrije Universiteit Brussel), Belgium
Dr.T.Vandendriessche

UKL-FR (Universitaetsklinikum Freiburg), Germany
Prof. T. Cathomen

FCSR (Fondazione Centro San Raffaele), Italy
Dr. E. Montini