3. Safety Assessment of SIN LVs Harboring Chromatin Insulators in the Sensitive *Cdkn2a-/- In Vivo* Genotoxicity Assay Show Enhancer-Blocking Activity of Specific Insulator Sequences

Monica Volpin,[1,2] Andrea Calabria,[1] Daniela Cesana,[1] Erika Tenderini,[1] Fabrizio Benedicenti,[1] Koyel Mitra,[4] Odile Cohen-Haguenauer,[3] Luigi Naldini,[1,2] Jack Lenz,[4] George Stamatoyannopoulos,[5] Eugenio Eugenio.[1]
[1]Telethon Institute for Gene Therapy, Milan, Italy; [2]Università Vita-Salute San Raffaele, Milan, Italy; [3]Ecole Normale Supérieure de Cachan, Cachan, France; [4]Albert Einstein College of Medicine, New York; [5]University of Washington, Washington.

Chromatin insulators (CI) have been proposed as safety features to increase the safety of self-inactivating (SIN) lentiviral vectors (LV) for gene therapy applications.

By taking advantage of an *in vivo* genotoxicity assay based on the systemic injection of LVs in newborn tumor-prone *Cdkn2a-/-* mice we were able to measure vector-induced genotoxicity as an accelerated tumor onset that was proportional to the genotoxic potential of the tested LV. Importantly, we took advantage of integration sites (IS) analysis to qualitatively characterize CI that were shown by other *in vitro* and *ex vivo* studies to function as insulators. Recently we showed for the first time that a CAAT-box binding Nuclear factor 1 (CTF/NF1)-based CI, when cloned in the LTRs of a SIN.LV with a strong SFFV enhancer-promoter in internal position, significantly reduced the frequency of tumors harboring integrations activating *Map3k8* oncogene accompanied by a marked skewing towards tumors harboring inactivating insertions targeting *Pten*.

Here by using this stringent *in vivo* genotoxicity assay and IS analysis in tumors we expanded our studies towards other CI sequences whose function is regulated by the binding of the CCCTC-binding factor (CTCF), the best characterized insulator protein in vertebrates.

Each CTCF-based insulating cassette was cloned in the LTRs of a LV construct containing the SFFV promoter in internal position (CTCF.SIN.LVs) and injected in *Cdkn2a-/-* mice. Interestingly, mice treated with some of the CTCF.SIN.LVs tested displayed an increased median survival time (ranging from 193.5 to 214 days) compared to mice treated with the uninsulated parental SIN.LV (186 days). Importantly, our preliminary IS analysis in tumors (881 IS) showed that two CTCF.SIN.LVs did not target *Map3k8* oncogene while *Pten* was often disrupted by exonic insertions, an escape genotoxicity mechanism on which CI cannot act.

These data confirm that the inclusion of two novel CTCF-based CIs of human origin completely abrogated the formation of tumors caused by enhancer-mediated activation of an oncogene *in vivo*.

The ability of these two new insulator elements to block the crosstalk between powerful vector enhancers and cellular regulatory elements increase the safety of SIN LVs and justify their prompt adoption in future gene therapy applications.

4. Developing an Engineered Nipah Virus Glycoprotein Based Lentiviral Vector System Retargeted To Cell Surface Receptors of Choice

Ruben R. Bender,[1] Anke Muth,[1] Irene C. Schneider,[1] Andrea Maisner,[2] Christian J. Buchholz.[1]
[1]Molecular Biotechnology and Gene Therapy, Paul-Ehrlich-Institut, Langen, Germany; [2]Institute of Virology, Philipps University Marburg, Marburg, Germany.

Delivery of genes selectively into specific cell types is a major bottleneck in successful gene therapy. Receptor-targeted lentiviral vectors (RT-LVs) can be an effective tool to achieve selective transfer of genes to cell types of choice, *ex vivo* and *in vivo*. So far, many different target receptors specific for cell types present in many different tissue types have been successfully addressed. RT-LVs based on engineered measles virus (MV) glycoproteins are most selective but can only be produced at moderate vector titers and suffer from susceptibility towards neutralising antibodies induced by vaccination against measles. In this study, LVs have been pseudotyped with modified Nipah virus (NiV) glycoproteins in order to generate LVs with high specificity for target cell populations and enhanced titers. The glycoproteins were engineered to make them deficient for the use of EphrinB2/3, the natural NiV receptor, and truncated in their cytoplasmic tails to allow efficient incorporation into LV particles. Designed ankyrin repeat proteins (DARPins) or single chain antibodies (scFv) specific for the cell surface receptor of choice were fused to the G glycoprotein to mediate receptor binding and subsequent cell entry. All G-DARPin/-scFv fusion proteins were efficiently expressed at the cell surface and incorporated into LV particles. The particles exhibited selective entry into their particular target cells and showed stable transgene expression. Importantly, NiV-LVs were at least 250-fold less effectively neutralized than MV glycoprotein pseudotyped LVs if incubated with pooled human intravenous immunoglobulin (Intratect®). So far, EpCAM, CD4, and CD8 served as functional receptors for NiV glycoprotein based RT-LVs. The CD8-targeted vector selectively transduced $CD8^+$ lymphocytes when added to human PBMCs *in vitro*. Remarkably, these NiV-based RT-LVs could be produced at 8- to 68-fold higher titers compared to the corresponding MV glycoprotein-based RT-LVs. Current efforts focus on the quantification of these observations and identification of the underlying mechanisms. In addition, the capability of NiV-based RT-LVs to transduce target cell populations *in vivo* is being analyzed.

5. Newly Developed Measles Virus Vector Can Simultaneously Transfer Multiple Genes Into Human Hematopoietic Cells and Induce Ground State Like Pluripotent Stem Cells

Takafumi Hiramoto,[1,2] Maino Tahara,[3] Chika Sakamoto,[1] Yuichiro Nakatsu,[3] Toru Kubota,[3] Hiroaki Ono,[1,4] Hiroshi Kohara,[1] Makoto Takeda,[3] Kenzaburo Tani.[1,5]
[1]Division of Molecular and Clinical Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; [2]Division of Hematology, University of Washington Medical Center, Seattle, WA; [3]Department of Viology 3, National Institute of Infectious Diseases, Tokyo, Japan; [4]Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [5]Department of Advanced Molecular and Cell Therapy, Kyushu University Hospital, Fukuoka, Japan.

We reports newly developed virus vector, measles virus vector can transfer multiple genes into human hematopoietic cells effectively and induce ground state pluripotent stem cells from somatic cells without affecting the host genome.

Measles virus (MV) which belongs to negative single strand RNA viruses has been known to have high affinity for human peripheral immune cells including monocytes, B cells and T cells. We recently have developed novel MV gene transfer vector which is non-transmissible, can transfer multiple genes simultaneously. The MV vector which carries 5 genes (GFP, human OCT3/4, SOX2, KLF4, and L-MYC) (MV-dF-OSKL-EGFP) could express these genes in various human cells with differential expression levels depending on the arrangement of the gene in the vector. Especially, MV-dF-OSKL-EGFP was able to transduce genes into more than 80 % of hematopoietic cells besides natural killer cells. Naive and stem cell memory T cells were also transduced by MV-dF-OSKL-EGFP.