“MicroRNA-regulated Viral Vectors”

Background and Description of Invention. MicroRNAs are a family of small non-coding RNAs involved in downregulating gene expression by recognizing in a sequence-specific manner target mRNAs.
The present invention describes a gene transfer vector system that utilizes the microRNA post-transcriptional gene silencing machinery for regulating transgene expression. Lentiviral vectors for transgene expression for gene therapy can be engineered with microRNAs target sequence in order to be recognized by endogenous microRNAs which are cell-type specific. Thus, regulation of transgene expression in a defined subset of cells can be achieved. Moreover, a combinations of miRNA target sequences can be used to obtain vectors with highly specific cell expression patterns. Remarkably, the invention could be employed to prevent immune-mediated rejection of the transferred gene.
As proof of concept, the inventors demonstrated that transgene expression from a ubiquitously expressed promoter can be prevented precisely in a hematopoietic cell line by using a vector that displays miR-142 target sequence at the transgene’s 3’UTR, as shown in the Figure 1.

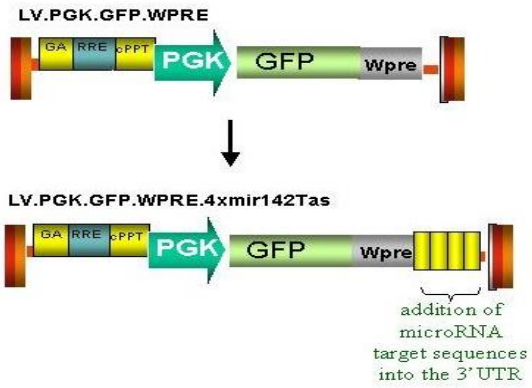

Figure 1. In cells expressing miR-142, the latter specifically recognizes its target sequence and therefore inhibits transgene expression. Since miR-142 has a cell-type specific expression pattern confined to hematopoietic tissues, transgene inhibition is restricted to those tissues without reduction of transgene expression in other cell types.

Upon vector administration *in vivo*, such special features would avoid transgene expression in antigen presenting cells (APC) of the immune system, which are part of the hematopoietic system, thereby preventing the initiation of an immune response against the transgene. Conceivably, when applied to a tissue-specific promoter which targets expression to hepatocytes, this system would allow suppression of ectopic transgene expression in transduced APC. This would potentially solve a major hurdle and long-standing problem in gene transfer; namely, immune-mediated rejection of the transferred gene (*Brown et al., Nat Med.* 2006*; Brown et al., Blood.* 2007).

Patent information. The international patent application was published as WO2007000668.
Patent applications pending in India, Singapore, US. Patents granted in China, Canada, S. Korea, Europe, Japan, Israel, US. Available for out-licensing in specific fields only.

Stage of Development. In vivo studies have been performed to validate the concepts presented herein.
LV.PGK.GFP.WPRE is a lentiviral vector in which transgene expression is controlled by the ubiquitously expressed PGK Promoter. By adding microRNA target sequences (<23bp) to the transgene’s 3’UTR, which are targeted by tissue specific miRNA, we can create a vector with tissue-restricted expression. Moreover, we can add combinations of miRNA target sequences to obtain vectors with highly specific cell expression patterns.
In addition, the inventors are carrying out studies to determine if this invention enables establishment of long-term transgene expression, and therapeutic activity, after systemic gene transfer in the absence of an immune response. If so, this invention would constitute an important component of any vector system intended for systemic delivery of a therapeutic transgene.

Potential Applications and Competitive Advantages. Current vector transcription control approaches mostly rely on the delivery of enhancer-promoter elements taken from endogenous genes. Using these approaches, reconstitution of highly specific gene expression patterns, as often required for gene transfer and therapy applications, is limited by the delivery system, the vector capacity, and the positional effects of insertion (for integrating vectors). By developing new vectors which take advantage of endogenously expressed microRNAs for their regulation, the inventors have added a layer of control to the vectors that did not previously exist.

- This new approach allows specific repression of gene expression in selected cell types and lineages. Furthermore, vectors with highly specific cell expression pattern may be useful in screening protocols as research tool.
- With this system we can reach much more stringent control of transgene exprssion than is current possible with existing technologies.
- When applied to integrating vectors, it can circumvent problems of transgene dysregulation, which can occur as a result of insertional position effects (integration next to strong promoter/enhancer sequences that override the transcriptional control of the vector-internal promoter) and enable highly cell-specific patterns of transgene expression.

Relevant Publications.

- 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. 2011*
- Matrai J et al., Hepatocyte-targeted expression by integrase-defective lentiviral vectors induces antigen-specific tolerance in mice with low genotoxic risk. *Hepatology. 2011*
- Naldini L. Ex vivo gene transfer and correction for cell-based therapies. *Nat Rev Genet. 2011*
- Gentner B et al., Identification of hematopoietic stem cell-specific miRNAs enables gene therapy of globoid cell leukodystrophy. *Sci Transl Med. 2010*
- Sachdeva R et al., Tracking differentiating neural progenitors in pluripotent cultures using microRNA-regulated lentiviral vectors. *Proc Natl Acad Sci U S A. 2010*
- Annoni A et al., In vivo delivery of a microRNA-regulated transgene induces antigen-specific regulatory T cells and promotes immunologic tolerance. *Blood. 2009*
- Brown BD, Naldini L. Exploiting and antagonizing microRNA regulation for therapeutic and experimental applications. *Nat Rev Genet. 2009*
- Brown BD et al., Endogenous microRNA can be broadly exploited to regulate transgene expression according to tissue, lineage and differentiation state. *Nat Biotechnol. 2007*
- Brown BD et al., A microRNA-regulated lentiviral vector mediates stable correction of hemophilia B mice. *Blood. 2007*
- Brown BD et al., Endogenous microRNA regulation suppresses transgene expression in hematopoietic lineages and enables stable gene transfer. *Nat Med. 2006*

We seek a potential commercial partner with a strong pipeline either in human gene therapy interested in avoiding immune mediated rejection of the transgene or/and in screening procedures.

For further information on this project please contact:

Business Contact
Paola Vella
Head, Office of Biotechnology Transfer
San Raffaele Hospital and Scientific Institute
Tel: +39 02 2643 4281
Fax: + 39 02 2643 8138
E-mail: vella.paola@hsr.it

Scientific Contact
Prof. Luigi Naldini
Director San Raffaele Telethon Institute for Gene Therapy
San Raffaele Hospital and Scientific Institute
Tel: +39 02 2643 4681
Fax: +39 02 2643 4621
E-mail: naldini.luigi@hsr.it