

Propositions belonging to the thesis:

## Gene Therapy of Liver Disease with Lentiviral Vectors Preclinical Studies in Models of Crigler-Najjar Disease and Hepatitis C

1. Intravenous administration of a UGT1A1 expressing lentiviral vector in juvenile Gunn rats, an animal model for the human condition of Crigler-Najjar disease, is able to normalize the hyperbilirubinemic phenotype of this rat strain.

*This thesis, Chapter 2*

2. Local delivery of lentiviral vector to the liver by isolated hepatic perfusion can be an appealing alternative to systemic intravenous vector administration, with potentially added transduction efficiency and safety.

*This thesis, Chapter 3*

3. Kupffer cell depletion prior to vector delivery by isolated hepatic perfusion increases the transduction efficiency to the liver with comparable vector dosages by at least a factor of two.

*This thesis, Chapter 3*

4. Delivery of a lentiviral vector expressing up to three different short hairpin RNA sequences from a single vector simultaneously inhibits HCV replication and expression of its entry receptors in human hepatoma cells *in vitro*.

*This thesis, Chapter 4*

5. The hydroxyethyl starch present in the UW solution (a preservation solution for liver transplantation) enhances the transduction of hepatocyte-like cells by lentiviral vectors *in vitro* and facilitates hypothermic transduction of the liver under conditions that resemble liver transplantation *in vivo*.

*This thesis, Chapter 5*

6. The further development of pre-clinical applications and toxicity testing of lentiviral vectors in animals larger than mice will make it necessary to develop an efficient and cost effective method for large scale vector concentration and purification.

7. The future clinical application of lentiviral vectors for gene therapy of inherited liver disease will depend on the outcome of current clinical trials with the transplantation of lentiviral transduced hematopoietic stem cells, and continued liver-directed gene therapy trials based on adeno-associated viral vectors.

8. Optimization of the expression level of gene therapy vectors by the incorporation of stronger transcriptional units results in more efficient but not necessarily safer gene therapy vectors.

*Zychlinski et al. (2008) Mol Ther 16:718-725*

9. The preferential design of lentiviral transgene expression cassettes for liver directed gene therapy purposes probably consists of a liver-specific promoter in combination with a downstream microRNA target sequence that specifically suppresses transgene expression in antigen presenting cells.

*Brown et al. (2006) Nat Med 12:585-591*

*Brown et al. (2007) Blood 110:4144-4152*

10. When compared to SIN gamma-retroviral vectors, SIN lentiviral vectors display an intrinsically safer genomic integration pattern.

*Montini et al. (2006) Nat Biotechnol 24: 687-696*

*Montini et al. (2009) J Clin Invest 119: 964-975.*

11. The proposition: "All things are poison and nothing is without poison; only the dose makes that a thing is no poison." also applies to gene therapy vectors.

*After: Paracelsus (1493-1541, Swiss physician, botanist, alchemist, astrologer, and occultist*