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Optimized lentiviral gene therapeutic vectors for prolonged and stable transgene expression

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Regarding innovative treatment options in medicine, gene therapy (GT) via viral vectors represents a powerful and versatile tool. *MDR1* gene transfer via lentiviral vectors displays an attractive method to confer radioprotection and chemoprotection to myeloid hematopoietic stem cells (HSCs) in cancer patients undergoing radiotherapy and/ or chemotherapeutic treatment. However, limitations such as transgene silencing or events of insertional mutagenesis represent major difficulties of lentiviral gene transfer into HSCs.

To address these issues, in this doctoral thesis optimized self-inactivating (SIN) lentiviral gene therapeutic vectors were constructed that contained elements such as the ubiquitous chromatin opening element (UCOE) and/ or the DNase I-hypersensitive site 4 (5'HS4) and scaffold/matrix attachment region (S/MAR) elements. For these elements not only the ability to avoid transgene silencing but also the capacity to improve the biosafety of viral vectors had been shown. Constructed vectors (LV-SIN-SEW-UCOE, LV-SIN-SEW-S/MAR-HS4, and LV-SIN-SEW-S/MAR-HS4-UCOE) as well as the control vector HR'SINcPPT-SEW comprised the enhanced green fluorescent protein (EGFP) as transgene and were used to produce viral particles in order to transduce HeLa, human myeloid HL-60 and mouse embryonic carcinoma P19 cells. The effect of the vector elements on long-term EGFP-transgene expression was monitored *in vitro* for up to three months post-transduction.

Already during the production of viral particles, LV-SIN-SEW-S/MAR-HS4 and LV-SIN-SEW-S/MAR-HS4-UCOE rendered lower viral titers than LV-SIN-SEW-UCOE or HR'SINcPPT-SEW. Furthermore, transduction efficiency and long-term EGFP-expression levels in HeLa cells were lower for both these vectors compared to LV-SIN-SEW-UCOE or the control vector, suggesting that inclusion of the 5'HS4 and S/MAR elements within the vector 3'LTR might compromise virus production, transduction and/or integration into genome of the host cell.

The UcoE-containing LV-SIN-SEW-UCOE, in turn, displayed a clear anti-silencing effect in P19 cells which represent an established *in vitro* model for transgene silencing. For the whole observation period the amount of EGFP-positive cells was higher for LV-SIN-SEW-UCOE-transduced P19 cells than for cells transduced with the control HR'SINcPPT-SEW vector. Thus, this study confirms the role of UCOE as promising and suitable regulatory element to reduce transgene silencing in the context of lentiviral HSC gene therapy. Overall, obtained results represent a further step towards clinical application of lentiviral vector mediated myeloprotective GT.