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EFFICIENT AND SUSTAINED GENE TRANSFER IN TERMINALLY DIFFERENTIATED CELLS WITH A LENTIVIRAL VECTOR

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The identification of monogenic and complex genes responsible
for disorders requires new approaches for delivering therapeutic protein genes to significant numbers of cells *in vivo*. A lentiviral-based vector pseudodtyped with the VSV.G protein capable of infecting dividing and quiescent cells, was investigated *in vivo* by injecting highly concentrated viral vector stock into the striatum and hippocampus, of adult rats. Control brains were injected with a Moloney murine leukemia viral (MLV), adenoviral (ADV) or adeno-associated viral (AAV) vectors. Transduced volumes and cell densities were stereologically determined to provide a basis for comparison among different viral vectors and variants of the viral vector stocks. The lentiviral vector system was able to efficiently and stably infect quiescent cells in the primary injection site with transgene expression over the time of six months. Triple labeling showed that 88,7% of striatal cells transduced by the lentiviral vector were terminally differentiated neurons. The efficiency of this lentiviral vector in other organ systems, like liver and muscle are currently under investigation.

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GENE AD CELLULAR THERAPY AT NOVARTIS PHARMA

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Novartis Pharma has recently been created by a merger of SANDOZ and CIBA, pharmaceutical companies with a strong committment to molecular therapies. SANDOZ has been the first big company to invest considerably in gene therapy. The acquisition of Genetic Therapy Inc. (GT) provided the company with a broad gene therapy technology base including the corresponding regulatory knowhow, the cooperation with and later integration of Systemix Inc. established hematopoietic stem cells as targets for gene therapy at Novartis. This contribution is intended as a short survey of the collaborative network inside and outside Novartis concerning gene and cellular therapy with its implications for clinical research. A high unmet medical need defined oncology as an early focus for the development of gene-based therapies; clinical studies in this area will be outlined.

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DESIGN AND EVALUATION OF A NOVEL FRIEND VIRUS RETROVIRAL VECTOR FOR GENE THERAPY

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A defective retroviral vector has been derived from FB29 Fr-MuLV strain which harbours high infectivity and pathogenicity in all haematopoietic lineages. A neoR high producer clone (over 107 cfu/ml) supernatant has been used to transduce CD34+ cells [from cord blood, bone marrow or peripheral blood (patients + solid tumors)]. Initial transduction efficiency of 40 to 90% CFU-GEMM (CB) or CFU-GM (BM and PBSC) generating cells could be evidenced by PCR ; 35% colonies proved resistant to G418 on average. Efficient transduction of LTC-IC was demonstrated on the basis of both resistance to G418 and PCR which tested positive in 25 to 60% late CFU colonies. A correlation was observed between virus titers and transduction efficiency ; a threshold otherwise limiting transduction potential might have been reached. Two cocktails of growth factors were applied in parallel (prestimulation): high (SCF, IL3, IL6, G-CSF, LIF & Epo) and low (SCF & LIF) concentrations ; both lead to similar results. A significant loss in the number of CFUs was evidenced in both instances. Shortening of transduction procedure was investigated. A single direct overnight exposure to supernatant was applied in parallel with 4 cycles over 48 hours and 40 hours conditioning. The percentage of colonies PCR-positive and G418-resistant proved similar. Interestingly, the absolute number of G418-resistant CFUs was twice higher in the case of shortened transduction. A further step consisted in testing the Fr-MuLV vector (FOCH) versus a reference Mo-MuLV-vector, i.e. MFG-Neo. Use of FOCH results in at least as good transduction and expression efficacy in haematopoietic progenitors. In those cases where a single exposure to virus-supernatant is applied, FOCH29 proves systematically better ; twice or three times more CFU-GM generating cells being transduced at once. Since more colonies proved PCR-positive as compared to neomycine resistance pattern, FOCH29-backbone has been optimized towards improving translation/expression potential. Two different versions have been derived FOCHMgag & FOCHA). Expression of novel marker genes will be presented ; as well as our strategy towards gene therapy of Fanconi Aneamia A currently underway (European network) making use of the optimised vector-constructs.

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A NOVEL HERPES VECTOR FOR THE HIGH EFFICIENCY TRANSDUCTION OF PRIMARY HEMATOPOIETIC CELLS

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Hematopoietic cells are attractive target cells for a variety of gene therapy applications, yet so far have proven very difficult to transduce with adequate efficiency. We have employed a genetically inactivated form of herpes simplex virus that is restricted to a single cycle of replication (disabled single-cycle virus, DISC-HSV) in permissive cells, but appears to have little direct cytologic effect on hemopoietic cells. Using β-GAL as a marker gene, we have transduced primary hematopoietic cells as well, as myeloid and lymphoid leukemia blasts with efficiencies ranging from 80% to 100%, in the absence of growth factors or stromal support. Toxicity was low, with 70-100% of cells surviving the transduction process. We have also utilized a DISC HSV amplicon system, in which the gene of interest is packaged as a multicopy plasmid in an otherwise empty HSV shell. These amplicons are more readily generated than conventional HSV vector which require complex recombinal steps. The amplicons also deliver more gene copies per particle to the target cell. Transduction of leukemic cells with these DISC-related amplicon vectors was as efficient as the DISC system. In both systems, peak expression of transferred genes occurred at 24-48 hours after transduction, declining to near background levels by 7 days. However, for many applications such as the generation of leukemia "vaccines" transient expression is adequate. Hence transduction of primary human AML and ALL blasts with a GM-CSF DISC-HSV vector induced secretion of levels between 300-2000pg/10^6cells/24hrs. The cytokine produced by this vector system is immunostimulatory. Murine A20 leukemia produced 3000pg/10^6cells/24hrs GM-CSF and stimulated a potent antitumor response in mice against preexisting leukemia, significantly delaying tumor progression in the GM-CSF group compared to the control ($p<0.005$). Thus the DICS system allows efficient genetic manipulation of human hemopoietic cells facilitating future biological and clinical investigation.