little is known about how this could affect beneficial effector immune responses. In the current study, we used a recombinant adeno-associated virus (AAV) expressing IL-2 to permanently release IL-2 and assess its effect on immune responses during vaccination, infection, cancer developement and pregnancy. We used single injections of AAV IL-2 at 2 doses. 10^9 virus particles (v.p.) increased CD25 and Foxp3 expression in Tregs, had minimal effects on Treg numbers and delayed but did not prevent the occurrence of type 1 diabetes (T1D) in NOD mice; 10^{10} v.p. enabled sustained stimulation and expansion of Tregs without inducing Teff activation and prevented T1D in NOD mice. After several weeks of IL-2 production at these two doses, mice could normally (i) eradicate a viral challenge with flu, (ii) mount immune responses to vaccination, and (iii) have normal pregnancies with pups that developed normally. They also had no change in occurrence and growth of chemically induced tumors, as well as in growth of transplanted tumors. Altogether, chronic low doses IL-2 treatment appears safe and does not affect usefull effector immune responses.

OR015

AAV gene therapy for Alzheimer disease: Consequences of AAV-mediated CHOLESTEROL 24-HYDROXYLASE overexpression in THY-TAU22 mouse model.

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Alzheimer's disease (AD), the major cause of dementia, is characterized by two hallmarks, amyloid pathology and neurofibrillary tangles of hyperphosphorylated Tau protein. We recently described the therapeutic effect of cerebral cholesterol 24-hydroxylase (CYP46A1) overexpression on amyloid pathology of APP23 mice (Hudry et al.).

The aim of this work was to investigate the consequences of CYP46A1 overexpression on the Tau component of AD.

CYP46A1 overexpression was induced in the THY-Tau22 transgenic mouse model by stereotactic injection of an AAV vector carrying the human CYP46A1 gene in cortex and hippocampus. An AAV vector coding a mutated inactive CYP46A1 enzyme was used as a control. Injected animals were compared to normal control littermates.

In contrast to THY-Tau22 mice treated with inactive CYP46A1, THY-Tau22 mice overexpressing functional CYP46A1 enzyme showed normal memory abilities in two behavioral tasks (Morris Water Maze and Y-maze). Thus CYP46A1 overexpression rescues the main cognitive dysfunction of this model.

Hyperphosphorylation of Tau and astrogliosis that characterize this THY-Tau22 model were not modified by CYP46A1 overexpression. Lipidomic analysis evidenced modified lipid profile in THY-Tau22 mice. CYP46A1 overexpression allowed complete restoration of this profile and increased expression of genes implicated in cholesterol synthesis in both hippocampus and cortex.

Mechanisms that underlie the neuroprotective effect of CYP46A1 overexpression in THY-Tau22 mouse are under investigation. Overall, phenotypic improvement of both APP23 and THY-Tau22 mice suggest that CYP46A1 is a relevant therapeutic target on AD.

OR016

Exon skipping gene therapy for Dystrophic Epidermolysis Bullosa

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Dystrophic Epidermolysis Bullosa (DEB) is a group of genetic skin disorders inherited in a dominant (DDEB) or recessive (RDEB) manner and characterised by severe skin and mucosae blistering after mild traumas. DEB is caused by mutations in *COL7A1* encoding type VII collagen that assembles into anchoring fibrils forming key dermo-epidermal adhesion structures. To date, there is no specific treatment for DEB. Exon skipping strategy consists in modulating the splicing of a pre-messenger RNA to induce the skipping of a mutated exon. Exons 73, 74 and 80 of *COL7A1* are of particular interest because they carry several recurrent mutations and their excision preserves the open reading frame. We first demonstrated the dispensability of these exons for type VII collagen function in an *in vivo* xenograft model using RDEB cells transduced with retroviral vectors containing *COL7A1* cDNAs deleted of the sequences of these exons. We then transfected primary RDEB keratinocytes and fibroblasts with antisense oligoribonucleotides (AONs) targeting key splicing regulatory elements (exonic splicing enhancers and/or acceptor sites) to induce efficient skipping of these exons (50% up to 90%). Western blot and immunocytochemistry analyses demonstrated significant collagen VII re-expression in cells from two RDEB patients, one homozygous for a nonsense mutation in exon 80, the other compound heterozygous for frameshift mutations in exon 73 and 80. We now aim to demonstrate the feasibility of this approach *in vivo* using animal models. If successful, this approach would offer potential for treating both RDEB and DDEB patients using local or systemic administration of AONs.

OR017

SMN rescue by using oligonucleotides of tricyclo-DNA to induce exon 7 inclusion in SMN2 mRNA

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Spinal muscular atrophy is a recessive disease caused by mutations in the SMN1 gene, which encodes a protein (SMN) involved in RNA processing whose absence dramatically affects the survival of motor neurons. In Man, the severity of the disease correlates with the SMN2 gene copy number, which varies from individual to individual.

SMN2 differs from SMN1 by 5 nucleotides, only one of which is in the coding sequence: it is a silent C to T change located at the sixth nucleotide of exon 7. Thus, SMN2 encodes the same SMN protein as SMN1. However, the single nucleotide change affects the definition of exon 7 during splicing such that about 90% of SMN2 mRNAs lack this exon.

Here, we show that SMN activity can be restored in SMA cells by using tricyclo-DNA (Tc-DNA) antisense oligonucleotides

annealing either the exon 7 terminal stem loop (TSL) or a nearby intron 7 splice silencer (ISS) of the SMN2 pre-mRNA. RT-PCR showed that about 30% and 60% of SMN2 mRNAs were rescued after treatment with Tc-DNA analogues annealing the TSL and ISS, respectively. For Tc-DNA[ISS], SMN levels were close to normal in Western blot analysis. Finally, immuno-staining revealed that rescued SMN was properly located in nuclear gems.

OR018

Lentiviral vectors designed for liver-directed gene therapy do not display detectable genotoxicity in sensitive *in vivo* assays

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We developed and validated sensitive genotoxicity assays based on *Cdkn2a*$^{-/-}$ or wild type (WT) mice to assess the biosafety of lentiviral vectors (LV) designed for liver gene transfer. As a positive control of insertional mutagenesis, we used a LV carrying Enhanced Transthyretin enhancer/promoter (ET) cloned in the Long Terminal Repeats (LV.ET.LTR) which induced hepatocellular carcinoma (HCC) in 30% of *Cdkn2a*$^{-/-}$ mice ($p < 0.01$) and 75% of WT mice in combination with CCl_4 treatment ($p < 0.01$).

We exploited these mouse models to test the safety of Self-Inactivating LVs developed for the therapy of haemophilia B which express factor IX under the control of ET (SINLV.ET.FIX). Systemic administration of SINLV.ET.FIX did not induce HCCs neither in tumor prone *Cdkn2a*$^{-/-}$ ($N = 39$) nor in WT mice + CCl_4 ($N = 23$). We retrieved a total of 9215 unique insertion sites from LV.ET.LTR- and SINLV.ET.FIX-treated mice. None of the previously validated cancer genes recurrently targeted by the oncogenic LV.ET.LTR was hit by SINLV.ET.FIX-integrations. However, SINLV.ET.FIX-insertions clustered at different Common Insertion Sites (CIS). Importantly, we found that: 1) SINLV.ET.FIX-CIS had a lower CIS power compared to LV.ET.LTR-CIS ($p < 0.01$); 2) SINLV.ET.FIX-CIS were represented by a lower percentage of sequence reads compared to LV.ET.LTR-CIS ($p < 0.001$); 3) SINLV.ET.FIX-CIS did not show any skewing towards genes involved in cancer; 4) SINLV.ET.FIX-CIS were embedded in large genomic areas with high LV integration incidence. These findings indicate that SINLV.ET.FIX-CIS are the result of LV-intrinsic integration biases rather than the result of selection.

Our data indicate that SINLV.ET.FIX represents a safe vector design for hepatocytes gene transfer for different gene therapy applications.

OR019

High-throughput monitoring of bone marrow clonality in pre-clinical and clinical gene therapy studies

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Clonal dominance and leukemia are potential side effects of hematopoietic stem cell gene therapy. High-throughput methods enable fast identification of individual stem cell-derived clones. Quantitative real-time PCR (QRT-PCR) has been shown to be an accurate method to quantify individual transduced cell clones. However, due to frequently scarce target material large scale clonal analysis of multiple clones by QRT-PCR is hardly achievable.

The aim of this study was to explore the potential of highly sensitive techniques for vector insertion site analysis (LAM-PCR, nonrestrictive LAM-PCR) to describe clonal compositions in gene therapy compared to QRT-PCR. Therefore, we designed artificial insertion sites (*arIS*) of different sizes which were mixed for mimicking defined clonal situations in clinical settings ranging from balanced clonality to monoclonality in an *in vitro* setting. We subjected all *arIS* mixes to either linear amplification-mediated PCR (LAM-PCR) or nonrestrictive LAM-PCR (nrLAM-PCR) both combined with 454 sequencing compared to QRT-PCR. By performing this important first technical comparison we showed that nrLAM-PCR/454-based clonal assessment is in the same range as the results obtained with QRT-PCR. To prove whether nrLAM-PCR/454 can prospectively facilitate clonality analyses in clinical gene therapy studies, we followed clonal kinetics of two clones detected in a patient enrolled in a clinical trial using both, nrLAM-PCR/454 and QRT-PCR. In line with the previous *in vitro* data, nrLAM-PCR/454 has shown to tightly reflect QRT-PCR-measured clonal contributions. We show for the first time a feasible high-throughput strategy to reliably monitor clonality in large-scale analyses of gene-marked cells in clinical gene therapy trials.

OR020

Tracking T-memory stem cells in humans by retroviral tagging

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Vector integrations in ADA (adenosine deaminase) deficient-SCID patients treated with hematopoietic stem cells (HSC) or mature lymphocytes (PBL) gene therapy (GT) introduce a univocal tag in each infused target cell. These studies allow tracking of single T-cell clones and studying the survival potential and hierarchical relationships of naive and memory subpopulations directly in vivo in humans. We analyzed at high-throughput level the integrome of sorted T cell subtypes from PBL-GT patients years after last infusion of gene corrected lymphocytes. Strikingly, we found that transduced T cells with an apparent naïve phenotype (CD45RA+/62L+) share the highest percentage of insertions (41.2%) with other T subpopulations while still surviving in vivo 10 years after infusion. A novel T-cell type (T memory stem cell, TSCM) with long-term survival capacity coupled with naive-like plasticity, has been recently identified as CD45RA+/62L+/CD95+. Interestingly, we found that the vast