compared to placebo (30.5 ml ± 12 ml) while wall motion score index (WMSI) significantly improved in treated animals (-0.17± 0.08) compared to placebo treated animals (0.12 ± 0.03, p ≤ 0.032). Biodistribution of the plasmid was primarily in the heart at 3 days and cleared from all non-injected organs by 60 days.

Conclusions: In a porcine model of heart failure, JVS-100 delivered via retrograde infusion demonstrated safety and improvement in cardiac function and remodeling compared to placebo. Based on these results, we initiated a 72 patient Phase I/ II trial to test safety and efficacy of delivery JVS-100 plasmid via retrograde CS infusion to treat heart failure.

375. Apolipoprotein B Knock-Down by Chitosan/ApoB-siRNA Nanoparticles Lowers Plasma Cholesterol in Atherosclerosis Mouse Model

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Purpose : Low-density lipoprotein (LDL) transports cholesterol in the bloodstream and is associated with the development of cardiovascular diseases, in particular atherosclerosis. Apolipoprotein B (ApoB) is mainly found in low density lipoproteins and is an essential structural component of this biochemical assembly. In order to inhibit expression of the structural protein of LDL cholesterol, ApoB, specific siRNAs targeting sequences in mouse ApoB mRNAs were used for in vitro and in vivo inhibition studies. Polymer/ApoB-siRNA nanoparticles (NP) using the natural polysaccharide chitosan (CS) were used for specific delivery of ApoB siRNA in vitro and in vivo.

Methods: Nanoparticles were prepared by mixing siRNA (chemically modified or non-modified) with different chitosan formulations, named [DDA-MW-N:P ratio], according to their specific molecular weight (Mn), degree of deacetylation (DDA) and ratio of chitosan amine to nucleic acid phosphate (N:P ratio).). Physicochemical characterization by ESEM, DLS, electrophoresis and in vitro analysis in HepG2 cells by FACS, confocal microscopy, qRT-PCR were performed in order to evaluate the nanoparticle potential to achieve ApoB gene specific knock-down. In vivo experiments aim to assess by histology, IHC, ELISA, qRT-PCR, the activity, specificity and safety of chitosan/ApoB-siRNA NPs mediated inhibition of ApoB gene expression in atherosclerosis, C57BL/6, mouse model injected intravenously.

Results: Chitosan-siRNA-ApoB NP size and ζ-potential varied from 75 to 135 nm and 20 to 30 mV respectively. Chitosan/ApoB-siRNA NP were stable for at least 20 hours and were able to protect the RNA cargo when challenged with supra-physiological concentration of nucleases. In vitro tests showed that NP uptake efficiency is ranged from 50 to 99% and ApoB gene silencing reached 80% (Fig.1A). In vivo, the ApoB mRNA levels of NP treated atherosclerotic mice reached those of healthy groups and led to a decrease of circulating ApoB level of 30 % in concordance with a decrease of circulating LDL/VLDL cholesterol level of 20%. According to histology and hepatotoxicity analysis, NP injections could reduce steatosis and lymphocytic infiltration without affecting the mice hepatic functions (Fig.1B).

Conclusion : Our results indicate a strong knock-down of ApoB by chitosan/ApoB-siRNA nanoparticles in the atherosclerotic murine liver and thereby support further development of chitosan/ApoB-siRNA nanoparticles as therapy for the treatment of hypercholesterolemia.

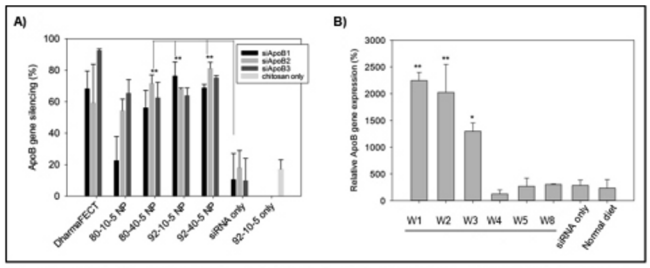

Figure 1: Quantitative real time PCR (qRT-PCR) analyzes **A)** ApoB gene silencing in HepG2 cells transfected with chitosan /ApoB-siRNA nanoparticles. ($p^* < 0.05$; $p^{**} \leq 0.01$) compared with siRNA only. **B)** Relative ApoB gene expression in C57BL/6 mice treated with chitosan 92-10-5/ ApoB-siRNA nanoparticles at different weeks (W1-W8) following the beginning of the high fat diet. siRNA only and Normal diet groups correspond to week 4 (W4). $p^* < 0,05$; $p^{**} \leq 0,01$) compared with Normal diet.

376. Helper Dependent-Adenoviral Vector Expressing Transferrin-LDL Receptor Fusion Protein for Gene Therapy of Familial Hypercholesterolemia

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Familial hypercholesterolemia (FH) is the most common and most characterized genetic disease that ultimately leads to coronaropathy and heart disease. FH is due to mutations in the gene coding for the LDL receptor (LDLR) that cause the increase of total and LDL cholesterol levels, with the consequent deposition in several tissues including arteries. The actual therapeutic options for FH are still not ideal, therefore we focused our attention on the development of a safe and effective innovative therapeutic strategy with the objective of reducing cholesterol levels in these patients. At this aim, we developed an helper dependent-adenoviral vector (HD-Ad) vector for the liver-specific expression of high levels of a soluble form of human LDLR fused in frame with rabbit transferrin (LDL-R/Tf): this is a particular fusion protein capable of mimicking the function of the LDLR, the protein absent in the patients. We, at first, evaluated the efficacy of the vector in cellular models as 293, COS and CHOldla7 (a cell lacking LDLR expression) observing expression of the fusion protein and restoration of the ability of incorporate fluorescent LDL in the CHOldla7 cell line. We also evaluated this strategy in LDLR-deficient mice, the mouse model of FH, and observed a reduction in total and LDL cholesterol levels in mice treated with the above-mentioned vector. Our results demonstrated the safety and efficacy of this novel approach in vitro and in LDLR-deficient mice; additional studies will address the possibility of applying the TF-LDLR fusion protein approach using different route of administration at the final aim of defining a protocol compatible with clinical applications.