Cell Therapy-2013

OMICS Group Conferences
Accelerating Scientific Discovery

2nd International Conference and Exhibition on
Cell & Gene Therapy

October 23-25, 2013 Holiday Inn Orlando International Airport, USA

Novel therapeutic nanoparticles for *in vivo* delivery of low dose siRNA in liver cells and for the treatment of liver fibrosis associated nonalcoholic steatohepatitis

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Abstract

siRNA is poised to be the next therapeutic drug. Potent siRNA can silence any gene, including non-druggable genes, at picomolar concentration. As a result, there is now a great deal of interest in using siRNA *in vivo* to better understand diseases but also to be use as a therapeutic molecule. The goal of this study was to develop new *in vivo* delivery nanoparticles to deliver siRNA in liver cells by screening a library of lipid based formulations.

Methods: An siRNA targeting FactorVII (FVII) was complexed with each formulation and injected intravenously at a 1mg/kg to 0.0125 mg/kg doses. Bid 3nd protein silencing was evaluated 48 hours after injection. The nanoparticles resulting in initial FVII knockdown were further optimized by design of experiment (mixture DOE) and evaluated for their ability to deliver other type of RNAi molecules. For NASH study, plasma and liver tissue were collected for determination of NASH features by histopathology, cell death assessment including TUNEL assay and immunoblotting using mitochondrial fractions. Hepatic stellate cell (HSC) activation was determined by real time PCR and liver fibrosis quantitated by image analysis of Sirius-red stained sections.

Results: After a single intravenous injection of FVII siRNAs (0.05mg/kg) complexed with this new reagent (formulation 401), we observed more than 90% mRNA and protein level reduction in liver cells for more than 2 weeks and this silencing was dose dependent with an ED50 < 0.02mg/kg. We also observed a reduction of Cholesterol and LDL after silencing the APOB gene with this reagent. In addition, by mixing the siRNAs together, we were able to knockdown at least 4 genes at the same time after a single injection.

Finally we showed that we can use these formulations to deliver a siRNA against a key pro-apoptotic gene (Bid 3) for treatment of fibrosis in NASH mice model. C57BL/6 mice were placed on choline-deficient L-amino acid defined (CDAA) diet for NASH mice model. After 19 weeks of CDAA diet, mice with severe fibrotic-NASH were injected with Bid 3siRNA/Formulation 401, weekly for three weeks at1.5 mg/kg (week1) and 0.15 mg/kg (week 2 and 3). At the end of the treatment, Bid 3 mRNA was suppressed to 50% ($p<0.003$) and Bid 3 protein was reduced to 10% ($p<0.002$). In Mice treated with Bid 3siRNA, liver fibrosis was improved as assessed by Sirius red quantitation and mRNA expression of fibrosis genes such as TIMP-1 ($p<0.03$) or CTGF ($p<0.05$). These changes were associated with marked reduction on TUNEL-positive cells and reduction on mitochondrial BAX.

Conclusion: We have identified novel therapeutic lipid nanoparticles for the delivery of siRNA with an ED50 of less than 0.02mg/kg. This study also demonstrates that these formulations with a pro-apoptotic gene (Bid 3) siRNA can be used to improve liver fibrosis associated with experimental NASH Gene. These findings are consistent with evidence that apoptosis triggers HSC activation and liver fibrosis and suggest that Bid 3 inhibition may be useful as an antifibrotic NASH therapy.

Biography

Xavier de Mollerat du Jeu, Ph.D. is a Senior Staff Scientist and lead the R&D *transfection* group at Life Technologies, in Carlsbad, California, working on creating and improving siRNA *in vivo/in vitro* delivery methods for both research and therapeutic applications. Xavier is also working on identifying new DNA delivery approaches for Hard to transfect cell lines and primary/stem cells. Xavier studied molecular biology and plant physiology at the University of Montpellier II in France, and received his Ph.D. in human genetics in 2003 from Clemson University in South Carolina. His thesis work involved identifying the gene(s) responsible for Split Hand/Split Foot Malformation 3 (SHFM 3). Hispost-doctoral fellowship research was in the laboratory of Dr. Michael G. Rosenfeld at UCSD, where he studied the roles of microRNAs in pituitary gland development. He joined Invitrogen (Life Technologies) in 2005.