Non-Viral Gene Delivery for Cystic Fibrosis with LPD Complexes

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Cystic Fibrosis (CF) is the UK's most common life-threatening, inherited disease and affects more than 7500 babies, children and young adults. The causative gene cystic fibrosis transmembrane conductance regulator (*CFTR)* has been identified and mutations in this gene lead to imbalanced ion and water movement across the airway epithelium, resulting in thickened mucus, chronic bacterial infection and inflammation.

Gene therapy involves adding a healthy gene to the body in order to treat or cure a disease. Developing a delivery or "vector" system to bypass the body's natural defences and the extra barriers of dense mucus and inflammation presented by CF is the key to the success of this research. Several clinical trials have been performed to date but they showed rather poor results. So although it has been proved that it can work and it appears to be safe, to make it effective the success rate must be increased making it stronger and longer lasting.

The main technical challenge for gene therapy remains the choice of vector for delivery of the therapeutic gene to the appropriate cells with high efficiency and safety. Non-viral vectors generally, exhibit much lower efficiency of gene transfer than viral vectors although they possess several compelling advantages in other areas for use in the lung as they are generally non-immunogenic, permitting repeated delivery at regular intervals to maintain the therapeutic effect of epithelial transgene expression. Hence, there is a need for a non-viral vector that induces sufficient gene transfer in order to confer a protective response.

The aim of this study is to develop synthetic vector systems for clinical gene therapy applications and to optimise receptor-targeted vectors comprising formulations of peptides that contain both a targeting ligand and an oligolysine, DNA-complexing moiety, a cationic liposome and DNA (LPD) using a systematic strategy first *in vitro* (human airway epithelial cells) and then *in vivo*. In previous work the peptide component of the vector was enhanced for targeting epithelial cells by identifying novel targeting peptides using phage display technology. This development significantly enhanced the efficiency and specificity of gene delivery in the target cells. Next, the cationic lipid component was selected by comparing the transfection efficiency *in vitro* of varying lipid chain lengths and different cationic head groups.

We have selected a number of complexes comprised of cationic lipids and peptides and these were tested *in vivo* by intra-tracheal instillation of the LPD system in C57BL6 mice with 8 or 16 μg of plasmid encoding luciferase.to assess the transfection efficiency of the lungs. High efficiency transfection in bronchial epithelium was shown with our new LPD systems with minimal inflammation or toxicity. The vector was also administered three times and results suggested that repeated administration would be feasible clinically without induction of vector-neutralising antibodies, a frequent problem for viral vectors. The inflammatory response to the vector was low as determined by histology and by cytokine levels in lavage fluids in mice. These data are very encouraging and the next step is to test these vectors in a large animal model, namely the pig, which mimics the human lung pathophysiology followed by a phase I clinical trial in human patients.