Towards Gene Therapy for Cystic Fibrosis Airway Disease: Development of Single-Dose Lentiviral Gene Transfer for Lifetime Airway Expression

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April 2010
I dedicate this thesis to
my family, particularly my husband Dave,
for their constant support and unconditional love.
I love you all dearly.
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Abstract

Cystic Fibrosis (CF) is the most common, fatal autosomal recessive disorder affecting the Caucasian population with a frequency of 1 in 2500 live births and has a current median survival age of approximately 33 years. Characteristics of CF include abnormalities in sweat glands, malnutrition, pancreatic disease and infertility. It is however, severe and chronic lung disease that currently accounts for greater than 95% of morbidity and mortality in CF patients. The CF transmembrane conductance regulator gene was discovered in 1989 and *in vitro* correction of the defect soon followed, providing the basis for gene therapy as a potential cure for CF lung disease. To date, the lack of an efficient gene transfer vector system combined with the physical barriers of the airway epithelium limit the successful application of CF gene therapy.

The work described in this thesis utilised a unique gene therapy approach developed by the CF Gene Therapy Research Group, which involved airway pre-treatment followed by gene delivery. Pre-treatment was with the natural detergent lysophosphatidylcholine (LPC), followed by a single-dose of a HIV-1 based lentivirus (LV) vector *in vivo*. Previously studies found significant gene expression within airway tissues, but areas of cell damage were also sometimes evident.

Initial work included examining the relationship between gene transfer, LPC dose and timing parameters, and airway epithelial damage. This study found that 0.3% LPC followed 60 minutes later with the LV produced significant gene expression within the airway, with only mild airway epithelial disturbance observed.

The longevity of LV-mediated gene expression was then evaluated in the nasal airway of C57Bl/6 mice using the LacZ marker gene. Treatment of mouse nasal airway
epithelium with the LPC prior to instillation of a single dose of an LVLacZ vector produced significant LacZ gene expression in many mice for at least 18 months. The finding of gene expression in one mouse after 24 months indicated essentially lifetime gene expression had been achieved.

We found that a single dose of LVLacZ produced immediate as well as lifetime mouse airway expression, confirming our hypothesis that use of an integrating vector extends transgene expression. Importantly, LVCFTR dosing achieved at least 12 months of CFTR expression, representing partial functional correction of the CFTR defect in CF knockout mice. These findings provide evidence that a single-dose Lentiviral gene transfer method may offer a novel *in vivo* therapeutic paradigm in the pursuit of a cure for CF airway disease.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Alice Stocker and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Alice Stocker
17 April 2010
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Working on this PhD has been a wonderful and often overwhelming experience. It is difficult to say where the real learning experience lie; in the research topic itself, or in learning to write papers and proposals, present talks, staying up until the birds started to sing and having to stay focused! In any case, I am indebted to many people for making the time working on my PhD an unforgettable experience. Here is a small tribute to all those people.

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To Lynn Scarman and Lesley Jenkins-White, thank you for your advice and technical assistance with animal handling, many times I would have been at a loss without you. Also a big thank you to Ruth Williams, I am indebted to you for your assistance and advice with all things histology, especially over the last few months of my PhD where time was such a pressing issue, you always came through.

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back for all those long boozy lunches in the sunshine. No more social isolation by me!!

Finally, to my incredible husband Dave. Life and what one achieves become insignificant if you can’t share it with someone very special. Dave, you have been a true and great supporter and have unconditionally loved me during the ups and downs of the PhD process. You have faith in me (and my intellect) even when I felt like digging a hole and crawling into it because I didn’t have faith in myself. To be the partner of a PhD student is not an easy ride and I want to thank Dave for putting up with me, even when I was being a truculent harridan and I love you for it!! I am really looking forward to our post-PhD life together. Tack och jag älskar dig.
Publications


A Stocker, P Cmielewski, DS Anson, DW Parsons. “Disturbance of airway epithelium is reduced, whilst Lentivirus-Mediated Gene Transfer is maintained, after Low Dose Lysophosphatidylcholine Pre-Treatment in vivo.” American Society For Gene Therapy, June 2005

A Stocker, P Cmielewski, DS Anson, DW Parsons “Lentivirus-Mediated Gene Transfer Efficiency in vivo is maintained when using Low Dose Lysophosphatidylcholine Pre-Treatment.” European Cystic Fibrosis Conference, June 2005


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Faculty of Health Sciences Postgraduate Travelling Fellowship (2006)

Semi-finalist for the Women’s & Children’s Hospital Young Investigator of the Year Award (2006)

Australian Cystic Fibrosis Research Trust PhD Grant Supplement (2004 – 2007)

Australian and New Zealand Cystic Fibrosis Conference “New Investigator” Award (2005)

Semi-finalist for the Women’s & Children’s Hospital Young Investigator of the Year Award (2005)
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<th>Definition</th>
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<tr>
<td>AB/PAS</td>
<td>Alcian Blue-Periodic Acid-Schiff</td>
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<tr>
<td>AdV</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>AAV</td>
<td>Adeno-Associated Virus</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>ASL</td>
<td>Airway Surface Liquid</td>
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<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<tr>
<td>β-Gal</td>
<td>Beta-Galactosidase</td>
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<tr>
<td>bp</td>
<td>Base Pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<tr>
<td>°C</td>
<td>Degrees Celsius</td>
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<tr>
<td>cAMP</td>
<td>Adenosine 3‘, 5’-cyclic monophosphate</td>
</tr>
<tr>
<td>cDNA</td>
<td>Copy DNA</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
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<tr>
<td>CFRD</td>
<td>Cystic Fibrosis Related Diabetes</td>
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<tr>
<td>CFTR</td>
<td>Cystic Fibrosis Transmembrane Conductance Regulator</td>
</tr>
<tr>
<td>cfltr&lt;sup&gt;tm1UNC&lt;/sup&gt;</td>
<td>CFTR knock-out Mouse Model</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>Chloride Ion</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>CYWHS</td>
<td>Child, Youth &amp; Women’s Health Service (formally the Women’s &amp; Children’s Hospital)</td>
</tr>
<tr>
<td>DAPI</td>
<td>4’,6-diamidino-2-phenylindole</td>
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<tr>
<td>ΔF508</td>
<td>In frame deletion of phenylalanine at position 508 of exon 10 of the CFTR gene</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>ATPD</td>
<td>The difference between the TPD value recorded under basal + amiloride conditions and the TPD recorded under low Cl⁻ + amiloride conditions</td>
</tr>
<tr>
<td>dL</td>
<td>Decilitre</td>
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<tr>
<td>DMEM</td>
<td>Dulbecco's Modified Eagle's Medium</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DNase</td>
<td>Deoxyribonuclease</td>
</tr>
<tr>
<td>DPX</td>
<td>Distyrene-tricresyl-phosphate-xylene</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunoabsorbent Assay</td>
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<tr>
<td>EnaC</td>
<td>Amiloride Sensitive Epithelial Sodium Channel</td>
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<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
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<tr>
<td>FCS</td>
<td>Foetal Calf Serum</td>
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<td>FITC</td>
<td>Fluoroscein Isothiocyanate</td>
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<td>H&amp;E</td>
<td>Haematoxylin and Eosin</td>
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<tr>
<td>HDAdV</td>
<td>Helper-Dependant Adenovirus</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>hr</td>
<td>Hour</td>
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<tr>
<td>HS</td>
<td>Hypertonic Saline</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish Peroxidase</td>
</tr>
<tr>
<td>i.m.</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
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<tr>
<td>IRT</td>
<td>Immuno-Reactive Pancreatic Trysonogen</td>
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<tr>
<td>K⁺</td>
<td>Potassium</td>
</tr>
<tr>
<td>kb</td>
<td>Kilobase</td>
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<td>L</td>
<td>Litre</td>
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<td>LacZ</td>
<td>Beta-Galactosidase</td>
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<td>LPC</td>
<td>Lysophosphatidylcholine</td>
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<tr>
<td>LRC/s</td>
<td>Label Retaining Cell/s</td>
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<td>LV</td>
<td>Lentivirus</td>
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<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>MCC</td>
<td>Mucociliary Clearance</td>
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<tr>
<td>Min/s</td>
<td>Minute/s</td>
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<tr>
<td>mL</td>
<td>Millilitre</td>
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<tr>
<td>MLV</td>
<td>Murine Leukaemia Virus</td>
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<tr>
<td>μL</td>
<td>Microlitre</td>
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<tr>
<td>μm</td>
<td>Micrometre</td>
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<tr>
<td>MQ-H2O</td>
<td>Milli Q Water</td>
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<tr>
<td>mV</td>
<td>Millivolts</td>
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<tr>
<td>MW</td>
<td>Molecular Weight</td>
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<tr>
<td>Na⁺</td>
<td>Sodium Ion</td>
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<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
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<tr>
<td>ng</td>
<td>Nanograms</td>
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<tr>
<td>NSS</td>
<td>Normal Swine Serum</td>
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<tr>
<td>o/n</td>
<td>Overnight</td>
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<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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<td>Periciliary Liquid</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PD</td>
<td>Potential Difference</td>
</tr>
<tr>
<td>PFA</td>
<td>Paraformaldehyde</td>
</tr>
<tr>
<td>PI</td>
<td>Pancreatic Insufficiency</td>
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<tr>
<td>RCR</td>
<td>Replication Competent Retrovirus</td>
</tr>
<tr>
<td>RT</td>
<td>Room Temperature</td>
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<tr>
<td>Saf-O</td>
<td>Safranin-O</td>
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<tr>
<td>SCID-X1</td>
<td>Severe Combined Immunodeficiency, X linked</td>
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<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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<tr>
<td>TJ</td>
<td>Tight Junction</td>
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<td>TPD</td>
<td>Transepithelial Potential Difference</td>
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<tr>
<td>TU</td>
<td>Transducing Units</td>
</tr>
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<td>University of North Carolina</td>
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<tr>
<td>v</td>
<td>Volume</td>
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<tr>
<td>w</td>
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<td>X-Gal</td>
<td>5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside</td>
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