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

Alice Stocker

School of Medical Sciences

The University of Adelaide

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.

Table of Contents

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.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the internet, *via* the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Alice Stocker

17 April 2010

Acknowledgements

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.

Firstly, I would like to gratefully acknowledge the expert guidance, advice and support of my supervisors, Dr David Parsons and Associate Professor Don Anson over the course of my PhD. In particular I would like to extend countless thanks to David for his tremendous assistance, encouragement, advice, cheerfulness and for being prepared to always offer assistance, this thesis would have been a mess without you. I also wish to thank my Postgraduate Coordinator, Mike Nordstrom for his support and assistance in general.

I would like to thank all the past and present members of the Adelaide Cystic Fibrosis Gene Therapy Research Group for listening to my numerous lab meeting talks, practice presentations and for giving endless guidance and advice, especially Karlea, with whom I shared many disasters and triumphs. Thanks must also go to the entire Department of Pulmonary Medicine and everyone in the Child Health Research Institute (especially my fellow PhD Student Room comrades) for their encouragement throughout my PhD and for making it a truly enjoyable and rewarding experience. I would also like to acknowledge Trish Cmielewski who contributed to the experiment "Histological Analysis of the Effect of LPC on the Airway Epithelium" described in Chapter 3 (section 3.2.3).

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.

I also would like to thank the Adelaide Cystic Fibrosis Gene Therapy Research Group fundraising team. Your devotion to finding a cure and your continued support are an inspiration.

I would like to acknowledge Tavik, graphics extraordinaire, without whom my photos and diagrams would have been a complete disaster! Also, my brother and his mate Pat Parisi who formatted my ugly word document into a professional and impressive looking thesis. I know how much time and effort you put into this and I am truly grateful, thank you both.

Most importantly I must thank my family for their tremendous support, encouragement, love and for believing in me throughout the past five years. I was raised to believe I could achieve anything I set my mind to as long as I had persistence. Well persistence has paid off!! Mum, Dad, my brother David, Nanna and Poppa, your support has been treasured. You have all contributed irreversibly to the person I have become. I can't express how much your love and support means to me, my PhD is just another item on the long list of things that I would never have achieved without you.

I wish to thank my truly amazing friends, too many to list here but you know who you are! I am so thankful for all the times you've scraped me off the ground and reassembled me back into a human. You have been my constant supporters and I would not be the person I am without your friendship. I promise that now I have finished that I will be

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, DS Anson, DW Parsons. "Half a Lifetime of Gene Expression: Validation for a One-Dose Gene Transfer Paradigm in Mouse Airways" Thoracic Society of Australia & New Zealand, March 2007

A Stocker, DS Anson, DW Parsons. "Single Dose Lentivirus Gene Transfer *in vivo* maintains Airway Gene Expression for Over Eighteen Months"." American Society For Gene Therapy, June 2007

A Stocker, DS Anson, DW Parsons. "Single-Dose Lentivirus Gene Transfer *in-vivo* Maintains Airway Gene Expression for over Six Months." American Society For Gene Therapy, June 2006

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

P Cmielewski, DW Parsons, **A Stocker** and DS Anson "*In vivo* Airway Conditioning For Improved Gene Delivery: Mucosubstance Release and Lentiviral Gene Transfer. Australasian Gene Therapy Conference, April 2005

A Stocker, P Cmielewski, DS Anson, DW Parsons. "Using Lysophosphatidylcholine Pre-Treatment to Boost Lentiviral Airway Gene Therapy Without Injury." Australian and New Zealand Cystic Fibrosis Conference, August 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." Journal of Cystic Fibrosis, (2005), 4 (Supplement):29 Abstract 107

A Stocker, P Cmielewski, DS Anson, DW Parsons. "*In vivo* Optimisation of Lentiviral Gene Transfer When Enhanced by Lysophosphatidylcholine Pre-Treatment. North American Cystic Fibrosis Society Conference, October 2005

R. Koldej, P. Cmielewski, **A Stocker**, D.W. Parsons, D.S. Anson. "Optimisation of a multipartite human immunodeficiency virus based vector system; control of virus infectivity and large-scale production." Journal of Gene Medicine, November 7 (11), pages 1390-1399, 2005.

Sponsorships & Awards

Solvay Pharmaceuticals Cystic Fibrosis Travel Scholarship (2006)

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)

List of Figures

List of Tables

Glossary of Terms

AB/PAS	Alcian Blue-Periodic Acid-Schiff
AdV	Adenovirus
AAV	Adeno-Associated Virus
ADP	Adenosine Diphosphate
ANOVA	Analysis of Variance
ASL	Airway Surface Liquid
ATP	Adenosine Triphosphate
β-Gal	Beta-Galactosidase
bp	Base Pairs
BSA	Bovine Serum Albumin
^{o}C	Degrees Celcius
cAMP	Adenosine 3', 5'-cyclic monophosphate
cDNA	Copy DNA
CF	Cystic Fibrosis
CFRD	Cystic Fibrosis Related Diabetes
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
cftr^{tm1UNC}	CFTR knock-out Mouse Model
Cl^-	Chloride Ion
cm	Centimetre
CYWHS	Child, Youth & Women's Health Service (formally the Women's & Children's Hospital)
DAPI	4',6-diamidino-2-phenylindole
ΔF508	In frame deletion of phenylalanine at position 508 of exon 10 of the CFTR gene

ΔTPD	The difference between the TPD value recorded under basal + amiloride conditions and the TPD recorded under low Cl^- + amiloride conditions
dL	Decilitre
DMEM	Dulbecco's Modified Eagle's Medium
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
DPX	Distyrene-tricresyl-phosphate-xylene
ELISA	Enzyme-Linked Immunoabsorbent Assay
EnaC	Amiloride Sensitive Epithelial Sodium Channel
ER	Endoplasmic Reticulum
FCS	Foetal Calf Serum
FITC	Fluoroscein Isothiocyanate
H&E	Haematoxylin and Eosin
HDAdV	Helper-Dependant Adenovirus
HIV	Human Immunodeficiency Virus
hr	Hour
HS	Hypertonic Saline
HRP	Horseradish Peroxidase
i.m.	Intramuscular
i.p.	Intraperitoneal
IRT	Immuno-Reactive Pancreatic Trysonogen
K^+	Potassium
kb	Kilobase
L	Litre
LacZ	Beta-Galactosidase
LPC	Lysophosphatodylcholine
LRC/s	Label Retaining Cell/s
LV	Lentivirus

M	Molar
MCC	Mucociliary Clearance
Min/s	Minute/s
mL	Millilitre
MLV	Murine Leukaemia Virus
µL	Microlitre
µm	Micrometre
MQ-H2O	Milli Q Water
mV	Millivolts
MW	Molecular Weight
Na^+	Sodium Ion
NaCl	Sodium Chloride
ng	Nanograms
NSS	Normal Swine Serum
o/n	Overnight
PBS	Phosphate Buffered Saline
PCL	Periciliary Liquid
PCR	Polymerase Chain Reaction
PD	Potential Difference
PFA	Paraformaldehyde
PI	Pancreatic Insufficiency
RCR	Replication Competent Retrovirus
RT	Room Temperature
Saf-O	Safranin-O
SCID-X1	Severe Combined Immunodeficiency, X linked
SEM	Standard Error of the Mean
TJ	Tight Junction
TPD	Transepithelial Potential Difference

TU	Transducing Units
UNC	University of North Carolina
v	Volume
VSV-G	Vesicular Stomatitis Viirus Glycoprotein G
w	Weight
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside