

# **Cystic Fibrosis Gene Therapy: Methods for the optimisation of CFTR gene delivery.**

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Submitted April 2010

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# Abbreviations

|                  |   |
|------------------|---|
| $\mu\text{g}$    | microgram   |
| $\mu\text{L}$    | microlitre  |
| AAV              | Adeno-associated virus                              |
| AdV              | Adenovirus  |
| ARO              | anaplastic thyroid cancer cell line                 |
| ASL              | Airway Surface Liquid                               |
| ATP              | Adenosine Triphosphate                              |
| Baf-A1           | Bafilomycin A1                                      |
| BAL              | Bronchoalveolar lavage                              |
| BPB              | Bromophenol Blue                                    |
| $\text{Ca}^{++}$ | Calcium ion   |
| cAMP             | Cyclic Adenosine Monophosphate                      |
| CF               | Cystic Fibrosis                                     |
| CFTR             | Cystic Fibrosis Transmembrane Conductance Regulator |
| CHOK-1           | Chinese Hamster Ovary cells                         |
| CIP              | Calf Intestinal Phosphatase                         |
| $\text{Cl}^-$    | Chloride Ion  |
| CT               | Computed Tomography                                 |
| Ct               | Cycle Threshold                                     |
| DGGE             | Denaturing gradient gel electrophoresis             |
| DMSO             | Dimethyl sulfoxide                                  |
| DNA              | Deoxyribonucleic acid                               |

|                |   |
|----------------|---|
| EF1 $\alpha$   | Elongation factor alpha                     |
| ENaC           | Epithelial sodium channel                   |
| EtOH           | Ethanol                                     |
| EYFP           | Enhanced Yellow Fluorescent Protein         |
| FABp           | fatty acid binding protein                  |
| FDA            | Food and Drug Administration                |
| FEV1           | Forced Expiratory Volume in one second      |
| gDNA           | Genomic Deoxyribonucleic acid               |
| H & E          | Haematoxylin and Eosin                      |
| HCl            | Hydrogen Chloride                           |
| HeBS           | Hepes Buffered Saline                       |
| HIV-1          | Human Immunodeficiency Virus type 1         |
| I.P            | Intraperitoneal                             |
| IFN- $\gamma$  | Interferon Gamma                            |
| Ig             | Immunoglobulin                              |
| IL-            | Interleukin-                                |
| IMVS           | Institute of Medical and Veterinary Science |
| IRT            | Immunoreactive Trypsinogen                  |
| K <sup>+</sup> | Potassium Ion                               |
| K18            | Cytokeratin-18                              |
| kbp            | kilobase pairs                              |
| kDa            | kilodalton                                  |
| LiCl           | Lithium Chloride                            |
| LMB            | Leptomycin B                                |
| LPC            | Lysophosphatidylcholine                     |

|                 |   |
|-----------------|---|
| LTR             | Long Terminal Repeat                    |
| LV              | Lentivirus                              |
| MDS             | Membrane Spanning Domains               |
| MeOH            | Methanol                                |
| MHC             | Major Histocompatibility Complex        |
| mL              | Millilitre                              |
| mm              | Millimetre                              |
| MRI             | Magnetic Resonance Imaging              |
| mRNA            | Messenger Ribonucleic Acid              |
| Na <sup>+</sup> | Sodium ion                              |
| NaCl            | Sodium Chloride                         |
| NaOAc           | Sodium Acetate                          |
| NaOH            | Sodium Hydroxide                        |
| NBD1            | Nuclear Binding Domain 1                |
| NBD2            | Nuclear Binding Domain 2                |
| NBF             | Neutral Buffered Formalin               |
| NFQ             | Non Fluorescent Quencher                |
| nM              | nanomolar                               |
| NO              | Nitric Oxide                            |
| OCT             | Optimal Cutting Temperature             |
| OGTR            | Office of the Gene Technology Regulator |
| OLA             | Oligonucleotide Ligation Assay          |
| ORCC            | Outwardly Rectifying Chloride Channel   |
| PAMP            | Pathogen-associated Molecular Patterns  |
| PBS             | Phosphate Buffered Saline               |

|               |   |
|---------------|---|
| PCL           | Periciliary Liquid  |
| PCR           | Polymerase Chain Reaction                                 |
| PD            | Potential Difference                                      |
| PEG           | Polyethylene glycol                                       |
| PET           | Positron Emission Tomography                              |
| PFT           | Pulmonary Function Testing                                |
| Pgk           | Phosphoglycerate kinase                                   |
| PKC           | Protein Kinase C  |
| pM            | picomolar   |
| PMA           | phorbol 12-myristate 12-acetate                           |
| PRR           | Pathogen Recognition Receptors                            |
| RSV           | Respiratory Syncytial Virus                               |
| RT-PCR        | Real Time Polymerase Chain Reaction                       |
| Saf O         | Safranin O  |
| SDS           | Sodium dodecyl sulphate                                   |
| SELEX         | Systematic Evolution of Ligands by Exponential Enrichment |
| SeV           | Sendai Virus  |
| SSC           | Saline sodium citrate                                     |
| SSCP          | Single Strand Conformation Polymorphism                   |
| Th1           | T-helper type 1   |
| TLR           | Toll Like Receptor  |
| TNF- $\alpha$ | tumor necrosis factor- $\alpha$                           |
| TPD           | transepithelial potential difference                      |
| UNC           | University of North Carolina                              |
| v/v           | volume for volume   |



|       |   |
|-------|---|
| Vif   | virion infectivity factor                 |
| Vpr   | viral protein r                           |
| VSV-G | vesicular stomatitis virus G glycoprotein |
| WCH   | Women's and Children's Hospital           |
| w/v   | Weight for weight                         |
| Z-LLL | Z-leu-leu-leu                             |

# Abstract

Gene therapy potentially holds the key for the treatment and cure of many genetic diseases, including cystic fibrosis. A number of delivery methods have been developed for the integration of a functional gene into the host genome, one of which is the use of a HIV-1 derived lentivirus, as is used in this thesis. However, a large number of issues need to be addressed before an effective gene therapy protocol can be developed, and some of these are described further in this thesis.

One such issue is that the response initiated by cells to the gene transfer vector need to be addressed, as organelles such as the proteasome and lysosome that break down foreign peptides and proteins may be involved in the degradation of our gene transfer vector, ultimately limiting the amount of gene transfer vector that is able to successfully integrate into the genome. Therefore, the potential use of proteasome and lysosome inhibitors for facilitating higher levels of gene transduction *in vivo* was investigated.

As this project uses a HIV-1 derived lentivirus for gene transfer, the use of an inhibitor of the IN1/PML innate antiretroviral response (Leptomycin B) was also assessed, again with the aim of increasing the efficiency, and hence level, of gene transfer obtained.

Using a robust animal model of disease is essential for testing lentivirus constructs containing the therapeutic gene and analysing phenotypic changes in disease. A mouse model of cystic fibrosis without gastrointestinal disease was bred to obtain a robust colony of mice that efficiently produce affected mice (CFTR knockout).

Visual analysis of therapeutic gene transfer in cystic fibrosis is often difficult due to the lack of antibodies available. Short DNA molecules that adopt a specific 3-D shape known as aptamers hold

much potential as agents that can be developed to bind to the CFTR gene product. These can then be labelled and used in the same way as antibodies to probe tissues excised from animals treated with the therapeutic CFTR gene.

Essential to gene therapy is the development of methods for the consistent determination of lentivirus titre. As the production of lentivirus becomes more sophisticated with the use of multiple transgenes in a single virus preparation, the need for multiple assays to determine the titres of each individual virus component are required. Real time PCR assays were developed for each individual transgene for titre determination. A real time PCR assay for use with CHOK-1 cells was also developed for comparison of real time PCR titre of a LacZ virus to the titre obtained using the traditional LacZ titre achieved *via* a staining assay in CHOK-1 cells. The use of a standard real time PCR assay for the determination of titre for all viruses- containing any transgene is essential to allow comparison by titre.

Determination of the level of gene expression required to achieve a therapeutic outcome in a cystic fibrosis mouse model is an important factor to consider, as high level expression in all cells may not achieve the best outcomes, and low level, ciliated-cell specific expression may in fact achieve a superior result. A range of different strength and cell targeted promoters (EF1 $\alpha$ , pgk and K18) were tested for their effect on phenotypic correction of the cystic fibrosis knockout mouse model.

Lastly, targeting therapeutics to the disease affected areas is essential in achieving the best patient outcomes. As the main target organ in cystic fibrosis are the conducting airways of the lungs, delivery of our gene transfer vectors to the lungs as an aerosol is a necessary step towards moving this gene therapy protocol to the clinic. Aerosolisation of the treatment protocol was investigated in the rat lung for evidence of whether this is a viable means of lentiviral mediated gene delivery to the airways.

# Declaration

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# Acknowledgements

I would like to thank my supervisors Associate Professor Donald Anson and Dr David Parsons for their support and patience throughout my PhD, and for ensuring my work is of a high standard.

I would also like to thank the members of the Adelaide Cystic Fibrosis Gene Therapy group and the staff of the departments of Genetic and Pulmonary Medicine- Trish Cmielewski, Julie Bielicki, Alice Stocker, Rachel Koldej, Martin Donnelley, Richard Bright, Edward Wong, Chantelle McIntyre, Sue Lim, Sin Lay Kang and Stanley Tan. Thankyou all for your support and willingness to help during my PhD.

Thankyou to the University of Adelaide for providing me an opportunity to undertake my PhD and supporting me with a Divisional Scholarship through the Faculty of Health Sciences.

I would like to thank my family- Mum, Dad, Alyssa and Brad for their love and support and always asking questions and being interested in my research.

Last, but by no means least, I would like to thank my husband Adam. Without your constant belief in me and support I would not have been able to get this far- Thankyou.