CYSTIC FIBROSIS: THE ROLE OF AIRWAY STEM CELLS IN SUSTAINED GENE EXPRESSION BY LENTIVIRAL DIRECTED GENE THERAPY

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This Thesis is dedicated to my daughter

Ella Farrow.

My very special little girl, your determination to enjoy life to its fullest despite having the insidious disease Cystic Fibrosis is a source of inspiration which drives me every day. Darling this is for you.

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Patricia Cmielewski, **Nigel Farrow**, Chantelle McIntyre, Harsha Padmanabhan, Martin Donnelley, Tim Kuchel, and David W Parsons." Lentiviral airway gene transfer in normal ferrets" American Society for Gene and Cell Therapy, Washington, USA.

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2012

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2011

D Parsons, **N Farrow**, D Miller, S Le Blanc, R Bright , P Cmielewski and D S Anson, "One Year Persistence From a Single HIV-1 Lentiviral Vector Delivery Into Marmoset Lung: LacZ and Vector Gene Presence" presented at the American Society of Gene & Cell Therapy conference, Seattle, North America, 2011

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Synopsis

In this thesis transduction of airway stem cells (basal cells) in the nasal and tracheal airways was investigated to determine the causality of sustained transgene expression following a gene therapy protocol that utilised an LPC pre-treatment and a HIV-1 VSV-G pseudotyped lentivirus vector treatment, as previously published. To assess stem cell transduction and epithelial regrowth a forced-injury model was employed at a number of time points after the gene therapy protocol.

Epithelial remodelling in cystic fibrosis and normal airways of mice was also assessed. Airway stem cell hyperplasia and goblet cell hyperplasia and hypertrophy, and epithelial mucin content were assessed in the trachea and in some instance the nasopharynx in the nasal airways of CF and normal mice.

Additionally, the effectiveness of the LPC / lentiviral gene therapy protocol was assessed in lung airways of normal ferrets and the marmoset, a non-human primate, to determine if airway transduction of both differentiated ciliated cells and stem cells reflected observations noted in previously-published mouse-based studies. These ferret and marmoset animal studies have been published prior to thesis submission.

Airway stem cells transduction was confirmed in the trachea and nasal airways of mice following pre-treatment with LPC and subsequent treatment with a HIV-1 VSV-G pseudotyped lentiviral vector carrying the LacZ marker gene. A forced injury model was employed to force regeneration of the airway epithelium after vector treatment. Following the ablation and subsequent regeneration of the airway epithelium, clusters of LacZ positive were observed in both the trachea and nasal airways suggesting transduction of the airway stem cells and the passing of the

transgene to their progeny upon differentiation.

Airway epithelial remodelling was demonstrated in both airway stem cells and goblet cells in CF mice. Hyperplasia of airway stem cells and goblet cells in CF mice was observed. Hypertrophy and change in mucin acidity of goblet cells was also observed. Additionally, remodelling of the cartilage rings in the trachea was observed in CF mice. This is the first study to demonstrate the presence of goblet cell hyperplasia, hypertrophy and change in mucin acidity in the presence of airway stem cell hyperplasia. Importantly, the hyperplasia of airway stem cells in CF airways had previously been proposed however, this is the first study to directly quantitate the airway stem cell compartment using a novel flow cytometry and clonogenic assay approach.

Finally, the transduction of airway stem cells and ciliated cells is shown in normal ferrets and marmosets, a non-human primate. Validation of transducing relevant airway cell type in these animals adds to the gene therapy proof of principle foundation previously demonstrated in the airways of mice.

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