LONGEVITY OF AIRWAY GENE THERAPY FOR CYSTIC FIBROSIS: SINGLE AND REPEAT LENTIVIRAL DOSING

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LIST OF ABBREVIATIONS

293T cells (HEK)	Human embryonic kidney cells
A549 cells	Carcinomic human alveolar basal cells
AAV	Adeno-Associated virus
ABC	Adenosine triphosphate binding cassette
ADA-SCID	Adenosine deaminase deficiency–severe combined immunodeficiency disorder
AdV	Adenovirus
AIDS	Acquired immunodeficiency syndrome
Amil	Amiloride
ANOVA	Analysis of variance
APC	Antigen presenting cell
ASL	Airway surface liquid
ATP	Adenosine triphosphate
AUS	Australia
BMI	Body mass index
°C	Degrees Celsius
^{13}C	Isotopic labelled carbon
cAMP	cyclic adenosine monophosphate

CAUV	Congenital absence of the uterus and vagina
CBAVD	Congenital bilateral absence of the vas deferens
CF	Cystic fibrosis
CFLD	Cystic fibrosis liver disease
CFRD	Cystic fibrosis related diabetes
CFTR	Cystic fibrosis transmembrane conductance regulator
cGMP	cyclic guanosine monophosphate
CHO-K1 cells	Chinese Hamster Ovary – K1 epithelial cells
Cl^-	Chloride
cm	centimetre
CMV	Cytomegalovirus
CO_2	Carbon dioxide
CpG	Cytosine-phosphate-guanine
Ct	Cycle threshold
CTL	Cytotoxic T Lymphoctes
ΔF508	Delta F508
Da	Daltons
DIOS	Distal intestinal obstruction syndrome
DMEM	Dulbecco's Modified Eagle's Medium
DMF	N, N, Dimethyl formamide
DNA	Deoxyribonucleic acid

ΔPD	Change in Potential Difference
DPX	Distyrene-tricresyl-phosphate-xylene
EDTA	Ethylene diamine tetraacetic acid
EF1-α	Human elongation factor 1-*alpha*
EGTA	Ethylene glycol tetraacetic acid
EIAV	Equine infectious anaemia virus
ELISA	Enzyme linked immunosorbent assay
ENaC	Epithelial sodium channel
env	Envelope
EYFP	Enhanced yellow fluorescent protein
FABp	Fatty acid binding protein
FCS	Fetal calf serum
FE-1	Faecal elastase-1
FEV_1	Forced expiratory volume in 1 second
FIV	Feline immunodeficiency virus
g	grams
gag	Group-specific antigen
Glut	Glutaraldehyde
GTP	Guanosine triphosphate
dH_2O	Deionized water
HCl	Hydrochloric acid

$HCO3^-$	Bicarbonate
HD-AdV	Helper-dependent adenovirus
H&E	Haematoxylin and Eosin
Het	Heterozygote (-/+)
HIV	Human immunodeficiency virus
hr	Hour
HRP	Horseradish peroxidase
Ig	Immunoglobulin
i.n.	Intranasal
i.p.	Intraperitoneal
i.u.	Infectious units
K^+	Potassium
KRB	Krebs-ringers buffer
L	Litre
LacZ	β-galactosidase
LPC	Lysophosphatidylcholine
LTR	Long terminal repeat
Luc	Luciferase
LV	Lentivirus
$\bar{X}$	Mean
m	month(s)

M	Molar
mA	milliamps
mM	millimolar
MCC	Mucociliary clearance
MCT	Mucociliary transport
MHC	Major histocompatibility complex
MI	Meconium ileus
min	minutes
ml	millilitre
μl	microlitre
mRNA	messenger ribonucleic acid
MSD	Membrane-spanning domain
MT	Empty vector
mTransferrin	mouse transferrin
mV	millivolts
MW	Molecular weight
Na^+	Sodium
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NBD	Nucleotide-binding domain
NBF	Neutral buffered formalin

Nef	Negative regulatory factor
NFQ	Non-fluorescent quencher
NIH3T3 cells	Mouse embryonic fibroblast cells
nls	Nuclear localised
nm	nanometres
OPD	o-Phenylenediamine dihydrochloride peroxidase substrate
ORCC	Outwardly rectifying chloride channel
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline + Tween
PC2	Physical containment level 2
PCL	Periciliary Layer
PD	Potential difference
PEG	Polyethylene glycol
Pen-Strep	Penicillin/Streptomycin
PFA	Paraformaldehyde
Ph	Photons
PI	Pancreatic Insufficiency
PKA	Protein kinase A
PKC	Protein kinase C
pol	Polymerase
PS	Pancreatic Sufficiency

qPCR	Real time quantitative polymerase chain reaction
Rev	Regulator of virion protein expression
RM	Repeated Measures
ROI	Region of Interest
ROMK	Renal outer medullary potassium channel
rpm	Revolutions per minute
RSV	Respiratory syncytial virus
RT	Room temperature
Rx	Treated
SafO	Safranin O
SCID-X1	X-linked severe combined immunodeficiency disorder
SEM	Standard error of the mean
SeV	Sendai virus
SFM	Serum free medium
SIN	Self inactivating
SIV	Simian immunodeficiency virus
SKMPBST	Skim milk PBS + Tween
SMG	Submucosal gland
SV-40	Simian virus type 40
T	Temperature
Tat	Trans-activator of transcription

TPD	Transepithelial potential difference
Treg	T -Regulatory cell
TU	Transducing units
Tween 20	Polyoxyethylene sorbitan monolaurate
UnRx	Untreated
USA	United States of America
UV	Ultraviolet
v	Volume
Vif	Virion infectivity factor
Vpr	Viral protein R
Vpu	Viral protein U
VSV-G	Vesicular stomatitis virus glycoprotein G
w	Weight
WCH	Women's and Children's Hospital
wk	Week(s)
Xgal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

ABSTRACT

The promise of gene therapy as a treatment and/or cure for cystic fibrosis (CF) airway disease is yet to be fulfilled. Lentiviral (LV) vectors possess many of the properties that would satisfy the requirements for an effective clinical gene correction treatment; the capacity to hold the large CF transmembrane conductance regulator (CFTR) gene, pseudotyped envelopes that provide broad tropism for a range of cells and tissue types, the ability to transduce dividing and non-dividing cells, the potential for long-term gene expression from genomic integration, and the lack of pre-existing blocking antibodies for majority of the CF population.

To determine the persistence of LV gene expression, the same mice were repeatedly assessed throughout their lifetimes. The utilization of the biological compound lysophosphatidylcholine (LPC) as a pre-treatment enhanced nasal airway gene expression of the HIV-1 based LV vector containing reporter genes, or the functional CFTR gene, in normal and CF mice *in vivo*.

Nasal luciferase (Luc) gene expression from a single LPC/LV nasal dose was sustained for the life time of normal mice, possibly suggesting an involvement of stem/progenitor cells or long-lived terminally differentiated cells. In contrast, stable long-term Luc gene expression was detected in the lung airways without the requirement of LPC pre-treatment. The loss then re-emergence of lung luminescence in CF mice demonstrated that stem/progenitor cells were transduced.

This was the first examination of persistence of LV reporter gene and functional gene expression, in individual CF mice over their lifetimes. CF mice treated with LPC/LV-CFTR demonstrated a significant

partial functional correction of the nasal CFTR electrophysiological defect that was sustained for up to 1 year. Importantly, this significantly increased survival, close to that observed in normal mice.

Since the level of functional expression diminished over time in CF mice the ability to re-dose and evade blocking host immune responses was addressed. Multiple doses of a LV vector over a short time frame were feasible but did not significantly increase expression compared to a single dose. Circulating antibodies to both the vector envelope and the transgene protein were detected after repeat dosing conducting over a longer time frame. The timing of additional LV vector doses may be crucial for effective boosting of waning gene expression.

The addition of a transient immunosuppressive treatment did not significantly enhance the level of gene expression produced by a single dose, but did reduce circulating antibodies to both the delivered foreign transgene and to the pseudotyped envelope protein.

The demonstration of longevity of gene expression, the functional correction of the CFTR defect, the substantial increase in CF animal survival, the ability to re-dose and the use of immune-suppression to reduce antibody production provides strong and specific support for the continued investigation of LV CFTR gene transfer towards a clinical gene therapy treatment 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 Patricia Cmielewski 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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