

GENE THERAPY FOR PULMONARY ARTERIAL
HYPERTENSION WITH BONE MORPHOGENETIC
PROTEIN RECEPTOR TYPE-2 MODULATION VIA
ENGINEERED ENDOTHELIAL PROGENITOR CELLS
OR A TARGETED ADENO-VIRAL CONSTRUCT:
CHANGES IN SMAD AND NON-SMAD SIGNALLING
CONTRIBUTED TO AMELIORATION OF DISEASE

BY

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To my love, Ninh.

And to all the rural kids who dare to look beyond the horizon.

ABSTRACT

Pulmonary arterial hypertension (PAH) is a rare but devastating disease and despite available therapeutics, survival remains at 3-5 years. Reduced expression of the bone morphogenetic protein receptor type 2 (BMPR2) is causally linked to hereditary, idiopathic and secondary forms of PAH. Thus, we proposed that up-regulation of BMPR2 may be therapeutic. As proof of concept, we've previously attenuated PAH in animal models through BMPR2 targeted gene delivery using Adenoviral (Ad) vectors. However, further understanding of the cell signalling mechanisms involved, as well as overcoming limitations with viral vector approaches is required to progress this approach to the clinic. Endothelial progenitor cells (EPCs) may be the key to avoiding the shortcomings of Ad-vector technology. EPCs are important for angiogenesis as well as tissue repair and have been shown to have altered function and abundance in patients with PAH. Manipulating these cells may be an alternate means to up-regulate BMPR2 in lungs affected by PAH, thereby avoiding some of the limitations of viral gene delivery techniques and enabling easier clinical translation.

Herein, I confirmed disease reversal in the rat monocrotaline (MCT)-induced PAH model following targeted gene delivery of BMPR2 to the pulmonary vascular endothelium and assessed the relevant BMPR2 mediated Smad pathways in whole lung tissue, 10 days following treatment. Microarray technology was utilised to identify any novel molecular targets, with results from this indicating that a peak Smad signalling effect was missed at this 10 day time-point. However, the microarray did indicate potential changes in BMPR2 mediated non-Smad signalling. PAH reversal was then assessed 2 days following targeted gene delivery of BMPR2 to the pul-

monary endothelium and further assessment of BMPR2 mediated Smad and non-Smad pathways were analysed in the subsequent whole lung tissue.

Moving towards a more clinically applicable therapy, cell therapy using *ex vivo* engineered EPCs to deliver BMPR2 to the pulmonary endothelium was investigated in the rat MCT-induced PAH model. To do this, the technique to isolate and culture rat bone marrow derived EPCs (r-EPCs) was developed. Successful transduction of these cells to over-express BMPR2 was optimised and these now engineered cells were used as a vehicle to deliver BMPR2 to the pulmonary vasculature via intravenous injection into rats with MCT-induced PAH. Amelioration of PAH was confirmed 10 days following the cell therapy treatment and subsequent protein analysis of BMPR2 mediated Smad pathways in the whole lung tissue saw changes activated Smad1/5/8.

The development of new therapies for PAH is critical. BMPR2 modulation is a novel therapeutic strategy which addresses the well known underlying pathology of BMPR2 deficiency that occurs not only in hereditary PH, but secondary PH and most PAH animal models. The success of our highly novel pre-clinical BMPR2 cell therapy may lead the way for further development of other BMPR2 therapies, as well as give significant insight into the pathophysiology of this devastating disease.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Rebecca L Harper,

July 25, 2016

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 10. R L Harper, A M Reynolds and P R Reynolds, *Gene Delivery of Bone Morphogenetic Protein Receptor Type 2 Ameliorates PAH via Changes in Smad Signalling*, in proceedings of Respirology, vol 17, pg. 19, 2012. Presented as an oral presentation at the TSANZ Annual Scientific Meeting, Canberra, 2012
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 12. S Pradeepan, R Harper, A Thornton, S Johnston and H Greville, *β -Blocker Usage by Patients Referred For Lung Function Testing: An Observational Study*, in proceedings of Respirology, vol. 16, pg. TP-155, 2011.

13. R Harper, S Johnston and A Thornton, *Six Minute Walk Test: Compliance with ATS Guidelines*, in proceedings *Respirology*, vol. 15:1, pg. A8, March, 2010. Poster presented at Australian and New Zealand Society of Respiratory Science (ANZSRS) Annual Scientific Meeting, Brisbane, March, 2010.
14. R Harper, P Roger, S Johnston and A Thornton, *15 Years of Inter-Laboratory Quality Control*, in proceedings, *Respirology*, vol. 14:1, pg. A5, April, 2009. Poster presented at Australian and New Zealand Society of Respiratory Science (ANZSRS) Annual Scientific Meeting, Darwin, April, 2009.

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AWARDS AND SCHOLARSHIPS

1. **Ann Woolcock Young Investigator of the Year 2015**, Thoracic Society of Australia and New Zealand.
A single prestigious annual award open to both Australia and New Zealand.
2. **Asia Pacific Young Investigator Award 2015**, Asia Pacific Respiratory Society.
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ACRONYMS

Alpha-SM Actin	Alpha-Smooth Muscle Actin
5-HT	Hydroxytryptamine or Serotonin
5-HTT	Hydroxytryptamine (Serotonin) Transporter
6MWD	6 Minute Walk Distance
AAV	Adeno-associated Virus
ACE	Angiotensin Converting Enzyme
Ad	Adenovirus
ADA-SCID	Adenosine Deaminase Deficiency
ALK-5	Activin Receptor-Like Kinase-5
ALK	Activin-Like Receptor Kinase-1
ANG-1	Angiopoietin-1
APC	Antigen Presenting Cell
BMPR₂	Bone Morphogenetic Protein Receptor Type 2
BSA	Bovine Serum Albumin
cAMP	cyclic AMP
CAR	Coxsackie and Adenovirus Receptor
CCB	Calcium Channel Blockers
cGMP	cyclic Guanosine Monophosphate

CPE	Cytopathic Effect
CsCl	Cesium Chloride
CXCL ₁₀	CXC-chemokine Ligand 10
DMEM	Dulbecco's Modeified Eagle Medium
EC	Endothelial Cell
ECM	Extracellular Matrix
EGF	Epidermal Growth Factor
EHS	Engelbreth-Holm-Swarm
eIF ₂	Eukaryotic Initiation Factor 2
EM	Electron Microscopy
EMA	European Medicine Agency
EMT	Epithelial to Mesenchymal Transition
EndoMT	Endothelial to Mesenchymal Transition
ENG	Endoglin
EPC	Endothelial Progenitor Cell
ERA	Endothelin-Receptor Antagonists
ESC	Embryonic Stem Cells
ET ₁	Endothelin-1
ETA	Endothelin-1 A
ETB	Endothelin-1 B
FCS	Foetal Calf Serum

FDA	Food and Drug Administration
FDR	False Discovery Rate
FH Rats	Fawn-Hooded Rats
FI	Fulton Index
FKBP	FK506 Binding Protein
FLAP	5-lipoxygenase Activating Protein
GFP	Green Florescent Protein
HIF-1alpha	Hypoxia Inducible Factor -1alpha
HIF-1beta	Hypoxia Inducible Factor -1beta
HMVEC-L	Lung Derived Human Microvascular Endothelial Cell
HPAH	Hereditary Pulmonary Arterial Hypertension
HRQOL	Health Related Quality of Life
IPA	Ingenuity Pathway Analysis
IPAH	Idiopathic Pulmonary Arterial Hypertension
Luc	Luciferase
LV	Left Ventricle
MAPK	Mitogen Activated Protein Kinase
MBP	Myeloid Binding Protein
mcDNA	Minicircle DNA
MCP-1	Monocyte Chemoattractant Protein-1
MHD	Mad-homology Domain

mPAP	Mean Pulmonary Arterial Pressure
NHMRC	National Health and Medical Research Council
NMD	Nonsense Mediated Decay
NO	Nitric Oxide
NT-proBNP	N-terminal Prohormone of Brain Natriuretic Peptide
NYHA	New York Heart Association
p-Smad	Phosphorylated-Smad
PA	Pulmonary Artery
PAEC	Pulmonary Arterial Endothelial Cell
PAH	Pulmonary Arterial Hypertension
PASMC	Pulmonary Arterial Smooth Muscle Cell
PAWP	Pulmonary Arterial Wedge Pressure
PBS	Phosphate Buffered Solution
PCA	Principle Component Analysis
PDE-5	Phosphodiesterase-5
PDGF	Platelet-Derived Growth Factor
pDNA	Naked Plasmid DNA
PFU	Plaque Forming Units
PGI₂S	Prostacyclin Synthase
PH	Pulmonary Hypertension
PI₃K	Phosphoinositide 3-Kinase

PVR	Pulmonary Vascular Resistance
rEPCs	Rat Derived Endothelial Progenitor Cell
RHC	Right Heart Catheterisation
Rho	Ras Homologous
RIN	RNA Integrity Score
ROCK	Rho Kinase
RT	Room Temperature
RVOT	Right Ventricle Outflow Tract
S	Septum
SDF-1	Stromal Derived Factor-1
sGC	Soluable Guanylate Cyclase
SMAD	Some Mothers Against Decapentaplegic
SMC	Smooth Muscle Cell
SVC	Superior Vena Cava
TCID₅₀	Tissue Culture Infectious Dose 50
TGF	Transforming Growth Factor- β
TNS	Trypsin Neutralising Solution
Treg Cells	High Regulatory T Cells
V/Q	Ventilation and Perfusion
VEGF	Vascular Endothelial Growth Factor
WSPH	World Symposium on Pulmonary Hypertension