# 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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# PUBLICATIONS

## JOURNAL ARTICLES

*Accepted*

1. R L Harper, A M Reynolds and P N Reynolds, *Changes in Smad Signalling Leads to Amelioration of PAH Following Gene Delivery of BMPR2* , accepted Respirology, September, 2015.

2. F Feng, R L Harper and P N Reynolds, *BMPR-2 gene delivery reduces mutation-related PAH and counteracts TGFβ mediated pulmonary cell signalling*, accepted Respirology, September, 2015.

*In preparation*

1. R L Harper, C S Bonder and P N Reynolds, *Endothelial Progenitor Cells Over-expressing BMPR2 Ameliorates PAH in A Rat MCT Model*.

2. R L Harper, C S Bonder and P N Reynolds, *Biodistribution of Engineered Endothelial Progenitor Cells Following Intraveneous Injection into Rats*.

3. R L Harper, C S Bonder and P N Reynolds, *Identification of Novel Therapy Targets following a Microarray Study of rat lungs from a disease reversal model of MCT-induced PAH*.

## CONFERENCE PRESENTATIONS

1. R L Harper and P N Reynolds, *Smad and Non Smad Signalling in PAH Following BMPR2 Gene Delivery*, presented as a poster presentation at the 2015 ATS Annual Scientific Meeting, Denver, CO, May 2015

2. R L Harper and P N Reynolds, *Up-regulation of BMPR2 in Rat Derived Endothelial Progenitor Cells Leads to the Attenuation of PAH in a MCT Rat Model*, presented as a poster presentation at the 2015 ATS Annual Scientific Meeting, Denver, CO, May 2015.
   **Recipient of ATS Scholarship**

3. R L Harper and P N Reynolds, *BMPR2 Replacement Therapy via In Situ Gene Delivery or Engineered Endothelial Cells Alleviates PAH in a Rat Model*, presented as a poster presentation at the 2015 American Society of Gene and Cell Therapy, Annual Scientific Meeting, New Orleans, LO, May 2015.

4. R L Harper and P N Reynolds, *BMPR2 Upregulation Via in situ Gene Delivery or Via Engineered Endothelial Progenitor Cells Alleviates Pulmonary Arterial Hypertension (PAH) in a Rat Model*, presented as an oral presentation at the 2015 TSANZ, Annual Scientific Meeting, Gold Coast, QLD, May 2015.
   **Winner of the Ann Woolcock Young Investigator of the Year**

5. R L Harper and P N Reynolds, *Identification of Novel Cellular Signalling Pathways Following Gene Delivery of Bone Morphogenetic Protein Receptor Type-2: A Microarray Study*, presented as an oral presentation at the 2014 TSANZ Annual Scientific Meeting, Adelaide, April, 2014.

6. R L Harper and P N Reynolds, *Identification of Novel Cellular Signalling Pathways Following Gene Delivery of Bone Morphogenetic Protein Receptor*

*Type-2: A Microarray Study*, presented as an oral presentation at the 2014 TSANZ Annual Scientific Meeting, Adelaide, April, 2014.

7. R L Harper, C S Bonder and P N Reynolds, *Isolation and Characterisation of a Defined Populations of Endothelial Progenitor Cells from the Left Ventricle: A Pilot Study*, presented as a poster presentation at the 2014 TSANZ Annual Scientific Meeting, Adelaide, April, 2014.

8. R L Harper, A M Reynolds and P N Reynolds, *BMPR2 Gene Delivery Shifts Intracellular Smad Activation Profile*, presented as a poster presentation at the 2012 European Respiratory Society Annual Congress, Vienna, September, 2012.

9. R L Harper, A M Reynolds and P R Reynolds, *Changes in Smad Signalling Leads To Amelioration of PAH Following Gene Delivery of Bone Morphogenetic Protein Receptor Type 2*, Am J Respir Crit Care Med, vol. 185, pg. A6517, 2012. Presented as an oral presentation at the 2012 ATS Annual Conference, San Francisco, 2012.

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

11. R L Harper, A M Reynolds and P R Reynolds, *Amelioration Of PAH Following Gene Delivery Of BMPR2 Via Changes in Smad Signalling*, in proceedings of Respirology, vol 17, pg. 19, 2012. Poster presented at the South Australian Cardiovascular Research Forum, 2012.

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.

### INVITED PRESENTATIONS

1. Japanese Respiratory Society, ASM, Tokyo. April, 2015.

2. Asia Pacific Respiratory Society, ASM, Kuala Lumpur.
   December, 2015.

### 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.
   A single award open to all nations in the Asia Pacific region to represent the region at the Japanese Respiratory Society, ASM, Tokyo.

3. **South Australia/ NT Branch, Young Investigator of the Year** 2015, Thoracic Society of Australia and New Zealand.
   A single annual award open to candidates from South Australia and Northern Territory.

4. **Abstract Scholarship** 2015, Pulmonary Circulation Committee, American Thoracic Society.
   A $500 award given to outstanding ATS ASM submissions.

5. **Dawes Top-up Scholarship**. 2013-2015.
   $5,000 pa scholarship, selected by the Royal Adelaide Hospital Research Committee.

6. **Lions Medical Research Foundation Top-up Scholarship**. 2012-2015.
   One of three $10,000 pa scholarships for PhD projects, selected by the Lions Medical Foundation.

7. **Australian Postgraduate Award** 2012-2015.
   Scholarships awarded to top honours students to undertake their PhD.

8. **Scicchitano Award** 2012.
   Annual $5,000 award given to outstanding research in the field of Thoracic Medicine.

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# ACRONYMS

| | |
|---|---|
| Alpha-**SM Actin** | Alpha-Smooth Muscle Actin |
| **5-HT** | Hydroxytrypamine or Serotonin |
| **5-HTT** | Hydroxytrypamine (Serotonin) Transporter |
| **6MWD** | 6 Minute Walk Distance |
| **AAV** | Adeno-associated Virus |
| **ACE** | Angiotenisin 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 |
| **CXCL10** | CXC-chemokine Ligand 10 |
| **DMEM** | Dulbecco's Modeified Eagle Medium |
| **EC** | Endothelial Cell |
| **ECM** | Extracellular Matrix |
| **EGF** | Epidermal Growth Factor |
| **EHS** | Engelbreth-Holm-Swarm |
| **eIF2** | 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 |
| **ET1** | 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-1**alpha | Hypoxia Inducible Factor -1alpha |
| **HIF-1**beta | 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** | Phosphoinsitide 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 |