# Optimal Delivery of Therapeutic Genes to Pancreatic Islets

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**Doctor of Philosophy**

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## Table of Contents

## Thesis Abstract

Islet transplantation is a promising therapeutic option for Type 1 Diabetic (T1D) patients, with the ability to improve glycometabolic control and in select cases achieve insulin independence. Intraportally transplanted islets must reside in the hostile environment of the liver, where they are exposed to the instant blood mediated inflammatory reaction (IBMIR), alloimmunity, recurrence of islet specific autoimmunity, a highly toxic pro-inflammatory cytokine storm (e.g. IL-1β, IFN-α, IFN-γ and TNF-α) and hypoxia due to inadequate revascularization post-transplantation. The early loss of functional islet mass (50-70%) due to apoptosis following clinical transplantation contributes to islet allograft failure. Strategies to prevent apoptosis are therefore highly desirable to enhance islet survival for transplantation.

In **Chapter 3**, the ability of Adenoviral (Ad) and Adeno-Associated Viral (AAV)-based vectors expressing a green fluorescent protein (GFP) reporter gene to transduce isolated human and rat pancreatic islets was investigated. Specific interest was placed on tyrosine mutant AAV-based vector types, which have not been previously explored in human and rodent pancreatic islets. Ad efficiently transduced isolated human and rat pancreatic islets while AAV failed to transduce human islets and showed a varied ability to transduce rat islets. The results in this chapter demonstrate that Ad vectors are more efficient at transducing isolated islets than AAV-based vector types.

**Chapter 4** aimed to characterise an Ad-based vector encoding an anti-apoptotic molecule termed Insulin-like Growth Factor-II (Ad-IGF-II). Ad-IGF-II effectively transduced rat pancreatic islets without affecting islet viability or function and did not induce uncontrolled islet cell proliferation. The results in this chapter suggest that Ad-IGF-II is an effective and non-toxic vector type for use in an islet gene therapy setting.

In **Chapter 5** and **Chapter 6**, the influence of local human IGF-II over expression on rat pancreatic islet cell survival *in vitro* and *in vivo* was examined, respectively. Over expression of IGF-II in islets resulted in enhanced islet survival *in vitro* and in an *in vivo* marginal mass islet transplant model. Transplantation of IGF-II over expressing islets under the kidney capsule of diabetic NOD-SCID mice restored euglycemia in 78% of recipients, compared to 46% and 18% of untransduced and Ad-GFP transduced control islet recipients, respectively.

In summary, this thesis demonstrated that compared to AAV, Ad is currently the optimal vector for use in an islet gene therapy setting. Moreover, over expression of IGF-II did not affect the viability or insulin secreting capacity of islets. Finally, the induced expression of anti-apoptotic IGF-II led to enhanced islet survival *in vitro* and improved transplant outcomes in an *in vivo* marginal mass islet transplant model, indicating that IGF-II gene transfer is a potentially powerful tool to improve islet survival post-transplantation.

## Thesis Declaration

I certify that 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 Amy Hughes 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 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. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis (as listed below*) resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

***Hughes A**, Jessup C, Drogemuller C, Mohanasundaram D, Milner C, Rojas D, Russ GR, Coates PT. Gene therapy to improve pancreatic islet transplantation for Type 1 diabetes mellitus. Curr Diabetes Rev. 2010 Sep;6(5):274-84.

Signed Amy Hughes

## Publications, Presentations and Awards

### *Publications*

Invited Reviews

1. **Hughes, A**, Jessup, C, Drogemuller, C, Mohanasundaram, D, Milner, C, Rojas, D, Russ, G.R and Coates, P.T, Gene Therapy to Improve Pancreatic Islet Transplantation for Type 1 Diabetes Mellitus, 2010 Curr Diabetes Rev, 6, 274-84

Published Manuscripts (1) and Manuscripts in Preparation (2)

1. **Hughes A**, Mohanasundaram D, Kireta S, Jessup C, Drogemuller C, Coates PTH. Insulin-like Growth Factor-II Prevents Proinflammatory Cytokine-Induced Apoptosis and Significantly Improves Islet Survival After Transplantation. *Transplantation*. 2013;95: 00-00.

2. **Hughes, A,** Jessup CF, Drogemuller, CJ, and Coates PTH, Tyrosine mutations in AAV2 and AAV8 Capsids is Insufficient to Enhance Gene Delivery to Isolated Human Pancreatic Islets

Published Abstracts

1. **Hughes, A**, Mohanasundaram, D, Drogemuller, CJ, Jessup, CF, Coates PTH, Anti-Apoptotic Insulin-like Growth Factor-II Gene Therapy Protects Islets from Cytokine Induced Cell Death, American Journal of Transplantation, Volume 12, Issue Supplement s3, pages 27 – 542, May 2012
2. **Hughes, A**, Jessup, CF, Drogemuller, Mohanasundaram, D, Milner, CR, Rojas, D, Russ, GR, Coates, PTH, Transduction of Rat Pancreatic Islets with Wildtype Adeno-Associated Virus (AAV) Serotype 2, Pseudotype AAV2/8. AAV2/1 and Surface-Exposed Tyrosine Mutant AAV Vectors – A comparative study, American Journal of Transplantation, Volume 90-Supplement, pp:1-1078, July 2010
3. **Hughes, A**, Jessup, CF, Drogemuller, Mohanasundaram, D, Milner, CR, Rojas, D, Russ, GR, Coates, PTH, Adenovirus-Mediated Transduction of Isolated Pancreatic Islets Using Insulin-Like Growth Factor-II to Promote Islet Survival Post-Transplantation, American Journal of Transplantation, Volume 90-Supplement, pp:1-1078, July 2010

4. **Hughes, A**, Jessup, CF, Drogemuller, Mohanasundaram, D, Milner, CR, Rojas, D, Russ, GR, Coates, PTH, Transgenic Expression of Insulin-like Growth Factor-II (IGF-II) in Pancreatic Islets to Prevent Apoptosis, Xenotransplantation, Vol. 16, Issue 6, page 553 December 2009

*Presentations*

**A. Hughes**, A.J. Kupke, C.J. Drogemuller, D.M. Mohanasundaram, C. Mee, C.R. Milner, C.F. Jessup, P.T.H. Coates. Adenovirus-Mediated Transduction of Pancreatic Islets using Insulin-like Growth Factor-II (IGF-II) to Prevent Apoptosis. Oral presentation. Australian Society for Medical Research, Annual Scientific Conference, 2009

**A. Hughes**, A.J. Kupke, C.J. Drogemuller, D.M. Mohanasundaram, C. Mee, C.R. Milner, C.F. Jessup, P.T.H. Coates. Adenoviral-Mediated Transduction of Pancreatic Islets using Insulin-like Growth Factor-II (IGF-II) to Prevent Apoptosis. Oral presentation. Transplantation Society of Australia and New Zealand Annual Scientific Meeting, Canberra, 2009

**A. Hughes**, A.J. Kupke, C.J. Drogemuller, D.M. Mohanasundaram, C. Mee, C.R. Milner, C.F. Jessup, P.T.H. Coates. Transgenic expression of Insulin-like Growth Factor-II in pancreatic islets to prevent apoptosis. Oral Presentation. Australian Diabetes Society (ADS) and the Australian Diabetes Educators Association (ADEA) Annual Scientific Meeting, Adelaide, 2009

**A. Hughes**, A.J. Kupke, C.J. Drogemuller, D.M. Mohanasundaram, C. Mee, C.R. Milner, C.F. Jessup, P.T.H. Coates. Investigation of cytokine-induced early apoptosis in isolated islets of langerhans. Oral Presentation. Annual Immunology Retreat, Australasian Society for Immunology, Adelaide, 2009

**A. Hughes**, C.J. Drogemuller, C.J. Jessup, P.T.H. Coates. Optimal Delivery of Therapeutic Genes to Pancreatic Islets. Invited Oral Presentation, T cell Laboratory, Weatherall Institute of Molecular Medicine, Oxford, UK, 2009

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Transgenic expression of insulin-like growth factor-II (IGF-II) in pancreatic islets to prevent apoptosis. Poster Presentation. Joint International Pancreas and Islet Transplantation Association (IPITA) and International Xenotransplantation Association (IXA) Meeting, Venice, Italy, 2009

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Investigation of cytokine-induced early apoptosis in isolated islets of langerhans. Oral Presentation. The Queen Elizabeth Hospital Annual Research Day, Adelaide, 2009

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Comparison of wildtype adeno-associated virus (AAV) serotype 2 vectors to pseudotyped AAV vectors for the transduction of rat pancreatic islets. Oral Presentation. Transplantation Society of Australia and New Zealand Annual Scientific Meeting, Canberra, 2010

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Transduction of Rat Pancreatic Islets with Wildtype Adeno-Associated Virus (AAV) Serotype 2, Pseudotype AAV2/8, AAV2/1 and Surface-Exposed Tyrosine Mutant AAV Vectors – A Comparative Study. Poster Presentation. XXIII International Congress of the Transplantation Society (TTS), Vancouver, Canada, 2010

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Adenovirus-Mediated Transduction of Isolated Pancreatic Islets Using Insulin-Like Growth Factor-II to Promote Islet Survival Post-Transplantation. Poster Presentation. XXIII International Congress of the Transplantation Society (TTS), Vancouver, Canada, 2010

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Introduction to the Renal and Transplantation Immunobiology Laboratory. Invited Oral Presentation. Diabetes Research Institute (DRI), University of Miami, Florida, 2010

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Evaluation of wildtype adeno-associated virus (AAV) serotype 2, pseudotype AAV2/8, AAV2/1 and surface-exposed tyrosine mutant AAV vectors and their ability to transduce isolated rat pancreatic islets. Oral Presentation. The Queen Elizabeth Hospital Annual Research Day, Adelaide, 2010

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Adenovirus-Mediated Transduction of Pancreatic Islets using Insulin-like Growth Factor-II (IGF-II) to Prevent Apoptosis. Oral presentation. Australian Society for Medical Research, Annual Scientific Conference, 2011

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Optimal Delivery of Insulin-Like Growth Factor-II to Rat Pancreatic Islets using a Replication Deficient Adenoviral Construct. Poster Presentation. Faculty of Health Sciences, Postgraduate Research Conference, Adelaide, 2011

**A. Hughes**, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, P.T.H. Coates. Adenoviral Overexpression of Insulin-like growth factor II Protects Pancreatic Islets from Pro-Inflammatory Cytokine Induced Apoptosis and Necrosis. Poster Presentation. Australasian Society of Immunology, Adelaide, 2011

**A. Hughes**, D. Mohanasundaram, C. J. Drogemuller, C. F. Jessup P.T.H. Coates. Anti Apoptotic Insulin-Like Growth Factor-II Gene Therapy Protects Islets from Cytokine Induced Cell Death. Poster Presentation. American Transplant Congress 2012, Boston, USA, 2012

**A. Hughes**, D. Mohanasundaram, C. J. Drogemuller, C. F. Jessup P.T.H. Coates. Transgenic Expression of Insulin-Like Growth Factor-II (IGF-II) in Pancreatic Islets Offers a Novel Therapeutic Strategy to Improve Islet Cell Survival Post-Transplantation. Oral Presentation. Australian Society for Medical Research, Annual Scientific Conference, 2012

**A. Hughes**, D. Mohanasundaram, C. J. Drogemuller, C. F. Jessup P.T.H. Coates. Anti-Apoptotic Insulin-Like Growth Factor-II (IGF-II) Gene Transfer Offers a Novel Therapeutic Strategy to Improve Islet Cell Survival Post-Transplantation. Oral Presentation. Transplantation Society of Australia and New Zealand Annual Scientific Meeting, Canberra, 2012

**A. Hughes**, D. Mohanasundaram, C. J. Drogemuller, C. F. Jessup P.T.H. Coates. Insulin-Like Growth Factor-II Decreases Islet Apoptosis *In Vitro* and Improves Islet Transplant Function in a Minimal Mass Model. Poster Presentation. 24th International Congress of The Transplantation Society, Berlin, Germany, 2012

*Awards*

2012 Pfizer Young Investigator Award, Transplantation Society of Australia and New Zealand, Annual Scientific Meeting, Canberra

2012 Medical Staff Society Research Prize, Medical Grand Round, Royal Adelaide Hospital

2012 Faculty of Health Sciences Postgraduate Travelling Fellowship, University of Adelaide

2010 International Travel Grant, The Transplantation Society of Australia and New Zealand

2009 Trevor Prescott Memorial Scholarship, The Freemasons Foundation, Adelaide

2009 Amgen Young Investigator Award, The Transplantation Society of Australia and New Zealand, Annual Scientific Meeting, Canberra

2009 Faculty of Health Sciences Postgraduate Divisional Scholarship, University of Adelaide

2008 The Queen Elizabeth Research Foundation Honours Scholarship, Adelaide

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## Abbreviations

°C - Degrees celcius

1x PBS - 1 x Phosphate Buffered Saline

1xHBSS - 1x Hanks Buffered Salt Solution

4E-BP1 - Eukaryotic initiation factor binding protein

4E-eIF4E - Eukaryotic initiation factor

AAV - Adeno-associated viruses

Ad - Adenovirus

Ad-GFP - Adenoviral-Green Fluorescent Protein

Ad-IGF-II - Adenoviral-Insulin like Growth Factor-II

ALS - Acid-labile subunit

Apaf-1 - Apoptosis-protease activating factor-1

APC - Antigen presenting cell

BAD - Bcl-associated death promoter

Bcl-2 - B-cell lymphoma 2

BGL - Blood glucose levels

BLAST - Basic local alignment search tool

bp - Base pairs

CAR - Coxsackie Adenovirus Receptor

CITR - Collaborative Islet Transplant Registry

cm - Centimeter

CPE - Cytopathic effects

DAPI - 4',6-diamidino-2-phenylindole

DISC - death-inducing signaling complex

ELISA - Enzyme linked immunosorbent assay

Expect-value - E-value

FADD - Fas-associated death domain

FasL - Fas-Fas ligand

FCS - Foetal calf serum

FOXO - Forkhead transcription factor

GAD65 - Glutamic acid decarboxylase

GFP - Green fluorescent protein

GLUT2 - Glucose transporter 2

GSIS - Glucose stimulated insulin secretion

GSK-3β - Glycogen synthase kinase 3β

HEK - Human Embryonic Kidney

hIL-1Ra - Human Interleukin-1 Receptor Antagonist

h - Hour

HPRT-1 - Hypoxanthinephosphoribosyltransferase 1

HSPG - Heparan sulphate proteoglycan

HSV - Herpes Simplex Virus

i.p - Intra peritoneal

IBMIR - Instant blood mediated inflammatory reaction

IEQ - Islet equivalents

IFN-γ - Interferon-gamma

IGF - Insulin-like Growth Factor

IGF-1R - Insulin-like Growth Factor-I receptor

IGF-1R/IR - Insulin-like Growth Factor-I receptor/Insulin receptor

IGFBP - Insulin-like Growth Factor binding protein

IGF-I - Insulin-like Growth Factor-I

IGF-II - Insulin-like Growth Factor-II

IGF-IIR - Insulin-like Growth Factor-II receptor

IL-10 - Interleukin-10

IL-1β - Interleukin-1β

IL-4 - Interleukin-4

iNOS - Inducible nitric oxide synthase

IR - Insulin receptor

IRS-2 - insulin receptor substrate 2

kbp - Kilo base pairs

kDa - kilo dalton

lamR - Laminin receptor

M - Molar

MAPK - Mitogen activated kinase

ml - Millilitre

mm - Millimeter

mM - Millimolar

MOI - Multiplicity of infection

mRNA - messenger RNA

mTOR - Mammalian target of rapamycin

NF-κB - nuclear factor kappa B

nm - Nanometer

NO - Nitric oxide

NOD - Non-Obese Diabetic

NOD-SCID - Non-Obese Diabetic Severe combined immune deficiency

p70S6K - ribososmal protein S6 kinase

pAkt - Phospho-Akt

PCR - Polymerase Chain Reaction

PDK - phosphoinositol dependent kinase-1

Pfu - Plaque forming units

PI - Propidium Iodide

PI3K - Phosphoinositide-3-kinase

PKB - Protein kinase B

Post-tx - Post-transplant

Pre-tx - Pre-transplant

PS - Phosphatidyl serine

PVDF - Polyvinyl difluoride

rIGF-II - Recombinant IGF-II

RIP - Receptor-interacting protein

RT - Room temperature

RT-PCR - Real-time PCR

SCID - Severe combined immune deficiency

SDS-PAGE - Sulfate polyacrylamide gel electrophoresis

SEM - Standard error of the mean

SFA - Sulphation factor activity

SI - Stimulation index

STAT-1 - Signal transducer and activator of transcription-1

STZ - Streptozotocin

SW - Starting weight

T1D - Type 1 Diabetes

T2D - Type 2 diabetes

TNF - Tumour necrosis factor

TRADD - TNF receptor-associated death domain

TRAF2 - TNF-R-associated factor 2

TSC - Tuberous sclerosis gene product

TUNEL - Terminal deoxynucleotidyl transferase dUTP nick end labelling

VEGF - Vascular endothelial growth factor

vg - Vector genome

μg - Microgram

μl - Microliters