The development of targeted adenoviral vectors for gene therapy of vascular disease, with emphasis on the pulmonary vasculature.

A body of work submitted for the
Degree of Doctor of Medicine.

University of Adelaide, Department of Medicine.

by

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Copies of Publications

Introductory Chapters

Methods Chapter

Original Articles

3) *Reynolds PN*, Zinn KR , Gavrilyuk VD, Balyasnikova IV , Rogers BE, Buchsbaum DJ, Wang MH, Miletich DJ, Grizzle WE, Douglas, JT, Danilov SM and Curiel DT. A targetable, injectable adenoviral vector for gene delivery to pulmonary endothelium in vivo. Molecular Therapy 2 (6): 562-578, 2000.

..... **P75-91**

4) Nicklin SA, *Reynolds PN*, Brosnan MJ, White SJ, Curiel DT, Dominiczak AK, Baker AH. Analysis of cell-specific promoters for viral gene therapy targeted at the vascular endothelium. Hypertension. 38: 65-70, 2001.

..... **P92-97**

5) *Reynolds PN,* Holmes MD, Adachi Y, Kaliberova, L and Curiel DT. A novel system for mitigation of ectopic transgene expression induced by adenoviral vectors. Gene Therapy, 8(16):1271-5, 2001.

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6) *Reynolds PN,* Nicklin SA, Kaliberova L, Grizzle WE, Baker AH, Danilov SM, Curiel DT. Combined transductional and transcriptional targeting improves the specificity of transgene expression *in vivo*. Nature Biotechnology, 19(9): 838-842, 2001.

..... **P103-107**

7) Work LM, Ritchie N, Nicklin SA, *Reynolds PN*, Baker AH. Dual targeting of gene delivery by genetic modification of adenovirus serotype 5 fibers and cell-selective transcriptional control. Gene Therapy, Aug;11(16):1296-300, 2004.

..... **P108-112**

8) Work LM, *Reynolds PN*, Baker AH. Improved gene delivery to human saphenous vein cells and tissue using a peptide-modified adenoviral vector. Genet Vaccines Ther. Oct 08;2(1):14, 2004

..... **P113-116**

9) Miller WH, Brosnan MJ, Graham D, Nicol CG, Morecroft I, Channon KM, Danilov SM, *Reynolds PN*, Baker AH, Dominiczak AF. Targeting endothelial cells with adenovirus expressing nitric oxide synthase prevents elevation of blood pressure in stroke-prone spontaneously hypertensive rats. Mol Ther. Aug;12(2):321-7, 2005.

..... **P117-123**

10) Everts M, Kim-Park SA, Preuss MA, Passineau MJ, Glasgow JN, Pereboev AV, Mahasreshti PJ, Grizzle WE, *Reynolds PN*, Curiel DT Selective induction of tumor-associated antigens in murine pulmonary vasculature using double-targeted adenoviral vectors. Gene Ther. Jul;12(13):1042-8, 2005.

..... **P124-130**

11) Izumi M, Kawakami Y, Glasgow JN, Belousova N, Everts M, Kim-Park S, Yamamoto S, Wang M, Le LP, *Reynolds PN*, Curiel DT. In vivo analysis of a genetically modified adenoviral vector targeted to human CD40 using a novel transient transgenic model. J Gene Med. 7 (12) 1517-25, 2005.

..... **P131-139**

Abstract / Summary

The development of gene therapy for clinical use continues to face many hurdles. A major issue is the limitation of gene delivery technology. This body of work describes strategies for improving the selectivity and efficacy of gene delivery to vascular endothelium, with emphasis on delivery to pulmonary vasculature in vivo. Several important principles were established which continue to be of relevance to the field. The work progresses from vector development through to the use of new vector strategies in the application of novel gene delivery approaches in disease models. Work in gene therapy and vector development began in the Division of Human Gene Therapy, University of Alabama at Birmingham, under the mentorship of Prof David T Curiel and has continued through international collaborations and the establishment of my own laboratory in the Hanson Institute with affiliate links to the University of Adelaide.

The work presented in this thesis consists entirely of published material, either as book chapters (three) or peer reviewed journal articles (twelve). The sequence of material progresses from a broad introduction to the field on Gene Therapy, more specific chapters dealing with pulmonary gene delivery including a detailed methodology chapter. The peer reviewed works contain an evolution of work dealing with the development of strategies to target adenoviral gene delivery vectors to the pulmonary vascular endothelium. This work encompasses the use of bi-specific conjugates, genetic modification of viral capsid (outer coat) proteins and the use of cell-specific promoters. The work progresses to a demonstration of the therapeutic gains achieved with the use of targeted over non-targeted vectors in animal models and culminates with a highly novel application of modulation of the bone morphogenetic protein pathway in pulmonary hypertension. A component of the work focuses on enhanced gene delivery to vein grafts ex-vivo.

There are many key original contributions encompassed within the work, including 1) first use of conjugate-based retargeting to vascular cells, 2) first demonstration that tropism modification could alter in vivo biodistribution of virus, 3) first demonstration of cell-specific retargeting of adenoviral vector after systemic vascular injection in vivo (a technique still unsurpassed in the field), 4) first demonstration of the in vivo selectivity gains achieved by combined cell-specific promoters with viral retargeting, 5) first demonstration of therapeutic gains achieved by targeting in a vascular context and 6) first demonstration that modulation of the BMPR2 pathway can have a therapeutic impact in pulmonary hypertension. Importantly, the targeting work I have developed has been adapted and used by others and laid a foundation for further vector improvements.

Detailed Curriculum Vitae

Associate Professor Paul N. Reynolds MBBS PhD FRACP

Current Appointment and Address

Current Rank/Title:	**Senior Consultant Respiratory / Sleep Physician** Department of Thoracic Medicine Division of Internal Medicine
	Director Basic Research Program Lung Research Laboratory, Hanson Institute
	Clinical Associate Professor and NHMRC Practitioner Fellow Department of Medicine University of Adelaide
Business Address:	Royal Adelaide Hospital Chest Clinic 275 North Terrace Adelaide South Australia, 5000
Phone:	(08) 8222 5376
Fax:	(08) 8222 5957
Email:	Paul.Reynolds@adelaide.edu.au

Previous Appointments

1986	**Medical and Surgical Intern** Royal Adelaide Hospital, Adelaide, Australia
1987-89	**Resident (FRACP Training)** Royal Adelaide Hospital, Adelaide, Australia.
1990-92	**Senior Registrar (FRACP Advanced Training, Pulmonary Medicine)** Royal Adelaide Hospital, Thoracic Medicine Department. Supervisors: P. Robinson, R. Antic.
1992-4	Clinical Tutor, Department of Medicine, University of Adelaide
1993-96	**Ph. D. Candidate** University of Adelaide/Royal Adelaide Hospital (Hanson Centre for Cancer Research). Thesis title: "The role of tachykinins in airway inflammation and bronchial hyper-responsiveness". Supervisors: M. Holmes, R. Scicchitano.
1993-96	**Consultant Physician in Internal and Pulmonary Medicine** Burnside Memorial Hospital, Adelaide.
1993-96	**Emergency Room Physician:** Royal Adelaide and Lyell McEwin Hospitals, Adelaide.
1997-99	**Post-Doctoral Fellow:** Gene Therapy Center, University of Alabama at Birmingham, U.S.A. Supervisor: David T. Curiel.
1999-2000	**Research Associate, Gene Therapy Center Associate Director for Clinical Affairs:** Gene Therapy Center, University of Alabama at Birmingham, U.S.A. Supervisor: David T. Curiel.
2000-2001	**Research Assistant Professor, Gene Therapy Center Associate Director for Clinical Affairs:** Division of Human Gene Therapy, Department of Medicine, University of Alabama at Birmingham, U.S.A.
2002-4	**Clinical Senior Lecturer:** University of Adelaide.

Detailed Curriculum Vitae

Summary Statement

I am a Respiratory Physician-Scientist and Clinical Associate Professor. I have devoted a substantial component of my career to research, having completed a PhD, a five-year Post-Doc / Research Assistant Professor position in the USA and then established a productive research group upon my return to Australia (currently heading a group of 8 laboratory staff). I was a winner of the Thoracic Society of Australia and New Zealand (TSANZ) National Young Investigator Award and the A&H/TSANZ Respiratory Research Fellowship. I am currently the recipient of an NHMRC Practitioner Fellowship (which extends to the end of 2011). I have managed to successfully integrate a productive research output with relevant clinical practice.

I currently have over 60 publications, many in journals with impact factor > 5 (e.g. Cancer Research (IF 8.3), Gene Therapy (IF 5.9), Molecular Therapy (IF 7.13), Am J of Respiratory and Critical Care Medicine (IF 8.9), European Resp Journal (IF 5.076)), as well as numerous conference presentations and abstracts. This includes a landmark achievement with novel combination of transcriptional and transductional targeting of adenoviral vectors which remains the best illustration of vector targeting in vivo worldwide and was published in Nature Biotechnology (IF 22). This work formed the basis of a non-provisional patent application in US. I have now progressed this technology locally to novel approaches for pulmonary hypertension therapy with NHMRC funding. I was intimately involved in the planning and preparation of data for submission for novel clinical trials, since approved by Recombinant DNA advisory committee (RAC) in the US. I established an international reputation in vector targeting and gene delivery to pulmonary vasculature. Invitations to speak at plenary sessions in international venues including the USA, The Netherlands, Germany, Japan, England and Scotland. In 2005 my work was accepted for oral presentations at the American Thoracic Society Scientific meeting, American Society of Gene Therapy Scientific Meeting and the European Respiratory Scientific meeting. In 2007 I was an invited speaker to the Japanese Respiratory and Asian Pacific Society meetings. My publications include several book chapters including a chapter on Gene Therapy in Cecil's Textbook of Medicine, a prominent undergraduate text.

Since my return to Australia I have been awarded five NHMRC project grants plus a Clinical Career Development Award and Practitioner Fellowship. I have overseen the rapid expansion of the department's research activities in COPD and Lung Transplantation while expanding Gene Therapy research into replicative viruses and immunotherapy for mesothelioma. Our group is recognized worldwide for pioneering work in apoptosis and impaired macrophage function in COPD and I have facilitated the development of Sandra Hodge, a former PhD student, into a world-class scientist in the field. I have vigorously promoted translation of our science to the clinic, as indicated by our recent investigator-initiated trial of azithromycin to improve macrophage function in COPD. The Lung Transplantation activities also enjoy a rapidly growing reputation. I have obtained grants from agencies including the Medical Research and Compensation Foundation, Cancer Council, CHATA, CARG, Australian Lung Foundation, Australian Heart Foundation, RAH and University of Adelaide, and was instrumental in the success of an NIH RO1 grant from which I established a subcontract. I have greatly assisted my colleagues in obtaining their own grant funds. I have supervised several students (two completed PhDs, one MD, one honours). Four of my students have won the TSANZ (SA) Young Investigator Award and one has won prestigious Australian Lung Foundation and TSANZ fellowships. I am heavily involved in the advancement of the discipline of respiratory medicine – I am a member of the National Executive of the TSANZ, Chairman of the Central Programming Committee (organisation of national conferences and courses), Convener for the 2007 Asian Pacific Respiratory Society/ ACCP conference, Member of the executive of the APSR, Secretary of the SA branch of the TSANZ, Member of the Curriculum Development Committee for Respiratory Medicine of the RACP, and Chairman of the Scientific Advisory Committee (and board Director) of the Asthma Foundation of SA. Peer review activities include reviewing for numerous granting agencies including the NHMRC – I was a member of the Respiratory/Sleep Grant Review Panel in 2005 and 2006 and a member of the Scholarships review panel in 2004. Also a frequent reviewer for many scientific journals. I am also a member of the IMVS Animal Ethics Committee.

Detailed Curriculum Vitae

Academic Qualifications

Bachelor of Medicine and Bachelor of Surgery (MBBS) University of Adelaide 1980-1985. Graduated ranked 13th in a class of 120.
Fellow of the Royal Australasian College of Physicians (FRACP). 1987-92 (Respiratory Medicine)
Doctor of Philosophy (PhD) University of Adelaide 1993-6 (conferred 2000) Thesis titled "The role of tachykinins in airway inflammation and bronchial hyper-responsiveness
Accredited Sleep Physician, RACP, 2006.

Licensure/Certification

Medical Board of South Australia. Specialist in General Internal and Thoracic Medicine.

Honours and Awards

Young Investigator Award – Allen and Hanburys / Thoracic Society of Australian and New Zealand, 1993, a single prestigious award open to investigators in both countries.

Respiratory Research Fellowship by the Thoracic Society of Australia and New Zealand and Allen and Hanburys, 1997-8, a two year research fellowship, only one offered on a competitive basis per year and open to investigators in both countries.

Young Investigator Grant-in-Aid Award - AstraZeneca / Australian Lung Foundation, 2003, a single Australasian Award for researchers returning to Australia after training overseas.

Clinical Career Development Award, NHMRC, 2003-7. A highly prestigious national award to foster the development of Physician-Scientists. Awarded based on my current plans for gene therapy development.

Practitioner Fellowship, NHMRC 2007-11. A highly prestigious fellowship aimed at integrated research into clinical translation.

Scientific Discipline Involvement

- Thoracic Society of Australia and New Zealand:
  - Member, National Executive Committee (2004-8)
  - National Chairman, Central Programming Committee (2004-8)
  - Committee member, SA Branch. (2002-present)
  - Chairman, Local organising committee for 2009 National TSANZ annual scientific meeting (2008-9)
  - Member, Local Organising Committee, TSANZ Annual Scientific Meeting, Adelaide 2003.

- Royal Australasian College of Physicians:
  - Fellow since 1992
  - Member, Respiratory Curriculum Development Committee (2007 – present)

- Asian Pacific Society of Respirology:
  - Convenor of 2007 Joint Scientific Congress of APSR and American College of Chest Physicians.
  - Australian representative and Executive Member (2007-present)
  - Editorial Board member for the journal Respirology (official Journal of the Asian Pacific Society of Respirology)

- Asthma Foundation of SA / Breathe Better Centre:
  - Vice President, Board of Directors (2006-present)
  - Chairman, Medical and Scientific Advisory Committee, (2006-present)

Detailed Curriculum Vitae

- Institute of Medical and Veterinary Science Animal Ethics Committee
  - Member (2007-present)
- Australian Lung Foundation – Member
- Australian Lung Cancer Trials Group – Member
- Australian Sleep Association – Member
- American Society of Gene Therapy
  - Member,
  - Respiratory Tract Committee 2001-4
- Australian Gene Therapy Society -Member.
- American Thoracic Society – Member, Cell Biology and Pulmonary Circulation Assemblies
- Hanson Institute of South Australia – Full Member
- Bioinnovations SA – Member of in vivo models working party (2004).
- Royal Adelaide Hospital Special Purposes Fund - Committee Member (2003-present).
- National Health and Medical Research Council
  - Grant Review Panel 2005 and 2006 – Respiratory/Sleep Medicine. Member.
  - NHMRC National Reference Group for Lung Cancer. Member (2007-present)
- Scientific Review Committee of the CRC for Asthma and Airways. Member (2007-present)
- Drug Safety Monitoring Committee, AMG317 (AMGEN). Member (2006)
- Drug Safety Monitoring Committee, Alpha-1 anti-trypsin deficiency (Baxter) Member (2006-8)

Detailed Curriculum Vitae

Current Clinical Activities

Inpatient responsibilities
I currently provide service as Duty Consultant for the Thoracic Medicine unit for a total of 2 months per year. This roster is organised into 2-week blocks, during which time I am effectively on call 24hrs/day for the full two weeks (this includes providing the after-hours consult service to the rest of the hospital). There is an additional two months rostered as "consult" physician, during which time advice is provided to other units about respiratory issues. These formal rostered attachments are either equivalent to or exceed those of the other physicians in the department.

The inpatient workload varies with seasonal fluctuations but typically there are 15 inpatients with perhaps up to 30 in mid-winter. The patient load consists of all aspects of thoracic medicine including –

Cystic Fibrosis.
Frequently 5-6 inpatients at any one time. The department has a highly effective multi-disciplinary team for CF management, headed by Dr Hugh Greville. Inpatient work largely revolves around management of acute or sub-acute respiratory deteriorations and it is the Duty Consultant that has primary responsibility for this. Other issues particularly GI tract or psycho-social issues frequently need to be addressed. End of life considerations, the role of palliative care versus pre-transplant work-up are also frequent issues to be dealt with.

Pulmonary malignancy
While much of our lung cancer service is now conducted on an ambulatory basis, there would typically be 2-3 inpatients with cancer-related problems at any time. A broad range of issues are covered, including initial diagnosis (co-ordinating and performing invasive investigations), liaison with other health professionals (e.g. Radiotherapy, Medical Oncology, Thoracic Surgery, Palliative Care), management of acute complications (e.g. infection, respiratory failure, thrombo-embolism) and discussions of end-of life issues. My experience includes the inpatient and ambulatory management of chemotherapy for small cell lung cancer. In addition to primary lung cancer, the diagnosis and management of mesothelioma is a significant part of the workload (e.g. pleural biopsies, therapeutic pleural drainage, talc pleurodesis, liaison with medical and radiation oncology and addressing medico-legal issues). The problem of mesothelioma is a major part of my current research program and at present we are assessing primary tumour tissue obtained from patients in the laboratory (see discussion under current research). My goal is to achieve meaningful clinical translation of our research developments.

Post-Lung Transplant care
The SA lung transplant service headed by A/Prof Mark Holmes currently has a cohort of 40 post-lung transplant patients. The management of these patients can be highly complex, encompassing issues of rejection, infection and immunosuppressive agent toxicities. Typically there would be 1-2 such patients in hospital at any one time and these can consume a considerable degree of time and resources. There is a multi-disciplinary approach to management, but the Duty Consultant has primary responsibility for managing the acute deterioration, which not infrequently requires urgent bronchoscopy to help distinguish infection from rejection.

COPD
COPD is a major component of the work-load for Thoracic Medicine, and the department, in conjunction with the General Medicine, Emergency and Intensive Care units is engaged in the development of a new comprehensive multi-disciplinary approach to management encompassing all aspects from emergency care through to primary care outreach. I am extensively involved in the management of inpatient COPD, which now increasingly incorporates the application of Non-Invasive Ventilation (NIV). In that regard, I have completed an advanced course in NIV, held in conjunction with the Australian Sleep Association conference in 2005. I also attended and was a member of the organising committee for the Thoracic Society of Australia and New Zealand's Advanced Course in 2005, which focussed on the Physiology of

Exercise in Health and Disease. Leading international speakers provided an intense physiology course, much of which was directly relevant to COPD management. I was also an attendee and guest speaker at the National "Airways 2004" conference held in Glenelg which focussed on COPD management. In 2005 I have personally screened over 100 volunteer subjects with COPD for involvement in research studies, many of whom I have gone on to perform bronchoscopy on as part of that project. More detail concerning my NHMRC-funded COPD research program is given in a later section.

Asthma
The management of complex and life – threatening asthma is also a frequent inpatient problem, often in conjunction with COPD and other co-morbidities. Establishment of an appropriate long term management plan and good patient education is critical. Liaison with the units highly proficient nursing staff, including the designated respiratory nurse helps to ensure an ongoing effective plan is reinforced and understood by the patient. My involvement in this process now extends to membership of the **Asthma Foundation of SA where I am Chairman of the Medical and Scientific Advisory Committee, Vice President and Member of the Board of Directors.** I am currently overseeing a strategy to bring together the voices of community advocacy for respiratory disease under the umbrella of the "Breathe Better Centre". Currently the plan involves coordinating data from various sources under the project heading "Defining the Respiratory Health Status of South Australia". This project extends beyond asthma to other important respiratory conditions including COPD especially. The project will raise the profile of respiratory disease in the community, thereby facilitating government lobbying and sponsorship. In this way we will define and address the unmet community needs in respiratory health.

Other
Numerous additional respiratory problems arise, as might be expected in a major tertiary referral centre. This includes the full spectrum of inflammatory and auto-immune interstitial lung diseases, infectious disease, including the inpatient management of tuberculosis (frequently 1-2 inpatients in the ward). Simple and complicated pleural disease is a frequent issues (e.g. pneumothorax, empyema).
I have developed a particular interest in pulmonary hypertension, discussed further under outpatients

Consult Service
Activities in the consult service cover a broad range of activities. Frequently this involves the management of severely immunosuppressed Haematology or Oncology patients, which often requires urgent bronchoscopic assessment. As a proceduralist, I am frequently called upon to perform or supervise these investigations in addition to broader management advice.

Outpatient service

I am currently seeing 20-25 outpatients per week, covering all aspects of general respiratory medicine. 20-25% of my outpatient work directly relates to Sleep Medicine.

Pulmonary Hypertension. I have developed a particular interest in this area, which complements one of my major research programs. A core interest group has developed at the RAH which includes Rheumatology (Dr Susannah Proudman) and Cardiology (Dr Peter Steele). The group has evolved around the need for rational implementation of a number of advances in therapy for PH, and currently around 40 patients have been work-up in accord with Commonwealth guidelines and subsequently approved for PBS-funded PH medication. I have attended intensive international workshops and symposia (Giessen and Berlin, 2005) to be familiar with the latest developments in the area, and have presented clinical symposia to SA physicians on the issue. I am currently serving on an advisory board to CSL in regard to the introduction of Sitaxsentan under the Pharmaceutical Benefits Scheme.

Detailed Curriculum Vitae

Lung Function test reporting

I personally report and/or supervise the reporting of well over 500 pulmonary function tests per year. These include Spirometry, Lung Volumes, 6MWT, Blood Gases (I am an Approved Pathology Provider for this) and other, less common tests (e.g. altitude simulation, shunt studies).

Sleep Medicine

I have undertaken intensive Post-FRAP training in Sleep Medicine and am an accredited Level One Sleep Physician. Currently I report approximately 200 sleep studies per year, predominantly full laboratory polysomnography, but also including maintenance of wakefulness tests and sleep latency tests. Recently this has included home-based studies (Somte). I am currently managing over 200 outpatient attendances per year for sleep-related issues. I am acutely aware of the resource issues that impact upon the clinical management of sleep disorders, as well as the sensitive issues concerning fitness to drive in patients with OSA. I am a regular attendee at the department's weekly sleep review meetings and monthly multidisciplinary meetings (involving Dental surgeons and ENT surgeons). There is a huge unmet need for diagnostic sleep services and publicly funded therapy for sleep disorders. This subject will be is a major problem and will be a further focus of the activities of the Breathe Better Centre.

Procedures

I currently perform 2-3 bronchoscopies per week (>100/year), which covers a broad range of indications – endobronchial lesions, parenchymal lesions etc, requiring either endobronchial or transbronchial biopsy, or transbronchial needle aspiration. I also perform research bronchoscopies on normal volunteers and volunteers with COPD. I perform or supervise a number of pleural procedures including Argyle and pig-tail tube insertion, blind pleural biopsy, streptokinase management of empyema and talc pleurodesis for malignant effusions. During my FRACP training I obtained experience in endobronchial laser resection, stent placement, balloon bronchoplasty and thoracoscopy. I am not currently performing these procedures (departmental need is typically only 1-2 per month in total), but have maintained a good knowledge of the developments and techniques involved, which includes completion of a "hands-on" interventional pulmonology workshop run in conjunction with the Thoracic Society of Australia and New Zealand's Annual Scientific Meeting in 2005. This workshop included hands-on exposure to techniques currently not available at the RAH such as endobronchial ultrasound.

Management of Respiratory Failure

The management of respiratory failure has been a core component of my clinical practice throughout the pre- and post-FRACP phases, both in terms of addressing the underlying cause and in managing the physiological derangements *per se*. This latter aspect is enhanced by my intensive post-FRAP training in sleep medicine and extensive involvement in the TSANZ's continuing education activities (e.g. Physiology Advanced Course, NIV Advanced Course).

Several disease processes that underlie the development of respiratory failure (including COPD, lung transplant-related graft failure and pulmonary hypertension) are currently major research interests of mine.

Quality Improvement, Departmental Peer Review

I am an active participant in the department's weekly clinico-radio-pathology review meetings, weekly sleep meetings (monthly multi-disciplinary meetings), monthly senior staff and clinical staff meetings, monthly journal club, fortnightly Thoracic Medicine grand rounds, weekly Medical grand rounds. I am also a current participant in the RACP MOPS program.

Detailed Curriculum Vitae

Current Research Support: (Total value of awards for all years is shown)

NHMRC Project Grant Monitoring of leucocyte cytokine/chemokines to improve morbidity and rejection rates in lung transplant patients
Reynolds PN, Holmes MD, Hodge G, Hodge S. 2008-10 $358,950

Cancer Council Grant: Combined Oncolytic and immune-therapy for mesothelioma
Reynolds PN, Holmes MD 2008 $85,000

National Heart Foundation (Australia): Mechanisms underlying the link between endothelial BMPR2 expression and pulmonary hypertension.
Reynolds PN, Reynolds AR. 2008-9 $126,000

Australian Lung Foundation / Slater and Gordon Research Foundation: Pre clinical evaluation of Conditionally Replicative Adenovirus Ad5/3Delta24 as a therapy for mesothelioma
Reynolds PN 2008 $30,000

NHMRC Practitioner Fellowship 2007-2011 $396,375
Novel Therapeutic Approaches for Pulmonary Disease

NHMRC National Asbestos Diseases Research Institute Project
Robinson BWS, Reynolds PN, Currie A, van der Most R, Lake R, Nowak A, Scott B.
2007-9 $773,115

NHMRC Project Grant: Improved Gene Therapy Strategies for Pulmonary Hypertension
Reynolds, PN, Reynolds AM 2006-8 $362,625

Past Support:

Australian Lung Foundation / Slater and Gordon: Does replicative viral therapy synergize with immunotherapy in mesothelioma?
Reynolds PN, Holmes MD 2007 $30,000

NHMRC Project Grant: The Role of Apoptosis and Macrophage Function in Chronic Obstructive Pulmonary Disease. Reynolds, PN. Holmes, MD. Hodge, G Hodge, S 2005-2007 $481,950

NHMRC Project Grant: Conditionally replicative adenoviruses for mesothelioma
Reynolds PN, Lake R, Holmes MD 2004-7 $256,000

Medical Research and Compensation Foundation
Strategies to improve virotherapy of mesothelioma
Reynolds PN , Lake R, Holmes MD, Curiel DT 2004-7 $300,000

Cancer Council Grant: TIMP gene delivery for pulmonary metastases
Reynolds PN, Holmes MD 2006-7 $76,000

Pfizer Pharmaceuticals – unrestricted grant
Reynolds PN. Investigator Initiated Study of Azithromycin in COPD 2006-7 $47,000

NHMRC Career Development Award 2003-7 $417,500
Development of a Gene Therapy Program (relinquished end of 2006 for Practitioner Fellowship)

CellCept Australia Research Grant (CARG) T-cell cytokines and drug levels in BAL and blood of lung transplant patients relates to prognosis Paul N. Reynolds, Mark D. Holmes, Greg Hodge, Sandra Hodge
2005 $20,000

RAH Research Committee The role of granzyme B and IL15 in the pathogenesis of lung-transplant-related bronchiolitis obliterans syndrome. Chien-Li Liew, Paul N. Reynolds, Mark D. Holmes, Greg Hodge, Sandra Hodge.
2005 $15,000

Detailed Curriculum Vitae

CHATA Harry Windsor Grant: Infection versus Rejection in Lung Transplant-Related Bronchiolitis Obliterans Syndrome: Can intracellular cytokines help? Paul N. Reynolds, Mark D. Holmes, Greg Hodge, Sandra Hodge
2005 $50,000

RAH Research Committee A new non-invasive strategy for monitoring of patients post lung transplantation
Greg Hodge, Paul N. Reynolds, Mark D. Holmes. 2005 $10,000

University of Adelaide Research Development Award. Improved Gene Therapy Strategies for Pulmonary Hypertension. PN Reynolds 2005 $12,000

E. R. Dawes Scholarship (RAH) 1993-4 $AU43,000

National Health and Medical Research Council Post-Graduate Scholarship 1995-6 $AU45,000 total

Alan and Hanburys / Thoracic Society of Australia and New Zealand Respiratory Research Fellowship 1997-8 $AU80,000

American Cancer Society Institutional Pilot Grant ("Conditionally replicative adenoviral vectors for lung cancer") 2000 $US20,000

American Heart Association Scientist Development Grant
("Targeted gene delivery to pulmonary endothelium") 2001-4 $US260,000
Support ceased at end of 2001 due to relocation to Australia

Avon Products Faculty Grant
("Transcriptional and transductional gene delivery for breast cancer therapy") 2001 $US80,000

National Institutes of Health (P30 component of Genetic Diseases Institutional Core Grant)
("Targeted BMPRII gene delivery for pulmonary hypertension") 2001-2 $US100,000
Support ceased at end of 2001 due to relocation to Australia

Cancer Council of Australia (SA Branch)
Targeting TIMP gene delivery as a strategy against pulmonary metastases. 2003 $60,926

RAH Research Committee
Prospective analysis of adenoviral receptors in clinical bronchogenic carcinoma and correlation to infectivity with adenoviral vectors. 2003 $20,000

Australian Lung Foundation Grant-in-Aid 2003 $24,000
Conditionally replicative adenoviruses combined with Extracellular matrix digestion for lung cancer therapy.

NHMRC Project Grant: The role of bone morphogenetic proteins in the pathogenesis of pulmonary hypertension
Reynolds PN (sole CI) 2002-4 $235,000

National Institutes of Health
(RO1, co-investigator with Australian subcontract) 2001-4 $US129,600
("Endothelial Gene Delivery for Pulmonary Hypertension" Curiel DT - PI) (subcontract component)

University of Adelaide B1 funding (NHMRC "near miss") 2004 $15,000
Gene Delivery of Tissue Inhibitors of Matrix Metalloproteinases for Pulmonary Metastases. Reynolds, PN. Holmes MD

Australian Lung Foundation
COPD Research Fellowship (S. Hodge Recipient) 2003-4 $82,000
Reynolds PN and Holmes MD associated senior scientists.

Detailed Curriculum Vitae

Selected Presentations

International Oral Presentations

1) Asian Pacific Society of Respirology / American College of Chest Physicians Annual Scientific Congress. Gold Coast Australia, December, 2007. Gene and Viral Therapies.
2) Japanese Respiratory Society Meeting. Tokyo May 2007. Gene and Viral Therapy for Respiratory Disease.
3) American Society of Gene Therapy Annual Scientific Meeting, St Louis, USA June 2005. ACE-targeted eNOS and BMPR2 gene therapy attenuates pulmonary hypertension in a chronic hypoxia rat model.
4) American Thoracic Society Meeting. Targeted Adenoviral Vector Gene Transfer of Endothelial Nitric Oxide Synthase (eNOS) Attenuates Hypoxic Pulmonary Hypertension in Rats, San Diego, USA May 2005.
5) British Society for Cardiovascular Research Spring 2003 Meeting, Glasgow Scotland. Gene Therapy for Pulmonary Disease. March 2003. Invited Speaker.
6) Japanese Respiratory Society, 43rd annual meeting, Fukuoka, Japan. Towards Gene Therapy for Pulmonary Vascular Disease. March 2003. Invited Speaker.
7) American Society of Gene Therapy, annual scientific meeting, Boston, USA, June, 2002. Gene Delivery for pulmonary vascular disease. Invited Speaker and Workshop Moderator.
8) Third International Symposium on Genetic Anticancer Agents. Amsterdam, The Netherlands. Feb 28 – Mar 1, 2002. In vivo targeting. Invited Speaker.
9) University of Glasgow, Department of Medicine and Therapeutics. Glasgow, Scotland. Mar 2-4, 2002. Targeting gene delivery to pulmonary vasculature. Invited Speaker.
10) University of Cambridge, Respiratory Medicine Dept., Addenbrooks Hospital, England. Mar 4 –8, 2002. Targeted gene delivery for primary pulmonary hypertension. Invited Speaker.
11) American Society of Gene Therapy International Conference. Seattle, USA, May 2001. Combined transcriptional and transductional targeting improves the specificity of transgene expression *in vivo*.
12) American Thoracic Society International Conference, Gene Therapy Symposium. San Francisco, USA. May 2001. Targeted gene delivery to vascular endothelium. Invited Speaker.
13) Cold Spring Harbor Laboratory Meeting- Vector Targeting Strategies for Therapeutic Gene Delivery. Cold Spring Harbor, USA. March 2001. Combined transductional and transcriptional targeting improves the specificity of transgene expression *in vivo*.
14) Institute for Environmental Medicine Seminar Series. University of Pennsylvania, USA. December 1 2000. Targeting Gene Delivery to Pulmonary Vascular Endothelium. Invited Speaker.
15) Keystone Symposium: Gene Therapy in The Next Millennium. Keystone, Colorado, USA. January 6-12 2000. Tropism Modified Adenoviral Vectors for Cell-Specific Gene Delivery. Invited Speaker.
16) Biological Therapy of Cancer (European Organization for Research and Treatment of Cancer) Munich, Germany. October 1999. Targeted adenoviral vectors.
17) American Thoracic Society International Conference, Gene Therapy Symposium. San Diego, USA. 1998. Strategies to adapt adenoviral vectors for gene therapy applications.
18) Chicago Institute of Neurosurgery and Neuroresearch Annual Meeting, Chicago, Illinois, USA. 1997. Targeted adenoviral vectors. Invited Speaker.
19) International Business Communications Gene Therapy Conference, Bethesda, USA. 1997 Strategies for targeted delivery of adenoviral vectors and host cell integration of adenovirally delivered genes.

National

1) Thoracic Society of Australia and New Zealand Annual Scientific Meeting, Melbourne, Australia. March 2008. A "tutorial" on EGFR mutations.
2) Australian Society of Microbiology Annual Scientific Meeting Sydney, September 2004. Development of Targeted Adenoviral Vectors
3) National Air Group Meeting, Glenelg, October , 2004. The Role of Apoptosis in COPD
4) Thoracic Society of Australia and New Zealand Annual Scientific Meeting, Adelaide, Australia. April 2003. New discoveries in the molecular pathogenesis of pulmonary hypertension and how these may be used to design new therapies. Invited Speaker.
5) Thoracic Society of Australia and New Zealand Annual Scientific Meeting, Cairns, Australia. 2002. Combined transductional and transcriptional targeting achieves pulmonary endothelial specific transgene expression in pulmonary hypertensive rats.
6) Thoracic Society of Australia and New Zealand Annual Scientific Meeting, Hobart, Australia. 1995. Substance P and Airway Epithelial Inflammation.
7) Thoracic Society of Australia and New Zealand Annual Scientific Meeting, Hamilton Island, Australia. 1994. Human Bronchial Epithelium Expresses mRNA For NK1, NK2 And NK3 Tachykinin Receptors, Neutral Endopeptidase And Preprotachykinin A. (*Winner TSANZ Young Investigator Award*)

8) Australian Society of Experimental Pathologists Annual Scientific Meeting - The Wilhelm Symposium Adelaide, Australia1994. Tachykinins and Airway Epithelial Inflammation.
9) Thoracic Society of Australia and New Zealand Annual Scientific Meeting, Sydney, Australia. April 1993 - Cell Biology Special Interest Group. An Ovine Model of Allergic Asthma.
10) Thoracic Society of Australia and New Zealand Annual Scientific Meeting, Canberra 1992. Update On A Hospital Based Lung Cancer Registry.

State

1) Flinders Medical Centre Grand Round. 2006. Gene Therapy for Cancer
2) PRISM State Physicians Meeting April 2006. Pulmonary Hypertension
3) Flinders Medical Centre Respiratory meeting May 2004. Development of Replicative Viruses for Cancer.
4) Hanson Centre Research Symposium. April 2003. Conditionally Replicative Adenoviruses for Cancer Therapy.
5) Thoracic Society of Australia and New Zealand (S.A. Branch) Scientific Meeting March 2003. Towards Gene Therapy for Pulmonary Hypertension
6) Presentations to Oncology Department, Infectious Diseases Department, Nuclear Medicine Department during 2002. Vector Development for Gene Therapy.
7) GSK / TSANZ Air Group meeting. Gene Therapy for Lung Disease. September 2002.
8) Royal Adelaide Hospital Grand Round. April 2002. Strategies to improve the prospects for human gene therapy.
9) Thoracic Society of Australia and New Zealand (S.A. Branch) Annual Scientific Meeting March 1995. Substance P increases interleukin 8 gene expression in freshly harvested human airway epithelial cells
10) Thoracic Society of Australia and New Zealand (S.A. Branch) Annual Scientific Meeting March 1994. The Role of Airway Epithelium in the Modulation of The Inflammatory Effects of Tachykinins. Tachykinin Involvement in Allergic Bronchoconstriction - An Ovine Model
12) Royal Adelaide Hospital Grand Round - Hanson Centre Mini-Symposium July 1994. Human Bronchial Epithelium Expresses mRNA For NK1, NK2 And NK3 Tachykinin Receptors, Neutral Endopeptidase And Preprotachykinin A.
13) Thoracic Society of Australia and New Zealand (S.A. Branch) Annual Scientific Meeting March 1993. Tachykinin Receptor Expression in Ovine Airways
14) Thoracic Society of Australia and New Zealand (S.A. Branch) Annual Scientific Meeting March 1992. Steroid Resistant Asthma - The Role of Methotrexate, Gold and other Immunosuppressive Therapies.

Selected Poster presentations (Details listed in Publications List))

American Thoracic Society Meetings - 1995, 1996, 1998, 1999, 2000. 2007
American Society of Gene Therapy Meetings - 1999, 2000, 2001, 2002, 2003, 2004
Cancer Gene Therapy Meeting, San Diego. - 1997
Keystone Symposium – Molecular and Cellular Biology of Gene Therapy - 1998
Thoracic Society of Australia and New Zealand - 1992,1993,1994,1995,1996, 2002, 2003, 2004, 2005, 2006,2007,2008
Asian Pacific Society of Respirology 2007
European Respiratory Society Meeting – 2004.

Detailed Curriculum Vitae

Teaching.

Clinical Tutor, University of Adelaide 1992-4. Provision of didactic undergraduate tutorials covering topics in general internal and respiratory medicine, practical demonstrations of history taking, clinical examination, interpretation of laboratory and radiological investigations. Participation in Problem-Based Learning Program, University of Adelaide, recommenced 2002. Higher degree supervision of Honours, MD and PhD students. Ongoing student teaching as **Clinical Senior Lecturer** since 2002-4. Presenter in 5th year "Respiratory Common Program" 2003, 2004. **Clinical Associate Professor** 2005- present. Ongoing activities as above, including serving as an examiner for viva assessments.

Postgraduate and FRACP training
Provision of didactic tutorials covering topics in general internal and respiratory medicine on a monthly rotating basis, practical demonstrations of history taking, clinical examination, interpretation of laboratory and radiological investigations. Preparation for FRACP written and viva voce examinations. Assistance with trial examinations. 1990-97, 2002-present. Seminars presenting respiratory medicine topics to the general hospital and trainees.

RACP curriculum development committee 2004-present for Respiratory Medicine.

Lecturer in UAB Postgraduate Special Topics Program

Convener, Adelaide Lung Club 1993-4.

Convener, Cardio-pulmonary and replicative vector weekly meetings, DHGT, UAB 2000-2002

Research Supervision: Training and supervision of postdoctoral fellows, graduate students, medical fellows and research assistants. 1993-present.

Currently supervising:
Sandra Hodge, PhD. Research Fellow. Principal Supervisor for PhD, graduated 2003, University of Adelaide
Wei Xia, Principal Supervisor, PhD student (Adelaide University International Scholarship) Thesis submitted April 2008.
Ann Reynolds PhD, Research Assistant
Geoff Matthews PhD, Research Assistant
Jessica Ahern BSc, Research Assistant
Joseph Tan, PhD, Research Assistant.
Chien Li Liew, MBBS FRACP, Master of Medical Science Candidate.

Selected Previous Supervision:
Katherine Finan, Principal Supervisor, MD Student, (Studies in Hanson Centre, enrolled via Dublin University) Graduated 2006
Sheree Broznya, Principal Supervisor, Honours Student, Graduated 2005.
Tracy McNamara, PhD Student, University of Adelaide. Graduated 2005.
A/Prof Mark Holmes – Seven month sabbatical. Senior Consultant Physician, RAH.
Brian Boatman (Clinical Research Fellow, Cardiology)- now Interventional Fellow, Cornell University
Koichi Takayama, Post-Doc –now Associate Professor, Fukuoka University, Japan
Yasuo Adachi, Post-Doc – now Research Scientist, Kyoto, Japan
Ludmila Kaliberova, Research Assistant, UAB
Baogen Liu, Research Assistant, UAB
Sharon Moey, B Med Sci Student, University of Adelaide
Melissa Armstrong, Year 11 work experience student
Alexandra Matthews, Year 10 work experience student
Intermittent assistance to a broad range of other post-docs and students was also provided.

Peer review activities

Reviewer of grants for NHMRC (5-6/yr), Cancer Council, Local and Interstate hospital pilot grant schemes

NHMRC Grant Review Panel Respiratory/Sleep 2005 and 2006 (Invitee for 2007 and 2008)

Reviewer for many journals including "Gene Therapy", "Molecular Therapy", "Cancer Research", Human Gene Therapy.

Judging Panel, Department of Medicine Research Symposium 2001, UAB.

Advisor/reviewer of applications to RAH Institutional Review Board for proposals using genetically modified organisms.

Institute of Medical and Veterinary Science, Animal Ethics Committee. 30+ applications reviewed in each six week cycle.

Clinical Trial Involvement

The Respiratory Clinical Trials Unit located in the Royal Adelaide Hospital Chest Clinic conducts a large number of pharmaceutical industry sponsored clinical trials. I have been involved as an investigator on a large number of these.

Recent highlights:

Principal Investigator – Build 3: Bosentan Use in Interstitial Lung Disease. Effects of Bosentan on morbidity and mortality in patients with Idiopathic Pulmonary Fibrosis. A multicenter, double-blind, randomised, placebo-controlled, parallel group, event-driven, group sequential, Phase III study.

Co-Investigator – AIR2 Trial: Safety and efficacy of the Alair system for the treatment of asthma: a multicenter randomised clinical trial (Asthma Intervention Research (AIR2) Trial). A trial of bronchial thermoplasty for asthma, including a sham control arm.

1. Books / Monographs / Symposia

1.1 Sole Author Works

1) *Reynolds PN.* Targeted Adenoviral Vectors. *In* Gene Therapy in Lung Disease. Marcel Dekker. (S. Albelda Ed) Lung Biology in Health and Disease series, Vol. 169.119-144. 2002.

2) *Reynolds PN.* Delivery of DNA to pulmonary endothelium using adenoviral vectors. In Gene Delivery to Mammalian Cells: Methods and Protocols. (Heiser, W. Ed.). Humana Press Inc. Volume 246. 69-89. 2004.

1.2 Joint Author Works

1) *Reynolds PN* and Curiel DT. Strategies to adapt adenoviral vectors for gene therapy applications - targeting and integration. *In* "The Development of Gene Therapy" Cold Spring Harbor Laboratory Press. (Ed. T. Friedmann) 111-130, 1998.

2) *Reynolds, PN* and Olman, M. Gene therapy for fibroproliferative phase ARDS. *In* Molecular Interventions: Gene Therapy for the ICU. (Factor, PH Ed.) Kluwer Academic Publishers. 85-106. 2001.

3) *Reynolds PN,* Hemminki, A and Curiel DT. Gene Therapy. *In* Cecil's Textbook of Medicine. 22nd Edition. Goldman L, and Ausiello D Eds. WB Saunders. 202-206, 2003.

4) *Reynolds, PN* and Danilov SM. Targeting gene delivery to pulmonary endothelium. *In* Mechanisms of Drug Delivery. (Muzykantov, V. Ed.). Kluwer Academic Publishers. 173-190. 2002.

5) Hodge G, Hodge S, Reynolds PN, Holmes M Measurement of intracellular cytokines to improve therapeutic monitoring of immunosuppressive drugs following lung transplantation. In Transplantation Immunology Research Trends. Nova Science Publishers, 2007 Ed. Ulricker ON

6) Hodge S, Hodge G, Holmes MD, Reynolds PN. New Apoptosis Research in Respiratory Disease In Cell Apoptosis Research Progress Nova Science Publishers, 2008 Eds Fenton RH and Burnside CV

7) Reynolds PN, Hodge S. The impact of defective clearance of apoptotic cells in the pathogenesis of chronic lung diseases: chronic obstructive pulmonary disease, asthma and cystic fibrosis. In: Phagocytosis of dying cells: from molecular mechanisms to human diseases. Editors: Dmitri V. Krysko and Peter Vandenabeele. Springer publisher. (In Press).

1.3 Editorial Works

N/A

2. Journal Articles

2.1 Refereed Journals

2.1.1 Sole Author Articles

N/A

2.1.2 Joint Author Articles

1) *Reynolds PN*, Rice AJ, Reynolds AM, Thornton, AT, Holmes MD and Scicchitano, R. Tachykinins contribute to the acute airways response to allergen in sheep actively sensitized to *Ascaris suum*. Respirology. 2: 193-200, 1997.

2) *Reynolds PN*, Holmes MD and Scicchitano, R. The role of tachykinins in bronchial hyper-responsiveness. Clinical and Experimental Pharmacology and Physiology. 24 (3-4): 273-280, 1997.

3) Reynolds AM, *Reynolds PN*, Holmes MD and Scicchitano, R. Tachykinin NK2 receptors predominantly mediate tachykinin-induced contractions in ovine trachea. Eur J Pharmacol 341(2-3): 211-224, 1998.

4) *Reynolds PN*, Miller CR, Goldman CK, Doukas J, Sosnowski BA, Rogers BE, Gomez-Navarro J, Pierce GF, Curiel, DT and Douglas JT. Targeting adenoviral infection with basic fibroblast growth factor enhances gene delivery to vascular endothelial and smooth muscle cells. Tumor Targeting 3: 156-168. 1998.

5) *Reynolds PN* and Curiel DT. Viral vectors show promise in Colorado. Nature Biotechnology 16(5):422-3. 1998.

6) Miller CR, Buchsbaum DJ, *Reynolds PN*, Douglas JT, Gillespie GY, Mayo MS, Raben D and Curiel DT. Differential susceptibility of primary and established human glioma cells to adenovirus infection: Targeting via the epidermal growth factor receptor achieves fiber receptor-independent gene transfer. Cancer Research 58: 5738-5748, 1998.

7) *Reynolds PN*, Dmitriev I, Curiel DT. Insertion of an RGD motif into the HI loop of adenovirus fiber protein alters the distribution of transgene expression of the systemically administered vector. Gene Therapy. 6: 1336-1339, 1999.

8) Blackwell JL, Miller CR, Douglas JT, Li, H, *Reynolds PN*, Caroll WR, Peters GE, Strong TV, and Curiel DT. Retargeting to EGFR enhances adenovirus infection efficiency of squamous cell carcinoma. Archives of Otolaryngology – Head and Neck Surgery 125: 856-863, 1999.

9) *Reynolds PN* and Curiel DT. Chimeric Vectors – The best of both worlds? Molecular Medicine Today. 5 (1) 25-31, 1999.

10) Kasano K, Blackwell JL, Douglas JT, Dmitriev I, Strong TV, *Reynolds PN*, Krops DA, Caroll WR, Peters GE, Bucy RP, Curiel, DT and Krasnykh V. Selective gene delivery to

head and neck cancer cells via an integrin targeted adenoviral vector. Clinical Cancer Research. 5: 2571-2579, 1999.

11) Adachi, Y, *Reynolds, PN*, Yamamoto, M, Grizzle WE, Overturf, K, Matsubara, S, Myramatsu, T, Curiel DT. Midkine promoter based adenoviral vector gene delivery for pediatric solid tumors. Cancer Research. 60(16):4305-10, 2000.

12) Schneider H, Groves M, Muhle C, *Reynolds PN*, Knight A, Themis M, Carvajal J, Scaravilli F, Curiel DT, Fairweather NF, Coutelle C. Re-targeting of adenoviral vectors to neurons using the Hc fragment of tetanus toxin. Gene Therapy. 7: 1584-92, 2000.

13) *Reynolds PN*, Zinn KR , Gavrilyuk VD, Balyasnikova IV , Rogers BE, Buchsbaum DJ, Wang MH, Miletich DJ, Grizzle WE, Douglas, JT, Danilov SM and Curiel DT. A targetable, injectable adenoviral vector for gene delivery to pulmonary endothelium in vivo. Molecular Therapy 2 (6): 562-578, 2000.

14) Rice AJ, *Reynolds PN*, Reynolds AM, Holmes MD and Scicchitano, R. Tachykinin-induced bronchoconstriction in sheep is NK-1 receptor mediated and exhibits tachyphylaxis. Respirology 6(2):113-123, 2001.

15) Suzuki, K, Fueyo, J, Krasnykh, V, *Reynolds, PN*, Curiel DT, Alemany, R. A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. Clinical Cancer Research, 7(1):120-6, 2001.

16) Nicklin SA, *Reynolds PN*, Brosnan MJ, White SJ, Curiel DT, Dominiczak AK, Baker AH. Analysis of cell-specific promoters for viral gene therapy targeted at the vascular endothelium. Hypertension. 38: 65-70, 2001.

17) *Reynolds PN*, Scicchitano, R and Holmes MD. Preprotachykinin A mRNA is expressed in airway epithelium and upregulated in smokers with chronic bronchitis. Respirology 6: 187-197, 2001.

18) *Reynolds PN,* Holmes MD, Adachi Y, Kaliberova, L and Curiel DT. A novel system for mitigation of ectopic transgene expression induced by adenoviral vectors. Gene Therapy, 8(16):1271-5.

19) Wesseling JG, Bosma PJ, Krasnykh V, Kashentseva EA, Blackwell JL, *Reynolds PN*, Li H, Parameshwar M, Vickers SM, Jaffee EM, Huibregtse K, Curiel DT, Dmitriev I. Improved gene transfer to primary and established human pancreatic carcinoma target cells via epidermal growth factor receptor and integrin-targeted adenoviral vectors. Gene Therapy, 8(13):969-76, 2001.

20) *Reynolds PN,* Nicklin SA, Kaliberova L, Grizzle WE, Baker AH, Danilov SM, Curiel DT. Combined transductional and transcriptional targeting improves the specificity of transgene expression *in vivo*. Nature Biotechnology, 19(9): 838-842, 2001.

21) Wesseling JG, Yamamoto M, Adachi Y, Bosma PJ, van Wijland M, Blackwell JL, Li H, *Reynolds PN*, Dmitriev I, Vickers SM, Huibregtse K, Curiel DT. Midkine and cyclo-

oxygenase 2: two tumor specific promoters for adenoviral vector gene delivery in pancreatic carcinoma. Cancer Gene Ther. 2001 8(12): 990-6.

22) Adachi A, *Reynolds PN*, Yamamoto M, Wang M, Takayama K, Matsubara S, Muramatsu T, Curiel DT. A midkine promoter-based conditionally replicative adenovirus for treatment of pediatric tumors and bone marrow purging. Cancer Res. 2001 1;61(21): 7882-8

23) Adachi A, Matsubara S, Muramatsu T, Curiel DT, *Reynolds PN*,. Midkine promoter-based adenoviral suicide gene therapy for midkine-positive pediatric tumors. Journal of Pediatric Surgery. 2002 Apr;37(4):588-592.

24) *Reynolds PN*, Curiel DT. New generation adenoviral vectors achieve enhanced transduction efficacy via CAR-independent entry mechanisms. Kidney International. 2002 Suppl 1:24-31 (70%)

25) Baker AH, *Reynolds PN*. Development of Vascular Gene Therapy: Targeting Concepts and Therapeutic Opportunities. Cardiology News and Reviews. Jan. 2002.

26) McKay T, *Reynolds PN*, Jezzard S, Curiel D, Coutelle C. Secretin-mediated gene delivery, a specific targeting mechanism with potential for treatment of biliary and pancreatic disease in cystic fibrosis. Mol Ther. 2002 5(4): 447-54.

27) Bauerschmitz GJ, Nettelbeck DM, Kanerva A, Baker AH, Hemminki A, *Reynolds PN*, Curiel DT. The flt-1 promoter for transcriptional targeting of teratocarcinoma. Cancer Research; 62(5):1271-4. 2002

28) Hodge S, Hodge G, Flower R, *Reynolds PN*, Scicchitano R, Holmes MD. Upregulation of production of TGF-β and IL-4 and downregulation of IL-6 by apoptotic human bronchial epithelial cells. Immunology and Cell Biology. 2002 Dec;80(6):537-43. (20%)

29) Richard JC, Zhou Z, Ponde DE, Dence CS, Factor P, *Reynolds PN*, Luker GD, Sharma V, Ferkol T, Piwnica-Worms D, Schuster DP. Imaging Pulmonary Gene Expression With Positron Emission Tomography (PET). Am J Respir Crit Care Med. 2003 May 1;167(9):1257-63.

30) Rogers BE, Chaudari T, *Reynolds PN*, Della Manna D, Zinn KR. Non-invasive gamma camera imaging of gene transfer using an adenoviral vector encoding an epitope-tagged receptor as a reporter. Gene Therapy. 2003 Jan;10(2):105-14.

31) Overturf K, LaPatra S *Reynolds PN*. The effectiveness of adenoviral vectors to deliver and express genes in rainbow trout, Oncorhynchus mykiss (Walbaum). Journal of Fish Diseases. 2003. 26 (2) 91-101.

32) Hodge SJ, Hodge GL, *Reynolds PN*, Scicchitano R, and Holmes M. Am J Physiol Lung Cell Mol Biol. Increased production of TGF-β and apoptosis of T-lymphocytes isolated from peripheral blood in COPD. Am J Physiol. Lung Cell Mol Biol. 285(2):L492-9. 2003 (30%)

33) Hodge SJ, Hodge GL, Scicchitano R, *Reynolds PN,* and Holmes M. Alveolar macrophages from subjects with COPD are deficient in their ability to phagocytose apoptotic airway epithelial cells. Immunology and Cell Biology. 81(4):289-96. 2003 (30%)

34) Takayama K, Reynolds PN, Short JJ, Kawakami Y, Adachi Y, Glasgow JN, Rots MG, Krasnykh V, Douglas JT, Curiel DT. A mosaic adenovirus possessing serotype Ad5 and serotype Ad3 knobs exhibits expanded tropism. Journal of Virology. 10;309(2):282-93, 2003 (20%)

35) Nicklin SA, Dishart KL, Buening H, *Reynolds PN,* Hallek M, Nemerow GR, Von Seggern DJ, Baker AH. Transductional and transcriptional targeting of cancer cells using genetically engineered viral vectors. Cancer Lett. 2003 Nov 25;201(2):165-73.

36) Work LM, Ritchie N, Nicklin SA, *Reynolds PN*, Baker AH. Dual targeting of gene delivery by genetic modification of adenovirus serotype 5 fibers and cell-selective transcriptional control. Gene Therapy, 2004 Aug;11(16):1296-300.

37) Hodge S, Hodge G, Holmes M, *Reynolds PN.* Flow-cytometric characterisation of cell populations in bronchoalveolar lavage (BAL) and bronchial brushings from patients with chronic obstructive pulmonary disease (COPD), Cytometry, 2004 Sep;61B(1):27-34.

38) Hodge S, Hodge G, *Reynolds PN.* Holmes M, Intracellular cytokines in blood T cells in lung transplant patients--a more relevant indicator of immunosuppression than drug levels.
Clin Exp Immunol. 2005 Jan;139(1):159-64.

39) Work LM, *Reynolds PN*, Baker AH. Improved gene delivery to human saphenous vein cells and tissue using a peptide-modified adenoviral vector. Genet Vaccines Ther. 2004 Oct 08;2(1):14.

40) Ono HA, Davydova JG, Adachi Y, Takayama K, Barker SD, Reynolds PN, Krasnykh VN, Kunisaki C, Shimada H, Curiel DT, Yamamoto M. Promoter-controlled infectivity-enhanced conditionally replicative adenoviral vectors for the treatment of gastric cancer. J Gastroenterol. 2005 Jan;40(1):31-42

41) Hodge *G, Hodge* S, Nairn J, Tippett E, Holmes M, *Reynolds PN* (2005) Poststorage leuko-depleted plasma inhibits T-cell proliferation and Th1 response in vitro: characterization of TGFbeta-1 as an important immunomodulatory component in stored blood. Transplantation. 2005 Jul 15;80(1):95-101.

42) Hodge S, Hodge G, Holmes M, *Reynolds P. N.* (2005) Increased peripheral blood T-cell apoptosis and decreased Bcl-2 in Chronic obstructive pulmonary disease (COPD). Immunology and Cell Biology 2005 Apr;83(2):160-6.

43) Hodge S, Hodge G, Holmes M, *Reynolds P. N.* (2005) Increased airway epithelial and T-cell apoptosis in COPD remains despite smoking cessation. European Respiratory Journal 2005 Mar;25(3):447-54.

44) Hodge S, Hodge G, *Reynolds PN*, Holmes M. (2005) Differential rates of apoptosis in BAL and blood of lung transplant patients. Journal of Heart and Lung Transplantation 2005 Sep;24(9):1305-14.

45) Hodge S, Hodge G, *Reynolds PN*, Holmes M. (2005) Upregulation of IL-8, IL-10, MCP-1 and MCP-3 in peripheral blood monocytes in stable lung transplant patients- are immunosuppression regimes working? Transplantation - 2005 Feb 27;79(4):387-391.

46) Miller WH, Brosnan MJ, Graham D, Nicol CG, Morecroft I, Channon KM, Danilov SM, Reynolds PN, Baker AH, Dominiczak AF. Targeting endothelial cells with adenovirus expressing nitric oxide synthase prevents elevation of blood pressure in stroke-prone spontaneously hypertensive rats. Mol Ther. 2005 Aug;12(2):321-7.

47) Everts M, Kim-Park SA, Preuss MA, Passineau MJ, Glasgow JN, Pereboev AV, Mahasreshti PJ, Grizzle WE, Reynolds PN, Curiel DT Selective induction of tumor-associated antigens in murine pulmonary vasculature using double-targeted adenoviral vectors. Gene Ther. 2005 Jul;12(13):1042-8

48) Hodge S, Hodge G, Holmes M, Reynolds P. N. Apoptosis in COPD Current Respiratory Medicine Reviews, 2005, 1, 33-41

49) Izumi M, Kawakami Y, Glasgow JN, Belousova N, Everts M, Kim-Park S, Yamamoto S, Wang M, Le LP, Reynolds PN, Curiel DT. In vivo analysis of a genetically modified adenoviral vector targeted to human CD40 using a novel transient transgenic model. J Gene Med. 2005, 7 (12) 1517-25.

50) Breidenbach M, Rein DT, Schondorf T, Khan KN, Herrmann I, Schmidt T, Reynolds PN, Vlodavsky I, Haviv YS, Curiel DT. A new targeting approach for breast cancer gene therapy using the Heparinase promoter. Cancer Lett. 2006 240(1) 114-22.

51) Hodge G, Hodge S, Reynolds PN, Holmes MD. Increased intracellular pro- and anti-inflammatory cytokines in bronchoalveolar lavage T cells of stable lung transplant patients. Transplantation. 2005 80 (8): 1040-5.

52) Finan KM, Hodge G, Reynolds AM, Hodge S, Holmes MD, Baker AH, Reynolds PN. In vitro susceptibility to the pro-apoptotic effects of TIMP-3 gene delivery translates to greater in vivo efficacy versus gene delivery for TIMPs-1 or-2. Lung Cancer 2006 53(3) 273-84.

53) Zhu ZB, Makhija SK, Lu B, Wang M, Wang Shuyi, Takayama K, Seigal GP, Reynolds PN, Curiel DT. Targeting Mesothelioma Using an Infectivity Enhanced Survivin-CRAd. Journal of Thoracic Oncology 2006, Sep; 1(7): 701-11.

54) Hodge G, Hodge S, Reynolds PN, Holmes M. Increased intracellular pro- and anti-inflammatory cytokines in bronchoalveolar lavage T cells of stable lung transplant patients. Transplantation. 2005 Oct 27, 80(8): 1040-5.

55) Hodge S, Hodge G, Broznya S, Jersmann H, Holmes M, Reynolds PN. Azithromycin increases phagocytosis of apoptotic bronchial epithelial cells by alveolar macrophages. Eur Resp J 2006 Sep;28(3):486-95.

56) Hodge G, Hodge S, Reynolds P, Holmes M. Compartmentalisation of intracellular proinflammatory cytokines in bronchial intraepithelial T cells of stable lung transplant patients. Clin and Exp Immunol 2006 Sep; 145(3): 413-9.

57) Hodge S, Hodge G, Nairn J, Holmes M, Reynolds PN. Increased airway granzyme b and perforin in current and ex-smoking COPD subjects. COPD. 2006 Dec; 3(4):179-87.

58) Takayama K, Reynolds PN, Adachi Y, Kaliberova L, Uchino J, Nakanishi Y, Curiel DT. Vascular endothelial growth factor based conditionally replicative adenovirus for pan-carcinoma application. Cancer Gene Ther 2007. 14 (1): 105-16.

59) Reynolds AM, Xia W, Holmes MD, Hodge SJ, Danilov S, Curiel DT, Morrell NW, Reynolds PN. Bone morphogenetic protein type 2 receptor (BMPR2) gene therapy attenuates hypoxic pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2007. May; 292(5): L1182-92.

60) Hodge G, Nairn J, Holmes M, Reynolds PN, Hodge S. Increased intracellular Th1 pro-inflammatory cytokine production in peripheral blood, bronchoalveolar lavage and intraepithelial T cells of COPD subjects. Clin and Exp Immunol. 2007 Oct; 150(1): 22-9.

61) Hodge S, Hodge G, Ahern J, Jersmann H, Holmes M, Reynolds PN. Smoking Alters Alveolar Macrophage Recognition and Phagocytic Ability: Implications in COPD. Am J Respir Cell Mol Biol. 2007 37(6):748-55

62) Hodge G, Hodge S, Chambers D, Reynolds PN, Holmes M. Acute lung transplant rejection is associated with localised increase in T-cell IFNgamma and TNFalpha proinflammatory cytokines in the airways. Transplantation 2007 Dec 15;84(11):1452-8

63) Southwood M, Jeffery T, Yang X, Upton P, Hall S, Atkinson C, Haworth SG, Stewart S, Reynolds PN, Long L, Trembath RC, Morrell NW. Regulation of bone Morphogenetic protein signaling in human pulmonary vascular development. Journal of Pathology 2008 Jan:214(1):85-95

64) Hodge G, Hodge S, Reynolds PN, Holmes M Airway infection in stable lung transplant patients is associated with decreased intracellular T-helper type 1 pro-inflammatory cytokines in bronchoalveolar lavage T-cell subsets. Transpl Infect Dis. 2008. Apr; 10(2): 99-105.

65) Hodge S, Hodge G, Jersmann H, Matthews G, Ahern J Holmes M, Reynolds PN. Azithromycin improves macrophage phagocytic function and expression of mannose receptor in COPD. Am J Respir Crit Care Med. Accepted Feb 2008 In press.

2.2 Unrefereed Journals etc

2.2.1 Sole Author Articles
N/A

2.2.2 Joint Author Articles

1) Robinson P and *Reynolds PN*. Unexplained Cough.: Modern Medicine, 16-24. May 1992.

2) *Reynolds PN* and Scicchitano R. Neuropeptides - Targets for asthma therapies? Current Therapeutics 37 (3) 13-15, 1996

3) Holmes MD and *Reynolds PN*. Cystic Fibrosis Gene Therapy. Science and Medicine.6 (1) 4-5, 1999.

4) *Reynolds, PN* and Curiel, DT. Targeted Adenoviral Vectors. Helix. Jan. VIII (2) 35-41, 1999.

5) *Reynolds PN* and Curiel DT. Gene therapy death highlights the remaining challenges. Helix. Jan. IX (2) 20-25, 2000.

3. Other (Conference Papers/Abstracts)

1) Young L., Bauman A., McGirr J., Richardson N., Rigby B., Meek R., Humphrey P., Fernando R., Teruci S., *Reynolds P.*, Antic R., Landau L., Geelhood G., Harris P., Musk B. A Multicentre Study Of Asthma Attendances At Accident And Emergency Departments. Proceedings TSANZ Annual Scientific Meeting, 1993.

2) Rice A, *Reynolds PN*, Holmes MD, Thornton A, and Scicchitano R. Phosphoramidon Enhances and the NK1 Receptor Antagonist CP 96,345 Inhibits Early Asthmatic Responses To Inhaled Ascaris In Sheep. Australian and New Zealand Journal of Medicine. 24 (4); 443,1994. Also Canadian Journal of Physiology and Pharmacology 72 Suppl 2 p3 1994. Peptides and their antagonists in tissue injury.

3) Scicchitano R, *Reynolds PN*, Usher S and Holmes MD. Substance P Is Present In Bronchoalveolar Fluid And Cells. Australian and New Zealand Journal of Medicine. 24 (4); 443, 1994. Also Canadian Journal of Physiology and Pharmacology 72 Suppl 2 p3 1994. Peptides and their antagonists in tissue injury.

4) *Reynolds PN*, Scicchitano R and Holmes MD. Human Bronchial Epithelium Expresses mRNA For NK1, NK2 And NK3 Tachykinin Receptors, Neutral Endopeptidase And Preprotachykinin A. Australian and New Zealand Journal of Medicine. 24 (4); 458, 1994. Also Canadian Journal of Physiology and Pharmacology 72 Suppl 2 p3 1994. Peptides and their antagonists in tissue injury.

5) Rice A, Holmes MD, Thornton A, *Reynolds PN* and Scicchitano R. The Effect of Infused Substance P in Sheep. Australian and New Zealand Journal of Medicine. 25 (4); 428, 1995.

6) *Reynolds PN*, Scicchitano R and Holmes MD. Substance P and Airway Epithelial Inflammation. Australian and New Zealand Journal of Medicine 25 (4); 414, 1995.

7) Rice A, Holmes MD, Thornton A, *Reynolds PN* and Scicchitano R. The Effect of Infused Substance P in Normal Sheep. Am J Respir Crit Care Med. 151 (4) 1995 A107

8) Rice A, Holmes MD, Thornton A, *Reynolds PN* and Scicchitano R. Tachykinins contribute to immediate allergic airways responses in sheep. Am J Respir Crit Care Med. 151 (4) 1995 A346

9) *Reynolds PN*, Scicchitano R and Holmes MD. Glucocorticoid Modulation of Tachykinin Receptor Expression in Human Airway Epithelium. Am J Respir Crit Care Med. 151 (4) 1995 A174

10) Shabani F, McNeil JD, *Reynolds PN*, Scicchitano R and Holmes MD. Transforming Growth Factor-β regulates the expression of collagenase and tissue inhibitor of metalloproteinases genes in human synovial fibroblasts. Proceedings of the Canadian Federation of Biological Societies 39th Annual Meeting.

11) Shabani F, McNeil JD, *Reynolds PN*, Scicchitano R and Holmes MD. Effect of gold sodium thiomaleate on in vitro collagenase gene expression in human synovial fibroblasts. Proceedings of the Canadian Federation of Biological Societies 39th Annual Meeting.

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The development of targeted adenoviral vectors for gene therapy of vascular disease, with emphasis on the pulmonary vasculature.

Gene therapy has the potential to have a major impact on human disease, but as yet has not realised its potential. Much work is still required before this technology enters mainstream therapeutics. The basic concepts, applications and challenges of gene therapy are discussed in Introductory Chapter 1.

This collection of original work has established several important principles in the field of gene therapy. The work began shortly after the landmark Orkin-Moltulsky National Institutes of Health report into gene therapy (1995), which lamented the fact that despite considerable investment and a number of human clinical trials, very little progress towards a meaningful application of this technology to human disease had been achieved. Probably the most important issue identified was the basic limitations in gene delivery technology. For success to be achieved, gene delivery vehicles (vectors) had to be capable of delivering adequate genetic material to specific target cells, to achieve adequate levels of gene expression for an appropriate period of time, and to do this safely. Partly in response to this assessment and partly as a result of realisations already occurring amongst those working in the field, much greater emphasis has been placed on the improvement of vector technology. In this regard, my work has focused largely on the development of targeting strategies to improve gene delivery. The work has focussed on adenoviral (Ad) vectors, which at the time were clearly the most efficacious agents for gene delivery in vivo, and still remain the most widely used vector platform. Initially work followed from the demonstration by others that Ad vectors could be retargeted using bi-specific conjugates and my first contribution here was to extend this concept to vascular cells (Original Article 1).

Strategies to retarget Ad vectors are based on an understanding of the natural infection pathway. Infection of cells is primarily mediated by a two-step process whereby the knob domain of the Ad fibre protein interacts with the Coxsackie and Adenoviral receptor (CAR), a component of the normal cell-cell adhesion complex. CAR is quite widely expressed and explains the fairly widespread tropism of Ad vectors for many cell types. On the other hand in many circumstances the reliance of Ad binding to CAR is a limitation. For example for gene delivery to airway epithelium for cystic fibrosis gene therapy, Ad vectors were found to be very poorly efficient due to CAR being located at the basolateral region of airway epithelial cells below tight junctions and thus relatively inaccessible via the airway route. For cancer applications, it was found that CAR is frequently downregulated in primary tumours (there is some evidence it may act as a tumour suppressor), thus reducing the efficacy of Ad in this setting also. After the Ad binds to CAR, internalisation of the virion is mediated by an interaction between cell surface integrins and an RGD motif located in the penton base protein at the base of the fibre. It was found that initial binding and subsequent internalisation are independent, thus the virion could be engineered to bind to non-CAR receptors and still

undergo efficient internalisation and subsequent intracellular steps including endosomal escape and delivery of the viral genome to the cell nucleus. The basic concepts and pathways underlying the retargeting strategy are expanded upon in some detail in Introductory Chapter 2 and the Methods Chapter.

The conjugate based approach with which I first worked uses a bi-specific conjugate. One of the two components consists of the Fab fragment of an antibody (designated 1D6.14) which binds to the receptor-recognising knob domain of the fibre. This component thus neutralises binding to CAR, thereby reducing uptake by non-target cells. For my initial studies I used a conjugate between the 1D6.14 Fab fragment and basic fibroblast growth factor (FGF) which is upregulated on many proliferating cells including tumour and vascular cells. This work established that conjugate-based retargeting could indeed enhance delivery to vascular endothelial and smooth muscle cells that had relatively low CAR expression, and some correlation between the level of FGF receptor expression and transduction efficiency was seen (Original Article 1).

The real challenge however, was to determine whether modifications of vectors could lead to useful improvements in gene delivery to specific cells in the highly stringent setting of systemic vascular administration. When I began this work there was no prior demonstration that vectors could be successfully engineered to improve cell – specificity of gene delivery in vivo. The challenge was to establish the principle in a general sense, which would have broad implications for gene therapy, as well as to establish principles for vascular gene delivery and my particular area of interest of pulmonary vascular gene delivery. Initial studies focused on the basic question of whether tropism modification of any kind would alter in vivo bio-distribution. At the time, there was a high degree of scepticism in the field that conjugate-based approaches would have sufficient affinity between the virus and the conjugate to function after systemic injection. Largely for this reason, I used a genetically modified virus constructed by a colleague at UAB for my initial studies. This virus was engineered to contain a small peptide sequence containing an integrin-binding RGD motif in one of the exposed loops of the knob domain (the so-called HI loop). In tissue culture, this vector had substantially increased infectivity for cells expressing low CAR, although it did not have any improved selectivity and still bound via CAR in addition to integrins. After systemic injection, this vector achieved relatively greater gene expression in some organs compared to an unmodified vector, particularly lung and kidney (Original Article 2). As such, the principle of engineered tropism modification leading to biodistribution changes was established.

However, because the particular vector (Ad-RGD) was "infectivity enhanced" rather than specificity-enhanced, true targeting required further developments. To this end, a collaboration was established to evaluate the use of a pulmonary vascular targeting antibody as a means to retarget Ad in vivo (Original Article 3). This antibody (mAb-9B9) binds to angiotensin converting enzyme (ACE) and had been show to target to pulmonary vasculature (specifically pulmonary endothelial cells) after systemic injection in a number of studies including work in human subjects. I constructed a bi-specific conjugate which linked 9B9 to

the Fab fragment the 1D6.14 anti-Ad antibody, the idea being that the bi-specific conjugate (hereafter called Fab-9B9) would simultaneously neutralise native Ad tropism and impart new tropism for ACE. Extensive in vitro validation of the conjugate and retargeting to ACE-expressing cells in culture was conducted, confirming the ACE-specificity of the targeting approach. Subsequently, the retargeting properties were assessed in vivo by administering various Ad + Fab-9B9 combinations by tail vein injection into rats. A significant increase in pulmonary gene expression was achieved. A very detailed methodology is provided in the published Methods Chapter, which could serve as a useful protocol for others to follow. A great deal of effort was required to provide convincing evidence that the redirected gene expression was truly in the target pulmonary endothelial cells. Several reporter genes were used and techniques included standard immunohistochemistry, immunofluoresence with dual staining for the reporter gene and endothelial makers and even electron-microscopy and immunogold staining to tell for sure that gene expression was in alveolar endothelial cells and not in the adjacent type I alveolar epithelial cells. Further validation of in vivo specificity was demonstrated by blocking the retargeting effect by prior injection into the animal of an excess of free mAb-9B9. These studies went beyond gene expression and included analysis of biodistribution of viral DNA, using the relatively new technology (at the time) of real time PCR. Yet another novel aspect was the inclusion of non-invasive imaging technology of gene expression (the first ever use of this technology for assessment of vector retargeting) – an area which has now developed into a huge field in its own right. This manuscript was the first demonstration that retargeting could be achieved in vivo and the results obtained in that regard have really not been surpassed even now. There was a degree of scepticism at the time that conjugate based technologies could be used in this way – many researchers had felt that the complex would simply "fall apart" after injection. Much more attention was being given to genetically modified vectors rather than conjugate-based approaches, but even now the genetic approaches have not achieved the degree of retargeting hoped for (although work still continues actively in this area). The principles I established have been built upon through further collaborative work to develop improved conjugate targeting and extend the principles to novel murine models (Original Articles 10, 11).

Despite the successes noted above, there were still shortcomings with regard to non-specific vector uptake and gene expression in non-target organs, most importantly the liver. It is now known that much of the liver uptake is not mediated by the CAR but in large part relates to heparin-binding domains in the viral fibre shaft and interactions of the viral capsid protein with blood coagulant factors such as factor X. The physiology of the fenestrated capillaries of the hepatic sinusoids also plays a major role. There is much work going on at present to incorporate this new knowledge into better vector design by mutating the regions of the viral capsid responsible for these interactions. Importantly, the Fab-9B9 conjugate I developed is still being used in conjunction with these approaches (this work is yet to be published). After demonstrating the basic principle of retargeting, my next major contribution was to show that unwanted non-target organ gene expression could be dramatically reduced by using cell-specific promoters to control gene expression. In this case putative endothelial specific promoters were the obvious choice. It should be noted that at the time this approach was not

as "obvious" as it may seem – many putative cell-specific promoters had been evaluated in Ad vectors and found to lose their specificity when placed in this context. Many also suffered from very low levels of activity. A new collaboration was established and a range of endothelial-specific promoters were assessed (Original Article 4). My specific role in this project and the experiments I personally conducted focussed on in vivo evaluation. This manuscript identified the flt-1 promoter as an excellent candidate for vascular gene delivery in vivo. The task then was to develop new Ad vectors containing the flt-1 promoter, and to evaluate these in conjunction with the Fab-9B9 conjugate. These studies were highly successful in establishing the principle that transductional and transcriptional strategies could be combined to improve specificity in vivo – a principle with implications beyond the specific application of pulmonary vascular delivery. The paper also included an additional highly stringent experiment in which vector-conjugate complex was injected into the heart (left ventricle) to further confirm that the pulmonary targeting effects being seen were not simply a first-pass effect. This work was published in the highly prestigious journal Nature Biotechnology (Original Article 6). Other strategies to reduce non-target expression were also explored by using the Cre-Lox system to de-activate unwanted expression in the liver. This strategy was initially embarked upon in part due to the uncertainty of how the endothelial-specific promoters would perform in vivo. Because the flt-1 promoter approach worked so well, the Cre-Lox approach was not further progressed (Original Article 5).

The work identifying the utility of the flt-1 promoter and the principles of combined transductional and transcriptional control that I established have been carried forward in a number of other vascular-related collaborative projects, particularly in regard to ex-vivo delivery to vein grafts, using vectors that I constructed in which the flt-1 promoter was combined which infectivity enhancement through the incorporation of the integrin-binding RGD motif in the Ad fibre knob. The flt-1 promoter has also been used in vectors containing novel vascular-targeting peptides in the knob domain (Original Articles 7, 8). Refinements to the conjugate strategy are also being developed using recombinant molecules and single-chain antibodies (Original Articles 10, 11). These technical improvements are increasing the feasibility of progressing conjugate-based strategies to human clinical trial. It is quite noteworthy that when I began this conjugate work 10 years ago, the use of conjugates was generally seen as a "stop-gap" measure, and there was much more enthusiasm for genetic modification of the vector. At this time, no genetically modified vector has achieved the degree of targeting I have achieved with the conjugate approach, and my work has laid the foundation for ongoing developments in the conjugate area.

The foregoing work achieved substantial progress in vector technology, but of course the real issue then becomes whether the use of this technology can achieve anything of physiological relevance when putatively "therapeutic" genes are delivered in disease models. I have pursued the physiological questions in pulmonary hypertension and have continued to collaborate with others with regard to systemic hypertension. With regard to the latter, a collaborative project with the University of Glasgow established that use of my conjugate to enhance gene delivery of endothelial nitric oxide synthase was much more effective at

reducing blood pressure in a spontaneously hypertensive rat model than if untargeted vector was used (Original Article 9). Importantly, these studies confirmed the utility of my reagents in the hands of other researchers in a separate lab to my own. This paper was the first ever demonstration of improved physiological outcomes using a targeted versus untargeted vector – another important milestone.

Upon returning to Australia I have set up pulmonary hypertension rat models. Over the last 5 years or so a great deal has been learned about the molecular and genetic basis of pulmonary hypertension, with the major discovery being that mutations in the gene for Bone Morphogenetic Receptor Type 2 (BMPR2) are the underlying basis for most cases of Familial Pulmonary Arterial Hypertension (previously called Familial Primary Pulmonary Hypertension). Mutations are also being discovered in many supposedly "sporadic" cases and there is growing evidence that BMPR2 is involved in some cases of secondary pulmonary hypertension. BMPR2 is predominantly expressed on pulmonary vascular endothelial cells. I thus developed new Ad vectors containing the BMPR2 gene with the aim of investigating the role of modulating BMPR2 expression in the pulmonary vascular endothelium in vivo, and to explore whether manipulation of this pathway might have therapeutic potential. The new vectors were validated for protein expression and functionality of delivered receptors in vitro, then administered to rats using Fab-9B9 targeting. We found that upregulation of BMPR2 attenuated the pulmonary hypertensive response to hypoxia (Original Article 12). This study is the first to show that BMPR2 might be manipulated for therapeutic gains, and also provides further evidence for the notion that BMPR2 is important in pulmonary hypertension beyond those cases of genetic mutations (given that the rats we used had normal BMPR2). The mechanisms underlying the link between BMPR2 expression and the development of pulmonary hypertension still remain unclear and is the subject of ongoing studies using the Fab-9B9 targeting approach as well as in vitro studies.

In summary I believe the provided body of work represents a cohesive and substantial contribution, progressing from the establishment of key principles in vector development through to specific novel applications in pulmonary disease. The concepts and reagents I have developed continue to be of relevance and have been adapted and utilised by other researchers in the field. There is no doubt that clinical application of gene therapy still faces many hurdles, but continued improvement in vector technology will certainly be critical if success is to be achieved.

In accord with the guidelines given, I also enclose a curriculum vitae which gives a broader overview of my contribution to medicine, and it can be seen that I have also been active in COPD (a major theme being the role of macrophage dysfunction in this disease – several landmark papers and another story in its own right), development of replicating viral therapy for cancer, and the pathogenesis of lung transplant rejection. The COPD work has been particularly fruitful and my group has pioneered the appreciation of the role of macrophage dysfunction and failure to clear apoptotic cells in the pathogenesis of COPD. The work has had a major impact in the field and has just recently culminated in the completion of an

investigator-initiated clinical study which I designed and executed. The study established that the macrolide antibiotic azithromycin can improve lung macrophage function when administered in low dose to COPD subjects. This latter study has just recently been accepted by the American Journal of Respiratory and Critical Care Medicine. This work is a significant contribution to the growing appreciation of the utility of low-dose macrolides for pulmonary disease and has immediate clinical translation possibilities.

I also have made substantial contributions outside of research, including involvement in International, National and Local Respiratory organisations including via the Thoracic Society of Australia and New Zealand, Asian Pacific Society of Respirology, College of Physicians and National Health and Medical Research Council.

Declaration

The publications presented in this Thesis Document have not been submitted to the University of Adelaide or any other university for any other degree.

To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis being made available in the University Library.

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Preface to the published works

Introductory Chapters

1) *Reynolds PN*, Hemminki, A and Curiel DT. Gene Therapy. In Cecil's Textbook of Medicine. 22nd Edition. Goldman L, and Ausiello D Eds. WB Saunders. 2003. (90%)

An overview of the current status of gene therapy and future directions aimed at clinicians and medical students. I was responsible for the overall layout and wrote all the final text.

2) *Reynolds PN.* Targeted Adenoviral Vectors. In Gene Therapy in Lung Disease. Marcel Dekker. (S. Albelda Ed) Lung Biology in Health and Disease series, Vol. 169.119-144. 2002.

Review of targeting strategies for lung disease

Methods Chapter

Reynolds PN. Delivery of DNA to pulmonary endothelium using adenoviral vectors. In Gene Delivery to Mammalian Cells: Methods and Protocols. (Heiser, W. Ed.). Humana Press Inc. Volume 246. 69-89. 2004.

Detailed description of methods used to develop the first successful strategy to specifically target adenoviral vectors in vivo

Original Articles

1) *Reynolds PN*, Miller CR, Goldman CK, Doukas J, Sosnowski BA, Rogers BE, Gomez-Navarro J, Pierce GF, Curiel, DT and Douglas JT. Targeting adenoviral infection with basic fibroblast growth factor enhances gene delivery to vascular endothelial and smooth muscle cells. Tumor Targeting 3: 156-168. 1998. (85%)

First use of conjugate-based retargeting to improve gene delivery to vascular cells. All experiments conducted by me.

2) *Reynolds PN*, Dmitriev I, Curiel DT. Insertion of an RGD motif into the HI loop of adenovirus fiber protein alters the distribution of transgene expression of the systemically administered vector. Gene Therapy. 6: 1336-1339, 1999. (85%)

First demonstration that tropism modification could alter the in vivo biodistribution of virus. All experiments conducted by me.

3) *Reynolds PN*, Zinn KR , Gavrilyuk VD, Balyasnikova IV , Rogers BE, Buchsbaum DJ, Wang MH, Miletich DJ, Grizzle WE, Douglas, JT, Danilov SM and Curiel DT. A targetable, injectable adenoviral vector for gene delivery to pulmonary endothelium in vivo. Molecular Therapy 2 (6): 562-578, 2000. (85%)

A landmark paper being the first demonstration of specific retargeting of adenovirus by in vivo systemic injection. This paper remains a milestone in the field and the conjugate-based approached developed is still being used by my current laboratory and by collaborators, and is yet to be surpassed for its efficacy. All experiments conducted by me.

4) Nicklin SA, *Reynolds PN*, Brosnan MJ, White SJ, Curiel DT, Dominiczak AK, Baker AH. Analysis of cell-specific promoters for viral gene therapy targeted at the vascular endothelium. Hypertension. 38: 65-70, 2001. (40%)

A collaborative project to investigate the utility of putative endothelial specific promoters for use in Ad vectors. My role here was to investigate the lead candidate (flt-1 promoter) in vivo with a view to further improving the selectivity of pulmonary vascular gene expression. A critical finding by me was the low activity of the promoter in vivo in the liver (a key non-target hurdle to overcome for systemic delivery approaches).

5) *Reynolds PN,* Holmes MD, Adachi Y, Kaliberova, L and Curiel DT. A novel system for mitigation of ectopic transgene expression induced by adenoviral vectors. Gene Therapy, 8(16):1271-5, 2001. (70%)

This paper illustrates a novel attempt to limit gene expression in non-target organs. Some technical success was achieved, but in the vascular context, the use of an endothelial promoter proved to be more useful. All experiments directly supervised or conducted by me.

6) *Reynolds PN,* Nicklin SA, Kaliberova L, Grizzle WE, Baker AH, Danilov SM, Curiel DT. Combined transductional and transcriptional targeting improves the specificity of transgene expression *in vivo*. Nature Biotechnology, 19(9): 838-842, 2001. (85%)

A landmark paper showing for the first time the synergistic gains in gene expression achieved using a combined transcriptional and transductional approach. The conjugate-based targeting strategy developed by me was combined with new reporter vectors constructed by me using the flt-1 promoter. All experiments conducted by me.

7) Work LM, Ritchie N, Nicklin SA, *Reynolds PN*, Baker AH. Dual targeting of gene delivery by genetic modification of adenovirus serotype 5 fibers and cell-selective transcriptional control. Gene Therapy, Aug;11(16):1296-300, 2004. (20%)

The genetic tropism modification used in paper 2) was combined with the flt-1 promoter in new vectors constructed by me and used in a collaborative project for in vitro gene expression in endothelial cells. The aim was to develop a translational approach for ex vivo modification of vein grafts. This paper is the first description of combined transcriptional and transductional targeting using genetic tropism modification.

8) Work LM, *Reynolds PN*, Baker AH. Improved gene delivery to human saphenous vein cells and tissue using a peptide-modified adenoviral vector. Genet Vaccines Ther. Oct 08;2(1):14, 2004 (15%)

The vector I constructed was used in ex vivo studies of human saphenous vein grafts as part of the further development toward a clinical program.

9) Miller WH, Brosnan MJ, Graham D, Nicol CG, Morecroft I, Channon KM, Danilov SM, *Reynolds PN*, Baker AH, Dominiczak AF. Targeting endothelial cells with adenovirus expressing nitric oxide synthase prevents elevation of blood pressure in stroke-prone spontaneously hypertensive rats. Mol Ther. Aug;12(2):321-7, 2005 (15%)

This paper is the first to show the physiological gains obtained by using a targeted vector system over untargeted vectors in a vascular disease model. I provided the key reagents needed for the demonstration of this effect and critical intellectual input. Importantly this paper also served as confirmation of the efficacy of the targeting strategy I developed when used in the hands of a separate group of investigators.

10) Everts M, Kim-Park SA, Preuss MA, Passineau MJ, Glasgow JN, Pereboev AV, Mahasreshti PJ, Grizzle WE, *Reynolds PN*, Curiel DT Selective induction of tumor-associated antigens in murine pulmonary vasculature using double-targeted adenoviral vectors. Gene Ther. Jul;12(13):1042-8, 2005 (10%)

11) Izumi M, Kawakami Y, Glasgow JN, Belousova N, Everts M, Kim-Park S, Yamamoto S, Wang M, Le LP, *Reynolds PN*, Curiel DT. In vivo analysis of a genetically modified adenoviral vector targeted to human CD40 using a novel transient transgenic model. J Gene Med. 7 (12) 1517-25, 2005 (10%)

These two papers illustrate ongoing vector development principles using key reagents and concepts that I developed. I have ongoing cognitive input into this program and continue to supply key reagents.

12) Reynolds AM, Xia W, Holmes MD, Hodge SJ, Danilov S, Curiel DT, Morrell NW, *Reynolds PN.* Bone morphogenetic protein type 2 receptor (BMPR2) gene therapy attenuates hypoxic pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 292: L1182–L1192, 2007. (85%)

In my current laboratory I have established models of pulmonary vascular disease. This paper is the first to demonstrate therapeutic benefits for pulmonary hypertension using endothelial – targeted adenoviral vectors, and indicates how targeted vector technology can be used to achieve benefits from the expanding knowledge of the molecular pathogenesis underlying pulmonary vascular disease. This is the first study to show that manipulation of the BMPR2 pathway has potential for therapeutic gains in pulmonary hypertension, and thus has implications beyond gene therapy approaches per se.

Preface to the published works

The works presented herein commenced during my tenure as a post-doctoral fellow and subsequently as research assistant professor in the Division of Human Gene Therapy, University of Alabama at Birmingham, and then as Director, Lung Research Laboratory, Hanson Institute, Adelaide South Australia.

A summary of the actual work and its contribution to science has been provided in the "Account of the research" section, along with specific reference to the individual manuscripts presented here.

I would especially like to thank Professor David T. Curiel, my friend and gene therapy mentor. I will forever be indebted to David for his warm welcome to Birmingham, Alabama, and his inspiring leadership during my time there, and I am especially grateful for our ongoing friendship and collaboration.

I would also like to thank Associate Professor Mark Holmes for inspiring me to undertake molecular biology research in the first place, supervising my PhD studies and providing the link with David Curiel. He has also been instrumental in facilitating my return to Australia, helping me establish the research program here and we are enjoying a productive ongoing research program.

I would also like to thank all my colleagues at the University of Alabama at Birmingham for teaching and supporting me during my time there. I especially want to thank Joanne Douglas, Victor Krasnykh, Lioudmilla Kaliberova, Igor Dmitriev and Alex Pereboev in regard to the work presented here.

I especially thank Sergei Danilov at the University of Illinois at Chicago for his initial and ongoing support and his unique expertise in the development of the ACE-targeting approach.

Equally, I thank Professor Andrew Baker at the University of Glasgow for inspiring and ongoing collaborative support, and colleagues in Andrew's department especially Stuart Nicklin and Lorraine Work.

I also thank Professor Nick Morrell for his expertise and intellectual input in regard to BMPR2.

And, now in my laboratory in Australia, I especially thank Ann Reynolds (no relation) for her outstanding technical expertise in the establishment of our animal model systems and her dedicated work with the BMPR2 projects, and Sandy and Greg Hodge for their tireless and inspirational devotion to science which has underpinned the expansion of our program into world class work in the COPD and Lung Transplantation areas.

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Concluding remarks

Conclusion

Continued advances in the understanding of the molecular basis of human disease will provide many new avenues for treatment. Gene – based therapies are a logical approach to exploit this new knowledge. Since first conceived, the application of gene therapies to correct inherited genetic defects has been a highly attractive proposition. There is no doubt that such an approach can provide substantial therapeutic advances as has been seen with gene therapy for severe combined immunodeficiency. Children with a universally fatal disease have been saved through the application of this technology. However, this application not only serves to highlight the potential of the approach, but illustrates some of the ongoing challenges in view of the development of leukemia in some of the treated children. Gene therapy for cystic fibrosis was one of the first goals of this technology for pulmonary disease, but despite major investment, no significant clinical progress has been achieved. To translate understanding of the genetic basis of disease to a therapy requires much more than identification of the responsible genes alone. It is now well-understood that the technology required to effectively deliver putatively therapeutic genes is in itself frequently the major stumbling block to progress. This recognition has lead to major investments in the development and assessment of vector technology. It is no longer acceptable for phase one human studies of gene therapies to merely provide safety data. Accurate assessment of gene delivery efficacy must be incorporated into early phase studies, and novel non-invasive imaging strategies to provide this data are an integral part of many ongoing research programs. As the field has advanced, the concept of gene therapy has moved well beyond the relatively simple notion of replacing an inherited defective gene with its normal counterpart, to the use of genes "as medicines" in a broad range of disorders. This expansion in scope increases both the potential for clinical benefit and the challenges involved. The most fundamental challenge is getting enough genetic material delivered to the key target cells.

To address the key challenge of efficient and selective gene delivery to target cells, many groups began to structurally modify gene delivery vectors. The adenovirus has proven to be a very useful vector platform for the development of this new generation of vectors, and David Curiel is the preeminent pioneer in this area. There is no doubt that adenovirus has limitations but major investments into the re-engineering of the basic vector have been made and this agent still remains the predominant system advancing to human clinical trial. Apart from targeting issues a great deal of effort is being applied to overcome the immune-stimulatory and pro-inflammatory effects of the vector, including approaches such as the use of helper-dependant ("gutless") vectors capable of long-term expression. Such new approaches will need to be combined with targeting strategies for optimal efficacy.

When I began my studies, there was a reasonable understanding of the pathway used by adenovirus to infect cells, although at the time, the cellular receptor (since identified as CAR) was unknown. There was one published paper that had demonstrated the feasibility of using bi-specific conjugates to retarget Ad-mediated gene delivery, using an in vitro model only. My goal from the outset was to establish the feasibility of targeting Ad vectors in the much

more challenging and clinically relevant setting of in vivo administration, in particular via the vascular route.

The demonstration of the success of the in vivo conjugate-based retargeting approach has provided an impetus for further developments in systemic targeting. Unfortunately, progress with genetically modifying the Ad vector has been slow in this regard for in vivo use. However, recently the group of Andrew Baker and colleagues, with whom I still collaborate, has identified motifs in the Ad capsid region which interact with blood clotting factors, specifically factor X, and this interaction subsequently mediates uptake of the vector by the liver. Overcoming the sequestration of vector into the liver has been the single biggest hurdle in the advancement of a systemically targeted agent. There is a degree of optimism that this new discovery will pave the way for significant advances. In the first instance we are planning studies to combine the Fab-9B9 conjugate with a capsid-modified virus, because at the present time the conjugate approach I developed still remains the best illustration of in vivo retargeting.

The combination of transcriptional targeting with an endothelial-specific promoter with the Fab-9B9 transductional targeting was a logical step to improve the fidelity of targeting of gene expression. This was the first demonstration of the improvements one could achieve in vivo with this approach, and will continue to be a model for a wide range of applications.

Coinciding with my vector development work has been the enormous growth in the understanding of the pathogenesis of pulmonary arterial hypertension, with the key new discovery being that BMPR2 mutations can cause the disease. BMPR2 mutation alone is insufficient however, as only approximately 20% of people with the mutation get the disease. Nevertheless, the role of BMPR2 in a wide range of conditions leading to pulmonary hypertension is being appreciated. The BMPR2 receptor and ligand pathway is thus an attractive target for new therapies, whether gene-based or conventional pharmaceuticals. Such an approach could complement the excellent progress that has been made recently with endothelin receptor antagonists, PDE5 inhibitors and new prostacyclin analogues. My work has been the first to show that this pathway could be manipulated for therapeutic gains, but this is still at a very early stage and the mechanisms linking BMPR2 mutations to the clinical disease require greater understanding. We are continuing our gene delivery studies in animal models to help address these questions.

In summary, I believe the work presented herein represents some critical milestones in vector development that not only have implications for pulmonary vascular disease but for the field of gene therapy more widely.