Institution: University of Warwick

Unit of Assessment: A1 – Clinical Medicine

Title of case study: Successful Clinical Translation and Optimisation of Antibody-Incompatible Renal Transplantation

1. Summary of the impact: Kidney disease affects about 10% of the population and 10% of these patients develop established kidney failure (ERF). Transplantation is a better treatment for ERF than dialysis but is limited by acute and chronic graft rejection. Treatment of rejection mediated by the recipient's T-lymphocytes is now remarkably successful, but antibody-mediated rejection (AMR) remains challenging. A principal cause of AMR is recipient antibodies targeting human leukocyte antigen (HLA, also known a tissue type) on the transplant organ. The presence of such antibodies previously vetoed transplantation but in the last ten years it has become increasingly feasible to transplant across HLA antibody barriers. Research at the University of Warwick (UoW) by Dr Daniel Zehnder and Professor Robert Higgins has facilitated and accelerated this process. Their research includes the first detailed monitoring of antibody levels after transplantation, showing how these affect graft function, and the development of new techniques to remove antibodies from patients. This resulted in over 100 HLA-mismatched renal transplants taking place in Coventry giving a net saving to the NHS of over £5M. Their research and its clinical translation encouraged the performing of another 350 such transplants across the UK and initiation of the National Case Registry.

2. Underpinning research: When kidney transplantation first took place in the 1950's, T-cell-mediated rejection was the key barrier to success. The drugs available to treat this have improved enormously. However, it was recognised in the 1960's that severe rejection could also be mediated by the recipient's antibodies against Human Leukocyte Antigen (HLA, also known as 'tissue type'; the name given to the Major Histocompatibility Complex in humans) expressed by the donor organ. Although transplantation across HLA antibody barriers has become feasible in the 21st century, the success rate for recipients with higher levels of antibodies continues to be poor, with an early mortality of up to 15% at two years and graft survival about 50% at five years.

The clinical programme at University Hospital Coventry and Warwickshire NHS Trust (UHCW) was started in 2003-4 by Higgins, Briggs and Zehnder, and was designed to be research intensive. The researchers have accumulated the world's largest bank of research specimens from AIT patients. The initial treatment protocol was based on practice at King's College London and the Johns Hopkins University, USA. Over the last 10 years their research led to significant changes in the clinical protocol[1-4]. This includes intensive therapy targeted at high-risk patients to enable transplantation, and marked reductions in the intensity of immunosuppression in patients without rejection so that mortality is reduced.

The research is a collaboration between Dr Daniel Zehnder, Associate Professor 2004-present and Dr Dan Mitchell, Associate Professor 2005-present at Warwick Medical School (WMS): Professor Robert Higgins, Consultant Nephrologist 1995-present at University Hospital Coventry and Warwickshire NHS Trust (UHCW), also WMS Honorary Professor 2004-present): and Professor David Briggs at NHS Blood and Transplant (NHSBT), Honorary Professor, University of Birmingham) [1-7].

- ***Antibody removal pre-transplant: High volume double filtration plasmapheresis (DFPP)****:* Standard treatment for the removal of HLA antibody was traditionally plasma exchange, where all plasma components are removed and then replaced with plasma and albumin free of HLA antibodies. Adverse effects limit the amount of antibody that can be removed. DFPP was developed clinically in this context by Higgins, Zehnder and Briggs between 2004 and 2009[1,a]. In this technique, plasma is filtered a second time rather than being discarded. The pore size of the second filter traps plasma proteins above a certain molecular weight, including antibodies. Plasma that passes through the second filter is returned to the patient. DFPP removes fewer non-immunological plasma proteins than plasma exchange and is better tolerated by the patient's cardiovascular system.
- ***Cryofiltration DFPP****:* Between 2008 and 2010, Higgins, Zehnder and Briggs performed the world's only plasma cryofiltration for the removal of HLA antibody in individuals with cardiovascular frailty. Recipient's plasma is filtered and then chilled to 0°C for six minutes, resulting in the

antibodies clumping together and enabling their removal by filtration. This method reduces intravascular fluid shifts and minimises the risk of hypotension. It was possible to use cryofiltration safely and effectively in patients who experienced life threatening hypotension with DFPP[2, a].

- ***HLA protein containing columns (HLA columns)****:* Since 2008 Zehnder, Mitchell, Higgins and Briggs have collaborated with Pure Transplant Solutions, an American company that produces a wide range of high quality HLA proteins[b]. A device that immobilises HLA proteins in a column has been developed. Three prototype devices have been tested successful in UoW laboratories, proving efficient removal of HLA antibodies from patient plasma. The next step will be first patient use with this patent protected device. This will be a major advance over all other techniques, which are non-selective and have limited ability to remove large amounts of HLA antibody.
- ***Real time clinical HLA antibody monitoring:*** Briggs, Higgins and Zehnder, together with others, have between 2003 and 2012 refined the clinical application of transplant recipient antibody quantification and monitoring with HLA protein-coated microbeads using fluorescence labelled anti-human IgG antibody on the liquid array (Luminex) platform. This method allows more sensitive and specific measurement of HLA antibody levels than possible previously. Daily pre-transplant antibody monitoring enabled the team to deliver optimal doses of DFPP before transplantation. Post-transplant antibody monitoring has shown that the relationship between antibody levels and occurrence of rejection is complex[3], but we have improved markedly the prediction of the clinical risk of AMR[3, 4]. This has allowed more effective targeting of treatment before and during AMR, and also holding off treatment when it is not necessary.
- ***Histological characterisation of AMR:*** The understanding of the histological changes that take place during acute AMR has been changed by our research[5]. It has been shown that the internationally accepted definition of AMR was wrong, placing too much reliance on staining for a blood component called complement C4d in tissues, and that C4d could be produced locally in renal tissues, rather than being deposited from the blood.
- ***Surface plasmon resonance (SPR) assessment of HLA antibody affinity to patient HLA protein****:* Recent results have shown that AMR is not associated simply with the blood level of antibody. Since 2009, Mitchell, Zehnder and Higgins have used SPR to characterise the affinity of recipient HLA antibodies. SPR is a real-time, label-free, sensitive and high throughput method to quantify binding of HLA antibodies to HLA proteins. Together with the UoW Department of Engineering (Dr Neil Evans, Associate Professor; Dr Mike Chappel, Associate Professor), using their skills in mathematical modelling[6], they have developed numerical quantification of this biological process. The results have changed our perception of antibody binding from a purely concentration effect to a potency paradigm where multiple factors affect antibody binding to tissues and the clinical outcomes.

3. References to the research

1. Higgins R, *et al.* Double filtration plasmapheresis in antibody-incompatible kidney transplantation. *Ther Apher Dial.* 2010; 14:392-9. DOI: 10.1111:j.1744-9987.2010.00821.x.
2. Sinha D, *et al.* Cryofiltration in the treatment of cryoglobulinemia and HLA antibody-incompatible transplantation. *Ther Apher Dial.* 2012; 16:91-6. DOI: 10.1111:j.1744-9987.2011.01004.x.
3. Higgins R, *et al.* Rises and falls in donor-specific and third-party HLA antibody levels after antibody incompatible transplantation. *Transplantation.* 2009; 87:882-8. DOI: 10.1097:TP.0b013e31819a6788.
4. Higgins R, *et al.* Blood levels of donor-specific human leukocyte antigen antibodies after renal transplantation: resolution of rejection in the presence of circulating donor-specific antibody. *Transplantation*. 2007; 84:876-84. DOI: 10.1097/01.tp.0000284729.39137.6e.
5. Higgins R, *et al.* The histological development of acute antibody-mediated rejection in HLA antibody-incompatible renal transplantation. *Nephrol Dial Transplant.* 2010; 25:1306-12. doi/ 10.1093:ndt:gfp610.
6. N.D. Evans, *et al.* Structural identifiability of surface binding reactions involving heterogeneous analyte: Application to surface plasmon resonance experiment. *Automatica.* 2013*;* 49:48–57. DOI: 10.1016/j.automatica.2012.09.015.
7. Higgins R, *et al.* Human leukocyte antigen antibody-incompatible renal transplantation: excellent medium-term outcomes with negative cytotoxic crossmatch. *Transplantation.* 2011; 92:900-6. DOI: 10.1097:TP.0b013e31822dc38d.

Associated Research Grants

- **Daniel Zehnder (PI)**, Associate Professor, WMS. Improvement in left ventricular geometry and cardiovascular functional capacity after restitution of the failing kidney through transplantation: a prospective non-randomised concurrent control study. British Heart Foundation, 05/2011 - 04/2014. £220,713 (PG/11/66/28982).
- **Daniel Zehnder (PI)** (Research Fellowship WMS David Lowe). Characterisation of immunological risk in antibody incompatible transplantation. NIHR, Doctoral Research Fellowship, 10/2010 - 09/2013. £284,270 (DRF-2010-03-045).
- **Daniel Zehnder (PI)** (Clinical Research Fellowship for Sunil Daga), WMS. Clinical characterisation and mathemathical modelling of donor kidney directed antibody specificity and affinity to determine risk of graft rejection. NIHR CRN WM South, Clinical Mentorship Program, 09/2011 - 08/2013. £105,000
- **Daniel Zehnder (PI)**, WMS. Characterisation of serum HLA antibody in patients undergoing an antibody incompatible renal transplant. NIHR CRN WM South, Clinical Research Nurse support, 04/2009 - 03/2010. £80,000
- **Robert Higgins (PI)**, Professor, WMS (Clinical Research Fellowship WMS Rizwan Hamer). Donor specific antibodies and complement in HLA-antibody incompatible renal transplantation. University Hospital Coventry and Warwickshire NHS Trust, 04/2006 - 03/2009. £200,000

4. Details of the impact

- ***Impact on patients*****:** An increasing number of patients are waiting for a kidney transplant, and those with HLA antibodies are waiting disproportionately longer and have more health problems. Our research on AIT has enabled the transplantation of more than 100 of these immunologically (most patients have multiple HLA antibodies) and physiologically complex (patients may have cardiovascular insufficiency and rigidity) cases since 2008 in Coventry alone. Their complexity is illustrated by an average time of renal failure of 14 years before we transplanted them; 71% had prior transplants, and 35% first developed renal failure as children. The majority of these would probably not have received a transplant without the use of the new tools and techniques resulting from our research[7, c, d, e]. Shared knowledge from our research has resulted in the transplantation of over 350 other similarly complex patients across the UK since 2008[c,d, and f]. The Associate Medical Director for Organ Donation at NHSBT has stated in supporting evidence, 'The impact of their research has allowed greater access to transplantation for this group of patients who have hitherto been disadvantaged and the lessons learnt have been extrapolated into other areas of solid organ transplantation so leading to better outcomes in terms of quality and length of life…'[c].
- ***Impact on the community:*** A long wait for transplantation is not only a burden for the patient, but also impacts on relationships, family and work, resulting in emotional, social and financial suffering[g]. The benefits of successful transplantation of our patients are illustrated by media features (over 20 TV, radio, and national media features, see source f for example).
- ***Impact on health care professionals:*** The value of the research for the day-to-day clinical practice of the NHS is shown by the significant changes in clinical protocol, which we[1-4], and others, have made since the 2003 protocol. This includes marked reductions in the intensity of immunosuppression in patients who do not develop rejection and this may account for the UHCW mortality rate being half that reported from the USA[7, h]. The research work, with the involvement of Higgins as chair of the guideline panel and Briggs, has led to the publication of national clinical guidelines for AIT by the British Transplantation Society[i]. These are the first such guidelines in the world and are important for the provision of standardised, high-quality care. UK Commissioners expect transplant units to follow these guidelines[c]. As a result of our clinical research, UoW, NHSBT together with UHCW, have helped other transplantation units implement our AIT transplant protocol and business model for obtaining funding through the NHS, including DFPP and cryofiltration[i]. Specialist NHS Renal Centres that have benefited directly from our research in this way include Guy's, St Georges, Oxford, Cambridge, Cardiff, Bristol, Leeds, Manchester, Dublin and Portsmouth[d]. Two international clinical workshops in AIT at Warwick for the dissemination of knowledge to the patient benefit in 2008 (80 participants) and 2012 (100 participants)[j], together with presentations at international meetings[h], and citations in the top clinical journal[i] further illustrate the international impact of our work. Our research has been instrumental in the generation of a national Registry for AIT in 2008 as a research and audit tool. This Registry was proposed and initiated by Higgins, who presented the data nationally and internationally. Other units such as

Bristol and Cambridge have used Registry data provided by Higgins when assessing and developing their clinical programmes.

- ***Impact on NHS:*** The financial impact of our programme locally has been highlighted by the Chief Executive of the University Hospital Coventry, who stated 'the UHCW has received over £6 million income for these transplants, and savings to the NHS from these transplants exceeds £5 million over and above the extra costs of the programme' [e]. In addition, this cost benefit goes up by around £1 million a year as grafts continue to function and patients do not need dialysis[k]. The impact is also reflected in the support from the National Director for Kidney Care at the UK Department of Health who stated that, 'This research has enabled not only the transplant unit at UHCW to be extremely productive, but has facilitated the development of Antibody Incompatible Transplantation nationally. The unit led the first NHS commissioned service for these transplants; national guidelines produced by the British Transplantation Society, and proposed the National Registry of cases that is run by NHS Blood and Transplant '[d].
- ***Commercial impact and new innovation:*** The hardware for these techniques is provided through L.IN.C. Medical Systems Ltd, whose Managing Director has stated that '…DFPP has become established as the treatment of choice for such patients in many transplant centres. [Professor Higgins] was also the first physician to perform cryofiltration DFPP outside of North America and Japan… L.IN.C. Medical Systems is an independent UK company and is now one of the key suppliers of extra-corporeal therapies providing innovative technologies that bring cost effective solutions to the NHS…'[a]. Despite the advantages of DFPP and cryofiltration over other therapies, they remain non-selective. In a formal collaboration with Pure Transplant Solutions and Pure Protein LLC, Oklahoma City, we have successfully tested a pre-clinical device to selectively remove antibodies against HLA on the donor kidney. The Director of Pure Protein has stated in supporting evidence, 'This is a well-developed collaboration between our biotechnology company… and the University of Warwick and University Hospital Coventry… with their academic and clinical credentials in AIT. Much progress has been made and … discussions with investors have started to take this device into clinical patient testing' [b]. Additionally, we are collaborating on the development of a novel assay to measure HLA antibody potency, with a first use preproduction product and data presented at the 2013 American Society of Histocompatibility meeting.

5. Sources to corroborate the impact

a. **Statement**: Managing Director, LINC Medical Systems LTD, Leicestershire, UK – Linc Medical. (Identifier 1).
b. **Statement**: Director, Pure Protein, LLC; Oklahoma City. US PureProtein. (Identifier 2).
c. **Statement**: Associate Medical Director, Organ Donation & Transplantation Directorate, NHS Blood & Transplant, Bristol, UK – NHSBT. (Identifier 3).
d. **Statement**: (Until 1 April 2013) National Director for Renal Care, Department of Health, Department of Renal Medicine, Salford Royal NHS Foundation Trust, Salford, UK (Identifier 4).
e. **Statement**: Chief Executive Officer, University Hospital Coventry & Warwickshire NHS Trust, Coventry, UK – UHCW. (Identifier 5).
f. National and International impact: XXIII international Transplant Society 17 August 2010, Vancouver, Canada. Presentation: UK Registry of Antibody Incompatible Kidney Transplantation 2001-2010 (http://tinyurl.com/o5z5fp8)
g. Patient testimonial: accessed 24 September 2013 (http://tinyurl.com/qbg6spk)
h. International impact: Montgomery RA et al, Desensitization in HLA-Incompatible Kidney Recipients and Survival. *N Engl J Med 2011; 365:318-326* DOI: 10.1056/NEJMoa1012376.
i. Guidelines: British Transplant Society, National Guidelines for Antibody Incompatible Transplantation: http://tinyurl.com/pc4hmtn
j. Teaching and training: 2nd International Meeting on Antibody Incompatible Transplantation. http://tinyurl.com/ney96d2
k. Cost effectiveness of renal transplantation: *http://www.organdonation.nhs.uk/newsroom/fact_sheets/cost_effectiveness_oftransplantation.asp).*