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Horizon Scanning Technology Horizon Scanning Report

Desensitisation protocols for human leukocyte antigen antibodies in renal transplantation

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The production of this *Horizon scanning report* was overseen by the Health Policy Advisory Committee on Technology (HealthPACT), a sub-committee of the Medical Services Advisory Committee (MSAC). HealthPACT comprises representatives from health departments in all states and territories, the Australia and New Zealand governments; MSAC and the New Zealand District Health Boards. The Australian Health Ministers' Advisory Council (AHMAC) supports HealthPACT through funding.

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Executive Summary

There is currently a severe shortage of available donor kidneys for patients with end stage renal disease. The average waiting time for these patients is four years. For sensitised patients with preformed antibodies against the human leukocyte antigens of their potential donors (positive crossmatch patients), the potential donor pool is much smaller and the waiting times can be much longer. In an attempt to expand the possibilities of increasing the donor pool for these patients and reducing their waiting times, positive crossmatch kidney transplantation using pre-emptive desensitisation techniques has been developed.

Positive crossmatch kidney transplantation was developed following the discovery that plasmapheresis and intravenous immunoglobulin administration are able to rescue patients who develop a positive crossmatch after transplantation. The technique involves the pre-transplant desensitisation of sensitised, positive crossmatched patients with the aim of achieving a negative crossmatch or sufficient reduction in sensitisation to proceed to transplantation. Currently, there exists two commonly used protocols to perform positive crossmatch kidney transplantation, a high-dose intravenous immunoglobulin based protocol and a plasmapheresis with low-dose intravenous immunoglobulin protocol.

From the retrieved studies there is some evidence suggesting that reasonable successful desensitisation rates are attainable using either desensitisation protocol. Once desensitised and transplanted, the evidence indicates that both protocols offer similar graft outcomes to one another. However, when compared to negative crossmatch patients who do not require desensitisation, graft rejection occurs more frequently in desensitised patients, regardless of the protocol. In terms of kidney function, there appears to be no difference between the different desensitisation protocols or with negative crossmatch patients undergoing kidney transplantation without desensitisation.

The included studies highlighted the potential role that the degree of sensitisation prior to undergoing desensitisation may play in determining not only the occurrence of successful desensitisation but also in post-transplant graft outcomes. Factors including the level of donor specific anti-human leukocyte antigen antibodies and transplant history were noted in various studies as playing a role in outcomes such as desensitisation and rejection rates.

Overall, the currently available evidence on positive crossmatch kidney transplantation suggests encouraging results for patients who would otherwise not have the possibility of receiving a kidney transplant. Despite this however, positive crossmatch kidney transplantation faces substantial challenges before the technique can be more widely adopted, namely the determination of an optimal desensitisation protocol and determination of long term graft outcomes for desensitised patients receiving kidney transplantation.

Although positive crossmatch kidney transplantation has been performed in Australia for many years, its use in the context of this report is a newer application and its ongoing development is important in order to increase the availability of donor organs. This technique is not an ideal means of providing donors who would otherwise be unsuitable, but it offers some hope for patients who would otherwise be faced with lifelong dependence on dialysis.

Introduction

The Australian Safety and Efficacy Register of New Interventional Procedures - Surgical, on behalf of the Medical Services Advisory Committee (MSAC), has undertaken a Horizon Scanning Report to provide advice to the Health Policy Advisory Committee on Technology (Health PACT) on the state of play of the introduction and use of positive crossmatch kidney transplantation.

Positive crossmatch kidney transplantation has the potential to expand the donor pool and address the current shortage of transplant kidneys available in Australia and New Zealand. In particular, positive crossmatch kidney transplantation has the potential to increase the opportunity for highly sensitised patients, who would generally otherwise remain on wait lists indefinitely, to undergo transplantation. Positive crossmatch kidney transplantation is currently in the investigational stage in Australia, and is still in the early stages of development elsewhere.

This Horizon Scanning Report is intended for the use of health planners and policy makers. It provides an assessment of the current state of development of positive crossmatch kidney transplantation, its present use, the potential future application of the technology, and its likely impact on the Australian health care system.

This Horizon Scanning Report is a preliminary statement of the safety, effectiveness, cost-effectiveness and ethical considerations associated with positive crossmatch kidney transplantation.

Background

The condition

Healthy kidneys function as a filter, controlling the level of water and chemicals within the human body, as well as producing hormones and clearing waste products. A number of diseases, including glomerulonephritis, hypertension, polycystic kidney disease and diabetes, can compromise kidney function. This can cause a dangerous build up of waste products and a loss of chemical balance (AIHW 2005).

Kidney failure (end stage renal disease) occurs when the kidneys have less than 10% of normal function and are no longer able to sustain life. This condition is fatal unless patients receive renal replacement therapy in the form of either ongoing haemodialysis or kidney transplantation. Haemodialysis replaces some kidney function by slowly pumping the patient's blood through an artificial kidney (dialyser) to remove waste and excess fluid. However, prolonged dialysis is associated with a reduced quality of life, due to the patient's dependence on it, and increased mortality rates, largely due to cardiovascular complications (McDonald and Russ 2002). Transplantation is the preferred option for patients with end stage renal disease (ESRD), but the supply of donor kidneys is limited and many patients are ineligible for the operation (Beimler and Zeier 2007). Consequently, methods have been developed to increase the number of people eligible for kidney donation by reducing the likelihood of tissue rejection in the recipient.

Sensitisation

One barrier to kidney transplantation is recipient sensitisation (presence of primary antibodies) to the human leukocyte antigen (HLA) of a potential donor. Patients sensitised to a potential donor's HLA possess previously formed antibodies which, in the case of transplantation, would result in graft rejection. Sensitisation to HLA can occur through various mechanisms, but the most common are a history of failed transplantation(s), pregnancy, and receiving a transfusion of blood products containing fragments of leukocytes (Dean et al 2005; Magee 2006).

During sensitisation, the potential transplant recipient develops immunoglobulin G (IgG), and also potentially IgA and IgM antibodies, against the two classes of HLA molecules (class I and class II) which may be either cytotoxic (complement[1]-activating) or non-cytotoxic (non-complement-activating) (Schonemann et al 2004). Antibodies specific to HLA class I molecules react with B- and T-lymphocytes, whereas, antibodies specific to HLA class II molecules react with monocytes and B-lymphocytes, but not T-lymphocytes (Fuggle and Martin 2004). Transplant recipients with preformed antibodies that are reactive to

[1] Complement refers to small proteins in the blood that form part of the immune system.

donor lymphocytes are at an increased risk of graft injury and hyperacute or accelerated rejection (Fuggle and Martin 2004). Therefore, prior to transplantation, a crossmatch test is performed to determine if the recipient has preformed antibodies against a donor's HLA molecules (Holechek et al 2003). A positive crossmatch result indicates that the recipient has preformed antibodies and that transplantation is likely to lead to hyperacute rejection (Holechek et al 2003).

Description of the technology

Positive crossmatch kidney transplantation developed following the observation that transplant patients with negative crossmatch who developed a positive crossmatch after transplantation could be rescued using plasmapheresis to remove the responsible antibodies from their blood (Holechek et al 2003). The discovery that intravenous immunoglobulin (IVIG) or cytomegalovirus hyperimmune globulin, in conjunction with immunosuppression, could prevent resynthesis of anti-HLA antibodies provided further support to the concept of positive crossmatch transplantation (Holechek et al 2003). Based on these observations, it was hypothesised that positive crossmatch transplantation could be performed pre-emptively using the above desensitisation techniques to obtain a negative crossmatch prior to transplantation.

This report will focus on positive crossmatch kidney transplantation utilising pre-emptive (pre-transplant) protocols as a technique to desensitise kidney transplant recipients prior to transplantation. Intraoperative and postoperative initiated desensitisation protocols exist and are also reported; however, they are not considered in detail due to time constraints.

The procedure

Two main pre-emptive desensitisation protocols currently exist: high-dose IVIG-based protocol and plasmapheresis[2] plus low-dose IVIG-based protocols. High-dose IVIG-based protocols involve the intravenous administration of high-dose (usually 2 g/kg of body weight) immunoglobulin prior to transplantation (Magee 2006). The proposed mechanisms by which these protocols achieve desensitisation are various and include blockage of Fc receptors on mononuclear phagocytes, direct neutralisation of alloantibodies (antibodies directed against tissue from another organism of the same species), inhibition of CD19 expression on activated B-cells, inhibition of complement and inhibition of alloreactive T-cells (Magee 2006). High-dose IVIG-based protocols do not suppress the immune

[2] Plasmapheresis is a process whereby blood is removed from the recipient and centrifuged to separate the cells from the plasma. The plasma, which contains the antibodies to the donor HLA, is discarded and the cellular components are reinfused into the patient, usually in a saline solution (Holechek et al 2003). Plasma exchange is a similar procedure to plasmapheresis with the additional step of replacing the removed plasma with a suitable substance. Both plasmapheresis and plasma exchange are categorised as apheresis. A consequence of apheresis includes the loss of clotting factors, as well as necessary antibodies to bacteria, viruses and fungi (Holechek et al 2003).

system, are relatively non-toxic and easy to administer, and do not require a live donor (Montgomery and Zachary 2004; Magee 2006). However, high-dose IVIG-based protocols are expensive, albeit less expensive than prolonged dialysis, have unpredictable efficacy and are associated with various adverse effects (Montgomery and Zachary 2004; Magee 2006). Typical high-dose IVIG-based protocols involve *in vitro* IVIG-inhibition testing to determine if sensitised patients are likely to become desensitised (Magee 2006). If the test shows that IVIG will be effective, the patient is offered monthly treatments with high-dose IVIG until a negative crossmatch is achieved (Magee 2006).

In plasmapheresis plus low-dose IVIG-based protocols, patients undergo approximately three pre-transplant plasmapheresis treatments weekly to remove anti-HLA antibodies from their blood (Holechek et al 2003). The number of treatments required to achieve a negative crossmatch (sufficient desensitisation) and proceed to transplantation can vary from one to seven, depending on the amount of antibody present (Holechek et al 2003). After each plasmapheresis treatment, patients receive low-dose IVIG (usually 100 mg/kg of body weight) on the assumption that, even at low concentrations, IVIG confers a beneficial effect (Magee et al 2006). Once a negative crossmatch is obtained, transplantation occurs within 24 hours. In some centres, additional plasmapheresis and low-dose IVIG are administered postoperatively to maintain the negative crossmatch. The advantages of this protocol include the ability to predict time to negative crossmatch and transplant (to some extent) and the removal of antibodies that cause blood group (ABO) incompatibility (Magee 2006). However, the protocol is costly and labour intensive. It also requires a living donor, because donor-specific antibody levels can rebound if cessation of desensitisation is not closely followed by a transplant (Montgomery and Zachary 2004; Magee 2006).

While other desensitisation protocols using alternative technologies exist, they are not currently used as commonly as the two mentioned desensitisation protocols. As a result, desensitisation using high-dose IVIG and plasmapheresis with low-dose IVIG-based protocols form the focus of this report. Other desensitisation protocols will be included for the purpose of discussion but they will not be analysed in detail.

Crossmatch detection

Currently, two main techniques exist to identify positive crossmatch: complement-dependent cytotoxicity (CDC) and flow cytometry (FC). A third technique, enzyme-linked immunosorbent assay (ELISA) is also becoming popular.

When a recipient is waiting to receive a cadaveric donor kidney, it is not possible to test for the presence of anti-HLA antibodies against a specific donor. Instead, a panel reactive antibody test is performed to determine the initial sensitivity of the recipient. This test measures recipient serum reactivity against an array of lymphocytes that represent selected HLA types commonly found in the population (Dean et al 2005). The resulting number of anti-HLA antibodies

detected, known as panel reactive antibodies (PRA), is expressed as a percentage; the higher the percentage, the more likely it is that the recipient will be a positive crossmatch with the cadaveric donor (Myers 2008). CDC, FC and ELISA may be used to perform PRA analysis by substituting the living donor's lymphocytes with a sample of lymphocytes likely to be representative of a potential deceased donor's.

CDC and FC are the most widely used crossmatching techniques; however, neither test is 100% effective. Patel and Terasaki (1969), demonstrated in their landmark study that only 80% of patients with positive CDC crossmatch experienced graft failure (Akalin and Bromberg 2005). Furthermore, CDC assays are unable to detect non-complement activating antibodies or to discriminate between HLA-specific IgG molecules and other lymphocytotoxic antibodies (Schonemann et al 2004). FC, on the other hand, is more sensitive and is capable of detecting these differences (some of which may not be relevant), but is a less specific test than CDC (Schonemann et al 2004). Consequently, ELISA is often used in conjunction with FC to confirm the specificity of FC results (Akalin and Bromberg 2005).

Although these detection methods are valuable in identifying patients at risk of rejection, they are not standardised across kidney transplant centres. As a result, depending on the detection method employed, patients identified as having a positive crossmatch with their potential donor may not be homogenous in terms of the amount and specificity of donor specific anti-HLA antibodies.

Intended purpose

Positive crossmatch kidney transplantation offers a potential alternative to long-term dialysis by increasing the available donor pool for sensitised patients who would otherwise be unlikely to receive an appropriate donor organ (Jordan et al 2003).This would reduce the number of patients on wait lists, as well as the length of time that potential recipients wait for an organ. It may also reduce the financial and emotional costs involved with dialysis for sensitised patients awaiting transplantation and improve their quality of life, whilst reducing their morbidity and mortality.

Clinical need and burden of disease

The target population for positive crossmatch kidney transplantation includes patients with ESRD who have preformed sensitisation to potential donor organs due to previous exposure to foreign HLA molecules. These patients generally remain on dialysis for the remainder of their lives, waiting to receive an appropriate cadaveric kidney, which often does not occur.

The incidence of chronic kidney disease in Australia remains unclear due to limitations in data collection. The 2001 National Health Survey indicated that less than 0.5% of responders were suffering from long-term kidney disease. However,

this is likely to be a considerable underestimation because mild to moderate kidney disease is often asymptomatic. Symptoms usually develop over several years and may not be apparent until the later stages of the disease (AIHW 2005). The National Health Survey also failed to collect the biomedical data required to determine the prevalence of chronic kidney disease in accordance with the US Kidney Disease Quality Outcome Initiative (K/DOQI) definition (AIHW 2005). The Australian Diabetes, Obesity and Lifestyle (AusDiab) study, on the other hand, used biochemical measures to determine the prevalence of chronic kidney disease from 1999–2000 in a national survey of non-institutionalised Australians aged over 25 years. The study found that 11.2% of participants had a glomerular filtration rate of less than 60 mL/minute/1.73m^2 and 5.1% of participants had protein or blood in their urine without significant reduced kidney function (Chadban et al 2003). If these conditions had persisted for a further three months, 16.3% of participants would have met the K/DOQI criteria for chronic kidney disease. Chronic kidney disease is associated with various other complications and comorbidities, including cardiovascular disease, respiratory infection, anaemia and musculoskeletal problems (AIHW 2005).

The Australian and New Zealand Dialysis and Transplant Registry (ANZDATA) reported that 16,751 (797 per million) were receiving some form of kidney replacement therapy at the end of 2007, with 7,109 (338 per million) receiving a kidney transplant and 9,642 (459 per million) undergoing dialysis (ANZDATA 2009).

Haemodialysis does not cure kidney failure, and in 2007 the death rate per 100 patient-years for dialysis-dependent patients was 15.4, in contrast to 2.2 for recipients of functioning kidney transplants. In 2007, 36% of the 1452 deaths occurring in dialysis-dependent patients were due to cardiovascular disease, in comparison with 33% of the 151 deaths in the kidney transplant recipients. Withdrawal from treatment was the second most common cause of death in dialysis patients (35%). Infection caused 10% of deaths in the dialysis group, compared with 17% in the kidney transplant group. Malignancy was responsible for 5% and 25% of deaths, respectively.

In January 2008, 1,388 Australians were waiting for a kidney transplant (Australian Donate 2009), and the average wait time for a cadaveric organ was four years (AIHW 2005). According to the Scientific Registry of Transplant Recipients, approximately 16% of patients on deceased donor allograft waiting lists are highly sensitised (defined as ≥ 80% PRA) (Myers 2008). The wait time for these individuals is significantly longer (Jordan et al 2003b).

Since 2000, the donor pool has expanded thanks to the growing acceptance of living donor transplants. However, the number of patients awaiting kidneys still outweighs the number of donor kidneys available, particularly as 35% of living donors are excluded because of blood group incompatibility (Crew and Ratner

2005). If antibody barriers can be overcome by desensitisation and immunosuppression methods, more patients with ESRD can be treated.

Stage of development

Flowing on from the discovery that antibodies developed after transplantation could be removed with plasmapheresis and their resynthesis prevented using immunoglobulin and immunosuppression was the development of pre-emptive protocols utilising the same modalities to remove antibodies in patients with a positive crossmatch (Holechek et al 2003). Since this time, a number of transplantation centres, primarily throughout the United States, have undertaken positive crossmatch kidney transplantation using variations of the two main desensitisation protocols, high-dose IVIG and plasmapheresis plus low-dose IVIG.

Several isolated trials, using variations of the same desensitisation protocol pioneered in the United States, have been conducted elsewhere, including Austria, Brazil, Canada, China, Egypt, England, France, Germany, India, Italy, Japan, Korea, Philippines, Portugal, Sweden, Switzerland, Turkey. Positive crossmatch kidney transplantation is in the investigational stages in Australia, with three documented case reports.

Existing comparators

There are no direct comparators to positive crossmatch kidney transplantation. However, the technique is often compared with conventional negative crossmatch kidney transplantation to determine whether graft and patient survival rates are comparable.

Clinical Outcomes

A total of eight comparative (one level II and seven level III) and two case series (level IV) studies investigating positive crossmatch kidney transplantation were selected for inclusion in this report. Evidence reported in the included studies was generally of good quality, however some lacked detail with regard to the desensitisation and subsequent transplantation protocol. The majority of studies did not specifically report on safety outcomes.

There were substantial differences among the studies with respect to the desensitisation protocols and types of drugs used (induction and immunosuppression therapy), patient characteristics and the definition of what constituted a positive and negative crossmatch, which limited inter-study comparisons. Nevertheless, the clinical outcomes presented overall should provide a useful insight into the viability of desensitisation protocols for positive crossmatch kidney transplantation.

Baseline patient characteristics, including recipient and donor age, race and gender, were generally comparable in all eight comparative studies, although the significance of these findings was not always reported. In studies comparing positive crossmatch patients with control (negative crossmatch) patients, donor specific alloantibody (DSA) and PRA levels were significantly higher in positive crossmatch patients as expected. In the study by Burns et al (2008), the mean number of prior transplantations was significantly higher in the high DSA group, compared with the low DSA group ($P = 0.022$). The same was seen in the study by Mai et al (2009), where patients with high PRA levels (> 20%) had significantly more previous transplants than those with PRA levels < 20% ($P < 0.01$).

Three of the eight included comparative studies and one of the two case series studies were prospective (Jordan et al 2004; Akalin et al 2008; Gloor et al 2006; Jordan et al 2003). None of the level III studies comparing positive crossmatch kidney transplantation with negative crossmatch kidney transplantation had historical controls.

Clinical outcomes are presented according to the type of desensitisation protocol utilised.

A) High-dose IVIG

One randomised controlled trial and one case series study investigated the use of a high-dose IVIG-based protocol (Table 1). Both studies were from the Cedars-Sinai Medical Centre, although the study populations were different.

Jordan et al (2004) performed a randomised, double blind, placebo-controlled study to investigate the effectiveness of a high-dose IVIG protocol on anti-HLA antibody levels in highly sensitised patients, most of whom were receiving cadaveric kidney transplants. While a strict crossmatch to confirm positive crossmatch status was not possible, the baseline PRA levels of patients (~80%) indicated they were highly sensitised and therefore at high risk of poor graft outcomes.

Patients were randomly assigned in a 1:1 ratio, yielding a study group of 48 patients and a placebo group of 50. Study group patients received pre-emptive IVIG infusions (2 g/kg of body weight) up to a maximum dose of 180 grams over a period of four months, during which the patient was expected to undergo transplantation. Placebo group patients received equal treatment with a placebo solution.

The case series study by Jordan et al (2003) reported on 45 patients (43% with confirmed anti-HLA antibodies) who underwent high-dose IVIG desensitisation and renal transplantation. Patients received a similar desensitisation protocol to that reported in Jordan et al (2004). However, unlike the 2004 study, this study included a larger proportion of living donor transplants (n = 28) and made use of preoperative *in vitro* testing to determine the likely effectiveness of the high-dose IVIG. The decision to transplant was based on the effectiveness of high-dose IVIG to reduce *in vitro* PRA (patients with deceased donors) or produce CDC-negative or acceptable crossmatches in previously positive patients with living donors.

Effectiveness

Jordan et al (2004) reported transplantation rate (presumably from successful desensitisation) as an outcome of interest; however, each participating centre decided independently whether or not patients would undergo transplantation following desensitisation. Therefore, although the transplantation rate was significantly (P = 0.048) better with high-dose IVIG, compared with placebo (35% versus 17%), the lack of transparency in deciding which patients underwent transplantation makes the significance of this finding questionable. Nevertheless, the study reported that high-dose IVIG desensitisation resulted in transplantation rates in patients with no history of prior transplantations that were approximately double those of patients who had previously received transplants (50% versus 22%; P value not reported). This finding suggests that prior transplantation, and perhaps degree of sensitisation, may influence the effectiveness of high-dose IVIG.

Another major outcome of interest was the reduction in anti-HLA antibodies. PRA changes were analysed for IgG plus IgM and IgG levels alone at 2, 4, 6, 8, 10 and 12 months (it was not specified if this analysis was performed on all patients or only those who underwent transplantation). The analysis revealed that at each time point, high-dose IVIG recipients had lower levels of IgG plus IgM and IgG levels (P = 0.033 and P = 0.0007, respectively). Despite this, the PRA level was >40% at each time point in the high-dose IVIG group. Additionally, after 6 months, the PRA levels returned toward baseline values in the high-dose IVIG group, which suggests that IVIG only temporarily reduces the levels of anti-HLA antibodies.

In transplant recipients (17 high-dose IVIG and 10 placebo patients), the graft failure rate at the end of the 30-month study period (as well as at the end of the first, second, and third years) was slightly higher in the placebo group, although this differences was not statistically significant. Serum creatinine levels (an indicator of renal function) at 24 months were also similar between both groups. The rate of allograft rejection in all patients, however, was significantly higher (P = 0.042) in the high-dose IVIG patients. A total of 12 deaths occurred during the study period in both groups; however no deaths were attributed to either the IVIG or placebo infusions.

In the study by Jordan et al (2003), successful desensitisation was observed in 93% of patients. Sixty-three percent of living donor patients achieved complete CDC negativity and 37% achieved acceptable crossmatch (negative T-/B-cell CDC, positive FC), with most patients (92%) requiring only a single dose of IVIG.

Thirty-one percent of the 42 transplanted patients experienced a rejection episode, with 11 occurring between the second and fourth postoperative week. All 13 received rescue therapy, of which eight responded and five needed treatment with OKT3[3]. Of these five patients, three experienced graft loss at 2, 6 and 7 months. Serum creatinine levels throughout the 2-year study period remained relatively constant and were similar to those reported in the randomised controlled trial.

Although only one death was reported, patient survival at 24 months was 97.6%, however only nine patients reached the 12 month follow-up time point. Similarly, the 24 month graft survival was 89.1% with only nine patients available at this time point. Therefore, significant follow-up attrition was apparent.

Both of the studies reported by Jordan et al suggested that high-dose IVIG infusions reduce anti-HLA antibody levels (and hence sensitisation) in deceased donor transplant recipients. Although this desensitisation led to an increased number of transplantations, without significantly compromising graft function in desensitised patients, the possibility of rejection (above 30% in both studies)

[3] OKT3 or muromonab is a monoclonal antibody directed against the CD3 antigen on peripheral human T-cells and effectively blocks all T-cell function (Wilde and Goa 1996).

suggests that its long term effectiveness may not be optimal. Overall, the randomised controlled trial by Jordan et al (2004) indicated that patients in the placebo group had better graft survival.

Safety

Jordan et al (2004) reported headaches due to the desensitisation protocol in both the high-dose IVIG group and the placebo group, but the difference between the groups was not statistically significant. Despite this however, the proportion of patients experiencing ≥ 1 headache both at the end of desensitisation and one hour after desensitisation was significantly higher in the high-dose IVIG group (P value not reported). Two high-dose IVIG patients experienced a serious desensitisation reaction; however, both went on to receive further infusions without reaction.

Table 1 High-dose IVIG-based protocol study results

Study	Patient details	Procedure	Effectiveness outcomes	Safety outcomes
Jordan et al (2004) Level II intervention evidence	**High-dose IVIG group** n = 48 **Placebo group** n = 50 **Preoperative PRA (mean ± SE)** High dose IVIG: 3 months 81.3 ± 2.2, 2 months 79.8 ± 2.2, 1 month 80.2 ± 2.5 Placebo group: 3 months 83.6 ± 2.0, 2 months 82.5 ± 2.1, 1 month 84.6 ± 2.1	**Preoperative desensitisation** High dose IVIG: Patients received IVIG (2 g/kg of body weight, maximum dose 180g) monthly for 4 months. Additional IVIG at 12 and 24 months if transplant had not yet occurred. Placebo group: Patients received placebo (2 g/kg of body weight, maximum 180g) monthly for 4 months. Additional placebo at 12 and 24 months if transplant had not yet occurred. **Induction/immunosuppression** Post-transplant immunosuppression as per centre protocol. **Postoperative** Blinded IVIG or placebo infusions given monthly for 4 months after transplant. **Rescue therapy** NR **Follow-up duration (median)** 24 months (study period: 30 months)	**Transplantation rate (n, %)** High dose IVIG: 17/48 (35%) – 2 LD Placebo group: 10/50 (20%) – 4 LD P = 0.069 **Adherent group transplantation rate (n, %)** High dose IVIG: 16/46 (35%) Placebo group: 8/46 (17%) P = 0.048 **Adherent group/prior transplant transplantation rate (n, %)** High dose IVIG: 10/34 (22%) Placebo group: 3/28 (7%) **Adherent group/no prior transplant transplantation rate (n, %)** High dose IVIG: 6/12 (50%) Placebo group: 5/18 (28%) **Estimated projected mean time to transplantation (years)** High dose IVIG: 4.8 Placebo group: 10.3 P = 0.049, P = 0.034 (when adjusted for previous transplant) **Graft failure (transplanted adherent group) at 30 months (n, %)** High dose IVIG: 4/16 (25%) Placebo group: 3/8 (38%) **Graft failure (transplanted adherent group) in first year (n, %)** High dose IVIG: 2/16 (13%) – 2 and 9 months (AMR and recurrent GN respectively) Placebo group: 1/8 (13%) – 17 days (diabetic hyperglycaemia, functioning graft) **Graft failure (transplanted adherent group) in second year (n, %)** High dose IVIG: 1/16 (6%) – 14 months (chronic rejection) Placebo group: 1/8 (13%) – 20 months (malignancy, functioning graft) **Graft failure (transplanted adherent group) in third year (n, %)** High dose IVIG: 1/16 (6%) – 27 months (chronic rejection) Placebo group: 1/8 (13%) – 26 months (hyperkalaemia, functioning graft) **Allograft rejection (all transplants) at 24 months (n, %)** High dose IVIG: 9/17 (53%) – 6 within first six months Placebo group: 1/10 (10%) P = 0.042	**Rate of headache due to desensitisation** High dose IVIG: 52% Placebo group: 30% P = 0.056 **Rate of moderate or severe headache due to desensitisation** High dose IVIG: 24% Placebo group: 13% **Patients experiencing ≥1 headache at end of desensitisation** High dose IVIG: 50% Placebo group: 24% P = NR (significant) **Patients experiencing ≥1 headache 1 hour after desensitisation** High dose IVIG: 45% Placebo group: 20% P = NR (significant) **Serious desensitisation reaction** High dose IVIG: 2/48 Placebo group: 0/50

			Serum creatinine at 24 months (mean mg/dL ± SE) High dose IVIG: 1.68 ± 0.28 Placebo group: 1.28 ± 0.13 P = 0.29 **Patient deaths during study period (n)** High dose IVIG: 4 Placebo group: 8 – 2 additional deaths after study period P = 0.22	
Jordan et al (2003) Level IV intervention evidence	n = 45 (28 LD) Included 2 patients waiting for heart transplantation and 15 patients on dialysis for mean† of 4.39 ± 4.49 years. **Crossmatch status** 28 LD patients positive CDCXM **Preoperative PRA>50% (%)** 40%	**Preoperative desensitisation** LD patients: IVIG incubated *in vitro* with titrated patient sera plus donor lymphocytes. If sufficient inhibition, single high-dose IVIG (2 g/kg of body weight, maximum dose 140g) given on dialysis during 4-hour period. If CDCXM after IVIG negative or acceptable, transplant within 24–72 hours. DD patients: IVIG PRA test performed. If *in vitro* inhibition seen, high-dose IVIG (2 g/kg of body weight, maximum dose 140g) given on dialysis monthly for 4 months, followed by transplantation. **Postoperative** Transplanted patients: high-dose IVIG infusion (2 g/kg of body weight) 1 month after transplant. **Induction/immunosuppression** Immunosuppression (post-transplant) included induction with humanised monoclonal anti-CD25 (1 mg/kg of body weight, 2 doses), Cellcept (500-750mg 2x day) or tacrolimus to maintain level at 10–12ng/mL. **Rescue therapy** Pulse steroids ± high-dose IVIG (2 g/kg of body weight). If no response, OKT3. **Follow-up duration** 24 months	**Sufficient *in vitro* PRA/positive XM reduction (n, %)** 42/45 (93%) – included 26 LD patients and 16 DD patients **DD patients IVIG desensitisation results (n, %)** Complete abrogation of XM positivity: 10/16 (63%) Acceptable XM (negative T/B CDC, positive FC): 6/16 (37%) **IVIG doses required to eliminate positive XM, LD patients (n, %)** 1 dose: 24/26 (92%) 3 doses: 2/26 (8%) **Rejection episode (n, %)** 13/42 (31%) - 11/13 occurred within 2–4 weeks of transplant **Requirement for rescue therapy (n, %)** Pulse steroids ± high-dose IVIG: 13 (31%) – 8 responded OKT3: 5 (12%) **Graft loss (n, %)** 3 (7%) - due to recalcitrant acute rejection at 2, 6.5 and 7 months **Serum creatinine (mean† mg/dL)** 1 month: 1.7 ± 1.4 3 months: 1.5 ± 1.5 6 months: 1.5 ± 1.0 12 months: 1.4 ± 0.5 24 months: 1.4 ± 0.4 **Deaths (n, %)** 1/42 (2.4%) - due to myocardial infarction, with normal graft function. **Graft survival at 24 months (%)** 89.1% - Only 9 patients reached the 24 month follow-up time point.	NR

† The authors did not specify what measure of dispersion was used, i.e. standard deviation or standard error.

AMR: antibody-mediated rejection; CDC: complement-dependent cytotoxicity; CDCXM: complement-dependent cytotoxicity crossmatch; DD: deceased donor; ESRD: end stage renal disease; FC: flow cytometry; GN: glomerulonephritis; IVIG: intravenous immunoglobulin; LD: living donor; NR: not reported; PKD: polycystic kidney disease; PRA: panel reactive antibody; SE: standard error; XM: crossmatch.

B) Apheresis plus low-dose IVIG

Two non-randomised comparative studies investigated the use of apheresis and low-dose IVIG-based desensitisation protocols (Table 2).

Haririan et al (2008) investigated the use of a pre-emptive plasma exchange and low-dose IVIG desensitisation protocol in a group of highly sensitised (mean PRA 54.7%) T-cell, B-cell and T-/B-cell FC crossmatch positive patients and compared their results with those of FC crossmatch negative patients. Desensitisation consisted of alternate day total plasma exchange followed by low-dose IVIG infusion (100 mg/kg of body weight). A crossmatch assay after each plasma exchange/IVIG session was used to determine any reduction in crossmatch positivity. Transplantation was permitted when the median FC channel shift was within two standard deviations of the mean.

West-Thielke et al (2008) reported the use of plasmapheresis plus IVIG desensitisation in T-cell FC positive crossmatch patients compared with FC crossmatch negative patients, with a focus on African American (AA) patients. Patients underwent plasmapheresis followed by low-dose IVIG every other day starting 1 week before the scheduled transplant. Transplantation occurred if a previously positive anti-human globulin-CDC crossmatch became negative or if the FC channel shift was <40. Postoperatively, desensitised patients continued to receive plasmapheresis and low-dose IVIG every other day for 1 week.

Effectiveness

In the study by Haririan et al (2008), desensitisation patients required a mean of 3.6 (standard deviation 2.2) sessions before sufficient desensitisation was achieved to undergo transplantation. Following desensitisation, the FC crossmatch status of patients was T-cell positive (n = 18), B-cell positive (n = 17), T-/B-cell positive (n = 15) and FC crossmatch negative (n = 16). A total of 41 patients underwent living donor kidney transplantation. During the period shortly after transplantation, the clinical progression (incidence of delayed graft function, slow graft function and acute cellular rejection) of both groups was comparable. Acute antibody-mediated rejection within 10 days of operation, however, was significantly more common in the study group (5 versus 0; $P < 0.03$).

Long term graft survival at 1 and 5 years was significantly ($P < 0.04$) better in the control group, with 69.4% of plasma exchange/IVIG group grafts surviving at five years versus 80.6% of grafts in the control group. Throughout the study period, graft loss was reported 14 times in the plasma exchange/IVIG group and seven times in the control group. However, serum creatinine levels remained comparable between groups over 5 years, with a substantial increase in serum creatinine in both the plasma exchange/IVIG group and the control group between the third an fifth year (1.5 ± 0.6 mg/dL to 3.4 ± 2.4 mg/dL and 1.7 ± 0.8 mg/dL to 2.0 ± 1.2 mg/dL, respectively). There were no between group differences in patient survival rates, and no deaths were attributed to desensitisation.

Various methods with differing levels of accuracy were employed throughout the study period to detect donor specific anti-HLA antibodies. However, the results presented provide some indication of the potential role of anti-HLA antibody levels on the effectiveness of plasma exchange/IVIG desensitisation. Delayed or slowed graft function, transplant history and acute rejection were identified as predictors of poor outcomes (Haririan et al 2008).

Of the 56 patients who underwent desensitisation in the study by West-Thielke et al (2008), 48 patients (79.4% of AA patients) and 95.5% of the non-AA patients) achieved successful desensitisation. Subsequently, 50 patients[4] underwent transplantation.

Results focused on desensitised AA patients and their comparison to various patient subgroups. The results indicated that in the first year, the rate of acute rejection in the AA IVIG group was significantly (P = 0.014) higher than in the AA control group, but comparable to non-AA IVIG patients (P = not significant). The 1-year mean estimated glomerular filtration rate was also significantly (P = 0.007) higher in the AA IVIG group than the AA control group, but comparable to non-AA IVIG patients. Other estimated glomerular filtration rate related outcomes followed the same pattern (Table 2). Despite the differences, however, 1-year patient and graft survival rates remained similar among all subgroups.

An analysis to identify factors associated with successful desensitisation was performed. The results showed that the mean T- and B-cell crossmatch channel shifts were significantly related to successful desensitisation (P < 0.001 and P = 0.012 respectively). This suggests an association between the degree of prior sensitisation and desensitisation success. Further support for this notion is provided in that the peak PRA of non-responders was 90.2% versus 66% in desensitised patients (West-Thielke et al 2008).

Safety

Haririan et al (2008) was the only study to report safety-related outcomes, such as the occurrence of transplant glomerulopathy, thrombotic microangiography and BK-virus-induced interstitial nephritis (BK nephropathy), which were comparable between groups.

[4] One positive crossmatch patient in each race group did not convert to negative crossmatch, but both were transplanted.

Table 2 Apheresis and low-dose IVIG-based protocol study results

Study	Patient details	Procedure	Effectiveness outcomes	Safety outcomes
Haririan et al (2008) Level III-2 intervention evidence	**PE/IVIG group** n = 41 (41 LD) **Control group** n = 41 (41 LD) **Crossmatch status (n)** PE/IVIG: T-FCXM positive 33, B-FCXM positive 35, T- and B-FCXM positive 27 Control group: All patients negative FCXM. **Peak PRA level (mean ± SD)** PE/IVIG: 54.7 ± 30.1 Control group: 15.6 ± 27.4 P = 0.03	**Preoperative desensitisation** PE/IVIG: Alternate day total PE followed by low-dose IVIG infusion (100 mg/kg of body weight). Plasma volume replaced with 5% albumin solution; fresh frozen human plasma given on preoperative day. Crossmatch repeated after PE/IVIG sessions. If FC median channel shift reduced within 2 SD from mean, transplant scheduled for following day. Patients received tacrolimus and MMF 2 weeks before transplant. Control group: no desensitisation **Induction/immunosuppression** PE/IVIG: Induction with OKT3 (prior to 2002) or thymoglobulin (post 2002). Tacrolimus and MMF maintained after surgery (adjusted for side effects). Intraoperative dose of methylprednisolone (500mg) given and additional postoperative doses (250 and 125mg) given daily. Tapering oral prednisone initiated so that by 3 months dosage was 10mg and by 1 year dosage was 5 mg. Control group: similar maintenance regimen to PE/IVIG. Less than half did not receive antibody induction. **Postoperative** No additional PE/IVIG postoperatively. Patients did not receive rituximab unless acute AMR developed. **Rescue therapy** Intravenous steroid, a thrice weekly course of PE + low dose IVIG and rituximab given to patients with biopsy-proven acute AAMR (in addition to optimisation of maintenance regimen). **Follow-up duration (mean ± SD)** PE/IVIG: 3.9 ± 2.2 years Control group: 5.2 ± 2.0 years	**Number of desensitisation sessions required (mean ± SD)** PE/IVIG: 3.6 ± 2.2 - 3 required > 6 sessions Control group: N/A **Crossmatch desensitisation results (pre-transplantation)** PE/IVIG: T-FCXM positive 18, B-FCXM 17, T- and B- FCXM positive 15 (16 achieved negative crossmatch) Control group: N/A **Delayed graft function (n)** PE/IVIG: 1 Control group: 3 **Slow graft function (n)** PE/IVIG: 2 (1 primary non function due to acute AMR) Control group: 4 **ACR (at least 1 episode; n)** PE/IVIG: 5 Control group: 6 P = 0.5 **Acute AMR within 10 days (n)** PE/IVIG: 5 - 2 of these grafts lost Control group: 0 P < 0.03 **Serum creatinine levels at 1, 3 and 5 years (mean mg/dL ± SD)** PE/IVIG: 1.7 ± 0.8, 1.5 ± 0.6, 3.4 ± 2.4 Control group: 1.5 ± 0.6, 1.7 ± 0.8, 2.0 ± 1.2 P = 0.3 (1 year), P = 0.5 (3 year), P = 0.09 (5 year) **Graft loss (n)** PE/IVIG: 14 (5 due to death with functioning graft) Control group: 7 (4 due to death with functioning graft) **Graft survival at 1 and 5 years (%)** PE/IVIG: 89.9%, 69.4% Control group: 97.6%, 80.6% P < 0.04 **1 and 5 year graft survival rate in T-FCXM and B-FCXM positive patients (%)** T-FCXM positive: 90.6% (1 year), 69.2% (5 year) B-FCXM positive: 87.5% (1 year), 72.9% (5 year)	**Transplant glomerulopathy (n)** PE/IVIG: 3 Control group: 4 P = 0.5 **Thrombotic microangiopathy (n)** PE/IVIG: 2 Control group 2 **BK nephropathy (n)** PE/IVIG: 2 Control group: 1

			(P = 0.96) **Deaths (n)** PE/IVIG: 6 (1 sepsis, 1 trauma, 4 unclear) Control group: 4 (2 gastrointestinal bleeding and pulmonary embolism, 2 unclear) Patient survival comparable (HR 1.96, P = 0.29; 95% CI 0.6 – 7.0) **Graft survival rates, PE/IVIG group with and without DSA (%)** With DSA: 83.3% (1 year), 70.5% (5 years) Without DSA: 90.5% (1 year), 68.3% (5 year) P = 0.44 (1 year)	
West-Thielke et al (2008) Level III-2 intervention evidence	**PP/IVIG group** n = 56 Group included 34 (28 LD) AA and 22 (22 LD) non-AA patients. **Control group** n = 426 Group included 198 (113 LD) AA and 228 non-AA patients. **Crossmatch status** PP/IVIG: All patients T-FCXM positive. Control group: All patients negative FCXM. **Peak PRA% (mean ± SD)** AA PP/IVIG: 66.18 ± 31.84 Non-AA PP/IVIG: 64.23 ± 34.68 AA control group: 10.09 ± 23.77	**Preoperative desensitisation** PP/IVIG: PP with plasma replacement with 5% human albumin and saline (2:1 volume ratio). If repeated PP prolonged coagulation, replacement fluids included fresh frozen plasma. PP occurred every other day starting 1 week prior to scheduled transplant (1–4 sessions). Low-dose IVIG (100 mg/kg of body weight) given after each PP session. 18 patients (10 AA and 8 non-AA) received 1–2 doses of rituximab. If AHG-CDC crossmatch became negative or FC channel shift was less than 40 transplant performed. Last PP on morning of operation. **Induction/immunosuppression** PP/IVIG: Patients received induction with antithymocyte globulin (1.5 mg/kg of body weight), starting intraoperatively and continuing on days PP/IVIG not given (total 4 doses). Maintenance immunosuppression included tacrolimus (target trough level 8–12ng/mL for first 2 months and 5–10ng/mL thereafter), MMF (1g dose x2/day). Methylprednisolone (500mg) was given intraoperatively to all patients. During the first 2 years protocol, prednisone tapered over a 6 month period from 1 mg/kg of body weight to 0.2 mg/kg of body weight daily. For a series of patients early steroid discontinuation protocol was used in which prednisone was tapered over first 5 days.	**Successful desensitisation by FC** PP/IVIG: 48/56 - 27/34 (79.4%) AA patients and 21/22 (95.5%) non-AA patients (2 unsuccessful AHG-CDC XM became negative). P = 0.130. **Patients transplanted (n, %)** AA PP/IVIG: 28 (82.4%)* Non-AA PP/IVIG: 22 (100%)* *Includes 1 patient with unsuccessful desensitisation but negative AHG-CDC. **Acute rejection within first year (n, %)** AA PP/IVIG: 14 (50%) AA control group: 47 (27%) - P = 0.014 AA control group (LD): 27 (27.3%) - P = 0.023 AA control group (DD): 20 (26.7%) - P = 0.025 Non-AA PP/IVIG: 8 (36.4%) – P = 0.335 **1 year eGFR, mL/min/1.73m² (mean ± SD)** AA PP/IVIG: 46.24 ± 25.24 AA control group: 60.56 ± 22.35 - P = 0.007 AA control group (LD): 60.92 ± 23.03 - P = 0.011 AA control group (DD): 60.09 ± 21.58 - P = 0.016 Non-AA PP/IVIG: 51.7 ± 22.47 – P = 0.495 **1 year eGFR <30mL/min/1.73m² (n, %)** AA PP/IVIVG: 7 (33.3%) AA control group: 7 (4.5%) - P < 0.001 AA control group (LD): 4 (4.5%) - P = 0.001 AA control group (DD): 3 (4.5%) - P = 0.001 Non-AA PP/IVIG: 2 (11.8%) – P = 0.148 **1 year eGFR <30mL/min/1.73m² - acute rejection patients (n, %)** AA PP/IVIG: 5 (45.5%) AA control group: 4 (12.5%) - P = 0.034 AA control group (LD): 3 (15%) - P = 0.095 AA control group (DD): 1 (8.3%) - P = 0.069	NR

		Control group: Antithymocyte globulin daily for 0–4 days (five doses) and underwent steroid avoidance (discontinued on day 6). **Postoperative** PP/IVIG: PP and low-dose IVIG every other day for one week. Control group: N/A **Rescue therapy** AMR treated with 3–5 session PP ± 3 day course antithymocyte globulin. ACR treated with intravenous methylprednisolone (500mg) for 3 consecutive days. **Duration of follow-up** 1 year	Non-AA PP/IVIG: 1 (16.7%) – P = 0.333 **1 year eGFR <30mL/min/1.73m² - non-acute rejection patients (n, %)** AA PP/IVIG: 2 (20%) AA control group: 3 (2.5%) - P = 0.047 AA control group (LD): 1 (1.5%) - P = 0.042 AA control group (DD): 2 (3.8%) - P = 0.115 Non-AA PP/IVIG: 1 (9.1%) – P = 0.586 **1 year patient survival (%)** AA PP/IVIG: 90.5% - 1 death at 12 days (severe vascular complication related to vascular surgery during transplant), 1 at ~7 months (secondary to sepsis and multi-organ failure) AA control group: 93.5% - P = 0.641 AA control group (LD): 95.4% - P = 0.331 AA control group (DD): 90.9% - P = 1.000 Non-AA PP/IVIG: 100% - P = 0.495 **1 year graft survival (%)** AA PP/IVIG: 82.6% AA control group: 89.9% - P = 0.289 AA control group (LD): 92.4% - P = 0.226 AA control group (DD): 87% - P = 0.732 Non-AA PP/IVIG: 94.1% - P = 0.373	

† The authors did not specify what measure of dispersion was used, i.e. standard deviation or standard error.

AA: African American; AAMR: Acute antibody-mediated rejection; ACR: acute cellular rejection; ADPKD: autosomal dominant polycystic kidney disease; AHG-CDC: antihuman globulin complement-dependent cytotoxicity; AMR: antibody-mediated rejection; AR: acute rejection; CI: confidence interval; DD: deceased donor; DGF: delayed graft function; DSA: donor-specific alloantibody; eGFR: estimated glomerular filtration rate; ESRD: end stage renal disease; FC: flow cytometry; FCXM: flow cytometry crossmatch; GN: glomerulonephritis; IVIG: intravenous immunoglobulin; LD: living donor; MMF: mycophenolate mofetil; PE: plasma exchange; PP: plasmapheresis; PRA: panel reactive antibody; SD: standard deviation; SGF: slow graft function.

C) Plasmapheresis/low-dose IVIG versus high-dose IVIG

One level III study by Stegall et al (2006) compared (Table 3) a high-dose IVIG protocol with two plasmapheresis-based protocols: plasmapheresis plus low-dose IVIG and anti-CD20 (an antibody that binds to B-cells) and plasmapheresis plus low-dose IVIG, anti-CD20 and post-transplant donor-specific alloantibody monitoring) in anti-human globulin-CDC T-cell positive patients. Patients receiving plasmapheresis plus low-dose IVIG, anti-CD20 (plasmapheresis/IVIG group; n = 32) desensitisation received a single dose of rituximab between 4 and 7 days before the scheduled transplant, followed by daily plasmapheresis and low-dose (100 mg/kg of body weight) IVIG sessions (19 also received splenectomy). Patients receiving plasmapheresis plus low-dose IVIG, anti-CD20 and post-transplant monitoring (plasmapheresis/IVIG/monitor group; n = 16) received similar treatment, with additional administration of a pre-transplant immunosuppressant (anti-thymocyte globulin) and post-transplant monitoring of donor-specific alloantibody levels. Patients in the high-dose IVIG group received between 2.1 and 3.0 g/kg of body weight IVIG over 3 days prior to the operation. The goal of the desensitisation protocols was to obtain an anti-human globulin-CDC T-cell negative crossmatch at the time of transplantation.

Effectiveness

The rates of successful desensitisation were similar in the two plasmapheresis groups, but were significantly ($P < 0.05$) better than those of patients who received high-dose IVIG. Three patients who were not successfully desensitised with high-dose IVIG underwent successful desensitisation with the plasmapheresis/IVIG.

Humoral rejection in the patients successfully desensitised and transplanted (including the three high-dose IVIG non-responders) was significantly ($P < 0.05$) more common in the high-dose IVIG group, compared with the plasmapheresis-based protocols although the time to diagnosis was comparable between the groups.

The number of pre- and post-transplant sessions of plasmapheresis plus low-dose IVIG was significantly ($P < 0.01$) lower in patients who did not receive pre-transplant immunosuppressant treatment (plasmapheresis/IVIG group). While the higher number of postoperative treatments observed in transplant patients may be explained by the monitoring itself, the investigators did not address the significance of the increased number of pre-transplant treatments. It is possible that pre-transplant administration of anti-thymocyte globulin may impact the effectiveness of the desensitisation protocol used.

The 1-year actuarial patient and graft survival rates for all patients were 93% and 82%. Rates for each of the three patient groups were not presented. In desensitised patients with functioning grafts, the 6-month serum creatinine levels did not significantly differ between groups.

Although the plasmapheresis/IVIG/monitor patients received a pre-transplant immunosuppressant to augment desensitisation, it was found that desensitisation success correlated with baseline antibody titers. The authors reported that patients with baseline crossmatch titers of less than 1:4 were able to achieve a negative crossmatch regardless of the protocol applied. In contrast, 1 out of 10 patients with a baseline titer of more than 1:32 was able to attain a negative crossmatch regardless of the protocol.

This study, unlike previous studies, did not perform an in vitro screening assay to determine the potential effectiveness of the high-dose IVIG treatment. As a result, it is possible that patients with higher levels of donor-specific anti-HLA antigen received the high-dose IVIG treatment when in other circumstances they would not have. This may have had consequences on the results reported. However, given that some high-dose IVIG non-responders were able to achieve a negative crossmatch using a plasmapheresis-based protocol, it is possible that in some cases at least, plasmapheresis may offer an alternative desensitisation method for high-dose IVIG non-responders. The study also found that the majority of patients experienced a decrease in levels of donor-specific alloantibody after IVIG treatment, thus suggestive that the effectiveness of IVIG is linked to the levels of this antibody.

Both the plasmapheresis, low-dose IVIG and high-dose IVIG based desensitisation protocols allowed transplantations in patients with confirmed positive crossmatches to take place. However, due to various factors, namely the differences in patient characteristics, definition of positive crossmatch, baseline PRA, accuracy of assays and outcomes reported, it is difficult to ascertain which of these two protocols provides the best combination of safety and effectiveness.

Saftey
There was no safety data reported in this study.

Table 3 Plasmapheresis and low-dose IVIG-based protocol versus high-dose IVIG-based protocol study results

Study	Patient details	Procedure	Effectiveness outcomes	Safety outcomes
Stegall et al (2006) Rochester, United States Level III-3 intervention evidence	**PP + low-dose IVIG + antiCD20** n = 32 **PP + low-dose IVIG + antiCD20 + monitoring** n = 16 **High-dose IVIG** n = 13 **Crossmatch status** All patients AHG-CDC T cell positive with DSA by flow beads or too many specificities to determine.	**Preoperative desensitisation** PP/IVIG: Single dose (375mg/m^2) rituximab 4–7 days prior transplant followed by daily 1-plasma volume exchange with 5% human albumin and low-dose (100 mg/kg of body weight) IVIG (4 PP sessions minimum). Negative XM determined morning of transplant after last PP. 19/32 patients underwent splenectomy. PP/IVIG/monitor: As above, except rabbit anti-human T-cell polyclonal antibody (1.5 mg/kg of body weight/day) given. IVIG: 11 patients received 2.1–3 g/kg of body weight and 2 received 3 g/kg of body weight IVIG over 1–3 days immediately prior to transplantation. XM repeated after treatment. If negative LD transplant performed. If positive, repeat XM on following day. If consistently positive, considered non-responder and treated with PP/IVIG protocol. **Postoperative** All patients: Daily PP/IVIG on postoperative days 1-3. PP/IVIG: as above plus post-transplant DSA monitoring. If DSA increased, additional PP/IVIG given. **Induction/immunosuppression** All patients: Thymoglobulin (1.5 mg/kg of body weight/day x 5–7 doses) induction. Maintenance immunosuppression: tacrolimus, MMF and prednisone (tapered to 5mg by 3 months). **Rescue therapy** All rejections treated with high-dose methylprednisolone and reinstitution of PP/IVIG. For severe rejection with marked allograft dysfunction, high-dose IVIG (2 g/kg of body weight) also administered. **Duration of follow-up** 127–1915 days	**Successful desensitisation (n, %)** PP/low dose IVIG/anti-CD20: 27/32 (84%) PP/low dose IVIG/monitor: 14/16 (88%) High dose IVIG: 5/13 (36%) *P* < 0.05 High dose IVIG versus PP/low dose IVIG/anti-CD20 **Humoral rejection successfully desensitised patients at transplant (n, %)** PP/low dose IVIG/anti-CD20: 11/30 (37%) – includes 3 IVIG non-responders PP/low dose IVIG/monitor: 4/14 (29%) High dose IVIG: 4/5 (80%) P < 0.05 (High dose IVIG vs PP groups) **Time to diagnosis of humoral rejection (days)** PP/low dose IVIG/anti-CD20: 4.7 PP/low dose IVIG/monitor: 6.4 High dose IVIG: 2.5 P = NS (High dose IVIG vs PP groups) **Humoral rejection in PXM patients (unsuccessful desensitisation) at transplant (n, %)** High dose IVIG and PP/low dose IVIG/anti-CD20 non-responders: 7/10 (70%); graft loss 5/10 (50%) **Pre-transplant PP/low dose IVIG (mean† sessions)** PP/low dose IVIG: 4.0 ± 1.6 PP/low dose IVIG/monitor: 9.8 ± 3.2 *P* < 0.001 **Post-transplant PP/low dose IVIG (mean† sessions)** PP/low dose IVIG: 3.5 ± 3.1 PP/low dose IVIG/monitor: 9.4 ± 7.7 *P* < 0.001 **Overall 1 year actuarial patient and survival – all patients (%)** 93% (patient), 82% (graft) **Serum creatinine in desensitised patients with functioning grafts at 6 months (mean†, mg/dL)** PP/low dose IVIG: 1.09 ± 0.71 PP/low dose IVIG/monitor: 1.64 ± 0.38 High dose IVIG: 1.75 ± 0.65 P = NS for all comparisons	NR

† The authors did not specify what measure of dispersion was used, i.e. standard deviation or standard error.
AHG-CDC: antihuman globulin complement-dependent cytotoxicity; DSA: donor specific alloantibody; IVIG: intravenous immunoglobulin; LD: living donor; MMF: mycophenolate mofetil; NS: not significant; PP: plasmapheresis; PXM: positive crossmatch; XM: crossmatch.

D) Mixed protocol

For the purpose of this report, a 'mixed protocol' refers to the use of two or more of the above protocols in a population of patients, where results are reported collectively so that a particular outcome cannot be attributed to a specific protocol.

Two studies, one non-randomised comparative study (Gloor et al 2006) and one case series (Vo et al 2008), used a mixed protocol for the desensitisation strategy (Table 4). These studies were included to provide additional safety and effectiveness data for desensitisation in general due to the low volume of studies fitting the inclusion criteria ($n \geq 20$) for the two main protocols: high-dose IVIG and plasmapheresis with low-dose IVIG.

Gloor et al (2006) assessed the allograft histology and function in positive crossmatch, ABO incompatible and conventional (negative crossmatch and ABO compatible) living donor kidney transplants. For the purposes of this report, only positive crossmatch and conventional study groups' data is presented. Patients were described as having a positive crossmatch if evaluation with flow beads found them to have HLA specificity.

Desensitisation included plasmapheresis and low-dose IVIG (100 mg/kg of body weight) in 23 patients with high DSA levels or high-dose IVIG (2 g/kg of body weight) without plasmapheresis in 14 patients with low DSA levels. The goal of desensitisation was to achieve negative T-cell antihuman globulin-CDC) crossmatch on the day of transplantation.

Vo et al (2008) featured the use of Alemtuzumab, which offers an additional immunosuppressive effect on B-cells. The authors hypothesised the use of Alemtuzumab would improve outcomes in highly sensitised patients and reduce the rate of antibody-mediated rejection. Alemtuzumab was administered subcutaneously to avoid the adverse events associated with its infusion.

In this study, patients were classified as positive crossmatch by either positive CDC crossmatch or positive FC crossmatch with their donors. Twenty-nine patients received kidneys from living donors and 25 received kidneys from deceased donors. Preoperative desensitisation for patients receiving living donor kidneys included either high-dose IVIG (2 g/kg of body weight) monthly for four months or until an acceptable crossmatch was achieved, or two doses of IVIG (2 g/kg of body weight) and one dose rituximab. Patients receiving deceased donor organs received the same protocol with additional high-dose IVIG (2 g/kg of body weight) given at transplantation. An acceptable crossmatch, facilitating transplantation, was defined as a negative CDC crossmatch but a positive FC crossmatch for T-, B-cells or both. All patients were given induction and maintenance immunosuppression, rescue therapy varied in intensity depending on the type and severity of rejection.

Effectiveness

In the study by Gloor et al (2006), biopsies were taken at 0 and 12 months to assess any histological changes. It was demonstrated that most patients in each group had normal biopsies or mild changes at 12 months. The incidence of acute humoral rejection was significantly higher in the positive crossmatch (38%) group compared with the control (14%) group ($P < 0.0001$). Antibody-mediated rejection also occurred more frequently in positive crossmatch patients (89%) compared with control patients (19%; $P < 0.0001$). The total number of rejections was 19 in the positive crossmatch group and 32 in the control group ($P = 0.001$).

Overall, in the study by Gloor et al (2006), the histology of positive crossmatch allografts were similar to those of conventional kidney transplants at 12 months follow-up, indicating histologically comparable results for positive crossmatch kidney transplantation.

In the study by Vo et al (2008), acute rejection occurred at an overall rate of 35%, with the majority of these occurring in patients who had a positive CDC and FC crossmatch (13%) or a negative CDC and positive FC crossmatch (13%) at the time of transplant. One acute rejection (6%) occurred in a patient with a negative CDC and FC crossmatch and no acute rejections occurred in patients with a positive CDC and negative FC crossmatch.

Overall, patient and graft survival at 12 months were 98% and 96%, respectively, which indicates good short term prospects for positive crossmatch kidney transplantation with Alemtuzumab. The subgroup analysis regarding acute rejection and crossmatch status at transplantation depicts negative FC crossmatch as a more important predictor of reduced rejection risk than negative CDC crossmatch, which is inconsistent with the study's forecast of an acceptable crossmatch. Because the number of patients representing each crossmatch status at transplantation was only small the validity of this observation may be affected, therefore, in order to substantiate this finding larger studies performing the same analysis on this new drug should be cited.

Safety

In the study by Gloor et al (2006), the distribution and occurrence of interstitial fibrosis, tubular atrophy, vasculopathy and arteriolar hyalinosis was similar between groups. Glomerulopathy did not appear to have an impact on renal function at one year follow-up. The primary event associated with chronic glomerulopathy was prior humoral rejection.

Vo et al (2008) reported bone marrow suppression as the main adverse event associated with Alemtuzumab in its patient population, which was seen in most patients at 2 to 4 weeks postoperative, requiring a reduction or elimination of mycophenolate mofetil maintenance immunosuppression. There were also eight (15%) infectious complications experienced in the study population.

In the case of both of these studies, it is important to note the intrinsic weakness of the mixed protocol study design. Studies which employ mixed protocol cannot provide evidence for a particular desensitisation modality because their results are combined; therefore, they can not specify the safety or effectiveness for one protocol alone, but for desensitisation collectively.

Table 4 Mixed protocol study results

Study	Patient details	Procedure	Effectiveness outcomes	Safety outcomes
Gloor et al (2006) Rochester, United States Level III-3 intervention evidence	**PXM** n = 37 (37 LD) **Control** n = 198 (198 LD) **Peak PRA (mean ± SD)** PXM: 53 ± 36 Control: 3.3 ± 14 *P* < 0.0001 (PXM vs ABOi and control) **Crossmatch status** PXM: T-cell AHG-CDC (n = 23) and T-/B- FCXM (n = 14) positive Control: NXM	**Preoperative desensitisation** PXM: Patients with high DSA levels (23 T-cell AHG-CDC patients) received preoperative de-sensitisation consisting of PP followed by low dose IVIG (100 mg/kg of body weight). Patients with low DSA levels (14 T-/B- cell FCXM patients) received high-dose IVIG (2 g/kg of body weight) immediately prior transplantation, without PP. **Induction/immunosuppression** All patients: induction with rabbit polyclonal antilymphocyte antibodies (1.5 mg/kg of body weight/day, 4–10 doses), tacrolimus (trough levels of 10–12ng/mL during first 4 months and 6–8ng/mL thereafter), MMF (750–1000mg 2x/day) and prednisone (tapering doses reaching 5mg/day by 3 months). **Rescue therapy** NR **Duration of follow-up (mean months ± SD)** PXM: 35 ± 17 Control: 25 ± 11 *P* < 0.0001	**Acute humoral rejection (n, %)** PXM: 14 (38%) Control: 27 (14%) *P* < 0.0001 **Total rejections (n)** PXM: 19 Control: 32 P = 0.001 **Antibody-mediated rejections (n, %)** PXM: 17 (89%) Control: 4 (13%) *P* < 0.0001 **12 month FC crossmatch in 28/37 patients in PXM group (n, %)** Positive FCXM: 11/28 (39%); 3/11 had TG Negative FCXM: 17/28 (61%); 4/17 had TG **12 month peritubular capillary C4d deposition in 33/37 patients in PXM (n, %)** PXM with TG: 2/7 (29%) PXM without TG: 5/26 (19%) **Serum creatinine at 12 months (mean ± SD, mg/dL)** PXM: 1.44 ± 0.33 (n=37) Control: 1.53 ± 0.45 (n=198) **Calculated GFR at 12 months (mean ± SD, mL/min/SA)** PXM: 52.0 ± 15 (n=37) Control: 49.5 ± 14.0 (n=198) **GFR at 12 months (mean ± SD, mL/min/SA)** PXM: 53 ± 18 (n=20) Control: 54.8 ± 18 (n=159) **Urine protein (mean ± SD, mg/24 hour)** PXM 822 ± 1590 (n=30) Control 425 ± 962 (n=190)	**TG at 12 months** PXM: 22% Control: 8% P = 0.026 **CG score at 12 months (mean ± SD)** PXM: 0.41 ± 0.89 Control: 0.10 ± 0.42 P = 0.26 **Factors associated with TG development at 12 months** PXM (P = 0.012; OR 1.84 [1.1-2.9]) Higher PRA (P = 0.018 OR: 1.02 [1.003-1.03]) Transplant, history (P = 0.03; OR: 2.07[1.07-4.03]) Acute AMR (P < 0.0001, OR: 11.5 [4.4-29.5])
Vo et al (2008) Los Angeles, United States Level IV	n = 54 (25 DD/29LD) **PRA levels (n, %)** <20%: 3 (5%) 20–50%: 11 (20%) >50%: 40 (75%)	**Preoperative desensitisation** LD: High-dose IVIG (2 g/kg of body weight, maximum dose 140g) monthly for up to 4 months until negative or acceptable XM (negative CDC but positive FCXM for T-, B-cell or both). OR, 2 IVIG doses (2 g/kg of	**Patient survival (%)** 98% **Graft survival (%)** 96% (2 graft losses, 1 with primary non-function and 1 with filgrastim-associated AMR approximated 4 months postoperatively)	**Infectious complications (n, %)** Total: 8 (15%) PBK viremia: 5 (9%) CMV and PBK viremia: 1 (2%) CMV: 2 (4%)

intervention evidence	**Crossmatch status** All patients positive CDC or FC XM.	body weight) + 1 dose rituximab (700–1000mg). DD: as above plus IVIG dose (2 g/kg of body weight) given at transplant time. **Induction/immunosuppression** Immediately postoperative, Alemtuzumab (30mg) induction. Premedications: acetaminophen 650 mg po, diphenhydramine 50 mg po and methylprednisolone 40 mg IVP. Maintenance immunosuppression: prednisone tapered to 5mg/day by 2 weeks, MMF (500mg) and tacrolimus to maintain 7–9ng/mL target for 3 months and 5–7ng/mL after 6 months. **Rescue therapy** Rejection treated with pulse methylprednisolone (10 mg/kg of body weight/day for 3 days) and/or rabbit anti-thymocyte globulin for cell-mediated rejections. Patients with AMR that were C4d positive pulse methylprednisolone (10 mg/kg of body weight/day for 3 days), one dose IVIG (2 g/kg of body weight) and rituximab (375mg/m^2) was given initially. Patients with severe AMR or thrombotic microangiopathy had PP (3–5 sessions) followed by repeat IVIG and rituximab was initiated. **Duration of follow-up (mean† months)** 13.9 ± 7.13	**Crossmatch status at transplant (n, %)** CDC+/FC+: 21 (39%) CDC+/FC-: 2 (4%) CDC-/FC+: 15 (27%) CDC-/FC-: 16 (30%) **AR episodes (%)** 35% (20% were C4d+ AMR) **AR by crossmatch at transplant (n, %)** CDC(+)/FC(+): 3 (13%) CDC(+)/FC(-): 0 (0%) CDC(-)/FC(+): 2 (13%) CDC(-)/FC(-): 1 (6%) **Serum creatinine (mean mg/dL)** 1 month: 1.8 3 months: 1.5 6 months: 1.5 12 months: 1.4 **Absolute lymphocyte counts (mean† 1000/µL)** Baseline: 1.68 ± 1.7 Day 1: 0.17 ± 0.19 Day 7: 0.09 ± 0.11 Day 3 0:0.18 ± 0.2 Day 180: 0.24 ± 0.29 Day 365: 0.14 ± 0.26	

† The authors did not specify what measure of dispersion was used, i.e. standard deviation or standard error.

ABOi: ABO incompatible; AHG-CDC: anti human globulin complement dependent cytotoxicity; AMR: antibody-mediated rejection; AR: acute rejection; CDC: complement-dependent cytotoxicity; CG: chronic glomerulopathy; CMV: cytomegalovirus; DD: deceased donor; DSA: donor-specific alloantibody; ESRD: end stage renal disease; FC: flow cytometry; FCXM: flow cytometry crossmatch; GFR: glomerular filtration rate; IVIG: intravenous immunoglobulin; LD: living donor; MMF: mycophenolate mofetil; NR: not reported; NS: not significant; NXM: negative crossmatch; PBK: polyoma BK; PP: plasmapheresis; PRA: panel reactive antibody; PXM: positive crossmatch; SA: surface area; SD: standard deviation; TG: transplant glomerulopathy; XM: crossmatch.

Other protocol

Protocols classified as 'other' for the purposes of this report refer to those which make use of modified desensitisation and therefore cannot be classified as high-dose IVIG or plasmapheresis based. These studies were included due to the strength of their patient cohort ($n \geq 20$) and because they provide additional comparative evidence for positive crossmatch kidney transplantation, despite their inconsistencies with the 'standard' high-dose IVIG or plasmapheresis and low-dose IVIG protocols. Furthermore, these studies assist in determining if desensitisation (in any form) is effective in reducing the risks associated with positive crossmatch patients. Three non-randomised comparative studies, using other protocol, were included.

Burns et al (2008), monitored donor specific alloantibody levels over 28 days following transplantation searching for any relationship with acute humoral rejection in positive crossmatch patients with confirmed HLA specificity. Patients were divided into two groups depending on DSA levels, high DSA (n = 41) and low (n = 29). High DSA patients received pre-emptive plasmapheresis only while low DSA patients were only monitored and only received desensitisation in response to a rejection episode. The aim was to achieve T- and B-cell FC crossmatch channel shift < 300 in high DSA patients on the day of transplantation.

Akalin et al (2008), enrolled 35 antihuman globulin-CDC (B cell) and/or FC (T and/or B cell) positive patients. Patients were divided into three groups based on the strength of their DSA. Group 1 patients had weak or moderate DSA levels (n = 12), group 2 patients had strong DSA levels (n = 9) and group 3 also had strong DSA levels (n = 14). In this study, desensitisation was performed preoperatively, intraoperatively and postoperatively. All patients received high-dose IVIG (1 g/kg of body weight) intraoperatively and low-dose IVIG (500 mg/kg of body weight) on postoperative days 1 and 2. Group 3 patients awaiting a living donor kidney received four to eight additional plasmapheresis treatments over a 2-3 week period prior to surgery until DSA levels were reduced to a moderate or weak level. Patients who received deceased donor kidneys in that group received three additional sessions of plasmapheresis every other day starting on postoperative day 1. The goal of desensitisation in this study was for patients to have a negative CDC T-cell crossmatch before transplantation.

Mai et al (2009) included 94 patients with antihuman globulin-CDC negative crossmatch separated into three groups depending on their PRA levels. Group 1 consisted of 58 patients with PRA levels <20% receiving primary transplants (low risk group), group 2 consisted of 16 patients with PRA levels >20% and negative crossmatch receiving retransplants and group 3 consisted of 20 patients with PRA levels >20% and positive crossmatch receiving retransplants (high risk group). Desensitisation involved induction and maintenance in all groups with group 3 patients receiving additional low-dose IVIG (500 mg/kg of body weight) intraoperatively and on postoperative days 1 and 2. The goal of desensitisation in

this study was negative antihuman globulin-CDC T-cell crossmatch at the time of transplantation.

Effectiveness

In the study by Burns et al (2008), most high DSA patients achieved crossmatch channel shifts <300 prior to transplant. Acute humoral rejection occurred in 40% of patients in the high DSA group and 31% of patients in the low DSA group (P value not reported). Overall actuarial graft and patient survival was 91% and 96%, respectively at one year and 84% and 94%, respectively at three years.

The time course of serum DSA levels was stratified into four groups: low DSA patients who experienced acute humoral rejection (n = 7), low DSA patients without acute humoral rejection (n = 5), high DSA patients who experienced acute humoral rejection (n = 12) and high DSA patients without DSA (n = 12). In patients who did not experience acute humoral rejection (both low and high DSA), B-cell FC crossmatch channel shifts remained below 300 postoperatively. Low DSA patients who experienced acute humoral rejection had an elevated mean B-cell FC crossmatch channel shift on postoperative day 10, which subsequently fell below 300 by day 28, probably in response to rescue therapy. All but one high DSA patient experienced a B-cell FC crossmatch channel shift <300 at baseline, by day four, four patients had channel shifts >300 (despite pre-emptive plasmapheresis) and three of these were diagnosed with acute humoral rejection. By day 10, 11/12 patients had acute humoral rejection and by 28 days only three had B-cell FC crossmatch channel shifts <300. In this group, five patients required splenectomy due to refractory rejection that did not respond to initial rescue therapy (plasmapheresis alone). Despite splenectomy, B-cell FC crossmatch channel shifts for these patients were still >300 (mean 353 ± 94.2) at 28 days compared to a mean of 301 ± 103.6 for those who responded to initial rescue therapy (Burns et al 2008).

Therefore, it appears patients with high DSA levels, regardless of pre-emptive treatment, experience worse results than positive crossmatch patients with low DSA levels. Despite this, the usefulness of the standard plasmapheresis protocol (with low-dose IVIG) cannot be discounted in patients with high DSA levels as the immunomodulatory effects of IVIG may play an important role in these patients.

In the study by Akalin et al (2008), rejection occurred with the greatest frequency in group 2 where patients had strong DSA levels and did not receive pre-emptive desensitisation. Although the statistical significance of this observation was not reported, the margin by which the frequency of rejection was increased by in group 2 was considerable. Patient survival was 100% in groups 1 and 2 at median follow-up of 16 and 22 months, and 93% in group 3 at a median follow-up of 12 months. Graft survival at the same time was 100% in group 1, 78% in group 2 and 86% in group 3.

Addition of pre-transplant plasmapheresis in living donor recipients prevented the development of antibody-mediated rejection. Similarly, post-transplant plasmapheresis in deceased donor recipients significantly reduced the incidence of antibody-mediated rejection. This study also demonstrated that in patients with strong DSA, there exists a higher risk for developing acute antibody-mediated rejection compared with patients that have low DSA levels, despite intraoperative high-dose IVIG desensitisation. Furthermore, the addition of peri-transplantation plasmapheresis to a high-dose IVIG intraoperative treatment has the ability to decrease the incidence of acute rejection. The results from this study highlight the importance of determining the strength of DSA prior to transplantation to determine appropriate desensitisation.

In the study by Mai et al (2009), acute rejection occurred in significantly more patients with PRA levels >20% and positive crossmatch (50%) compared with patients with low PRA levels (12%) or high PRA levels and negative crossmatch (25%; $P < 0.01$). Antibody-mediated rejection also occurred significantly more in patients with high PRA and positive crossmatch (60%) compared with patients with low PRA (2%) or high PRA and negative crossmatch (16%; $P < 0.01$). Steroid resistant rejection was also highest in group 3 (high PRA and positive crossmatch); however, the significance of this was not reported. Two-year serum creatinine and glomerular filtration rate, as well as 3-year patient and graft survival were comparable between the groups.

These results show that rejection rate is higher in patients with positive crossmatch, regardless of PRA levels. Overall, all three groups exhibited similar renal function and graft survival indicating the suitability of kidney transplantation in high risk patients.

Safety

Akalin et al (2008) reported chronic allograft nephropathy and transplant glomerulopathy occurred at a greater rate in patients in group 2 compared with the other groups indicating the importance of pre-emptive desensitisation in patients with high DSA levels.

In the study by Mai et al (2009), the rate of infection with Cytomegalovirus, BK viremia and BK viruria was similar between the groups and did not exceed 10%.

Although these studies, utilising other protocol, cannot add to the evidence base of standard desensitisation protocols, they do provide useful evidence in regards to desensitisation in general. It appears that elevated DSA prior to transplantation indicates an increased risk of rejection and should be considered (independent from crossmatch) when deciding on a desensitisation regime. As well as this, these studies demonstrate desensitisation is capable of facilitating similar results to conventional (negative crossmatch) kidney transplants in patients with positive crossmatch, namely those with low DSA levels.

Table 5 Other protocol study results

Study	Patient details	Procedure	Effectiveness outcomes	Safety outcomes
Burns et al (2008) Rochester, United States Level III-2 intervention evidence	**PP (high DSA) group** n = 41 (41 LD) **Monitor (low DSA) group** n = 29 (27 LD, 2 DD) **Baseline DSA (channel shift, B-cell FC)** High DSA: 203 ± 64 (range: 200–565) Low DSA: 375 ± 66 (range: 73–288) P < 0.001 **Crossmatch status** High DSA: T-FCXM and B-FCXM channel shift ≥ 300. 11 (27%) had positive T cell AHG CDC at baseline. Low DSA: T-FCXM or B--FCXM channel shift <300	**Preoperative desensitisation** High DSA group: Mean 6.5 ± 3 (range 1-15) PP treatments. Patients also received 4-7 postoperative PP treatments. Low DSA group: no PP treatment. **Induction/immunosuppression** Induction with antithymocyte globulin (1.5 mg/kg of body weight/ day for 5–7 days) and maintenance immunosuppression with tacrolimus, MMF and prednisone. **Rescue therapy** AHR treated with PP. Patients with severe AHR not responsive to PP (n = 10) underwent laparoscopic splenectomy. **Duration of follow-up** 3 years	**Achievement of crossmatch channel shifts <300 (n, %)** High DSA group: 39/41 (95%). Two patients underwent transplantation with B-XM channel shift of 338 and 328. Low DSA group: N/A **AHR episodes (n, %)** High DSA group: 16/40 (40%) Low DSA group: 9/29 (31%) **Actuarial graft survival for all patients (%)** 1 year: 91% 3 year: 84% **Actuarial patient survival for all patients (%)** 1 year: 96% 3 year: 94% **Time course of serum DSA levels stratified to 4 groups (n = 36)** *Low DSA/AHR- (n=5)* No patient required PP. B-FCXM channel shift <300 maintained. *Low DSA/AHR+ (n=7)* B-FCXM channel shift (mean†)at baseline, day 4, day 10 and day 28: 240 ± 30, 228 ± 127, 486 ± 63, 261 ± 63 *High DSA/AHR- (n=12)* B-FCXM channel shift (mean†)at baseline, day 4, day 10 and day 28: 387 ± 63, 236 ± 108, 211 ± 72, 197 ± 78 *High DSA/AHR+ (n=12)* 11 patients B-FCXM channel shift <300 on transplant day. Five patients B-FCXM channel shift >300 by day 4 (despite postoperative PP) and three with AHR. By day 10, 11 patients had B-FCXM channel shift > 300 and AHR. One patient achieved maximum DSA by day 28 with AHR. 3/11 patients <300 on day 28. Five required splenectomy due to refractory rejection (no response to PP rescue). Mean channel shift in patients with splenectomy at 28 days 353 ± 94.2 compared with 301 ± 103.6 for those who responded to treatment.	NR
Akalin et al (2008) New York, United States Level III-3 intervention evidence	**Group 1 (High-dose IVIG)** n = 12 (12 LD) Patients with weak/moderate DSA. **Group 2 (High-dose IVIG)** n = 9 (5 LD)	**Desensitisation** All patients: 1 g/kg of body weight IVIG given during transplant surgery. Group 3: LD patients 4–8 sessions of pre-transplant PP over 2–3 weeks until DSA reduced to moderate or weak followed by	**Acute rejection (%)** Group 1: 0 (0%) Group 2: 6 (66%) – 4 DD and 2 LD patients Group 3: 1 (7%) – 1 DD patient **AMR (%)**	**Non-tissue invasive cytomegalovirus disease** Group 1: 1 (8%) responded to treatment Group 2: 1 (11%) responded to treatment

	Patients with strong DSA. **Group 3 (PP + high-dose IVIG)** n = 14 (4 LD) Patients with strong DSA. **Crossmatch status** All patients: AHG-CDC T cell negative. AHG-CDC B cell or FC T and/or B cell positive. **PRA levels (median)** Group 1: 54% (range 14 to 83) Group 2: 87% (14 to 100) Group 3: 83% (27 to 100)	transplant. DD patients received 3 sessions of PP every other day starting on postoperative day 1. **Induction/immunosuppression** All patients: Thymoglobulin (1.5 mg/kg of body weight/day, for 5 days) induction therapy, with tacrolimus, MMF and steroid taper. Methylprednisolone (500mg) initiated intraoperatively, followed by oral prednisone taper to 10mg/day by 2–3 months and 5mg/day by 4–6 months. **Postoperative treatment** All patients: 500 mg/kg of body weight IVIG on postoperative days 1 and 2. **Rescue therapy** AMR treated with pulse methylprednisolone (250mg, 3 days); 4–8 sessions PP (each session followed by 500 mg/kg of body weight IVIG); single dose rituximab (375mg/m^2) following PP and IVIG. ACR (Ia and Ib) treated with pulse methylprednisolone (250mg, 3 days), grade ≥IIa with thymoglobulin (1.5 mg/kg of body weight for 5–7 days). **Duration of follow-up (median months)** Group 1: 16 (range: 8–35) Group 2: 22 (range: 8–31) Group 3: 12 (range: 6–18)	Group 1: 0 (0%) Group 2: 4 (44%) Group 3: 1 (7%) **ACR (%)** Group 1: 0 (0%) Group 2: 2 (22%) Group 3: 0 (0%) **Patient survival (n, %)** Group 1: 12 (100%) Group 2: 9 (100%) Group 3: 13 (93%) 1 death (gastrointestinal bleeding with stable kidney graft) **Graft survival (n, %)** Group 1: 12 (100%) Group 2: 7 (78%) 1 acute AMR at 3 months, 1 sepsis and acute tubular necrosis at 10 months Group 3: 12 (86%) 1 due to donor related factors **Patients who lost all DSA at last clinic visit (n, %)** Group 1: 7/10 (70%) Group 2: 4 (44%) Group 3: 6 (43%) **Serum creatinine (median, mg/dl)** Group 1: 1.1 (range: 0.6–2.8) Group 2: 1.2 (range: 1.0–4.5) Group 3: 1.2 (range: 0.7–1.9)	Group 3: 0 (0%) **Biopsy-proven polyoma nephropathy** Group 1: 0 (0%) Group 2: 0 (0%) Group 3: 1 (7%) at 14 months with stable renal function **Cryptococcal meningitis** Group 1: 1 (8%) at 9 months and responded to treatment Group 2: 0 (0%) Group 3: 0 (0%) **Chronic allograft nephropathy (n, %)** Group 1: 0 (0%) Group 2: 4 (44%) Group 3: 1 (14%) **Transplant glomerulopathy (n, %)** Group 1: 0 (0%) Group 2: 2 (22%) with AMR Group 3: 1 (7%) with AMR
Mai et al (2009) Jacksonville, United States Level III-2 intervention evidence	**Induction + maintenance (group 1)** n = 58 (5 LD) – low risk group **Induction + maintenance (group 2)** n = 16 (2 LD) **Induction + maintenance + low-dose IVIG (group 3)** n = 20 (5 LD) – high risk group **PRA levels** Group 1: < 20% all patients Group 2: > 20% all patients Group 3: > 20% all patients	**Desensitisation** Group 3: Low dose IVIG (500 mg/kg of body weight) intraoperatively and on days 1 and 2. **Induction/immunosuppression** All patients induced with Thymoglobulin 1.5 mg/kg of body weight intraoperatively (day 0) and 1-1.5 mg/kg of body weight on days 1, 2 and 4 (total dose 4.5–6 mg/kg of body weight). Maintenance immunosuppression consisted of tacrolimus (10–15ng/mL in first 3 months, 5–10ng/mL thereafter), MMF (1000mg 2x/day) and corticosteroids (tapered to 20mg by day 6 and beginning on	**Acute rejection (n, %)** Group 1: 7 (12%) Group 2: 4 (25%) Group 3: 10 (50%) *P* < 0.01, group 3 versus group 1 or 2 **AMR (n, %)** Group 1: 1 (2%) Group 2: (16%) Group 3: 3 (60%) *P* < 0.01, group 3 versus group 1 or 2 **Steroid resistant rejection (n, %)** Group 1: 1 (2%)	**Cytomegalovirus infection (n, %)** Group 1: 4 (7%) Group 2: 1 (6%) Group 3: 2 (10%) **BK viruria (n, %)** Group 1: 6 (10%) Group 2: 1 (6%) Group 3: 2 (10%) **BK viremia (n, %)** Group 1: 6 (10%) Group 2: 0 (0%)

	Crossmatch status Group 1: AHG-CDC T negative Group 2: AHG-CDC T/FC negative Group 3: AHG-CDC T negative, FC positive	day 30 tapered to 5mg by day 110). **Rescue therapy** ACR treated with methylprednisolone (5 mg/kg of body weight, maximum dose 500mg) intravenously for 5 days. Steroid resistant rejection treated with Thymoglobulin or muromonabCD3 or both. AMR treated with PE (one volume exchange with albumin replacement daily for 3 days and then every other day as needed) and IVIG (100 mg/kg of body weight after each PE session) for 3 days. Persistent AMR treated with one dose anti-CD20 antibody (375mg/m^2) in addition to another 3 day cycle of PE and IVIG. **Duration of follow-up** Minimum 1 year	Group 2: 1 (6%) Group 3: 3 (15%) **Graft loss (n, %)** Group 1: 11 (19%) Group 2: 2 (13%) Group 3: 2 (10%) **Dialysis post-transplant (n, %)** Group 1: 23 (40%) Group 2: 5 (31%) Group 3: 6 (30%) **Length of say (mean days ± SD)** Group 1: 7.31 ± 0.42 Group 2: 7.63 ± 0.89 Group 3: 7.45 ± 1.2 **Serum creatinine at 4, 12 and 24 months (mean mg/dL ± SD)** Group 1: 1.68 ± 0.89, 1.57 ± 0.13, 1.55 ± 0.12 Group 2: 1.15 ± 0.11, 1.64 ± 0.20, 1.45 ± 0.25 Group 3: 1.46 ± 0.11, 1.47 ± 0.10, 1.18 ± 0.29 **Glomerular filtration rate 4, 12 and 24 months (mean mL/min ± SD)** Group 1: 54.4 ± 3.2, 61.3 ± 3.9, 55.8 ± 3.6 Group 2 50.1 ± 3.5, 50.9 ± 5.7, 54.0 ± 11.5 Group 3 50.1 ± 4.0; 50.3 ± 7.1, 50.2 ± 11.6	Group 3: 0 (0%)

† The authors did not specify what measure of dispersion was used, i.e. standard deviation or standard error.
ACR: acute cellular rejection; AHG CDC: antihuman globulin complement-dependent cytotoxicity; AHR: acute humoral rejection; AMR: antibody-mediated rejection; DD: deceased donor; DSA: donor-specific alloantibody; FC: flow cytometry; FCXM: flow cytometry crossmatch; IVIG: intravenous immunoglobulin; LD: living donor; MMF: mycophenolate mofetil; NR: not reported; PE: plasma exchange; PP: plasmapheresis; PRA: panel reactive antibody;, SD: standard deviation; XM: crossmatch.

Cost Analysis

An economic analysis performed by Higgins et al (2006) found that standard (negative crossmatch, blood group compatible) kidney transplantation was more cost effective than dialysis by approximately £15,000 per annum over a 10-year period (Higgins et al 2006). Similarly, a meta-analysis conducted using studies published from 1968 to 1998, found the cost of in-centre haemodialysis to be between US$55,000 and US$80,000 per life-year saved, compared with kidney transplantation at US$10,000 per life-year saved. This resulted in a saving of US$45,000 to US$70,000 per patient per year, in favour of transplantation (Higgins et al 2006). While the above data do not strictly relate to positive crossmatch kidney transplantation, they at least give some indication of the expense of dialysis in comparison to kidney transplantation.

The John Hopkins University estimated the additional cost of plasmapheresis and IVIG on top of standard transplantation was US$45,000 (Higgins et al 2006). Information provided by another study supports this estimate, costing IVIG at approximately US$35/g; therefore, for a 70kg person, receiving a 2 g/kg of body weight dose, the cost would be US$4,900 and a total of US$19,600 for a 4-day course (Jordan et al 2004). Despite this, transplantation, even across the antibody barrier, was considerably more cost-effective than prolonged dialysis.

The John Hopkins group also estimated that by removing half of the highly sensitised patients from dialysis and performing successful transplantation, Medicare (the sole provider of ESRD services in the US) would experience a cost saving of US$1.4 billion over a three year period (Jordan et al 2004).

It is not only the financial costs of maintaining sensitised patients on dialysis for many years that are significant, the emotional effects are also great. Therefore, early transplantation, facilitated by desensitisation, would result in considerable cost savings, reduced morbidity and mortality and improved quality of life (Jordan et al 2003).

Costs must also be factored in for living donors. Even in the case of a successful transplantation the organ donor is likely to encounter medical costs and possible loss of income during their convalescence.

The infrastructure required to perform positive crossmatch kidney transplantation are significant but within reach of most major transplantation centres (Dean et al 2005).

Ethical Considerations

Informed Consent

The general consensus of the available positive crossmatch kidney transplantation literature is that pre-transplant patient education is paramount, as well as donor education in the case of living kidney donation. Patients should be aware of the risks of the procedure, including graft loss due to non-compliance with desensitisation and immunosuppression regimens, as well as the benefits, including the opportunity of regained kidney function and independence from dialysis. Living donors should also be aware of the morbidity they face, including medical costs and loss in income, as well as the likelihood of unsuccessful transplantation.

Access Issues

Due to the complexity of positive crossmatch kidney transplantation, the procedure should be conducted in specialised hospitals with adequate equipment and experienced personnel. In particular, the hospital should be equipped with a histocompatibility laboratory capable of monitoring donor-specific antigen levels and a renal pathologist experienced in the diagnosis of antibody-mediated rejection (Montgomery and Zachary 2004).

There is also the ethical question of what graft failure threshold is acceptable for a sensitised patient to come off dialysis and be given access to a kidney. Ideally, studies comparing the long-term outcomes of sensitised patients remaining on dialysis and those undergoing desensitisation and transplantation should be used to determine this.

Training

Because positive crossmatch kidney transplantation is not in use in Australia there is currently no nationally standardised protocol for the procedure. The same appears to be the case for positive crossmatch kidney transplantation in other countries where its use is more widespread. For example, in the United States various transplantation centres perform the procedure but the desensitisation protocol and standard immunosuppression regimen used varies per centre.

Essential requirements for the performance of positive crossmatch kidney transplantation are listed previously and include a histocompatibility laboratory capable of monitoring donor specific antigen levels and a renal pathologist experienced in the diagnosis of antibody-mediated rejection (Montgomery and Zachary 2004).

Clinical Guidelines

At the time of writing, there were two British guideline documents available for positive crossmatch kidney transplantation. One addressed antibody incompatible transplantation, including positive crossmatch kidney transplantation and ABO incompatible kidney transplantation (Higgins et al 2006) and the other living donor kidney transplantation in high risk adult recipients (British Transplantation Society consensus group 2008), where high risk recipients are defined as patients with a higher risk of death, complications or graft failure due to pre-existing co-morbidities or immunological status.

The first guideline document, by the British Transplantation Society, made recommendations for transplant units, histocompatibility and haematology laboratories, commissioners, audit and research pertaining to kidney transplantation across the antibody and ABO barrier. The second document was produced by the British Transplantation Society in conjunction with clinicians, nurses and patients, including the Renal Association and the Clinical Affairs Board (for review of the guidelines). These recommendations focussed on the recipient's pre-transplant agreement and understanding of the procedure and risks involved, rather than the desensitisation protocol.

Guidelines for the use of positive crossmatch kidney transplantation in Australia are needed. The existing UK documents can be used as a backbone, as they cover much of the procedure and patient care involved with it. Ideally, a more rigid description of the ideal protocol should be included; however, until high quality studies comparing desensitisation modalities are published this is not possible.

Limitations of the Assessment

Methodological issues and the relevance or currency of information provided over time are paramount in any assessment carried out in the early life of a technology.

Horizon Scanning forms an integral component of Health Technology Assessment. However, it is a specialised and quite distinct activity conducted for an entirely different purpose. The rapid evolution of technological advances can in some cases overtake the speed at which trials or other reviews are conducted. In many cases, by the time a study or review has been completed, the technology may have evolved to a higher level leaving the technology under investigation obsolete and replaced.

A Horizon Scanning Report maintains a predictive or speculative focus, often based on low level evidence, and is aimed at informing policy and decision makers. It is not a definitive assessment of the safety, effectiveness, ethical considerations and cost effectiveness of a technology.

In the context of a rapidly evolving technology, an Horizon Scanning Report is a 'state of play' assessment that presents a trade-off between the value of early, uncertain information, versus the value of certain, but late information that may be of limited relevance to policy and decision makers.

This report provides an assessment of the current state of development of positive crossmatch kidney transplantation, its present and potential use in the Australian public health system, and future implications for the use of this technology.

Search Strategy used for the Report

The sources utilised in this assessment are listed in Table 6. The medical literature was searched with the search terms outlined in Table 7 to identify relevant studies up to March 2009 in English only. In addition to this, major international health technology assessment databases and clinical trial registers were searched.

Table 6: Literature sources utilised in assessment

Source	Location
Electronic databases	
AustHealth	University of Adelaide library
Australian Medical Index	University of Adelaide library
CINAHL	University of Adelaide library

Cochrane Library – including Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, the Cochrane Central Register of Controlled Trials (CENTRAL), the Health Technology Assessment Database, the NHS Economic Evaluation Database	University of Adelaide library
Current Contents	University of Adelaide library
Embase	Personal subscription
Pre-Medline and Medline	University of Adelaide library
PyscINFO	Personal subscription
RACS electronic library	Personal subscription
Internet	
Blue Cross and Blue Shield Association's Technology Evaluation Center	http://www.bcbs.com/tec/
Canadian Agency for Drugs and Technologies in Health	http://www.cadth.ca
Current Controlled Trials metaRegister	http://www.controlled-trials.com/
EuroScan	http://www.euroscan.bham.ac.uk/
Health Technology Assessment International	http://www.htai.org/
International Network for agencies for Health Technology Assessment	http://www.inahta.org
Medicines and Healthcare products Regulatory Agency (UK)	http://www.mhra.gov.uk/
US Food and Drug Administration, Center for Devices and Radiological Health	http://www.fda.gov/cdrh/index.html
US Food and Drug Administration, Manufacturer and User Facility Device Experience Database	http://www.fda.gov/cdrh/maude.html
UK National Research Register	http://www.nrr.nhs.uk/
Websites of specialty organisations	http://www.anzdata.org.au

Table 7: Search terms utilised

Search terms
MeSH
Kidney transplantation; desensitization, immunologic
Text words
Kidney transplant*; renal transplant*; desensiti?*; positive cross?match; P?XM; +?XM
Limits
English, human

Availability and Level of Evidence

The medical literature (Table 6) was searched utilising the search terms outlined in Table 7 to identify relevant studies and reviews, until March 2009. In addition, major international health assessment databases were searched.

A total of 10 studies were retrieved for inclusion in this horizon scanning report; one was a randomised controlled trial, seven were non-randomised comparative studies and two were case series. The profiles of the included studies are summarised in Appendix B.

Sources of Further Information

Searches of clinical trial registers revealed two ongoing single-centre trials and one Australian multicentre randomised controlled trial. One single-centre trial commenced in November 2004 and anticipated testing the clinical and laboratory observations of IVIG in highly sensitised patients. The study intended to enrol 50 patients with an identified positive crossmatch and high PRA levels to be treated with cytomegalovirus-immune IVIG in combination with plasmapheresis at the Loma Linda University Medical Center, United States. The primary outcome of the trial is the conversion of a positive crossmatch to a negative crossmatch prior to transplantation. The estimated completion date of this trial is November 2012. Currently, the study is in the recruitment stage.

The second single-centre trial commenced in July 2007 and planned to study 17 highly-sensitised patients with established positive crossmatches with their potential donors at the Academic Medical Centre in the Netherlands. Patients were given one dose of rituximab, followed by a full course of IVIG infusions (2 g/kg of body weight, maximum dose 140g) over a four month period. If these patients' crossmatches were converted to negative at this time they received another two doses of Rituximab prior to undergoing transplantation. If their crossmatch remained positive 'rescue' plasmapheresis was given (maximum seven courses) until a negative crossmatch was achieved and transplantation performed, subsequent to two additional doses of rituximab. The anticipated completion date for this study is January 2010; the current status of the trial is ongoing.

The Australian multicentre randomised controlled trial commenced in April 2006 and proposed to test whether rituximab will safely lower antibody mediated rejection rate when added to "standard" therapy. The trial also aimed to test whether rituximab facilitates more transplants by enabling more patients to achieve negative crossmatch against their donor, compared with standard therapy alone. The trial is to take place in one centre in New South Wales and two centres in Victoria, with an estimated enrolment of 192 patients. Patients with positive T- and/or B-cell CDC or FC crossmatch and DSA identified by solid phase assay at screening were randomly allocated to receive a single dose of intravenous rituximab (375 mg/m^2) two weeks prior to transplantation in addition to standard therapy. Standard therapy included plasma exchange/IVIG plus mycophenolate mofetil before and immediately after transplantation, followed by standard care immunosuppressive regimen. Once the patient achieved negative CDC crossmatch they proceeded to live donor transplantation. The anticipated completion date of this trial was January 2009; however, the trial is still recruiting patients.

The Queen Elizabeth Hospital (Adelaide, South Australia) and University of Adelaide (Adelaide, South Australia) produced a poster describing their

experience with three positive crossmatch kidney transplantations. This poster was presented at the Annual Meeting of the Australian and New Zealand Society of Nephrology 2006 in Melbourne, Australia. All three patients (1 woman, 2 men) had multiple previous failed transplantations, and subsequent peak PRA of 99%, 83% and 60%, respectively. All three patients initiated desensitisation with rituximab followed by up to five successive cycles of plasmapheresis and low-dose IVIG (100mg/kg of body weight) and a final cycle of plasma exchange and high-dose IVIG (2g/kg of body weight) immediately prior to transplantation. Induction and maintenance immunosuppression occurred perioperatively. Transplantation occurred when patients became CDC crossmatch negative and DSA were not detectable by ELISA. Postoperatively, three cycles of plasmapheresis were undertaken (on days one, two and three), as well as administration of IVIG (100mg/kg of body weight) on day 30.

These three highly sensitised patients were desensitised using the above protocol and subsequently received renal transplantation with good short-term outcome. At the time this poster was presented, all patients had functioning allograft, persistent B-cell depletion, no infective complication particularly that of cytomegalovirus and BK virus and all post-transplant biopsies were negative for C4d deposition.

Other pertinent studies reporting the use of positive crossmatch kidney transplantation, that were excluded from the current review due to insufficient patient numbers or study design (i.e. case report), are listed below.

Gloor JM, Degoey SR, Pineda AA, Moore SB, Prieto M, Nyberg SL, Larson TS, Griffin MD, Textor SC, Velosa JA, Schwab TR, Fix LA, Stegall MD. Overcoming a positive crossmatch in living-donor kidney transplantation. *American Journal of Transplantation* 2003; **3**(8): 1017-1023.

Glotz D, Antoine C, Julia P, Suberbielle-Boissel C, Boudjeltia S, Fraoui R, Hacen C, Duboust A, Bariety J. Desensitization and subsequent kidney transplantation of patients using intravenous immunoglobulins (IVIg). *American Journal of Transplantation* 2002; **2**(8): 758-760.

Hamza A. Transduction of a positive crossmatch into a negative crossmatch through immunosuppressive therapy. *Transplantationsmedizin: Organ der Deutschen Transplantationsgesellschaft* 2005; **17**(1): 13-18.

Jordan SC, Vo AA, Nast CC, Tyan D. Use of high-dose human intravenous immunoglobulin therapy in sensitized patients awaiting transplantation: the Cedars-Sinai experience. *Clinical Transplants* 2003: 193-198.

Kim SM, Lee C, Lee JP, Kim EM, Ha J, Kim SJ, Park MH, Ahn C, Kim YS. Kidney transplantation in sensitized recipients; a single center experience. *Journal of Korean Medical Science* 2009; **24**(Suppl): S143-S147.

Thielke J. Highly successful living donor kidney transplantation after conversion to negative of a previously positive flow-cytometry cross-match by pretransplant plasmapheresis. *Transplantation Proceedings* 2005; **37**(2): 643-644.

Yoon HE, Hyoung BJ, Hwang HS, Lee SY, Jeon YJ, Song JC, Oh EJ, Park SC, Choi BS, Moon IS, Kim YS, Yang CW. Successful renal transplantation with desensitization in highly sensitized patients: a single center experience. *Journal of Korean Medical Science* 2009; **24**(Suppl): S148-S155.

Conclusions

Positive crossmatch kidney transplantation using plasmapheresis or IVIG based protocols offer the potential of kidney transplantation to sensitised patients who would otherwise remain on dialysis indefinitely.

The studies included in this report, presented an overview of the evidence for the two most commonly used desensitising protocols, high-dose IVIG and plasmapheresis plus low-dose IVIG. Unfortunately, due to various factors, namely the differences among the studies with respect to the definition of positive crossmatch status and what constitutes successful desensitisation, the drug protocols used and patient characteristics, it was not possible to conduct a meaningful comparison between the studies. Despite these shortcomings, the data from the included studies demonstrated that kidney transplantation in crossmatch positive and highly sensitised patients is potentially feasible.

The data available from one level II and one level IV study on the high-dose IVIG protocol suggested that high dose IVIG-based desensitisation produces a transient reduction in anti-HLA antibodies. While rejection rates were approximately 30% in both studies, and were significantly higher than for the placebo group in the comparative study, the overall graft failure rate did not differ from patients treated with placebo after 3 years.

Two level III studies compared the results of patients receiving plasmapheresis plus low-dose IVIG-based protocols with crossmatch negative controls. In terms of rejection rates, there appears to be significantly more acute rejection episodes in patients who have undergone desensitisation using plasmapheresis-based protocols as opposed to negative crossmatch patients who did not require desensitisation. The results for long-term (> 1 year) graft survival were equivocal.

Only one study (level III evidence) compared the plasmapheresis low-dose IVIG-based protocol with the high-dose IVIG-based protocol. The results suggested that the plasmapheresis-based protocols result in better outcomes. Namely, successful desensitisation rates and humoral rejection rates were better with the use of plasmapheresis low-dose IVIG protocols, although the patient and graft survival rates were not reported for the individual treatment groups. Furthermore, the study suggests that in patients who are non responders to the IVIG only protocol, desensitisation may be an option using a plasmapheresis based protocol.

In terms of safety and kidney function, the use of either desensitisation protocol did not appear to have any significant adverse events associated.

The inclusion of studies with mixed or other protocols added to the evidence available by confirming that the use of desensitisation is not associated with any major adverse events. Furthermore, these studies added support to data from the

previous studies suggesting that desensitisation success and the clinical outcomes following transplantation are associated with pre-operative sensitisation levels.

While positive crossmatch kidney transplantation may be a potentially useful strategy for expanding the availability of donor organs for sensitised patients, the desensitisation protocols used to facilitate this are still in a developmental stage. It is still not clear exactly how the protocols work and which blood factors are pre-eminent in the rejection of foreign tissue in transplant recipients. Thus, it is not completely clear which factors need to be targeted, and which factors affect the success of the protocols.

Appendix A: Levels of Evidence

Designation of levels of evidence according to type of research question

Level	Intervention [§]	Diagnosis [**]	Prognosis	Aetiology [†††]	Screening
I [*]	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard, [§§] among consecutive patients with a defined clinical presentation [††]	A prospective cohort study [***]	A prospective cohort study	A randomised controlled trial
III-1	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard, [§§] among non-consecutive patients with a defined clinical presentation[††]	All or none [§§§]	All or none [§§§]	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)
III-2	A comparative study with concurrent controls: Non-randomised, experimental trial [†] Cohort study Case-control study Interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for Level II and III-1 evidence	Analysis of prognostic factors amongst untreated control patients in a randomised controlled trial	A retrospective cohort study	A comparative study with concurrent controls: Non-randomised, experimental trial Cohort study Case-control study
III-3	A comparative study without concurrent controls: Historical control study Two or more single arm study [‡] Interrupted time series without a parallel control group	Diagnostic case-control study [††]	A retrospective cohort study	A case-control study	A comparative study without concurrent controls: Historical control study Two or more single arm study
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard) [‡‡]	Case series, or cohort study of patients at different stages of disease	A cross-sectional study	Case series

Tablenotes

* A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence.

§ Definitions of these study designs are provided on pages 7-8 *How to use the evidence: assessment and application of scientific evidence* (NHMRC 2000b*)*.

† This also includes controlled before-and-after (pre-test/post-test) studies, as well as indirect comparisons (ie. utilise A vs B and B vs C, to determine A vs C).

‡ Comparing single arm studies ie. case series from two studies.

** The dimensions of evidence apply only to studies of diagnostic accuracy. To assess the effectiveness of a diagnostic test there also needs to be a consideration of the impact of the test on patient management and health outcomes. See *MSAC (2004) Guidelines for the assessment of diagnostic technologies*. Available at: www.msac.gov.au .

§§ The validity of the reference standard should be determined in the context of the disease under review. Criteria for determining the validity of the reference standard should be pre-specified. This can include the choice of the reference standard(s) and its timing in relation to the index test. The validity of the reference standard can be determined through quality appraisal of the study. See Whiting P, Rutjes AWS, Reitsma JB, Bossuyt PMM, Kleijnen J. The development of QADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Medical Research Methodology*, 2003, 3: 25.

†† Well-designed population based case-control studies (eg population based screening studies where test accuracy is assessed on all cases, with a random sample of controls) do capture a population with a representative spectrum of disease and thus fulfil the requirements for a valid assembly of patients. These types of studies should be considered as Level II evidence. However, in some cases the population assembled is not representative of the use of the test in practice. In diagnostic case-control studies a selected sample of patients already known to have the disease are compared with a separate group of normal/healthy people known to be free of the disease. In this situation patients with borderline or mild expressions of the disease, and conditions mimicking the disease are excluded, which can lead to exaggeration of both sensitivity and specificity. This is called spectrum bias because the spectrum of study participants will not be representative of patients seen in practice.

‡‡ Studies of diagnostic yield provide the yield of diseased patients, as determined by an index test, without confirmation of accuracy by a reference standard. These may be the only alternative when there is no reliable reference standard.

*** At study inception the cohort is either non-diseased or all at the same stage of the disease.

§§§ All or none of the people with the risk factor(s) experience the outcome. For example, no smallpox develops in the absence of the specific virus; and clear proof of the causal link has come from the disappearance of small pox after large-scale vaccination.

††† If it is possible and/or ethical to determine a causal relationship using experimental evidence, then the 'Intervention' hierarchy of evidence should be utilised. If it is only possible and/or ethical to determine a causal relationship using observational evidence (ie. cannot allocate groups to a potential harmful exposure, such as nuclear radiation), then the 'Aetiology' hierarchy of evidence should be utilised.

Note 1: Assessment of comparative harms/safety should occur according to the hierarchy presented for each of the research questions, with the proviso that this assessment occurs within the context of the topic being assessed. Some harms are rare and cannot feasibly be captured within randomised controlled trials; physical harms and psychological harms may need to be addressed by different study designs; harms from diagnostic testing include the likelihood of false positive and false negative results; harms from screening include the likelihood of false alarm and false reassurance results.

Note 2: When a level of evidence is attributed in the text of a document, it should also be framed according to its corresponding research question eg. level II intervention evidence; level IV diagnostic evidence; level III-2 prognostic evidence etc.

Hierarchies adapted and modified from: NHMRC 1999; Lijmer et al 1999; Phillips et al 2001; Bandolier editorial 1999)

Appendix B: Profiles of studies

Profiles of included studies

Study	Location	Study design	Study population	Outcomes assessed
High-dose IVIG				
Jordan et al (2003)	Los Angeles, United States	Case series Level IV intervention evidence	**High-dose IVIG:** 43 patients (28 LD) Mean age: 42 (range: 1.5–75) *Inclusion criteria* Highly sensitised patients, positive CDCXM with donor *Exclusion criteria* Patients who showed no *in vitro* inhabitation of PXM or PRA activity *patients were recruited from a single centre. All patients requested the compassionate use of IVIG in an attempt to improve their chances of transplantation	Patient survival, graft survival, rejection-free graft survival
Jordan et al (2004)	Los Angeles, United States	Double-blinded RCT Level II intervention evidence	**High-dose IVIG:** 48 patients Mean age ± SE: 39 ± 1.6 (range: 20–73) **Placebo:** 50 patients Mean age ± SE: 42.5 ± 1.6 (range: 21-69) *Inclusion criteria* Highly sensitised patients (PRA ≥50% monthly for 3 months) with ESRD *Exclusion criteria* Patients who failed to initiate therapy or who received crossover therapy *patients were recruited from 12 centres. Centre-blocked randomisation occurred. Patients and assessors were blinded.	Adverse experiences (including infusion symptoms, such as headaches and reaction), transplantation rate, graft survival, allograft rejection, patient survival, PRA changes
Apheresis + low-dose IVIG				
Haririan et al (2008)	Baltimore, United States	Comparative cohort study Level III-2 intervention evidence	**PE followed by IVIG plus standard immunosuppressive drugs:** 41 (PXM) Mean age ± SD: 42.8 ± 12.2 **Standard immunosuppressive drugs only:** 41 (NXM) Mean age ± SD: 42.8 ± 12.4 *Inclusion criteria* Positive FCXM with a LD *Exclusion criteria* NR *patients were retrospectively recruited from a single centre	Graft survival, graft loss (including graft failure and death with a functioning graft), patient survival, serum creatinine levels at various time points, incidence of ACR and/or AAMR, BK nephropathy
West-Thielke et al (2008)	Chicago, United States	Non-randomised comparative study Level III-2 intervention evidence	**PP followed by IVIG plus standard immunosuppressive drugs:** 34 AA patients; 22 non-AA patients (PXM) Mean age ± SD: AA PP/IVIG 41.96 ± 9.68, non-AA PP/IVIG: 41.14 ± 12.03 **Standard immunosuppressive drugs only:** 198 AA patients; 228 non-AA patients (NXM) Mean age ± SD: AA 47.02 ± 13.10	Successful desensitisation rate, acute rejection rates, renal function at 1 year, percentage of patients with eGFR <30mL/min/1.73m^2 at 1 year, graft survival at 1 year, patient survival at 1 year

			Inclusion criteria Adult patients with PXM (particularly AA patients), AA patients with NXM *Exclusion criteria* NR *patients were retrospectively recruited from a single centre	
PP + low-dose IVIG *versus* high-dose IVIG				
Stegall et al (2006)	Rochester, United States	Non-randomised comparative study Level III-3 intervention evidence	**PP + low-dose IVIG + anti-CD20:** 32 Mean†: 46.1 ± 16.3 (range, 21–64) **PP + low-dose IVIG +anti-CD20 + monitoring:** 16 Mean†: 45.2 ± 12.8 (range, 22-63) **High-dose IVIG:** 13 Mean†: 44.7 ± 13.1 (range, 34-62) *Inclusion criteria* PXM against their potential LD *Exclusion criteria* Patients with PXM only by T- or B-cell FC techniques but negative CDCXM transplanted without pre-emptive desensitisation, patients with positive B-cell crossmatch and negative T-cell crossmatch *patients were retrospectively recruited from a single centre	Response to desensitisation protocol, humoral rejection rate, graft survival, patient survival
Mixed protocol				
Gloor et al (2006)	Rochester, United States	Non-randomised comparative study Level III-2 intervention evidence	**Induction immunotherapy + IVIG with or without PP:** 37 (PXM) Mean ± SD: 46 ±14 **Induction immunotherapy only:** 198 (ABOc, NXM) Mean ± SD: 50 ± 15 *Inclusion criteria* Recipients of LD kidneys *Exclusion criteria* NR *patients were recruited at a single centre	ACR, transplant glomerulopathy, AAMR, , humoral rejection, chronic histology scores, graft function, graft survival
Vo et al (2008)	Los Angeles, United States	Case series Level IV intervention evidence	**IVIG + Rituximab:** 54 (29 LD recipients) Age range: 16-75 years *Inclusion criteria* Highly sensitised patients with positive CDC or FC crossmatches with their LD, or patients on the United Network of Organ Sharing list >5years with PRA >50% *Exclusion criteria* NR	Patient survival, graft survival, acute rejection episodes, delayed graft function, renal function (mean serum creatinine), absolute lymphocyte counts, adverse events, infection
Other protocol				
Akalin et al (2008)	New York, United States	Non-randomised comparative study Level III-3 intervention evidence	**High-dose IVIG:** 12 (weak/moderate DSA levels) Median age: 51 (range, 24-75) **High-dose IVIG:** 9 (strong DSA) Median age: 51 (range, 34-65) **PP + high-dose IVIG:** 14 (strong DSA) Median age: 51 (range, 30-72) *Inclusion criteria* Patients with CDC T-cell NXM but CDC B-cell and/or FC PXM	Acute rejection rate, patient survival, graft survival, chronic rejection rate, complications

			Exclusion criteria NR	
Burns et al (2008)	Rochester, United States	Non-randomised comparative study Level III-2 intervention evidence	**PP:** 41 patients with baseline T- or B-cell FC crossmatch channel shift ≥300 (high DSA group) Mean†: 44 ± 12.7 (range, 15-65) **Monitoring:** 29 patients with baseline T- or B-cell FC crossmatch channel shift <300 (low DSA group) Mean†: 53 ± 12.1 (range, 26-78) *Inclusion criteria* Patients with PXM receiving ABO compatible kidneys *Exclusion criteria* NR *consecutive patients were retrospectively recruited from a single centre	Hyperacute rejection rate, graft loss, patient survival, DSA levels at various time points
Mai et al (2009)	Jacksonville, United States	Non-randomised comparative study Level III-2 intervention evidence	**RATG induction + maintenance immunosuppression:** 58 patients with PRA < 20% (low-risk) **RATG + maintenance immunosuppression:** 16 patients with PRA > 20%, negative FC crossmatch **RATG + maintenance immunosuppression + high dose IVIG:** 20 patients with PRA > 20%, positive FC crossmatch (high-risk) *Inclusion criteria* Patients with negative CDCXM *Exclusion criteria* Patients who received a liver-kidney transplant, kidney-pancreas transplant, kidney after another organ transplant or were enrolled in a RCT or steroid avoidance protocol, or did not receive RATG as induction *patients were retrospectively recruited at a single centre	Acute rejection, patient survival, graft survival, delayed graft function, graft loss, infection, hospital stay duration, kidney function

† The authors did not specify if mean was reported as ± standard deviation or standard error.
AA: African American; AAMR: acute antibody-mediated rejection; ABOc: ABO compatible, ABOi: ABO incompatible; ACR: acute cellular rejection; CDCXM: complement dependent cytotoxicity crossmatch; DSA: donor-specific alloantibody; eGFR: estimated glomerular filtration rate; ESRD: end stage renal disease; FCXM: flow cytometry crossmatch; IVIG: intravenous immunoglobulin; LD: living donor; NXM: negative crossmatch; PE: plasma exchange; PP: plasmapheresis; PRA: panel reactive antibody; PXM: positive crossmatch; RATG: Rabbit antithymocyte globulin; RCT: randomised controlled trial, SD: standard deviation, SE: standard error.

Appendix C: HTA Internet Sites

AUSTRALIA

- Centre for Clinical Effectiveness, Monash University
  http://www.med.monash.edu.au/healthservices/cce/evidence/

- Health Economics Unit, Monash University
  http://chpe.buseco.monash.edu.au

AUSTRIA

- Institute of Technology Assessment / HTA unit
  http://www.oeaw.ac.at/ita/welcome.htm

CANADA

- Agence d'Evaluation des Technologies et des Modes d'Intervention en Santé (AETMIS) http://www.aetmis.gouv.qc.ca/en/

- Alberta Heritage Foundation for Medical Research (AHFMR)
  http://www.ahfmr.ab.ca/publications.html

- Canadian Coordinating Office for Health Technology Assessment (CCOHTA)
  http://www.cadth.ca/index.php/en/

- Canadian Health Economics Research Association (CHERA/ACRES) – Cabot database http://www.mycabot.ca

- Centre for Health Economics and Policy Analysis (CHEPA), McMaster University http://www.chepa.org

- Centre for Health Services and Policy Research (CHSPR), University of British Columbia http://www.chspr.ubc.ca

- Health Utilities Index (HUI) http://www.fhs.mcmaster.ca/hug/index.htm

- Institute for Clinical and Evaluative Studies (ICES) http://www.ices.on.ca

DENMARK

- Danish Institute for Health Technology Assessment (DIHTA)
  http://www.dihta.dk/publikationer/index_uk.asp

- Danish Institute for Health Services Research (DSI)
  http://www.dsi.dk/engelsk.html

FINLAND

- Finnish Office for Health Technology Assessment (FINOHTA)
  http://finohta.stakes.fi/FI/index.htm

FRANCE

- L'Agence Nationale d'Accréditation et d'Evaluation en Santé (ANAES)

  http://www.anaes.fr/

GERMANY

- German Institute for Medical Documentation and Information (DIMDI) / HTA

  http://www.dimdi.de/dynamic/en/

THE NETHERLANDS

- Health Council of the Netherlands Gezondheidsraad
  http://www.gr.nl/adviezen.php

NEW ZEALAND

- New Zealand Health Technology Assessment (NZHTA)
  http://nzhta.chmeds.ac.nz/

NORWAY

- Norwegian Centre for Health Technology Assessment (SMM)

  http://www.kunnskapssenteret.no/

SPAIN

- Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud "Carlos III" / Health Technology Assessment Agency (AETS)

  http://www.isciii.es/htdocs/investigacion/Agencia_quees.jsp

- Catalan Agency for Health Technology Assessment (CAHTA)
  http://www.aatrm.net/html/en/dir394/index.html

SWEDEN

- Swedish Council on Technology Assessment in Health Care (SBU)

  http://www.sbu.se/www/index.asp

- Center for Medical Health Technology Assessment

  http://www.cmt.liu.se/

SWITZERLAND

- Swiss Network on Health Technology Assessment (SNHTA)

  http://www.snhta.ch/

UNITED KINGDOM

- NHS Quality Improvement Scotland

  http://www.nhshealthquality.org

- National Health Service Health Technology Assessment (UK) / National Coordinating Centre for health Technology Assessment (NCCHTA)

  http://www.hta.nhsweb.nhs.uk/

- University of York NHS Centre for Reviews and Dissemination (NHS CRD)

  http://www.your.ac.uk/inst/crd/

- National Institute for Clinical Excellence (NICE)

  http://www.nice.org.uk/

UNITED STATES

- Agency for Healthcare Research and Quality (AHRQ)

  http://www.ahrq.gov/clinic/techix.htm

- Harvard School of Public Health – Cost-Utility Analysis Registry

  http://www.tufts-nemc.org/cearegistry/index.html

- U.S. Blue Cross / Blue Shield Association Technology Evaluation Center (TEC)

  http://www.bcbs.com/tec/index.html

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