We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

The Evolution of HLA-Matching in Kidney Transplantation

Hung Do Nguyen, Rebecca Lucy Williams, Germaine Wong and Wai Hon Lim

Additional information is available at the end of the chapter

1. Introduction

In this chapter, we will explore the effect of human leukocyte antigen (HLA) matching on renal transplant outcomes. The importance of HLA matching has been clearly established in renal transplantation and the extent of HLA mismatches at the A, B and DR loci form an important part in the assessment of the immunological risk of potential transplant candidates. Increasing number of HLA mismatches has been shown to be associated with poorer graft and patient survival following kidney transplantation but the ongoing importance of this association in the era of more potent immunosuppression and improved donor selection remains unclear. Nevertheless, HLA mismatches remain a crucial component of deceased donor kidney allocation in most countries including the United States and Australia. As a result of major advances in technology, HLA-typing has evolved from serological-based typing to molecular HLA-typing and solid-phase anti-HLA-antibody-detection assays, which have had a major influence in both allocation and outcome of transplanted kidneys. The identification of donor-specific anti-HLA-antibody (DSA) has become standard practice and cross-matching assays to establish the presence of DSA has evolved from complement-dependent cytotoxicity (CDC) assay to the exquisitely sensitive flow-cytometric and solid-phase assays. The availability of these sensitive assays has enable clinicians to perform calculated panel reactive antibody and virtual cross-match, which has led to a more accurate assessment of immunological risk of potential transplant candidates and improvement in the allocation of deceased donor kidneys. Defining the appropriate threshold values for clinically relevant DSA assignment, the ongoing significance of HLA-matching in the presence of DSA and the importance of anti-HLA-Cw, HLA-DQ and HLA-DP antibodies remain poorly defined. Finally, we will discuss the process of identifying acceptable HLA-mismatches using HLAMatchmaker, which determines HLA-

compatibility at the level of polymorphic amino acid triplets or eplets in antibody-accessible regions, and the benefit of acceptable HLA-mismatch programs in improving the transplant potential of highly sensitized transplant candidates.

2. Basic transplant immunology

Immune protection against foreign antigens in humans relies on a coordinated response of both innate and adaptive immune system [1]. The innate system, comprising of anatomical barriers (e.g. skin), phagocytic cells (e.g. macrophages), and soluble compounds (e.g. complements and interferons [IFN]) provide an efficient initial defence against foreign antigens such as donor antigens in solid organ transplantation but this response lacks specificity. In contrast, subsequent adaptive immune response has the ability to create a large diversity of antigen-specific responses upon antigenic challenge to the host, with the development of immunological memory consequent on subsequent exposure to the same antigen. This response involves predominantly lymphocytes and antibodies, and is characteristically more intense, leading to a more rapid elimination of the foreign antigen (Figure 1).
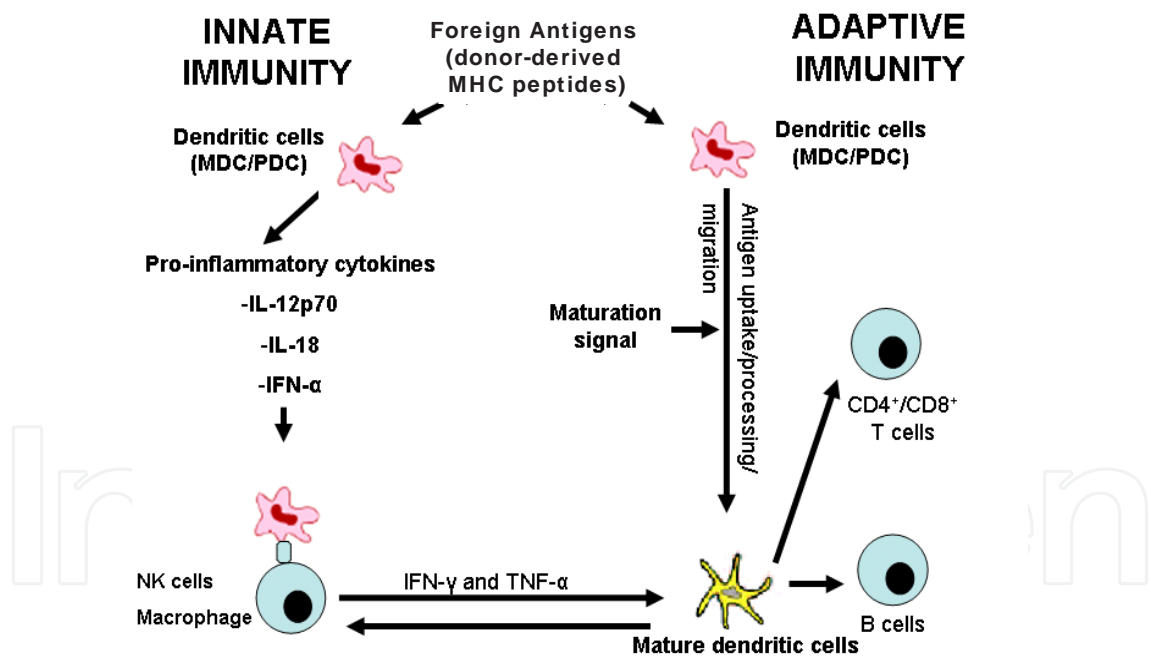

The ability of dendritic cells to coordinate innate and adaptive immune system. Upon exposure to foreign antigens, dendritic cells secrete pro-inflammatory cytokines ± cell-cell contact, activate effector cells including natural killer cells and macrophages (innate immunity). Immature dendritic cells capture and process antigens for presentation to T cells via major histocompatibility complexes. DC undergo maturation and migrate to secondary lymphoid tissues (enhanced by inflammatory cytokines produced by natural killer cells and CD40 ligand expressed by activated T cells). Mature dendritic cells drive the expansion of antigen-specific, major histocompatibility complex-restricted T and B cell responses and the development of immunologic memory (adaptive immunity).

Figure 1. Innate and adaptive immune response to foreign antigens.

2.1. Dendritic cells (Figure 2)

Dendritic cells (DC) are a group of rare, heterogenous population of professional antigen-presenting cells (APC) that can initiate primary immune responses, and hence have the ability to regulate both innate and adaptive immune responses [2-4]. Precursor DC (pre-DC), arising from bone marrow progenitors, enter tissues as immature DC with superior phagocytic capabilities. DC encounter foreign antigens such as donor antigens (in solid organ transplantation), bacteria and tumour antigens resulting in the secretion of cytokines (e.g. IFN) and activation of natural killer (NK) cells, macrophages and eosinophils. Following antigen capture and processing, DC undergo maturation and migrate to secondary lymphoid tissues where they present processed antigen/peptide coupled to major histocompatibility complexes (MHC) to T cells, allowing for selection and expansion of antigen-specific cluster designation (CD)4+ T-helper cells. These CD4+ T-helper cells subsequently amplify the immune responses by regulating antigen-specific (e.g. CD8+ cytotoxic T cells, B cells), and antigen non-specific (e.g. macrophages, NK cells, and eosinophils) effector cells.
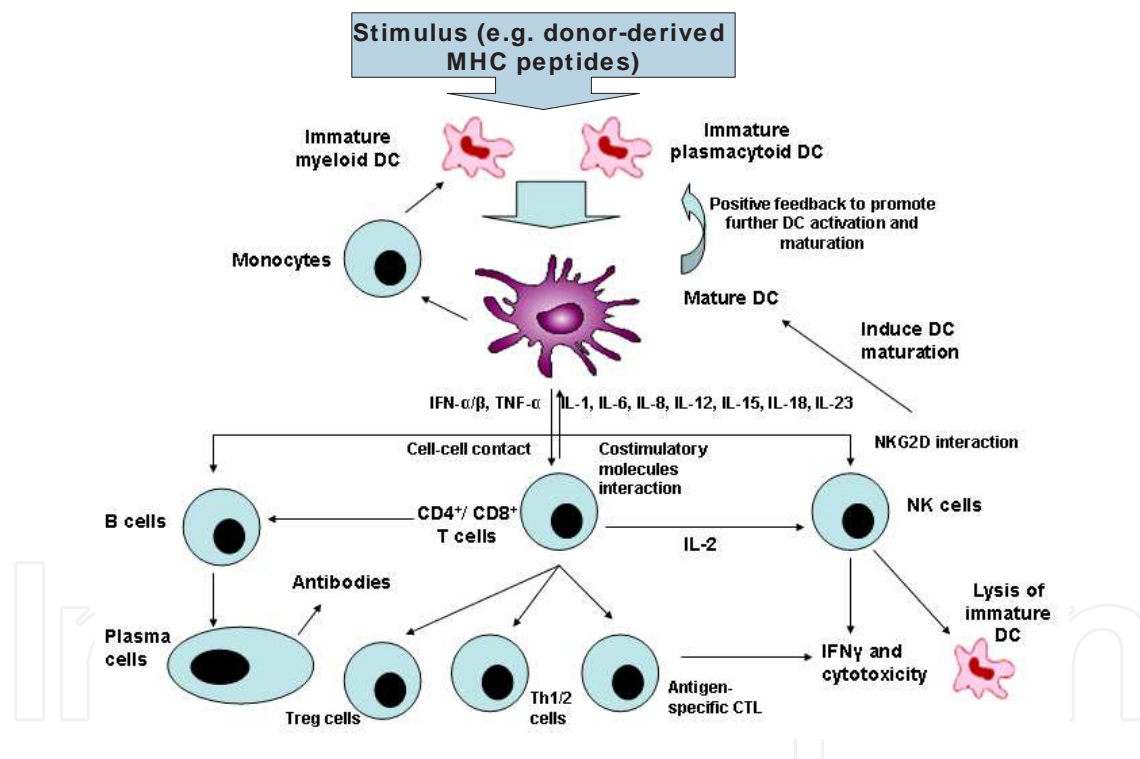
Overview of the complex relationship between dendritic cells and effector T and B cells. Immature DC (MDC and PDC) maturate in response to appropriate stimuli (e.g. microbial products, TLR ligands). Mature DC secretes immunoregulatory cytokines (including IFN-α and IL-12] and with cell-cell contact, modulates effector cell response including NK cells, B and T cells as well as providing a positive feedback to DC to initiate ongoing activation and maturation. Activated effector cells could in turn modulate DC activation, maturation, and survival as well as enhancing other effector cell functions through the production of cytokines (IFN-γ) and/or via cell-cell contact.

Figure 2. Interaction between dendritic cells and effector T and B cells.

DC play a critical role in the initiation and regulation of adaptive T cell responses, the maintenance of central and peripheral tolerance in normal steady-state and hence are essential in

regulating immune responses in solid organ and cellular transplantation. DC have dual roles in organ transplantation. They are responsible for allorecognition and presentation of foreign antigens to T cells, which may initiate allograft rejection; but are also involved in the promotion of transplant tolerance.

2.2. Role of T and B cells in allograft rejection

2.2.1. T cells

The most common form of acute rejection of allogeneic tissues and allografts involve the activation of recipient's T cells (i.e. adaptive immune response) directed against donor MHC antigens or MHC-derived peptides presented by either the donor's or recipient's APC [5]. DC are considered the most potent form of APC in humans through their capacity for antigen uptake and processing of foreign antigens into peptides which can then be presented to antigen-specific T cells via MHC complexes, leading to activation and clonal expansion of naïve and memory T cells (i.e. primary and secondary immune responses) [2]. During steady state, DC reside as functionally immature cells in most tissues. Following organ transplantation, the systemic effects of donor brain death and/or ischaemia-reperfusion injury are sufficient to generate an inflammatory response to mature these DC during their migration carrying donor antigens from the transplanted organ to the recipient's secondary lymphoid organs including the draining lymph nodes and spleen [6, 7]. DC may also be activated via CD40-CD40L interaction, with activated cells (e.g. platelets, T cells, mast cells) within transplanted allografts the potential source of CD40L. This interaction may regulate DC migration possibly via tumour necrosis factor (TNF)-α production by DC [8]. DC maturation and immunostimulatory capacity are dependent on nuclear factor kappa B (NF-κB)-dependent gene transcription including genes involved in the expression of adhesion molecules, chemotactic factors and the production of various cytokines [9]. Although DC are very efficient in presenting donor antigens to T cells, other cell types including tubular epithelial cells, endothelial cells, macrophages and also B cells can participate in T cell interaction, the latter by capturing and presenting foreign antigens via their surface immunoglobulins and MHC class II molecules [10-12].

Direct and indirect allorecognition of allogeneic antigens are mediated by donor-derived and recipient's DC respectively. Donor DC present donor peptide mounted on donor MHC molecules to recipient's T cells following migration of donor DC to T cell areas of lymphoid tissues ('passenger leukocytes') in response to surgery [13]. This mode of presentation is termed *direct allorecognition* and is particularly important in the initiation of acute rejection resulting from a powerful alloantigen-specific T cell response directed against allogeneic antigens [14]. The finding of >90% of infiltrating recipient's T cells involved in recognising donor-derived MHC molecule directly presented by donor DC during acute rejection of allogeneic skin graft in mice support the existence of this direct pathway [15]. Furthermore, the frequency of direct donor-specific hyporeactivity is similar between long-term renal transplant recipients with good graft function compared to those recipients with established chronic rejection suggesting that direct allorecognition is not the predominant response in

chronic rejection [16]. In contrast, recipient's DC may acquire allogeneic donor antigens following migration into the allograft in response to proinflammatory cytokines and chemokines. Recipient's DC present donor MHC-derived peptides (e.g. regions of MHC class II molecules) loaded to self-MHC molecule to recipient's T cells. This mode of presentation is termed *indirect allorecognition* and may be more important in establishing chronic rejection. Unlike direct allorecognition, indirect allorecognition involves a less potent T cell response with a reduced proportion of recipient's T cells involve in the immune response directed against the donor-derived antigens [17, 18]. The finding of a higher frequency of T cells with indirect anti-donor reactivity in transplant recipients with established chronic rejection support this finding [16]. Similarly, studies in non-human primates demonstrated that inhibition of direct anti-donor reactivity can prolong graft survival, but does not prevent late graft loss to chronic rejection [19]. In both direct and indirect allorecognition pathways, DC can internalise extracellular donor antigens, process them and present them to either CD4+ or CD8+ T cells through MHC class I or II molecules respectively. However, the contribution of direct and indirect pathway in acute and chronic allograft rejection remains controversial with studies demonstrating that indirect pathway may also be important in the initiation of acute rejection [20].

Following activation of naïve T cells, activated CD4+ T cells proliferate and differentiate into different cell types with distinct cytokine profiles. Subtypes of helper T cells include type I helper T (Th1], Th2 cells, Th17 cells and regulatory T (Treg) cells. Although Th1 cells may be more important in allograft rejection by producing inflammatory cytokines capable of driving a cellular immune response such as IFN-γ and interleukin (IL)-2, Th2 cells may also be involved in rejection through the activation of eosinophils and promoting a humoral immune response (via cytokines IL4, 5 and 13) [21, 22]. There is increasing evidence that Th17 cells contribute to allograft rejection although the susceptibility of these cells to immune regulation remains unclear [23]. Although Treg cells are capable of inducing immune tolerance in animal models of transplantation, the role of these cells in humans remains unclear [24, 25]. Both CD4+ and CD8+ T cells can mediate allograft injury either directly or indirectly through the production of cytokines or by activating vascular endothelial cells. CD8+ T cells can directly cause cell death by promoting caspase-induced cell apoptosis by releasing perforin and granzymes A and B intracellularly or via Fas-ligand/Fas-receptor interaction between CD8+T cells and allograft [26]. Similarly, CD4+ T cells can directly induce cell apoptosis via Fas-ligand/Fas-receptor interaction but they can also cause indirect cell damage by secreting TNF-α and TNF-β, which subsequently bind to TNF-receptors on endothelial or tubular cells resulting in cell apoptosis [27, 28].

2.2.2. B cells

There is increasing evidence that in solid organ transplantation, B cells play an important role in the immune response to an allograft through the production of antibodies (resulting in the development of acute and chronic antibody mediated rejection [AMR]), but these cells may also have an important role in the support of T cells (resulting in the development of acute cellular rejection) [29]. Most peripheral B cells are produced in the bone marrow and contin-

uously circulate as immature cells through secondary lymphoid organs until they encounter antigen. Once activated, B cells become efficient APC by capturing antigen via B-cell receptor, then interacts with naïve T cells through the presentation of antigen by MHC class II molecules to T-cell receptor respectively. Through this interaction coupled with the ability to produce cytokines such as IL-2, B cells are critical for optimal T cell activation and development of T cell memory [30, 31]. Activated B cells may also differentiate into memory B cells or plasma cells, a small proportion of the latter cell type may persist as long-lived plasma cells that reside in the bone marrow ± allografts indefinitely, continuously producing IgG antibodies [32]. APCs such as DC, monocytes and macrophages produce BAFF (B-cell-activating factor belonging to the tumour necrosis factor family), a cytokine which enhances B cell survival [33]. Antibodies produced by terminally differentiated B cells, especially directed against donor antigens, are critical mediators of AMR and associated graft damage through complement activation and Fc-receptor cross-linking, the latter resulting in proinflammatory cytokine release, DC maturation, macrophage phagocytosis and NK cell-mediated antibody-dependent cellular cytotoxicity [34]. Like Treg cells, there is a recently described subset of B cells in humans and mouse known as regulatory B cells, which are capable inhibiting T cell responses, possibly through the production of IL-10 [35]. The clinical significance of these regulatory B cells in organ transplantation remains unclear.

3. Human Leukocyte Antigen (HLA)

The HLA system is the name given to the human MHC, which was first described by Jean Dausset in 1952 after observing the development of alloantibodies to leukocytes following blood transfusions [36]. The HLA system comprises a group of cell-surface antigen-presenting proteins encoded by a region on the short arm of chromosome 6 and is divided into class I and class II molecules. Humans have three class I HLA (A, B, C) that are present on all nucleated cells and six class II HLA (DPA1, DPB1, DQA1, DQB1, DRA, DRB1) that are present only on antigen-presenting cells and lymphocytes. Class I HLA presents intracellular antigens while class II HLA present extracellular antigens. HLA are highly polymorphic with almost 6000 HLA Class I and over 1500 HLA Class II alleles having been identified [37]. Three of the seven heterodimers (HLA-A, -B, and -DRB1) contribute to the majority of the immunogenicity of mismatched antigens and therefore traditional HLA-typing methods have primarily focussed on these alleles.

HLA play an important role in the immune system by controlling immune responses through antigen presentation and distinguish "self" from "non-self". Since its introduction after the first International Histocompatibility Workshop (IHWS) in 1964, HLA matching has formed the cornerstone of deceased-donor kidney allocation policies worldwide [38]. By the first World Health Organization nomenclature meeting in 1970, 27 HLA antigens were identified. The discovery of new antigens on occasion splits previously known 'broad' antigens into two or more antigens, termed 'split' antigens. For example, the A9 broad antigen was split to A23 and A24 split antigens, whereas the DR2 broad antigen was split to DR15 and DR16 split antigens [39]. HLA matching criteria may vary with regards to consideration of broad or split

antigens. Split antigen matching appears to be more common and clinically important for HLA-A and-B antigens than for HLA-DR antigens [40]. Not surprisingly, utilization of matching for broad antigens increases the probability of identifying HLA-matched recipients for any given donor [41].

Although 0 HLA-mismatched grafts have been shown to have superior graft outcomes compared with grafts with ≥1 HLA-mismatch, a proportion of 0 HLA-mismatched grafts may be complicated by acute rejection, possibly reflecting potential allorecognition of incompatibilities at other minor HLA loci. On the contrary, many HLA-mismatched grafts have excellent graft outcomes without acute rejection, suggesting that under specific circumstances, certain HLA mismatches may be permissible, such as the lack of immunologic response against non-inherited maternal HLA antigens (NIMA) as a result of prenatal tolerance development. However, verification of this association between NIMA and graft outcomes remains inconclusive [42-44].

HLA compatibility has also been defined by mismatch acceptability known as acceptable HLA-mismatch. These are mismatched HLA antigens that do not result in a positive complement dependent cytotoxicity (CDC) crossmatch [42]. Identification of acceptable HLA-mismatches has been utilised to improve the transplant potential of highly sensitized patients, and this concept and application will be discussed in greater details later in this chapter.

In most countries worldwide including Australia, the number of HLA-mismatches is calculated by the sum of the total number of HLA-mismatches between donor-recipient at HLA-A, B, and DR loci. Large single centre and registry studies have consistently demonstrated an inverse association between increasing number of mismatches and graft and/or patient survival [43-45]. However, with the evolution from serological to molecular-based HLA-typing over time resulting in improved immunological risk stratification of transplant candidates, coupled with the availability of more potent immunosuppression and donor selection has created uncertainty regarding the ongoing clinical importance of HLA-mismatches in the modern era.

4. Effect of HLA-mismatches and renal transplant outcomes

Large registry reports including analysis from the Collaborative Transplant Study (CTS) and more recently from the Australia and New Zealand Dialysis and Transplant (ANZDATA) registry have consistently demonstrated a strong association between HLA-matching at the HLA-A, B and DR loci and graft and patient outcomes, independent of donor type, initial immunosuppression, transplant era and even the presence of DSA [46-48].

The advantage of improved HLA-matching in reducing acute rejection risk has been demonstrated predominantly in renal transplant recipients receiving cyclosporine-based immunosuppressive regimen [49, 50]. Recent retrospective single centre study of live and deceased donor renal transplants has demonstrated that HLA-mismatches remained an important determinant of acute rejection risk in renal transplant recipients receiving quadruple immu-

nosuppression involving the use of interleukin-2 receptor antibody induction, tacrolimus, mycophenolate mofetil and corticosteroids [51]. In this study, increasing number of HLA mismatches was an independent predictor of acute rejection (OR 1.65 for every single HLA-mismatch; 95% CI: 1.15 to 2.38; P=0.007), with HLA-mismatches at the HLA-DR locus associated with the highest risk of acute rejection compared to mismatches at the HLA-A and HLA-B loci in the adjusted model. Analysis of the CTS data of 135,970 deceased donor renal transplant recipients demonstrated that the effect of HLA-mismatches on acute rejection risk remained highly significant over two consecutive decades (1985-1994 vs 1995-2004), independent of 'intention to treat' immunosuppressive regimen [47]. Similarly, recent analysis of ANZDATA registry of live and deceased donor renal transplants between 1998 and 2009 demonstrated that the association between HLA-mismatches and acute rejection risk appeared to be independent of transplant era and initial immunosuppression, but this association appeared to be much stronger for live-donor transplants compared to deceased donor transplants (Figure 3A). The reduced benefit of 0-HLA-mismatched kidneys in recipients of deceased compared with live donor kidneys may be explained by the presence in unrelated deceased donors of apparently matched but actually mismatched splits of antigens, which is less frequently observed in biologically related living donors [46]. However, the association between HLA mismatches and rejection was not linear, with the greatest benefit of HLA matching appeared to be confined to those with <4 HLA mismatches [46, 47].

Large retrospective studies have consistently demonstrated the importance of HLA-matching in determining deceased donor renal allograft survival [52-54]. Analysis of the United Network for Organ Sharing (UNOS) registry between 1991 to 1997 demonstrated an 11% reduction in 3-year graft survival rate (p<0.001) between transplants involving 6 compared to 0 HLA-mismatches, with the most discernible difference in survival was observed between recipients with 0 to 1 HLA-mismatch [55]. In the UNOS study, the association between HLA-mismatches and reduced graft survival appeared to be related to mismatches at the HLA-DR locus within the first year post-transplant, whereas mismatches at the HLA-AB loci were more important beyond the first year post-transplant. However, the association between HLA-mismatches and graft survival in the era of modern immunosuppression remains contradictory [56]. Analysis of the CTS data demonstrated that the importance of HLA-matching on graft outcomes remained strong during the two decades of 1985-1994 and 1995-2004, suggesting that association between HLA-mismatches and graft survival remains robust in the era of modern immunosuppression [47]. Unlike the other large registry studies that had focused on deceased donor renal transplants, the study by *Lim WH et al* using ANZDATA registry data evaluated both live and deceased donor renal transplants. Similarly, the authors demonstrated a strong association between HLA-mismatches and overall graft survival for both live and deceased donor renal transplants (Figure 3B), especially between those receiving 0-HLA-mismatched kidneys compared to those receiving ≥1 HLA-mismatched kidneys [46]. In contrast, analysis of the UNOS data suggested that the relative importance of HLA-mismatches and reduced graft survival may have diminished in recent years, whereas other factors such as donor age retained their statistical significance over time prompting the suggestion that kidney allocation algorithms based predominantly on HLA-matching should be modified [57]. However, this study focused on era between 1994 and 1998 whereby the use of induction therapy and/or tacrolimus was limited.
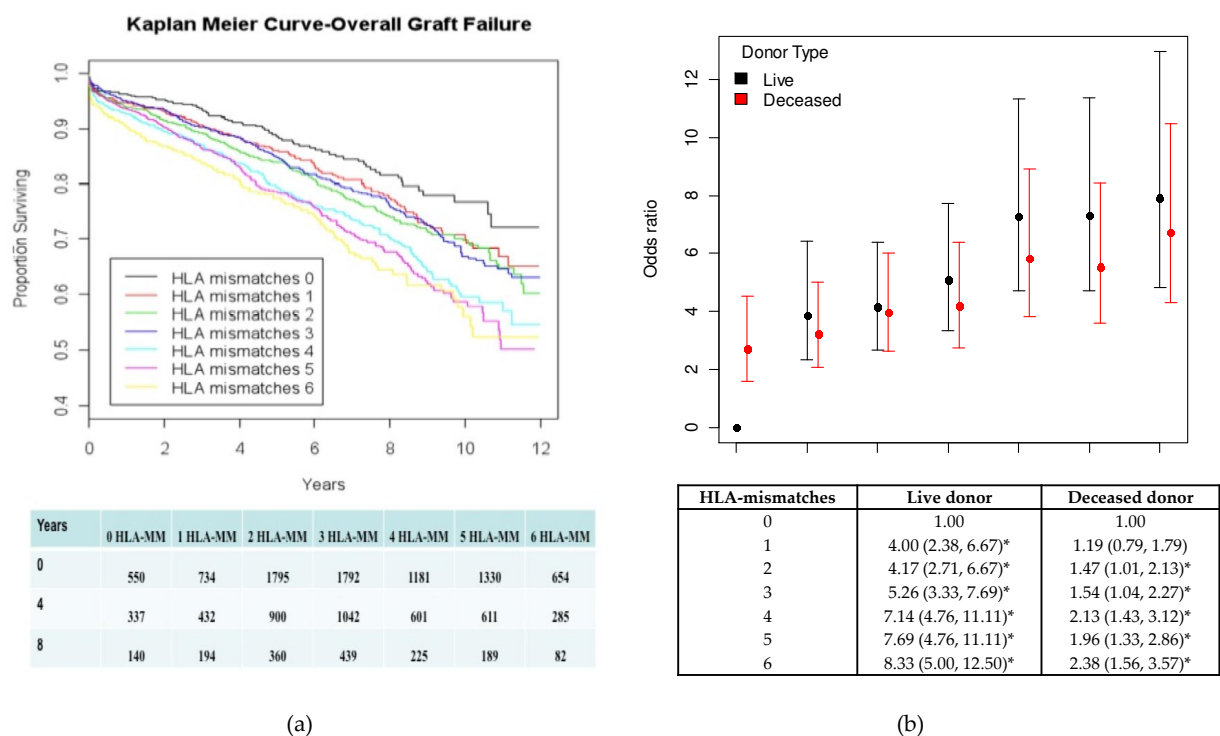
Kaplan Meier Curve-Overall Graft Failure

Years	0 HLA-MM	1 HLA-MM	2 HLA-MM	3 HLA-MM	4 HLA-MM	5 HLA-MM	6 HLA-MM
0	550	734	1795	1792	1181	1330	654
4	337	432	900	1042	601	611	285
8	140	194	360	439	225	189	82

HLA-mismatches	Live donor	Deceased donor
0	1.00	1.00
1	4.00 (2.38, 6.67)*	1.19 (0.79, 1.79)
2	4.17 (2.71, 6.67)*	1.47 (1.01, 2.13)*
3	5.26 (3.33, 7.69)*	1.54 (1.04, 2.27)*
4	7.14 (4.76, 11.11)*	2.13 (1.43, 3.12)*
5	7.69 (4.76, 11.11)*	1.96 (1.33, 2.86)*
6	8.33 (5.00, 12.50)*	2.38 (1.56, 3.57)*

(a)   (b)

Figure 3. (a) Odds ratio plot of HLA mismatches and acute rejection according to donor type (reference live donor 0 HLA mismatch) and corresponding table of the adjusted odds ratio between HLA mismatches and acute rejection according to donor type (reference live or deceased donor 0 HLA mismatch; adapted from *Lim WH et al* Clin Transplant 2012) [46]. (b) Kaplan–Meier survival curve of overall graft failure according to the number of HLA mismatches with corresponding numerical table of the number at risk at 0, 4 and 8 years post-transplant (adapted from *Lim WH et al* Clin Transplant 2012) [46].

The association between acute rejection and graft survival appears well established. In the study by *Wissing et al*, the authors had shown rejection within the first year post-transplant was independently associated with a significant reduction in overall (57% vs 83%; p=0.0004) and death-censored graft survival (63.5% vs 91.2%; p<0.0001) [51], a finding corroborated by ANZDATA registry analysis [46].

Although HLA-DR mismatches appear to be of greater importance in predicting graft outcomes compared to HLA-AB mismatches, the current kidney allocation algorithm in Australia specifically favours fully HLA-DR matched recipients but still takes into account HLA-AB matching, therefore confers an appropriate concession to allow satisfactory HLA-matching but avoiding discrimination to potential recipients with rare HLA combinations as HLA-DR locus has fewer polymorphisms compared to HLA-AB loci [58]. Previous studies have demonstrated that allocation based predominantly on HLA-DR matching, as implemented in the United States, may eliminate any advantage of HLA-AB matching but this remains controversial [59, 60]. Analysis of Scientific Registry of Transplant Recipients (SRTR) of 108,701 deceased donor renal transplant recipients demonstrated that the elimination of allocation priority for HLA-B mismatches improved the transplant potential of ethnic minorities and this policy had achieved comparable renal allograft survival compared to historical graft outcomes prior to the change in allocation policy [61]. Although the presence of HLA-
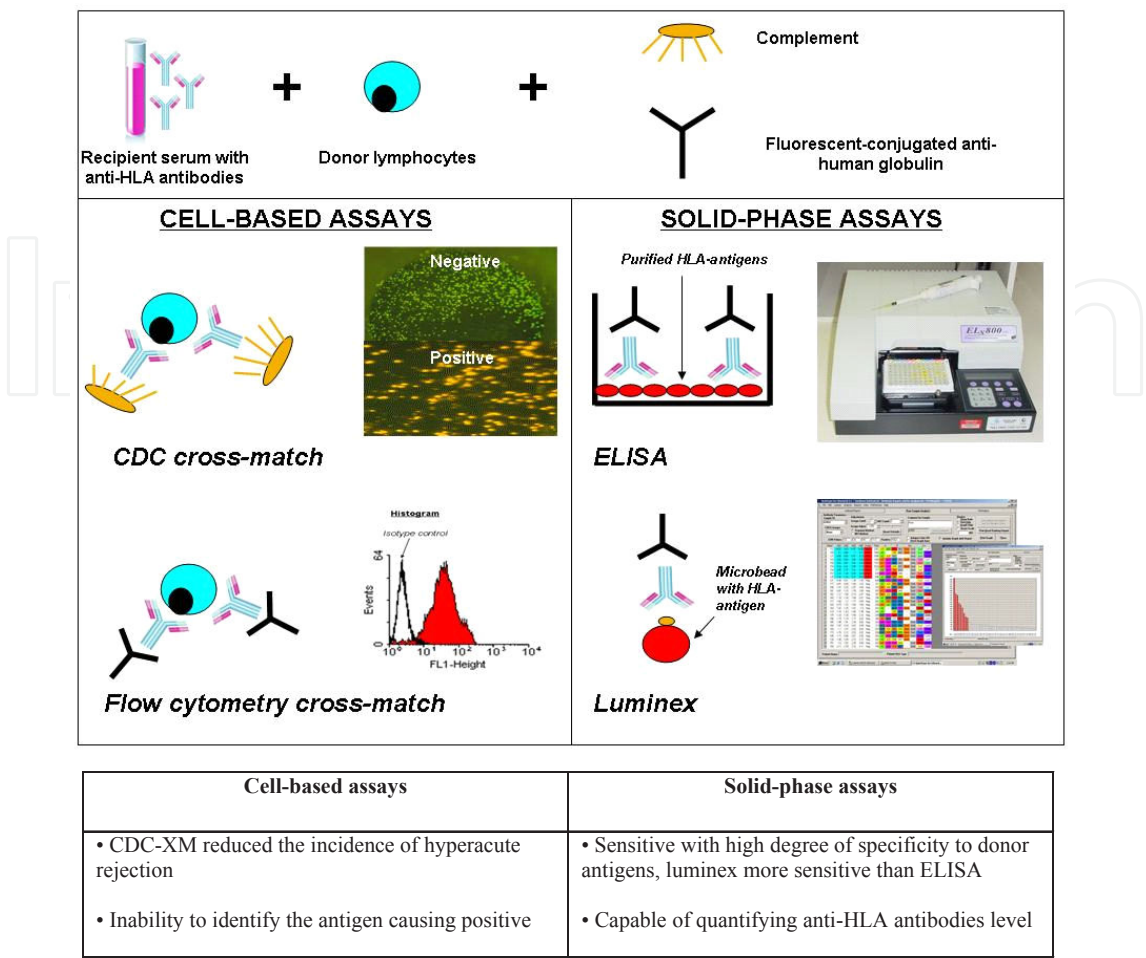
Cw, DP and DQ DSA have been shown to be associated with poorer graft outcomes [62, 63], matching at the HLA-Cw, DP and DQ loci are not routinely performed and therefore is not explicitly included in the allocation of deceased donor kidneys in any countries.

5. Serological and molecular HLA typing and the detection of donor-specific anti-HLA antibodies (Figure 4)

The evolution in our understanding of the HLA system is closely linked to advancements in technology. Traditional serological-based low resolution HLA typing methods can be completed relatively quickly but are dependent on the availability of specific cell types, viability and appropriate anti-sera that are capable of recognising HLA antigens. The emergence of molecular HLA typing techniques over the past two decades has allowed for a more specific, flexible and robust means of high resolution HLA typing. In 1982, *Wake et al* described restriction fragment length polymorphism (RFLPs), which eventually highlighted the shortcomings of serology-based methods ensuing the establishment of molecular-based HLA-typing for routine clinical practice [64]. Data generated via the genome project and the initiation of polymerase chain reaction (PCR) techniques through the 1980s further refined DNA-based techniques for HLA-typing, which has led to the development of a number of PCR-based techniques still in use to the present day.

Alongside advances in the typing of HLA alleles, the techniques used to detect anti-HLA antibodies has also evolved from CDC assays to more sensitive techniques including flow-cytometry and solid-phase assays (e.g. enzyme-linked immunosorbent assay [ELISA] or Luminex), which has allowed a more accurate assessment of transplant candidate's immunological risk pre-transplantation (e.g. calculated panel reactive antibodies to determine level of sensitization and application of virtual cross-match to determine transplant suitability) (Figure 4).

Since the recognition of the clinical importance of CDC assay in kidney transplantation in the 1960s, CDC cross-match has become the cornerstone of determining transplant suitability in both live and deceased donor renal transplantation [65]. The underlying principle of CDC cross-match is to detect clinically relevant donor-specific anti-HLA antibodies that could result in hyperacute rejection following transplantation. Donor T and B cells are incubated in the presence of recipients' sera and complements. If donor-specific anti-HLA antibodies are present, these will bind to donor cells and initiate the complement cascade resulting in lysis of donor lymphocytes. The percentage of lysis will be quantified and forms the basis of determining transplant candidate's suitability for transplantation. Many laboratories perform CDC assays in the presence of anti-human globulin (enhances the sensitivity of assay by enhancing the number of Fc receptors available to bind with complements) and/or dithio-threitol (breaks down the disulfide bonds in IgM antibodies of no clinical significance) to improve the accuracy and reduce the false negative rates associated with these assays [66, 67]. Initial data using the CDC assay revealed that 80% of CDC cross-match–positive transplants and 4% of CDC cross-match–negative transplants were associated with early graft loss (within 48 hours post-transplant), thereby establishing the clinical significance of anti-HLA antibodies

Cell-based assays	Solid-phase assays
• CDC-XM reduced the incidence of hyperacute rejection	• Sensitive with high degree of specificity to donor antigens, luminex more sensitive than ELISA
• Inability to identify the antigen causing positive	• Capable of quantifying anti-HLA antibodies level

HLA – human leukocyte antigen, CDC-XM – complement-dependent cytotoxicity cross-match, ELISA – enzyme-linked immunosorbent assay.

Figure 4. Detection of anti-HLA antibodies – differences between cell-based and solid-phase assays.

in renal transplantation. The inability to correlate all graft losses with anti-HLA antibodies has led to the development of more sensitive cross-match assays, including flow cytometric cross-match assays. It is noteworthy that 20% of patients transplanted across a positive cross-match did not lose their grafts [68]. Because T cells express class I antigens and B cells express both class I and II antigens, the interpretation of T cell together with B cell cross-match will help to establish whether class I and/or II anti-HLA antibodies are present. A positive B cell CDC cross-match invariably accompanies a positive T cell CDC cross-match but this may reflect either anti-HLA antibodies to class I antigens and/or multiple antibodies to class I and/or II antigens. However, a positive B cell CDC cross-match may occur in the absence of a positive T cell CDC cross-match and suggest the presence of class II antigens or low levels class I antigens. The presence of a positive T cell CDC cross-match is an absolute contraindication for transplantation whereas a positive B cell cross-match is a relative contraindication because of the uncertainty regarding the clinical significance and the possibility of false-positive results [69, 70]. In the allocation of deceased donor kidneys in Australia, the presence of a positive T cell CDC

cross-match is an absolute contraindication for transplantation whereas B cell cross-match is not routinely performed and therefore not utilized in the decision-making process for transplantation. With the increasing recognition of the potential importance of a positive CDC B cell cross-match, these results are now often interpreted in the context of solid phase assays.

The basic principle of flow cross-match technique is similar to CDC assay. Since the description of this assay in the early 1980s, this technique has been widely adopted to determine transplant suitability [71]. Similar to CDC assay, flow assay requires the addition of donor cells to recipients' sera, followed by the addition of a secondary fluorescein-labelled antibody allowing for the detection by flow cytometry and quantification of antibodies expressed as channel shifts. Unlike CDC cross-match, flow cytometric cross-match identifies both complement-fixing and non-complement-fixing anti-HLA donor-specific antibodies. However, the availability of different subtypes of detection antibodies has allowed for the differentiation between complement-fixing versus non-complement-fixing antibodies [72]. Although an universal cut-off value for a positive flow cross-match has not been determined, it is agreed that the use of a low cut-off point will result in increased sensitivity but reduced specificity for predicting graft outcomes (especially in the presence of negative CDC cross-match) as this may identify anti-HLA donor specific antibodies of no clinical significance. Nevertheless, renal transplant recipients with positive flow cross-match but negative CDC cross-match have a significantly greater risk of antibody-mediated rejection (AMR) and early graft loss with a positive predictive value for predicting AMR of 83% [72, 73].

To avoid problems associated with the viability of the donor cells, which could affect the accuracy of cell-based assays, the introduction of solid-phase assays have largely circumvented these problems and improved the sensitivity of detection of anti-HLA antibodies [74]. The identification of anti-HLA antibodies using ELISA was first described in 1993 where purified HLA antigens were directly immobilized on the surface of microtitre plates but the basic principle of antibody detection was similar to cell-based assays [75]. The Luminex platform is a solid-phase assay that utilizes polystyrene microspheres (beads), each embedded with fluorochromes of differing intensity attached to one (single-antigen beads) or several HLA molecules (screening beads) to determine anti-HLA antibody specificity. Similar to other assays, the addition of recipients' sera containing anti-HLA antibodies are added to the bead mix, these antibodies will bind to the appropriate beads expressing specific antigen(s). A second phycoerytherin-labelled anti-human IgG is then added to this mixture and these antibodies will bind to the primary anti-HLA antibody already attached to the beads. The sample is then passed through lasers, which would independently excite the beads and the phycoerytherin therefore allowing the laser detector to define antibody specificity [76, 77]. Unlike the CDC assays, Luminex assay detect both complement-fixing and non-complement-fixing anti-HLA antibodies but does not detect IgM autoantibodies or non-HLA antibodies. With the continued reliance on using cell-based cross-match assays, especially CDC cross-match assays to determine transplant suitability, a potential disadvantage of virtual cross-match is that transplants may be excluded based on antibody results with unknown clinical relevance [78]. It is generally accepted that solid phase virtual cross-match to identify anti-

HLA donor specific antibodies complements the results of cell-based assays to help inform decision-making process with regards to transplant suitability.

6. Clinical significance of anti-HLA donor-specific antibodies

It is well known that the presence of high levels of pre-transplant class I (HLA-A and B) ± II (HLA-DR) donor-specific antibodies (DSA; i.e. anti-HLA antibodies with reactivity against the potential donor leading to positive cross-match often as a result of prior sensitization events including previous HLA-mismatched transplants, blood transfusions or pregnancy) is associated with poorer graft outcomes, including the development of acute AMR, chronic AMR, transplant glomerulopathy and late graft loss (Table 1) [79-81]. However, few studies have suggested that the association between pre-transplant DSA and graft survival was restricted to recipients who had developed early AMR, within the first 30-days post-transplantation [82]. In addition, the authors queried the cost-effectiveness of pre-transplant screening for preformed DSA by demonstrating that the additional cost associated with quarterly screening for anti-HLA antibodies would be between 3200 to 6700 Euros, which would equate to an additional 83,000 to 130,000 Euros per avoided AMR because of preformed non-lymphocytotoxic DSA in transplant candidates on the transplant wait-list for >5 years [82]. There is also increasing evidence demonstrating that the development of *de novo* DSA (occurring post-transplantation), especially development of DSA directed against HLA-DQ graft molecules in HLA-class II incompatible graft transplantations, are both associated with acute and subclinical AMR and graft loss in kidney transplant only and/or simultaneous pancreas-kidney transplant recipients [80, 83-85]. Although there is no current consensus on the level of clinically significant DSA identified by flow cytometric or Luminex assays, most studies have demonstrated that increasing single, peak or total DSA levels were associated with an incremental risk of rejection and/or graft loss [86, 87]. Recent studies have suggested that the detection of C1q-fixing DSA (i.e. the potential to identify DSA that can activate complements by binding C1q) may be more specific in predicting acute rejection, biopsy C4d-deposition, transplant glomerulopathy and late graft failure following kidney transplantation but this remains controversial and not routinely performed in many transplanting centres [88, 89]. The clinical benefit of routine regular surveillance for *de novo* DSA in improving graft survival following kidney transplantation remains unclear although a recent study of 72 live-donor renal transplant recipients suggested that the appearance of *de novo* DSA was inversely proportional to the amount of maintenance immunosuppressive drugs (especially in the weaning phase of immunosuppression minimization particularly prednisolone) such that DSA monitoring may be highly effective for detecting escape from tolerance and reappearance of the immune response in weaned patients [90]. With the greater understanding of HLA antigens and anti-HLA antibodies, innovative techniques have been established to allow transplantation across positive CDC and/or flow cross-match barriers but this is beyond the scope of this chapter.

Study	Cohort	Rejection	Graft survival
Eng H et al (n=471 DD renal transplant recipients) [*79*]	83 T-B+ XM vs 386 T-B- XM; IgG HLA DSA in 33% of T-B+ XM patients	Vascular: 19% T-B- vs 32% T-B+ (p=0.01); DSA was a significant predictor for vascular or glomerular rejection	Graft loss: T-B+ - 44%; T-B- 27% (especially class I DSA)
Lefaucheur C et al (n=402 DD renal transplant recipients) [*80*]	83 (21%) positive DSA by Luminex by peak sera vs 76 (19%) by current sera	The presence of SAB HLA-DSA on the peak and current serum has a PPV for AMR of 35% and 32% respectively. Prevalence of AMR 1% in patients with MFI <465, 19% MFI between 466 and 3000, 36% MFI between 3001 and 6000, and 51% MFI "/>6000. Peak HLA-DSA Luminex MFI predicted AMR better than current HLA-DSA MFI.	5 and 8-year DCGS were 89% and 84% in non-sensitized patients, 92% and 92% in sensitized patients with no peak HLA-DSA, and 71% and 61% in patients with peak HLA-DSA. Relative risk (RR) for graft loss for patients who had an episode of AMR was 4.1 (95% CI 2.2 to 7.7) as compared with patients without AMR.
Lefaucheur C et al (n=237 LD and DD renal transplant recipients) [*81*]	All negative T and B-cell CDC-XM. 27% class I or II anti-HLA antibody with 52% DSA.	The incidence of AMR among patients with preformed DSA was 35%, 9-fold higher than in patients without DSA (3%) (p < 0.001).	Overall graft survival at 8 years was 68% in patients with DSA and 77% in those with no DSA. Graft survival of patients with DSA and AMR was significantly worse than in DSA patients without AMR and in non-DSA patients.
Amico P et al (n=334 LD and DD renal transplant recipients) [*105*]	332 negative T and B cell CDC-XM, 67 DSA vs 267 no DSA by Luminex	Overall incidence of clinical/subclinical rejection (i.e., AMR and acute T-cell mediated rejection) at day 200 post-transplant was significantly higher in patients with HLADSA (48/67; 71%) than in patients without HLA-DSA (94/267; 35%).	DCGS at 5 years was 89% in those without DSA, 87% with DSA but no AMR and 68% with DSA and AMR.

HLA – human leukocyte antigen, DD – deceased donor, LD – live-donor, CDC-XM – complement dependent cytotoxicity cross-match, DSA – donor-specific antibodies, SAB – single antigen bead, AMR – antibody mediated rejection, DCGS – death-censored graft survival, MFI – mean fluorescent intensity, PPV – positive predictive value.

Table 1. Association between donor-specific antibodies and graft outcomes.

7. HLA-matching in kidney allocation from deceased donors

Most renal transplant programs preferentially allocate kidneys from deceased donors to transplant candidates with favourable HLA compatibility. The current allocation of deceased-

donor kidneys in most countries, including Australia and the Eurotransplant group (Germany, The Netherlands, Belgium, Luxembourg, Slovenia, and Austria), is weighted largely on the degree of mismatched antigens at the HLA-A, -B and -DR loci, with less emphasis on other factors such as time on dialysis, prior sensitization and even ischaemic time. When a potential deceased-donor kidney is available in Australia, transplant candidates on the wait-list are ranked according to an allocation score calculated from a combination of factors including the number of HLA-mismatches, age of recipient, degree of sensitization and time on wait-list [91]. Approximately 20% of deceased donor kidneys are allocated on a national level to highly sensitized transplant candidates (around 20% of kidneys allocated) but the remaining 80% of deceased donor kidneys are allocated through individual state allocation algorithms.

Despite efforts to achieve equity of access to transplantation in many countries, the inclusion of HLA matching in the allocation of deceased donor kidneys is believed to disadvantage transplant candidates with uncommon HLA phenotypes [92]. Consequently, indigenous populations and ethnic minorities often have a much longer transplant wait-list time and are less likely to receive well-matched kidneys [97-100]. The elimination of the allocation priority for HLA-B mismatches has been shown to improve the transplant potential of ethnic minorities but this approach has not been widely adopted by other countries [61].

In Australia, unacceptable class I HLA-mismatches are defined using the Luminex platform and the presence of class I DSA against HLA-A and -B antigens with >2000 mean fluorescent intensity (MFI) excludes transplant candidates from receiving these donor kidneys, independent of the CDC-cross match results. At present, class II DSA is not explicitly considered in the allocation of kidneys from deceased donors in Australia but many centres have already adopted the policy of avoiding transplantation of kidneys into transplant candidates with high levels of class II DSA.

8. Acceptable HLA-mismatch and highly sensitized transplant candidates

Highly sensitised transplant candidates (defined as those having a panel reactive antibody [PRA] level of >80%) on the deceased donor transplant wait-list are less likely to receive donor kidneys (greater likelihood of obtaining a positive complement-dependent cytotoxicity [CDC]-cross-match result with any given donor) and have a much longer wait-list time compared to unsensitized transplant candidates, resulting in a greater risk of mortality whilst remaining on the transplant wait-list [93]. In Australia, highly sensitized transplant candidates represent approximately 5% of the wait-listed candidates and are more likely to wait on average twice as long as unsensitized transplant candidates despite an increase in the number of deceased donors over time (202 donors in 2006 compared to 309 donors in 2010) [6].

Although HLA matching has traditionally been performed at the broad antigen level, a model considering cross-reacting groups (CREGs) may increase the probability of identifying more compatible kidneys for ethnic minorities and highly sensitized transplant candidates. HLA antigens comprise of multiple serologic epitopes made of polymorphic amino acid residues, and it is these structures and their conformation and position that determine antibody
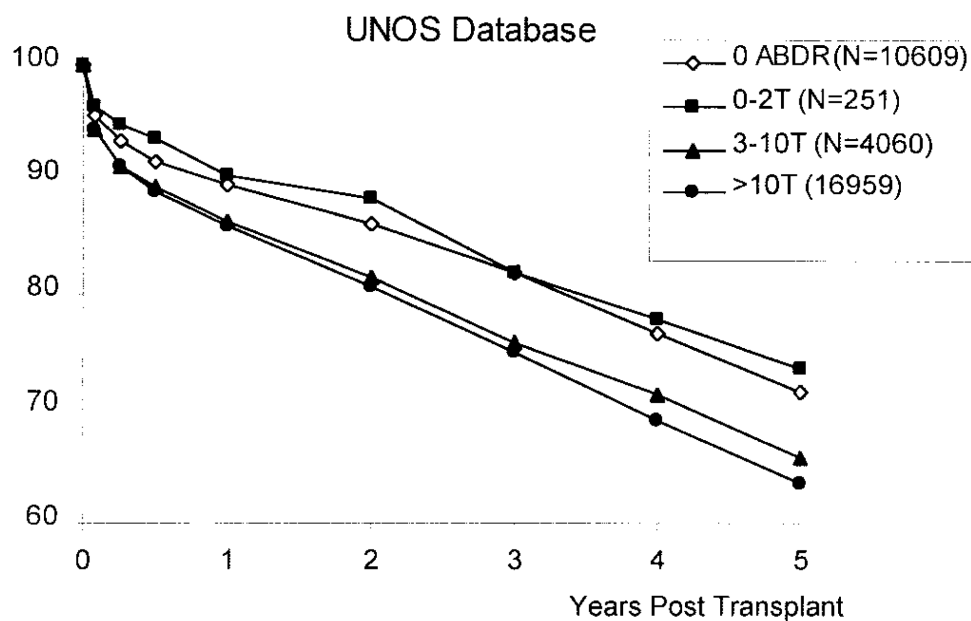
HLA – human leukocyte antigen, UNOS – United Nation Organ Sharing, T - triplets.

Figure 5. Impact of HLA-A, -B triplet (T) matching on 5-year graft survival rates in zero-HLA-DR-mismatched kidney transplants in a cohort of United Nation of Organ Sharing (UNOS) renal transplant recipients between 1987 and 1999 (adapted from *Duquesnoy et al* Transplantation 2003) [101].

accessibility, recognition, and subsequent reactivity [94]. Almost 200 class I and II epitopes have been defined by Luminex technology [95]. Some epitopes are shared across different HLA alleles while some are unique to single or more restricted numbers of HLA alleles. While there are considerable differences in HLA antigen frequencies between different ethnic groups, CREGs are more evenly distributed [96].

The concept of acceptable HLA-mismatch identifies mismatched HLA-antigens that could be considered as compatible at a structural or functional level. It is based on the principle that each HLA antigen is structurally unique and that an individual cannot mount an immuno-logical response against an epitope expressed by their own HLA, i.e. one cannot react against shared 'self' epitopes [105, 106]. It has been demonstrated that such acceptable HLA-mis-matches would result in a negative CDC cross-match and therefore allow transplantation to safely proceed [97, 98].

Acceptable HLA-mismatches can be identified using HLAMatchmaker or the Luminex platform. HLAMatchmaker is a computer algorithm that regards each HLA antigen as a string of polymorphic amino acid configurations in antibody-accessible positions (epitopes) formed by triplets or eplets [99, 100]. For any given set of HLA antigens, HLAMatchmaker can define the number of triplet or eplet mismatches present against any foreign HLA antigen and hence define the HLA antigens that are mismatched at the broad antigen level but matched at the eplet level, i.e. acceptable HLA-mismatches. Graft outcomes of HLAMatchmaker-identified 0-2 triplet-mismatched kidney transplant recipients are similar compared to recipients with 0 HLA-mismatch at the HLA-A, -B and –DR loci (Figure 5) [101]

The Luminex platform determines specificity and quantifies anti-HLA antibodies present in potential transplant candidates and is used in Australia to define unacceptable class I HLA-mismatches. Although it may be logical to consider Luminex-define DSA with MFI of <500 as acceptable mismatch, the utlilization of this technique or the appropriate thresholds of Luminex-determined acceptable mismatch remain unknown [102].

In highly sensitized transplant candidates, the identification of acceptable HLA-mismatch has been shown to improve their transplant potential by reducing the number of HLA-mismatches therefore identifying additional donors likely to produce a negative CDC cross-match.

9. Acceptable HLA-mismatch programs

Successful acceptable HLA-mismatch programs have been implemented in many countries, including Europe, United Kingdom and United States [45, 114-116]. Eurotransplant Acceptable Mismatch Program was established in mid 1970 to improve the transplant potential of highly sensitized transplant candidates. Over the ensuing decade, eleven other similar programs were introduced throughout Europe [103]. Although there is considerable variation in PRA cut-off to define highly sensitized transplant candidates, it is generally accepted that PRA >80% may be the most appropriate cut-off. Table 2 highlights the results of the established acceptable mismatch programs.

Scheme	Initiation	Reference	Eligibility	Outcomes/Activity
UK Transplant SOS Scheme (UKT:SOS)	Feb 1984	[106]	PRA"/>85% (historic or current sera)	• 65% graft survival at 1year • 42% transplanted within 1year
Collaborative Transplant Study Highly Immunized Trial (CTS:HIT)	1985	[107]	PRA"/>80% (current sera)	• 5-year graft survival comparable to unsensitized recipients (59% vs 60%)
Eurotransplant Acceptable Mismatch Program (ET:ACMM)	1985	[100]	PRA≥85% (historic or current sera)	• 2-year graft survival comparable to unsensitized recipients (87%) • 45% transplanted in 1year, mean 8.9 months reduction in mean wait time
South Eastern Organ Procurement Foundation High Grade HLA Match algorithm (SEOPF:HGM)	Jan 1994	[108]	PRA≥40% (current sera)	• 2-year graft survival comparable to unsensitized recipients (86% vs 88%)

PRA – panel reactive antibody, HLA – human leukocyte antigen

Table 2. Description of allocation schemes based on acceptable HLA-mismatch.

The Eurotransplant Acceptable Mismatch Program is the largest and most successful program and runs in parallel with the Eurotransplant Kidney Allocation System (ETKAS) to identify acceptable HLA-mismatches in potential highly sensitized transplant candidates through comprehensive serum screening for acceptable mismatches. The introduction of the acceptable mismatch program has significantly reduced waiting time for highly sensitized transplant candidates whilst achieving comparable short and long-term graft outcomes to unsensitized transplant recipients [100].

The deceased donor kidney allocation algorithm in Australia does not consider acceptable HLA-mismatches for highly sensitized transplant candidates. We are currently investigating the impact of identifying and incorporating acceptable mismatches into the deceased-donor kidney allocation model and our preliminary data suggest that an acceptable mismatch program could result in an improvement in transplant potential of 1 in 10 highly sensitized renal transplant recipients (PRA >80%) with a potential reduction in average transplant wait-list time of 33 months [104].

10. Conclusion

Despite the evolution of more sensitive molecular-based HLA-typing and the ability to detect DSA, there continues to be an important association between HLA-matching and graft and patient outcomes in kidney transplantation. Nevertheless, the application of molecular-based typing in kidney transplantation is already being mandated by most of the transplant community and may provide greater accuracy in the assessment of individual's immunological risk as well as improving transplant outcomes.

Author details

Hung Do Nguyen[1,2], Rebecca Lucy Williams[1], Germaine Wong[3] and Wai Hon Lim[1,2]

*Address all correspondence to: wai.lim@health.wa.gov.au

1 Department of Renal Medicine, Sir Charles Gairdner Hospital, Australia

2 School of Medicine and Pharmacology, University of Western Australia, Perth, Australia

3 Sydney School of Public Health, University of Sydney; Centre for Kidney Research, The Children's Hospital at Westmead; Centre for Transplant and Renal Research, Westmead Hospital, Sydney, Australia

References

[1]   Fearon D, Locksley R. The instructive role of innate immunity in the acquired im‐
mune response. Science 1996; 272 (5258): 50.

[2]   Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. Ann Rev Im‐
munol 2000; 18: 767.

[3]   Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature
1998; 392: 245.

[4]   Coates PTH, Thomson AW. Dendritic cells, tolerance induction and transplant out‐
come. Am J Transplant 2002; 2 (3): 299.

[5]   Morelli A, Thomson A. Dendritic cells: regulators of alloimmunity and opportunities
for tolerance induction. Immunol Rev 2003; 196: 125.

[6]   Gallucci S, Lolkema M, Matzinger P. Natural adjuvants: endogenous activators of
dendritic cells. Nat Med 1999; 5: 1249.

[7]   Gallucci S, Matzinger P. Danger signals: SOS to the immune system. Curr Opin Im‐
munol 2001; 13: 114.

[8]   Morelli A, Zahorchak A, Larregina A, et al. Regulation of cytokine production by
mouse myeloid dendritic cells in relation to differentiation and terminal maturation
induced by LPS or CD40 ligation. Blood 2001; 98 (5): 1512.

[9]   Rescigno M, Martino M, Sutherland C, Gold M, Ricciardi-Castagnoli P. Dendritic cell
survival and maturation are regulated by different signaling pathways. J Exp Med
1998; 188: 2175.

[10] Nankivell B, Alexander S. Rejection of the kidney allograft. N Engl J Med 2010; 363
(15): 1451.

[11] Hagerty D, Allen P. Processing and presentation of self and foreign antigens by the
renal proximal tubule. J Immunol 1992; 148: 2324.

[12] Kreisel D, Krupnick A, Balsara K, et al. Mouse vascular endothelium activates CD8+
T lymphocytes in a B7-dependent fashion. J Immunol 2002; 169 (11): 6154.

[13] Larsen C, Morris P, Austyn J. Migration of dendritic leukocytes from cardiac allog‐
rafts into host spleens. A novel pathway for initiation of rejection. J Exp Med 1990;
171: 307.

[14] Suciu-Foca N, Colovai A, Ciubotariu R, Cortesini R. Mapping of HLA-DR determi‐
nants recognized via the indirect pathway. Graft 1999; 2: 28.

[15] Benichou G, Valujskikh A, Heeger PS. Contributions of direct and indirect T cell al‐
loreactivity during allograft rejection in mice. J Immunol 1999; 162: 352.

[16] Baker R, Hernandez-Fuentes M, Brookes P, Chaudhry A, Cook H, Lechler R. Loss of direct and maintenance of indirect alloresponses in renal allograft recipients: implications for the pathogenesis of chronic allograft nephropathy. J Immunol 2001; 167 (12): 7199.

[17] Gould D, Auchincloss HJ. Direct and indirect recognition: the role of MHC antigens in graft rejection. Immunol Today 1999; 20: 77.

[18] Lechler R, Ng W, Steinman R. Dendritic cells in transplantation - friend or foe. Immunity 2001; 14 (4): 357.

[19] Montgomery S, Xu H, Tadaki D, et al. Combination induction therapy with monoclonal antibodies specific for CD80, CD86, and CD154 in nonhuman primate renal transplantation. Transplantation 2002; 74 (10): 1365.

[20] Auchincloss H, Lee R, Shea S, Markowitz J, Grusby M, Glimcher L. The role of "indirect" recognition in initiating rejection of skin grafts from major histocompatibility complex class II-deficient mice. Proc Natl Acad Sci U S A 1993; 90: 3373.

[21] Zhai Y, Ghobrial R, Busuttil R, Kupiec-Weglinski J. Th1 and Th2 cytokines in organ transplantation: paradigm lost? Crit Rev Immunol 1999; 19: 155.

[22] Goldman M, Le Moine A, Braun M, Flamand V, Abramowicz D. A role for eosinophils in transplant rejection. Trends Immunol 2001; 22 (5): 247.

[23] Chadha R, Heidt S, Jones N, Wood K. Th17: contributors to allograft rejection and a barrier to the induction of transplantation tolerance? Transplantation; 91 (9): 939.

[24] Wood K, Sakaguchi S. Regulatory T cells in transplantation tolerance. Nat Rev Immunol 2003; 3: 199.

[25] Wood K, Bushell A, Hester J. Regulatory immune cells in transplantation. Nat Rev Immunol 2012; 12 (6): 417.

[26] Barry M, Bleackley R. Cytotoxic T lymphocytes: all roads lead to death. Nat Rev Immunol 2002; 2: 401.

[27] Al-Lamki R, Wang J, Skepper J, Thiru S, Pober J, Bradley J. Expression of tumor necrosis factor receptors in normal kidney and rejecting renal transplants. Lab Invest 2001; 81: 1503.

[28] Jagger A, Evans H, Walter G, et al. FAS/FAS-L dependent killing of activated human monocytes and macrophages by CD4+CD25- responder T cells, but not CD4+CD25+ regulatory T cells. J Autoimmun 2012; 38 (1): 29.

[29] Caltworthy M. Targeting B cells and antibody in transplantation. Am J Transplant 2011; 11: 1359.

[30] Ng Y, Oberbarnscheidt M, Chandramoorthy H, Hoffman R, Chalasani G. B cells help alloreactive T cells differentiate into memory T cells. Am J Transplant 2010; 10: 1970.

[31] Lund F, Randall T. Effector and regulatory B cells: modulators of CD4+ T cell immunity. Nat Rev Immunol 2010; 10 (4): 236.

[32] Vinuesa C, Linterman M, Goodnow C, Randall K. T cells and follicular dendritic cells in germinal center B-cell formation and selection. Immunol Rev 2010; 237: 72.

[33] Parsons R, Vivek K, Redfield Rr, et al. B-lymphocyte homeostasis and BLyS-directed immunotherapy in transplantation. Transplant Rev 2010; 24 (4): 207.

[34] Smith K, Clatworthy M. FcgammaRIIB in autoimmunity and infection: Evolutionary and therapeutic implications. Nat Rev Immunol 2010; 10: 328.

[35] DiLillo D, Matsushita T, Tedder T. B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity, and cancer. Ann N Y Acad Sci 2010; 1183: 38.

[36] Dausset J. Les thrombo-anticorps: Acta Haematologica, 1958.

[37] EMBL-EBI. IMGT HLA sequence data.

[38] Bontadini A. HLA techniques: Typing and antibody detection in the laboratory of immunogenetics. METHODS 2012: 1.

[39] Terasaki PI. Histocompatibility testing, 1980, 1980.

[40] Hata Y, Cecka JM, Takemoto S, Ozawa M, Cho YW, Terasaki PI. Effects of changes in the criteria for nationally shared kidney transplants for HLA-matched patients. Transplantation 1998; 65 (2): 208.

[41] Takemoto S, Terasaki P. Equitable allocation of HLA-compatible kidneys for local pools and for minorities. New England Journal … 1994.

[42] Claas FHJ, Claas FH, Witvliet MD, Duquesnoy RJ, Persijn GG, Doxiadis IIN. The acceptable mismatch program as a fast tool for highly sensitized patients awaiting a cadaveric kidney transplantation: short waiting time and excellent graft outcome. Transplantation 2004; 78 (2): 190.

[43] Lim W, Chadban S, Clayton P, et al. Human Leukocyte Antigen Mismatches Associated with Increased Risk of Rejection, Graft Failure and Death Independent of Initial Immunosuppression in Renal Transplant Recipients. Unpublished: 1.

[44] Opelz G. Impact of HLA compatibility on survival of kidney transplants from unrelated live donors. Transplantation 1997; 64 (10): 1473.

[45] Paramesh AS, Zhang R, Baber J, et al. The effect of HLA mismatch on highly sensitized renal allograft recipients. Clinical transplantation 2010; 24 (6): E247.

[46] Lim W, Chadban S, Clayton P, et al. Human leukocyte antigen mismatches associated with increased risk of rejection, graft failure, and death independent of initial immunosuppression in renal transplant recipients. Clin Transplant 2012; e-pub.

[47] Opelz G, Dohler B. Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. Transplantation 2007; 84 (2): 137.

[48] Dunn T, Noreen H, Gillingham K, et al. Revisiting Traditional Risk Factors for Rejection and Graft Loss After Kidney Transplantation. Am J Transplant 2011; 11 (10): 2132.

[49] Beckingham I, Dennis M, Bishop M, Blamey R, Smith S, Nicholson M. Effect of human leucocyte antigen matching on the incidence of acute rejection in renal transplantation. Br J Surg 1994; 81 (4): 574.

[50] McKenna R, Lee K, Gough J, et al. Matching for private or public HLA epitopes reduces acute rejection episodes and improves two-year renal allograft function. Transplantation 1998; 66 (1): 38.

[51] Wissing K, Fomegné G, Broeders N, et al. HLA mismatches remain risk factors for acute kidney allograft rejection in patients receiving quadruple immunosuppression with anti-interleukin-2 receptor antibodies. Transplantation 2008; 85 (3): 411.

[52] Held P, Kahan B, Hunsicker L, et al. The impact of HLA mismatches on the survival of first cadaveric kidney transplants. N Engl J Med 1994; 331 (12): 765.

[53] Gjertson D, Terasaki P, Takemoto S, Mickey M. National allocation of cadaveric kidneys by HLA matching. Projected effect on outcome and costs. N Engl J Med 1991; 324 (15): 1032.

[54] Takemoto S, Terasaki P, Gjertson D, Cecka J. Twelve years' experience with national sharing of HLA-matched cadaveric kidneys for transplantation. N Engl J Med 2000; 343 (15): 1078.

[55] Schnitzler M, Hollenbeak C, Cohen D, et al. The economic implications of HLA matching in cadaveric renal transplantation. N Engl J Med 1999; 341 (19): 1440.

[56] Gillich M, Heimbach D, Schoeneich G, Müller S, Klehr H. Comparison of blood group versus HLA-dependent transplantation and its influence on donor kidney survival. Nephrol Dial Transplant 2002; 17 (5): 884.

[57] Su X, Zenios S, Chakkera H, Milford E, Chertow G. Diminishing significance of HLA matching in kidney transplantation. Am J Transplant 2004; 4 (9): 1501.

[58] Corte G, Damiani G, Calabi F, Fabbi M, Bargellesi A. Analysis of HLA-DR polymorphism by two-dimensional peptide mapping. Proc Natl Acad Sci 1981; 78 (1): 534.

[59] Doxiadis I, de Fijter J, Mallat M, et al. Simpler and equitable allocation of kidneys from postmortem donors primarily based on full HLA-DR compatibility. Transplantation 2007; 83 (9): 1207.

[60] Vereerstraeten P, Abramowicz D, Andrien M, Dupont E, De Pauw L, Kinnaert P. Allocation of cadaver kidneys according to HLA-DR matching alone would result in optimal graft outcome in most recipients. Transplant Proc 1999; 31 (1-2): 739.

[61]   Ashby V, Port F, Wolfe R, et al. Transplanting Kidneys Without Points for HLA-B Matching: Consequences of the Policy Change. Am J Transplant 2011; 11: 1712.

[62]   Frohn C, Fricke L, Puchta J-C, Kirchner H. The effect of HLA-C matching on acute renal transplant rejection. Nephrol Dial Transplant 2001; 16: 355.

[63]   Takemoto S, Port F, Claas F, Duquesnoy R. HLA Matching for kidney transplantation. Hum Immunol 2004; 65: 1489.

[64]   Wake C, Long E. Allelic polymorphism and complexity of the genes for HLA-DR β-chains—direct analysis by DNA–DNA hybridization. 1982.

[65]   Patel R, Terasaki P. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 1969; 280 (14): 735.

[66]   Bryan C, Martinez J, Muruve N, et al. IgM antibodies identified by a DTT-ameliorated positive crossmatch do not influence renal graft outcome but the strength of the IgM lymphocytotoxicity is associated with DR phenotype. Clin Transplant 2001; 15 (Suppl 6): 28.

[67]   Mulley W, Kanellis J. Understanding crossmatch testing in organ transplantation: A case-based guide for the general nephrologist. Nephrology 2011; 16: 125.

[68]   Gebel H, Bray R. Laboratory assessment of HLA antibodies circa 2006: making sense of sensitivity. Transplant Revs 2006; 20 (4): 189.

[69]   Eng H, Bennett G, Chang S, et al. Donor HLA Specific Antibodies Predict Development and Define Prognosis in Transplant Glomerulopathy. Hum Immunol 2011.

[70]   Le Bas-Bernardet S, Hourmant M, Valentin N, et al. Identification of the antibodies involved in B-cell crossmatch positivity in renal transplantation. Transplantation 2003; 75 (4): 477.

[71]   Garovoy M, Bigos M, Perkins H, Colombe B, Salvatierra O. A high technology crossmatch technique facilitating transplantation. Transplant Proc 1983; XV: 1939.

[72]   Limaye S, O'Kelly P, Harmon G, et al. Improved graft survival in highly sensitized patients undergoing renal transplantation after the introduction of a clinically validated flow cytometry crossmatch. Transplantation 2009; 87 (7): 1052.

[73]   Karpinski M, Rush D, Jeffery J, et al. Flow cytometric crossmatching in primary renal transplant recipients with a negative anti-human globulin enhanced cytotoxicity crossmatch. J Am Soc Nephrol 2001; 12 (12): 2807.

[74]   Schlaf G, Pollok-Kopp B, Manzke T, Schurat O, Altermann W. Novel solid phase-based ELISA assays contribute to an improved detection of anti-HLA antibodies and to an increased reliability of pre- and post-transplant crossmatching. NDT Plus 2010; 3: 527.

[75] Kao K, Scornik J, Small S. Enzyme-linked immunoassay for anti-HLA antibodies-an alternative to panel studies by lymphocytotoxicity. Transplantation 1993; 55: 192.

[76] Gibney E, Cagle L, Freed B, Warnell S, Chan L, Wiseman A. Detection of donor-specific antibodies using HLA-coated microspheres: another tool for kidney transplant risk stratification. Nephrol Dial Transplant 2006; 21 (9): 2625.

[77] Muro M, Llorente S, Gónzalez-Soriano M, Minguela A, Gimeno L, Alvarez-López M. Pre-formed donor-specific alloantibodies (DSA) detected only by luminex technology using HLA-coated microspheres and causing acute humoral rejection and kidney graft dysfunction. Clin Transpl 2006: 379.

[78] Lee P, Ozawa M. Reappraisal of HLA antibody analysis and crossmatching in kidney transplantation. Clin Transplant 2007: 219.

[79] Eng H, Bennett G, Tsiopelas E, et al. Anti-HLA donor-specific antibodies detected in positive B-cell crossmatches by Luminex predict late graft loss. Am J Transplant 2008; 8 (11): 2335.

[80] Lefaucheur C, Loupy A, Hill G, et al. Preexisting donor-specific HLA-antibodies predict outcome in kidney transplantation. J Am Soc Nephrol 2010; 21: 1398.

[81] Lefaucheur C, Suberbielle-Boissel C, Hill G, et al. Clinical relevance of preformed HLA donor-specific antibodies in kidney transplantation. Am J Transplant 2008; 8 (2): 324.

[82] Ziemann M, Schonemann C, Bern C, et al. Prognostic value and cost-effectiveness of different screening strategies for HLA antibodies prior to kidney transplantation. Clin Transplant 2012; e-pub.

[83] Cantarovich D, De Amicis S, Aki A, et al. Posttransplant donor-specific anti-HLA antibodies negatively impact pancreas transplantation outcome. Am J Transplant 2011; 11: 2737.

[84] Ntokou I-SA, Iniotaki A, Kontou E, et al. Long-term follow up for anti-HLA donor specific antibodies postrenal transplantation: high immunogenicity of HLA class II graft molecules. Transplant Int 2011; 24: 1084.

[85] Wiebe C, Gibson I, Blydt-Hansen T, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. Am J Transplant 2012; 12: 1157.

[86] Lefaucheur C, Loupy A, Hill G, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol 2010; 21 (8): 1398.

[87] Mujtaba M, Goggins W, Lobashevsky A, et al. The strength of donor-specific antibody is a more reliable predictor of antibody-mediated rejection than flow cytometry crossmatch analysis in desensitized kidney recipients. Clin Transplant 2011; 25 (1): E96.

[88] Yabu J, Higgins J, Chen G, Sequeira F, Busque S, Tyan D. C1q-fixing human leuko-cyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. Transplantation 2011; 91 (3): 342.

[89] Sutherland S, Chen G, Sequeira F, Lou C, Alexander S, Tyan D. Complement-fixing donor-specific antibodies identified by a novel C1q assay are associated with allog-raft loss. Pediatr Transplantation 2012; 16: 12.

[90] Hoshino J, Kaneku H, Everly M, Greenland S, Terasaki P. Using donor-specific anti-bodies to monitor the need for immunosuppression. Transplantation 2012; 93: 1173.

[91] Organ Transplantation from Deceased Donors: Consensus Statement on Eligibility Criteria and Allocation Protocols, 2011.

[92] Gaston R, Ayres I, Dooley L. Racial equity in renal transplantation. JAMA: the jour-nal of the … 1993.

[93] Smits JM, van Houwelingen HC, De Meester J, Persijn GG, Claas FH. Analysis of the renal transplant waiting list: application of a parametric competing risk method. Transplantation 1998; 66 (9): 1146.

[94] Duquesnoy RJ. Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation. Current Opinion in Immunology 2008; 20 (5): 594.

[95] Duquesnoy RJ, Marrari M. Correlations between Terasaki's HLA class I epitopes and HLAMatchmaker-defined eplets on HLA-A, -B and -C antigens. Tissue Antigens 2009; 74 (2): 117.

[96] Rodey GE, Neylan JF, Whelchel JD, Revels KW, Bray RA. Epitope specificity of HLA class I alloantibodies. I. Frequency analysis of antibodies to private versus public spe-cificities in potential transplant recipients. Human Immunology 1994; 39 (4): 272.

[97] Gebel HM, Bray RA, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: contraindication vs. risk. American jour-nal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2003; 3 (12): 1488.

[98] Lobashevsky AL, Senkbeil RW, Shoaf JL, et al. The number of amino acid residues mismatches correlates with flow cytometry crossmatching results in high PRA renal patients. Human Immunology 2002; 63 (5): 364.

[99] Duquesnoy RJ. HLAMATCHMAKER: a molecularly based donor selection algorithm for highly alloimmunized patients. Transplantation proceedings 2001; 33 (1-2): 493.

[100] Claas F, Witvliet M, Duquesnoy R, Persijn G, Doxiadis I. The acceptable mismatch program as a fast tool for highly sensitized patients awaiting a cadaveric kidney transplantation: short waiting time and excellent graft outcome. Transplantation 2004; 78 (2): 190.

[101] Duquesnoy RJ, Takemoto S, de Lange P, et al. HLAmatchmaker: a molecularly based algorithm for histocompatibility determination. III. Effect of matching at the HLA-

A,B amino acid triplet level on kidney transplant survival. Transplantation 2003; 75 (6): 884.

[102] Claas FHJ, Doxiadis IIIN. Human leukocyte antigen antibody detection and kidney allocation within Eurotransplant. Human Immunology 2009; 70 (8): 636.

[103] Gore SM, Bradley, Benjamin A. Renal transplantation : sense and sensitization. Strasbourg Dordrecht; Boston: Council of Europe; Kluwer Academic Publishers, 1988.

[104] Do Nguyen H, Fidler, S, Irish, A, D'Orsogna, L, Christiansen, FT, Martinez, P, Lim, W. The Idenfication of Acceptable HLA-Mismatches Improves Transplant Potential of Highly-Sensitised Renal Transplant Recipients: Sir Charles Gairdner Hospital, Royal Perth Hospital, University of Western Australia, 2012.

[105] Amico P, Hönger G, Mayr M, Steiger J, Hopfer H, Schaub S. Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads. Transplantation 2009; 87 (11): 1681.

[106] Klouda P, Corbin S, Ray T, Rogers C, Bradley B. Renal transplantation in highly sensitized pati... [Clin Transpl. 1990] - PubMed - NCBI. Clinical transplantation 1990: 69.

[107] Opelz G. Five-year results of renal transplantation in highly sensitized recipients. Collaborative Transplant Study. Transplant international : official journal of the European Society for Organ Transplantation 2007; 9 (Suppl 1): S16.

[108] Tardif GN, McCalmon RT. SEOPF high-grade HLA match algorithm: effective kidney sharing using ROP trays with HLA matching for highly sensitized patients. Transplantation proceedings 1997; 29 (1-2): 1406.