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APPLICATION OF AN EPITOPE-BASED ALLOCATION SYSTEM IN PAEDIATRIC KIDNEY TRANSPLANTATION.

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Catherine Quinlan had a substantial contribution to the study design, data interpretation and revision of the work.

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Amanda M Walker had a substantial contribution to the study design, data interpretation and drafting and revision of the work.

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ABBREVIATIONS

AMR, Antibody-mediated rejection

cPRA, Calculated panel-reactive antibody

CDC, Complement-dependent cytotoxicity

DD, Deceased donor

DD-Excl, DD after 2013 with eplet exclusions

DD-NoExcl, DD after 2013 without eplet exclusions

DD-Pre2014, DD between 2003-2013

dnDSA, De novo donor specific antibody

ESKD, End-stage kidney disease

KTR, Kidney transplant recipients

LD, Live donor

LD-NoExcl, Live donor after 2013

MFI, Mean fluorescence intensity

MM, Mismatch

PDE, Predicted donor exclusion

RCH, Royal Children's Hospital Melbourne

VTIS, Victorian Immunogenetics and Transplantation Service

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APPLICATION OF AN EPITOPE-BASED ALLOCATION SYSTEM IN PAEDIATRIC KIDNEY TRANSPLANTATION.

Pediatr Transplant.

ABSTRACT

Background

Donor-recipient HLA mismatch remains a leading cause for sensitisation and graft loss in kidney transplantation. HLA compatibility at an epitope level is emerging as an improved method of matching compared to current HLA antigen allocation.

Methods

A novel epitope-based allocation approach to prospectively exclude donors with high level mismatches was implemented for paediatric kidney transplant recipients (KTRs) on the deceased donor (DD) waiting list.

Results

19 consecutive transplants were followed for 12 months, including 8 DD KTRs listed with eplet exclusions, as well as 3 DD KTRs and 8 live donor (LD) KTRs without exclusions. KTRs with eplet exclusions had estimated GFR of 78.5ml/min/1.73m^2, no episodes of rejection and time to transplant 6.55 months. HLA-A,B,DR antigen mismatches were similar between all groups. KTRs with exclusions had significantly lower class II eplet mismatches (20.4) than the contemporary DD KTRs without exclusions (63.7) and DD KTRs transplanted in the preceding decade (46.9). De novo donor-specific antibodies (dnDSAs) were identified in 2 of 8 DD KTRs with exclusions, 2 of 3 DD KTRs without exclusions and 5 of 8 LD KTRs.

Conclusions

Epitope-based allocation achieved timely access to transplantation, low class II eplet mismatches and low rates of dnDSAs in the first year. This strategy requires longer follow-up and larger numbers, but has the potential to reduce anti-HLA sensitisation and improve both graft survival and opportunities for future re-transplantation.

Key words: Epitope, paediatric, transplant, kidney, donor specific antibody

Introduction

For children with end-stage kidney disease (ESKD) kidney transplantation provides the best outcomes for growth[1], cognitive development[2-4], social well-being[5] and patient survival[6,7], They therefore require early access to transplantation, but due to limited graft survival, the need for re-transplantation is inevitable. Strategies are

required to reduce anti-HLA sensitisation and improve both graft survival and access to re-transplantation. Most DD allocation algorithms acknowledge the need for paediatric access to transplantation by incorporating bonus points and/ or priority access to younger DDs and DDs with favourable HLA-matching.

There is compelling evidence for the associations between HLA mismatching (particularly at class II), the development of de novo donor specific antibodies (dnDSAs), incidence of antibody-mediated rejection (AMR) and ultimately, reduced graft survival in both children and adults[8-13]. Furthermore, with increasing number of HLA-DR mismatches (MMs), rates of sensitisation increase in paediatric KTRs, which in turn impairs access to second and subsequent transplants by as much as 20% and also reduces the survival of each subsequent graft[14,15]. The majority of kidney allocation programs base their HLA matching on HLA-A, HLA-B and HLA-DR antigens and this is predicated on the improved graft outcomes reported with better HLA matching[16]. With greater understanding of the HLA molecule, its allelic variants and its three dimensional structure, functional epitopes of antibody binding have been defined. These functional epitopes may not be specific to individual HLA molecules, but can be shared across different HLA molecules (previously referred to as “public” and “private” antigens)[17]. This provides a logical explanation for the scenario where exposure to one foreign HLA molecule can lead to the generation of HLA antibodies directed at multiple other HLA molecules, sharing the same functional epitopes[18-20].

HLAMatchmaker employs an algorithm, based on the amino acid sequence of an HLA molecule, to define its binding epitopes, called ‘eplets’, which can then be used to quantify the mismatched eplets between a donor and recipient[17]. The clinical outcome of a mismatched donor eplet will depend on whether it can react with antibody (antigenicity) and whether it can induce antibody production (immunogenicity). Eplet MMs in kidney transplantation have been demonstrated as a more accurate determinant of the risk of HLA antibody development for both class I[21,22] and class II[23] and have also been implemented in a successful program to facilitate identification of acceptable mismatched donors for highly sensitised patients in Europe, the Eurotransplant Acceptable Mismatch Program[24]. Reports of the use of HLAMatchmaker cases in the paediatric transplant literature are limited[25,26].

In 2011, the Transplantation Society of Australia and New Zealand (TSANZ) introduced a points bonus in the national allocation algorithm for any child under 18 years, who had been on dialysis for >12 months and progressively introduced this system in states across the country[27]. In order to maximise the benefits of this bonus for children transplanted at the Royal Children's Hospital Melbourne (RCH), a program was developed using HLA eplet matching criteria to minimise the possibility of transplanting kidneys likely to result in broad sensitisation, while simultaneously achieve timely access to transplantation. This paper describes the implementation of a paediatric-specific kidney allocation strategy using eplet loads and the outcomes for the first year's cohort of recipients transplanted under this program.

Materials and Methods

This was a single centre prospective cohort study performed at RCH, where 86 kidney transplants were performed between 2003-2014, including 59 LD transplants and 27 DD transplants. Following inception of the paediatric bonus into the local regional kidney allocation program on December 10, 2013 donor eplet loads were integrated into the donor acceptance criteria for DD-listed patients.

Patients were eligible for DD listing once they had completed detailed medical, surgical and psychological assessments and had commenced dialysis (patients are not entitled to pre-emptive DD transplantation in Australia). From January 2014, all DD listed patients had HLA typing at an allele level and HLAMatchmaker assessments by senior scientists at the Victorian Immunogenetics and Transplantation Service (VTIS).

The principles used to integrate eplet matching into kidney donor allocation, included the following:

- Any child requiring an urgent transplant was exempt from this program to ensure no delay in transplantation.
- An eplet threshold approach for class I and class II HLA was adopted based on data from studies addressing the association between the number of eplet MMs and risk of antibody development or graft survival, primarily observational studies of adult KTRs [21-23,28].

- Eplet thresholds were determined to maximise the immunological benefits, without serving to exclude the vast majority of potential donors, thereby excessively extending waiting times. Listing criteria for younger/ healthy donors also remained, particularly for the youngest recipients. Based on the HLA typing of the preceding 1015 local donors, the predicted donor exclusion (PDE) was used to quantify the percentage of donors carrying the excluded HLA antigens and estimate the impact of any eplet-based exclusions on the donor pool.
- Specific eplet thresholds were determined, above which HLA antigens were deemed unacceptable. As distinct from previous studies, for HLA class II, the well-recognised increasing burden of late AMR and its association with non-DRB1 dnDSAs was considered important. Accordingly, the class II threshold was based on the cumulative eplets of that antigen and class II antigens carried in linkage disequilibrium across DRB1, DRB345, DQB1 and DQA1 (For a detailed explanation and worked case example, see Supplementary Figure). The final eplet thresholds for each antigen were as follows: HLA class I <10 eplets, HLA class II <30 eplets.
- All patients had eplet-based HLA exclusions formulated by the lead transplant clinician and scientist prior to listing. HLA antigens associated with eplet loads above these thresholds were entered on the allocation system as unacceptable and offers of donors with these antigens were automatically excluded. No decision on eplet mismatches would therefore be required at the time of a donor offer.
- As a safeguard against excessive delay in access to transplantation through the additional donor exclusions, the designated lead transplant clinician and scientist met three-monthly face-to-face to consider revision of each listed patient's exclusions.

Outcomes of DD KTRs transplanted at age <19 years through the RCH kidney transplant program from January 2014 to January 2015 were compared to LD KTRs during the same period, as well as to DD KTRs in the preceding 10 years. In summary, four groups of patients were studied:

1. DD January 2014 to January 2015, with eplet exclusions (DD-Excl)

2. DD January 2014 to January 2015, without eplet exclusions (DD-NoExcl)

3. LD January 2014 to January 2015, without eplet exclusions (LD-NoExcl) and

4. DD 2003-2013, inclusive (DD-Pre2014)

Routine immunosuppression for both DD and LD patients comprised induction with basiliximab and intravenous methylprednisolone followed by oral maintenance with prednisolone, mycophenolate mofetil and tacrolimus. However, one child in the DD-NoExcl group with severe osteoporosis had a steroid minimisation regimen incorporating thymoglobulin induction followed by rapid wean of prednisolone and maintenance mycophenolate mofetil and tacrolimus, while two LD KTRs received plasma exchange in the peri-transplant period; one with an ABO-incompatible and HLA incompatible transplant, the second with two weak donor specific antibodies. No patient received rituximab or any other cell-depleting therapy at any stage prior to or post-transplant.

Patients and donors were typed to an allele level: HLA A,B,DRB1, DPB1,DRB3/4/5 using Sequence Based Typing (Connexio, WA) and analysed using software Assign (Connexio, WA); HLA -C, DQA1,DQB1 were typed by SSO hybridisation using Luminex LABTYPE typing Kits (OneLambda, Canoga Park, CA) as previously published[29]. Post-transplant monitoring of HLA antibodies was performed as follows: Prior to 2014- only for indication; DD KTRs transplanted after January 2014- at 3, 6 and 12 months; LD KTRs- annual surveillance. Prior to 2014, sera were initially screened for HLA antibodies using in house CDC T cell panels and then either screened by GTI Quikscreen ELISA kits (2003-2007) or Luminex Mixed bead kits and Single Antigen bead kits (OneLambda, Canoga Park, CA) (2008-2013). From 2014 sera were tested for HLA Class I and II specific IgG antibodies using the LABScreen Mixed Class I and II antibody screening kit (OneLambda, Canoga Park, CA) and if positive, further evaluated using HLA Class I and/or Class II LABScreen single antigen kits (OneLambda, Canoga Park, CA). Post-transplant sera were pretreated to remove interference from IgM specific antibodies with hypotonic dialysis using Slide-A-Lyzer Mini Dialysis Units: 10,000 MWCO (Thermo Scientific). Eplet analysis was generated using ABC + DRDQDP Eplet Matching Macro v2.1: Duquesnoy.

Ethics approval for this study was granted by the human research ethics committee at RCH. Data collection and statistical analysis were performed using Microsoft Excel 2010 and GraphPad Prism (version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). For normally distributed data, unpaired t tests were performed, whereas for asymmetrically distributed data Wilcoxon rank sum test was used. A p-value <0.05 was accepted as a significant difference.

Results

The first patient to receive a DD transplant under this program was transplanted on February 24, 2014. In the first 12 months of the program, 19 patients were transplanted. This included 8 LD transplants and 11 DD transplants, of which 8 were listed with eplet exclusions (DD-Excl). Indications to list without eplet exclusions (DD-NoExcl) were as follows: one for medical urgency due to dialysis access failure; one for decompensating liver failure and failed second kidney transplant requiring combined liver-kidney transplant; while the third, aged 18 years was both ineligible for the paediatric bonus and awaiting a second transplant. During the study period 13 children were listed for DD transplant, of whom 11 were successfully transplanted. From the implementation of the paediatric bonus, the time to transplant for the 8 DD-Excl was 6.55 months (range 0.4-13.6 months), of which 5 donor offers resulted directly from the paediatric bonus and 3 resulted from standard allocation matching criteria. Of the 8 DD-Excl KTRs, five were transplanted from their first offer and three from their second offer.

Table 1, figure 1 and figure 2 detail demographic and baseline immunological data. The KTRs across all groups were largely unsensitised, with all DD-Excl KTRs having a pre-transplant PRA of <25%. There were no episodes of clinical or biopsy-proven rejection in any of the DD-Excl, DD-NoExcl or LD-NoExcl KTRs during the first 12 months. The institution of eplet-based exclusions in the DD-Excl group resulted in a PDE of 74.4% (SEM +/- 3.3%), Figure 1C. Paediatric Schwartz[30] estimated mean GFR (ml/min/1.73m^2) at 12 months revealed no significant differences: DD-Excl- 78.5 (range 46.45- 104.7), DD-NoExcl- 72.5 (range 33.65- 104), LD-NoExcl – 79.6 (range 53.5- 117.8), Fig 1D.

There was no difference in class I and class II HLA antigen MMs between the DD-Excl group and LD-NoExcl groups: DR and ABDR MMs for DD-Excl and LD-NoExcl groups were 1.13 vs 0.88 and 3.6 vs 3.0, respectively (Table 1 and Fig 2 A,B). Class I eplet MMs were very similar between all groups. By contrast, class II eplet MMs in the DD-Excl group (20.4) were maintained at an almost identical level to the LD-NoExcl group (20.1), but were significantly lower than both the DD-NoExcl (63.7, p=0.008) and DD-Pre2014 (46.9, p=0.037) groups, Fig 2 C,D. The distinction between antigen and eplet mismatches was well demonstrated by one KTR who was transplanted with the second of two suitable donor offers over the course of one month. The first offer emerged immediately prior to commencement of the prospective exclusion of high eplet MMs in this allocation program. The donor was a 4/6 antigen MM for HLA-ABDR, but was manually excluded based on a high eplet load DR53, an antigen not factored into standard allocation. The second, accepted donor offer had no high eplet MM antigens and despite complete 6/6 MM at HLA-ABDR antigens, was more favourable based on eplets. Eplet MMs for the first and second donors were 19 vs 18 for class I and 63 vs 28 for class II, respectively. He remained free of dnDSAs at 12 months.

Development of dnDSAs is summarised in Table 2. None of the DD-Excl KTRs developed dnDSAs in the first 6 months, but at 12 months, one had weak dnDSAs (MFI <2000) and one had moderate dnDSAs (MFI 2000-8000). By comparison, 5 of 8 LD-NoExcl KTRs had dnDSAs as did two of three DD-NoExcl KTRs. Data were available on 13 of the 16 DD-Pre2014 KTRs, 2 of whom had no dnDSAs, 2 had weak dnDSAs, whereas 9 carried high strength dnDSAs (MFI >8000).

Discussion

Sensitisation is well-recognised as a major barrier to transplantation, leading to extended waiting times on dialysis and increased mortality, for both adults and children[31-37]. Most DD allocation programs incorporate some strategy to expedite or prioritise their access to transplantation, such as occurred with ‘Share35’ in the USA, but with the burden of higher rates of HLA MMs[38]. Similarly in Australia[29], a comparison was reported between states with and without a paediatric bonus. States with a paediatric bonus had a higher rate of DD:LD transplant, reduced DD waiting

time, but greater HLA MMs, with one state reporting >3 HLA-A,B,DR MMs in 89.3% of DD KTRs and 2 HLA-DR MMs in 66.6% of DD KTRs.

A fundamental premise of epitope-based matching is that the immunological anti-HLA targets are not defined at an antigen level, but at the level of the molecular target of the anti-HLA antibody, the functional epitope or eplet. The HLA antigen MM may have either a low or high risk for immunogenicity, which can be predicted by an eplet-based HLA matching algorithm. The novel eplet-based allocation system used at the RCH allowed timely access to transplantation by accepting HLA antigenic mismatches, however, reduced risk of sensitisation (low eplet mismatch).

To our knowledge, this is the first reported prospectively implemented kidney allocation program incorporating eplet loads to minimise sensitisation in paediatric KTRs. By gaining the benefit of expedited access to DD kidneys, while minimising the risk of sensitisation of unsensitised paediatric KTRs by highly mismatched donors, the aim was not only to improve graft survival, but also improve access to re-transplantation.

There were excellent outcomes amongst all the contemporary groups, with no graft losses, no rejection episodes and GFR >78ml/min/1.73m^2 for the DD-Excl and LD-NoExcl groups and 72ml/min/1.73m^2 for the higher risk, DD-NoExcl group. These findings at 12 months post-transplant require longer-term follow-up to evaluate any effect on graft survival. The specific eplet threshold target was innovated based on a small body of evidence from the published literature, but also on a pragmatic compromise of maintaining a sufficient access to the donor pool. The data from Wiebe et al[23] concluded that class II dnDSA production was much more accurately predicted by eplet rather than antigen matching. They found a threshold of eplet mismatches associated with reduced rates of dnDSA production: DR<10 (0% dnDSA) and DQ<17 (2.7% dnDSA). These data emerged contemporaneously with the initiation of our study, but it is interesting that their cumulative threshold risk of 27 is so closely aligned to the targeted threshold in our study of 30. Imposing eplet thresholds on these unsensitised patients significantly impacted on access to HLA-compatible donors, with predicted exclusion of 74.4% of the donor pool. Nonetheless, 8 of 10 DD-Excl wait-listed patients received a transplant during the

study period, with a mean time to transplant of 6.5 months. The paediatric bonus proved essential, being directly responsible for the offer in 5 of the 8 patients.

It was hypothesised that the risk of inducing dnDSAs, could be more accurately predicted/ prevented based on HLA-eplet MMs rather than the traditional HLA-A,B,DR antigens used for allocation. This report is limited by the small numbers of patients and short follow-up. Neither HLA antigen nor class I eplet MMs yielded statistically significant differences between the four groups, but there were significant findings with respect to class II eplet MMs. Class II eplet MMs were similar between DD-Excl and LD-NoExcl groups, albeit with a trend to higher HLA antigen MMs at both A,B,DR combined and DR alone (Table 1). However, both the DD-NoExcl and DD-Pre2014 groups had significantly higher class II eplet MMs.

The key early outcome measure of this strategy was development of dnDSAs, which has been reported in approximately 35% paediatric KTRs at a mean of 3 months[13]. Two of the 8 patients in the serially monitored DD-Excl group had detectable dnDSAs by 12 months post-transplant. Interestingly, in the study by Kim et al[13], the majority of dnDSAs were indeed found to be directed against HLA class II (70%), which in turn were primarily HLA-DQ antibodies (45%). Similarly, in our study, of 20 patients with dnDSAs, 16 had class II dnDSAs, either alone or combined with class I (Table 2). The strong linkage disequilibrium between HLA-DR and HLA-DQ is not taken into consideration by standard HLA-A,B,DR allocation, but was integrated into the RCH eplet exclusion strategy (see Supplementary Figure) and the resulting lower class II eplet loads, despite equivalent or higher DR MMs, are a plausible explanation for the low rate of dnDSAs. This may have important implications, given the emerging evidence supporting the importance of class II MMs and the risk of AMR, graft loss and sensitisation (particularly with HLA-DQ, rather than HLA-DR DSAs)[8,9,11,12,14].

Due to the constraints of this clinical observational study using standard of care management, intensive monitoring for dnDSAs was only performed for the DD-Excl group; the LD group had annual surveillance or as clinically indicated; and the DD-Pre2014 KTRs were only tested as clinically indicated. Acknowledging that interpretation of data from the comparator groups is limited by the less stringent antibody surveillance than the DD-Excl group, one would expect any difference in

dnDSA results would be greater if detection of dnDSAs, which emerged at an earlier time-point and subsequently resolved, were missed at 12 months in the LD-Excl KTRs. Irrespective of frequency of testing, of 16 routinely wait-listed DD KTRs transplanted between 2003- 2013, dnDSAs were detected in 11 of 13 tested. Furthermore, 9 of these KTRs now carry at least one high level dnDSA (MFI >8000), likely to make accordingly typed potential donors incompatible for future transplantation.

The success of any strategy based on eplet matching requires several critical innovations to standard practice. It is a prerequisite that allele level typing is performed for HLA class I and II, an aspiration for which there has been strong advocacy[39]. Additionally, there were two key principles guiding the development of this program. Firstly, the prospective assignment of eplet threshold exclusions to minimise the complexity at time of donor offer for the multiple nephrology staff, with varying knowledge of the individual recipients and HLAMatchmaker. Secondly, timely access to transplantation was addressed by 3 monthly reviews of each individual's exclusions and access to potential donors. Having paediatric bonus allocation points to expedite access to donors and employing these principles make eplet-based allocation ideally suited to the characteristically small, single-centre paediatric transplant programs. More data are required before this strategy can be recommended for allocation at a national level for children or adults, or for implementation in other allocation systems.

This study reports data from the inception of a novel program. Its strength lies in the demonstration of the practical implementation of epitope-based allocation with no adverse effect on early clinical outcomes. Several limitations have been identified, including the small number of patients, short follow-up and lack of a direct comparator group. Nonetheless, the low rates of dnDSAs are promising, given their development within this time-frame is well-established and early development of dnDSAs is predictive of graft loss[13,40]. The comparator groups, LD-NoExcl, DD-NoExcl and DD-Pre2014, served to provide some insight into the baseline rates of eplet MMs and development of dnDSA, appreciating the many potential differences between these groups, including era of transplantation, specific indications for transplantation and limited longitudinal data on dnDSAs. Finally, it must be acknowledged that without a precedent for this approach, the eplet loads set in this

strategy were empiric and there was no consideration for immunogenicity of individual eplets, an area for which data is eagerly awaited[41]. Matching on targeted HLA class epitopes (such as DR and DQ) and/ or key immunogenic epitopes are promising potential strategies to further refine the use of epitope matching.

In summary this program pioneers the methodical application of DD kidney allocation to paediatric recipients, using an eplet-based MM load. These early data suggest that with the benefit of a mechanism to facilitate expedited access to the donor pool (paediatric bonus points in the Australian setting) this strategy allows transplantation to occur within an acceptable waiting time, achieving the targeted eplet MMs, with excellent early graft function and a low rate of dnDSAs. Longer-term follow-up is intended as this program continues to be implemented and 5, 10 and 20 year outcomes for graft survival, rates of rejection, dnDSAs and access to retransplantation will provide the definitive evidence as to the success of this approach.

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Table 1: Baseline demographic and immunologic data.

	DD-Excl (n=8)	DD-NoExcl (n=3)	LD-NoExcl (n=8)	DD-Pre2014 (n=16)
Gender (M,F)	4,4	3	3,5	9,7
Age at Tx (SEM)	11.2 (1.3)	17.8 (0.5)*	11.4 (2.1)	11.7 (1.1)
Donor age (SEM)	40.1 (5.6)	45 (10.1)	47 (4.6)	36.6 (3.4)
Transplant number:				
1	8	1	6	13
2	0	1	2	3
3	0	1	0	0
Cause of kidney disease:				
ARPKD		2	1	3
Dysplasia	2		5	4
Glomerulonephritis	1			1
Nephronophthisis	1		1	3
Obstructive uropathy	1		1	2
Other	3	1		2
Pre-transplant PRA (n):				
0%	5	1	6	14
>0-25%	3	1	1	
>25-50%		1	1	2
>50%				
HLA-AB MM (SEM)	2.5 (0.4)	2.7 (0.9)	2.13 (0.3)	2.06 (0.3)
HLA-DR MM (SEM)	1.13 (0.2)	1.7 (0.3)	0.88 (0.2)	1.25 (0.2)
HLA-ABDR MM (SEM)	3.6 (0.6)	4.3 (1.2)	3 (0.4)	3.3 (0.4)
Class I Eplet MM (SEM)	15.4 (3.1)	11.7 (5.2)	14.6 (2.1)	16 (2.0)
Class II Eplet MM (SEM)	20.4 (5.4)	63.7 (16.2)**	20.1 (5.0)	46.9 (7.9)*
Class I and II Eplet MM (SEM)	35.8 (7.4)	75.3 (18.8)*	34.8 (5.6)	62.9 (8.8)

Other includes focal and segmental glomerulosclerosis, atypical haemolytic uraemic syndrome, cortical necrosis and Denys-Drash syndrome. ARPKD- autosomal recessive polycystic kidney disease, MM- mismatch, SEM- standard error of the mean. *$p<0.05$, **$p<0.01$ (comparison to DD-Excl group).

Table 2: Development of de novo donor specific antibodies (dnDSAs).

	DD-Excl (n=8)	DD-NoExcl (n=3)	LD-NoExcl (n=8)	DD-Pre2014 (n=13)
Strength of dnDSA				
Negative	6	1	3	2
Low	1	0	3	2
Moderate	1	2	2	0
High	0	0	0	9
Class of dnDSA				
Class I			2	2
Class II	1	1	1	1
Class I & II	1	1	2	8

Note, development of dnDSAs was prospectively recorded at 3, 6 and 12 months for DD-Excl and DD-NoExcl KTRs; at 12 months for LD KTR and retrospective data was recorded from any stage post-transplant for DD-Pre2014. Three KTRs from the DD-Pre2014 group did not have antibody testing post-transplant. Antibody strength based on mean fluorescence intensity (MFI): Low <2000; Moderate >2000- 8000; High >8000.

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Figure 2: Donor-recipient HLA matching data.