Kidney paired donation in the presence of donor-specific antibodies

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Incompatible donor/recipient pairs with broadly sensitized recipients have difficulty finding a crossmatch-compatible match, despite a large kidney paired donation pool. One approach to this problem is to combine kidney paired donation with lower-risk crossmatch-incompatible transplantation with intravenous immunoglobulin. Whether this strategy is non-inferior compared with transplantation of sensitized patients without donor-specific antibody (DSA) is unknown. Here we used a protocol including a virtual crossmatch to identify acceptable crossmatch-incompatible donors and the administration of intravenous immunoglobulin to transplant 12 HLA-sensitized patients (median calculated panel reactive antibody 98%) with allografts from our kidney paired donation program. This group constituted the DSA(+) kidney paired donation group. We compared rates of rejection and survival between the DSA(+) kidney paired donation group with a similar group of 10 highly sensitized patients (median calculated panel reactive antibody 85%) that underwent DSA(−) kidney paired donation transplantation without intravenous immunoglobulin. At median follow-up of 22 months, the DSA(+) kidney paired donation group had patient and graft survival of 100%. Three patients in the DSA(+) kidney paired donation group experienced antibody-mediated rejection. Patient and graft survival in the DSA(−) kidney paired donation recipients was 100% at median follow-up of 18 months. No rejection occurred in the DSA(−) kidney paired donation group. Thus, our study provides a clinical framework through which kidney paired donation can be performed with acceptable outcomes across a crossmatch-incompatible transplant.

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Sensitization to human leukocyte antigens (HLAs) can be a devastating condition that makes the hurdle to receiving a kidney transplant even more difficult to surmount. Finding a suitable organ for a highly sensitized patient represents one of the most daunting challenges for transplant providers. The significance of antibodies as effectors of transplant rejection has been recognized since the early years of transplantation.[1] Patients with end-stage renal disease may be fortunate to have a willing and medically suitable living donor, only to be told that they are incompatible either through blood-type or donor-specific HLA antibodies (DSA). Historically, patients in this predicament were told that they would have to wait for a deceased-donor kidney, or go through a desensitization protocol to attempt to perform the transplant, despite their greater risk of rejection.[2–11]

In the past decade, kidney paired donation (KPD) has become a powerful method for facilitating living-donor transplantation for incompatible donor/recipient pairs. Incompatible donors, in essence, are exchanged to a compatible recipient. What started as a method for transplanting living-donor pairs across blood-type incompatibilities in simple two-way exchanges has expanded to kidney transplant chains involving up to 30 transplants.[12–19] Ideally, KPD achieves a completely negative crossmatch for the recipient, with no detectable DSA or blood-type incompatibility. Finding such matches remains extraordinarily difficult for patients who are broadly sensitized.

Although it is not always possible to find an ideal, negative crossmatch through KPD, a multicenter donor/recipient pool can be used to exchange sensitized patients away from a donor with an unacceptable positive crossmatch to a donor with whom they have persistent DSA, but at an 'acceptable' level. Our program prospectively defined crossmatch criteria by both the flow cytometric crossmatch (FXM) and by DSA strength by median fluorescence intensity (MFI) at which we would accept an organ from the KPD donor pool.

In this report, we present the outcomes of our experience with sensitized patients in our KPD program who were able to receive a kidney transplant with persistent DSA, but at a level that resulted in an acceptable crossmatch.

RESULTS

Patient characteristics

Between July 2008 and July 2011, a total of 22 living-donor kidney transplants facilitated by KPD were performed in sensitized recipients. Patient characteristics are shown in Tables 1 and 2. This was a highly sensitized group of patients with a median calculated panel reactive antibody (cPRA) of 98% (range 27–100) for the DSA(+)KPD group, and 85% (range 27–98) for the DSA(−)KPD group ($P=0.20$). All recipients had DSA to their intended donor with whom they entered into the KPD program. A total of 12 patients (55%) underwent DSA(+)KPD as they could not find a donor to whom they had no DSA. Patient 4 in the DSA(+) KPD group was also ABO incompatible with his matched KPD donor; this patient had been on hemodialysis for over 6 years since his first transplant had failed, with a cPRA of 100%. We were able to find a completely DSA(−) donor for 10 patients (45%) who underwent DSA(−)KPD.

No KPD chains at any participating medical centers were disrupted by DSA(+) transplants.

Dialysis time and KPD wait-time

Patients who underwent DSA(+)KPD had a median dialysis time of 31 months (range 0–94) and 41 months (range14–81) in the DSA(−)KPD group ($P=0.39$). For patients who underwent their second transplant, dialysis time was started from the date of return to dialysis. KPD wait-time was

Table 2 | Demographics comparison

Recipient variable	DSA(+)KPD ($n=12$)	DSA(−)KPD ($n=10$)	*P*-value
Age (years), median (range)	60 (28-69)	46 (34–57)	0.06
KPD donor age, median (range)	48 (41-60)	52 (31-64)	0.72
Sex, (male/female)	8M/4F	7M/3F	0.99
Peak cPRA%, median (range)	98 (27–100)	85 (27–98)	0.20
Dialysis time (months), median (range)	31 (0–94)	41 (14–81)	0.39
KPD wait-time (months), median (range)	6 (2–17)	3 (0–16)	0.15
Diabetes (yes/no)	2/10	3/7	0.62

Abbreviations: cPRA, calculated panel reactive antibody; DSA, donor-specific antibody; F, female; KPD, kidney paired donation; M, male.

Table 1 | Detailed patient characteristics

Patient no./sex/age (years)	Race/ethnic group[a]	ESRD cause	No. of previous transplants	KPD donor age (years)	HLA mismatch[b]	Blood group	cPRA%[c]	Dialysis time[d] (months)	KPD wait-time[e] (months)
DSA(+) KPD (n = *12*)									
1/F/49	Black	Lupus	0	56	4/6	O+	97	74	5.5
2/M/57	White	Diabetes	1	56	4/6	B+	100	22	6
3/M/66	White	FSGS	1	44	6/6	A+	68	54	14
4/M/38	White	FSGS	1	48	5/6	B+	100	73	3.5
5/F/62	White	PCKD	0	41	5/6	A+	99	94	6
6/F/67	White	IgA	0	60	6/6	O+	63	29	11
7/F/69	White	FSGS	0	50	6/6	O+	99	12	15
8/F/66	White	PCKD	0	42	5/6	O+	69	0	4.5
9/F/51	Hispanic	PCKD	0	60	3/6	O+	98	32	7
10/F/28	Hispanic	HTN	0	42	5/6	O+	27	0	2
11/F/44	White	HTN	0	47	4/6	O−	89	4	5
12/F/68	Asian	HTN	0	43	4/6	B+	99	37	3
DSA(−)KPD (n = *10*)									
13/F/52	Asian	Lupus	0	44	2/6	A+	92	18	2
14/F/42	Black	HTN	0	31	3/6	O−	76	60	5
15/M/45	Asian	IgA	0	55	6/6	AB+	27	42	To wait-list[f]
16/F/43	White	HTN	0	56	4/6	AB+	88	65	To wait-list[f]
17/M/43	Asian	Diabetes	1	60	6/6	O+	87	23	7
18/F/38	Asian	Diabetes	0	37	3/6	A+	98	39	15
19/F/47	White	FSGS	0	46	5/6	B+	65	14	6
20/F/46	Other	MGN	0	64	3/6	AB−	75	45	To wait-list[f]
21/M/34	Asian	HTN	1	56	3/6	O+	98	38	2
22/F/57	Hispanic	Diabetes	0	49	4/6	O+	82	81	3

Abbreviations: cPRA, calculated panel reactive antibody; DSA, donor-specific antibody; ESRD, end-stage renal disease; F, female; FSGS, focal segmental glomerulosclerosis; HLA, human leukocyte antigen; HTN, hypertension; IgA, immunoglobulin A; KPD, kidney paired donation; M, male; MGN, membranous glomerulonephritis; PCKD, polycystic kidney disease.
[a]Patient race/ethnic group is self-reported.
[b]HLA mismatch based on HLA-A, B, and DR loci.
[c]cPRA based on unacceptable antigens using the UNOS calculator (http://optn.transplant.hrsa.gov/resources/allocationcalculators.asp?index=78).
[d]Dialysis time for repeat-transplant patients is measured from time of renal transplant failure to transplant date. Patients with '0' dialysis time were transplanted preemptively.
[e]KPD wait-time is measured from recipient list-date in to the National Kidney Registry matching database to the actual date of transplantation.
[f]Patient without willing living donor who received living-donor renal transplant from KPD pool while on deceased-donor waiting list.

calculated as the time from patient entry in to the matching databases to the date of transplantation. Three patients who underwent DSA(−)KPD received a living-donor kidney from a KPD donor while waiting on the deceased-donor list and were considered to have '0' KPD wait-time. KPD wait-time was a median of 6 (range 2–17) months for DSA(+) KPD patients versus 3 (range 0–16) months in the DSA(−) KPD patients ($P=0.15$).

Immunological data

Each of the DSA(+)KPD patients received a kidney from a donor with a lower perceived immunological risk than their intended donor. Detailed immunologic data for the DSA(+) KPD group is shown in Tables 3 and 4. In respect to the DSA(+)KPD transplants performed, 12 recipients (100%) had a positive virtual crossmatch based on the DSA identified on solid-phase testing. Four recipients (33%) had a positive T-FXM (>50 median channel shift (MCS)), and 8 recipients (67%) had a positive B-FXM (>100 MCS). The 10 (100%) patients who underwent DSA(−)KPD had DSA to their intended donor, but obtained a negative virtual crossmatch to their matched KPD donor.

Transplant outcomes and rejection episodes

Transplant outcomes are detailed in Table 5. Median follow-up time for the DSA(+) and DSA(−) groups was 22 (range 10–29) and 18 months (range 9–36), respectively ($P=0.74$). Patient and graft survival in both groups was 100% at all follow-up intervals. Delayed graft function did not occur in the DSA(+)KPD patients, and occurred in 1 of 10 (10%) of the DSA(−)KPD patients ($P=0.46$).

Serum creatinine levels were higher in the DSA(+) group at all follow-up intervals, but there was no statistically significant difference at 1 week, 6 months, and 1 year. Because of less follow-up at longer intervals, we did not calculate for significance after 1 year. (Table 5)

Three patients were biopsied for decline in renal function in the DSA(−)KPD group, but none was found to have rejection. Three patients (25%) who underwent DSA(+) KPD experienced acute rejection ($P=0.22$). Antibody-mediated rejection (AMR) occurred in all three cases, and one patient also had acute cellular rejection (Table 3). All three episodes of AMR were associated with positive C4d staining on biopsy. No patients that obtained a negative pre-transplant B-FXM (<100 MCS) in the DSA(+)KPD group experienced rejection. All three patients who rejected had HLA class II DSA pre-transplant and a positive B-FXM to their matched DSA(+)KPD donor. The occurrence of AMR was statistically associated with a positive B-FXM when comparing the 8 patients (three with AMR) with positive FXM with the 14 negative FXM patients (none with AMR)($P=0.04$).

Patient number 1 was treated with intravenous immune globulin (IVIG; 1 g/kg daily for 2 days) and five sessions of plasmapheresis; single-antigen bead (SAB) testing for DSA was not conducted during this episode. Patient number 7 had biopsy findings consistent with both AMR and acute cellular rejection and three *de novo* DSAs (DR9, DR10, and DQ5) with a MFI range of 8000–15000 MFI on SAB testing. Patient 7 was treated with IVIG (1 g/kg daily for 2 days) and antithymocyte globulin (125 mg/daily for 5 days). At the time of rejection, patient number 9 was found to have one known DSA (DR8) at 3429 MFI in addition to a *de novo* DSA (B39) at 1132 MFI. Patient 9 required eight plasmapheresis sessions, IVIG (1 g/kg daily for 6 days), and one dose of rituximab 750 mg/m^2. All three rejection episodes were successfully treated with recovery of allograft function.

Adverse events

There were no major surgical complications, and no complications related to allograft biopsies. Adverse events related to IVIG were minimal. There were no major infectious complications. One patient experienced mild airway irritation and pruritis during IVIG infusion that was rapidly improved with intravenous methylprednisolone.

DISCUSSION

In this report, we utilized KPD to obtain an acceptable crossmatch that involved DSA to achieve kidney transplantation for 12 broadly sensitized patients. Recipients of living-donor kidney transplants who participated in DSA(+)KPD experienced 100% overall survival and 100% graft survival at a median follow-up of 22 months.

Despite being unable to find an negative crossmatch donor through KPD, patients in the DSA(+)KPD group derived an immunological benefit from being exchanged away from their intended donor with whom they had a crossmatch that would require more intensive desensitization with the possibility of not reaching an acceptable crossmatch. The reduction in DSA levels achieved through DSA(+)KPD allowed crossmatch-incompatible living-donor transplantation. The reduction in T- ($P<0.003$) and B-cell ($P<0.008$) FXM levels, class II DSA strength ($P<0.02$), and overall DSA strength ($P<0.003$) was statistically significant for the DSA(+) recipients. (Table 4)

Patients who underwent DSA(+)KPD had an AMR rate of 25% compared with 0% in those who underwent DSA(−) KPD ($P=0.22$). Previous studies concerning HLA-incompatible transplantation have reported AMR rates ranging from 20 to 80%, depending on the strength of the positive crossmatch.[2–11,20–22] Reinsmoen *et al.* have shown that recipients with a FXM greater than 200 MCS are at higher risk of AMR despite pretreatment with IVIG.[6] In our immunogenetics laboratory, a FXM <200 MCS was consistently achieved with DSA strengths of <8000 normalized MFI on SAB tests, with the exception of HLA-Cw locus antibodies, which had an even higher threshold likely due to a lower expression on cells compared with HLA-A and -B antigens.[23] None of the cases here involved HLA-Cw-directed DSA. We have used these parameters when deciding to accept a KPD match that involves DSA.

Table 3 | Transplant immunological data for patients who underwent DSA(+)KPD

Patient no.	Crossmatch and DSA to intended donor[a]	Crossmatch and DSA to matched DSA(+)KPD donor[b]	Benefit from DSA(+)KPD match[c]	Immunosuppression	Follow-up DSA to matched DSA (+)KPD Donor	Rejection type, time from transplant and DSA present
1	T-FXM (17) B-FXM (341) DR7 (12557) , DQ2 (11667)	T-FXM (8) B-FXM (123) DQ2 (11667)	B-FXM <200 ↓DSA	IVIG/alemtuzumab FK/MMF/prednisone	12 Months: DQ2 (11421) A1 (2075) A11 (1739) B35 (5693)	AMR 17 Days 6 Days DQ2 (5982) 17 Days no serum 40 Days DQ2 (7628)
2	ABO-I T-FXM (419) B-FXM (379) A3 (4512), A33 (7188), DR11 (11993), DR13 (11331), DR52 (13732)	T-FXM (42) B-FXM (60) B71 (2613)	ABO-C T-FXM <50 B-FXM <100 ↓DSA	IVIG/ ATG FK/MMF/prednisone	No DSA at 6, 12, and 24 months	No rejection
3	T-FXM (44) B-FXM (247) DR51 (8587)	T-FXM (15) B-FXM (159) DQA 0501 (3578)	B-FXM <200 ↓DSA	IVIG/ ATG Sirolimus/prednisone	6 Months: no DSA 12 Months: DQA 0501 (2200)	No rejection
4	ABO-I T-FXM (136) B-FXM (412) DR14 (11796), DQ5 (9955) DR52 (2279)	ABO-I T-FXM (85) B-FXM (142) A1 (6123), DR52 (2279)	B-FXM <200 ↓DSA	ABO-I protocol/ ATG FK/MMF/prednisone	No DSA at 1 and 2 months	No rejection
5	T-FXM (201) B-FXM (381) A24 (14575), DR11 (4528) DR 13 (7746), DQ6 (7422)	T-FXM (161)[d] B-FXM (172) DQ5 (5452)	B-FXM <200 ↓DSA	IVIG/ ATG FK/MMF/prednisone	No DSA at 6,12, and 24 months	No rejection
6	ABO-I T-FXM (45) B-FXM (36) A2 (6328)	ABO-C T-FXM (20) B-FXM (77) A3 (2605)	ABO-C ↓↓DSA	IVIG/ daclizumab FK/MMF/prednisone	No DSA at 24 months	No rejection
7	CDC(+) T-FXM (226) B-FXM (340) B58 (7793) DR16 (14341), DQ5 (2688)	CDC(−) T-FXM (69) B-FXM (222) B44 (3139), DP17 (2658).DQ5 (2688)	(−)CDC B-FXM < 300 ↓DSA	IVIG/ alemtuzumab FK/MMF/prednisone	12 Months: DR9 (1308), DR10 (2428), DQ5 (3745) 18 Months: DR10 (2005), DQ5 (2362)	AMR + ACR 6 Months DR9 (8000), DR10 (15000), DQ5 (13000)
8	T-FXM (33) B-FXM (363) DR4 (15741), DQ7 (3716)	T-FXM (6) B-FXM (27) DQ7 (3716), DR9 (3489)	B-FXM <100 ↓DSA	IVIG/ ATG FK/MMF/prednisone	No DSA at 6 and 12 months	No rejection
9	T-FXM (45) B-FXM (327) DR51 (14397), DR15 (9719)	T-FXM (27) B-FXM (170) DR8 (3345)	B-FXM <200 ↓DSA	IVIG/ ATG FK/MMF/prednisone	No DSA at 3, 6, and 9 months	AMR 6 DAYS DR8 (3429), B39 (1132)
10	ABO-I T-FXM (106) B-FXM (115) B44 (3174), B45 (5335)	ABO-C T-FXM (88) B-FXM (107) B44 (3174)	ABO-C ↓DSA	IVIG/ ATG FK/MMF/prednisone	2 Weeks: B44 (8083) 2 Months: B44 (2757) 9 Months: B44 (1212) 12 Months: B44 (1164)	No rejection
11	ABO-I T-FXM (49) B-FXM (79) DR13 (3478), DR52 (2044)	ABO-C T-FXM (2) B-FXM (102) DR17 (2960), DR52 (2044)	ABO-C	IVIG/ ATG FK/MMF/prednisone	6 Months: DR17 (1210), DR52 (1447) 12 Months: DR17 (1300), DR52 (3547)	No rejection
12	T-FXM (202) B-FXM (202) B48 (7359)	T-FXM (38) B-FXM (14) B52 (1209)	T-FXM < 50 B-FXM <100 ↓DSA	IVIG/ ATG FK/MMF/prednisone	3 Months: B52 (2396) 6 Months: B52 (1736)	No rejection

Abbreviations: ABO-C, ABO blood group compatible; ABO-I, ABO blood group incompatible; ACR, acute cellular rejection; AMR, antibody-mediated rejection; ATG, antithymocyte globulin; B-FXM, B-cell flow cytometric crossmatch; CDC, complement-dependent cytotoxic crossmatch; DSA, donor-specific antibody; FK, tacrolimus; IVIG, intravenous immune globulin; KPD, kidney paired donation; MFI, median fluorescence intensity; MMF, mycophenolate mofetil; T-FXM, T-cell flow cytometric crossmatch.
↓DSA indicates that donor specific antibodies to the matched KPD donor are fewer in number and/or have lower MFI.
Except where noted, the complement-dependent cytotoxic crossmatch was negative.
(−) CDC indicates that a negative complement-dependent crossmatch was achieved.
[a]MFI for DSA is shown in parentheses following the specific antibody ( ).
[b]B-FXM and T-FXM values are expressed in median channel shift.
[c]KPD benefit indicates match parameters before administration of IVIG. A T-FXM <50, and B-FXM <100 are considered negative. A B-FXM <200 or <300 denotes that DSA(+)KPD facilitated a positive, but significantly weaker B-FXM than the crossmatch with the original, intended donor.
[d]Patient 5 had a positive auto T-cell flow crossmatch.

Multiple DSAs that individually fall below the unacceptable threshold are problematic in assessing risk. Our general approach has been to limit the number of DSAs to three or fewer and to sum the average MFIs for each using the 8000 MFI threshold as a relative limit. Only one of the five patients transplanted across multiple DSAs (patient 7 in Table 3) experienced AMR at 6 months with increased anti DQ5 and two *de novo* DSAs directed against DR9 and DR10. On the basis of this limited experience, there was no indication that multiple DSAs increased the risk of AMR.

We did not conduct additional crossmatch or SAB testing after the administration of high-dose IVIG (2 g/kg) because the increased immunoglobulin levels can interfere with these tests, but previous studies support single high-dose IVIG administration for HLA-incompatible kidney transplantation in patients with similar crossmatch parameters.[5,6,9]

There was no statistically significant difference in renal function as measured by serum creatinine at 1 week, 6 months, and 1 year, but there is a trend toward higher serum creatinine levels in our patients who underwent DSA(+) KPD. It is possible that patients in the DSA(+)KPD group have higher incidence of subclinical AMR or transplant glomerulopathy, but this cannot be proven without biopsy evidence.

Negative crossmatch kidney transplantation, such as facilitated by DSA(−)KPD, is superior to crossmatch-incompatible transplantation in terms of rejection rates, and finding a DSA(−) crossmatch should remain a priority. Haririan *et al.*[2] reported that positive crossmatch kidney transplants have a 69.4% graft survival rate at 5 years compared with 80.6% for negative crossmatch controls. An expanded national, or even international, KPD pool of

Table 4 | Immunologic benefit derived from DSA(+)KPD

	Intended donor	Matched DSA(+)KPD donor	*P*-value
Flow cytometric crossmatch (MCS)			
T-cell, median (range)	78 (17–419)	32 (2–161)	<0.003
B-cell, median (range)	334 (36–412)	115 (14–222)	<0.008
Single-antigen bead (summed MFI)[a]			
Class I, median (range)	0 (0–14,575)	604 (0–6123)	0.18
Class II, median (range)	12,808 (0–37,056)	3462 (0–11,667)	<0.02
Class I + II median (range)	16,098 (5522–48,756)	4291 (1209–11,667)	<0.003

Abbreviations: DSA, donor-specific antibody; KPD, kidney paired donation; MCS, median channel shift; MFI, median fluorescence intensity.
[a]Values represent the summed MFI values for all DSA found on single-antigen testing for each individual patient.

Table 5 | Transplant outcomes

DSA(+)KPD (patient no.)	Cold ischemia time[a] (h)	DGF?[b]	Rejection?		Total transplant follow-up (months)	Creatinine level (1 week)	Creatinine level (6 months)	Creatinine level (12 months)	Creatinine level (18 months)	Creatinine level (24 months)
1	1	No	Yes		29	1.7	1.4	1.7	1.7	1.9
2	10.4	No	No		28	1.4	1.5	1.2	1.2	1.2
3	14.5	No	No		24	1.4	1.1	1.2	1.2	1.3
4	8.8	No	No		24	1.4	1.5	1.6	1.5	1.5
5	9.7	No	No		24	1.3	1.5	2.3	1.6	2.0
6	1	No	No		24	1.0	1.2	1.5	1.3	1.3
7	8.7	No	Yes		19	0.7	3.3	1.1	1.0	NA
8	14.5	No	No		17	1.0	1.1	1.1	NA	NA
9	14.4	No	Yes		14	1.9	1.8	1.7	NA	NA
10	12	No	No		13	1.1	1.0	1.0	NA	NA
11	12.5	No	No		12	0.9	1.0	1.2	NA	NA
12	1	No	No		10	0.6	0.6	NA	NA	NA
DSA(−)KPD (patient no.)	**Median: 10 range (1–15)**	**0/12**	**3/12**	**Median (range)**	**22 (10–29)**	**1.2 (0.6–1.9)**	**1.3 (0.6–3.3)**	**1.2 (1.0–2.3)**	**1.3 (1.0–1.7)**	**1.4 (1.2–2.0)**
13	1	No	No		36	1.0	0.9	0.9	0.9	1.0
14	1	No	No		30	1.0	0.9	1.0	1.3	1.1
15	1	No	No		28	1.6	1.7	1.7	1.6	1.5
16	1	No	No		24	0.7	0.9	0.9	0.8	1.0
17	1	No	No		19	1.5	1.0	0.9	0.9	NA
18	16	No	No		17	0.8	0.9	0.9	0.9	NA
19	8.2	No	No		12	1.1	1.7	2.0	NA	NA
20	1	No	No		10	1.0	1.1	NA	NA	NA
21	14.8	No	No		10	1.2	1.0	NA	NA	NA
22	15.1	Yes	No		9	3.4	1.2	NA	NA	NA
	Median: 1 range (1–16)	**1/10**	**0/10**	**Median (range)**	**18 (9–36)**	**1.0 (0.8–3.4)**	**1.0 (0.9–1.7)**	**0.9 (0.9–2.0)**	**0.9 (0.8–1.6)**	**1.0 (1.0–1.5)**
P-value	0.41	0.46	0.22		0.74	0.95	0.13	0.08	Not calculated[c]	Not calculated[c]

Abbreviations: DGF, delayed graft function; DSA, donor-specific antibody; KDP, kidney paired donation; NA, not applicable.
[a]Cold ischemia time (CIT) reported for living-donor kidneys that were shipped from an outside medical center. For non-shipped kidneys, CIT = 1 h.
[b]DGF as defined by the need for dialysis within 1 week of transplantation.
[c]Statistical significance was not calculated because of small patient numbers in both groups.

incompatible living recipient/donor pairs would likely increase the chances of performing negative crossmatch transplants.[19,24–28]

Finding a balance between waiting for a compatible organ and minimizing dialysis time needs to be better elucidated. Patient 10, with a cPRA of 27%, O blood type, and only 2 months of waiting time in the KPD pool illustrates this conundrum; she underwent pre-emptive DSA(+)KPD as she was adamant about proceeding with transplantation before starting dialysis. Given the increased morbidity and mortality of dialysis, it has been our preference to proceed with DSA(+)KPD in sensitized patients who have difficulty finding a negative crossmatch within the KPD pool. As the demographics of a KPD pool are constantly changing, it remains difficult to predict how long a broadly sensitized patient should wait for a completely negative crossmatch.

Most previous reports of crossmatch-incompatible transplants in the context of KPD have been performed at single centers with control over the logistics of desensitization and transplantation. However, success with compound matching logistics, and difficult-to-transplant patients, requires a large potential pool of donors, and extensive trust and cooperation between transplant centers. Patients in this report received living-donor kidneys from 11 transplant programs. Some hospitals are thousands of miles from each other, requiring shipping of living-donor organs. A recent report from the Australian National KPD Program[29] included three patients who were successfully transplanted with low-level DSA and had uneventful early outcomes, but these transplants occurred in one match run when the standard MFI cutoff used by all five centers to avoid disruption in multicenter matches was temporarily lifted.

No chain transplants at partnering medical centers were delayed or disrupted by the DSA(+)KPD transplants in our experience. Extended tissue-typing techniques and characterization of HLA antibodies for the virtual crossmatch has improved the predictability of actual crossmatch results among participating transplant centers. As each center in the KPD program defined its acceptable level of DSA, the risk of disruption because of an unexpectedly strong, or unacceptable, crossmatch was minimized; however, to maintain chains, University of California, Los Angeles Medical Center (UCLA) adhered to realistic acceptable limits for DSA strength and FXM positivity. The low rate of delayed graft function, despite significant cold ischemia times, provides additional evidence that shipping can be tolerated even in patients who are at a higher risk for delayed graft function and rejection.

This report does have limitations. Larger patient groups will be needed to achieve statistical power. Longer follow-up and participation by additional institutions are required to confirm our initial findings. We did not use a specific protocol for immune monitoring and management of post-transplant DSAs. It is also important to recognize that the quantitation of anti-HLA antibodies using solid-phase or cell-based crossmatch tests may vary widely within and among laboratories and the transplant centers they serve. It has been reported that the concentrations of antigens can vary from lot to lot and for antigens of the different HLA loci,[30] and results from different laboratories may differ.[31] Thus, it is important that these tests be validated and correlated with other tests and with outcomes at each transplant center. As this was not a prospective study with a defined induction and immunosuppression protocol, there is some heterogeneity in the regimens our patients received. Finally, the utility of single-dose IVIG in the DSA(+)KPD recipients cannot be evaluated fully in this report. Nonetheless, it is our center's preference to augment the immunosuppression of patients being transplanted with known DSA in this manner. The authors recognize that some centers may choose to transplant similar patients with low levels of DSA without the addition of IVIG.

On the basis of our early results, multi-regional DSA(+) KPD should be considered as a modality for transplanting our most immunologically challenging patients. DSA(+) KPD results in a significant reduction in DSA that allows for living kidney transplantation with minimal desensitization. Incompatible donor/recipient pairs should be considered candidates for DSA(+)KPD if they cannot find a match without DSA in a large pool of potential donors.

MATERIALS AND METHODS

Patients

Institutional review board approval was granted to search the UCLA kidney transplantation database for this retrospective report. From July 2008 through July 2011, 58 living-donor kidney transplants were facilitated by KPD at the Ronald Reagan UCLA Medical Center. Twenty-two KPD recipients (38%) had DSA against their intended living donor and entered the KPD program with the intent of finding a HLA-compatible donor. Twelve of 22 patients did not find a match without DSA and constituted the DSA(+)KPD group. Ten of 22 patients found a donor with no DSA and constituted the DSA(−)KPD group.

The remaining 36 (62%) KPD recipients had only ABO incompatibility with their intended donor, without any DSA, and were not included in this report.

HLA typing and evaluation of HLA antibodies

Recipient and donor pairs were typed using molecular methods for HLA-A, -B, -DRB1,-DRB3/4/5, and -DQB1 loci by LABType SSO DNA typing, according to the manufacturer's specifications using HLA Visual software (One Lambda, Canoga Park, CA). Donors were further typed for HLA-Cw, DQA1, and DP loci (LABType SSO DNA typing) to permit accurate virtual crossmatching.

Pre-transplant serum samples were analyzed for antibodies directed against HLA class I (A, B, and C) and class II (DR, DQ, and DP) antigens using the Gen-Probe Luminex PRA and antibody specificity reagents, according to the manufacturer's specifications (San Diego, CA). Particle florescence was assessed by Luminex 100 IS (Luminex, Austin, TX). Additional Luminex-based single-antigen antibody identification assays (One Lambda) were run on positive sera to confirm the antibody specificity assignment and strength as indicated by the MFI. The sensitivities of the different lots of PRA and SABs used for HLA antibody testing were routinely

monitored for significant variance using HLA reference sera and determined to be comparable. Antibodies were considered positive when the normalized MFI value on SABs was >1000 and their target antigens were considered unacceptable in a donor when the MFI value was >8000, with the exception of antibodies directed against HLA-Cw antigens, which were considered positive when the MFI value was >7000 and unacceptable when >10 000 MFI. These cutoffs were established by empirical testing when we compared MFI values with actual flow crossmatches in a series of more than 80 positive T- and 80 positive B-cell FXMs for patients with class I and class II antibodies, respectively. Although the correlation between antibody strengths estimated from SAB tests and FXM strengths was not always consistent, our initial analyses revealed no cases when an antibody that registered <8000 MFI on SAB resulted in a flow crossmatch using T- or B-cell targets of >200 MCS. About 25% of patients with DSA and an FCXM<200 MCS in both T- and B-cell analyses had SAB strengths >8000 MFI.

These established cutoffs have been monitored and validated through continuing review of crossmatch tests. The FXM strength of <200 MCS is well above the level of our positive crossmatch (>50 MCS). This strategy was established to avoid disadvantaging sensitized patients by involving the transplant team in reviewing weak or moderately positive crossmatch donors rather than excluding those donors *a priori*.

HLA antibodies were tested every 3 months from the time of listing. For patients who underwent DSA(+)KPD, DSAs were periodically monitored following transplant by Luminex-based SAB identification assays.

Crossmatching and KPD matching

All recipients had a negative complement–dependent lymphocytotoxicity assay without AHG augmentation or wash modification. Three-color T- and B-cell flow-cytometric IgG crossmatches (FXM) were performed on a FACS caliber flow cytometer as previously described.[32] The flow crossmatch was considered positive when the MCS were >50 for T cells, and >100 for B cells using a 1024-channel scale.

Patients with suitable, but incompatible, living donors who wished to participate in our KPD program were enrolled in the National Kidney Registry (NKR).[17,19] Beginning in October 2010, all recipient/donor pairs were simultaneously entered into the KPD database administered by the United Network for Organ Sharing (UNOS) KPD pilot program, in addition to the NKR database.

All 22 transplants were performed as part of nonsimultaneous extended altruistic donor chains as previously described.[14–19] Three recipients did not have a suitable living donor with whom they could participate in KPD, but received a living-donor kidney transplant from a KPD donor, whereas on the transplant wait-list, ending that individual transplant chain. The remaining 19 patients were matched by the NKR, as no matches were identified through the UNOS pilot program.

The UCLA Positive-Crossmatch Committee, consisting of transplant surgeons, immunogeneticists, nephrologists, and nurse coordinators reviewed matches involving HLA class I and II antigen DSA to determine whether the crossmatch was acceptable for DSA(+)KPD with single-dose IVIG. The committee reviewed each case individually, but sought to achieve ABO compatibility, and T- and B-cell FXMs of <200 MCS each. Thus, although unacceptable antigens were initially based on antibody strength <8000 MFI on the basis of the SAB test, unacceptable antigen thresholds were dynamic and could be modified on the basis of patient parameters, potential donor offers, surrogate crossmatches, and actual crossmatch results.

Shipping of living-donor kidneys and transplant operations

Thirteen of 22 (59%) laparoscopic donor nephrectomies, 9 in the DSA(+)KPD group and 4 in the DSA(−)KPD group were performed at 11 partnering centers and the kidneys were shipped to UCLA for transplantation. Distances between UCLA and partnering centers ranged from ~300 miles to 2600 miles. Living-donor kidneys were shipped by commercial airline. Handling and logistics were facilitated by the local organ procurement organization (OneLegacy) and a national courier service (Sterling Courier). All 22 recipient operations were performed at UCLA.

Immunosuppression

Induction immunosuppression for DSA(+)KPD patients included antithymocyte globulin (1.5 mg/kg daily for 5 days), alemtuzumab, or daclizumab. All patients received methylprednisolone 500 mg intravenous as part of their induction. Maintenance immunosuppression in both groups included tacrolimus (0.05 mg/kg daily, divided in two doses), mycophenolate mofetil (2 g daily, divided in two doses), and prednisone (5 mg daily) for all patients, except for patient 3 who received sirolimus (6 mg daily) and prednisone only. Specific immunosuppression information for the DSA(+)KPD patients appears in Table 3.

Eleven patients in the DSA(+)KPD group received augmented immunosuppression with IVIG (2 g/kg up to 140 g, over two infusions) within 2 weeks before their scheduled transplant. Final crossmatch testing (within 30 days of the transplant) was conducted before the administration of IVIG. We did not recheck for DSA after the administration of IVIG. Pre-transplant plasmapheresis was not routinely performed in the DSA(+)KPD group. Patients who underwent DSA(−)KPD did not receive IVIG.

Patient 4 had DSA in addition to blood-type incompatibility (ABOi) with his matched DSA(+)KPD donor; this patient received anti-CD20 monoclonal antibody (rituximab 375 mg/m^2 intravenous × one dose), IVIG (2 g/kg in two infusions), and underwent six preoperative plasmapheresis sessions until acceptable titers (anti-A1 1:8) were reached. One postoperative plasmapheresis sessions was performed.

Diagnosis of rejection

Renal biopsies were carried out only for clinical suspicion of allograft rejection. Rejection was characterized by the Banff classification.[33] Protocol biopsies were not carried out.

Statistical analysis

Statistical analysis was performed with STATA 12.1 (ref 34) Fisher's exact test was used for categorical data. Wilcoxon's signed-rank test was used for paired continuous data. All *P*-values are two-sided, and a $P \leqslant 0.05$ was considered to be statistically significant.

DISCLOSURE

All the authors declared no competing interests.

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