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Use of Donor-derived Cell-free DNA to Inform Tapering of Immunosuppression Therapy in Kidney Transplant Recipients: An Observational Study

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Background. Immunosuppression therapy (IST) is required for allograft survival but can cause significant adverse effects. Donor-derived cell-free DNA (dd-cfDNA) is a validated noninvasive biomarker for active rejection in kidney transplant (KTx). Evidence supporting dd-cfDNA testing use in IST management is limited. **Methods.** In this single-center observational study, dd-cfDNA testing was performed in 21 KTx patients considered good candidates for mycophenolic acid (MPA) reduction. Patients with dd-cfDNA <1% at the first visit (enrollment) had their MPA dosage reduced; those with dd-cfDNA ≥1% had their MPA dosage maintained. Patients were monitored with dd-cfDNA for 6 additional visits. **Results.** Of 21 patients enrolled in the study, 17 were considered low risk for rejection by dd-cfDNA and underwent MPA reduction; 4 patients were considered high risk for rejection by dd-cfDNA and had their initial MPA dosage maintained. Of the 4 patients considered high risk for rejection by dd-cfDNA, 1 experienced chronic allograft nephropathy and graft loss, and another received an indication biopsy that showed no evidence of rejection. Of the 17 patients considered low risk for rejection by dd-cfDNA, none experienced allograft rejection. dd-cfDNA was used for surveillance in a 6-mo period following MPA reduction; no untoward results were noted. **Conclusions.** This proof-of-concept study reports the use of dd-cfDNA to directly inform IST management in a cohort of KTx who were candidates for IST reduction.

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Although advances in immunosuppressive therapy (IST) management have dramatically improved short-term survival after organ transplant,[1,2] its accompanying morbidity continues to pose a substantial challenge.[3,4] Balancing IST side effects with allograft rejection risk is one of the ultimate goals in posttransplant care.

Conventional noninvasive markers of kidney transplant (KTx) function, such as serum creatinine (SCr), are neither specific nor predictive of future rejection.[5] Renal allograft biopsies are invasive with an attendant risk of allograft injury, and their interpretation is highly subjective.[6-8] Posttransplant IST care requires frequent visits to transplant centers, posing challenges to patients in rural areas with limited access to such facilities.[9-11] These challenges highlight the unmet need for reliable, noninvasive, specific, and early indicators of allograft rejection to help optimize IST dosing while minimizing rejection risk.

Circulating donor-derived cell-free DNA (dd-cfDNA) is an established noninvasive biomarker for ongoing allograft injury and rejection in solid organ transplant patients.[12-15] The Prospera test measures dd-cfDNA fraction in KTx recipients: patients with dd-cfDNA ≥1% are considered at a high risk of rejection and those with dd-cfDNA <1% are considered at low risk of rejection.

Emerging evidence shows that dd-cfDNA may be a leading indicator, rising before clinical symptoms of rejection are apparent.[16] This has spawned interest around its use to directly inform IST management; however, equipoise concerns have hindered research especially in scenarios involving increasing IST based on elevated dd-cfDNA. We felt that using dd-cfDNA to help inform IST reduction would allow for a low-risk trial design. Recently, a small case series successfully

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explored the use of dd-cfDNA as part of tailored IST reduction in a limited number of stable patients (n = 5),[17] where each patient received a tailored intervention including different kinds of IST regimens. In contrast, our study incorporated a larger cohort of patients (n = 21) who underwent a standardized intervention of one kind of IST.

This observational study recruited patients who (1) were initially on high doses of MPA, (2) were deemed to be at low risk of rejection, (3) were good candidates for IST reduction, and (4) who used dd-cfDNA to inform IST reduction decisions. Given the low clinical risk of continuing patients on their IST regimen outside the first year post-KTx, we felt this study design maintained equipoise. To our knowledge, this is the first study describing the use of dd-cfDNA testing to inform IST management directly with a standardized intervention.

MATERIALS AND METHODS

Study Population

An observational study of adult KTx patients on maintenance IST was performed at Victoria Kidney and Dialysis Associates, Victoria, TX (IRB00013544). Patients on high doses of MPA, considered at low risk for rejection, and good candidates for IST reduction were offered enrollment between March 2020 and November 2021. Demographic information was obtained at visit 1 (enrollment). Clinical information, such as dd-cfDNA, SCr, estimated glomerular filtration rate, and MPA dosages and tacrolimus levels, was collected over 7 monthly visits. Patients were excluded if they were younger than 18 y, were with an organ donated from an identical twin, underwent multiorgan transplants, were not on MPA, or were noncompliant with the study design, were excluded from the study.

Measurement of dd-cfDNA Fractions

Blood was collected from patients for dd-cfDNA fraction measurement at each of the 7 visits and sent to a central laboratory for processing (Prospera, Natera Inc, Austin, TX). Massively multiplexed polymerase chain reaction was used to amplify cfDNA in plasma samples, targeting 13 926 single nucleotide polymorphisms, followed by amplicon measurement with next-generation sequencing as described previously.[18] dd-cfDNA levels were reported as a fraction of total cfDNA (%) and were used to stratify patients into low risk of rejection (dd-cfDNA <1%) and high risk of rejection (dd-cfDNA ≥1%) at the first visit and then monitor them over 6 monthly visits.

IST Dosages

At visit 1, dd-cfDNA levels were measured, and then decisions to update IST regimens were made on the basis of (1) the patient's initial dosage, (2) the patient's initial dd-cfDNA fraction at visit 1, and (3) the center's control protocol based on risk assessment. IST regimens entailed twice daily (documented as total daily dose) dosing of MPA and tacrolimus, with 2 patients also receiving prednisone. For patients prescribed mycophenolic sodium (n = 2), dosages were adjusted to the equivalent dosage of the active ingredient in mycophenolate mofetil (MMF).

Patients were stratified on the basis of dd-cfDNA fraction measurements taken at visit 1. For patients considered low risk by dd-cfDNA, MPA dosage was reduced subject to the control protocol of the transplant center and physician discretion. For patients considered high risk by dd-cfDNA, their IST regimen was maintained throughout the remainder of the study based on physician discretion. The serum tacrolimus levels of all patients were measured at each visit and maintained to be between 5 and 7 ng/mL. Biopsies were performed as appropriate to assess any suspicion of rejection.

Statistical Analyses

The Wilcoxon rank-sum test was used for pairwise comparisons of continuous variables, whereas the Fisher exact test was used for categorical variables. Analyses were conducted using R programming (https://www.r-project.org/).

RESULTS

Patient Demographics and Clinical Information at Visit 1 (Enrollment)

Patients who were seen at the clinic and were on high doses of IST for any reason, were selected for this study. Most of the patients enrolled in this study faced challenges in maintaining a regular follow-up schedule with their transplant centers posttransplant (due to distance), and thus, they were on high IST doses before enrollment. Of the 30 patients who were eligible to enroll in the study, 3 were excluded because they were not taking MPA (n = 2) or were not selected by the physician for IST reduction despite being otherwise eligible (n = 1). Six patients dropped out due to death (n = 1), retransplantation (n = 1), or noncompliance with protocol (n = 4). The demographic and clinical information of the remaining 21 patients is detailed in **Table 1**. At visit 1, the patients of the cohort had a median age of 54.0 y (interquartile range [IQR], 52.0–62.0) and a median time posttransplant of 60.0 mo (IQR, 36.0–90.0). The cohort was primarily comprised Hispanic (66.7%; 14/21) and male patients (61.9%; 13/21; **Table 1**). None of the patients had prior KTx rejection.

dd-cfDNA% Measurements at Visit 1

At visit 1, 17 patients had dd-cfDNA <1%, thus were considered at low risk for rejection and were approved for IST reduction by the physician; 4 patients had dd-cfDNA ≥1%, thus were considered at high risk of rejection and continued their original IST regimen (**Table 1**). The median dd-cfDNA fraction among the low-risk patients was 0.2% (IQR, 0.1–0.3), compared with 2.2% (IQR, 1.7–3.2) in the high-risk patients ($P = 0.003$). There were no statistically significant differences in the median levels of SCr or estimated glomerular filtration rate between the high-risk and low-risk patients ($P = 0.45$ and 0.90, respectively).

IST Regimen and MPA Tapering

The IST regimen for all patients in this study included tacrolimus and MPA, with a target tacrolimus trough level of 5 to 7 ng/mL and MPA dosage ranging from 1000 to 2000 mg/d (in MMF equivalents) before visit 1. One patient in the high-risk group was on prednisone (5.0 mg/d) through all 7 visits, along with 1 patient in the low-risk group (10.0 mg/d). The median initial MPA dosage among the low-risk cohort was 2000 mg/d and was 1250 mg/d in the high-risk cohort. MPA dosing was not weight-based. Most of the patients were on high MPA doses at visit 1 because of (1) inadequate follow-up visits to their transplant centers after completing 1 y

TABLE 1.

Patients' demographic and clinical information at visit 1 (enrollment) and outcomes at last visit (visit 7)

Demographic and clinical data for the patients at visit 1				
Characteristic	**All patients (N = 21)**	**Low-risk patients (dd-cfDNA <1%) (N = 17)**	**High-risk patients (dd-cfDNA ≥1%) (N = 4)**	***P***
Age, y, median (25%ile–75%ile)	54 (42–62)	55 (42–64)	50.5 (42.2–55)	0.37
Sex				1.00
Female	8 (38.1%)	7 (41.2%)	1 (25.0%)	
Male	13 (61.9%)	10 (58.8%)	3 (75.0%)	
Race/ethnicity				0.23
Hispanic	14 (66.7%)	11 (64.7%)	3 (75.0%)	
White	5 (23.8%)	5 (29.4%)	0 (0.0%)	
African American	2 (9.5%)	1 (5.9%)	1 (25.0%)	
Other	0 (0%)	0 (0%)	0 (0%)	
BMI, median (25%ile–75%ile)	33.2 (26.8–35.4)	31.6 (26.8–35.4)	34.1 (31.4–37.6)	0.76
Serum creatinine, mg/dL, median (25%ile–75%ile)	1.4 (1.1–1.6)	1.4 (1.0–1.5)	1.6 (1.4–2.0)	0.45
eGFR (CKD-EPI), mL/min/1.73 m^2, median (25%ile–75%ile)	57.3 (44.6–72.1)	57.3 (44.6–72.1)	56.4 (44.5–69.2)	0.90
dd-cfDNA, %, median (25%ile–75%ile)	0.2 (0.1–0.7)	0.2 (0.1–0.3)	2.2 (1.7–3.2)	0.003
Time since transplant, mo, median (25%ile–75%ile)	60 (36–90)	60 (36–90)	60 (36–114)	0.86
Study outcomes at visit 7				
Rejection				
Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	
No	20 (95.2%)	17 (100.0%)	3 (75.0%)	
Graft loss	1 (4.8%)	0 (0.0%)	1 (25.0%)	
Functioning graft				
Yes	20 (95.2%)	17 (100.0%)	3 (75.0%)	
Yes: preparing for dialysis	1 (4.8%)	0 (0.0%)	1 (25.0%)	
No	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Clinical stability				
Stable	19 (90.5%)	17 (100.0%)	2 (50.0%)	
Unstable	2 (9.5%)	0 (0.0%)	2 (50.0%)	

BMI, body mass index; CKD-EPI, chronic kidney disease- epidemiology collaboration; dd-cfDNA, donor-derived cell-free DNA; eGFR, estimated glomerular filtration rate.

posttransplant (mostly due to travel distance barriers), which limited the ability of transplant centers to adjust IST doses, and (2) lack of comfort having the IST doses adjusted by the primary nephrologist rather than transplant centers. At visit 2, the MPA dosages were reduced in the low-risk group, with an average reduction of 50%. The MPA dosage for the high-risk patients was maintained from visit 1 throughout the study duration. The MPA dosages in MMF equivalents at the time of each visit for all 21 patients are depicted as a heat map in **Figure 1**.

At visit 4, the MPA dosage was reduced for a patient in the high-risk cohort, per patient preference, as the patient was being listed for kidney retransplant because of graft loss. At visit 6, a patient in the low-risk cohort stopped taking prednisone and, therefore, had concomitant increase in MPA dosage in the subsequent visits. At visit 7, a patient in the low-risk cohort underwent a further reduction in MPA dosage because of a urinary tract infection.

Clinical Stability and Graft Outcomes

Table 1 lists the patients' clinical and graft outcomes at the end of the study for the low- and high-risk groups. Throughout the study, 100% (17/17) of the low-risk patients who underwent MPA reduction were clinically stable, with rejection-free, surviving grafts. Half (2/4) of the high-risk patients were clinically unstable at the end of the study. One of these patients declined a physician-recommended biopsy, and the other one had elevated SCr due to chronic allograft nephropathy, experienced graft loss before visit 3, and was listed for retransplant. The remaining 2 high-risk patients, one of whom underwent a biopsy, did not experience rejection or graft loss.

dd-cfDNA Surveillance

For study visits 2 to 7, dd-cfDNA fractions were measured to monitor the risk of allograft rejection. Among the low-risk patients, 100% (17/17) of patients had dd-cfDNA <1% at all visits. Among the high-risk patients, 100% (4/4) of patients had dd-cfDNA >1% at all visits.

DISCUSSION

This observational study describes the direct use of dd-cfDNA to help inform IST decisions and monitor a cohort of 21 patients who were considered eligible for IST reduction. Patients who were initially on high doses of MPA for their transplant vintage and considered at low risk for graft rejection at visit 1 (dd-cfDNA <1%) had their IST regimens reduced by the physician, whereas those considered at high risk for graft rejection at visit 1 (dd-cfDNA ≥1%) had their IST regimens maintained. Over the remaining 6 monthly visits, no rejections were observed in the low-risk group, and 2 patients (50%) in the high-risk group experienced reduced graft function, including 1 patient with graft loss.

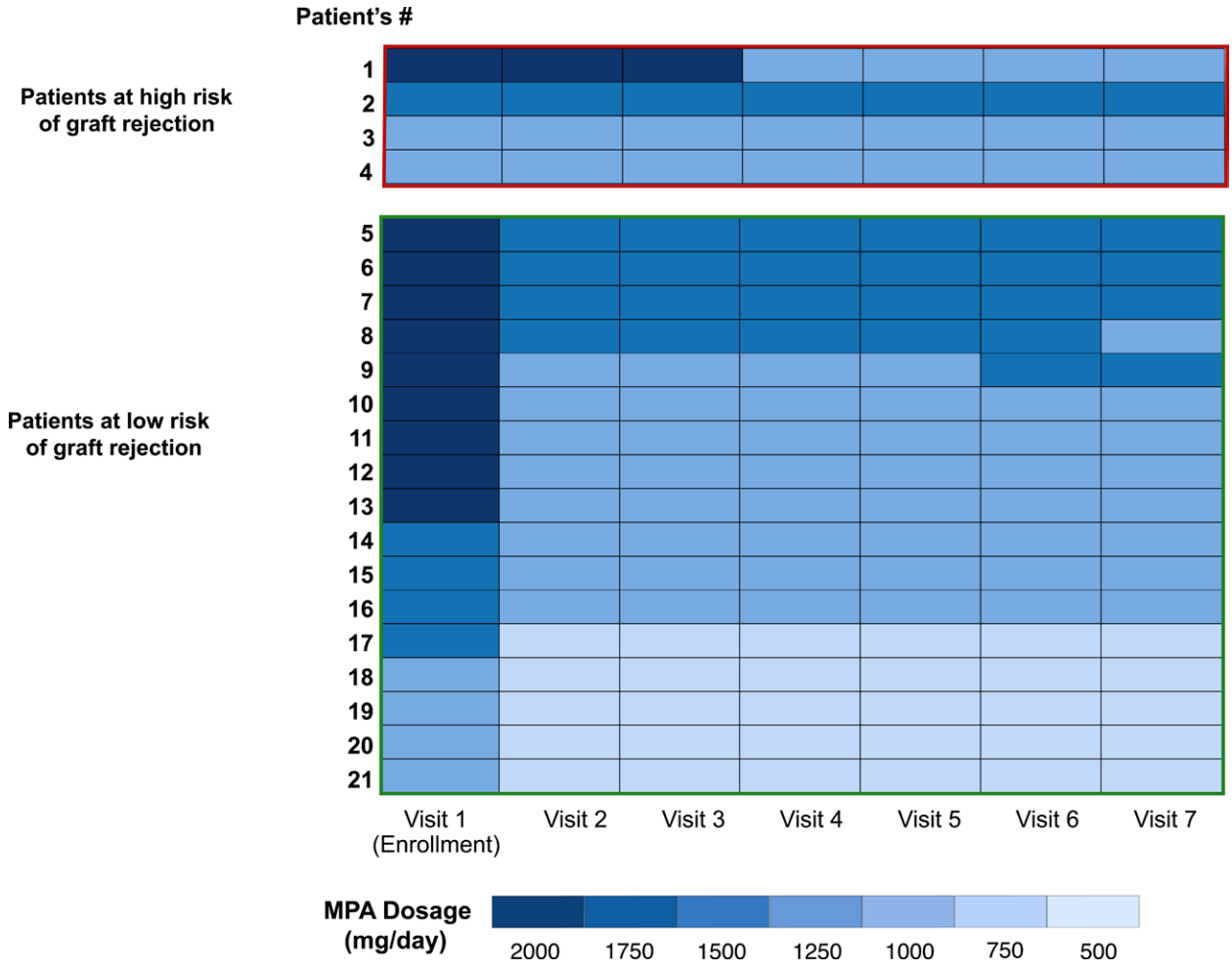

FIGURE 1. Heat map representing MPA dosages (milligram/day) throughout the course of the study (7 monthly visits) for each patient (n = 21). Each row represents a patient, and each column represents the MPA dosage at each of the 7 visits. Patients at high risk and low risk of KTx rejection are highlighted by red and green boxes, respectively. Patients in the high-risk group maintained their IST dose from visit 1 through visit 7, excluding patient 1 who was listed for kidney retransplant because of graft loss at visit 4, at which point the MPA was reduced for the remainder of the study, per the patient preference. Patients in the low-risk group had their MPA dose reduced from visit 2 through the end of the study, with 2 exceptions. The MPA dose of patient 8 was further reduced at the last visit because of a urinary tract infection. The MPA dose for patient 9 was increased at visit 6 to compensate for discontinuation from prednisone. IST, immunosuppressive therapy; KTx, kidney transplantation; MPA, mycophenolic acid.

dd-cfDNA is a validated noninvasive biomarker for use in detecting allograft rejection in transplant patients,[12,19] and a number of studies have demonstrated the ability of dd-cfDNA to assess the risk of future rejection.[20,21] Recently, thoughts have turned to using dd-cfDNA in the context of IST management and patient graft surveillance.[8,12,15,22,23] This study successfully demonstrates the potential of dd-cfDNA to help inform immunosuppression management. dd-cfDNA identified candidates for IST reduction, with none of the patients in the low-risk group experiencing untoward effects following IST reduction. Additionally, elevated dd-cfDNA identified 2 patients whose apparent clinical stability belied impending allograft dysfunction, 1 of whom subsequently went on to experience allograft loss.

This study opens the doors into exploration of the use of dd-cfDNA to inform IST management, as a rule-out indicator for IST reduction in stable patients who are otherwise candidates for reduction. We hope such an approach to IST management guidance will be particularly useful for patients with limited access to advanced transplant testing centers, such as those in this study cohort, many of whom encountered geographic obstacles preventing regular visits to transplant centers.[10,11,24]

There are several limitations to this study. First, the small cohort size necessitates more studies to confirm the effects observed herein. Second, the study was not controlled, and no protocol biopsies were performed, with the designation of clinical stability relying on traditional noninvasive markers. Third, the starting MPA dosages varied across the cohort. Although this is reflective of the situation typically experienced in clinical care, it is possible that the impact of MPA dose reduction on dd-cfDNA differs depending on the starting dose. Additionally, dd-cfDNA was only used to inform IST decisions at the first visit, and no subsequent management changes were made based on dd-cfDNA results. Fourth, the follow-up period of our study was short, which may not be sufficient to capture long-term outcomes, such as chronic rejection and graft survival. One study investigating MMF withdrawal effects found that some adverse outcomes did not manifest for >1 y after discontinuation of MMF.[25]

Future studies are needed to consider the value of serial dd-cfDNA testing for IST optimization. Additionally, absolute quantification of dd-cfDNA could be considered, because some reports have indicated that dd-cfDNA quantity may be more representative of organ status as it is not affected by host cfDNA factors (eg, infection) and has superior accuracy in KTx rejection detection compared with dd-cfDNA fraction alone.[26,27]

In summary, this proof-of-concept observational study shows that dd-cfDNA, a noninvasive biomarker for rejection risk, can be used to help inform physician decisions regarding IST reduction in KTx patients, along with patient evaluation and other clinical factors. As the patients in this study cohort were at a median of 5 y post-KTx, future studies are required to assess this approach in the first year posttransplant, when physicians routinely make decisions about IST reduction.

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