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Analysis of biomarkers within the initial 2 years posttransplant and 5-year kidney transplant outcomes: results from Clinical Trials in Organ Transplantation-17

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Abstract

Background—An early posttransplant biomarker/surrogate marker for kidney allograft loss has the potential to guide targeted interventions. Previously published findings, including results from the Clinical Trials in Organ Transplantation (CTOT)-01 study, showed that elevated urinary

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chemokine CXCL9 levels and elevated frequencies of donor-reactive interferon gamma-(IFNγ)-producing T cells by enzyme linked immunosorbent spot (ELISPOT) assay associated with acute cellular rejection within the first year and with lower 1-year posttransplant estimated glomerular filtration rate (eGFR). How well these biomarkers correlate with late outcomes, including graft loss, is unclear.

Methods—In CTOT-17, we obtained 5-year outcomes in the CTOT-01 cohort and correlated them with a) biomarker results and b) changes in eGFR (Chronic Kidney Disease Epidemiology Collaboration formula) over the initial 2 years posttransplant using univariable analysis and multivariable logistic regression.

Results—Graft loss occurred in 14/184 subjects (7.6%) 2 to 5 years posttransplant. Neither IFNγ ELISPOTs nor urinary CXCL9 were informative. In contrast, a ≥40% decline in eGFR from 6 months to 2 years posttransplant independently correlated with thirteen-fold odds of 5-year graft loss [adjusted odds ratio (aOR) 13.1; 95% CI 3.0–56.6], a result that was validated in the independent Genomics of Chronic Allograft Rejection cohort (n=165, aOR: 11.2).

Conclusions—We conclude that while pre and early posttransplant ELISPOT and chemokine measurements associate with outcomes within 2-years posttransplant, changes in eGFR between 3 months or 6 months and 24 months are better surrogates for 5-year outcomes, including graft loss.

Keywords

kidney transplant; eGFR; surrogate marker; biomarker

INTRODUCTION

Kidney transplantation is the preferred modality for renal replacement therapy in end stage kidney disease (ESKD). Whereas short-term kidney transplant outcomes have improved significantly over the past 3 decades as manifested by higher rates of 1-year patient and graft survival and lower rates of acute rejection, long-term outcomes remain suboptimal [1–3]. Ten-year rates of all-cause graft loss in the United States hover at ~40%, and are not substantially different from those reported during the previous decade [1]. Despite the increasing interest in preventing late allograft loss and the availability of a number of novel diagnostics and therapeutics that have the potential to improve transplant outcomes, the high costs and the impracticality of performing trials that last greater than 5 years remain significant barriers to identifying approaches capable of preventing late graft failure [4]. Identification and validation of early posttransplant biomarkers or surrogate endpoints for 5-year graft loss and/or poor kidney function is therefore of crucial importance. Such validated surrogates could be used to a) guide individualized therapeutic interventions aimed at protecting recipients with allografts at the highest risk, b) safely taper immunosuppression to limit off-target effects and improve patient health in subjects at lowest risk and c) select high-risk subjects for more aggressive intervention and/or inclusion in interventional clinical trials.

Collaborative analyses from the chronic kidney disease (CKD) literature have previously explored the possibility that a short-term percentage change in estimated glomerular filtration rate (eGFR) could function as a reliable surrogate for late development of dialysis-dependent ESKD [5,6]. The results of this previous work indicate that a 30–40% eGFR decline

over a 2–3 year time period functions as an acceptable surrogate endpoint in CKD clinical trials [5,6] raising the possibility that a similar decline could be indicative of a high risk for kidney allograft loss posttransplantation. Previous analyses of transplant outcomes from large databases have shown that changes in serum creatinine are associated with graft loss, although the sensitivity and specificity have been modest [7,8]. The relationship between changes in eGFR and late graft outcomes in prospective cohorts of kidney transplant recipients using modern methods of eGFR estimation and in comparison to other candidate biomarkers of transplant outcome is unknown.

Our research group has been performing prospective, multicenter, observational and interventional trials in kidney transplantation as part of the Clinical Trials in Organ Transplantation (CTOT) consortium. As part of this effort we previously reported findings from CTOT-01, an observational study of 280 first kidney transplant recipients from 7 sites in North America, in which 2-year outcomes were correlated with clinical variables and a panel of biomarkers [9,10]. These analyses revealed that urinary CXCL9 protein and/or gene expression was an excellent biomarker for detecting/ruling out acute cellular rejection (ACR). They also confirmed that elevated frequencies of donor-reactive IFNγ-producers in peripheral blood (ELISPOT) correlated with lower 12-mo eGFR. Whether these pre and early posttransplant markers relate to late outcomes, including 5-year graft loss has not been reported previously.

Herein we report the results of the CTOT-17 analysis in which we collected 5-year outcome data on the CTOT-01 cohort in an effort to identify early surrogates of 5-year graft loss and worsened kidney function. Our analyses showed that neither the results of T cell ELISPOT assays nor the urinary CXCL9 assays performed within 2-years posttransplant correlated with 5-year kidney transplant outcomes. In contrast, changes in eGFR over the initial 24-months posttransplant correlated with graft loss and 5-year eGFR in the CTOT-17 cohort, a finding that we independently corroborated within the distinct Genomics of Chronic Allograft Rejection (GoCAR) study cohort.

MATERIALS AND METHODS

Clinical Trials in Organ Transplantation (CTOT) Study

The details of the primary CTOT-01 study including CXCL9 results and ELISPOT assay results as correlates of outcomes within 2-years posttransplant have been published [9,10]. CTOT-01 was a prospective, multicenter, observational study that enrolled 280 adult and pediatric, crossmatch negative kidney transplant candidates and followed them for 2 years. CTOT-17 was designed to collect information on 5-year outcomes in this cohort. Eligible subjects were those who were enrolled in CTOT-01 between 2006 and 2009 and were 5 years ± 6 months posttransplant. The sole exclusion criterion was withdrawal of consent from CTOT-01 (37 subjects withdrew consent). CTOT-17 was designed as a retrospective chart review in which the following data were collected: a) subject survival (yes or no), if no, date and cause of death, graft functioning (yes or no) at the time of death, b) if subject alive, we collected information on graft loss (yes or no) including cause and date of graft loss (defined as return to dialysis). We also evaluated 5-year allograft function by calculation of eGFR (Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula for adults

and Schwartz formula for children]. IFNγ ELISPOT and urinary CXCL9 protein measurements were previously reported [9,10]. All data from CTOT-17 were reported using electronic case report forms to Rho, Inc. (Chapel Hill, NC). The study protocol underwent IRB review by each of the study sites. In each case the protocol was determined to be low risk and a new consent form was not required for retrospective data collection or reporting. Upon study closure the data were collated at Rho and transferred to Mount Sinai where all analyses performed.

Genomics of Chronic Renal Allograft Rejection (GoCAR) Study

The details of this prospective multicenter (5 clinical sites) observational study have been published previously [11,12]. The GoCAR study protocol included follow-up of patients for 2 years, with optional long-term follow-up per center. Data regarding renal function decline of GoCAR enrollees and graft survival from 1 of the clinical sites (Westmead Millennium Institute, Sydney, AU), was utilized for a different analysis of eGFR in transplant subjects (ANZDATA)[7] hence for the purposes of this analysis these patients were excluded to avoid duplication. Among the 448 enrollees at the US clinical sites, 165 patients had GFR decline data available between 6-months and 2 years as well as 5-year graft loss (return to dialysis) and were included in this analysis.

Endpoints

The primary endpoint for the CTOT-17 analysis and the GoCAR validation cohort was graft loss (return to dialysis) between 2 and 5 years posttransplant. We also considered a secondary endpoint of eGFR as a continuous variable at 5 years (only in the CTOT-17 cohort since we did not have eGFR at 5 years in the GoCAR cohort).

Exposure

Our primary exposures of interest were donor reactive IFNγ ELISPOT results, urinary CXCL9 protein, and a percentage change in eGFR at various time points before year 2 posttransplant.

Covariates

Covariates collected for the CTOT and GoCAR studies included demographics (age, sex, race, living vs. deceased donor, cold ischemia time for deceased donors), induction medication, acute rejection, immunologic risk measures [panel of reactive antibody (PRA), donor specific antibody (DSA), human leukocyte antigen (HLA) match/mismatch] and measures of kidney function (serum creatinine) at various time points.

CTOT Study

As noted, eGFR was calculated posttransplant by the CKD-EPI equation for adults and by the modified Schwartz equation (8) for subjects younger than 18 years. Estimated GFR in subjects who crossed the 18 year mark during the study were calculated by the modified Schwartz equation for all time-points <18 years of age and by CDK-EPI for time-points ≥18 years of age. Serum creatinine levels were assayed centrally using an IDMS-traceable analyzer (Hitachi Module D/P, Indianapolis, IN). All demographic variables were self-

reported. Allograft protocol biopsies were obtained at implantation and 6 months after transplantation. “For-cause” biopsies were obtained at the discretion of the subject’s physician. Biopsy-proven AR was defined as Banff 1997 grade ≥1A or Banff grade borderline and treated according to local practice.

GoCAR Study

Serum creatinine measurements in GoCAR enrollees were entered into the GoCAR database from the respective clinical care databases at the sites and eGFR was calculated from them using the CKD-EPI formula [13]. Protocol biopsies in GoCAR recipients were performed at 3-, 12-, & 24-months and were read centrally by a pathology core laboratory at Massachusetts General Hospital [12]. “For-cause” biopsies were similarly obtained at the discretion of the subject’s physician. For the purposes of this manuscript, we collated data on AR (≥Banff 1A) among the GoCAR cohort as reported by the pathology core including AR detected on surveillance biopsies.

Statistical Analyses

We used means (SD) and proportions for descriptions of patient characteristics and covariates in both the derivation and validation cohorts. We used univariable tests of correlation (Pearson’s correlation) and association (univariable linear regression/t test for continuous and chi^2 test for categorical variables) to assess important confounders between the outcomes (graft loss and 5-year continuous eGFR) and covariates. We adjusted for these variables depending on whether they were significant in univariable analyses as well as whether they were part of the confounding pathway in multivariable modeling. We tested IFNγ ELISPOT positivity [≥25 spots per 300,000 PBMC [14]]), positive urinary CXCL9 (>200 pg/ml) and various thresholds of percentage change in eGFR between various prespecified time points (3 months to 1 year; 3 months to 2 years and 6 months to 2 years) and their association with graft loss/5-year eGFR in the CTOT-17 cohort. We imputed a 5-year eGFR value of 10 ml/min in participants with graft loss.

We then used multivariable logistic regression (for graft loss) to calculate adjusted odds ratios (aOR) with 95% confidence intervals (CI) and multiple linear regression (for 5-year eGFR) to calculate coefficients and standard errors (SE). Time-to-event analysis was not performed as dates of graft loss were not collected. We used similar methods to compare association of eGFR change with risk factors in the Go CAR cohort. We considered a 2 tailed p value of <0.05 as statistically significant. We used STATA version 13, College Station, TX and SPSS for Windows, Version 16.0, Chicago, SPSS Inc. for all analyses.

RESULTS

Characterization of the CTOT-17 Cohort

Of 280 participants originally enrolled in the CTOT-01, 76 subjects died, lost their graft or were lost to follow-up at the end of the 2-year study period (Figure 1). Twenty additional CTOT-01 enrollees were lost to follow-up after 2 years (18 were lost to follow-up without ascertainment of death/graft loss and 2 did not have samples for eGFR calculations), and were excluded from the analyses. The CTOT-17 cohort was thus comprised of 184 subjects

alive at the end of CTOT-01 and for whom 5-year follow-up data were available (Figure 1). Of these 184, 3 subjects died (2 of whom experienced graft loss) and 12 were alive with graft loss between the end of CTOT-01 and the 5-year posttransplant time period for CTOT-17. The baseline clinical characteristics of the 184 subjects included in the CTOT-17 analysis were not different from those of the parent CTOT-01 cohort (Table 1) supporting nondifferential loss of subjects.

Univariable associations with clinical characteristics for 5-year graft loss or eGFR in CTOT-17 cohort

We initially tested the strength of relationships between various clinical/epidemiological characteristics and either graft loss that occurred between 2 and 5 years posttransplant or absolute 5-year eGFR, focusing on characteristics previously shown by others to be associated with worse transplant outcomes [15–17]. These analyses showed that self-identified African American (AA) race in both recipient and donor, male sex, and HLA mismatch associated with graft loss between 2 and 5 years posttransplant. DSA was persistently detected in 16/184 subjects (3 of whom had anti-class II DSA). DSA did not associate with either graft loss or eGFR (data not shown). Elevated donor age, recipient AA race, administration of rabbit anti-thymocyte globulin (ATG) as induction therapy, and acute rejection correlated with lower 5-year eGFR (Table 2).

Lack of association between early immune markers and 5-year graft loss or eGFR

We next tested relationships between IFNγ ELISPOT positivity or CXCL9 positivity and outcomes. We were able to recapitulate the primary findings from CTOT-01 [9,10,18] within the smaller CTOT-17 subset (184 of the original 280 enrolled subjects) regarding ELISPOT assays: within the subjects who did not receive ATG induction, pretransplant donor-reactive IFNγ ELISPOT positivity correlated with lower 6-month eGFR (67.0±2.8 ml/min/1.73m^2 in ELISPOT negative vs. 57.2±3.0 ml/min/1.73m^2 in ELISPOT positive, p=0.02) and 12-mo eGFR (68.8±3.0 in ELISPOT negative vs. 59.0±3.4 in ELISPOT positive, p=0.037). However, when we tested for relationships between ELISPOT positivity assessed pretransplant and at 1-, 2-, 3-, 4-, 6-, 12- or 24-mo posttransplant, and 5-year outcomes, we did not observe any consistent relationships, regardless of ATG induction (Table 3). Similarly, urinary CXCL9 positivity at any of the tested time points prior to 2 years posttransplant did not correlate with 5-year outcomes, even in those subjects with >2 positive tests over the initial 2 years (Table 3).

24-month eGFR associates with 5-year eGFR

We next attempted to verify and extend previously reported associations between estimated posttransplant eGFR and late kidney transplant outcomes [7,8]. We evaluated relationships between absolute eGFR (calculated by CKD-EPI) at various time points within the initial –2 years posttransplant and the 5-year eGFR. These analyses showed that absolute eGFR at 3, 6, 12 and 24 months each correlated with 5-year eGFR (p<0.001 for each), with the 24-month eGFR having the strongest correlation (correlation coefficient=0.68, Figure 2). However, we did not observe a significant relationship between differing thresholds of 24-month eGFR and graft loss that occurred between 2 and 5 years posttransplant (Table 4).

Changes in eGFR over the initial 2 posttransplant years associate with 5-year outcomes

In an effort to identify a stronger predictor of 5-year graft loss we next examined relationships between percentage decline in eGFR within the initial 2 years posttransplant and graft loss that occurred between 2 and 5 years. Percentage decline from baseline within 2 years have been shown to be strongly predictive of eventual ESRD in nontransplant patients [5,6]. Fourteen of the CTOT-17 participants lost their grafts between 2 and 5 years posttransplant. The causes of graft loss (Table 5) were chronic rejection, acute rejection/ nonadherence, cytomegalovirus (CMV) infection and recurrent focal segmental sclerosis.

A decline in eGFR between 3 and 24 months, or between 6 and 24 months posttransplant of 20%, 30% or 40% were all significantly associated with graft loss that occurred between 2 and 5 years. The strongest associations were with a 40% eGFR decline between 3–24 months (OR 9.5; 95% CI 3.6–74) and between 6–24 months (aOR 13.1; 95% CI 3 – 56.6). The specificities for all eGFR declines were high, with the highest specificity being 0.94 for a 40% eGFR decline between 6–24 months (Table 6). The sensitivities were generally moderate, with the highest being 45.5% for a 20% eGFR decline from 6–24 months (Table 6). Any magnitude of eGFR decline was associated with a lower eGFR at 5 years, with the lowest eGFRs being in those patients who underwent a 40% decline (lower eGFR by 28.3 ml/min for 40% decline 3–24 months and 32.9 ml/min for decline 6–24 months). Multivariable analyses showed that the associations were independent of recipient AA race and peak PRA among other variables (Table 6). Re-analysis removing the 2 subjects with graft loss due to documented nonadherence did not alter the results (data not shown).

Validation in the GoCAR cohort

In an effort to validate relationships between changes in eGFR and graft loss at 5 years we analyzed outcomes of the US participants in the GoCAR study, a prospective observational cohort of 165 kidney transplant patients from North America followed for 5 years. Baseline and outcome characteristics for the GoCAR subjects (Table 1) showed that in comparison to the CTOT-17 cohort, the GoCAR cohort was older (42.4 vs. 52.4 years); contained fewer females (41.5% vs. 31.5%) and fewer white participants (71.1% vs. 62.4%). Both cohorts contained a high proportion of living donors (69.9% and 49.1%). There were also significant differences in the donor race between the 2 cohorts. Mean eGFR was lower in the GoCAR cohort at all time periods compared to the CTOT-17 cohort (Table 7). The acute rejection rate (excluding borderline cases) and rate of graft loss were lower in the GoCAR cohort but the rate of death was similar.

Death-censored graft loss at 5 years in the GoCAR cohort occurred in 9 participants (alive at end of 5-year follow-up). We observed strong associations between graft loss and either 20% or 30% eGFR decline between 3 or 6 months and 24 months. The strongest association was with a 30% decline from 6–24 months (aOR 14.03; 95% CI 2.83–69.6) after adjusting for donor and recipient race, recipient gender and PRA (Table 8).

DISCUSSION

Among the key observations derived from this work is that while we (among others) have shown that IFNγ ELISPOT assays [9,10,18–24] and urinary CXCL9 assays [9,18,25–33] are informative regarding both risk of ACR within the initial 2 posttransplant years and kidney function at 1 and 2 years posttransplant, we were not able to detect meaningful relationships between either of these early biomarkers and 5-year outcomes in the CTOT-17 cohort. We speculate that the inability of these early markers of T cell immunity associated with ACR to predict late graft outcome in this observational cohort could be a result of multiple factors that were not measured as part of the original study. Immune factors include the development of pathogenic donor-reactive antibodies beyond 2 years. Nonimmune factors could include physician directed changes in immunosuppression or nonadherence with medications beyond 2 years, recurrent primary disease, or the effects of late viral or bacterial infections on allograft function. We thus suggest that serial analyses using multiple biomarkers capable of detecting varied, distinct mechanisms of allograft injury (including but not limited to those tested herein) are more likely to be informative than relying on individual test results [34].

In contrast to the ELISPOT and chemokine results, our analyses support the conclusion that a decline in eGFR by ≥30% between 6-months to 2-years posttransplant associates with a) graft loss (both in CTOT and US GoCAR cohorts) and b) lower eGFR at 5-years posttransplant (in CTOT cohort). Our results from these 2 prospectively followed cohorts suggest that the finding in patients with primary chronic kidney disease (CKD), that a ≥30% eGFR decline over 2 years is a viable endpoint for research studies of end stage renal disease (ESRD)[5,6], might be generalizable to kidney transplant recipients. They also support the results of the 2016 registry study from Australia and New Zealand, in which the authors demonstrated that a ≥30% decline in eGFR between 1–3-years posttransplant was associated with death–censored graft failure and death [7]. The fact that ≥30% decline in eGFR correlates with graft loss from a retrospective registry study of Caucasians in Australia/New Zealand and from our prospectively collected, contemporary, heterogeneous, US, derivation and validation cohorts, attests to the robustness of the prognostic biomarker. This change in eGFR-based surrogate endpoint is independent of standard clinical/epidemiological characteristics, including ACR, as a predictor of 5-year graft loss (Table 7). While we observed a correlation between 2-year eGFR and 5-year eGFR (Figure 2), no absolute threshold of 2-year eGFR correlated with 5-year graft loss in the CTOT cohort (Table 4). The 2-year eGFR of <30 ml/min did correlate with graft loss in the GoCAR cohort and in the published Australian cohort [7], and 2-year eGFR correlated with 5-year eGFR in a prior registry study [35], but the aORs were lower than those of the ≥40% decline in eGFR reported herein. Together, the findings support the conclusion that the ≥40% change in eGFR between 6 months and 2 years functions as a superior surrogate of 5-year outcome than the absolute 2-year eGFR.

Our data suggest that the ≥30% decline in eGFR could be considered as a surrogate endpoint, as it occurs frequently and is strongly correlated with an eventual graft outcome as much as 3 years later. Other proposed biomarkers such as an episode of acute rejection occur infrequently and are inconsistently associated with graft loss [36]. Similarly, the prevalence of

positive markers of alloimmunity and/or molecular biomarkers including donor-specific antibody (DSA) within the first 2 years posttransplant, is relatively low, and their respective associations with graft outcome are not sufficiently strong to be accepted as surrogate endpoints [16]. As patients are intensively followed during the initial 2 years posttransplant, a ≥30% decline in eGFR at 6- to 24-months posttransplant could identify at-risk subjects in whom interventions aimed at improving graft survival could be effective.

Despite some differences in key patient and study characteristics between the derivation and validation cohorts (Table), the ≥40% decrease in 6–24 month eGFR biomarker behaved similarly between groups (Table 6). We did not have histology data on the majority of participants and thus could not analyze histopathological correlates of eGFR decline. We did not have access to cause of graft loss data in the GoCAR cohort beyond 2 years and we acknowledge that the sample sizes are limited.

We also acknowledge that the confidence intervals for the associations were wide (albeit positive) due to limited sample size and only ~50% of study subjects with death-censored graft loss met the surrogate endpoint. It is notable, however, that this modest sensitivity is similar to studies in the nontransplant population [52% of subjects with ESKD met the same surrogate endpoint] and is better than any other reported marker from multicenter trials. Several of the CTOT subjects who lost their grafts without meeting the endpoint were likely nonadherent to their immunosuppressive medication beyond the 24-month timeline (Table 5), a cause that is may be detectable using alternative, noninvasive biomarkers [37–39].

In summary, we provide evidence that while ELISPOT and urinary CXCL9 values can detect early ACR, a ≥30% eGFR decline in the posttransplant period from 6-months to 2-years is a better surrogate of graft loss at 5-years. We suggest that future studies should incorporate early decline of eGFR as a biomarker, both to confirm its robustness as a predictor of later outcomes, and to determine whether basing therapeutic intervention on the presence or absence of this biomarker influences outcomes.

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Abbreviations

AA African American

ACR	acute cellular rejection
aOR	adjust odds ratio
CTOT	Clinical Trials in Organ Transplantation
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMV	cytomegalovirus
DSA	donor specific antibody
eGFR	estimate glomerular filtration rate
ELISPOT	enzyme linked immunosorbent spot
ESKD	end stage kidney disease
HLA	human leukocyte antigen
IFNγ	interferon gamma
GoCAR	Genomics of Chronic Allograft Rejection
MDRD	Modification of Diet in Renal Disease
NIAID	National Institute of Allergy and Infectious Diseases
PRA	panel of reactive antibody

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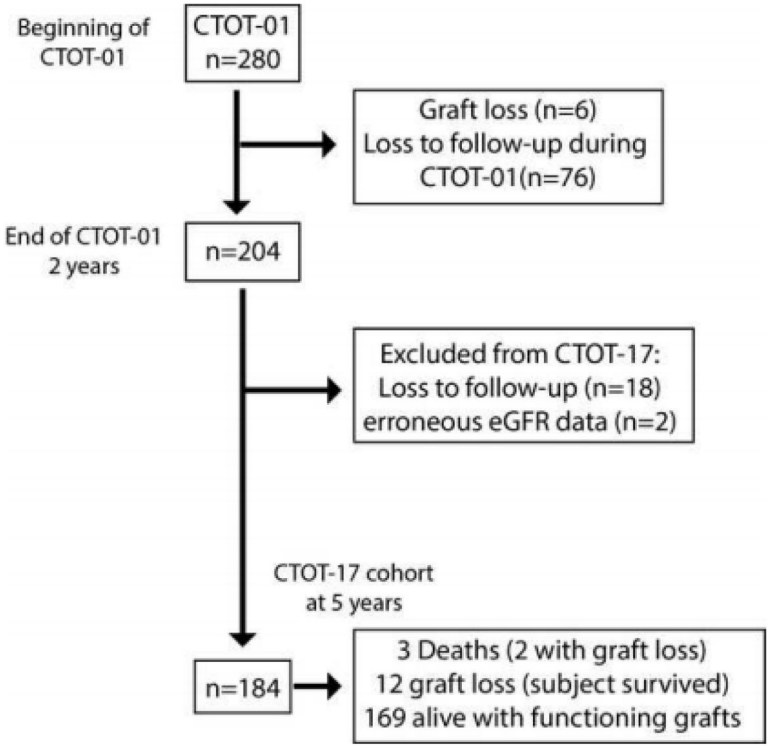

Figure 1.

Derivation of the primary study population. Consort diagram illustrating outcomes and follow-up of the parent CTOT-01 cohort that resulted in the CTOT-17 cohort analyzed herein.

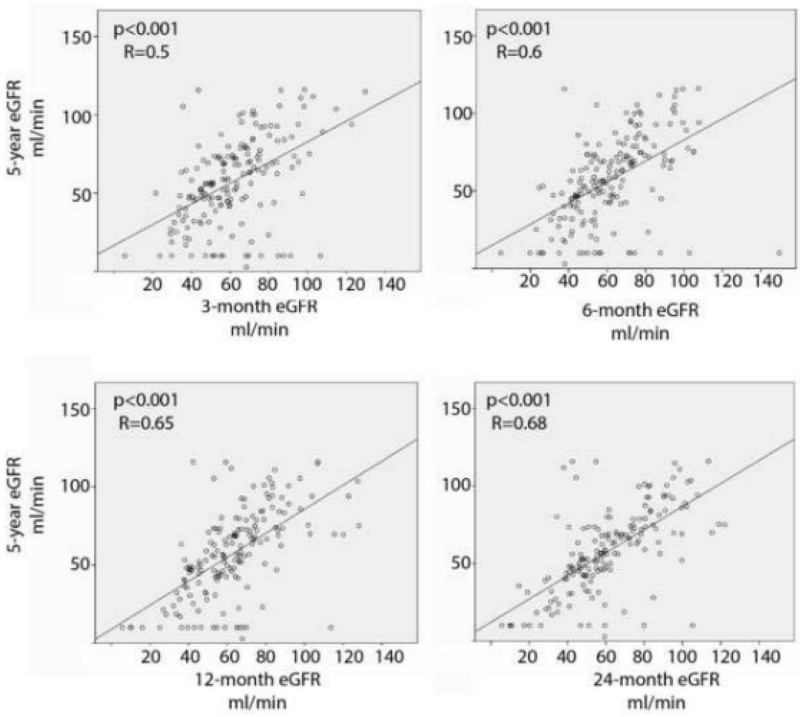

Figure 2.

Relationship between early and 5-year allograft function. Estimated GFRs for each study subject in CTOT-17 at 3-, 6-, 12 and 24-months posttransplant are correlated with their 5-year eGFR.

Table 1

Characteristics and Outcomes in the CTOT and GoCAR Cohorts

	CTOT-01 n=280	**CTOT-17 n=184**	**p-values CTOT-01 vs. CTOT-17**	**GoCAR n=165**	**p-values CTOT-17 vs. GoCAR**
Age, Mean (SD)	43.5 (17.6)	42.4 (17.5)	0.82	52.4 (12.7)	<0.01
Sex, n (%) Female	112 (40)	77 (41.8)	0.67	52 (31.5)	<0.01
Recipient Race, n (%)			0.13		0.49
White	179 (63.9)	120 (65.2)		103 (62.4)	
African American/Hispanic	80 (28.5)	47 (25.5)		46 (27.9)	
Asian	5 (1.9)	5 (2.7)		13 (7.9)	
Other/Missing	16 (5.7)	12 (6.5)		3 (1.8)	
Living donor	192 (68.6)	129 (70.1)	0.72	81 (49.1)	<0.01
Donor Race, n (%)			0.45		0.051
White	199 (71.1)	129 (70.1)		129 (78.2)	
African American/Hispanic	51 (18.2)	32 (17.4)		30 (18.2)	
Other/Missing	24 (8.6)	23 (12.5)		6 (3.6)	

Table 2

Univariable Associations with Graft Loss (n=178[a]) and 5 Year eGFR (n=184)

	Graft Loss[b]		5-year eGFR[b]	
	OR (95% CI)	**p-value**	**Beta Coefficient**	**p-value**
Recipient Age	0.71 (0.25–2.05)	0.63	−0.169 (0.1)	0.148
Donor Age	1.44 (0.44–5.34)	0.68	−0.83 (0.15)	<0.001
Male Gender in Recipient	0.9 (0.3 – 2.8)	0.85	5 (4.1)	0.219
Male Gender in Donor	0.6 (0.2 – 1.9)	0.38	5.1 (4)	0.2
African American Recipient	9.9 (2.9 – 33.5)	<0.001	−15 (4.6)	0.001
African American Donor	3.4 (1 – 11.2)	0.029	−10.3 (5.3)	0.054
Peak PRA>75%	1.8 (0.2 – 16)	0.47	−21 (9.3)	0.026
HLA Mismatch>4	1.6 (0.5 – 4.9)	0.28	−2.6 (4.4)	0.54
Living transplant[c]	0.5 (0.17 – 1.5)	0.25	4.8 (4.4)	0.274
Thymoglobulin Induction	2.1 (0.7 – 6.4)	0.17	−11 (5)	0.029
Acute Rejection	2.1 (0.5 – 8.5)	0.23	−14.9 (5.9)	0.013

[a] Six subjects lost their grafts prior to 2-years posttransplant and were thus excluded from the analysis of variables associated with graft loss between 2- and 5-years. These 6 subjects were included in the eGFR analysis at 5-years with an imputed value of 10 ml/min.

[b] All estimates are unadjusted odds ratios for the outcome of graft loss and unadjusted beta coefficients for the outcome of 5-year eGFR.

[c] 48 of the subjects were recipients of deceased donor transplants. Within that subset, there was a nonsignificant trend between cold ischemia time (CIT) and 5-year eGFR (p=0.085), and no correlation between CIT and either 20%, 30% or 40% decrease in eGFR.

Table 3

Relationships between ELISPOT/urine CXCL9 and outcomes

	Graft Loss between 2–5 years		eGFR at 5 years	
Variables	**Adjusted Odds Ratio (95% Confidence Interval)**	**p**	**Beta Coefficient [95% CI]**	**p**
CXLC9 Visit 2	0.92 [0.87 – 0.96]	0.12	7.0 [−4.8 – 18.9]	0.24
CXLC9 Visit 3	0.92 [0.80 – 0.97]	0.17	−1.1 [−13.4 – 11.2]	0.86
CXLC9 Visit 4	1.20 [0.22 – 6.00]	0.57	−2.4 [−13.5 – 8.6]	0.66
CXLC9 Visit 5	0.70 [0.07 – 6.60]	0.6	−0.2 [−10.7 – 10.2]	0.96
CXLC9 Visit 6	3.4 [0.96 – 12.2]	0.06	2.2 [−10.1 – 14.7]	0.72
CXLC9 Visit 7	0.8 [0.17 – 4.2]	0.6	11.0 [0.45 – 21.7]	0.04
CXLC9 Visit 8	1 [0.2 – 5.2]	0.6	5.20 [−7 – 17.9]	0.39
CXLC9 Visit 9	0.45 [0.05 – 3.8]	0.4	−0.60 [−13.6 – 12.3]	0.92
CXLC9 Visit 10	1.8 [0.4 – 7.7]	0.3	3.50 [−8.9 – 16]	0.57
CXLC9 Visit 11	0.7 [0.08 – 5.8]	0.59	5.20 [−10 – 20.6]	0.49
ELISPOT pretransplant	0.9 [0.25 – 3.2]	0.56	2.00 [−7.9 – 12]	0.68
ELISPOT Visit 4	0.6 [0.07 – 5.5]	0.57	−2.00 [−15.6 – 11.5]	0.76
ELISPOT Visit 5	1.7 [0.32 – 9.7]	0.4	−1.20 [−13.8 – 11.3]	0.84
ELISPOT Visit 6	0.67 [0.13 – 3.3]	0.47	6.80 [−4.5 – 18.3]	0.23
ELISPOT Visit 7	0.98 [0.19 – 5.1]	0.67	6.00 [−5.4 – 18.5]	0.28
ELISPOT Visit 8	2.4 [0.6 – 9.1]	0.17	3.90 [−8 – 15.9]	0.51
ELISPOT Visit 9	0.4 [0.04 – 3.2]	0.33	12.40 [−0.7 – 25.6]	0.06
ELISPOT Visit 10	1.4 [0.4 – 5.4]	0.39	−1.90 [−13.3 – 9.4]	0.73
ELISPOT Visit 12	1 [0.1 – 8.8]	0.66	2.30 [−16.6 – 21.2]	0.8
ELISPOT Visit 13	1.4 [0.16 – 13.4]	0.55	9.50 [−11.4 – 30.6]	0.37
ELISPOT Visit 14	0.9 [0.86 – 0.97]	0.62	−2.70 [−25.3 – 19.8]	0.8

Table 4

Adjusted Odds Ratios for graft loss with different eGFR cutoffs at 24 months

	Odds Ratio	P
eGFR<45 at 24 months	0.22 (0.03–1.55)	0.13
eGFR<40 at 24 months	0.15 (0.01–1.97)	0.15
eGFR<35 at 24 months	1.97 (0.21–18.1)	0.55
eGFR<30 at 24 months	4.37 (0.44–43.5)	0.21
eGFR<25 at 24 months	2 (0.55–48.5)	0.31

Table 5

Causes of Graft Loss in CTOT-17 Participants with Graft Loss

Time to Graft Loss (in Days)	Reason for graft loss
1959	Recurrence of FSGS
1878	Chronic Allograft Nephropathy
1826	Acute Rejection
1158	Chronic Rejection
1115	Chronic Rejection
1065	Chronic Rejection and infectious etiology
918	Cytomegalovirus Infection
1845	Chronic Rejection
1707	Nonadherence
1693	Metastatic prostate cancer
1685	Chronic Rejection
1680	Chronic Rejection
1296	Acute rejection
1112	Acute Rejection due to likely Nonadherence

Table 6

Multivariable regression with differing thresholds of eGFR decline at different time points and graft loss in CTOT

	Graft Loss after 2 years N=178				5 Year eGFR (REGRESSION) N=184	
Variables	**OR (95% CI)**	**P**	**Sensitivity**	**Specificity**	**Beta Coefficient (SE)**	**P**
20% eGFR decline between 3 months to 24 months	3 (1– 9.2)	0.04	40	86.3	−20.2 (4)	<0.001
20% eGFR decline between 6 months to 24 months	2.5 (0.8 – 7.6)	0.09	45.5	82.5	−18.5 (4.5)	<0.001
30% eGFR decline between 3 months to 24 months	4.7 (1.5 – 14.4)	0.004	40	91	−19.3 (4.7)	<0.001
30% eGFR decline between 6 months to 24 months	3.9 (1.2 – 13)	0.031	45.5	88	−20 (5.3)	<0.001
40% eGFR decline between 3 months to 24 months	9.5 (3.6–74)	<0.001	40	94	−28.3 (5.8)	<0.001
40% eGFR decline between 6 months to 24 months	13.1(3 – 56.6)	<0.001	27.3	94	−32.9 (7.4)	<0.001

Adjusted for African American race in recipient, peak PRA>75% and HLA mismatch

Table 7

Comparison between Outcomes and eGFR levels in CTOT-17 and GoCAR Cohort

	CTOT-17 n=184	**GoCAR n=165**	**P**
eGFR at time points, mean (SD)			
3 months	61.5 (20.5), n=173	53.9 (18.8) n=145	<0.001
6 months	61.6 (20.9) n=178	54.87 (16.8) n=165	0.002
12 months	63 (22) n=174	53.95 (15.8) n=135	<0.001
24 months	61.5 (24.6) n=170	57.26 (19.2) n=165	0.06
Outcomes, n (%)			
Acute Rejection	25 (13.7)	14 (8.48)	0.02
Graft Loss	20 (10.9)	9 (6.2)	0.009
Death	9 (4.9)	11 (6.66)	0.90

All eGFR values in ml/min; n for each time point varies to due availability of serum creatinine results at each time point within each cohort

Table 8

Multivariable regression with eGFR decline and death censored graft loss in the GoCAR cohort

	Adjusted Odds Ratio (95% Confidence Interval)
20% decline from 3–24 months	7.30 (1.65–32.2)
30% decline from 3–24 months	5.84 (1.44–23.7)
40% decline from 3–24 months	2.78 (0.63–12.4)
20% decline from 6–24 months	10.87 (2.54–46.6)
30% decline from 6–24 months	14.03 (2.83–69.6)
40% decline from 6–24 months	11.22 (0.77–163.2)

Adjusting for donor and recipient race, recipient gender and PRA.