



## Tacrolimus Elimination in Four Patients With a *CYP3A5*\*3/\*3 *CYP3A4*\*22/\*22 Genotype Combination

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### Abstract

Cytochrome P450 3A5 (*CYP3A5*) and cytochrome P450 3A4 (*CYP3A4*) are the predominate enzymes responsible for tacrolimus metabolism. The presence of *CYP3A4* and *CYP3A5* genetic variants significantly affects tacrolimus clearance and dose requirements. *CYP3A5*\*3 is a loss-of-function variant resulting in no *CYP3A5* enzyme production. *CYP3A4*\*22 is a variant that reduces production of functional *CYP3A4* protein. Caucasians commonly carry these variant alleles but are very rarely homozygous for both *CYP3A5*\*3 and *CYP3A4*\*22. This report describes four kidney transplant recipients who carry a rare genotype combination (*CYP3A5*\*3/\*3 and *CYP3A4*\*22/\*22). These patients were identified from a larger cohort of Caucasian kidney transplant recipients (n=1366). To understand the significance of this genotype combination on tacrolimus troughs and doses, we compared these patients to recipients without this combination. Patients homozygous for both variants are at risk for profound reductions in metabolism of *CYP3A* substrates. A 342% and a 90.6% increase in the median dose-normalized trough was observed, when the *CYP3A5*\*3/\*3 and *CYP3A4*\*22/\*22 genotype combination was compared to the *CYP3A5*\*1/\*1 and *CYP3A4*\*1/\*1 genotype combination and the *CYP3A5*\*3/\*3 and

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*CYP3A4\*1/\*1* genotype combination, respectively. These four individuals only required on average 2.5 mg/day of tacrolimus. Knowledge of these genotypes would be useful in selecting appropriate tacrolimus doses to avoid overexposure.

## Keywords

CYP3A5; CYP3A4; tacrolimus; single nucleotide polymorphisms; Transplant; kidney; pharmacogenetics

Tacrolimus is a highly effective immunosuppressant used as maintenance therapy after solid organ transplantation. Tacrolimus pharmacokinetics are variable mainly due to unpredictable bioavailability and metabolism requiring therapeutic monitoring of trough concentrations.<sup>1</sup> Low bioavailability is largely attributed to high first-pass metabolism and efflux from intestinal cells. It is extensively metabolized in the gut and liver with < 0.5% excreted unmetabolized in the feces.<sup>1</sup> Biliary excretion is responsible for approximately 95% of tacrolimus metabolite excretion, and urinary excretion accounts for < 3%.<sup>1</sup> Cytochrome P450 3A5 (CYP3A5) and cytochrome P450 3A4 (CYP3A4) are thought to be the predominate isozymes contributing to tacrolimus metabolism.<sup>2, 3</sup> Four major metabolites (13-*O*-desmethyl tacrolimus, 15-*O*-desmethyl tacrolimus, 31-*O*-desmethyl tacrolimus, and 12-*O*-desmethyl tacrolimus) account for the majority of tacrolimus clearance by CYP3A.<sup>4</sup> When CYP3A5 is expressed, it has twice the intrinsic clearance of CYP3A4.<sup>5</sup> The relative contribution of CYP3A5 versus CYP3A4 within an individual is highly dependent on the presence of CYP3A4 or CYP3A5 activity.<sup>6</sup>

The presence of *CYP3A4* or *CYP3A5* variants significantly influences the metabolism of tacrolimus.<sup>7</sup> A single-nucleotide polymorphism (SNP) in intron 3 of the *CYP3A5* gene (*CYP3A5\*3*, rs776746) significantly reduces tacrolimus clearance and lowers dosing requirements.<sup>8, 9</sup> *CYP3A5\*3* is a loss-of-function variant that leads to the production of a nonfunctional protein caused by creation of a cryptic splice site resulting in incorporation of exon 3B.<sup>10</sup> This leads to CYP3A5 nonsense-mediated mRNA decay.<sup>10, 11</sup> The genetic variant *CYP3A4\*22* (rs35599367), in which a C-to-T substitution in intron 6 deletes a SF2/AF2 binding site, has also been associated with reduced tacrolimus metabolism.<sup>12, 13</sup> It results in 255-base pair partial intron 6 retention and a splice variant that terminates before exon 7, leading to reduced production of functional mRNA and enzyme activity when compared to the wild-type. When the *CYP3A4\*22* variant was transfected into HepG2 cells, higher alternative splice variant formation was observed. No difference was observed when this variant is expressed in intestine-derived LS-174T cells, suggesting a tissue-specific effect.<sup>12, 13</sup>

A previous case report described tacrolimus troughs in a kidney transplant recipient with Alport syndrome who was homozygous for the *CYP3A5\*3* allele and homozygous for a variant in exon 5 of the *CYP3A4* gene (*802 C>T*, *CYP3A4\*26*) that results in a premature stop codon R268\*.<sup>14</sup> This patient had extremely high tacrolimus trough concentrations and was eventually stabilized on delayed-release tacrolimus 0.5 mg 3 times weekly, leading to trough concentrations between 5 and 10 ng/ml.

This case report describes four kidney transplant recipients who carry a rare genotype combination (*CYP3A5*\*3/\*3 and *CYP3A4*\*22/\*22). To understand the significance of this genotype combination on tacrolimus troughs and doses, we compared these four patients to kidney transplant recipients without this combination.

## Materials and Methods

Genotypes from 1366 Caucasian kidney transplant recipients enrolled in the multicenter Deterioration of Kidney Allograft Function (DeKAF) Genomics study ([www.ClinicalTrials.gov](http://www.ClinicalTrials.gov), NCT01714440) were evaluated. Deterioration of Kidney Allograft Function Genomics is a prospective, observational study to identify genetic variants associated with transplant outcomes through a genome-wide association study (GWAS). The 1366 Caucasian kidney transplant recipients were divided into 6 groups based on their *CYP3A5*\*3 and *CYP3A4*\*22 genotype combination. From this cohort, four transplant recipients who were homozygous for both *CYP3A5*\*3 and *CYP3A4*\*22 alleles were identified. These patients would be expected to have profoundly reduced tacrolimus clearance since tacrolimus is both a CYP3A4 and CYP3A5 substrate. To understand the influence of this genotype, tacrolimus troughs, doses, and dose-normalized troughs in the first 6 months post transplant of these four recipients were compared to patients not carrying this genotype combination. All 1366 patients received oral immediate-release tacrolimus therapy with mycophenolate for maintenance immunosuppression post transplant. Induction therapy varied among the patients. Most patients received a short course of induction corticosteroids at time of transplant and no steroid maintenance. Tacrolimus trough concentrations were obtained as part of routine clinical care in all patients and were measured from whole blood. Tacrolimus concentrations were measured with Clinical Laboratory Improvement Amendments (CLIA)-approved assays and 95% were measured by liquid chromatography–mass spectrometry. Two trough concentrations were obtained in each of weeks 1–8, and in each of months 3–6, for a maximum of 24 trough concentrations per subject. Tacrolimus doses were adjusted based on institution-specific trough goals; generally, the goals were 8–12 ng/ml in months 0–3 and 6–10 ng/ml in months 4–6. Tacrolimus troughs and doses were obtained from the medical record in all patients. Dose-normalized trough values were calculated using the trough concentration (ng/ml) divided by the total daily dose (mg). All patients were also evaluated for acute tacrolimus-related nephrotoxicity, which has been previously described.<sup>15</sup> Tacrolimus-related nephrotoxicity was defined as any rise in serum creatinine that resulted in lowering of the tacrolimus dose. The study was carried out in accordance with the Declaration of Helsinki and was approved by the institutional review boards of the enrolling centers. Statistical analyses were not conducted given the small number of patients in groups 1 and 6.

Concomitant use of angiotensin-converting enzyme inhibitors (ACEi), anti-cytomegalovirus (CMV) prophylaxis, dihydropyridine calcium channel blockers (CCBs, mainly amlodipine), and corticosteroids was recorded for all patients. In the four patients of interest, time on one of these concurrent therapies was calculated and defined as the percentage of time the patient reported taking a concurrent medication around the time of tacrolimus trough measurements.

The patients' genotypes were determined from DNA isolated from pretransplant peripheral blood lymphocytes. Genotypes were determined with the Affymetrix Transplant GWAS chip and have been previously described.<sup>16, 17</sup> The *CYP3A5\*3* (rs776746) and *CYP3A4\*22* (rs35599367) genotypes were taken from this chip.

## Results

The 1366 transplant recipients were divided into six groups based on their *CYP3A5\*3* and *CYP3A4\*22* allele status. Group 1 consisted of the four subjects with the *CYP3A5\*3/\*3* and *CYP3A4\*22/\*22* genotype combination. Groups 2–6 contained recipients with the *CYP3A5\*3/\*3* and *CYP3A4\*1/\*22*, *CYP3A5\*3/\*3* and *CYP3A4\*1/\*1*, *CYP3A5\*1/\*3* and *CYP3A4\*1/\*22*, *CYP3A5\*1/\*3* and *CYP3A4\*1/\*1*, and *CYP3A5\*1/\*1* and *CYP3A4\*1/\*1* genotype combinations, respectively. Table 1 describes the demographics, genotype distribution, dose-normalized tacrolimus trough concentrations, trough concentrations, and total daily dose for each group. Group 3 (*CYP3A5\*3/\*3* and *CYP3A4\*1/\*1*) and group 5 (*CYP3A5\*1/\*3* and *CYP3A4\*1/\*1*) had the most common genotypes, representing 76.7% and 11.8% of the study population, respectively. Group 1 accounted for only 0.29% of the population. Figure 1 displays the median dose-normalized trough concentrations for groups 1–6.

Group 1 (*CYP3A5\*3/\*3* and *CYP3A4\*22/\*22*) had the highest dose-normalized tacrolimus troughs (3.05 ng/ml/mg) and received the lowest dose (2.5 mg/day) of the six groups. Dose-normalized tacrolimus troughs incrementally increased with each addition of a variant allele (*CYP3A5\*3* and *CYP3A4\*22*). The median tacrolimus dose in group 1 (2.5 mg/day) was > 3-fold lower relative to groups 5 and 6 (8–9 mg/day). For the patients in group 1, the median tacrolimus trough was similar (8.25 ng/ml) to groups 2–5 and higher compared to group 6 (5.70 ng/ml) with no variants.

The number of *CYP3A5\*22* alleles present was important. The addition of a second *CYP3A4\*22* allele increased the median dose-normalized tacrolimus trough from 2.18 to 3.05 ng/ml/mg (40.2% increase) when comparing group 2 (*CYP3A5\*3/\*3* and *CYP3A4\*1/\*22*) to group 1 (*CYP3A5\*3/\*3* and *CYP3A4\*22/\*22*), respectively. A 90.6% increase (1.60 ng/ml/mg compared to 3.05 ng/ml/mg) in the median dose-normalized trough was observed when comparing group 3 (*CYP3A5\*3/\*3* and *CYP3A4\*1/\*1*, the most frequent allele combination) to group 1 (*CYP3A5\*3/\*3* and *CYP3A4\*22/\*22*). Furthermore, a 342% higher (3.05 ng/ml/mg vs 0.69 ng/ml/mg) median dose-normalized trough was observed in group 1 (*CYP3A5\*3/\*3* and *CYP3A4\*22/\*22*) relative to group 6 (*CYP3A5\*1/\*1* and *CYP3A4\*1/\*1*).

One of the four patients in group 1 experienced tacrolimus-induced nephrotoxicity (defined as any rise in serum creatinine that resulted in lowering of the tacrolimus dose) by day 25. In the overall cohort, acute tacrolimus-related nephrotoxicity occurred in 16% of the population. In group 1, one of the four patients received corticosteroids for maintenance therapy, whereas the three others reported no corticosteroids use except for a short course with induction. ACEi, anti-CMV prophylaxis, and CCB use varied among the four patients in group 1 (Table 2).

## Discussion

The median dose-normalized tacrolimus troughs increased with the accumulation of inactive or reduced activity *CYP3A5* and *CYP3A4* alleles (Figure 1). The individuals homozygous for both *CYP3A5\*3* and *CYP3A4\*22* had the lowest total daily tacrolimus dose requirements (2.5 mg/day) compared to all the other groups. This is a rare genotype combination and occurred in only 0.29% of our study population. The *CYP3A4\*22* allele frequency in African and European populations is approximately 0% and 5%, respectively ([phase3browser.1000genomes.org](http://phase3browser.1000genomes.org)). The *CYP3A5\*3* allele frequency in African and European populations is approximately 18% and 94%, respectively ([phase3browser.1000genomes.org](http://phase3browser.1000genomes.org)). We believe, after an extensive literature review, that this is the first in-depth description of patients who are homozygous for both the *CYP3A5\*3* and *CYP3A4\*22* alleles receiving tacrolimus. Therefore, little is known about this genotype combination. *CYP3A5\*3* and *CYP3A4\*22* allele status can explain up to 50% of dose-normalized tacrolimus trough concentration variability in kidney transplant recipients.<sup>18</sup> In a mixed-model analysis, dose-normalized tacrolimus trough concentrations were reported to be 43% higher in *CYP3A4\*22* carriers than in non carriers and 43.3% higher in *CYP3A5\*3* homozygotes than in *CYP3A5\*1* carriers after adjusting for age, sex, and ethnicity.<sup>19</sup> In another study, similar tacrolimus trough concentrations were achieved with a 33% lower daily tacrolimus dose in individuals with *CYP3A4\*1/\*22* genotype compared to the homozygote wildtype (*CYP3A4\*1/\*1*).<sup>19</sup> Individuals homozygous for the *CYP3A5\*3* allele, who also carried one *CYP3A4\*22* allele, were reported to have a 26% higher dose-adjusted tacrolimus trough concentrations compared to those who did not carry a *CYP3A4\*22* allele.<sup>20</sup> Other investigators also found that European white kidney transplant recipients who carried one *CYP3A4\*22* allele required lower tacrolimus doses.<sup>18, 19</sup> A study in Dutch and German kidney transplant patients found that individuals who were *CYP3A5\*3* homozygotes and also carried *CYP3A4\*22* alleles required lower mean tacrolimus doses than those who were *CYP3A5\*1* carriers who also carried one or two *CYP3A4\*22* alleles (4.8 and 12.5 mg/ day, respectively).<sup>21</sup> Researchers observed higher dose-normalized tacrolimus trough concentrations at 3 months post transplant in individuals carrying a *CYP3A4\*22* allele when compared to *CYP3A4\*1* homozygotes.<sup>22</sup> These studies highlight the important effect of *CYP3A5\*3* and *CYP3A4\*22* variants on reducing tacrolimus clearance.

An in vitro study evaluating the effect of *CYP3A5* polymorphisms on tacrolimus metabolic clearance showed that the formation of a major inactive tacrolimus metabolite, 13-*O*-desmethyl tacrolimus, was 13.5 times greater in human liver microsomes from *CYP3A5\*1* carriers compared to *CYP3A5\*3* homozygotes.<sup>5</sup> This suggests that metabolite formation may be altered in transplant recipients with variants. The effect of *CYP3A4\*22* on tacrolimus metabolite formation is not known. The clinical implication of differing metabolite formation has not been studied.

We predicted tacrolimus elimination would be nearly absent in the four individuals who carried the rare genotype combination (*CYP3A5\*3/\*3* and *CYP3A4\*22/\*22*); however, tacrolimus was eliminated in these patients, albeit substantially less than in the other groups. This may be explained by several possibilities: (i) despite two *CYP3A4\*22* alleles, partially

active enzyme is produced and facilitates clearance; (ii) compensatory tacrolimus metabolism via other CYP enzymes or tacrolimus glucuronidation occurs; (iii) increased elimination of non metabolized tacrolimus occurs in the presence of reduced CYP3A4 and CYP3A5 enzyme activity; and (iv) the *CYP3A4\*22* variant reduces only hepatic enzyme, leaving intact CYP3A4 intestinal metabolism.

Investigators have found that *CYP3A4\*22* variant splicing may be liver specific<sup>13</sup>; therefore, tacrolimus metabolism in our patients may have occurred through intestinal CYP3A4 activity. Our patients did not receive intravenous tacrolimus, so we were unable to distinguish intestinal epithelial cell metabolism versus hepatic metabolism in these four patients. Other enzymes also may contribute to tacrolimus metabolism, including UGT1A4-mediated glucuronidation and/or other CYP enzymes. Recently, the existence of a tacrolimus-glucuronide metabolite was confirmed in vivo in human bile, indicating that tacrolimus may undergo enterohepatic circulation.<sup>23</sup> In addition, tacrolimus is a known inhibitor of CYP2C8, and *CYP2C8* genotypes have been linked to nephrotoxicity and delayed graft function among patients receiving tacrolimus, suggesting it may be a substrate for CYP2C8.<sup>24, 25</sup> The impact of CYP2J2 on tacrolimus pharmacokinetics has also been studied, but was not linked to any clinical outcomes.<sup>24, 25</sup>

The seven patients identified in group 6 also present a unique subset as the *CYP3A5\*1/\*1* and *CYP3A4\*1/\*1* genotype combination was also rare (0.51%) in our patients of Caucasian descent. Individuals of African ancestry commonly carry the *CYP3A5\*1/\*1* genotype, and this explains why they have significantly lower tacrolimus trough concentrations than Caucasians. The *CYP3A5\*1/\*1* genotype is well known to lead to higher rates of tacrolimus clearance in humans<sup>17</sup> and in cell culture studies.<sup>17, 26</sup> These individuals are also of concern with a median tacrolimus trough of only 5.70 ng/ml (target generally > 8 ng/ml) that places them at higher risk for transplant rejection.

Drug-gene or drug-drug interactions may play a role in our reported observation. Exogenous glucocorticoid steroids are believed to induce CYP3A via the pregnane X receptor (PXR).<sup>27</sup> All patients received corticosteroids beginning at the time of transplant, and only one of the four patients in group 1 received corticosteroids throughout the first 6 months. Initially, corticosteroids may have induced CYP3A, resulting in a phenoconversion to a higher metabolism phenotype in the first 1–2 weeks post transplant. CYP3A induction may have allowed patients in group 1 to tolerate the higher tacrolimus doses they initially received while doses were titrated down.

Tacrolimus is therapeutically monitored to identify out-of-range blood concentrations and allow for dose adjustments accordingly. However, most CYP3A substrate drugs are not monitored. The clinical implication of no or dramatically reduced CYP3A5 and CYP3A4 activity may be serious as these enzymes are widely involved in drug metabolism. The frequency of individuals who do not express CYP3A5 and are homozygous for the *CYP3A4\*22* allele is rare (0.29% in our population), but the implications for this subset of patients could be serious.

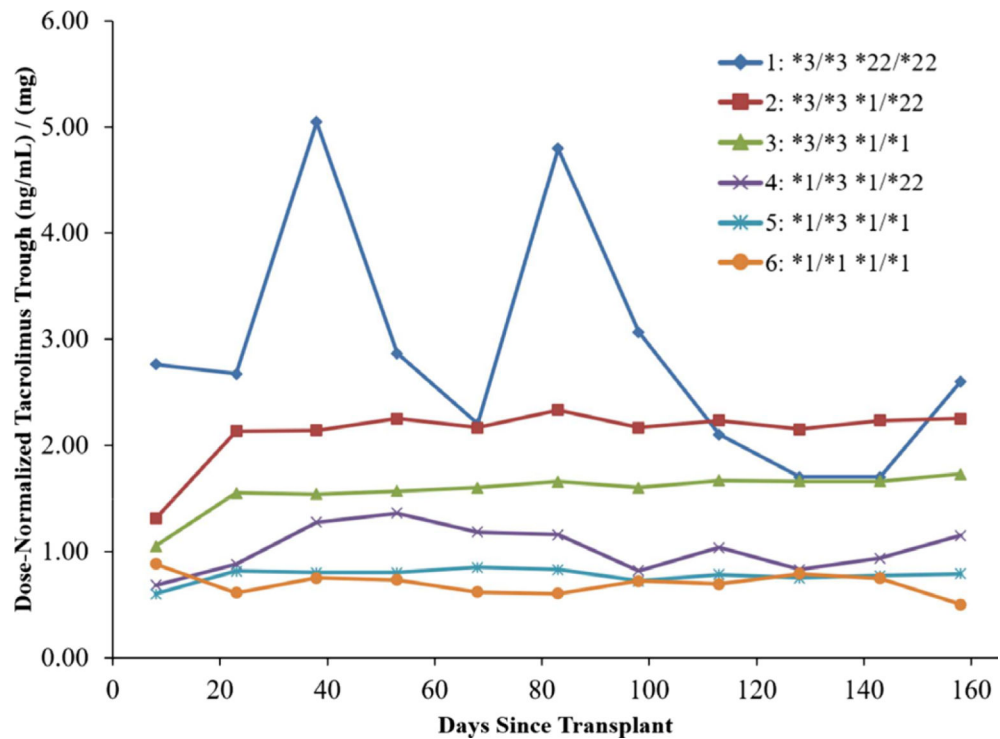
Definitive conclusions about the impact of two *CYP3A4\*22* alleles on drug metabolism will need to come from larger populations and additional patients, but this will likely be difficult due to the infrequency of this genotype combination. In addition, DeKAF Genomics did not collect information regarding hepatic function. Therefore, the influence of liver function on tacrolimus metabolism could not be accounted for in this case report. Our data suggest that individuals homozygous for both *CYP3A5\*3* and *CYP3A4\*22* display a reduced ability to eliminate tacrolimus compared to those heterozygous and homozygous for the wild-types. This case report contributes to the pharmacogenomic considerations in patients receiving tacrolimus and to understanding the metabolism phenotype produced by the combination of *CYP3A5\*3/\*3* and *CYP3A4\*22/\*22* genotypes.

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**Figure 1.**

Median dose-normalized trough concentrations for group 1 (*CYP3A5*\*3/\*3 and *CYP3A4*\*22/\*22, diamond, n=4), group 2 (*CYP3A5*\*3/\*3 and *CYP3A4*\*1/\*22, square, n=133), group 3 (*CYP3A5*\*3/\*3 and *CYP3A4*\*1/\*1, triangle, n=1048), group 4 (*CYP3A5*\*1/\*3 and *CYP3A4*\*1/\*22, "x," n=13), group 5 (*CYP3A5*\*1/\*3 and *CYP3A4*\*1/\*1, cross, n=161), and group 6 (*CYP3A5*\*1/\*1 and *CYP3A4*\*1/\*1, circle, n=7) calculated by 15-day intervals spanning the first 6 months post transplant. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**Table 1.**

Comparison of Caucasian Patients' Demographics, Dose-Normalized Tacrolimus Trough Concentrations, Tacrolimus Trough Concentrations, and Total Daily Tacrolimus Dose by *CYP3A5* and *CYP3A4* Genotype Status in the First 6 Months Post transplant

	Group 1 (n=4, 0.29%)	Group 2 (n=133, 9.7%)	Group 3 (n=1048, 76.7%)	Group 4 (n=161, 11.8%)	Group 6 (n=7, 0.51%)
Genotype	<i>CYP3A5</i> *3/*3 and <i>CYP3A4</i> *22/*22	<i>CYP3A5</i> *3/*3 and <i>CYP3A4</i> *1/*22	<i>CYP3A5</i> *3/*3 and <i>CYP3A4</i> *1/*1	<i>CYP3A5</i> *1/*3 and <i>CYP3A4</i> *1/*1	<i>CYP3A5</i> *1/*1 and <i>CYP3A4</i> *1/*1
Age, mean, SD (yr)	49.9, 13.6	51.9, 13.1	51.2, 12.8	47.4, 13.4	58.7, 13.4
BMI, mean, SD (kg/m <sup>2</sup> )	27.8, 3.7	27.9, 5.1	28.3, 5.6	28.4, 4.8	26.7, 5.6
Weight, mean, SD (kg)	84.2, 13.1	82.5, 17.9	83.9, 20.0	88.1, 19.0	73.5, 20.8
Living donor transplant (%)	75	63.2	66.5	53.9	71.4
Female (%)	25	37.6	37.2	23.1	57.1
Diabetes at time of transplant (%)	25	33.1	39.7	46.2	42.9
Simultaneous pancreas-kidney transplant (%)	0	6.0	9.0	7.7	0
Dose-normalized tacrolimus trough, median, IQR (ng/ml/mg of total daily dose)	3.05, 2.12–1.32	2.18, 1.50–3.33	1.60, 1.10–2.40	1.06, 0.65–1.50	0.69, 0.52–1.15
Tacrolimus trough, median, IQR (ng/ml)	8.25, 6.45–10.50	8.70, 7.00–10.60	8.50, 6.80–10.5	8.00, 6.20–9.50	5.70, 4.10–8.40
Daily tacrolimus dose,	2.50, 2.00–3.00	4.00, 2.50–6.00	5.00, 4.00–8.00	7.00, 5.00–12.00	8.00, 6.00–13.00

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	Group 1 (n=4, 0.29%)	Group 2 (n=133, 9.7%)	Group 3 (n=1048, 76.7%)	Group 4 (n=13, 0.95%)	Group 5 (n=161, 11.8%)	Group 6 (n=7, 0.51%)
median, IQR (mg)						

BMI = body mass index; IQR = interquartile range; SD = standard deviation.

**Table 2.**

Time on Concurrent Drug Therapies in Group 1 Patients in the First 6 Months Post transplant

Therapy	Group 1 – Time on Concurrent Drug Therapies (%)			
	Patient 1	Patient 2	Patient 3	Patient 4
ACEi	60	0	0	0
Anti-CMV	0	66.7	64.3	27.8
Maintenance corticosteroids	0	0	0	100
CCB	70	33.3	35.7	61.1

Concurrent drug therapies of ACEi, anti-CMV therapy, maintenance corticosteroids, and dihydropyridine CCBs were recorded at time of tacrolimus trough measurement. Time on a concurrent drug therapy was defined as the percentage of time the patient reported taking a medication in each drug category. ACEi = angiotensin-converting-enzyme inhibitors; CCB = calcium channel blocker; CMV = cytomegalovirus.

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