I, David K. Hooper, hereby submit this original work as part of the requirements for the degree of:

Master of Science

in Clinical and Translational Research

It is entitled:

The Impact of CYP3A5 Genotype on the Interaction Between Tacrolimus and Intravenous Nicardipine in Kidney Transplant Recipients

Student Signature: David K. Hooper

This work and its defense approved by:

Committee Chair: Paul Succop, PhD

Jens Goebel, MD

Mark Mitsnetes, MD

Erin Nicole Haynes, DrPH
The Impact of CYP3A5 Genotype on the Interaction Between Tacrolimus and Intravenous Nicardipine in Kidney Transplant Recipients

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David K. Hooper

M.D. University of Utah School of Medicine Salt Lake City, Utah 84112

B.A. Russian, University of Utah Salt Lake City, Utah 84112

Committee Chair: Paul Succop, Ph.D
ABSTRACT

Tacrolimus (TAC) is prescribed for immunosuppression in the majority of solid organ transplant recipients, yet overexposure can cause acute and chronic kidney injury. Continuous intravenous nicardipine (CIVN) for the treatment of post-transplant hypertension inhibits TAC metabolism by cytochrome P450 (CYP) 3A4. We hypothesized that CIVN in TAC-treated patients would lead to TAC overexposure in patients who genetically lack the alternative pathway for TAC metabolism, CYP3A5. We compared maximum 12-hour TAC trough (MaxC₀) and dose-adjusted MaxC₀ in 12 cases treated with CIVN immediately following kidney transplantation with 26 controls who were not treated with CIVN. CYP3A5 genotype was determined for all cases. The eight cases who do not express CYP3A5 (CYP3A5*3/*3) had higher median MaxC₀ (24.3 ng/ml) than the four cases who do express CYP3A5 (CYP3A5*1/*1) (13.9 ng/ml, p=0.28) and the 26 controls (14.6 ng/ml, p=0.003). Time to MaxC₀ was half as long in CYP3A5*3/*3 cases than in the other two groups combined (36 vs. 72 hours, p=0.002) and significantly more scheduled TAC doses were held per patient (1.75 vs. 0.4, p=0.007). Dose-adjusted MaxC₀ was likewise higher for CYP3A5*3/*3 cases than the two other groups combined (p=0.02). Six of eight (75%) CYP3A5*3/*3 cases had potentially toxic MaxC₀ (> 20 ng/ml) compared to none of four CYP3A5*1/*1 cases and three of 26 (11.5%) controls (p<0.001, CYP3A5*3/*3 cases vs. all others). Thus CYP3A5 non-expressors who are treated with CIVN are at increased risk for TAC levels in the toxic range. Well designed clinical studies should be carried out to further characterize the clinical implications of this interaction.
ACKNOWLEDGMENTS

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INTRODUCTION

Tacrolimus (TAC) is prescribed for immunosuppression in the majority of solid organ transplant recipients in the United States yet, its narrow therapeutic index and propensity to cause acute and chronic kidney injury in addition to other side effects require meticulous therapeutic drug monitoring and anticipation of drug-drug interactions(1). TAC is metabolized primarily by two isoforms of Cytochrome P450, CYP3A4 and CYP3A5(2). Whereas the majority of Black patients express both enzymes(3) and thus metabolize TAC equally via CYP3A4 and CYP3A5(2), 70-90% of White patients are homozygous for the CYP3A5*3 allele which encodes a truncated and consequently non-functional protein(3). These patients metabolize TAC almost exclusively via CYP3A4. Clinically, this manifests as dose-adjusted TAC trough levels in CYP3A5 non-expressors that are approximately twice as high as in patients expressing this enzyme(4). Furthermore, CYP3A4 is involved in the metabolism of more than 50% of all medications(1), suggesting that drug-drug interactions with TAC would be most common and severe in patients lacking TAC metabolism via CYP3A5(5-6).

Nicardipine is a dihydropyridine calcium channel blocker (CCB), administered orally or by continuous intravenous infusion, that inhibits CYP3A4 metabolism of other drugs(1, 7). In recent years, continuous intravenous nicardipine (CIVN) has gained in popularity for the nuanced management of post-operative hypertensive urgency in children following kidney transplantation, with some large pediatric transplant centers using it in more than 30% of patients (unpublished data). In vitro studies(1, 7) and clinical studies of other CCBs and calcineurin inhibitors in humans(8-10) suggest that intermittent oral nicardipine may impair TAC metabolism through inhibition of cytochrome P450 3A4 (CYP3A4), though the clinical significance of this specific interaction has never been reported. Furthermore, nothing has been reported regarding the impact of CIVN on TAC metabolism.
Based on clinical observations at our center, we hypothesized that CIVN inhibits CYP3A4 sufficiently to increase the risk of TAC over-exposure, especially in patients who lack CYP3A5 expression. We report here an analysis of TAC exposure according to CYP3A5 genotype in 12 patients treated with CIVN immediately following kidney transplantation compared to 26 controls who were not. Our data suggest that, indeed, CYP3A5 non-expressors treated with CIVN and TAC are at increased risk for TAC overexposure.
MATERIALS AND METHODS

Study Population

Following approval by our Institutional Review Board, we conducted a systematic chart review of patients who underwent kidney transplantation at our center from January 2003 through April 2010 to identify two cohorts: 1) cases on TAC-based immunosuppression who were treated with CIVN for more than twelve hours continuously in the first three days following transplantation and 2) consecutive controls on TAC-based immunosuppression who did not receive CIVN following transplantation. We excluded patients who 1) had liver failure or had undergone liver transplantation, 2) were on medications known to induce the cytochrome P450 3A enzymes such as rifampin or anti-convulsants, 3) received plasmapheresis within three days of kidney transplantation, 4) did not start taking TAC within 48 hours of transplantation (e.g. because they received thymoglobulin induction), or 5) had TAC discontinued sometime within the first week following kidney transplantation (delayed graft function). Two controls were included for each case. We invited all cases to participate in the pharmacogenetic component of the study and obtained written informed consent from all 12 patients. Each case submitted a single saliva specimen for analysis of CYP3A5 genotype.

All patients were treated according to our transplant immunosuppression protocol which includes 1) induction with intravenous methylprednisolone 10 mg/kg and basiliximab 12 mg/m² body surface area (BSA) (maximum 20 mg) intra-operatively and 2) maintenance immunosuppression with oral mycophenolate mofetil 450 mg/m² BSA twice daily (BID), TAC 0.1 mg/kg BID, and prednisone 0.75 mg/kg BID for the first seven days. Prior to July 2008, TAC and mycophenolate mofetil were started five to seven days prior to transplantation in patients with living donors. Since that time, only mycophenolate mofetil is started the week prior to kidney transplantation, whereas TAC and prednisone are started as soon as the patient is able to take oral medication following transplantation, usually in the evening following the procedure.
Treatment of post-kidney transplant hypertension was at the discretion of the attending nephrologist and intensive care unit physicians at the time of kidney transplantation. Because a significant proportion of patients transplanted at our institution come from locations up to thousands of miles away and are often discharged to the care of local physicians, we have data from only the transplant hospitalization for these patients. Thus, we limited follow up in this study to one week following kidney transplantation.

Data collection
We recorded TAC dose, time of administration and associated twelve-hour TAC trough level for the first seven days following transplantation on each patient in addition to the following data: age, race, gender, height, weight, whether this was the primary kidney transplant, all concurrent medications, and any scheduled TAC doses that were held. For cases we also recorded the time of initiation of CIVN, total duration, average hourly CIVN dose, total CIVN dose and any clinically important TAC toxicity (tremor, seizure, oliguria with stagnant creatinine). Data from our electronic medical record was compared with nursing records to ensure accuracy. Dose-corrected twelve-hour TAC trough levels were calculated for each time point by dividing the TAC trough by the corresponding TAC dose given twelve hours prior and adjusted for weight according to the following formula ($C_0/[mg/kg]$)(11). Intensive care unit nursing flow sheets were systematically reviewed to estimate total CIVN duration, total CIVN dose and average CIVN dose. CIVN dose in mcg/kg/minute is recorded at minimum each hour in these flow sheets and each dosing change is documented at the time of change. Average weight-based CIVN dose for each hour was recorded in a spreadsheet, totaled and then divided by the total number of hours to calculate the average dose of CIVN in mcg/kg/min. For adult dosing maximum mg/hr was also calculated by dividing the average weight-based CIVN dose by 1000 and multiplying by 60 and the patients weight according to the following: $mg/kg = [(mcg/kg/minute)*(60 min/hr)*(kg)]/(1000 mcg/mg)$. 
Prior to October 2009 TAC assays were performed in the clinical laboratory at our institution via fluorescence immunoassay (IMx) by Abbott Laboratories (Abbott Park, Illinois). Since October 2009 TAC assays were performed via chemiluminescent immunoassay (Abbott Architect, Abbott Park, Illinois) (7 controls). Our primary outcome variables were maximum twelve-hour TAC trough (MaxC₀) and dose-adjusted MaxC₀ (MaxC₀/[mg/kg]) in the 7 days following kidney transplantation. Secondary outcome variables included time to MaxC₀ and dose-adjusted MaxC₀ and TAC-related side effects.

Identification of Genotypes

Genomic DNA was isolated from saliva using Oragene DNA self collection kit (DNA genotek Inc. Ottawa, Ontario, Canada) in the genetics core of our institution. Tsuyoshi Fukuda PhD perform genetic analysis according to the following procedure: The presence of the CYP3A5*3 allele was determined using a mismatch PCR–restriction fragment length polymorphism (RFLP) analysis as previously reported(12). In brief, genomic DNA was amplified by PCR with CYP3A5 6956Fm (5-CTT TAA AGA GCT CTT TTG TCT CTC A-3) as a forward primer and CYP3A5 7155R (5-CCA GGA AGC CAG ACT TTG AT-3) as a reverse primer. After amplification, the 200-base pair (bp) PCR amplicon was digested with the restriction enzyme Ddel. Digestion of the 200-bp amplicon with Ddel yielded fragments of 107, 71 and 22 bp for the CYP3A5*3 allele, while digestion yielded fragments of 129 and 71 bp for the CYP3A5*1 allele (defined by the absence of CYP3A5*3). The results were confirmed for randomly selected individuals for each genotype by direct sequence analysis.

Statistical analysis

All continuous data were evaluated for normality using the Kolmogorov-Smirnov test and parametric and non-parametric tests were applied when appropriate for inferential statistics. All
data are presented as mean and standard deviation (SD) except where specified. Normally distributed data were compared using the unpaired student’s t-test, whereas non-normally distributed data were compared using the Mann-Whitney U test. Categorical variables are reported as percentages and were evaluated using Fisher’s exact test. For time to even analysis Kaplan Meier curves were generated for each group and compared using the log rank test.

For primary analysis, MaxC₀ and dose-adjusted MaxC₀ during the first week following transplantation were compared between cases and controls. \textit{A priori} we had explicit interest in whether cases who do not express CYP3A5 (CYP3A5*3/*3) would have higher TAC exposure than cases who do express CYP3A5 (CYP3A5*1/*1 or CYP3A5*1/*3) and controls. Therefore, subgroup analysis compared our outcome variables between CYP3A5*3/*3 cases and the other two groups. All analyses were conducted using two-tailed tests and p-values < 0.05 were considered statistically significant. Statistical analyses were carried out using SAS 9.2 statistical software (SAS Institute, Cary, North Carolina, USA) and GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

\section*{RESULTS}
\subsection*{Enrollment and Patient Characteristics of Comparison Groups}
Of 97 charts reviewed 19 patients (20\%) received some dose of CIVN, of which seven patients were excluded for the following reasons: CIVN < 12 hours (n=3), early TAC withdrawal (n=2), plasmapheresis (n=1), treatment with known CYP3A4 inducers (n=1). Thus twelve cases were included in the study. Forty-six consecutive charts were reviewed to identify 26 patients who met inclusion criteria as controls. We had originally identified 13 cases, yet one was excluded after further chart review, thus the 26 controls represent 2 controls for each case, prior to excluding the 13\textsuperscript{th} case. Twenty patients were excluded from the control group for the following
reasons: early TAC withdrawal (n=6), plasmapheresis (n=6), delayed TAC (n=4), liver transplant (n=3), treatment with known CYP3A4 inducers (n=1).

Patient characteristics by CIVN treatment status are presented in Table 1. Cases and controls were similar with regards to age, weight, gender, race and initial TAC dose. Cases differed significantly from controls in that they were more likely to have a living donor (83% vs. 42%, p=0.03). There was a trend toward fewer cases with a primary transplant though this did not reach statistical significance. Nearly all patients received oral dihydropyridines in the first week following transplant (100% of cases and 81% of the controls, p=0.15). Three cases and two controls received other continuous antihypertensives (labetalol and/or nitroprusside) during the study period. Of the twelve cases, eight where homozygous for CYP3A5*3 (CYP3A5*3/*3) and four were homozygous for CYP3A5*1 (CYP3A5*1/*1) (Table 2).

**Continuous Intravenous Nicardipine**

Altogether, cases received a mean CIVN dose of 2.0 (0.5) mcg/kg/min over a mean of 43.1 (11.3) hours. When stratified by CYP3A5 genotype, CYP3A5 expressors and non-expressors received similar mean CIVN doses (1.94 mcg/kg/min +/- 0.4 vs. 1.97 +/- 0.6, NS) for a similar period of time (39.8 +/- 15.0 hours vs. 44.8 +/- 9.8 hours, NS). No patient received more than 4.5 mcg/kg/min at any given time, however one patient did receive a dose of 19 mg/hr (adult maximum dose is 15 mg/hr) for several hours. Demographics and CIVN dosage of cases are presented in Table 2.

**Twelve-hour TAC Trough Levels (C0)**

When taken as a group, control patients experienced a relatively slow and controlled rise in TAC trough levels, compared to the cases, some of whom experienced a rapid and dramatic rise in 12-hour TAC trough levels within 48 hours of their initial dose while being treated with CIVN
(Figure 1). CYP3A5 genotype accurately predicted which patients were at highest risk for this interaction as depicted in Figures 2 and 3. CYP3A5*3/*3 cases experience a rapid increase in TAC-exposure within 36-48 hours of starting TAC, whereas CYP3A5*1/*1 appear to be protected from TAC over-exposure.

Our primary outcome measures for statistical analysis were MaxC\textsubscript{0} and dose-adjusted MaxC\textsubscript{0}. Median (inter-quartile range) MaxC\textsubscript{0} was 24.3 ng/ml (18.1-37.1) in CYP3A5*3/*3 cases compared to 13.9 ng/ml (9.2-17.5) (p=0.03) for CYP3A5*1/*1 cases and 14.6 ng/ml (10.8-17.7) (p=0.003) for controls (Figure 4), despite having significantly more scheduled doses that were held per patient (1.75 vs. 0.5 and 0.35 respectively, p=0.015). Dose adjusted MaxC\textsubscript{0} was likewise significantly higher in CYP3A5*3/*3 cases than controls (330 vs. 180, p=0.016) and all other patients combined (330 vs. 175, p=0.012) (Figure 5). Dose-adjusted MaxC\textsubscript{0} was numerically higher in CYP3A5*3/*3 cases than CYP3A5*1/*1 cases though this result didn’t reach statistical significance (330 vs. 156, p=0.07). CYP3A5*3/*3 cases also had significantly shorter time to MaxC\textsubscript{0} than the other two groups combined (36 vs. 72 hours, p=0.003). Six of Eight (75\%) of CYP3A5*3/*3 cases had 12-hour TAC trough levels > 20 ng/ml compared to none of the four CYP3A5*1/*1 cases and three of 26 controls (p<0.001, CYP3A5*3/*3 cases vs. all others).

**TAC-related toxicity**

TAC-related toxicity in CYP3A5*3/*3 cases (Table 2) included acute oliguria in one patient with extremely high TAC trough levels (MaxC\textsubscript{0} = 84 ng/ml). The oliguria resolved as the patient’s TAC levels decreased. One other patient had a fine tremor diagnosed by occupational therapy on post-KTX day five. There was no other reported TAC toxicity in any of the cases. Interestingly, however, one CYP3A5*1/*1 case experienced a rapid decline in TAC levels following discontinuation of CIVN and subsequently developed early acute cellular rejection in
the setting of sub-therapeutic TAC levels.
DISCUSSION

This observational study demonstrates a significantly increased risk of TAC over-exposure during the tenuous days following kidney transplantation in CYP3A5 non-expressors who are treated with CIVN. Despite clinicians’ best efforts to hold or decrease TAC doses, CYP3A5 non-expressors who were treated with CIVN experienced a rapid and dramatic rise in TAC levels well into the toxic range within 24 to 48 hours of kidney transplantation. While this study was not designed to examine clinical outcomes, at least one patient with extremely high TAC exposure experienced an abrupt decrease in urine output and stagnant creatinine 36 hours after his kidney transplant that improved when CIVN was discontinued and TAC exposure decreased. This is in contrast to one CYP3A5 expressor who was just starting to achieve therapeutic levels while on CIVN but experienced a rapid decline in TAC exposure to sub-therapeutic levels when CIVN was discontinued, resulting in early acute cellular rejection of his graft. Importantly, all the cases - CYP3A5 expressors and non-expressors alike - and nearly all controls received oral calcium antagonists, yet a rapid rise in TAC exposure was seen primarily in those treated with CIVN.

Our results highlight the well known difference in TAC metabolism in individuals who express CYP3A5 compared to those do not(13), a difference that appears to be exacerbated substantially in the presence of CIVN. Because TAC is metabolized almost exclusively by CYP3A4 and CYP3A5(1-2), individuals not expressing CYP3A5 would be at much higher risk for TAC over-exposure in the setting of the high sustained nicardipine levels and resulting CYP3A4 inhibition that is achieved with CIVN. Conversely, CYP3A5 expressors may not be at risk for elevated TAC exposure in the setting of continuous CYP3A4 inhibition because of their preserved ability to metabolize TAC via CYP3A5, but they may be at risk for low TAC exposure once the CYP3A4 inhibition is removed, i.e. when CIVN is discontinued. Others(6) have postulated a similar increase in toxicity risk based on CYP3A5 polymorphisms in patients
treated with vincristine, which is also metabolized by CYP3A enzymes. Furthermore, Kuypers et al. demonstrated that fluconazole, a CYP3A4 inhibitor, had significantly less impact on TAC metabolism in CYP3A5 expressors compared to CYP3A5 non-expressors(5).

These results are important in light of two decades of literature advocating the use of calcium channel blockers (CCBs) in patients treated with cyclosporine, another calcineurin inhibitor (CNI) metabolized by CYP3A4. Animal (14-15) and in vitro studies(16) have demonstrated the beneficial effect of CCBs on calcium-mediated renal vasoconstriction, which is at least partially responsible for CNI-induced nephrotoxicity and hypertension. Since then, numerous human studies(17-20) in solid organ transplant recipients taking cyclosporine have demonstrated improved renal blood flow and renal function in patients treated with CCBs. Furthermore, CCBs have been advocated as useful adjuncts to help lower immunosuppression drug costs specifically because they can impair CNI metabolism, resulting in a lower dose requirement(8-10). Yet, the vast majority of these studies were carried out in patients treated with cyclosporine and oral CCBs. Very few investigators have examined the effects of continuous intravenous CCBs on CNI metabolism(17), and none have specifically evaluated the simultaneous administration of CIVN and TAC.

In our study, nearly all patients were treated with oral calcium antagonists, yet primarily those treated with CIVN experienced a rapid increase in TAC exposure before clinicians could adequately compensate with decreased TAC dosage. This may be explained by the much higher and sustained drug levels and resulting CYP3A4 inhibition achieved with continuous rather than oral CCB administration(21). Along these lines, Neumayer and colleagues performed a randomized controlled trial in cyclosporine-treated patients(17). Ten patients treated with intravenous diltiazem for the first 48 hours after kidney transplantation and then with oral diltiazem were compared to eleven patients who received no such treatment. Similar to our
study, the authors found significantly increased cyclosporine exposure in patients treated with
diltiazem compared to controls. Unlike our study, the diltiazem group was reported to be free of
cyclosporine-related adverse events and to display a trend towards a lower incidence of delayed
graft function and better glomerular filtration rate in the first week following kidney
transplantation, though neither of these parameters reached statistical significance.
Interestingly, in a similar randomized controlled trial by the same authors that involved diltiazem
pretreatment of donors and grafts, followed by recipient therapy with diltiazem according to the
above mentioned protocol, statistically significantly decreased delayed graft function and
increased glomerular filtration rates were observed(17). While these authors had no knowledge
of the potential effect of CYP3A5 genotype on the interaction between CCBs and CNIs, their
study does raise the question as to whether the concomitant CCB therapy, including CIVN, may
itself also be partially protective against adverse effects of the high TAC exposure experienced
by patients in our study. Thus, well designed clinical studies of this CIVN-TAC interaction are
warranted in order to accurately define its clinical impact.

The present study highlights an important potential benefit to pharmacogenetic testing in solid
organ transplant recipients. While the difference in dose-adjusted TAC exposure between
CYP3A5 expressors and non-expressors has been well documented(4, 13), there has been
substantial disagreement in the literature as to whether knowledge of the CYP3A5 genotype
would improve clinical outcomes as long as rigorous therapeutic drug monitoring is applied (22).
In our study, we demonstrate that even in the setting of such standardized monitoring of TAC
levels, non-expressors of CYP3A5 treated with CIVN are at significantly increased risk of TAC
over-exposure in the immediate post-transplant period. Thus, knowledge of CYP3A5 genotype
may be required to anticipate and avoid adverse drug-drug interactions.
Perhaps the major limitation of this observational study is the lack of clinical outcomes data. Due to the small and heterogenous group of patients we have access to and the fact that we do not have follow-up data beyond one week in a substantial proportion of our patients, we were unable to examine clinical outcomes such as graft function with meaningful statistical power. As previously referenced, there is evidence to suggest that CIVN may at least partially counteract the nephrotoxicity of TAC over-exposure, though it would likely not mitigate other systemic adverse effects of TAC or the adverse impact of the erratic drug levels seen in our patients. It is also possible that we have failed to account for a confounder that would be associated with both CIVN administration and elevated TAC-exposure, though the physiologic basis for the CIVN-TAC interaction strongly argues against this. Moreover, we only assessed CYP3A5 genotype because of the compelling previously published data. It is, however, likely that other genetic polymorphisms and factors controlling gene expression also influence the CIVN-TAC interaction, which may explain some of the heterogeneity in TAC levels witnessed in the CYP3A5*3/*3 cases. Finally, due to low patient numbers and the retrospective nature of our data, we were also unable to make a meaningful assessment of additional factors (e.g. race, diet, other medications) that may influence this interaction.

In conclusion, we have demonstrated a significant interaction between CIVN and TAC that is modified by CYP3A5 genotype. Specifically, non-expressors of CYP3A5 are at significant risk for TAC over-exposure in the tenuous days following kidney transplantation if they are treated with CIVN to control post-operative hypertension. While decades of literature have advocated the use of CCBs in patients treated with CNIs, human studies of CCBs in TAC-treated patients are lacking and existing data do not address the potential for greater inhibition of CYP3A4 with continuously administered nicardipine or the potential for serious drug-drug interactions in patients who do not express CYP3A5. Thus, we recommend that well designed clinical studies be carried out to more fully characterize this CIVN-TAC interaction and the effect of CYP3A5.
genotype on it. Until such studies are completed, CIVN should be used with caution in TAC-treated individuals.
<table>
<thead>
<tr>
<th>Table 1. Patient Characteristics by CIVN Treatment Status</th>
</tr>
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<tbody>
<tr>
<td><strong>Controls (n=26)</strong></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td><em>Age in years</em></td>
</tr>
<tr>
<td><em>Weight in Kg</em></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Black</td>
</tr>
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<td><strong>Living donor</strong></td>
</tr>
<tr>
<td>Primary transplant</td>
</tr>
<tr>
<td><em>Initial TAC dose (mg/kg)</em></td>
</tr>
<tr>
<td>Pre-transplant TAC</td>
</tr>
<tr>
<td>Oral dihydropyridines</td>
</tr>
<tr>
<td>Continuous labetalol or nitroprusside</td>
</tr>
</tbody>
</table>

*Values reported as mean (standard deviation)*
### Table 2. Characteristics of Cases Treated with CIVN\(^1\) Separated by CYP3A5 Genotype

<table>
<thead>
<tr>
<th>Case #</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Race</th>
<th>CYP3A5 genotype</th>
<th>Initial TAC dose (mg/kg)</th>
<th>CIVN duration (hrs)</th>
<th>Average CIVN dose (mcg/kg/min)</th>
<th>Other CYP3A4 inhibitors</th>
<th>MaxC(_0) (ng/ml)</th>
<th>Dose-adjusted MaxC(_0) (ng/ml per mg/kg)</th>
<th>Time to MaxC(_0) (hrs)</th>
<th>Clinical signs of TAC over-/under-exposure</th>
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<tr>
<td>1</td>
<td>20.5</td>
<td>82.8</td>
<td>White</td>
<td>CYP3A5*3/*3</td>
<td>0.060</td>
<td>31</td>
<td>3.02</td>
<td>Nifedipine XL</td>
<td>25.3</td>
<td>419.0</td>
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<td></td>
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<tr>
<td>2</td>
<td>12.9</td>
<td>32.0</td>
<td>Asian</td>
<td>CYP3A5*3/*3</td>
<td>0.094</td>
<td>43</td>
<td>1.93</td>
<td>Nifedipine XL</td>
<td>13.9</td>
<td>148.3</td>
<td>48</td>
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</tr>
<tr>
<td>3</td>
<td>10.9</td>
<td>27.7</td>
<td>White</td>
<td>CYP3A5*3/*3</td>
<td>0.090</td>
<td>43</td>
<td>2.24</td>
<td>Amlodipine, metronidazole</td>
<td>39.8</td>
<td>551.2</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15.0</td>
<td>67.5</td>
<td>White</td>
<td>CYP3A5*3/*3</td>
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<td>56</td>
<td>1.40</td>
<td>Amlodipine</td>
<td>28.8</td>
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<td>CYP3A5*3/*3</td>
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<td>33</td>
<td>1.82</td>
<td>Nifedipine XL</td>
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<td>1539.1</td>
<td>48</td>
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<td>67.0</td>
<td>White</td>
<td>CYP3A5*3/*3</td>
<td>0.075</td>
<td>49</td>
<td>1.79</td>
<td>Nifedipine XL</td>
<td>20.2</td>
<td>270.7</td>
<td>36</td>
<td>Fine Tremor</td>
</tr>
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<td>7</td>
<td>19.3</td>
<td>58.6</td>
<td>White</td>
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<td>0.102</td>
<td>58</td>
<td>1.34</td>
<td>Nifedipine XL</td>
<td>23.3</td>
<td>227.6</td>
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<tr>
<td>8</td>
<td>6.7</td>
<td>18.0</td>
<td>Biracial</td>
<td>CYP3A5*3/*3</td>
<td>0.100</td>
<td>44</td>
<td>2.42</td>
<td>Nifedipine XL</td>
<td>17.4</td>
<td>174.0</td>
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</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td>16.1(^2) (5.5)</td>
<td>53.3(^2) (24.0)</td>
<td>0.083 (0.016)(^2)</td>
<td>44.8 (9.8)(^2)</td>
<td>1.97 (0.6)(^2)</td>
<td>24.3(^3) (18-37)</td>
<td>330(^3) (187-518)</td>
<td>36(^3) (18-48)</td>
</tr>
<tr>
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<td>12.0</td>
<td>36.2</td>
<td>Black</td>
<td>CYP3A5*1/*1</td>
<td>0.083</td>
<td>44</td>
<td>2.39</td>
<td>Nifedipine XL</td>
<td>18.0</td>
<td>419.0</td>
<td>48</td>
<td>Acute Rejection (POD(^4) #7)</td>
</tr>
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<td>116.0</td>
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<td>Nifedipine XL</td>
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<tr>
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<td>15.6</td>
<td>47.0</td>
<td>Black</td>
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<td>57</td>
<td>2.13</td>
<td>Nifedipine XL</td>
<td>8.3</td>
<td>78.0</td>
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<td>CYP3A5*1/*1</td>
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<td>none</td>
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<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td>13.8(^2) (2.9)</td>
<td>57.6(^2) (39.5)</td>
<td>0.086 (0.02)(^2)</td>
<td>39.8 (15.0)(^2)</td>
<td>1.94 (0.4)(^2)</td>
<td>13.9(^3) (9-18)</td>
<td>156(^3) (94-287)</td>
<td>72(^3) (36-108)</td>
</tr>
</tbody>
</table>

\(^1\) Continuous intravenous nicardipine  
\(^2\) NS between CYP3A5*3/*3 and CYP3A5*1/*1 cases  
\(^3\) Median (interquartile range)  
\(^4\) Post-operative day
Figure 1. 12-hour TAC trough levels ($C_0$) in patients treated with CIVN (cases) compared to patients not treated with CIVN (controls). Mean CIVN duration was approximately 42 hours. Values are depicted as mean and standard deviation.
Mean CIVN Duration: 44.8 hours

Figure 2: 12-hour TAC trough levels (C₀) by time from first dose in cases treated with CIVN who do not express CYP3A5 (CYP3A5*3/*3). Values are depicted as mean and standard deviation.
Figure 3: 12-hour TAC trough levels (C₀) by time from first dose in cases treated with CIVN who do express CYP3A5 (CYP3A5*1/*1). Values are depicted as mean and standard deviation.
Figure 4. Maximum TAC trough level (MaxC₀) in the first week following kidney transplantation in controls and cases (separated by CYP3A5 expressor status). CYP3A5*3/*3 cases experienced nearly two-fold higher maximum trough levels than the other two groups. The bold bar represents median with error bars for inter-quartile range.
Figure 5. Maximum dose-adjusted trough level in the first week following kidney transplantation in controls and cases (separated by CYP3A5 expressor status). CYP3A5*3/*3 cases experienced nearly two-fold higher maximum dose-adjusted TAC trough levels than the other two groups. The bold bar represents median with error bars for inter-quartile range.
REFERENCES

2. Iwasaki K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. Drug Metab Pharmacokinet 2007;22(5):328-335.