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Assessment of the Intestinal CYP3A Contribution to Drug Interactions with Extended-Release Tacrolimus (LCPT) Using Grapefruit Juice

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Abstract

Grapefruit juice (GFJ) is a known inhibitor of intestinal cytochrome P450 3A (CYP3A) metabolism leading to increased exposure to CYP3A substrates such as tacrolimus. The extended-release tacrolimus formulation Envarsus (LCPT) exhibits prolonged absorption throughout the entire GI tract. Although a clinically significant drug–drug interaction occurs with immediate-release tacrolimus formulations, this has not been evaluated with extended-release formulations. This study assessed the impact of GFJ on LCPT in adult kidney transplant patients. Eleven adult kidney transplant recipients on a stable dose of LCPT were enrolled in a randomized crossover study. Participants were administered either GFJ or water during each pharmacokinetic visit, with midazolam used as a positive control. A washout period of 2–4 weeks was included between visits. Tacrolimus concentrations were determined using validated LC-MS/MS methods. Tacrolimus AUC_{0–24} was 28% higher with GFJ (GMR = 1.28, 90% CI 1.12–1.44) and C_{max} was 73% higher (GMR = 1.73, 90% CI 1.46–2.00) compared to control. GFJ exhibited a clinically meaningful interaction with LCPT. However, the magnitude appears less than those reported with immediate-release formulations, suggesting the extended absorption profile of LCPT may affect susceptibility to drug interactions in the intestine.

Keywords

cytochrome P450 3A, drug–drug interactions, Envarsus, grapefruit juice, LC-MS/MS, midazolam, tacrolimus

Tacrolimus is a calcineurin inhibitor immunosuppressant widely used to prevent rejection in solid organ transplant patients. Tacrolimus has a narrow therapeutic range with high intra- and interindividual variability in pharmacokinetics that requires therapeutic drug monitoring to individualize dosing. Supratherapeutic tacrolimus exposure increases the risk of toxicities (such as nephrotoxicity), whereas subtherapeutic exposure increases the risk of rejection.^{1,2} Orally administered tacrolimus products are available as either immediate-release formulations (administered twice daily) and as extended-release formulations (administered once daily). Tacrolimus is subject to numerous clinically significant drug–drug interactions involving cytochrome P450 3A (CYP3A), an enzyme that is highly expressed in the small intestine and liver. Drug interactions mediated by the CYP enzyme system account for a large proportion of adverse drug events, hospitalizations, and increased morbidity and mortality leading to increased healthcare costs.³ CYP3A4 metabolizes more than 50%

of marketed drugs in the United States.⁴ For orally administered drugs, bioavailability depends in part on the

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extent of intestinal and hepatic metabolism prior to reaching the systemic circulation, commonly referred to as first-pass metabolism. Intestinal metabolism may significantly contribute to first-pass loss (as high as 93% with a range from 8% to 93%).⁵

Grapefruit juice (GFJ) has been shown to interact with many medications including tacrolimus.^{6,7} In a study of 30 liver transplant recipients, GFJ led to a clinically significant increase in whole blood tacrolimus concentrations by more than 10 ng/mL.⁸ GFJ down-regulates CYP3A intestinal protein content causing increases in concentrations of CYP3A substrate drugs and potential toxicity.⁹ The interaction with GFJ is exclusively an intestinal CYP3A-mediated interaction and does not involve hepatic CYP3A.¹⁰ In fact, GFJ has been suggested as a probe to interrogate intestinal CYP3A4-mediated drug interactions.⁵

The majority of drug interactions that have been documented with tacrolimus involve immediate-release formulations which are primarily absorbed in the small intestine, specifically the duodenum and ileum.^{8,11–14} The extended-release tacrolimus formulation Envarsus (LCPT) utilizes melt-dose technology to avoid the high peak concentrations associated with immediate-release tacrolimus (IR-Tac).^{15,16} By dispersing tacrolimus more distally in the intestine and throughout the colon, there may be less opportunity for metabolism by intestinal CYP3A, as enteric CYP3A enzymes are concentrated in the proximal intestine.^{17,18} A recent study in healthy volunteers who received a single oral dose of either IR-Tac or LCPT with or without voriconazole demonstrated a significantly less pronounced increase in tacrolimus exposure in the LCPT arm in comparison to the IR-Tac arm (2.6-fold vs 6-fold, $P < .01$).¹⁹ It is unclear if this decreased interaction is due to intestinal CYP3A inhibition as voriconazole inhibits both intestinal and hepatic CYP3A. The purpose of this study was to characterize the magnitude of the drug interaction between GFJ and LCPT. Given that CYP3A enzymes are concentrated in the proximal intestine, we hypothesized that this interaction would be significantly lower.

Materials and Methods

Adult participants with a kidney transplant provided informed consented (UC San Diego IRB #190617) prior to participation in this prospective, single-center, crossover pharmacokinetic study. The enrollment period was from October 2019 to December 2021. Inclusion criteria included age greater than 18 years, at least 3 months post-transplantation, receiving a stable dose (no dosage change in last month, last two trough concentrations within target range) of LCPT for immunosuppression while maintaining a trough concentration of 6–12 ng/mL, and BMI between 18 and 32 kg/m².

Exclusion criteria included recipients of multi-organ transplantation, concomitant use of mammalian target of rapamycin (mTOR) inhibitors, belatacept, medications affecting CYP3A metabolism or P-gp transport, hypersensitivity or intolerance to midazolam or LCPT, active diarrhea or constipation, BMI < 18 or >32 kg/m² or participants with clearance creatinine (Cockcroft–Gault) of less than 10 mL/min.

Participants were admitted to the UC San Diego Clinical and Translational Research Institute (CTRI) Phase I Unit for an intensive steady-state pharmacokinetic (PK) sampling of tacrolimus following once-daily dosing of LCPT. Each participant received LCPT with either a single 8 oz glass of GFJ (final dihydroxybergamottin (DHB) concentration 17,500 ng/mL or ~50 μ M) or water then, after a ≥ 2 and ≤ 4 weeks washout period, received the other treatment. Participants were randomly assigned to GFJ–Water or Water–GFJ. After fasting from midnight the night prior, participants took an observed LCPT dose with either GFJ or water. Participants also received 3 mg of oral midazolam as a positive control for the inhibitory effect of GFJ on intestinal CYP3A activity. Blood samples were taken pre-dose and post-dose at 0.25, 0.5, 1, 2, 4, 6, 8, 10 or 12, and 24 h. Each participant was fed the same low-fat meals to avoid variability due to food intake. Frozen GFJ concentrate was kindly provided by Ventura Coastal, LLC (Ventura, CA). After dilution of the GFJ concentrate with water, DHB concentrations were determined. GFJ was then frozen in glass bottles and thawed prior to each use. Blood samples were processed and analyzed by a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assay for tacrolimus²⁰ and midazolam. This study was exploratory and mechanistic in nature; therefore, no formal sample size calculation was performed. The crossover design minimized interindividual variability, and precision was assessed using 90% confidence intervals for key PK parameters.

Chemicals and Reagents

Tacrolimus traceable calibrators, quality controls (QC), and standard materials in lyophilized whole blood were from the Waters MassTrak Immunosuppressant Assay Kit with ascomycin internal standard (186010251IVD and 186010252IVD). Certified reference material for midazolam and midazolam-D4 were purchased from Cerilliant (M908, M918; Round Rock, TX) and spiked into blank plasma (MSG3200; Golden West) to generate matrix calibrators and QC's. The 0.1 M zinc sulfate solution was purchased from Honeywell Research Chemicals (Germany). LC-MS/MS grade methanol (A456), acetonitrile (A955), formic acid (A117), and ammonium acetate (A639) were all obtained from Fisher Scientific (Hampton, NH). Ultra purified water

at 18.2 M Ω -cm was sourced from a Siemens purification system (Elga, Purelab Ultra).

Sample Preparation

Fifty microliters of calibrators, quality controls, and participant sample (whole blood for tacrolimus and plasma for midazolam) were added to a 1.5 mL tube Eppendorf for liquid extraction with 200 μ L of 0.1 M ZnSO₄. Sample mixture was vortexed for 5 s followed by the addition of 500 μ L of 2 ng/mL internal standard in acetonitrile. Ascomycin was used as an internal standard for tacrolimus extraction while midazolam-d4 was used for midazolam extraction. The mixture was vortexed for 20 s and centrifuged at 13,400 g for 5 min. Supernatant was collected and directly used for LC-MS/MS analysis of tacrolimus while samples were diluted in water 1:1 for analysis of midazolam.

LC-MS/MS Methods

Analysis of tacrolimus and midazolam in whole blood and plasma, respectively, was performed on a Waters Acquity UPLC I-Class coupled to a Waters TQS-micro triple quadrupole MS with an electrospray ionization source. The analytical column (Acquity UPLC HSS C18 SB Column, 1.8 μ m, 2.1 $\times$ 30 mm) was maintained at 55°C. Chromatographic separation was achieved with a 0.4 mL/min flow rate using mobile phase (A) water + 2 mM ammonium acetate + 0.1% formic acid and mobile phase (B) methanol + 2 mM ammonium acetate + 0.1% formic acid. The total method was 1.8 min. All compounds were analyzed in positive ion mode with the multiple reaction monitoring (MRM) transitions, mass to charge transition of m/z 821.5 > 768.5 (tacrolimus), internal standard 809.5 > 756.5 (ascomycin), 326 > 208.8 (midazolam), and 330.1 > 294.9 (midazolam-d4). Concentrations were determined by the peak area ratio of quantifier ion to internal standard against the calibration curve included in each batch run.

Dihydroxybergamottin Method

GFJ was quantified for DHB concentrations. Calibration standards and quality controls were prepared using blank human plasma (Bioreclamation, Baltimore, MD) at concentrations ranging from 0.25 to 1000 and 0.8 to 800 nM, respectively. Ethyl acetate (500 μ L) containing 400 nM internal standard (psoralen) was added to thawed plasma (100 μ L) to precipitate proteins. Samples were vortexed for 3 min at room temperature then centrifuged (2000 $\times$ g, 10 min, 4°C). Supernatant (400 μ L) was transferred to 0.6-mL cluster tubes and dried under heated (50°C) nitrogen gas. Residues were reconstituted in 95% water:5% acetonitrile in 0.1% formic acid (v:v:v). Samples (10 μ L) were injected onto a Thermo Aquasil C18 column (3 μ m, 2.1 $\times$ 50 mm). Analytes were eluted using a gradient initially held at 95% mo-

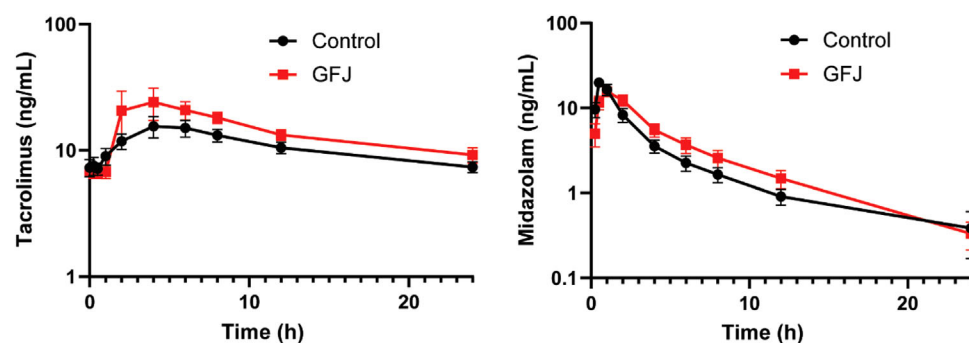
bile phase A (0.1% formic acid in water) and 5% mobile phase B (acetonitrile in 0.1% formic acid) for 0.4 min. Mobile phase B was increased linearly for 1.1 min to 95%, maintained for 0.2 min, then returned to initial conditions over 6 s. The column was equilibrated for 2 min. Eluent was directed to waste for the first 0.4 min then to a Sciex API 6500 hybrid triple quadrupole mass spectrometer (Framingham, MA) operated in MRM mode, with a source temperature of 250°C and ion spray voltage of 2500 V. MRM transitions for DHB and psoralen were 273.2 $\rightarrow$ 203.1 (collision energy, 25 mV) and 187.1 $\rightarrow$ 131.2 (collision energy, 32 mV), respectively. DHB was quantified using peak area ratios, calibration standards, and Multiquant software (v3.0, AB Sciex, Framingham, MA). The lower limit of quantification was 250 pM based on FDA guidelines. Mean DHB concentration in the diluted GFJ was 17,500 ng/mL.

Pharmacokinetic and Statistical Analysis

Maximum and minimum concentrations ($C_{\max}$ and $C_{\min}$) along with corresponding time points ($T_{\max}$ and $T_{\min}$) were observed directly. Area under the concentration versus time curve over 24 h (AUC_{0-24}) was calculated using non-compartmental analysis with the log-linear trapezoidal method as implemented in Phoenix WinNonLin. The terminal elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$, where λ_z is the elimination rate constant derived from the terminal slope of the log concentration versus time curve. Apparent oral clearance (CL/F) was calculated as dose divided by AUC . Pharmacokinetic analysis was performed using Phoenix WinNonLin version 8.3 (Certara, Radnor, PA). $C_{\max}$ and AUC_{0-24} data were log-transformed prior to statistical analyses. No effect boundary testing was performed for $C_{\max}$ and exposure parameters. An analysis of variance and a general linear model were performed that included subject and treatment effects and a calculated least squares geometric mean ratio (LS-GMR).²¹ Ninety-percent confidence intervals (90% CI) were calculated and expressed as a percentage relative to the LS-GMR during control (i.e., water) and GFJ administration phases for tacrolimus and midazolam. If the 90% CI is within the 0.8 to 1.25 interval, it is concluded that there is no significant CYP3A-mediated drug-drug interaction. In contrast, if the 90% CI is outside the 0.8 to 1.25 interval, it is concluded that an interaction was observed (M12 Drug Interaction Studies, Guidance for Industry, Food and Drug Administration, August 2024. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m12-drug-interaction-studies>).^{22,23} The desired sample size was 15 patients. Statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Table 1. Participant Demographics (Mean $\pm$ SD)

	All	Females	Males
N	11	5	6
Age (Years)	46.1 $\pm$ 11.2	46.8 $\pm$ 8.2	45.5 $\pm$ 13.2
Weight (kg)	77.3 $\pm$ 13.2	78.3 $\pm$ 15.8	76.4 $\pm$ 10.3
BMI (kg/m ²)	27.6 $\pm$ 3.7	29.34 $\pm$ 3.5	26.3 $\pm$ 3.3
LCPT daily dosage (mg)	4.8 $\pm$ 3.0	6.7 $\pm$ 3.5	3.4 $\pm$ 1.4
Time post-transplant (months)	68.5 $\pm$ 88.7	8.8 $\pm$ 4.3	139.8 $\pm$ 89.3
Race, n (%)			
White	6(55)	3(60)	3(50)
Asian	1(9)	1(20)	0(0)
Hispanic	4(36)	1(20)	3(50)

**Figure 1.** Pharmacokinetic profiles of extended-release tacrolimus (LCPT) and midazolam in participants over 24 h in the presence of grapefruit juice. Concentrations expressed as mean $\pm$ standard error of the mean ($n = 11$).**Table 2.** Tacrolimus Pharmacokinetic Parameters

Parameter	LCPT $n = 11$	LCPT + GFJ $n = 11$	LS Geometric Mean Ratio of (LCPT + GFJ/LCPT)	90% Confidence Interval
AUC _{0–24} (ng h/mL)	245.3 (36.7)	314.7 (44.8)	1.28	1.12–1.44*
C _{max} (ng/mL)	15.9 (49.7)	27.7 (78.9)	1.73	1.46–2.00*
T _{max} (h)	6.0 (2–8)	6.0 (2–8)	-	-
C ₂₄ (ng/mL)	7.04 (31.1)	9.2 (39.6)	-	-
CL/F (L/h)	7.2 (58.8)	5.7 (36.3)		
T _{1/2} (h)	20.6 (34.5)	17.9 (32.9)		

Data presented as geometric mean (%CV) except T_{max} which is presented as median (range).

*90% confidence interval is outside the 0.80–1.25 range.

Results

Eleven (five female and six male) adult kidney transplant participants were enrolled with an average age of 46 years. Participant demographics are shown in Table 1. Compared with paired data, tacrolimus AUC_{0–24} was 28% higher with GFJ ($n = 11$, geometric mean of ratio [GMR] = 1.28, 90% confidence interval [CI] 1.12–1.44) and C_{max} was 73% higher with GFJ ($n = 11$, GMR = 1.73, 90% CI 1.46–2.00). Geometric mean (%CV) tacrolimus C₂₄ increased from 7.04 (31.1) to 9.2 (39.6) with GFJ (NS). There was no change in tacrolimus half-life with GFJ (Figure 1 and Table 2).

Compared with paired data, midazolam AUC_{0–24} was 20% higher with GFJ ($n = 11$, GMR = 1.20, 90% CI 1.11–1.29) and C_{max} was 22% lower with GFJ ($n = 11$, GMR = 0.78, 90% CI 0.72–0.84) (Figure 1 and Table 3). No serious adverse effects were reported during the study.

Discussion

To our knowledge, this is the first study to evaluate the impact of GFJ on LCPT in kidney transplant patients. Tacrolimus exposure increased by 28% and C_{max} increased by 73% GFJ with no change in elimination half-life. These results suggest that GFJ increased LCPT

Table 3. Midazolam Pharmacokinetic Parameters

Parameter	Midazolam n = 11	Midazolam + GFJ n = 11	LS Geometric Mean Ratio of (MDZ + GFJ/MDZ)	90% Confidence Interval
AUC ₀₋₂₄ (ng h/mL)	51.3 (50.6)	61.5 (55.9)	1.20	1.11–1.29*
C _{max} (ng/mL)	19.7 (36.3)	15.4 (40.6)	0.78	0.72–0.84*
T _{max} (h)	0.5 (0.25–1)	1.0 (0.5–2)	-	-
C ₂₄ (ng/mL)	0.4 (112.5)	0.5 (49.8)	-	-
CL/F (L/h)	5.3 (66.4)	4.5 (73.5)	-	-
t _{1/2} (h)	5.0 (48.2)	4.4 (38.8)	-	-

Data presented as geometric mean (%CV) except T_{max} which is presented as median (range).

*90% confidence interval is outside the 0.80–1.25 range.

bioavailability. In comparison, the CYP3A4 model substrate, midazolam AUC increased by 20% and C_{max} decreased by 22% with GFJ. Since tacrolimus half-life was unchanged with GFJ compared to water, the increase in AUC is likely due to increased oral bioavailability secondary to intestinal inhibition of CYP3A by GFJ as opposed to a change in elimination. A previous study in liver transplant participants investigating the interaction between GFJ and IR-Tac demonstrated a much larger magnitude of change in trough concentrations. Trough concentrations increased by 10 ng/mL after GFJ which translated to a 110% increase from baseline. This study was conducted under different conditions as the GFJ source was fresh juice, the assay utilized was an immunoassay which is known to overestimate tacrolimus concentrations, and only trough concentrations were measured.⁸

It is possible that CYP3A interactions with LCPT are diminished compared to IR-Tac. For example, voriconazole, a strong CYP3A4 inhibitor, increased LCPT AUC by 2.6-fold and C_{max} by 2-fold which was significantly lower compared to IR-Tac (AUC 6.0-fold, $P < .001$; and C_{max} increased 2.7-fold, $P = .026$).¹⁹ With respect to CYP3A4 induction, St. John's wort (SJW) trended toward a less pronounced inductive effect with LCPT compared to IR-Tac although they were not statistically different (AUC decreased by 67% vs 73%, respectively).²⁴

The CYP3A4 inhibition with GFJ is likely due to the furanocoumarins in GFJ. Grapefruit and products derived from it, such as juices, are widely common products rich in flavonoids and furanocoumarins, especially bergamottin and dihydroxybergamottin which are known reversible and mechanism-based inhibitors of CYP3A4.²⁵ It has been demonstrated that the amount of DHB needed to inhibit 6-beta testosterone (a CYP3A4 substrate) by 50% (IC₅₀) is 25 μ M²⁶ which is equivalent to 8460 ng/mL. A clinical study in healthy volunteers demonstrated that 38 μ M (12,856 ng/mL) DHB in GFJ significantly increased felodipine (CYP3A4 substrate) AUC and

C_{max}.²⁷ The DHB concentration in GFJ in this study was 17,500 ng/mL or ~50 μ M which is above the IC₅₀ for CYP3A4 metabolism. It is important to note that GFJ composition varies significantly depending on the source (fresh-squeezed vs commercial), processing method (cloudy vs clarified), and storage conditions. The DHB content can range from <1000 to >20,000 ng/mL in different preparations. Our study used a standardized frozen concentrate with quantified DHB content (17,500 ng/mL), which may not reflect the interaction magnitude observed with other GFJ preparations commonly consumed by patients. Clinicians should be aware that the clinical significance of this interaction may vary depending on the type and amount of GFJ consumed.

The variability in the inhibition by GFJ with tacrolimus and midazolam could be due to genetic polymorphisms in the CYP3A4 or CYP3A5 gene. There is mechanistic and clinical data that demonstrates variable CYP3A inhibition based upon different metabolizer phenotypes. CYP3A5*1 carriers (expressors) exhibit faster tacrolimus clearance and may demonstrate altered susceptibility to CYP3A4 inhibition compared to CYP3A5*3/*3 non-expressors. CYP3A5*1 heterozygotes or homozygotes have been shown to be less susceptible to inhibition.²⁸ For example, the CYP3A5*3/*1 genotype was associated with reduced susceptibility of fluconazole inhibitory effects on tacrolimus metabolism,²⁹ and a 30% greater inhibition by ketoconazole in kidney transplant patients lacking the *1 allele.³⁰ The magnitude of an interaction may depend not only on CYP3A4/5 genotype but also on inhibitor selectivity, the CYP3A substrate, and the degree of intestinal and/or hepatic metabolism.^{30,31} The interindividual variability observed in our study (CV% ranging from 31% 79% for tacrolimus parameters) may partially reflect genetic differences in CYP3A expression.

It is unclear why midazolam C_{max} decreased when given together with GFJ; however, the pharmacokinetic variability of midazolam likely contributed to this

effect. Potential explanations include variability in midazolam absorption, delayed gastric emptying, or GFJ effects on intestinal transporters. Similar variability has been reported in prior studies.⁷ Further research is warranted to elucidate this phenomenon.

The small sample size was a study limitation, impacting the statistical power and generalization of study results. Previous studies in liver transplant patients receiving midazolam reported smaller sample sizes ($n = 7-10$).^{32,33} The enrollment of transplant patients into clinical studies is a constant challenge.³⁴ Regrettably, the enrollment period for this study was impacted by the COVID-19 pandemic and the decision was made to prematurely terminate the study. A second limitation was the lack of CYP3A5 genotyping. Consequently, the extent of contribution of CYP3A5 genetic polymorphisms on GFJ and tacrolimus pharmacokinetic variability is unknown. Future studies should incorporate comprehensive pharmacogenetic profiling to better predict individual patient susceptibility to drug–drug interactions with LCPT and enable more personalized therapeutic drug monitoring strategies.

In conclusion, GFJ caused a 28% increase in AUC of LCPT and 74% increase in C_{max} . In comparison, GFJ led to a 22% decrease in C_{max} and 20% increase in AUC of the CYP3A4 probe midazolam. Despite the prolonged absorption of LCPT throughout the GI tract, an interaction between GFJ exists, though the magnitude appears to be less than would be expected with IR-Tac. This is consistent with altered LCPT disposition in the proximal intestine (where CYP3A4 expression is more abundant), but not in the distal intestine. While the effect of GFJ on LCPT is modest, this would still be considered a clinically significant interaction given that tacrolimus is a narrow therapeutic range drug.

Author Contributions

Adekunle Alabi: Data analysis/interpretation; drafting article; critical revision of article; approval of article. **Janice S. Kerr:** Concept/design; drafting article; critical revision of article; approval of article. **Mita Shah:** Drafting article; critical revision of article; approval of article. **Adnan Khan:** Drafting article; critical revision of article; approval of article. **Joseph D. Ma:** Data analysis/interpretation; critical revision of article; approval of article. **Raymond T. Suhandynata:** Data analysis/interpretation; critical revision of article; approval of article. **Jeremiah D. Momper:** Concept/design; data analysis/interpretation; critical revision of article; approval of article. **Shirley M. Tsunoda:** Concept/design; data analysis/interpretation; critical revision of article; approval of article; funding secured.

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Conflicts of Interest

Shirley M. Tsunoda received funding from Veloxis Pharmaceuticals; all other co-authors declare no conflicts of interest.

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Data Availability Statement

The de-identified data that support the findings of this study are available from the corresponding author upon reasonable request.

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