The Journal of Clinical Pharmacology
2018, 58(3) 286–293

DOI: 10.1002/jcph.1014

Thorough QT/QTc Evaluation of the Cardiac Safety of Secnidazole at Therapeutic and Supratherapeutic Doses in Healthy Individuals

Borje Darpo, MD, PhD[1,2], Hongqi Xue, PhD[2], Nikki Adetoro, MS, MPH[3], Barbara G. Matthews, MD, MPH[4], and Helen S. Pentikis, PhD[3,5]

Abstract

SYM-1219, a novel oral granule formulation of secnidazole, is under development as single-dose treatment for bacterial vaginosis. This 4-way, randomized, crossover study evaluated the effects of SYM-1219 on electrocardiographic (ECG) parameters in 52 healthy subjects. Subjects were administered single doses of SYM-1219, 2 g (proposed therapeutic dose), 6 g (supratherapeutic dose), placebo, and moxifloxacin (positive control). Serial digital 12-lead ECGs were recorded pre- and postdose; blood samples were taken to determine plasma secnidazole concentrations. A high-precision QT technique measured ECGs. The primary end point was change from baseline QTcF (ΔQTcF); data were analyzed with the objective of excluding QT effects >10 milliseconds at postdosing time points and with exposure-response analysis. Safety and tolerability were assessed. Single doses of 2 g and 6 g SYM-1219 did not have a clinically relevant effect on the QTcF interval; an effect >10 milliseconds could be excluded at all postdosing time points. A shallow slope of the exposure-response relationship was seen (0.058 millisecond per μg/mL; 90%CI 0.042, 0.073); in this model, the effect on QTc can be predicted to be <10 milliseconds up to a secnidazole plasma concentration of $\sim$125 μg/mL, approximately 3.4-fold higher than anticipated peak therapeutic plasma levels. The moxifloxacin QT response demonstrated assay sensitivity. The most frequently reported treatment-emergent adverse events with SYM-1219 were headache, dizziness, and nausea. This thorough QT study demonstrated that SYM-1219 in doses and plasma concentrations up to 3-fold above therapeutically relevant levels does not have a clinically concerning effect on ECG parameters, including the QT interval.

Keywords

bacterial vaginosis, cardiac safety, QT interval, secnidazole, SYM-1219

Bacterial vaginosis (BV), with an estimated prevalence in the United States of approximately 29% in women 14-49 years old, is the most common vaginal condition among women of reproductive age.[1] The importance of BV as a public health issue arises from its association with an increased risk of sexually transmitted diseases, including both acquisition and transmission of the human immunodeficiency virus, as well as the potential for preterm births and low birth weight among pregnant women who have BV.[2]

Although the etiology of BV has still not been fully elucidated, it results from alterations in the vaginal microbiome.[2] These alterations are generally by replacement of *Lactobacillus* species with heterogeneous vaginal bacterial communities consisting of different species of Gram-positive or Gram-negative anaerobic bacteria that may vary among individuals and be associated with variability in clinical presentation.[3,4] Standard pharmacologic management consists of a 5- to 7-day regimen of drugs that include metronidazole or clindamycin, both of which are recommended as first-line therapy as either twice-daily oral administration (metronidazole) or once-daily intravaginal administration (metronidazole and clindamycin).[5] However, the extended dosing schedule, including a need for twice-daily dosing of oral metronidazole, impacts patient adherence,[6] which is generally highest with once-daily dosing and decreases as dose frequency increases.[7] Reduced adherence to metronidazole potentially contributes to low clinical effectiveness and an increased rate of recurrence.

SYM-1219 is a new oral, granule formulation of secnidazole [1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole; Figure 1], an antimicrobial drug in the

[1]Karolinska Institute, Division of Cardiovascular Medicine, Department of Clinical Sciences, Danderyd's Hospital, Stockholm, Sweden
[2]iCardiac Technologies, Inc, Rochester, NY, USA
[3]Symbiomix Therapeutics, LLC, Baltimore, MD, USA
[4]BioDirect, Inc, Silver Spring, MD, USA
[5]SAJE Consulting, Baltimore, MD, USA

Submitted for publication 19 June 2017; accepted 15 August 2017.

Corresponding Author:
Helen S. Pentikis, PhD, Symbiomix Therapeutics, LLC, 1101 E 33rd Street, Suite E306, Baltimore, MD 21218
Email: hpentikis@symbiomix.com

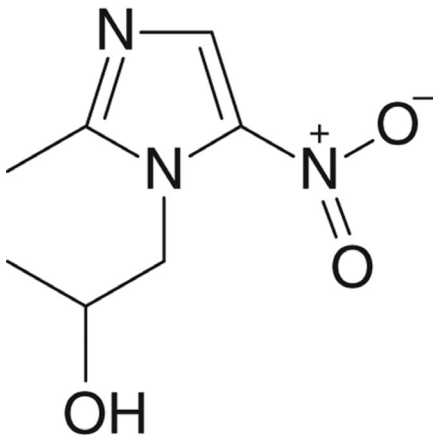

Figure 1. Molecular structure of SYM-1219 (secnidazole; 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole).

5-nitroimidazole class, which also includes metronidazole. The efficacy of secnidazole for the treatment of BV and other infectious pathogens has long been recognized.[8] The pharmacokinetic (PK) profile of the SYM-1219 granule formulation, which includes a half-life of 17 hours and its high potency, makes SYM-1219 amenable for use as a single-dose treatment for BV.[9,10] A single-dose regimen of SYM-1219, which can be sprinkled on food or in drinks for ease of administration in patients who may have problems swallowing pills, would be expected to be of benefit by increasing patient adherence with therapy in the clinical setting, thereby resulting in greater effectiveness. A phase 2 study demonstrated that SYM-1219 not only was well tolerated but also showed that the efficacy of a single dose of SYM-1219 2 g was significantly greater than placebo and numerically superior to a 1-g dose for the treatment of BV,[10] supporting 2 g as the therapeutic dose. The efficacy and safety of SYM-1219 2 g for the treatment of BV were further confirmed in a phase 3 trial.[11]

Appropriate identification of potential effects on cardiac physiology is an important criterion in determining the safety of new drugs. A recent in vitro study characterizing the effect of SYM-1219 on the *hERG* potassium current expressed in human embryonic kidney cells showed minimal inhibitory effects at clinically relevant concentrations, suggesting a low potential for cardiac toxicity.[12] In line with regulatory guidance,[13–15] this thorough QT (TQT) study was undertaken to evaluate electrocardiographic effects of SYM-1219 at therapeutic and supratherapeutic dose concentrations.

Methods

The protocol was approved by Schulman Institutional Review Board (Cincinnati, Ohio), and the study was performed in accordance with Guidelines for Good Clinical Practice and the revised Declaration of Helsinki; all subjects provided written informed consent before participation.

This was a 4-way crossover phase 1 study in healthy subjects to evaluate the electrocardiogram (ECG) effects of SYM-1219 compared with placebo, and with moxifloxacin as a positive control to demonstrate assay sensitivity.

Study subjects were healthy, nonsmoking, men or women, 18 to 65 years of age, inclusive, with body mass index between 18 and 32 kg/m^2, who met standard criteria for clinical pharmacology studies in healthy subjects. The ECG-related exclusion criteria were heart rate (HR) at rest <50 or >100 beats per minute, QRS >110 milliseconds, QTcF >440 milliseconds, or PR interval >200 milliseconds.

A total of 48 subjects (at least 40% of each sex) was planned for enrollment to ensure a minimum sample size of 40 evaluable subjects. This sample size was selected based on calculated 90% power to exclude a 10-milliseconds effect of SYM-1219 (ie, the upper bound of the CI below 10 milliseconds) at all postdosing time points under the assumption of an intrasubject standard deviation of 8 milliseconds for ΔQTc and a true mean difference of 3 milliseconds in ΔQTc between SYM-1219 and placebo.

Subjects were randomized in a double-blind manner except for moxifloxacin to receive a sequence that included all 4 treatment modalities: placebo, 2 different doses of SYM-1219, ie, the proposed therapeutic dose of 2 g and a supratherapeutic dose of 6 g, and moxifloxacin (400 mg). Dose selection was based on several factors, including that for the proposed therapeutic dose. Secnidazole is available either by prescription or over-the-counter in multiple countries worldwide, with available literature indicating that it is safe and well tolerated when used as a single-dose administration of 2 g for a variety of indications.[8] The supratherapeutic dose (6 g) was determined from safety results of a phase 1, randomized, placebo-controlled, single-blind study that assessed the PKs, safety, and tolerability of single oral administration of SYM-1219 granules containing either 4 g or 6 g of secnidazole.[16]

The study was performed on an inpatient basis at the clinic study site, with subjects admitted at least 12 hours before dosing on day 1 for each treatment period and remaining at the inpatient unit until completion of study assessments on day 2.

A single oral dose was administered in a fasted state (ie, after an overnight fast of at least 10 hours) on day 1 of each treatment period according to the randomization schedule, with a washout period of 8 ± 2 days between treatment periods. SYM-1219 and matching placebo were administered orally as granules sprinkled in 8 oz of applesauce for consumption by the subject within 5 minutes of preparation and followed

by 240 mL of water. Water was allowed ad libitum for up to 2 hours before dosing but was prohibited through 2 hours postdose except for that consumed with the dose. No food was allowed for 4 hours after SYM-1219 and placebo administration. Moxifloxacin was administered as an oral tablet and was followed by consumption of 8 oz of applesauce and 240 mL of water.

ECGs were recorded using a M12R ECG continuous 12-lead digital Holter recorder (Global Instrumentation, Manlius, NY). Data were stored on SD memory cards and were not reviewed until the time of analysis. All ECGs were read centrally (iCardiac Technologies, Inc, Rochester, New York) in a manner blinded to subject, visit, and treatment allocation, and using lead II as the primary analysis lead.

The central laboratory used TQT Plus® to extract 10-second digital ECG tracings from the protocol-specified ECG extraction windows. Ten 14-second replicate ECGs were extracted from the 5-minute window at the following time points: 45, 30, and 15 minutes before dosing and 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after dosing. Subjects remained in a supine position at rest from 10 minutes before to 5 minutes after each of the time points. ECG intervals were measured using the high-precision QT technique, which measures QT and RR intervals from up to 100 beats per timepoint.[17]

A mixed-effects model was used with change from baseline in Fridericia-corrected QTc (ΔQTcF) as the dependent variable; treatment, time, period, sequence, and time-by-treatment interaction as fixed factors; baseline QTc as a covariate; and subject within sequence as a random effect. The primary end point was ΔQTcF performed for each of the postdosing time points to determine whether the upper bound of the 2-sided 90%CI of the placebo-corrected ΔQTcF (ΔΔQTcF) exceeds 10 milliseconds; baseline was defined as the average of the measured QTc intervals from the 3 predose ECG time points for assessed dosing period. The same mixed-effects model used to determine assay sensitivity, controlled for multiplicity using the Hochberg procedure,[18] was applied to the primary analysis. Assay sensitivity was established by evaluating whether the least-squares mean difference between moxifloxacin and placebo of ΔQTcF (ΔΔQTcF) for at least 1 time point of 1, 2, and 3 hours postdose was significantly larger than 5 milliseconds on the 5% level, ie, the lower bound of the 2-sided 90%CI should be >5 milliseconds.

Outlier analysis was performed, and frequency tables were used to summarize the total counts and percentages for both the number of subjects and number of time points exceeding established threshold criteria. These thresholds included absolute QTcF values >500 milliseconds, >480 and ≤500 milliseconds, or >450 and ≤480 milliseconds, and increases in QTcF from baseline of 30-60 milliseconds as well as >60 milliseconds. Other secondary end points included descriptive statistics on the ECG interval parameters of HR, PR interval, QRS interval, T-wave morphology, and U-wave presence.

A secondary end point was to evaluate the pharmacodynamic relationship between the QTc interval and plasma concentrations of SYM-1219. Blood sampling was performed at predefined time points corresponding with the QTc sampling to correlate ECG findings with the PK profile. Blood samples (~4 mL per sample) were collected via direct venipuncture or by an indwelling catheter into tubes with K_2EDTA as anticoagulant at 6-10 minutes after each corresponding 12-lead Holter ECG time point, ie, 30 minutes before dosing and at 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after dosing. Samples were cooled to 0°C and processed within 60 minutes of collection by centrifugation at 1000-2000*g* for 10 minutes at 4°C to obtain the plasma, which was aliquoted into polypropylene tubes and stored at –20°C until analysis. Plasma secnidazole concentrations were determined by Celerion (Lincoln, Nebraska) using validated analytical procedures,[19] and standard PK parameters were estimated including maximum plasma concentration (C_{max}), time to C_{max}, and area under the concentration-time curve from 0 to 24 hours.

The relationship between plasma concentration levels of secnidazole following SYM-1219 administration and ΔΔQTcF was evaluated using a linear mixed-effects modeling approach. The following 3 linear models were considered: Model 1 was a linear model with an intercept; Model 2 was a linear model with mean intercept fixed to 0 (with variability); and Model 3 was a linear model with no intercept. Time-matched concentration was included in the model as a covariate and subject as a random effect for both intercept and slope, whenever applicable. The best model to fit the data was used to predict the population average ΔΔQTcF and its corresponding upper 95% 1-sided CI bound at the geometric mean maximum plasma concentrations for the therapeutic and supratherapeutic doses of SYM-1219. The plot of the observed quantile concentrations and associated mean ΔΔQTcF (90%CI) together with the mean (90%CI) predicted ΔΔQTcF was used to evaluate the adequacy of the model fit to the assumption of linearity and the impact on quantifying the concentration-response relationship.[20]

Safety and tolerability were evaluated based on the reporting of treatment-emergent adverse events (TEAEs) regardless of causality and the monitoring of vital signs and laboratory tests for abnormal values. TEAEs were assessed by study personnel using direct observation, spontaneous reporting, and nonspecific questioning of subjects. Safety ECGs were also performed to detect any immediate ECG effects.

All statistical analyses were performed using SAS Version 9.3 or higher (SAS Institute, Cary, North Carolina).

Results

Fifty-two subjects with a mean age of 45.0 years (range 20–65) and mean body mass index 26.9 kg/m^2 (range 19.9–32.0) were enrolled and randomized. These subjects were predominantly male (57.7%) and were white (86.5%) or black (13.5%), with the majority (96.2%) of Hispanic or Latino ethnicity. Of the 52 subjects, 47 completed the study. Among the 5 (9.6%) discontinuations, 2 subjects were discontinued for noncompliance with study procedures (unable to consume total dose); 1 subject withdrew consent, and another was lost to follow-up; and 1 subject was discontinued after the first treatment period due to a TEAE of atrial fibrillation that was considered not related to the study drug.

ECG parameters were well balanced at baseline for each of the treatment periods (data not shown). The time course and pattern of ΔQTcF on secnidazole followed that seen on placebo, and all mean values were negative or close to 0 after dosing with placebo and SYM-1219 2 g, and somewhat higher with SYM-1219 6 g (Figure 2A). Consequently, the placebo-corrected ΔQTcF was small for SYM-1219 2 g, with mean values <5 milliseconds at all time points (Figure 2B). After dosing with SYM-1219 6 g, mean placebo-corrected ΔQTcF reached 7.6 milliseconds (90%CI 5.5 to 9.7) 4 and 5 hours after dosing (Figure 2B, Table 1). The QT effect on moxifloxacin was clearly larger with a peak effect of 15.2 milliseconds (Figure 2B) and with the lower bound of the 90%CI >5 milliseconds at all prespecified time points (1, 2, and 3 hours; Table 1).

There were no subjects on any treatment who met the outlier criteria of absolute QTcF values >480 and ≤500 milliseconds, or >500 milliseconds, or ΔQTcF >60 milliseconds.

Table 2 presents the PK parameters for both SYM-1219 treatment groups and shows dose proportionality for C_{max} and area under the concentration-time curve for the first 24 hours, and a median time to C_{max} that was slightly longer for the 6-g dose group relative to the 2-g dose group (5.1 hours vs 4.1 hours).

A scatter plot of pairs of observations for placebo-corrected ΔQTcF and SYM-1219 plasma concentrations is shown in Figure 3A, and a goodness-of-fit plot in Figure 3B. The prespecified linear mixed-effects model with an intercept (Model 1) provided an acceptable fit to the data. The estimated slope of the exposure-response relationship was very shallow but statistically significant, 0.058 milliseconds per μg/mL (90%CI 0.042 to 0.073), with an intercept of 0.6 milliseconds. From this model, it can be predicted

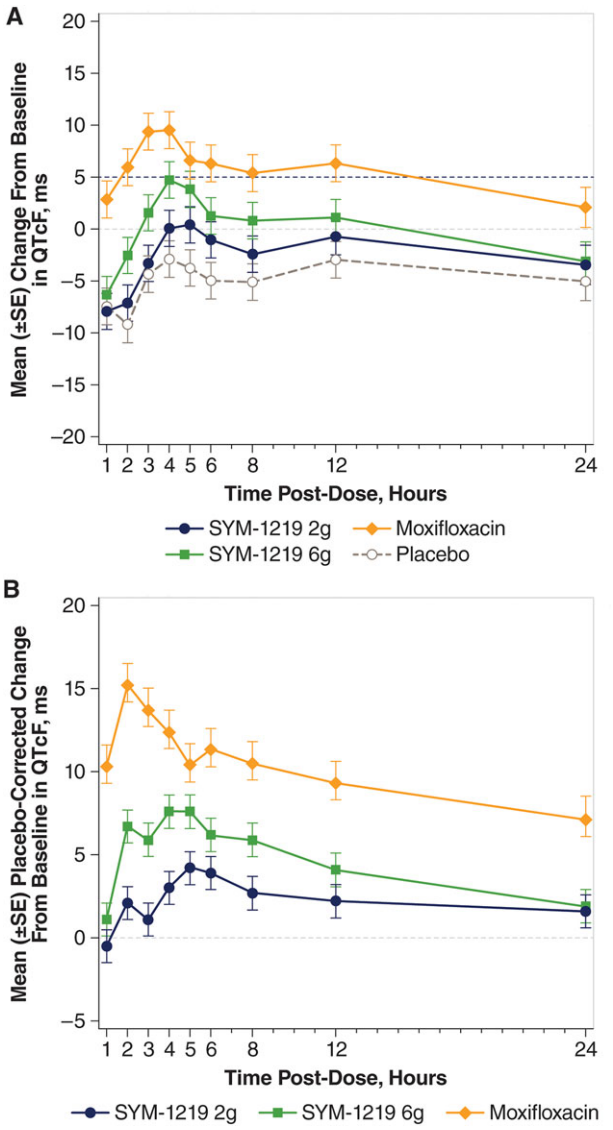

Figure 2. A, Change from baseline in QTcF (ΔQTcF, mean ± 90%CI; milliseconds) across treatments and time points postdosing. B, Placebo-corrected ΔQTcF for SYM-1219 2 g and 6 g, and 400 mg moxifloxacin. QTcF indicates QTc corrected for heart rate by the Fridericia method; ΔQTcF, change from baseline in Fridericia-corrected QTc.

that the QT effect (placebo-corrected ΔQTcF) will be below 10 milliseconds up to SYM-1219 plasma concentrations of ~125 μg/mL.

Single doses of SYM-1219 of 2 g and 6 g did not have a clinically relevant effect on HR, with all mean placebo-corrected change from baseline values within ±3 beats/min at all postdosing time points (data not shown). Similarly, the mean placebo-corrected change from baseline PR ranged between −1.7 milliseconds and 0.7 milliseconds after SYM-1219 2 g, and between –2.2 milliseconds and 1.1 milliseconds after SYM-1219 6 g; all mean placebo-corrected changes from baseline

Table 1. Placebo-Corrected Change From Baseline in QTcF (ΔΔQTcF) for SYM-1219, 2 g and 6 g, and 400 mg Moxifloxacin

	Least-Squares Mean (90%CI), ms		
Time Point Postdose (h)	SYM-1219, 2 g (n = 49)	SYM-1219, 6 g (n = 49)	Moxifloxacin, 400 mg (n = 47)
1	–0.5 (–2.6, 1.6)	1.1 (–0.9, 3.2)	10.3 (8.2, 12.4)
2	2.1 (–0.0, 4.1)	6.7 (4.6, 8.7)	15.2 (13.1, 17.3)
3	1.1 (–1.0, 3.1)	5.9 (3.8, 8.0)	13.7 (11.6, 15.8)
4	3.0 (0.9, 5.0)	7.6 (5.5, 9.7)	12.4 (10.3, 14.5)
5	4.2 (2.1, 6.2)	7.6 (5.5, 9.7)	10.4 (8.3, 12.5)
6	3.9 (1.9, 6.0)	6.2 (4.2, 8.3)	11.3 (9.2, 13.4)
8	2.7 (0.6, 4.8)	5.9 (3.8, 8.0)	10.5 (8.4, 12.6)
12	2.2 (0.2, 4.3)	4.1 (2.0, 6.1)	9.3 (7.2, 11.4)
24	1.6 (–0.7, 3.9)	1.9 (–0.3, 4.2)	7.1 (4.8, 9.4)

QTcF indicates QTc corrected for heart rate by the Fridericia method; ΔQTcF, change from baseline in Fridericia-corrected QTc; ΔΔQTcF, placebo-corrected ΔQTcF.

Table 2. Pharmacokinetic Parameters for SYM-1219 (0 to 12 Hours)

Pharmacokinetic Variable	SYM-1219, 2 g (n = 49)	SYM-1219, 6 g (n = 49)
C_{max}, μg/mL, mean ± SD (range)	38.33 ± 10.36 (20.7, 72.7)	113.08 ± 27.80 (70.7, 202)
T_{max}, h, median (range)	4.13 (3.12, 24.1)	5.12 (3.12, 8.13)
$AUC_{0\text{-}24}$, μg·h/mL, mean ± SD (range)	627.2 ± 142.1 (386.7, 877.5)	1982.6 ± 430.6 (1108, 2765)

$AUC_{0\text{-}24}$ indicates area under the concentration-time curve from 0 to 24 hours; C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration.

in the QRS interval were within ±1 millisecond in both SYM-1219 treatment periods.

In the safety analysis there were no deaths or serious TEAEs reported. Overall, a total of 41 TEAEs were reported among the 52 subjects. The incidence of TEAEs was higher after SYM-1219 administration than with placebo or moxifloxacin, although the proportion of subjects who reported TEAEs was similar between the 2 SYM-1219 doses (Table 3). The most common TEAEs (ie, occurring with an incidence ≥5% with any treatment) were headache, dizziness, and nausea, all of which were most frequent at the SYM-1219 6-g dose (Table 3). The investigator considered 23 events to be treatment related. The highest incidence of treatment-related TEAEs was reported at the supratherapeutic dose of SYM-1219, 6 g (20%), with a lower frequency (8%) in subjects receiving the therapeutic dose of SYM-1219, 2 g (Table 3). The single withdrawal due to a TEAE, a case of atrial fibrillation observed during the period 2 prescreen, occurred after completion of the SYM-1219 2-g treatment period and was deemed to be nonserious, moderate in intensity, and not related to the study drug. All TEAEs were graded by the investigator as mild to moderate in severity, with no TEAEs of severe intensity reported. Changes from baseline in all vital sign categories were similar in all treatment groups, and no notable differences were observed across treatments in changes for any of the laboratory parameters.

Discolored urine was observed in 23 of 49 subjects (46.9%) with administration of SYM-1219 6 g compared with 0% during other treatments; urine was green in 22 subjects and red in 1 subject. None of the urine abnormalities were reported as TEAEs, and all subjects with discolored urine had normal urine color at their subsequent visit. Further evaluation showed that although all subjects with green urine also had leukocyte esterase and nitrites present in their urine, only 1 subject may have had a urinary tract infection accounting for the positive urine leukocyte esterase and nitrate.

Discussion

The antimicrobial properties of secnidazole have long been recognized, and it is in widespread use in many countries for treatment of bacterial and parasitic conditions,[8] but its potential cardiac effects have not been determined. Development of SYM-1219, a new granule formulation containing 2 g of secnidazole for oral administration that is being evaluated as a single-dose treatment for BV, provided the opportunity to assess ECG parameters as part of its PK/pharmacodynamic profile. This TQT/QTc study of SYM-1219 is the first published analysis evaluating any formulation of secnidazole for its effects on cardiac physiology.

In the central tendency analysis of the ECG data, the SYM-1219 therapeutic dose of 2 g did not show any signals for threshold pharmacologic effects on cardiac repolarization, defined by 5 milliseconds and indicated by an upper bound of the 2-sided 90%CI around the mean effect on QTc of 10 milliseconds.

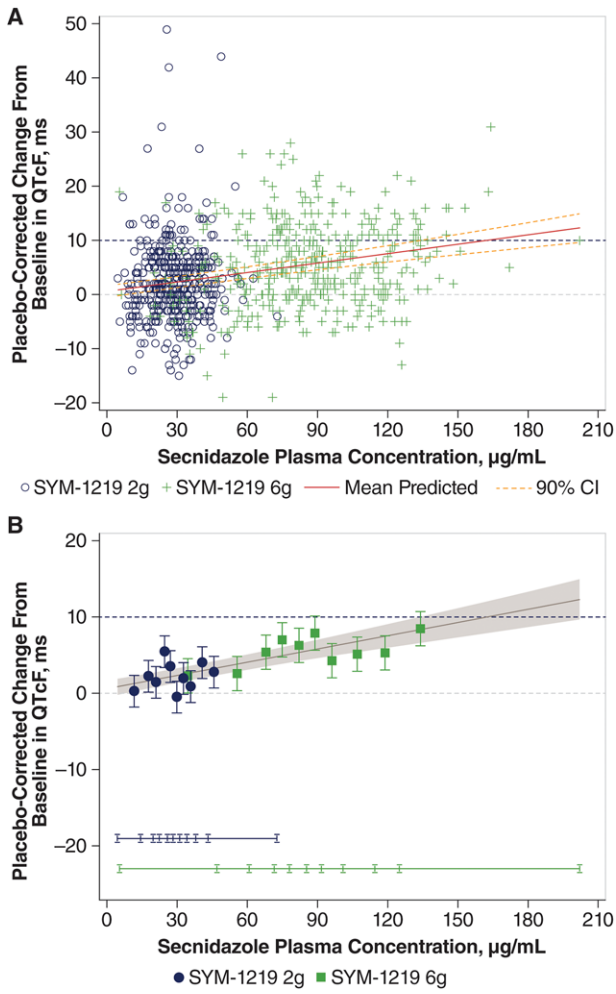

Figure 3. Exposure-response analysis. A, Scatter plot over pairs of observed secnidazole plasma concentrations vs placebo-corrected change from baseline in QTcF. B, Predicted mean (90%CI) of placebo-corrected change from baseline in QTcF across deciles of secnidazole plasma concentrations. Blue circles and green squares with vertical bars denote the observed mean placebo-corrected changes in QTcF from baseline at the median secnidazole plasma concentration within each decile after dosing with SYM-1219 2 g and SYM-1219 6 g, respectively. The horizontal blue and green lines with bars at the bottom show the range of concentrations divided into deciles for secnidazole after dosing with SYM-1219 2 g and SYM-1219 6 g, respectively. The solid gray line with gray shaded area denotes the model-predicted mean placebo-corrected change from baseline in QTcF with 90%CI. QTcF indicates QTc corrected for heart rate by the Fridericia method.

These results demonstrate the lack of clinically significant cardiac effects with respect to prolongation of the QTc interval. For SYM-1219 6 g, although the mean placebo-corrected change in QTcF was >5 milliseconds between 2 and 8 hours postdosing, with the highest value at 4 hours (7.6 milliseconds, 90%CI 5.5 to 9.7), an effect on QTcF >10 milliseconds can be excluded with this supratherapeutic dose. Furthermore, applying an exposure-response analysis to the linear model showed that a predicted QT effect exceeding 10 milliseconds (upper bound of the 2-sided 90%CI) can be excluded at secnidazole plasma concentrations up to ~125 μg/mL. At therapeutic dosing, the average maximum plasma concentration achieved is 44.5 μg/mL approximately 4 hours after dosing, with the long half-life of approximately 17 hours ensuring that plasma concentrations are maintained above the minimal inhibitory concentrations of BV-associated pathogens for longer than 72 hours.[9] Thus, the plasma concentrations obtained at the supratherapeutic dose used in the current study are more than 3-fold higher than the plasma concentrations at therapeutic dosing. A maximum plasma concentration exceeding therapeutic concentrations would also be unlikely in the clinical setting because secnidazole has no drug interactions via hepatic cytochrome pathways;[21] only 50% is excreted as unchanged drug via the renal route;[22] and SYM-1219 does not demonstrate a food effect (H. S. Pentikis, PhD, unpublished data, April 2017).

The absence of other alterations in ECG morphology supports the lack of a clinically meaningful effect on cardiac conduction at both doses of SYM-1219. Outlier analysis was consistent with these findings, and in the outlier analysis, no patients exceeded new thresholds of >480 and >500 milliseconds or increases ≥60 milliseconds; new QTcF >450 milliseconds and increases >30 milliseconds were generally similar between the SYM-1219 and placebo treatments.

These results are consistent with a recent analysis suggesting that SYM-1219 has minimal inhibitory effects on *hERG* potassium currents;[12] *hERG* encodes the major channel protein responsible for repolarization of the cardiac myocyte ventricular action potential.[23,24]

Both doses of SYM-1219 were safe and well tolerated. Although the overall incidence of TEAEs was higher after administration of SYM-1219 relative to placebo and moxifloxacin, all events were mild or moderate in severity. The most frequently reported events were headache, dizziness, and nausea, and these occurred with highest frequency in subjects receiving the supratherapeutic SYM-1219 6-g dose. Discolored urine, which mainly presented as a green color, was observed only in a substantial proportion of subjects receiving the supratherapeutic SYM-1219 6-g dose; discolored urine has long been recognized as a side effect of metronidazole[25–27] and is not likely to be of clinical significance. It should also be noted that the association of discolored urine with high-dose SYM-1219 was discovered after the study had been completed and was unblinded. Theoretically, this could have led to unblinding. However, the central ECG reader remained blinded until the ECGs had all been read. Thus, this individual was not aware of the discolored urine that was reported at the end of the study. Because the main objective of the study was to examine the effect of drug concentrations on ECG parameters, there was no

Table 3. Treatment-Emergent Adverse Events

Event	SYM-1219, 2 g (n = 50)	SYM-1219, 6 g (n = 50)	Placebo (n = 48)	Moxifloxacin, 400 mg (n = 47)
Incidence, n (%)				
Any TEAE	10 (20.0)	12 (24.0)	4 (8.3)	2 (4.3)
Serious TEAEs	0	0	0	0
Treatment-related TEAEs	4 (8.0)	10 (20.0)	1 (2.1)	1 (2.1)
Withdrawal due to TEAEs	1 (2.0)	0	0	0
Most common TEAEs[a]				
Headache	2 (4.0)	5 (10.0)	0	1 (2.1)
Dizziness	1 (2.0)	3 (6.0)	1 (2.1)	0
Nausea	0	3 (6.0)	0	0
Number of TEAEs				
Total TEAEs	11	22	6	2
Treatment-related TEAEs	4	17	1	1

TEAE indicates treatment-emergent adverse event.
[a]Occurring with a frequency ≥5% with any treatment.

unblinding of the independent reader, as this individual was separated from the clinical conduct of the study.

Conclusions

In conclusion, this TQT study demonstrated that SYM-1219 in doses and plasma concentrations up to 3-fold higher than therapeutically relevant levels does not have a clinically concerning effect on ECG parameters, including the QT interval, corresponding to a negative TQT study, as defined by FDA guidance.[13] Additionally, SYM-1219 was generally well tolerated even at the supratherapeutic dose.

Declaration of Conflicting Interests

Borje Darpo owns stock and is eligible for stock options in iCardiac Technologies, Inc. Hongqi Xue is an employee of and eligible for stock options in iCardiac Technologies, Inc. Nikki Adetoro is an employee and equity holder of Symbiomix Therapeutics, LLC. Barbara G. Matthews is an employee of BioDirect, Inc, and a consultant to Symbiomix Therapeutics, LLC. Helen S. Pentikis is a consultant and equity holder of Symbiomix Therapeutics, LLC.

Funding

Sponsorship for this study was funded by Symbiomix Therapeutics, LLC. The authors would like to acknowledge the significant contribution to this study of Carol Braun, MD, a Founder and former Chief Medical Officer of Symbiomix Therapeutics, LLC, who passed away in April 2016. The authors would also like to thank The Curry Rockefeller Group, LLC, for aid in manuscript preparation (funded by Symbiomix Therapeutics, LLC).

References

1. Koumans EH, Sternberg M, Bruce C, et al. The prevalence of bacterial vaginosis in the United States, 2001-2004; associations with symptoms, sexual behaviors, and reproductive health. *Sex Transm Dis.* 2007;34(11):864–869.
2. Centers for Disease Control and Prevention. Bacterial vaginosis - CDC fact sheet. Last updated February 16, 2017. https://www.cdc.gov/std/bv/stdfact-bacterial-vaginosis.htm. Accessed March 13, 2017.
3. Srinivasan S, Hoffman NG, Morgan MT, et al. Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS One*. 2012;7(6):e37818.
4. Onderdonk AB, Delaney ML, Fichorova RN. The human microbiome during bacterial vaginosis. *Clin Microbiol Rev*. 2016;29(2):223–238.
5. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines: bacterial vaginosis. Last updated June 4, 2015. https://www.cdc.gov/std/tg2015/bv.htm. Accessed March 13, 2017.
6. Bartley JB, Ferris DG, Allmond LM, Dickman ED, Dias JK, Lambert J. Personal digital assistants used to document compliance of bacterial vaginosis treatment. *Sex Transm Dis*. 2004;31(8):488–491.
7. Osterberg L, Blaschke T. Adherence to medication. *N Engl J Med*. 2005;353:487–497.
8. Gillis JC, Wiseman LR. Secnidazole. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic use in the management of protozoal infections and bacterial vaginosis. *Drugs*. 1996;51(4):621–638.
9. Pentikis HS, Adetoro N, Braun CJ. A phase 1 study to determine the single dose, safety and pharmacokinetics of SYM-1219 (secnidazole) in healthy female volunteers [abstract]. American Society for Clinical Pharmacology and Therapeutics (ASCPT); March 3-7, 2015; New Orleans, LA.
10. Hillier SL, Morgan FG, Walsbaum AS, et al. A phase 2 randomized, double-blind, placebo-controlled study to evaluate the effectiveness and safety of single, oral doses of SYM-1219, a granule formulation containing 1 and 2 gram doses of secnidazole, for the treatment of women with bacterial vaginosis [abstract]. Infectious Diseases Society for Obstetrics and Gynecology (IDSOG); August 6-8, 2015; Portland, OR.
11. Schwebke JR, Morgan FG, Koltun W, Pentikis HS, Adetoro N, Braun CJ. A phase 3 randomized, double-blind, placebo-controlled study to confirm the efficacy and safety of a single, oral dose of SYM-1219, a granule formulation containing 2 grams of secnidazole, for the treatment of bacterial vaginosis [abstract]. *Am J Obstet Gynecol*. 2016;215:821–822.
12. Darpo B, Adetoro N, Pentikis HS. The effect of SYM-1219 on hERG potassium channels *in vitro* and its implications on cardiac

function *in vivo*. Poster presented at the Annual Meeting of the American Society for Clinical Pharamacology and Therapeutics (ASCPT); March 15-18, 2017; Washington, DC.
13. US Food and Drug Administration. Guidance for industry. E14 clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. Last updated October 2005. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073153.pdf. Accessed March 12, 2017.
14. European Medicines Agency Committee for Medicinal Products for Human Use. ICH Topic E14. The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. Last updated November 2005. http://www.ema.europa.eu/docs/en_GB/document_library/Sciegntific_uideline/2009/09/WC500002879.pdf. Accessed March 12, 2017.
15. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2005. The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. Last updated May 12, 2005. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Guideline.pdf. Accessed March 12, 2017.
16. Symbiomix: Data on file. Study SYM-1219-104 clinical study report: a safety and tolerability study of a single dose of SYM-1219 containing 4 g or 6 g of secnidazole in healthy adult male and female volunteers; 2015.
17. Darpo B, Fossa AA, Couderc JP, et al. Improving the precision of QT measurements. *Cardiol J*. 2011;18(4):401–410.
18. Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika*. 1988;75(4):800–802.
19. Celerion: Data on file. Study CA18485-01. Bioanalysis of SYM-1219 in human plasma (K_2EDTA) in support of clinical protocol SYM-1219-105; April 21, 2016.
20. Tornoe CW, Garnett CE, Wang Y, Florian J, Li M, Gobburu JV. Creation of a knowledge management system for QT analyses. *J Clin Pharmacol*. 2011;51(7):1035–1042.
21. Lamp KC, Freeman CD, Klutman NE, Lacy MK. Pharmacokinetics and pharmacodynamics of the nitroimidazole antimicrobials. *Clin Pharmacokinet*. 1999;36(5):353–373.
22. Frydman A, Lemar M, Le Roux Y. A review of the pharmacokinetics of secnidazole in man, pp 12-26. In: Katz N, Willis A, eds. *Secnidazole: A New Approach in 5-Nitroimidazole Therapy*. In: Proceedings from the 16th International Congress of Chemotherapy; June 11-16, 1989; Jerusalem, Israel.
23. Sanguinetti M, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: hERG encodes the IKr potassium channel. *Cell*. 1995;81(2):299–307.
24. Sanguinetti MC, Keating MT. Role of delayed rectifier potassium channels in cardiac repolarisation and arrhythmias. *News Physiol Sci*. 1997;12(4):152–157.
25. Bruce TA. Dark urine related to metronidazole therapy. *JAMA*. 1971;218(12):1832.
26. Geeting GK, Roberts JR. Urine discoloration secondary to metronidazole. *Am J Emerg Med*. 2001;19(4):322.
27. Flagyl [prescribing information]. New York, NY: G.D. Searle LLC Division of Pfizer Inc; 2016.