Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Brannan SK, Sawchak S, Miller AC, Lieberman JA, Paul SM, Breier A. Muscarinic cholinergic receptor agonist and peripheral antagonist for schizophrenia. N Engl J Med 2021;384:717-26. DOI: 10.1056/NEJMoa2017015

Supplementary Appendix

Supplementary Files For: Brannan, SK, Sawchak, S, Miller, AC, et al., Muscarinic Cholinergic Receptor Agonist and Peripheral Antagonist for Schizophrenia

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Methods

Inclusion and Exclusion Criteria

Inclusion Criteria:

1. Participant is aged 18-60 years, inclusive, at screening.
2. Participant has a primary diagnosis of schizophrenia established by a comprehensive psychiatric evaluation based on the DSM-5 (American Psychiatric Association 2013) criteria and confirmed by Mini International Neuropsychiatric Interview for Schizophrenia and Psychotic Disorder Studies (MINI) version 7.0.2.
3. Participant is experiencing an acute exacerbation or relapse of symptoms, with onset less than 2 months before screening.
    a. The participant requires hospitalization for this acute exacerbation or relapse of symptoms.
    b. If already an inpatient at screening, has been hospitalized for less than 2 weeks for the current exacerbation at the time of screening.
4. Positive and Negative Syndrome Scale total score between 80 and 120, inclusive.
    a. Score of ≥ 4 (moderate or greater) for ≥ 2 of the following Positive Scale (P) items:
        i. Item 1 (P1; delusions)
        ii. Item 2 (P2; conceptual disorganization)
        iii. Item 3 (P3; hallucinatory behavior)
        iv. Item 6 (P6; suspiciousness/persecution)
5. There should not be a change (improvement) in PANSS total score between screening and baseline of more than 20%.
6. Participant will have been off lithium therapy for at least 2 weeks before baseline and free of all oral antipsychotic medications for at least 2 weeks before baseline.
7. Participants taking a depot antipsychotic could not have received a dose of medication for at least 1 and a half injection cycles before baseline (e.g., 3 or more weeks off for a 2-week cycle).
8. Participant is capable of providing informed consent.
    a. A signed informed consent form must be provided before any study assessments are performed.

b. Participant must be fluent (oral and written) in English to consent

9. Participant is willing and able to be confined to an inpatient setting for the study duration, follow instructions, and comply with the protocol requirements.
10. Participant has a CGI-S score of ≥ 4 at screening and baseline visits.
11. Body mass index (BMI) must be ≥ 18 and ≤ 40 kg/m^2.
12. Participant resides in a stable living situation and is anticipated to return to that same stable living situation after discharge, in the opinion of the investigator.
13. Both females of child-bearing potential and males with partners of child-bearing potential must be willing to use a double-barrier method of birth control (i.e., any double combination of male or female condom with spermicidal gel, diaphragm, sponge, or cervical cap with spermicidal gel) during the study and for 7 days after the last dose of study drug.
14. Participant has an identified reliable informant. An informant is needed at the screening and baseline visits as well as at the end of the study for relevant assessments. (Site staff may act as informant while the participant is an inpatient.) An informant may not be necessary if the participant has been the patient of the investigator for ≥ 1 year.

Exclusion Criteria

1. Any primary DSM-5 disorder other than schizophrenia within 12 months before screening (confirmed using MINI version 7.0.2 at screening).
2. History or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurologic, or oncologic disease or any other condition that, in the opinion of the investigator, would jeopardize the safety of the participant or the validity of the study results, to exclude participants with human immunodeficiency virus (HIV), cirrhosis, biliary duct abnormalities, hepatobiliary carcinoma, and/or active hepatic viral infections based on the liver function test results.
3. History or high risk of urinary retention, gastric retention, or narrow-angle glaucoma.
4. History of irritable bowel syndrome (with or without constipation) or serious constipation requiring treatment within the last 6 months.
5. Has a DSM-5 diagnosis of moderate to severe substance abuse disorder (except tobacco use disorder) within the 12 months before screening (confirmed using MINI version 7.0.2 at screening), or current abuse as determined by urine toxicology screen or alcohol test. A screening participant with mild substance abuse disorder within the 12 months before screening must be discussed and agreed upon with the medical monitor before

he/she can be allowed into the study. Use of cannabis at screening will result in screen failure with the allowance to rescreen at a later date if no moderate to severe substance use disorder is determined.

6. Risk for suicidal behavior during the study as determined by the investigator's clinical assessment and Columbia Suicide Severity Rating Scale (C-SSRS) as confirmed by the following:

    a. Answers "Yes" on items 4 or 5 (C-SSRS – ideation) with the most recent episode occurring within the 2 months before screening, or answers "Yes" to any of the 5 items (C-SSRS-behavior) with an episode occurring within the 12 months before screening. Nonsuicidal self-injurious behavior is not exclusionary.

7. Clinically significant abnormal finding on the physical examination, medical history, ECG, or clinical laboratory results at screening.

8. Participants cannot currently (within 2 weeks of baseline) be receiving oral antipsychotic medications, MAO inhibitors, anticonvulsants (e.g., lamotrigine, Depakote), tricyclic antidepressants (e.g., imipramine, desipramine), selective serotonin reuptake inhibitors, or any other psychoactive medications except for as needed anxiolytics (e.g., lorazepam, chloral hydrate).

9. Pregnant, lactating, or less than 3 months postpartum. Sperm donation is not allowed for 90 days after the final dose of study drug.

10. If, in the opinion of the investigator (and/or Sponsor), participant is unsuitable for enrollment in the study or participant has any finding that, in the view of the investigator (and/or Sponsor), may compromise the safety of the participant or affect their ability to adhere to the protocol visit schedule or fulfill visit requirements.

11. Participant has had psychiatric hospitalization(s) for more than 30 days (cumulative) during the 90 days before screening.

12. Participant has a history of treatment resistance to schizophrenia medications defined as failure to respond to 2 adequate courses of pharmacotherapy (a minimum of 4 weeks at an adequate dose per the label) or required clozapine within the last 12 months.

13. Risk of violent or destructive behavior.

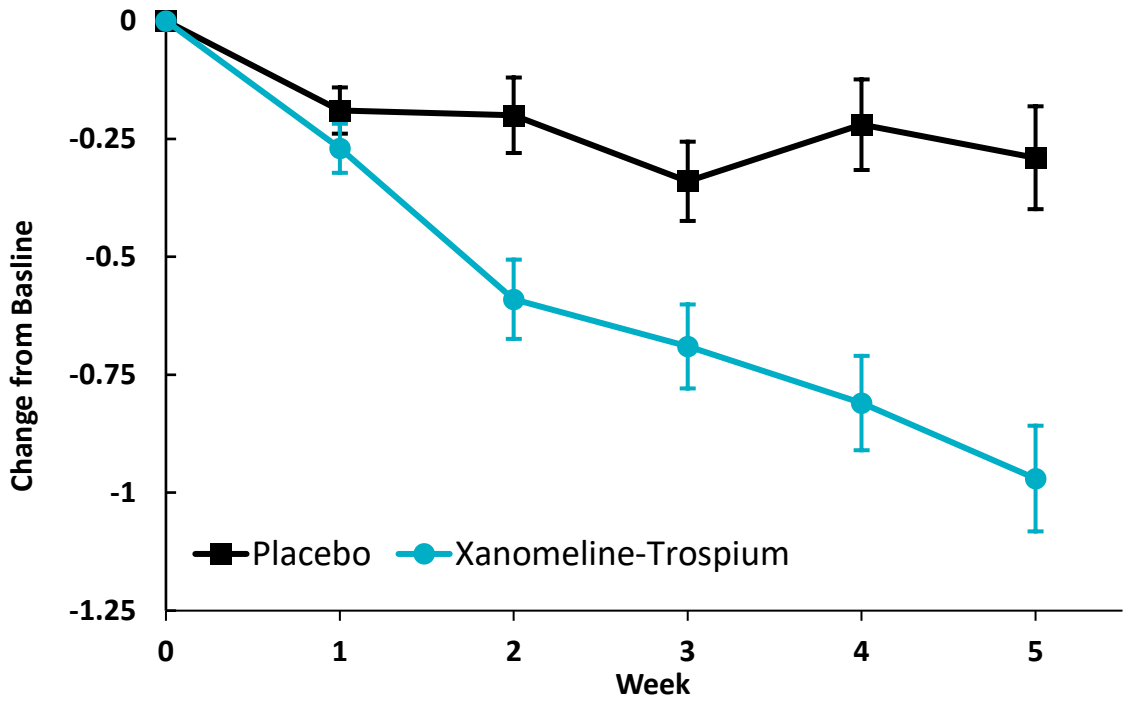
14. Current involuntary hospitalization or incarceration.

15. Participation in another clinical study in which the participant received an experimental or investigational drug agent within 3 months of screening.

Dosing Schedule

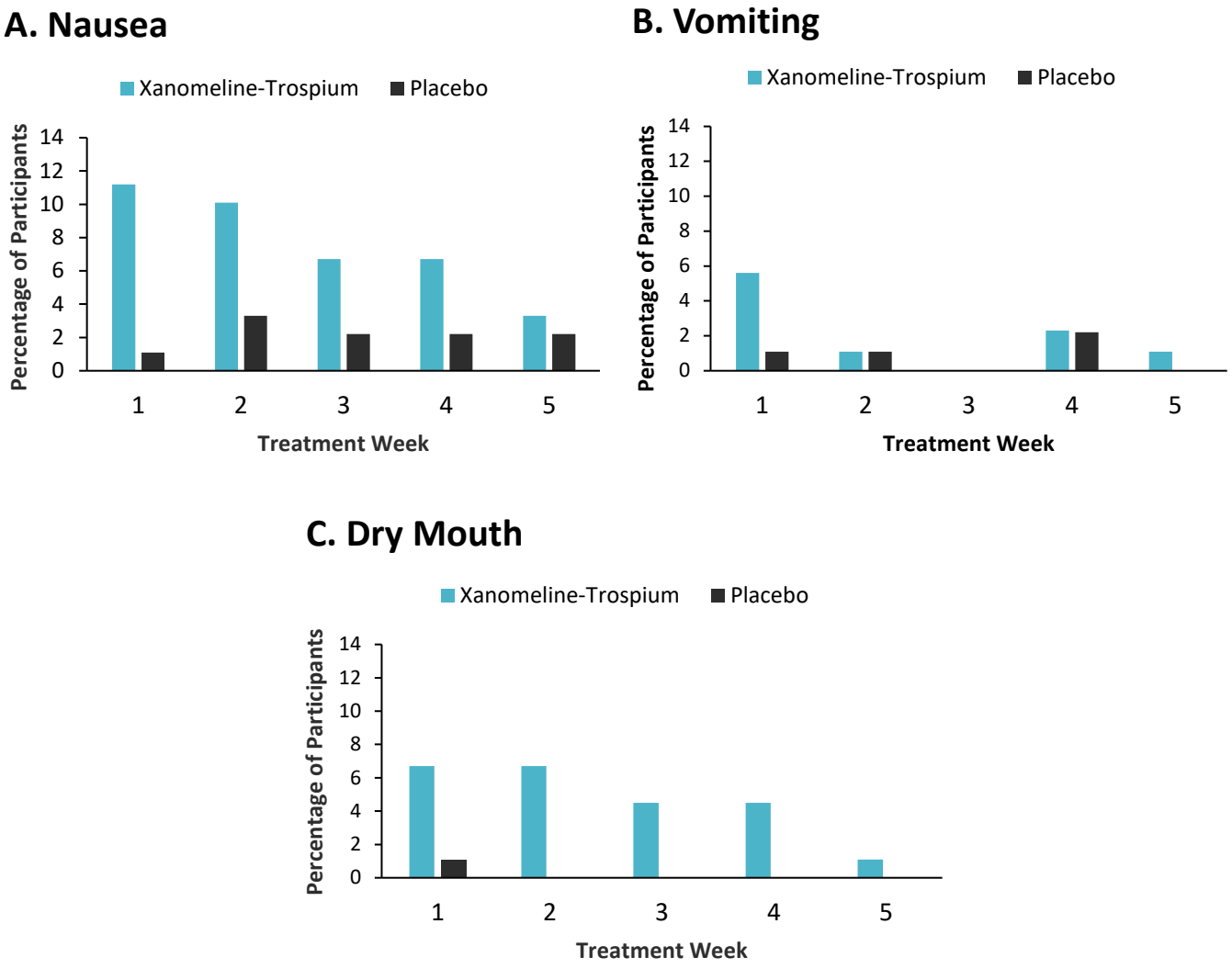
Xanomeline-trospium was initiated at a dose of 50 mg xanomeline/trospium 20 mg BID for the first 2 days, followed by xanomeline 100 mg/trospium 20 mg BID for the remainder of week 1 (Days 3 to 7). On Day 8 (week 2), dosing was titrated upwards to xanomeline 125 mg/trospium 30 mg BID, unless the participant experienced adverse events from their previous dose. The treating physician made all dose titration decisions. All participants who increased to xanomeline 125 mg/trospium 30 mg had the option to return to xanomeline 100 mg/trospium 20 mg BID, depending on tolerability, until day 21. No dose changes were allowed during the last 2 weeks of the trial.

Figure S1. *Post hoc* Analysis of Least-squares Mean Changes from Baseline in CGI-S Score



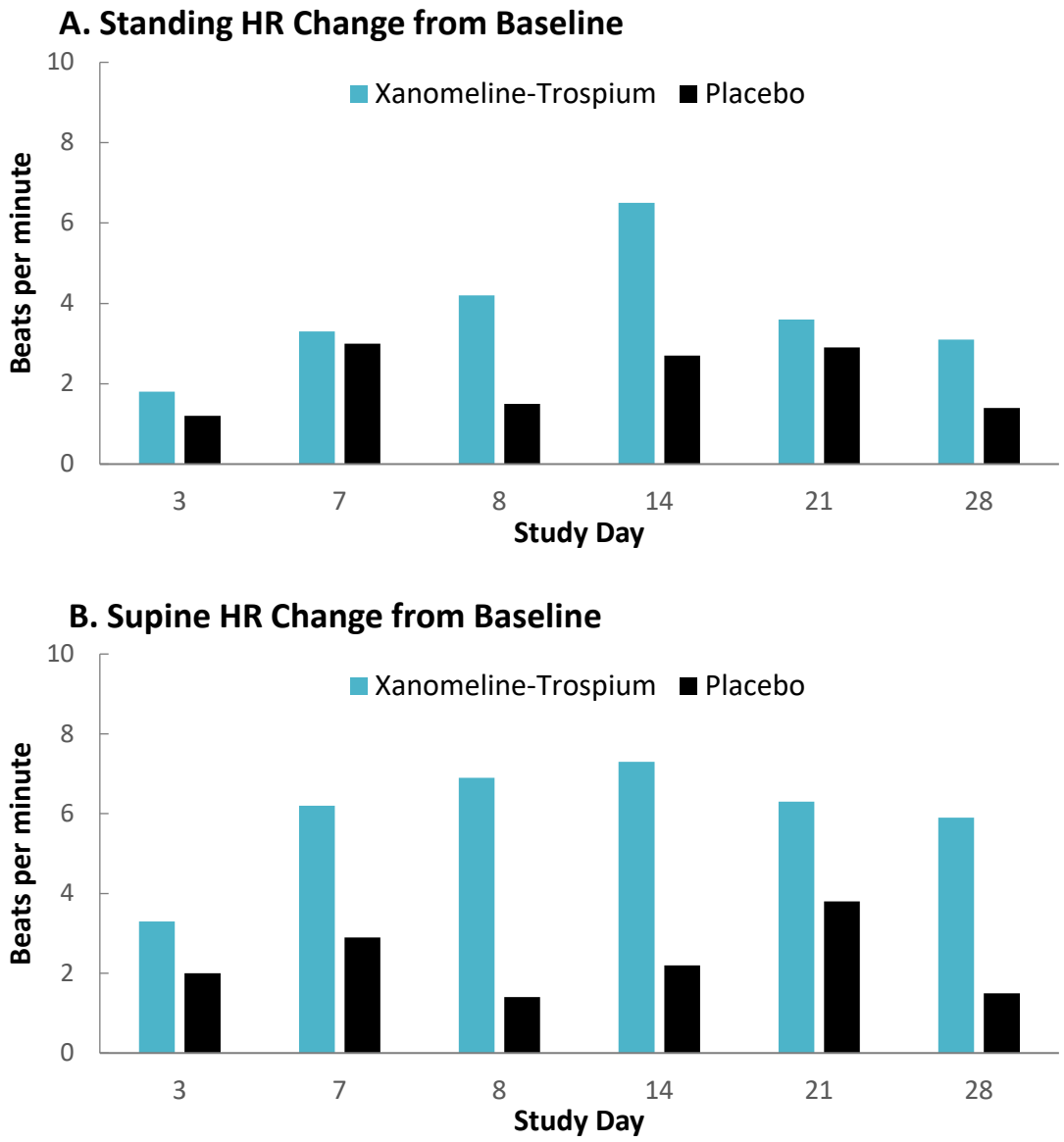
Least-squares mean change in CGI-S score[1] from baseline in xanomeline-trospium and placebo as function of treatment week. In a *post hoc* analysis, the least-squares mean change from baseline in CGI-S scores (± SE) for xanomeline-trospium compared to placebo at week 5 were -1.0 ± 0.1 vs -0.3 ± 0.1, corresponding to a least-squares mean difference (95% CI) of -0.7 points (-1.0 to -0.4). The modified intention-to-treat (mITT) population comprised all participants who underwent randomization and received at least one end point assessment. The mixed model for repeated measures (MMRM) included the treatment group (xanomeline-trospium or placebo), visit, and the interaction between the treatment group and visit as fixed factors. Site, age, gender, and baseline CGI-S total score were covariates in the model. CGI-S scores range from 1 to 7; higher scores reflect greater severity.

Figure S2. Percent of Participants with Nausea, Vomiting, and Dry Mouth as a Function of Treatment Week



Rates of nausea, vomiting, and dry mouth decreased over the course of the 5-week trial. Blue bars represent the percentage of participants treated with xanomeline-trospium and black bars represent the percentage of participants treated with placebo who experienced (**A**) nausea, (**B**) vomiting, or (**C**) dry mouth as a function of treatment week. Rates of constipation remained essentially constant over the course of the trial. To obtain values for percentage of participants, the number of participants with at least one recorded adverse event of each type listed above was summed for each week of the trial and divided by the number of participants in each respective arm of the safety population.

Figure S3. Mean Change from Baseline in Supine and Standing Heart Rate as a Function of Study Day



Changes from baseline in standing (A) and supine (B) heart rate as a function of study day. The difference in change from baseline between treatment groups peaked at 3.8 bpm on day 14 for standing HR (6.5 bpm in the xanomeline-trospium group vs. 2.7 bpm in the placebo group) and at 5.5 bpm on day 8 for supine HR (6.9 bpm in the xanomeline-trospium group vs. 1.4 bpm in the placebo group).

Abbreviations: HR= heart rate.

Table S1. Independent Institutional Review Board (IRB)

The following central institutional review board (IRB) was used by all investigators.

IRB	Committee Chair
Copernicus Group 5000 Centregreen Way STE 200 Cary, NC 27513	Glenn Veit, JD, CIP

Table S2. Least-Squares Mean Change in PANSS Total Score from Baseline to Week 5 (Sensitivity Analyses) *

Efficacy Measure	Least-Squares Mean Change from Baseline to Week 5		Least-Square Mean Difference (95% CI)	P-value
	Xanomeline-trospium	Placebo		
ITT Completer Analysis	N=72	N=73		
	-18.7 ± 1.8	-6.9 ± 1.7	-11.8 (-16.5 to -7.2)	P<0.001
Multiple Imputation Assuming Missing at Random	N=83	N=87		
	-17.6 ± 1.8	-5.5 ± 1.7	-12.1 (-16.6 to -7.6)	P<0.001
Multiple Imputation Assuming Not at Random	N=83	N=87		
	-17.2 ± 1.7	-5.9 ± 1.7	-11.3 (-15.8 to -6.9)	P<0.001

*Plus-minus values are means ± SE. Abbreviations: N=Number of participants. The total score on the Positive and Negative Syndrome Scale (PANSS)[2] ranges from 30 to 210; higher scores indicate greater severity of psychotic symptoms. Statistics are from a mixed model for repeated measures (MMRM) analysis, including the observed change from baseline PANSS total score at week 2, week 4, and week 5 as the response. The model included the treatment group (xanomeline-trospium or placebo), visit, and the interaction between the treatment group and visit as fixed factors, and baseline PANSS total score, site, age, and gender as covariates. Sensitivity analyses shown here were conducted using multiple imputation assuming data missing at random and multiple imputation assuming data not missing at random. The Multiple Imputation approaches replaced each missing value with a set of plausible values (random or not random) that represented the uncertainty about the right value to impute. Each of these analyses confirmed the primary analysis and had a P value <0.001.

Table S3. Longitudinal Cumulative Logit Regression Model of CGI-S Frequency Counts: Week 5 Result (mITT Population)

Time point	Odds Ratio- One Category Increase in CGI-S Score	95% Confidence Interval
Week 5	0.067	0.022-0.202

The odds ratio comparing a one-category increase in CGI-S score[1] for xanomeline-trospium versus placebo at week 5 favored xanomeline-trospium treatment, a result that was consistent with the primary ordinal categorical CGI-S frequency count analysis that employed the Mann-Whitney Wilcoxon test. The longitudinal cumulative logit regression model was fit to model an outcome of CGI-S score post-baseline, adjusted for baseline CGI-S value, visit, treatment arm, and visit-by-treatment arm interaction. CGI-S scores range from 1 to 7; higher scores reflect greater severity.

Table S4. Percentage of Participants with >7% Increase in Body Weight and Mean Change in BMI from Baseline to Week 5 (Safety Population) *

	Xanomeline-Trospium (N=89)	**Placebo (N=90)**
Participants with >7% increase in weight at Week 5 —no. (%)	2 (2.2)	5 (5.6)
Mean Change in BMI from baseline to Week 5—kg/m^2	0.5 ± 1.0	0.4 ± 1.2

*Plus-minus values are means ± SD.

Table S5. Number and Percent of Participants with Treatment-Emergent Increases in Liver Function Test Values

Analyte	**Xanomeline-Trospium (N=89)**	**Placebo (N=90)**
	No. (%)	**No. (%)**
ALT > 3X ULN	0	1 (1.1)
AST > 3X ULN	0	1 (1.1)
GGT > 2X ULN	2 (2.2)	1 (1.1)
ALP > 2X ULN	0	0
TB > 2X ULN	0	0

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyltransferase; ALP = alkaline phosphatase; TB=total bilirubin; ULN = upper limit of normal

Table S6. Mean Change Blood Pressure from Baseline to Week 5 *

Blood Pressure Measurement (mmHg)		Xanomeline-Trospium (N=89)	Placebo(N=90)
Orthostatic	Systolic	-0.4 ± 11.9	-1.0 ± 11.3
	Diastolic	-0.9 ± 7.8	-1.3 ± 10.4
Supine	Systolic	-3.9 ± 14.5	-0.2 ± 12.3
	Diastolic	-1.4 ± 9.3	0.5 ± 10.3
Standing	Systolic	-4.3 ± 14.8	-1.2 ± 12.0
	Diastolic	-2.3 ± 9.6	-0.8 ± 8.3

*Plus-minus values are means ± SD. Abbreviations: mmHg=millimeters of mercury

Table S7. QT Interval Data *

	Xanomeline-Trospium (N=89)			**Placebo (N=90)**		
QT Interval Measurement (msec)	Baseline	Week 5	Mean CFB to Week 5	Baseline	Week 5	Mean CFB to Week 5
Aggregate	376.1 ± 32.6	355.0 ± 24.9	-20.7 ± 33.5	377.8 ± 31.0	367.8 ± 24.4	-9.6 ± 32.4
QTcF	395.6 ± 22.7	392.3 ± 18.5	-2.7 ± 22.0	400.3 ± 18.1	396.6 ± 17.9	-3.8 ± 17.5

* No participants in either arm experienced a QTcF interval greater than 450 msec or an increase in the QTcF interval of more than 60 msec. Plus-minus values are means ± SD. Abbreviations: CFB=change from baseline; msec=milliseconds; SD=standard deviation; Min=minimum; Max=maximum

References

1. Haro JM, Kamath SA, Ochoa S, et al. The Clinical Global Impression-Schizophrenia scale: a simple instrument to measure the diversity of symptoms present in schizophrenia. Acta Psychiatr Scand Suppl 2003:16-23.
2. Kay SR, Opler LA, Lindenmayer JP. The Positive and Negative Syndrome Scale (PANSS): rationale and standardisation. Br J Psychiatry Suppl 1989:59-67.