

Concentration-QTc and cardiac safety analysis of single and multiple
zavegepant nasal spray doses in healthy participants to support approval

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Table S1. Concentration-QTc analysis of zavegepant and associated $\Delta\Delta\text{QTcF}$ prolongation (SAD and MAD studies pooled) (PK/QTc analysis population)

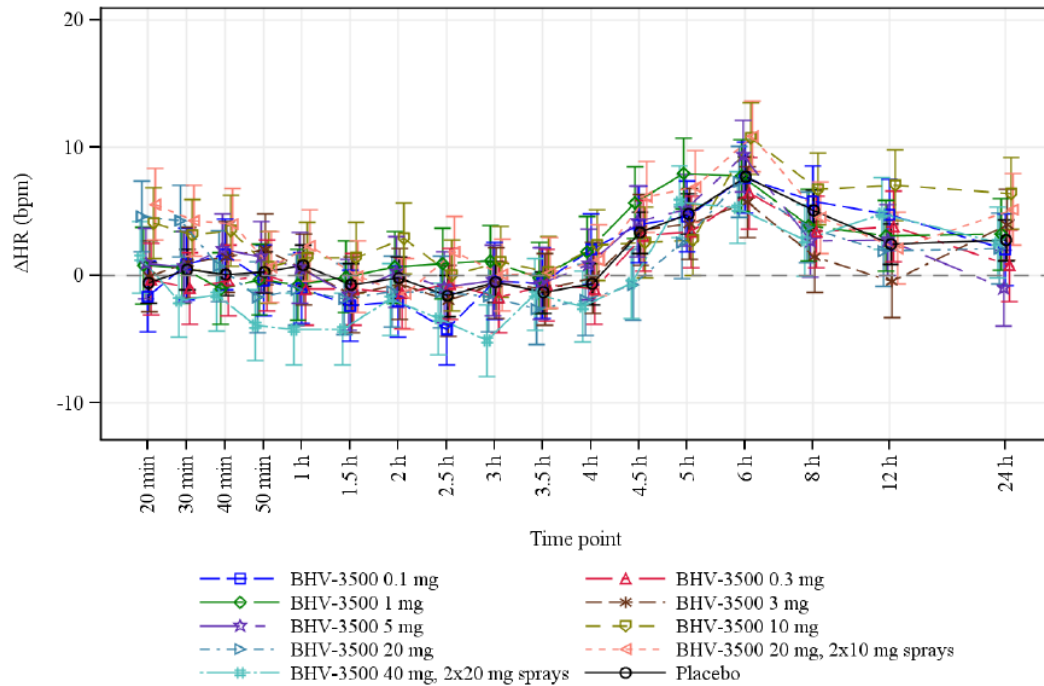
Parameter	Estimate	SE	df	t-Value	P Value	90% CI
Treatment effect (ms)	0.99	1.1783	116.7	0.84	0.4012	-0.961, 2.946
Zavegepant slope (ms per ng/mL)	-0.053	0.0246	22.2	-2.16	0.0415	-0.0955, -0.0110
Centered baseline effect (ms)	-0.056	0.0235	123.3	-2.37	0.0191	-0.0949, -0.0169
Day 1 post-dose effect						
Hours post dose	Estimate (ms)	SE	df	t-Value	P Value	90% CI
0.33	-0.49	1.1147	168.0	-0.44	0.6613	-2.333, 1.355
0.5	-1.82	1.1177	169.9	-1.63	0.1048	-3.671, 0.026
0.67	-2.11	1.1265	175.3	-1.88	0.0622	-3.977, -0.252
0.83	-2.46	1.1142	168.1	-2.20	0.0288	-4.300, -0.614
1	-1.71	1.1109	166.3	-1.54	0.1265	-3.544, 0.131
1.5	-1.10	1.1110	166.3	-0.99	0.3248	-2.935, 0.741
2	-1.77	1.1198	171.6	-1.58	0.1154	-3.624, 0.080
2.5	-1.79	1.1086	165.0	-1.61	0.1086	-3.622, 0.046
3	-2.20	1.1092	165.3	-1.99	0.0488	-4.037, -0.367
3.5	-1.78	1.1087	165.0	-1.60	0.1105	-3.613, 0.055
4	-0.87	1.1090	165.1	-0.78	0.4337	-2.705, 0.964
4.5	0.0039	1.1100	165.7	0.00	0.9972	-1.83216, 1.83993
5	-1.47	1.1096	165.4	-1.33	0.1866	-3.307, 0.364
6	-1.87	1.1101	165.6	-1.69	0.0935	-3.709, -0.037
8	-5.87	1.1107	165.9	-5.28	< 0.0001	-7.707, -4.032
12	-2.97	1.1112	166.2	-2.67	0.0083	-4.806, -1.130
24	-4.70	1.1188	170.8	-4.20	< 0.0001	-6.554, -2.854
Day 8 post-dose effect						
Hours post dose	Estimate (ms)	SE	df	t-Value	P Value	90% CI
-0.75	-9.46	1.8381	976.0	-5.15	< 0.0001	-12.486, -6.433
0.33	-5.55	1.8704	1051.5	-2.97	0.0031	-8.633, -2.474
0.5	-7.22	1.8187	973.6	-3.97	< 0.0001	-10.218, -4.229
0.83	-6.47	1.8186	971.3	-3.56	0.0004	-9.465, -3.477
1	-7.40	1.8179	967.2	-4.07	< 0.0001	-10.389, -4.403
1.5	-5.95	1.8197	965.9	-3.27	0.0011	-8.949, -2.957
2.5	-7.32	1.8227	981.9	-4.02	< 0.0001	-10.321, -4.319
3	-6.26	1.8197	971.6	-3.44	0.0006	-9.255, -3.263
3.5	-6.34	1.8176	964.9	-3.49	0.0005	-9.331, -3.346
4	-5.60	1.8182	963.1	-3.08	0.0021	-8.594, -2.607
4.5	-5.61	1.8204	963.1	-3.08	0.0021	-8.609, -2.615
5	-4.84	1.8219	963.9	-2.66	0.0080	-7.840, -1.840
6	2.37	1.8250	966.1	1.30	0.1951	-0.638, 5.371
8	-5.59	1.8311	970.7	-3.05	0.0023	-8.604, -2.574
12	-3.97	1.8349	973.8	-2.16	0.0307	-6.991, -0.949
24	-6.19	1.8385	976.1	-3.37	0.0008	-9.217, -3.164

Supplementary Information

Parameter	Estimate	SE	df	t-Value	P Value	90% CI
Day 14 post-dose						
Hours post dose	Estimate (ms)	SE	df	t-Value	P Value	90% CI
-0.75	-7.40	1.3473	348.9	-5.49	< 0.0001	-9.620, -5.176
0.33	-3.27	1.3389	341.7	-2.44	0.0150	-5.480, -1.064
0.5	-4.74	1.3460	348.2	-3.52	0.0005	-6.963, -2.523
0.67	-4.33	1.3460	348.6	-3.21	0.0014	-6.546, -2.106
0.83	-5.12	1.3422	345.7	-3.81	0.0002	-7.334, -2.907
1	-4.28	1.3380	342.1	-3.20	0.0015	-6.482, -2.068
1.5	-3.62	1.3344	338.6	-2.71	0.0071	-5.818, -1.417
2	-4.33	1.3336	337.6	-3.25	0.0013	-6.530, -2.131
2.5	-3.84	1.3338	337.5	-2.88	0.0042	-6.044, -1.644
3	-4.03	1.3344	337.8	-3.02	0.0027	-6.231, -1.829
3.5	-4.70	1.3349	338.1	-3.52	0.0005	-6.897, -2.494
4	-2.89	1.3354	338.4	-2.16	0.0312	-5.092, -0.687
4.5	-2.59	1.3359	338.7	-1.94	0.0531	-4.796, -0.390
5	-2.46	1.3363	339.0	-1.84	0.0663	-4.666, -0.258
6	-0.55	1.3370	339.5	-0.41	0.6835	-2.751, 1.660
8	-4.67	1.3379	340.1	-3.49	0.0005	-6.874, -2.461
12	-3.22	1.3386	340.6	-2.41	0.0166	-5.430, -1.014
24	-7.95	1.3569	358.4	-5.86	< 0.0001	-10.192, -5.717
<p>Based on a linear mixed-effects model with ΔQTcF as the dependent variable, time-matched zavegepant plasma concentration as an explanatory variate, centered baseline QTcF as an additional covariate, treatment (active = 1 or placebo = 0) and time as fixed effects, and a random intercept and slope per subject. ΔQTcF, change from baseline in corrected QT interval using Fridericia's formula; $\Delta\Delta$QTcF, placebo-corrected change-from-baseline in QT interval using Fridericia's formula. CI, confidence interval; df, degrees of freedom; MAD, multiple-ascending dose; PK, pharmacokinetics; SAD, single-ascending dose; SE, standard error.</p>						

Figure S1. Δ HR by time, dose, and study

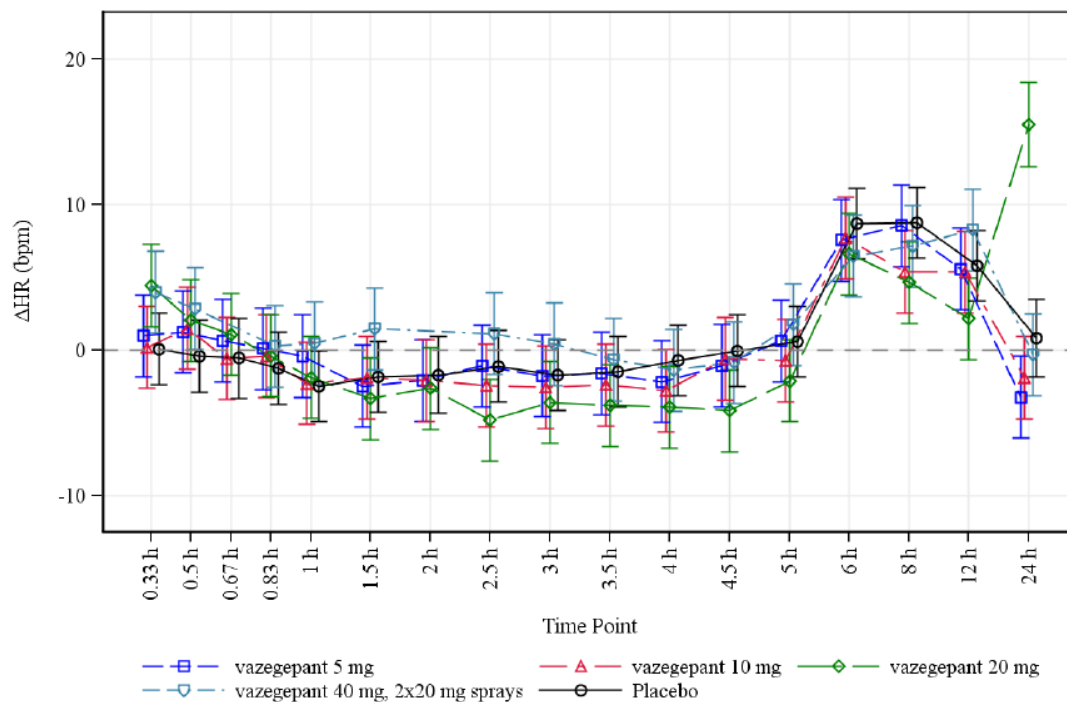
A. LS mean Δ HR across time points (SAD study QT/QTc analysis population)



Notes: LS mean and 90% CI based on a linear mixed-effects model.

Δ HR, change-from-baseline heart rate; BHV-3500, zavegepant; bpm, beats per minute; CI, confidence interval; LS, least squares; QTc, corrected QT interval; SAD, single-ascending dose.

B. LS mean Δ HR across time points on Day 1 (MAD study QT/QTc analysis population)

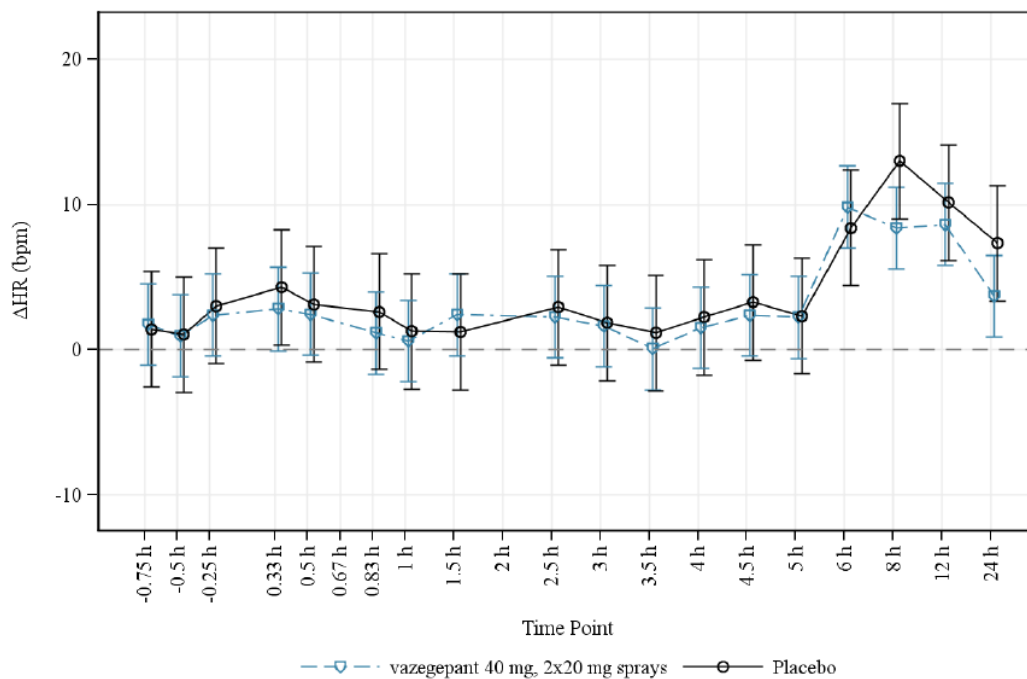


Notes: LS mean and 90% CI based on a linear mixed-effects model.

Δ HR = Time+Treatment+Time*Treatment+Baseline HR. A compound symmetry covariance structure was used to specify the repeated measures (time within subject).

Δ HR, change-from-baseline heart rate; CI, confidence interval; LS, least squares; MAD, multiple-ascending dose; QTc, corrected QT interval; vazegepant, vazegepant.

C. LS mean Δ HR across time points on Day 8 (MAD study QT/QTc analysis population)

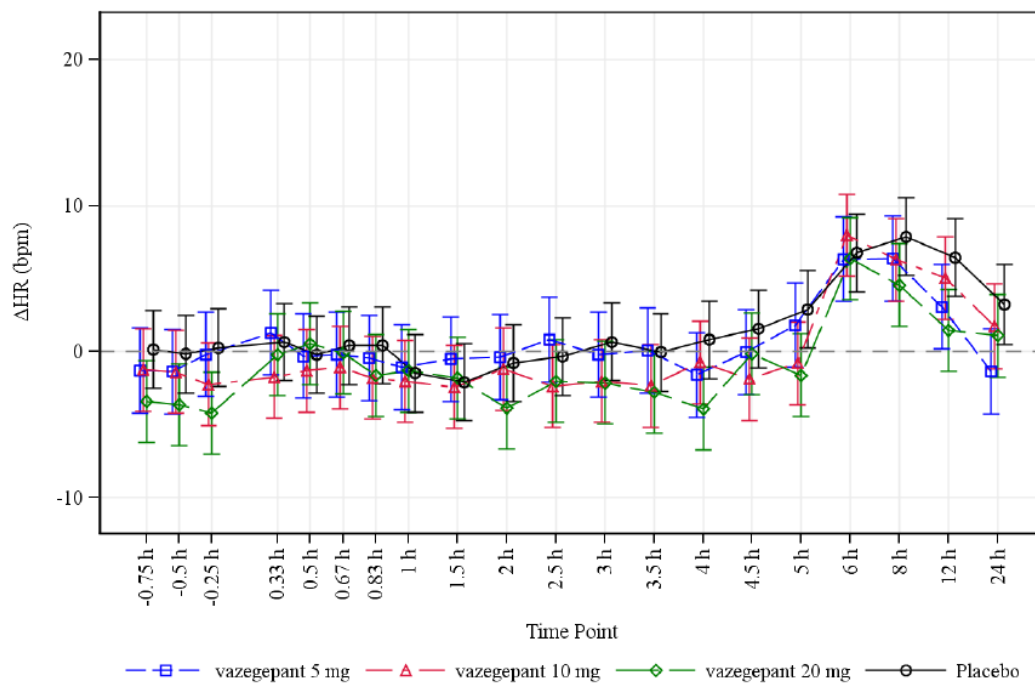


Notes: LS mean and 90% CI based on a linear mixed-effects model.

Δ HR = Time+Treatment+Time**Treatment*+Baseline HR. A compound symmetry covariance structure was used to specify the repeated measures (time within subject).

Δ HR, change-from-baseline heart rate; CI, confidence interval, LS, least squares; MAD, multiple-ascending dose; QTc, corrected QT interval; vazegepant, zavegepant.

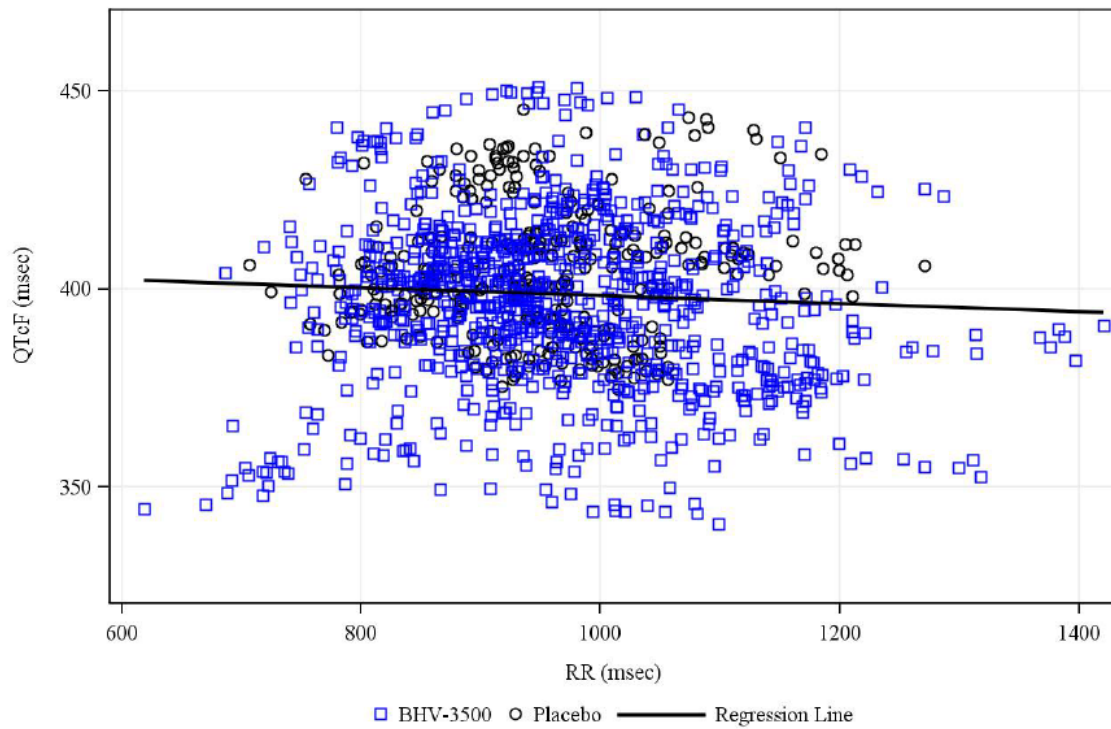
D. LS mean Δ HR across time points on Day 8 (MAD study QT/QTc analysis population)



Notes: LS mean and 90% CI based on a linear mixed-effects model.
 Δ HR = Time+Treatment+Time*Treatment+Baseline HR. A compound symmetry covariance structure was used to specify the repeated measures (time within subject).
 Δ HR, change-from-baseline heart rate; CI, confidence interval; LS, least squares; MAD, multiple-ascending dose; QTc, corrected QT interval; vazegepant, zavegepant.

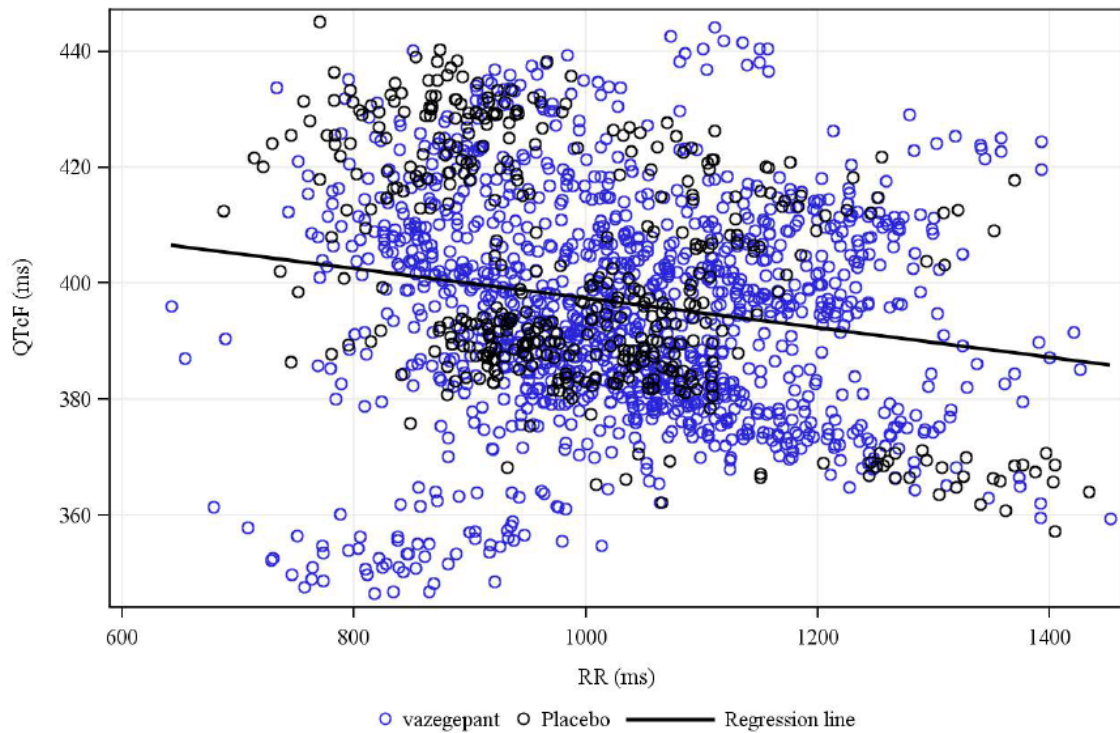
Figure S2. Scatter plots of QTcF and RR by treatment (QT/QTc population)

A. SAD study



Notes: The solid black line denotes the simple linear regression line across all pairs of (RR, QTcF) based on the equation $QTcF = 408.27 + -0.010 \cdot RR$
BHV-3500, zavegepant; QTc, corrected QT interval; QTcF, QT interval Fridericia's correction; SAD, single-ascending dose.

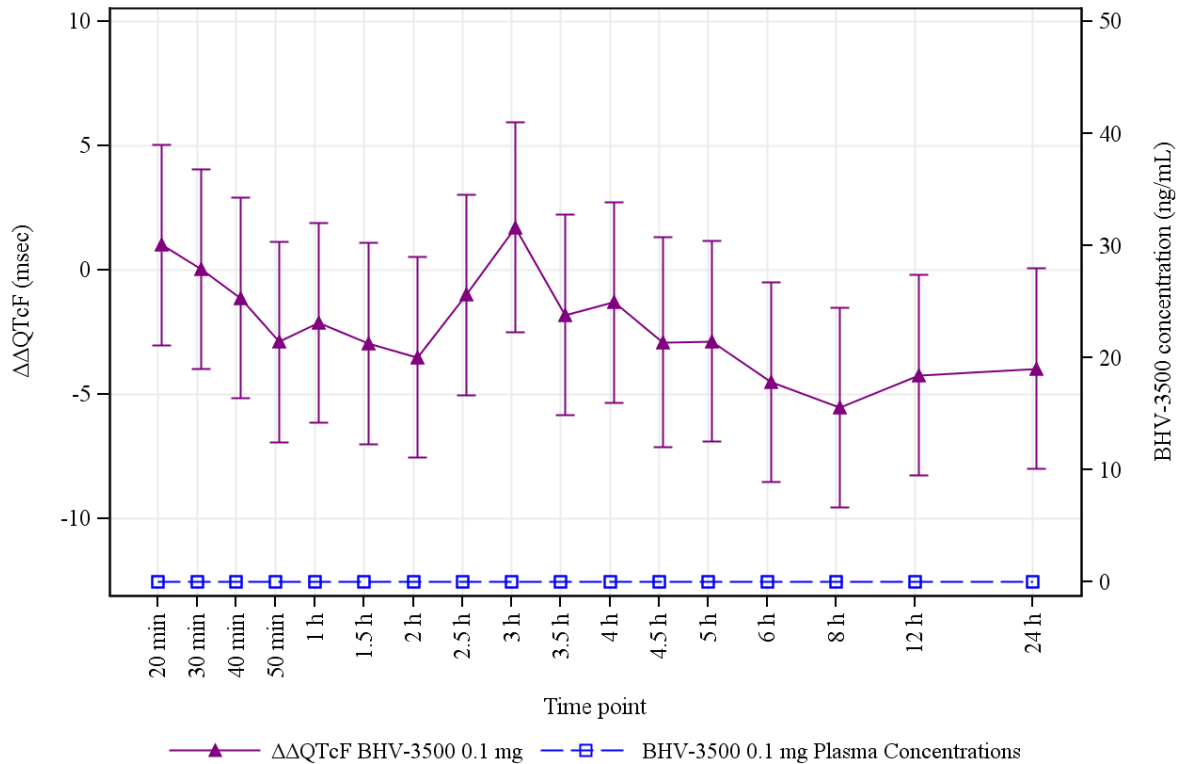
B. MAD study



Notes: The solid black line denotes the simple linear regression line across all pairs of (RR, QTcF) based on the equation $QTcF=422.89 + -0.026*RR$ with a P value of <0.0001 for the slope. BHV-3500, vazegepant; MAD, multiple-ascending dose; QTc, corrected QT interval; QTcF, QT interval Fridericia's correction.

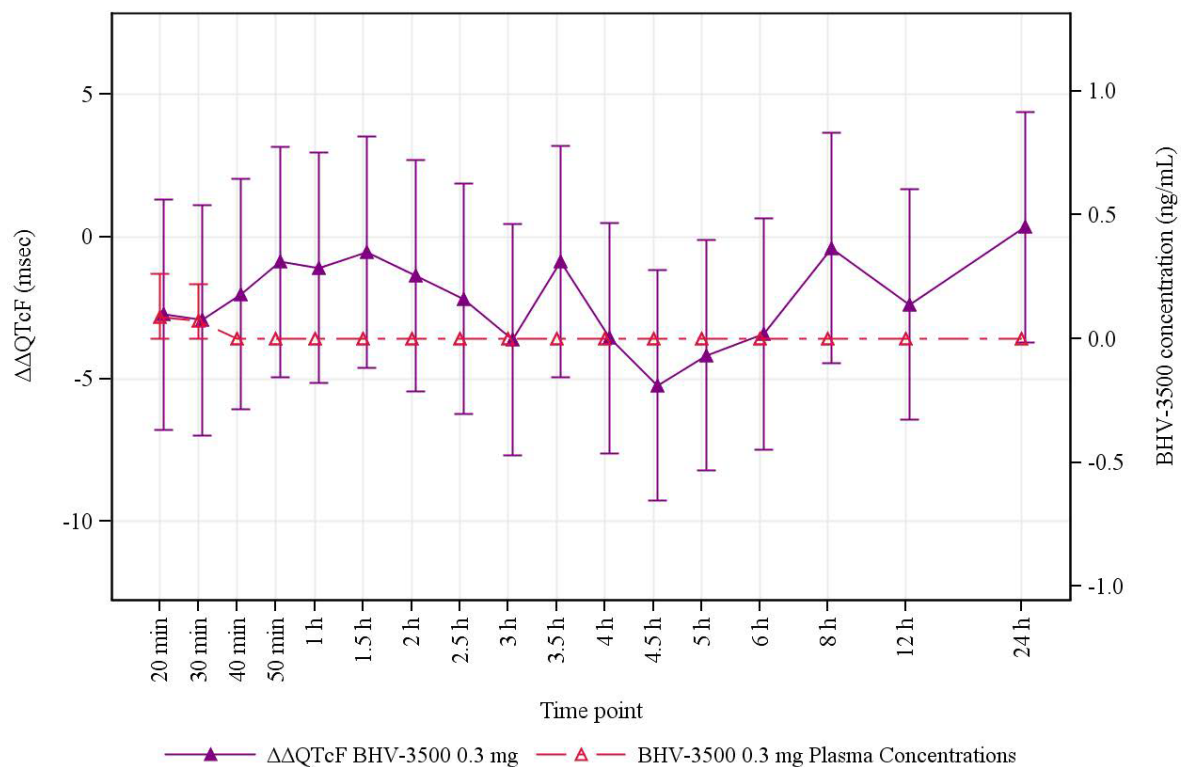
Figure S3. $\Delta\Delta$ QT joint plots stratified by dose (SAD Study: QT/QTc analysis population, PK/QTc analysis population)

A. Joint plot of zavegepant plasma concentrations and $\Delta\Delta$ QTcF over time (0.1 mg)



Notes: Error bars for $\Delta\Delta$ QTcF are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. A lower bound for concentration less than 0 was not biologically plausible and was therefore substituted as 0. $\Delta\Delta$ QT, ; placebo-corrected change-from-baseline in QT interval; $\Delta\Delta$ QTcF, placebo-adjusted change-from-baseline QT interval Fridericia's correction; BHV-3500, zavegepant; CI, confidence interval; PK, pharmacokinetics; QTc, corrected QT interval; SAD, single-ascending dose.

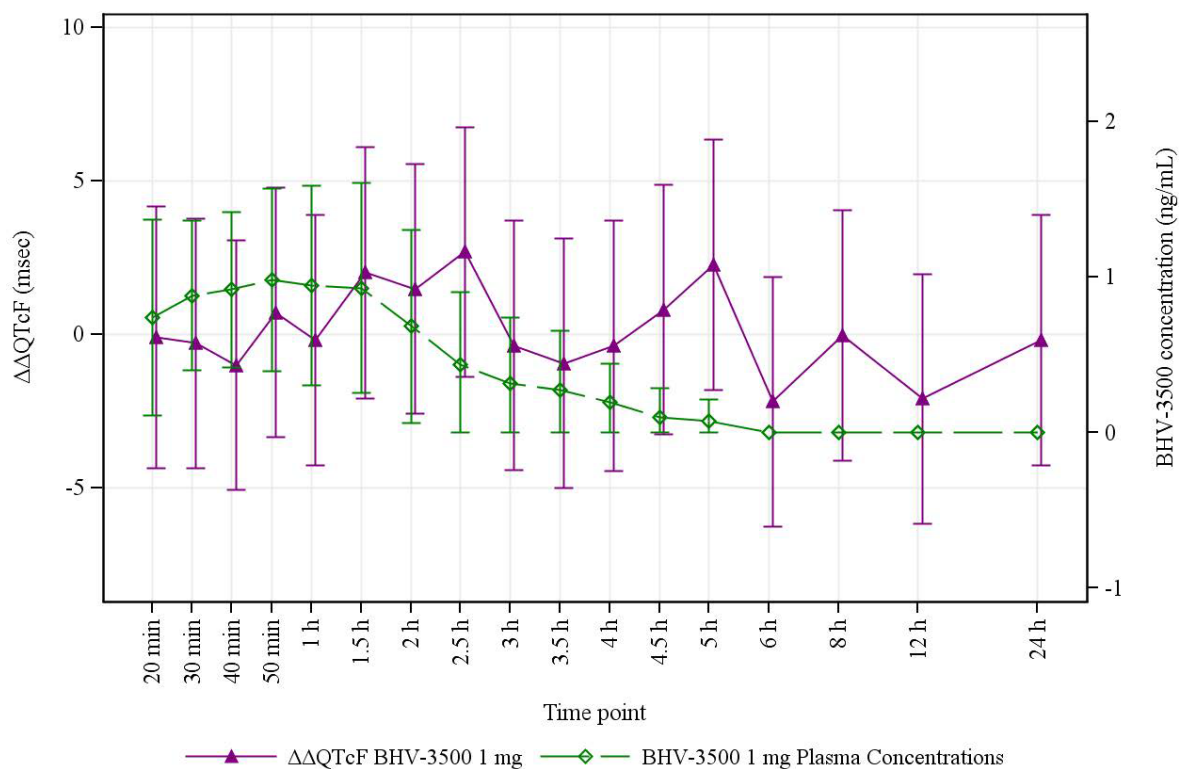
B. Joint plot of zavegepant plasma concentrations and $\Delta\Delta\text{QTcF}$ over time (0.3 mg)



Notes: Error bars for $\Delta\Delta\text{QTcF}$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. A lower bound for concentration less than 0 was not biologically plausible and was therefore substituted as 0.

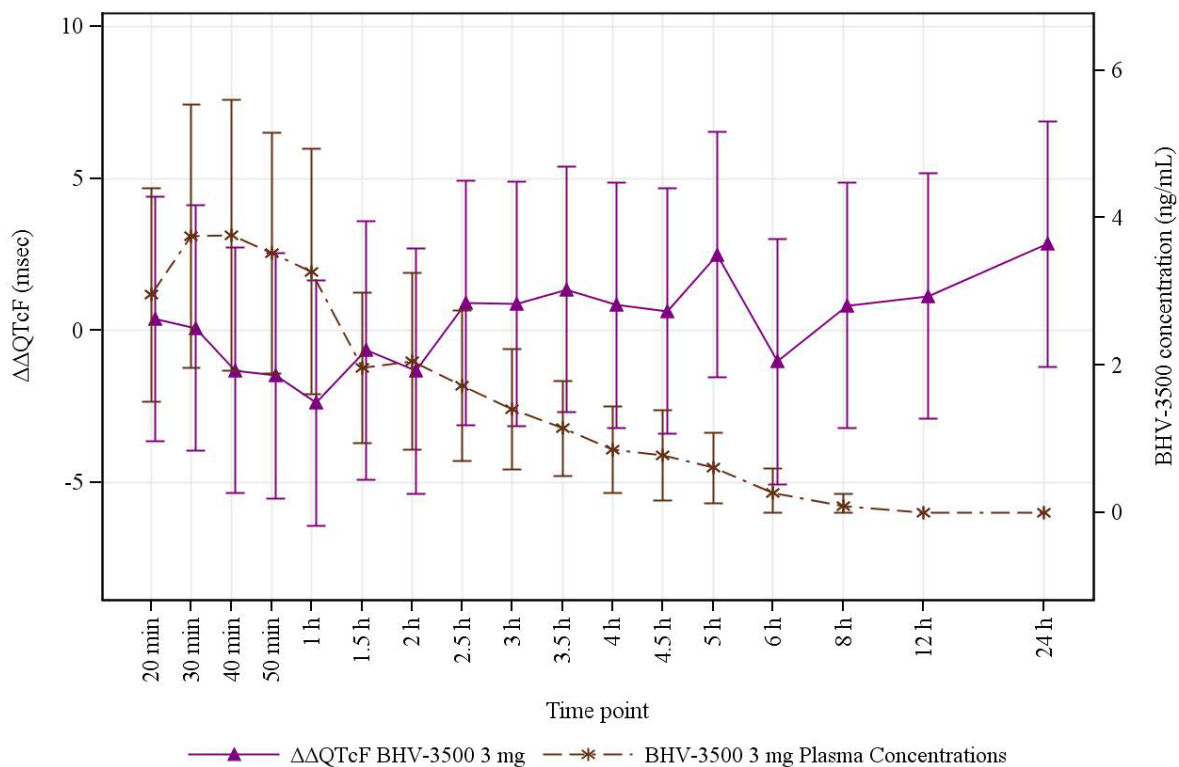
$\Delta\Delta\text{QTcF}$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; BHV-3500, zavegepant; CI, confidence, interval.

C. Joint plot of zavegepant plasma concentrations and $\Delta\Delta\text{QTcF}$ over time (1 mg)



Notes: Error bars for $\Delta\Delta\text{QTcF}$ are 90% CI from by-time point statistical modeling while the error bars for concentration are 90% CI from descriptive statistics. A lower bound for concentration less than 0 was not biologically plausible and was therefore substituted as 0. $\Delta\Delta\text{QTcF}$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; BHV-3500, zavegepant; CI, confidence interval.

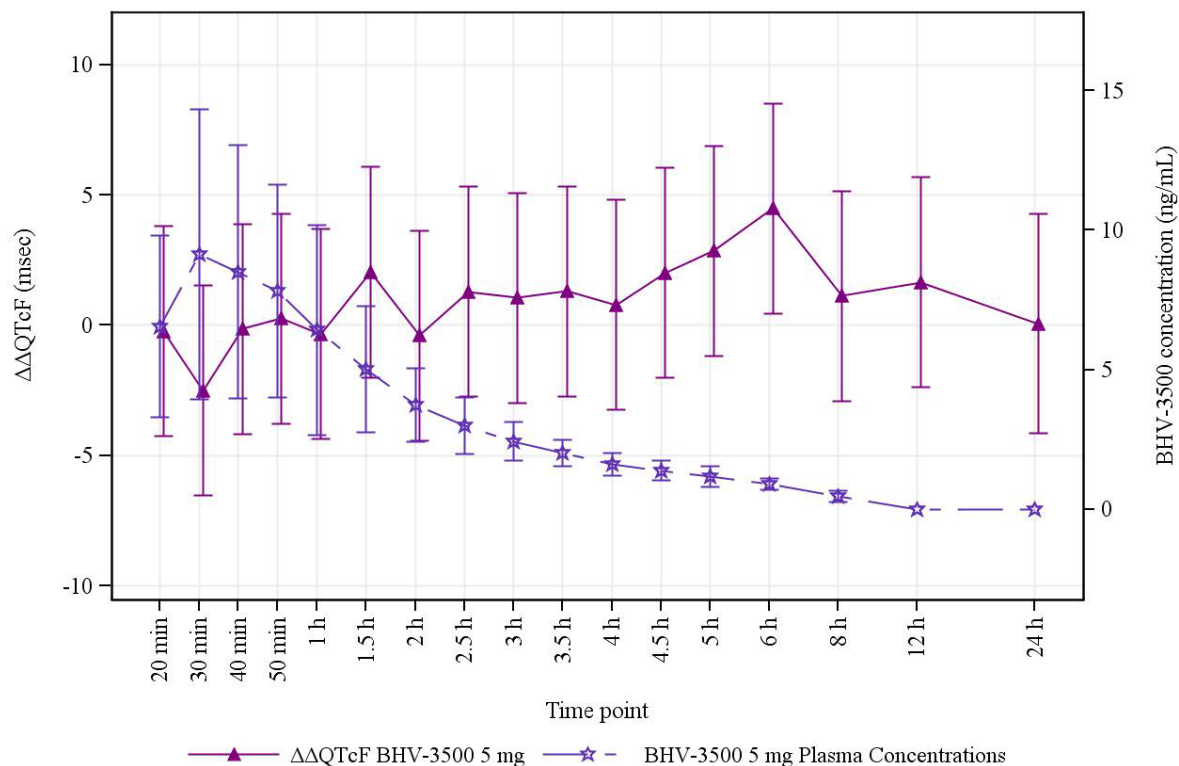
D. Joint plot of zavegepant plasma concentrations and $\Delta\Delta QTcF$ over time (3 mg)



Notes: Error bars for $\Delta\Delta QTcF$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. A lower bound for concentration less than 0 was not biologically plausible and was therefore substituted as 0.

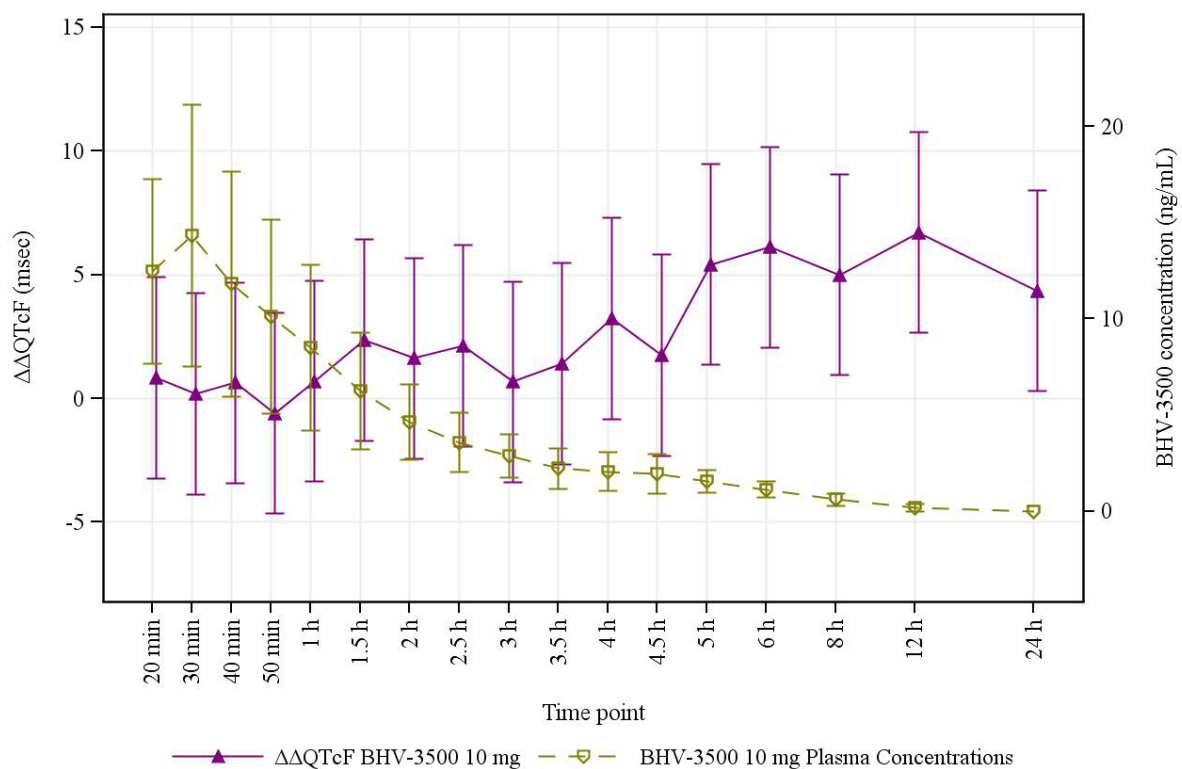
$\Delta\Delta QTcF$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; BHV-3500, zavegepant; CI, confidence interval.

E. Joint plot of zavegepant plasma concentrations and $\Delta\Delta\text{QTcF}$ over time (5 mg)



Notes: Error bars for $\Delta\Delta\text{QTcF}$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. A lower bound for concentration less than 0 was not biologically plausible and was therefore substituted as 0. $\Delta\Delta\text{QTcF}$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; BHV-3500, zavegepant; CI, confidence interval.

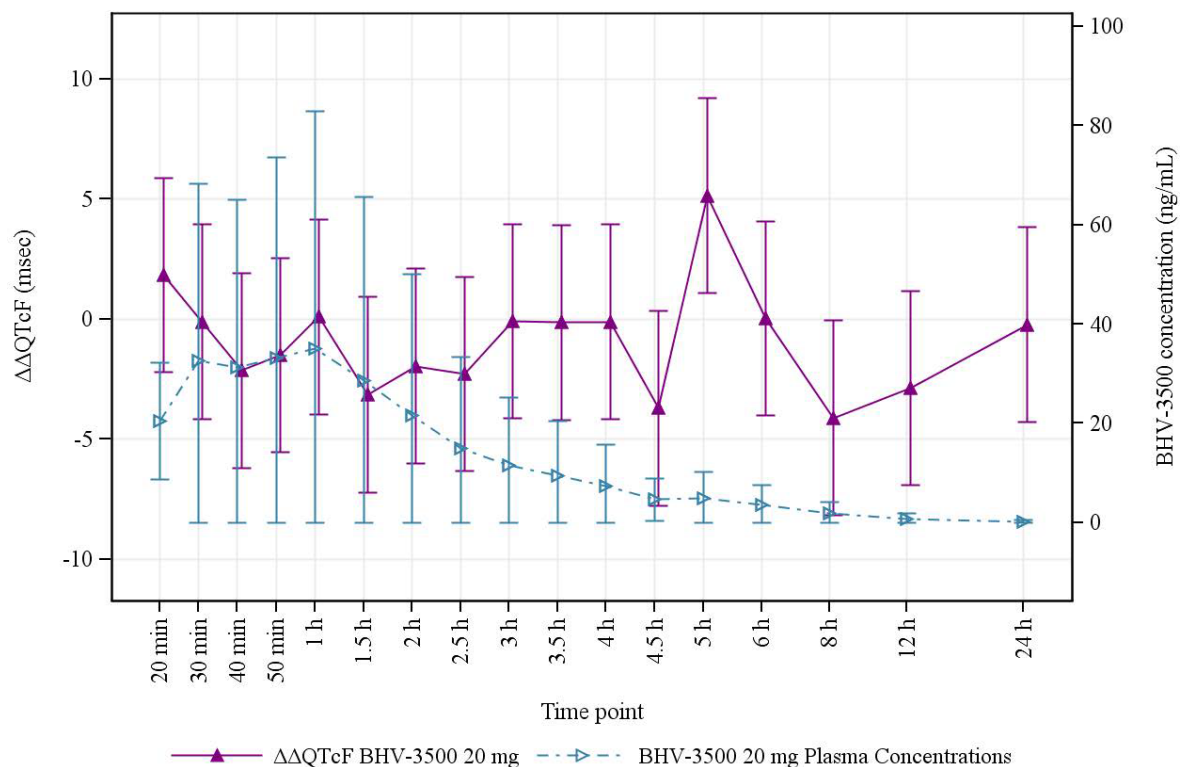
F. Joint plot of zavegepant plasma concentrations and $\Delta\Delta\text{QTcF}$ over time (10 mg)



Notes: Error bars for $\Delta\Delta\text{QTcF}$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. A lower bound for concentration less than 0 was not biologically plausible and was therefore substituted as 0.

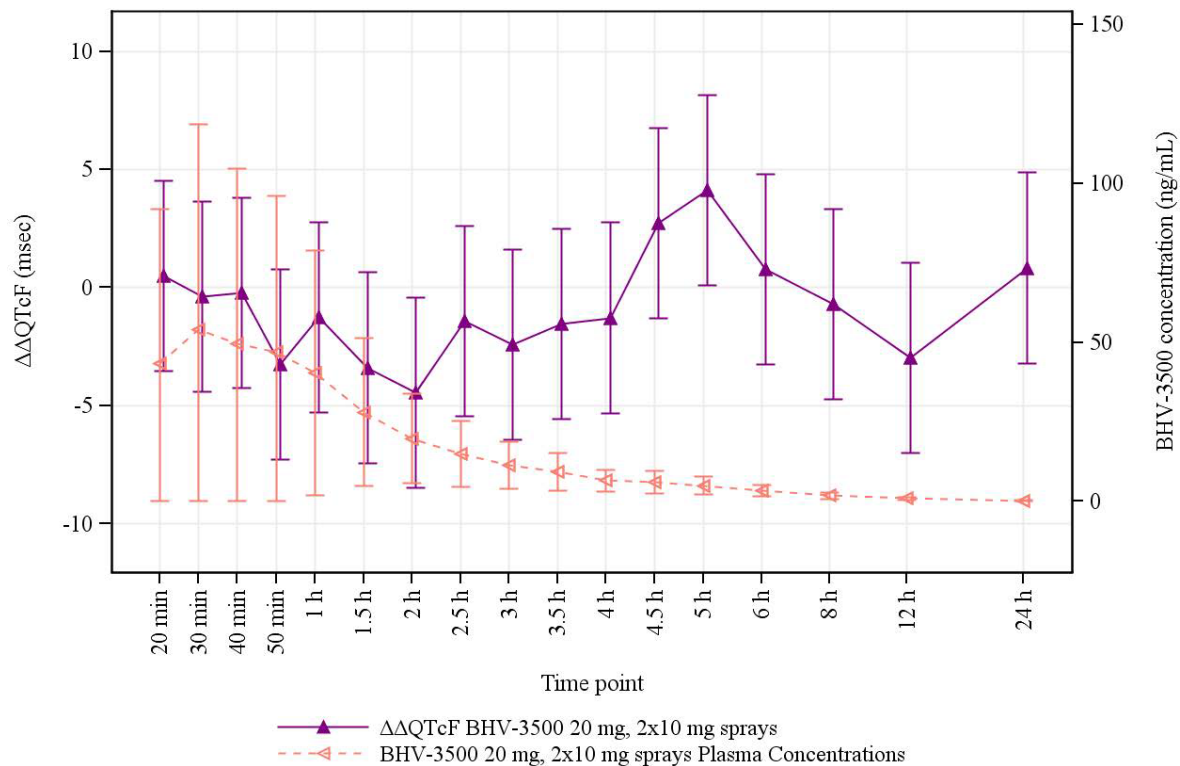
$\Delta\Delta\text{QTcF}$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; BHV-3500, zavegepant; CI, confidence interval.

G. Joint plot of zavegepant plasma concentrations and $\Delta\Delta\text{QTcF}$ over time (20 mg)



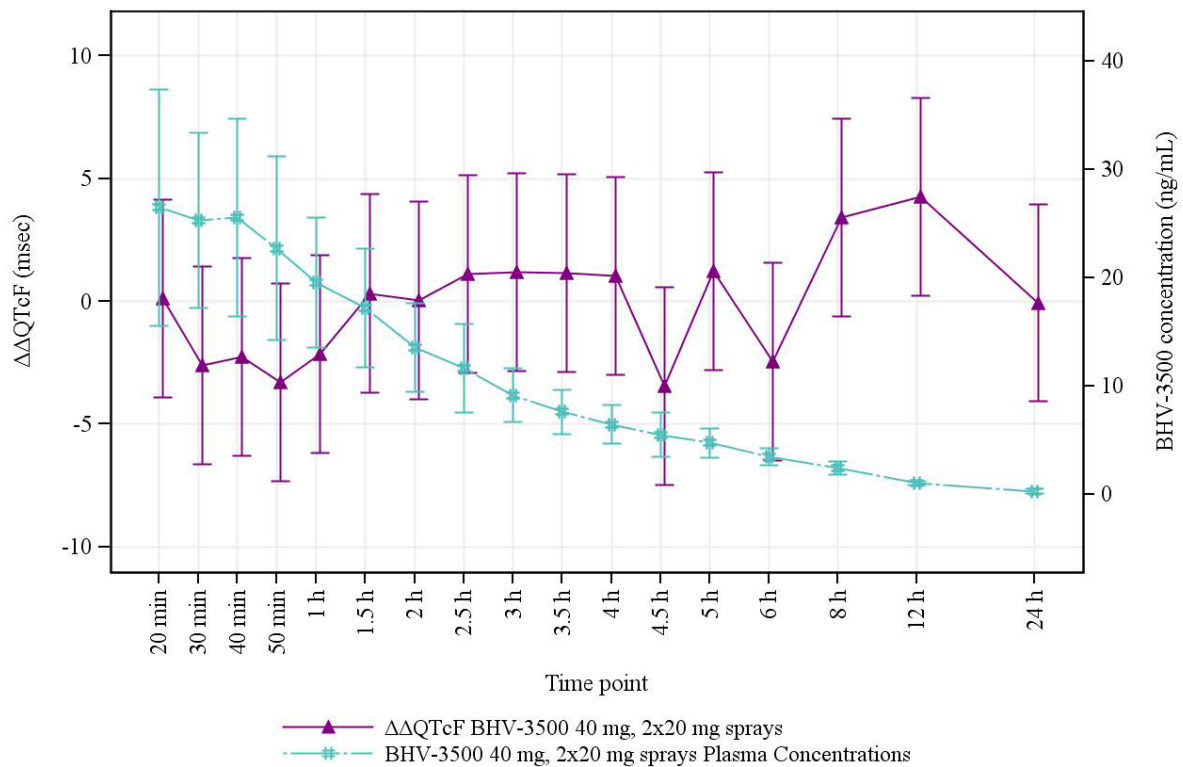
Notes: Error bars for $\Delta\Delta\text{QTcF}$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. A lower bound for concentration less than 0 was not biologically plausible and was therefore substituted as 0. $\Delta\Delta\text{QTcF}$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; BHV-3500, zavegepant; CI, confidence interval.

H. Joint plot of zavegepant plasma concentrations and $\Delta\Delta QTcF$ over time (20 mg, 2x10 mg sprays)



Notes: Error bars for $\Delta\Delta QTcF$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. A lower bound for concentration less than 0 was not biologically plausible and was therefore substituted as 0. $\Delta\Delta QTcF$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; BHV-3500, zavegepant; CI, confidence interval.

I. Joint plot of zavegepant plasma concentrations and $\Delta\Delta\text{QTcF}$ over time (40 mg, 2x20 mg sprays)



Notes: Error bars for $\Delta\Delta\text{QTcF}$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. A lower bound for concentration less than 0 was not biologically plausible and was therefore substituted as 0. $\Delta\Delta\text{QTcF}$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; BHV-3500, zavegepant; CI, confidence interval.

Figure S4. $\Delta\Delta QT$ joint plots stratified by dose (MAD Study: QT/QTc analysis population, PK/QTc analysis population)

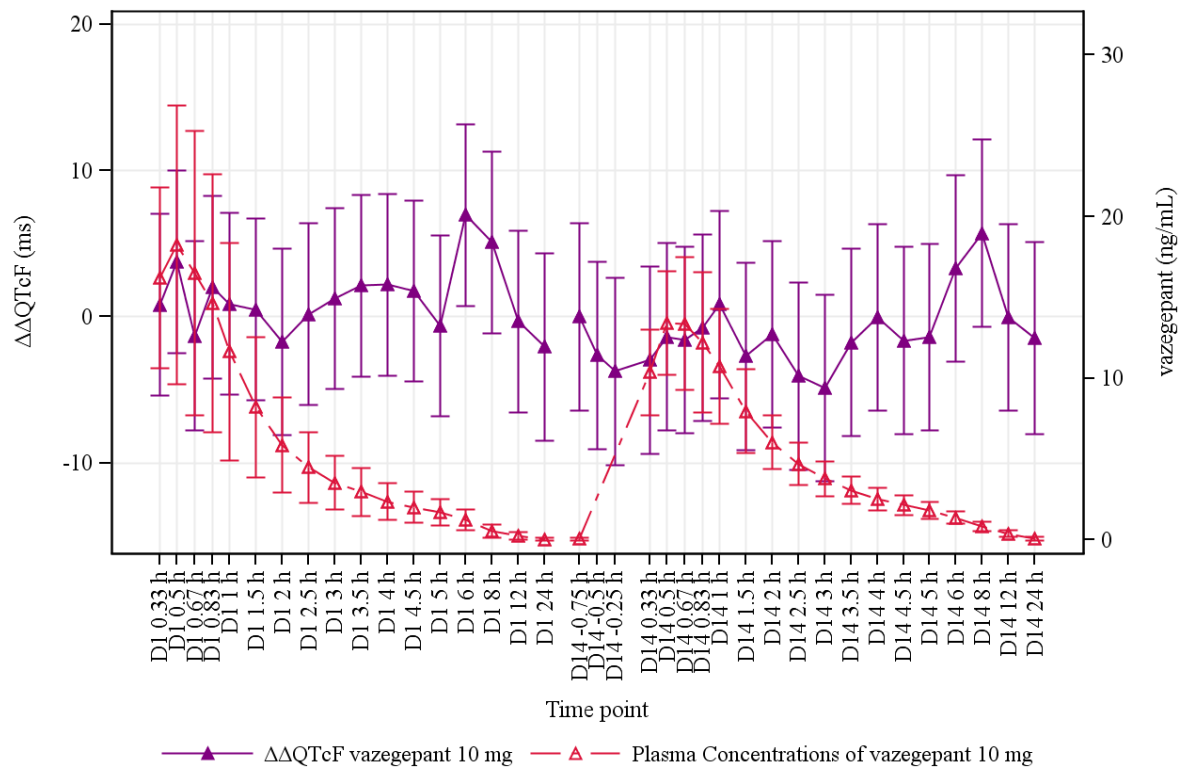
A. Joint plot of vazegepant plasma concentrations and $\Delta\Delta QTcF$ over time (5 mg QD)



Notes: Error bars for $\Delta\Delta QTcF$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics.

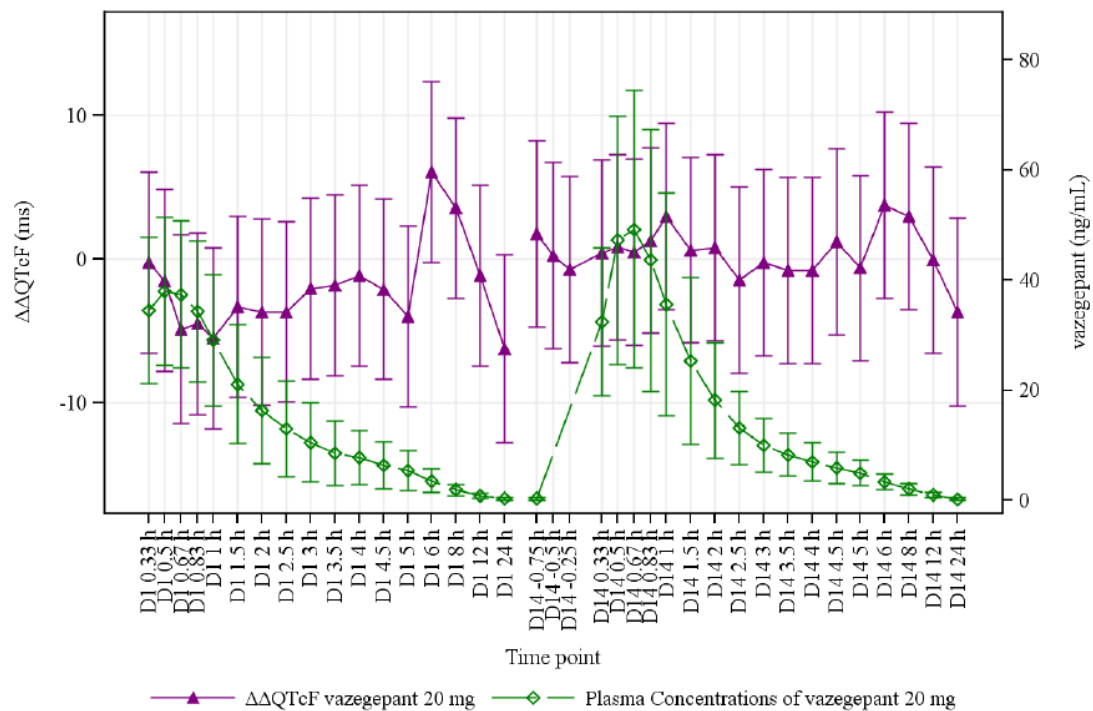
$\Delta\Delta QT$, placebo-corrected change-from-baseline in QT interval; $\Delta\Delta QTcF$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; CI, confidence interval; D, Day; MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; QD, once per day; vazegepant, vazegepant.

B. Joint plot of zavegepant plasma concentrations and $\Delta\Delta\text{QTcF}$ over time (10 mg QD)



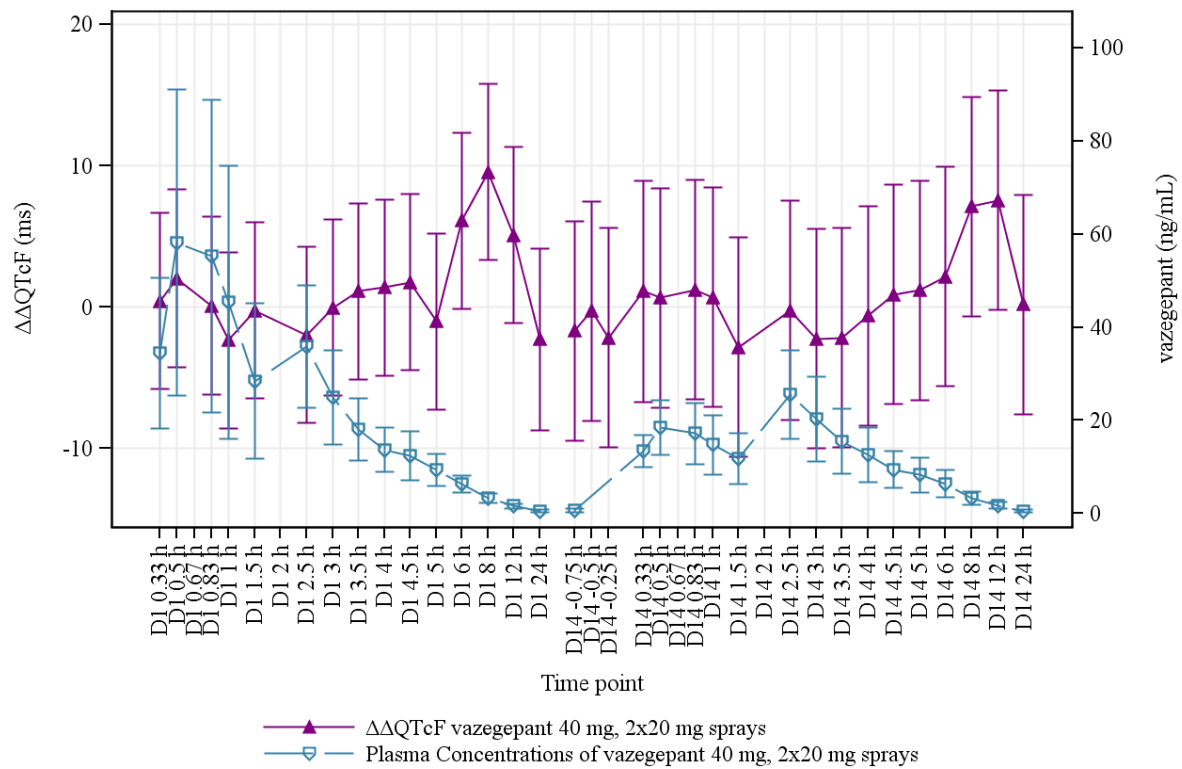
Notes: Error bars for $\Delta\Delta\text{QTcF}$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. $\Delta\Delta\text{QTcF}$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; CI, confidence interval; D, Day; QD, once per day; zavegepant, zavegepant.

C. Joint plot of zavegepant plasma concentrations and $\Delta\Delta\text{QTcF}$ over time (20 mg QD)



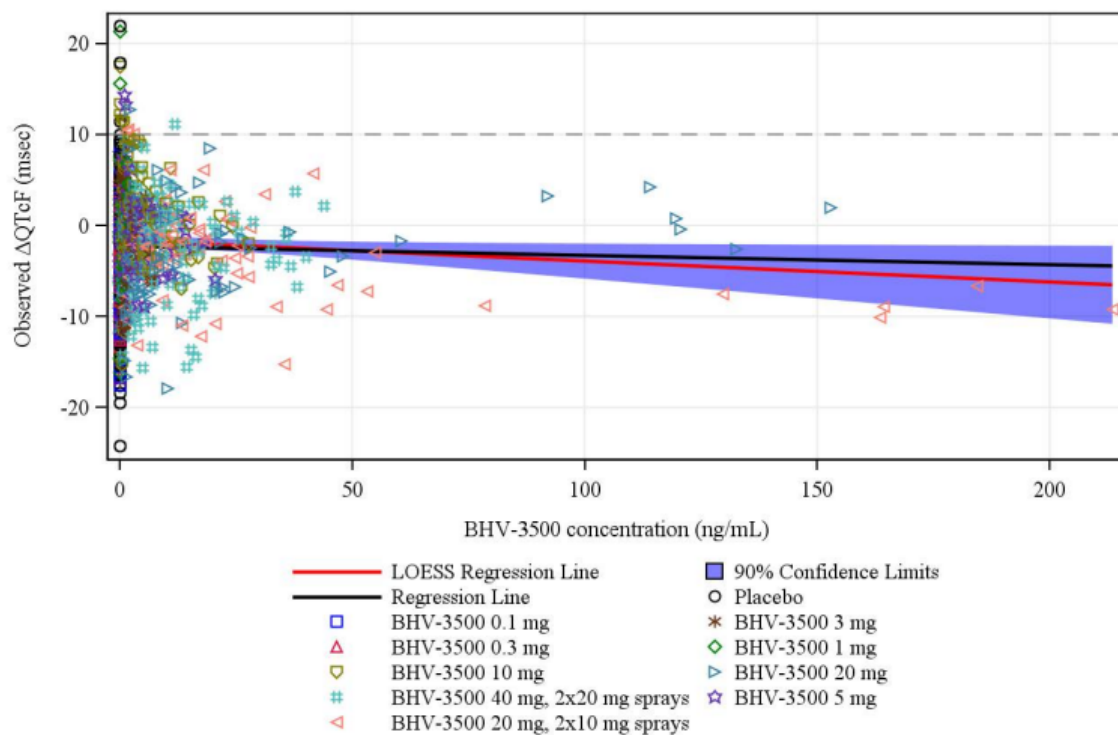
Notes: Error bars for $\Delta\Delta\text{QTcF}$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. $\Delta\Delta\text{QTcF}$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; CI, confidence interval; D, Day; QD, once per day; zavegepant, zavegepant.

D. Joint plot of zavegepant plasma concentrations and $\Delta\Delta\text{QTcF}$ over time (40 mg, 2x20 mg sprays QD)



Notes: Error bars for $\Delta\Delta\text{QTcF}$ are 90% CI from by-time point statistical modeling while the errors bars for concentration are 90% CI from descriptive statistics. $\Delta\Delta\text{QTcF}$, placebo-adjusted change-from-baseline QT interval Fridericia's correction; CI, confidence interval; D, Day; QD, once per day; zavegepant, zavegepant.

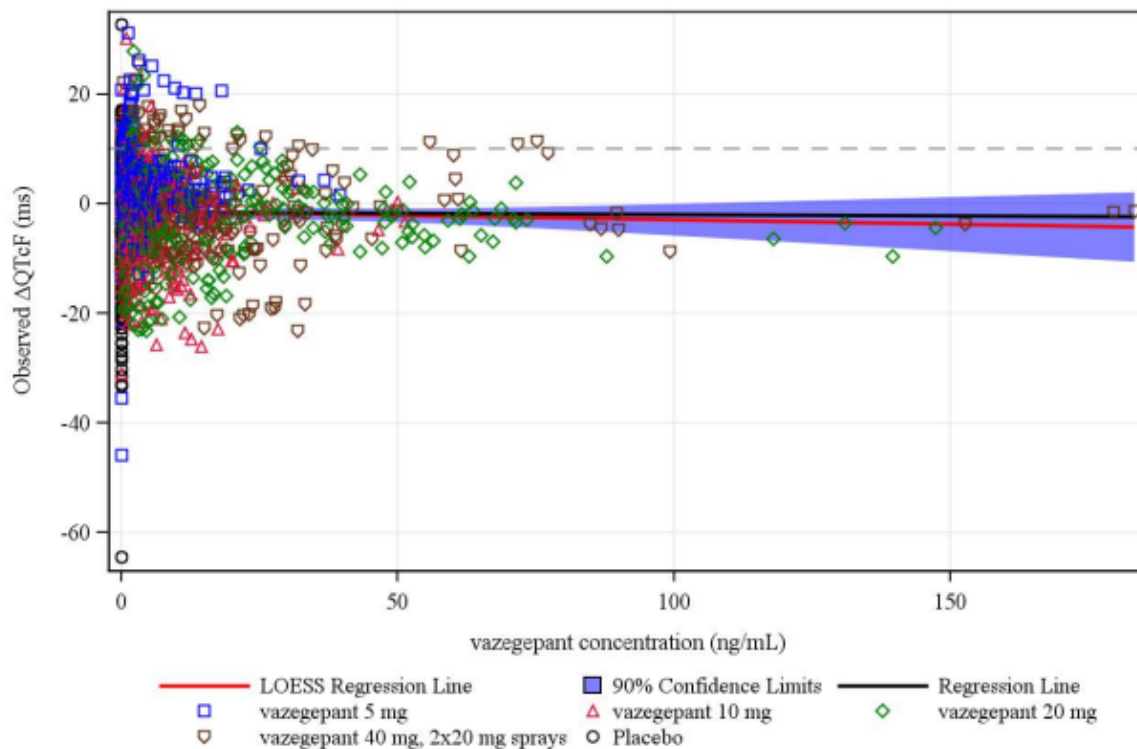
Figure S5. Scatter plot of observed zavegepant plasma concentrations and observed Δ QTcF (SAD study, PK/QTc analysis population)



Notes: The red line with the blue shaded area denotes the LOESS regression line and 90% confidence limits. The black solid line denotes the simple linear regression line.

Δ QTcF, change from baseline QTcF; BHV-3500, zavegepant; LOESS, locally weighted scatter plot smoothing; PK, pharmacokinetics; QTc, corrected QT interval; SAD, single-ascending dose.

Figure S6. Scatter plot of observed zavegepant plasma concentrations and observed Δ QTcF (MAD study, PK/QTc analysis population)

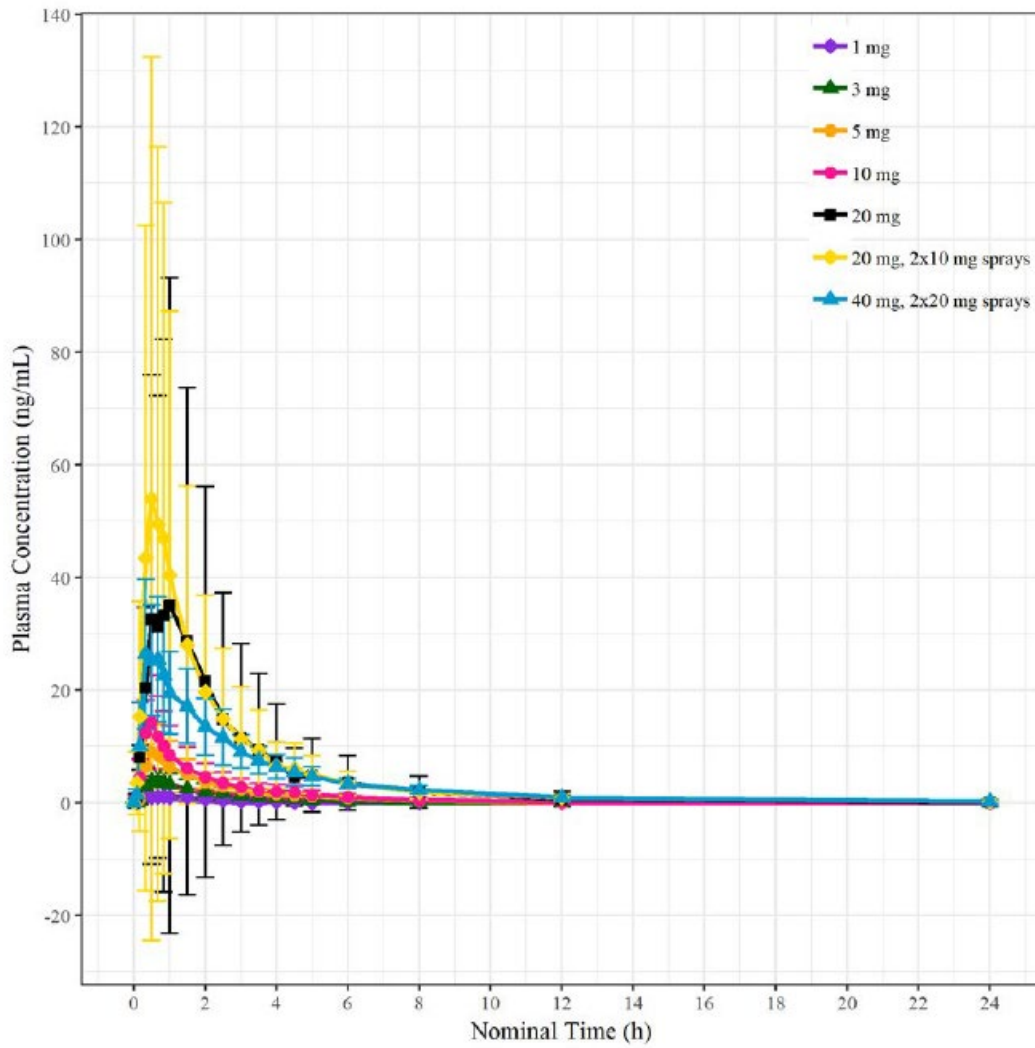


Notes: The red line with the blue shaded area denotes the LOESS regression line and 90% confidence limits. The black solid line denotes the simple linear regression line.

Δ QTcF, baseline QTcF; LOESS, locally weighted scatter plot smoothing; MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; zavegepant, zavegepant.

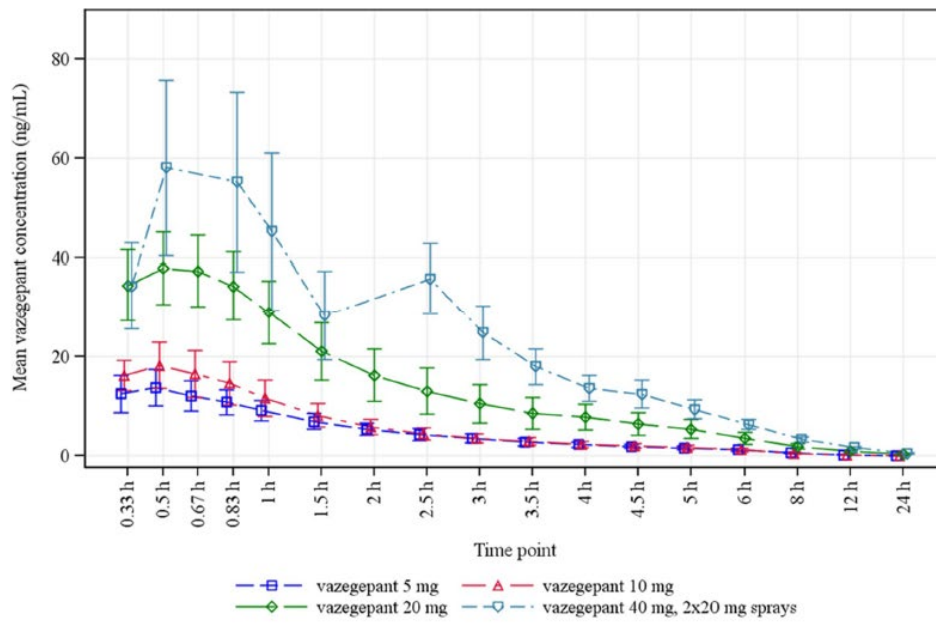
Figure S7. Concentration time plots by dose and study

A. Mean \pm SD zavegepant plasma concentration: SAD study



SAD, single-ascending dose; SD, standard deviation.

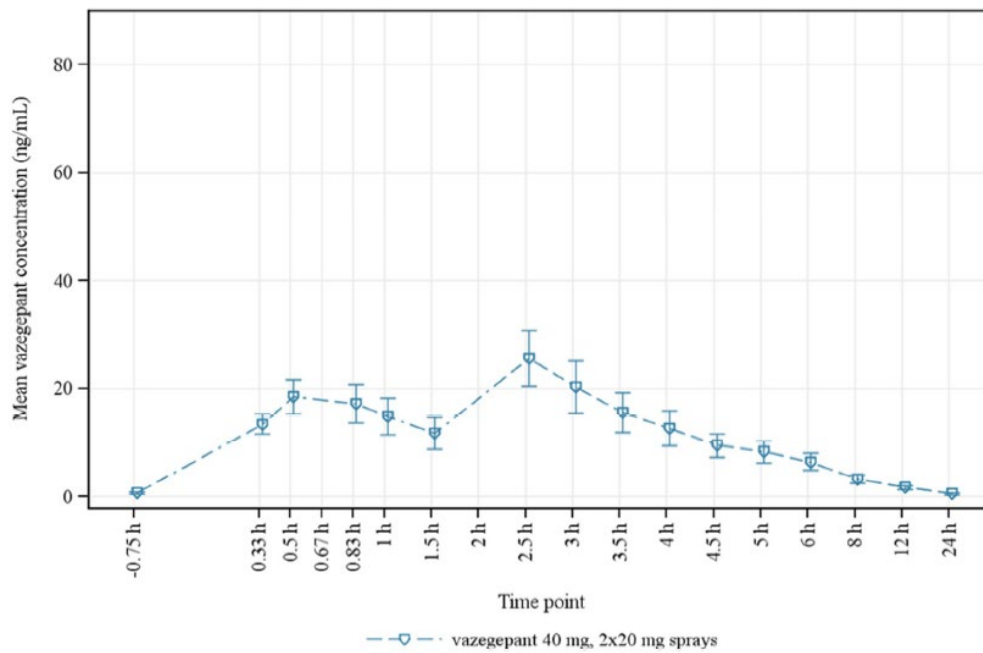
B. Mean \pm SE zavegepant plasma concentration: MAD study Day 1



Notes: Figure displays mean and \pm SE from descriptive statistics. If mean - SD was below 0, 0 was substituted, as concentrations below 0 are not biologically plausible.

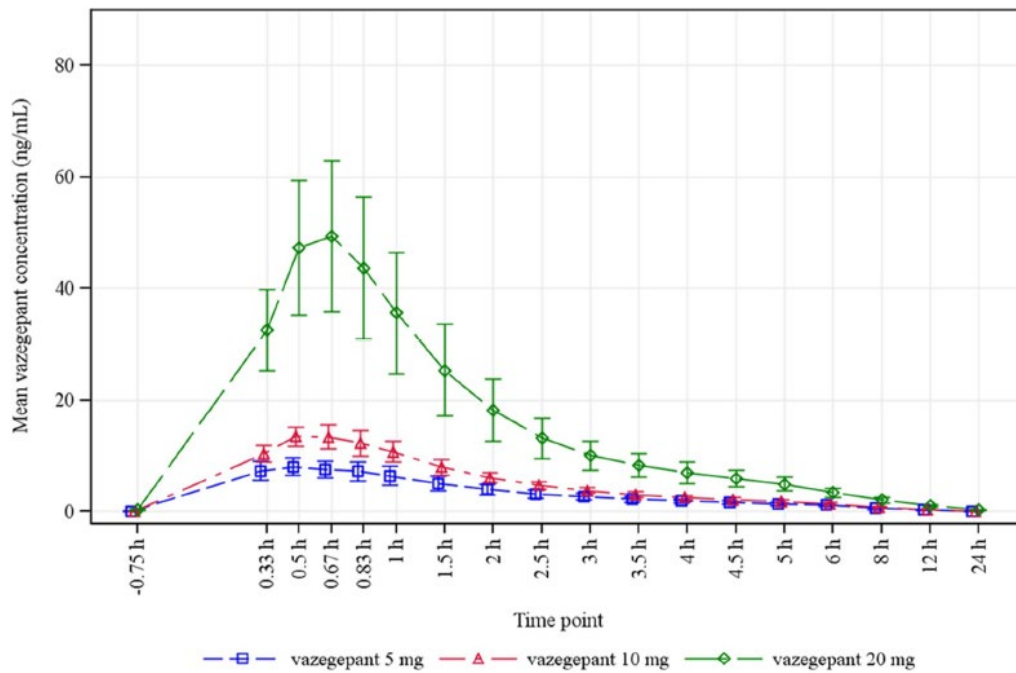
MAD, multiple-ascending dose; SD, standard deviation; SE, standard error; vazegepant, zavegepant.

C. Mean \pm SE zavegepant plasma concentration: MAD study Day 8



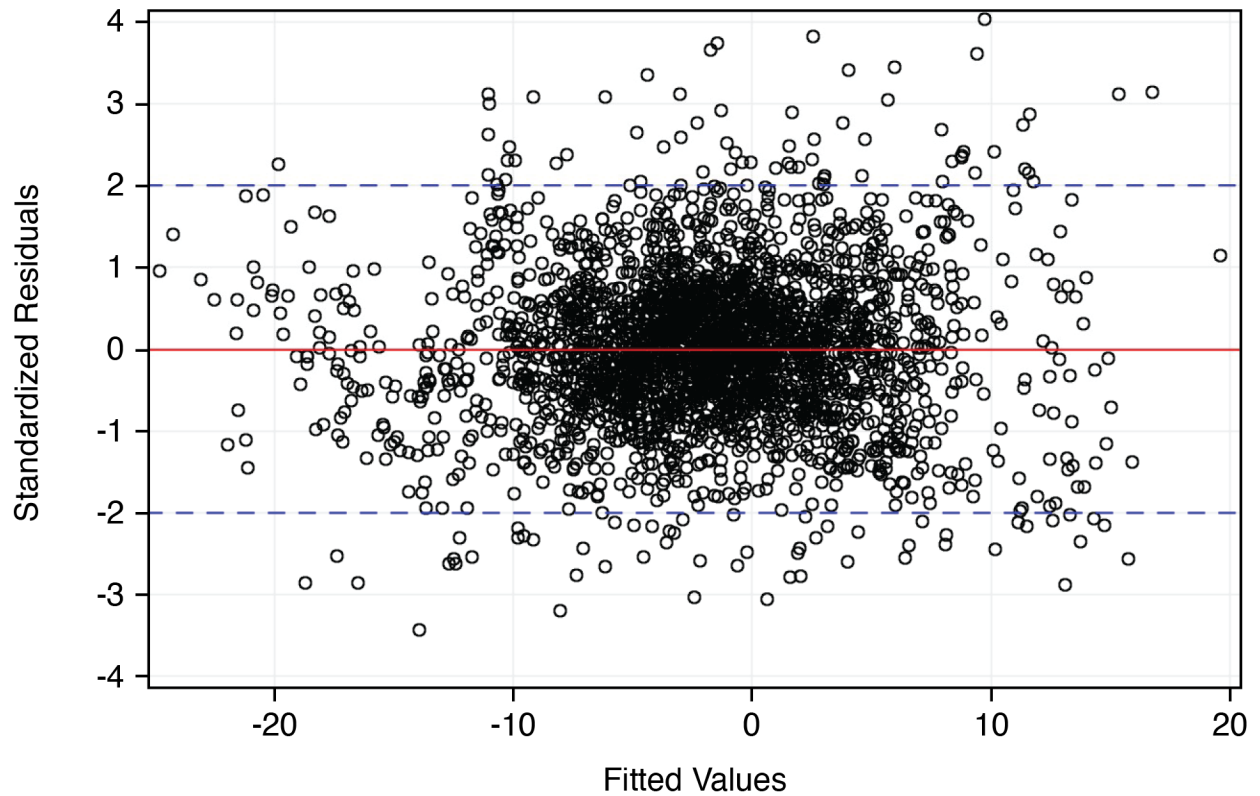
Notes: Figure displays mean and \pm SE from descriptive statistics. If mean - SD was below 0, 0 was substituted, as concentrations below 0 are not biologically plausible. MAD, multiple-ascending dose; SD, standard deviation; SE, standard error; vazegepant, zavegepant.

D. Mean \pm SE zavegepant plasma concentration: MAD study Day 14



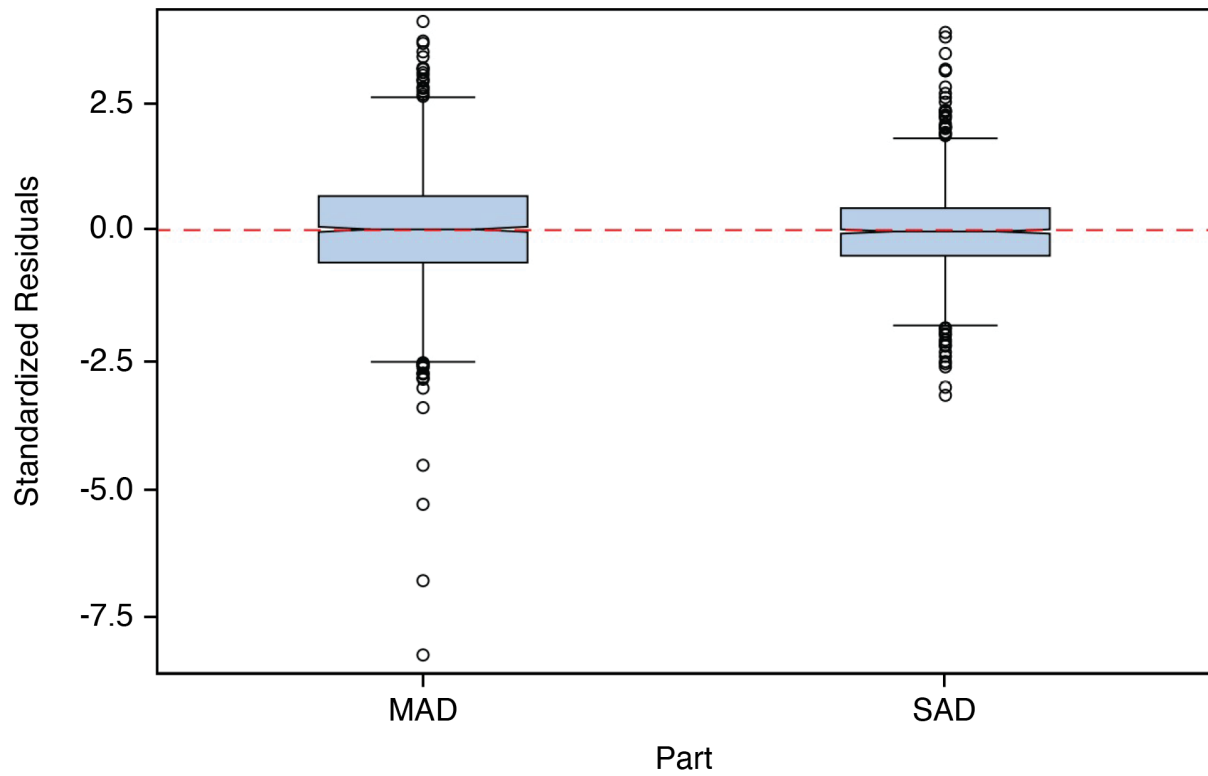
Notes: Figure displays mean and \pm SE from descriptive statistics. If mean - SD was below 0, 0 was substituted, as concentrations below 0 are not biologically plausible.
MAD, multiple-ascending dose; SD, standard deviation; SE, standard error; zavegepant, zavegepant.

Figure S8. Scatter plot of standardized residuals versus fitted values for zavegepant (SAD and MAD studies pooled) (PK/QTc analysis population)



Notes: The blue dashed lines are reference lines at ± 2 while the red solid line is a reference line at 0. MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; SAD, single-ascending dose.

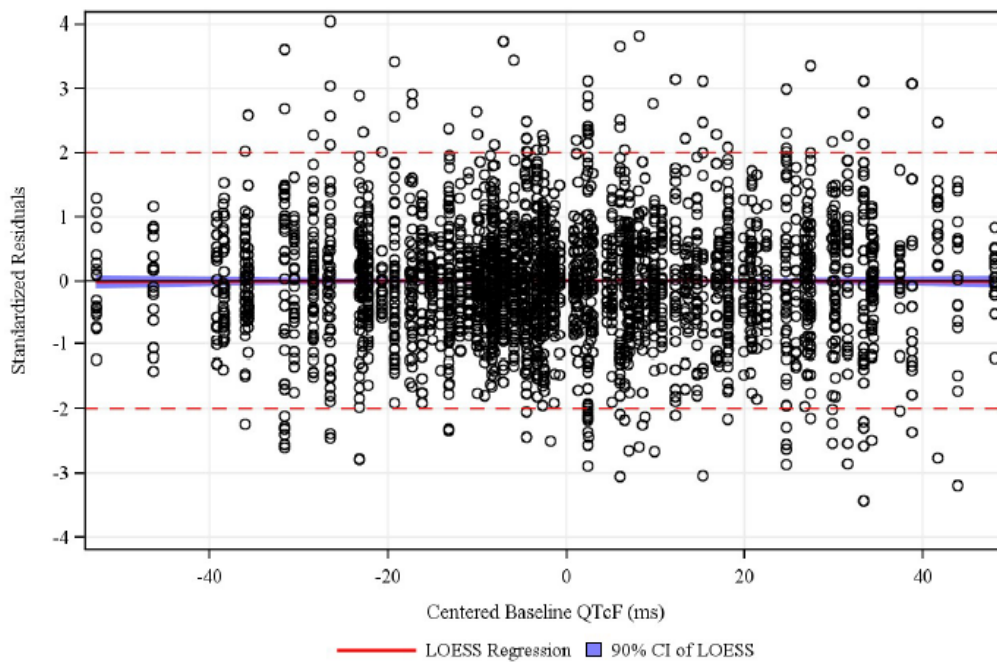
Figure S9. Boxplot of standardized residuals versus study (SAD and MAD studies pooled) (PK/QTc analysis population)



Notes: Notches calculated as $\text{median} \pm (1.58 \times \text{Interquartile range})/\sqrt{n}$.

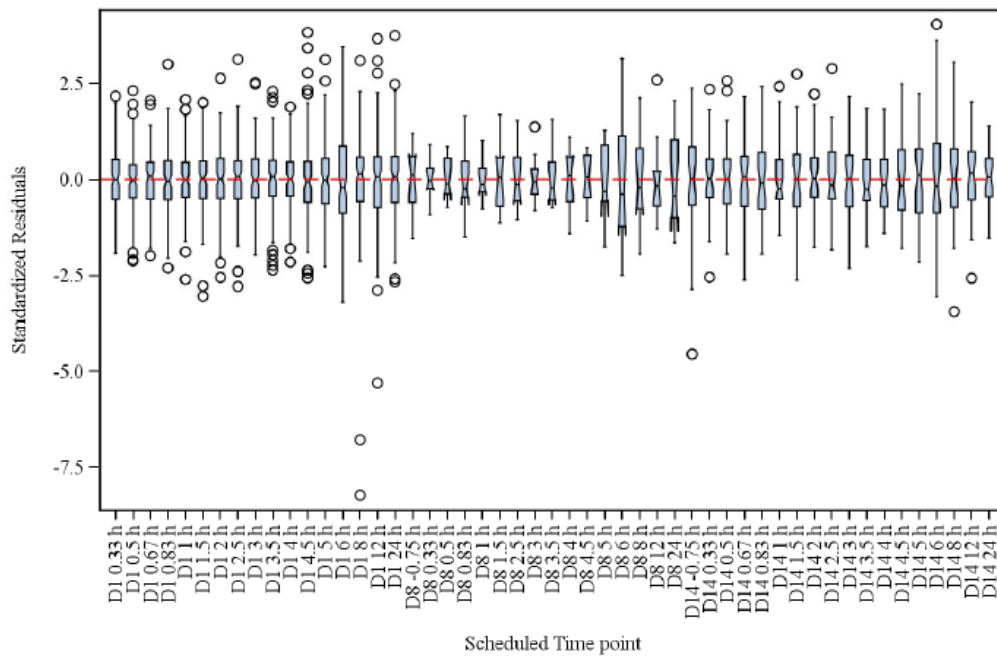
MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; SAD, single-ascending dose.

Figure S10. Scatter plot of standardized residuals versus baseline QTc for zavegepant (SAD and MAD pooled data, PK/QTc analysis population)



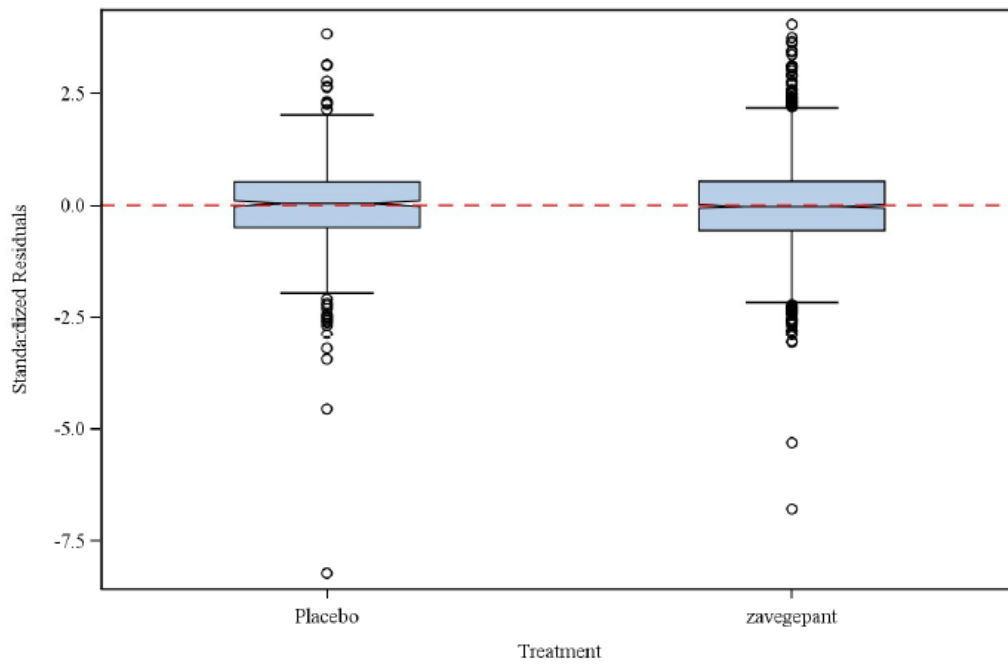
Notes: The red dashed lines are reference lines at ± 2 while the black solid line is a reference line at 0. The red solid line with blue shaded area denotes the LOESS regression line with 90% CI. CI, confidence interval; LOESS, locally weighted scatter plot smoothing; MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; QTcF, QT interval Fridericia's correction; SAD, single-ascending dose.

Figure S11. Boxplot of standardized residuals versus time for zavegepant (SAD and MAD pooled data, PK/QTc analysis population)



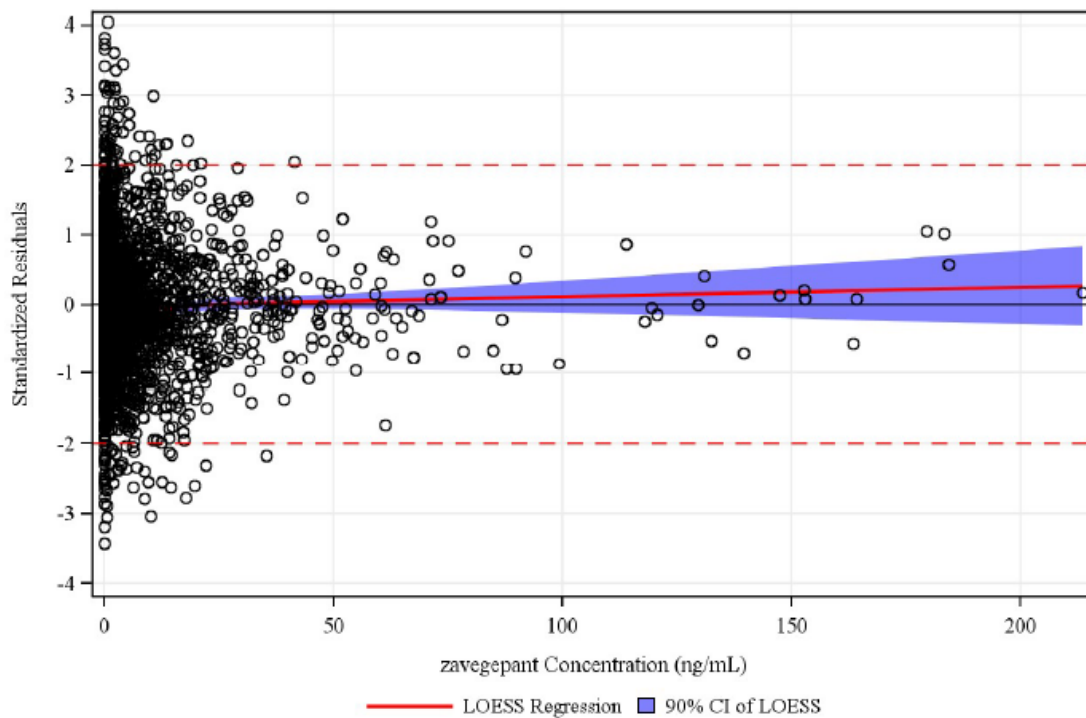
Notes: Notches calculated as $\text{median} \pm (1.58 \times \text{interquartile range})/\sqrt{n}$.
D, day; MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; SAD, single-ascending dose.

Figure S12. Boxplot of standardized Residuals versus active treatment for zavegepant or placebo (SAD and MAD pooled data, PK/QTc analysis population)



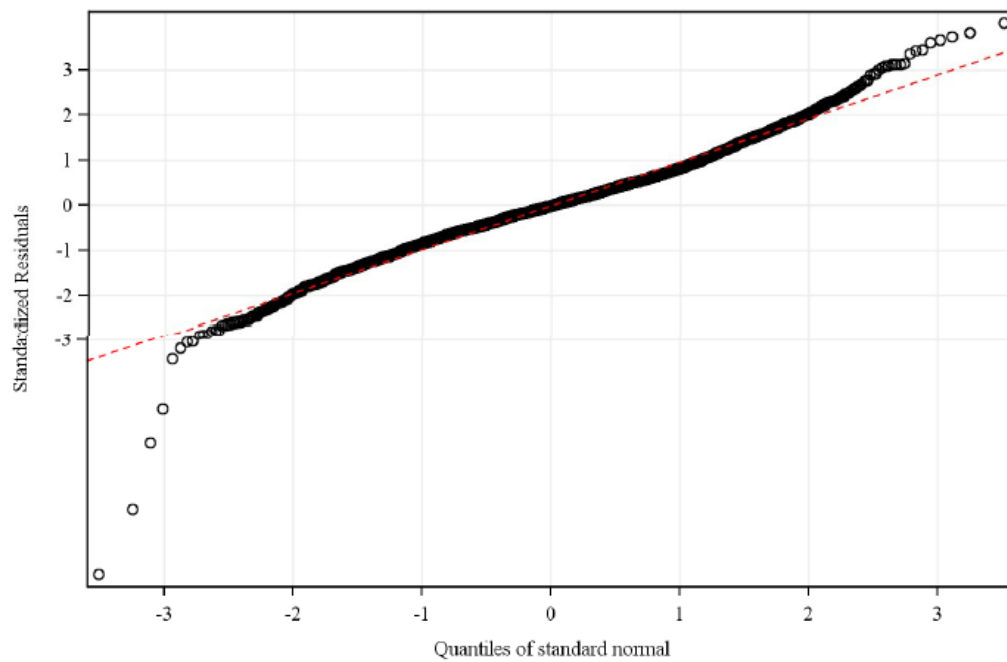
Notes: Notches calculated as $\text{median} \pm (1.58 \times \text{interquartile range})/\sqrt{n}$.
MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; SAD, single-ascending dose.

Figure S13. Scatter plot of standardized residuals versus zavegepant concentration (SAD and MAD pooled data, PK/QTc analysis population)



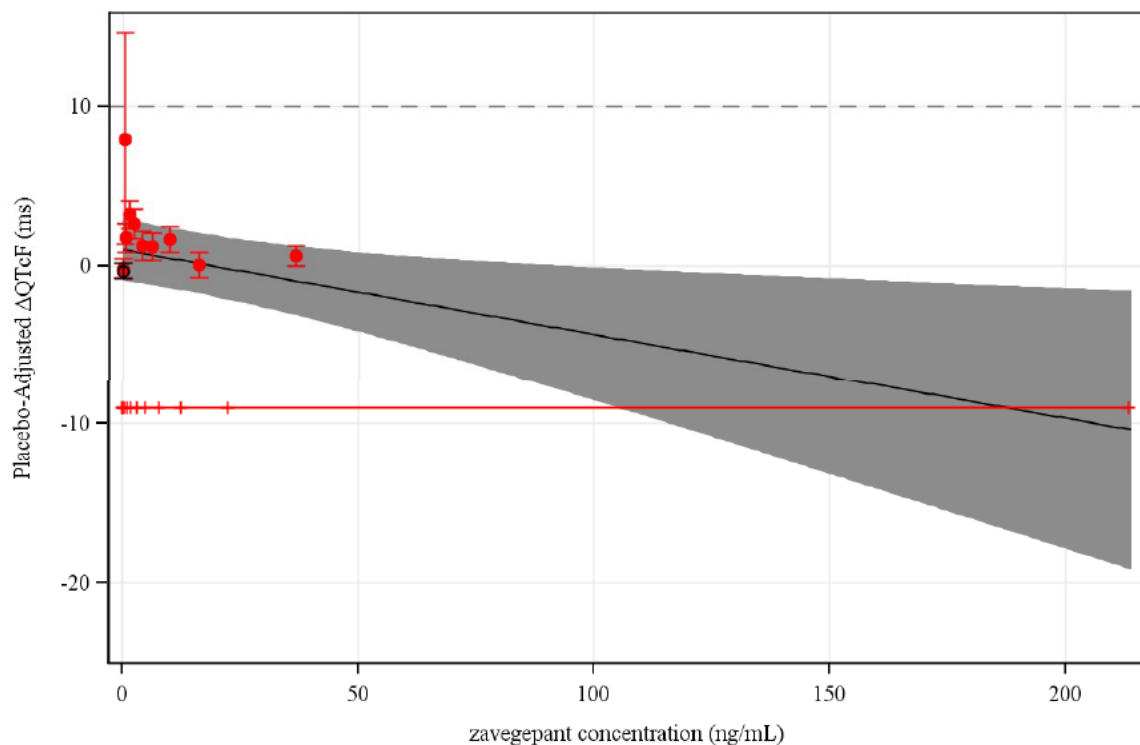
Notes: The red dashed lines are reference lines at ± 2 while the black solid line is a reference line at 0. The red solid line with blue shaded area denotes the LOESS regression line with 90% CI. CI, confidence interval; LOESS, locally weighted scatter plot smoothing; MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; SAD, single-ascending dose.

Figure S14: Normal quantile-quantile plot of residuals for zavegepant (SAD and MAD pooled data) (PK/QTc population)



Notes: The red dashed line denotes theoretical cumulative distribution function. MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; SAD, single-ascending dose

Figure S15. Model-predicted $\Delta\Delta\text{QTcF}$ (mean and 90% CI) and estimated $\Delta\Delta\text{QTcF}$ (mean and 90% CI) across deciles of zavegepant plasma concentrations (SAD and MAD studies pooled) (PK/QTc analysis population)



Notes: The solid black line with gray shaded area denotes the model-predicted mean $\Delta\Delta\text{QTcF}$ with 90% CI, which is calculated from the equation $\Delta\Delta\text{QTcF} \text{ (ms)} = 0.99 \text{ (ms)} - 0.053 \text{ (ms per ng/mL)} \times \text{zavegepant plasma concentration (ng/mL)}$. The red filled circles with vertical bars denote the estimated mean $\Delta\Delta\text{QTcF}$ with 90% CI displayed at the associated median plasma concentration within each decile for zavegepant, among which the individually estimated placebo-adjusted $\Delta\text{QTcF}_{i,k}$ ($\Delta\Delta\text{QTcF}_{i,k}$) equals the individual $\Delta\text{QTcF}_{i,k}$ for subject i administered with zavegepant at time point k minus the estimation of time effect at time point k . The black circle with vertical bars denotes the mean placebo-adjusted ΔQTcF with 90% CI for placebo at a concentration of 0. The horizontal red line with notches shows the range of concentrations divided into deciles for zavegepant. The area between each decile represents the point at which 10% of the data are present; the first notch to second notch denotes the first 10% of the data, the second notch to third notch denotes the 10-20% of the data and so on.

$\Delta\Delta\text{QTcF}$, placebo-corrected change-from-baseline in QT interval using Fridericia's formula; ΔQTcF , change from baseline in corrected QT interval using Fridericia's formula; CI, confidence interval; MAD, multiple-ascending dose; PK, pharmacokinetics; QTc, corrected QT interval; SAD, single-ascending dose;