Supplementary Materials

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Pharmacokinetics With and Without Food of Zilurgisertib from Single and Multiple Ascending Dose Phase 1 Studies in Healthy Adults

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Dose Escalation Rules

Participants in cohorts 1–8 of the single ascending dose (SAD) study were split into 2 groups to be dosed 24 h apart. Group 1 comprised 3 participants randomized to receive zilurgisertib (n = 2) or placebo (n = 1). Group 2 comprised 9 participants randomized to receive zilurgisertib (n = 7) or placebo (n = 2). The decision to dose group 2 was at investigator discretion based on outcomes from group 1. Dose escalation followed satisfactory review of safety and tolerability data of the previous cohort by both investigator and sponsor and review of pharmacokinetic data from the most recent cohort with available data. Every other cohort was assessed. Dose escalation only occurred following review of safety data from ≥9 participants (including ≥6 receiving zilurgisertib) from the lower dose cohort. Doses could be modified to lower or higher doses, dose level analysis could be repeated, and alternate dosing regimens could be explored based on evolving safety and pharmacokinetic data. Before the start of each successive dose cohort, an interim blinded safety report summarizing results from all available safety assessments was sent to the sponsor. Any significant results were discussed with the sponsor before continuation of dose escalation. To confirm the study design remained appropriate, interim blinded pharmacokinetic data from a previous dose cohort were reviewed in terms of dose escalation. In cases of disagreement between sponsor and investigator, the decision of the investigator would be upheld.

Dose escalation would be halted if any of the following occurred:

- ≥2 participants developed grade ≥3 adverse events (AEs) in the same organ system (excluding asymptomatic grade 3 elevations in blood pressure). For blood pressure, >160 mmHg systolic and >105 mmHg diastolic were used in place of grade 3 criteria to halt dose escalation.
- Development of a treatment-emergent serious AE not clearly attributed to some cause other than the study drug. Dose escalation could be resumed if risk mitigation was possible via implementation of additional safety measures, such as new screening tests, updated inclusion and exclusion requirements, or changes to study conduct.
- ≥3 participants receiving a particular dose of zilurgisertib met individual withdrawal criteria (excluding pregnancy) (i.e., consent withdrawn, further participation would be injurious to participant's health or well-being in the view of the investigator, unacceptable toxicity).

- ≥4 participants receiving a particular dose of zilurgisertib developed significant confirmed QTcF prolongation (>60-ms increases).
- ≥2 participants in a cohort of ≥5 participants receiving zilurgisertib had confirmed increases in hepatic transaminases (alanine aminotransferase or aspartate transaminase) >3.0 × upper limit of normal (ULN).
- ≥1 participant(s) receiving zilurgisertib had confirmed elevations in hepatic transaminases >5 × ULN (not including, for example, participants with normal transaminases with a single elevation >3.0 × ULN not confirmed on repeat testing the next day).

Dose escalation was not affected if another cause of liver injury was found (such as an intercurrent viral infection or other predisposing condition) and the elevation was clearly unrelated to zilurgisertib. However, the individual participant could be discontinued from dosing. If dose escalation was halted, the dose level could be repeated at the discretion of the investigator in consultation with the sponsor and with institutional review board notification. If the adverse finding was confirmed in a second cohort at the same dose level (e.g., severe toxicity in ≥2 participants), the study could be terminated. If the adverse finding was not confirmed, dose escalation could proceed.

Sample Collection and Preparation

Blood samples (4 mL) were collected for plasma pharmacokinetic analysis in 4-mL Vacutainer®-evacuated, dipotassium ethylene diamine tetraacetic acid (K2EDTA)-containing collection tubes (BD Biosciences, Franklin Lakes, NJ, USA). Where indwelling catheters were used, the first 1 mL of blood from each sample was discarded. Samples were gently mixed by inverting the tube 6–8 times, then stored in an ice bath or on ice. Samples were centrifuged ≤45 min after collection at approximately 2000 × g (or 2960 RPM) for 15 min at approximately 4°C using a refrigerated centrifuge, then split equally between 2 threaded polypropylene cryovials and stored on ice and frozen at –80°C (±15°C). In both studies, saliva was collected in sample tubes (Norgen Biotek Corporation, Thorold, ON, Canada) via an attached funnel. At least 1 mL saliva per sample was needed and typically 2 mL or 3 mL was collected per tube (<4 mL was collected per tube to avoid leakage after freezing/thawing). Each sample was contained within a single tube. Tubes were stored on ice and frozen at –80°C (±15°C) ≤45 min after collection.

Bioanalytical Methods

All bioanalytical methods were fully validated with standard operating procedures based on US Food and Drug Administration guidance and European Medicines Agency guidelines. The validations included calibration linearity, sensitivity, accuracy and precision, batch size, dilution factor verifications, matrix selectivity and matrix effect, extraction recovery, injection, carry-over, and short term and long-term stabilities. All parameters were verified acceptable prior to the analysis of clinical study samples.

Plasma and Urine Samples

Samples (25 µL) were placed into 2-mL tubes in a 96-well format; DMSO:methanol (25 µL, 50:50, v/v) was added to control blanks and the internal standard (a deuterium-labeled compound of zilurgisertib [zilurgisertib-d8]) solution in DMSO:methanol (25 µL, 50:50, v/v) was added to all other samples. Acetonitrile (300 µL) was added to all samples. Samples were vortexed (3 min, approximately 1500 rpm) and centrifuged (approximately 1640 × g for approximately 5 min). For plasma samples, supernatant (250 µL) was transferred to clean 2-mL tubes in 96-well format, dried under nitrogen, reconstituted with 300-µL reconstitution solution (methanol:water, 50:50, v/v), and the plate was vortexed for 2 min. For urine samples, supernatant (50 µL) was mixed with 450-µL reconstitution solution (50:50, v/v) in clean 2-mL tubes in 96-well format. For both plasma and urine samples, plates were placed in an autosampler tray and samples (5–25 µL) were injected into a liquid chromatography tandem mass spectrometry (LC-MS/MS) system. The LC-MS/MS system was composed of binary high-performance liquid chromatography (HPLC) pumps and an autosampler coupled to a SCIEX API 4000 tandem mass spectrometer (SCIEX, Concord, ON, Canada). The chromatographic separation was achieved using a Phenomenex Gemini-NX C18 column (50 × 2.0 mm, 5 µm; Phenomenex, Torrance, CA, USA) at flow rate 0.75 mL/min, using mobile phases of 1M ammonium formate:water (1:100, mobile phase A) and 1M ammonium formate:acetonitrile:methanol (1:50:50, mobile phase B) with gradient setting. The mass spectrometer was operated in positive electrospray ionization mode with multiple reaction monitoring mass/charge (m/z) 503.4 → 462.4 for zilurgisertib and m/z 509.4 → 468.4 for zilurgisertib-d8. The validated assay range for zilurgisertib in human plasma was 1–1000 nM with 100-fold dilution factor verified and a lower limit of quantification (LLOQ) of 1 nM, and the assay range for zilurgisertib in human urine was 10–10,000 nM with 100-fold dilution factor verified and an LLOQ of 10 nM.

Saliva Samples

Each saliva sample (50µL) was placed into a well of a 96-well plate. Internal standard (zilurgisertib-d8) solution (200 µL in acetonitrile) was added and the plate was then capped and vortexed for approximately 2 min and centrifuged for 5 min. Sample supernatant (100 µL) was transferred into a final collection plate that contained 200 µL of water per well. The final collection plate was capped and vortexed for approximately 1 min and placed in the autosampler tray. Each sample (0.5 µL) was injected into an LC-MS/MS. The LC-MS/MS analysis was carried out with a SCIEX Triple Quad 6500+ or QTRAP 6500 MS coupled with binary ultra HPLC pumps and an autosampler. The chromatographic separation was achieved for zilurgisertib on a Thermo Hypersil Gold C4 column (50 × 2.1 mm, 3 µm; Thermo Fisher Scientific, Waltham, MA, USA) with isocratic elution at 29% mobile phase B. The mass spectrometer was operated in positive electrospray ionization mode and the resolution setting used was "unit" for both Q1 and Q3. The multiple reaction monitoring transition was m/z 503.2 → 462.2 for zilurgisertib and 509.3 → 468.2 for the internal standard, zilurgisertib-d8. The validated assay range for zilurgisertib in human saliva was 5–3000 nM with 50-fold dilution factor verified and LLOQ of 5 nM.

Supplementary Table S1 Calibration standard performance, and accuracy and precision of quality control samples, for human plasma, urine, and saliva

Calibration standard performance in human plasma								
Concentration	1.00 nM	2.00 nM	7.50 nM	30.0 nM	100 nM	400 nM	800 nM	1000 nM
Mean	1.01	1.97	7.52	30.4	101	400	795	991
RSD (%)	5.3	3.4	2.3	1.7	1.1	2.2	1.0	2.2
Bias (%)	1.0	–1.5	0.3	1.3	1.0	0.0	–0.6	–0.9
n	6	6	6	6	6	6	6	6
Accuracy and precision of quality control sample in human plasma								
Concentration	1.00 nM	3.00 nM	40.0 nM	400 nM	750 nM	7500 nM		
	Dilution = 1	Dilution = 1	Dilution = 1	Dilution = 1	Dilution = 1	Dilution = 100		
Mean	1.05	3.03	40.6	403	740	7770		
RSD (%)	8	2.9	1.9	1.6	2	1.4		
Bias (%)	5	1	1.5	0.8	–1.3	3.6		
n	18	18	18	18	18	6		
Calibration standard performance in human urine								
Concentration	10.0 nM	20.0 nM	75.0 nM	300 nM	1000 nM	4000 nM	8000 nM	10,000 nM
Mean	9.95	20.2	75.8	300	1000	4030	7930	9810
RSD (%)	5.0	4.8	1.6	2.4	2.3	1.8	1.6	3.1
Bias (%)	–0.5	1.0	1.1	0.0	0.0	0.8	–0.9	–1.9
n	6	6	6	6	6	6	6	6
Accuracy and precision of quality control sample in human urine								
Concentration	10.0 nM	30.0 nM	400 nM	4000 nM	7500 nM	75,000 nM		
	Dilution = 1	Dilution = 1	Dilution = 1	Dilution = 1	Dilution = 1	Dilution = 100		
Mean	9.6	29.7	389	3870	7430	72,900		
RSD (%)	6.5	4.3	2.4	1.8	2.4	1.3		
Bias (%)	–4	–1	–2.8	–3.3	–0.9	–2.8		
n	18	18	18	18	18	6		
Calibration standard performance in human saliva								
Concentration	5 nM	12 nM	50 nM	120 nM	500 nM	1200 nM	2500 nM	3000 nM
Mean	5.04	11.7	51.0	118	520	1180	2480	3000
RSD (%)	2.5	2.5	3.8	3.1	3.3	3.7	2.1	2.3
Bias (%)	0.8	–2.5	2.0	–1.7	4.0	–1.7	–0.8	0.0
n	18	18	18	18	18	18	18	18

Accuracy and precision of quality control sample in human saliva					
Concentration	LLOQ 5 nM	Low 15 nM	Mid 180 nM	High 2400 nM	5000 nM
	Dilution = 1	Dilution = 1	Dilution = 1	Dilution = 1	Dilution = 10
Mean	5.02	14.5	173	2340	4310
RSD (%)	4.8	4.7	4.4	2.5	2.5
Bias (%)	0.4	–3.3	–3.9	–2.5	–13.8
n	18	18	18	18	6

LLOQ lower limit of quantification, *RSD* relative standard deviation. The extraction recoveries are 96.7% to 102.2% for plasma, 96.3% to 102.9% for urine, and 91.9% to 97.3% for saliva for the assay ranges.

Supplementary Table S2 Baseline demographics and characteristics of participants in the SAD study

Characteristic	Cohort (dose level QD)									
	Placebo (*n* = 21)	Cohort 1 10 mg (*n* = 9)	Cohort 2 25 mg (*n* = 9)	Cohort 3 50 mg (*n* = 9)	Cohort 4 100 mg (*n* = 7)	Cohort 5 175 mg (*n* = 9)	Cohort 6 250 mg (*n* = 9)	Cohort 7 500 mg (*n* = 6)	Cohort 9 100 mg (fasted and fed) (*n* = 12)	Total (*N* = 91)
Age, years, mean (SD)	35.7 (10.6)	34.9 (10.5)	35.7 (9.0)	35.9 (9.6)	37.9 (11.4)	34.8 (7.9)	37.6 (10.8)	34.0 (2.8)	37.8 (7.7)	36.1 (9.2)
Sex, *n* (%)										
Male	12 (57)	4 (44)	4 (44)	7 (78)	3 (43)	5 (56)	6 (67)	6 (100)	11 (92)	58 (64)
Female	9 (43)	5 (56)	5 (56)	2 (22)	4 (57)	4 (44)	3 (33)	0	1 (8)	33 (36)
Race, *n* (%)										
White	15 (71)	9 (100)	9 (100)	7 (78)	7 (100)	8 (89)	7 (78)	3 (50)	11 (92)	76 (84)
Black	6 (29)	0	0	1 (11)	0	0	0	3 (50)	1 (8)	11 (12)
Asian	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	1 (11)	0	1 (11)	2 (22)	0	0	4 (4)
Weight, kg, mean (SD)	68.5 (9.4)	71.7 (8.7)	73.2 (11.5)	75.1 (14.6)	75.0 (7.5)	76.0 (13.4)	75.4 (16.7)	76.7 (13.5)	76.5 (6.1)	73.4 (11.2)
BMI, kg/m^2, mean (SD)	24.8 (3.1)	26.2 (1.2)	26.7 (2.1)	25.6 (3.4)	25.9 (2.2)	26.7 (2.6)	25.7 (4.5)	25.7 (3.4)	25.6 (2.2)	25.8 (2.8)

BMI body mass index, *QD* once daily, *SAD* single ascending dose, *SD* standard deviation

Supplementary Table S3 Baseline demographics and characteristics of participants in the MAD study

Characteristic	Cohort (dose level)							
	Placebo (n = 20)	Cohort 1 50 mg QD (n = 9)	Cohort 2 100 mg QD (n = 9)	Cohort 3 150 mg QD (n = 9)	Cohort 4 200 mg QD (n = 9)	Cohort 5 400 mg QD (n = 9)	Cohort 6 300 mg BID (n = 14)	Total (N = 79)
Age, years, mean (SD)	35.6 (10.0)	32.0 (9.4)	38.7 (5.9)	32.6 (8.8)	35.3 (9.4)	37.6 (6.9)	35.4 (6.6)	35.3 (8.4)
Sex, n (%)								
Male	15 (75)	4 (44)	6 (67)	2 (22)	9 (100)	9 (100)	8 (57)	53 (67)
Female	5 (25)	5 (56)	3 (33)	7 (78)	0	0	6 (43)	26 (33)
Race, n (%)								
White	18 (90)	7 (78)	6 (67)	8 (89)	6 (67)	8 (89)	11 (79)	64 (81)
Black	1 (5)	2 (22)	2 (22)	1 (11)	2 (22)	1 (11)	2 (14)	11 (14)
Asian	1 (5)	0	1 (11)	0	0	0	0	2 (3)
Other	0	0	0	0	1 (11)	0	1 (7)	2 (3)
Weight, kg, mean (SD)	76.4 (10.7)	74.8 (12.7)	75.2 (10.7)	71.6 (10.4)	77.3 (10.7)	78.2 (9.7)	75.9 (12.2)	75.8 (10.8)
BMI, kg/m^2, mean (SD)	26.2 (2.1)	26.7 (2.9)	26.2 (3.3)	26.6 (2.2)	25.5 (2.9)	27.2 (2.0)	26.5 (2.9)	26.4 (2.5)

BID twice daily, *BMI* body mass index, *MAD* multiple ascending dose, *QD* once daily, *SD* standard deviation

Supplementary Table S4 Summary of TEAEs in the SAD study

n (%)	Cohort (dose level QD)										
	Placebo (*n* = 21)	Cohort 1 10 mg (*n* = 9)	Cohort 2 25 mg (*n* = 9)	Cohort 3 50 mg (*n* = 9)	Cohort 4 100 mg (*n* = 7)	Cohort 5 175 mg (*n* = 9)	Cohort 6 250 mg (*n* = 9)	Cohort 7 500 mg (*n* = 6)	Cohort 9 100 mg (fasted) (*n* = 12)	Cohort 9 100 mg (fed) (*n* = 12)	Total (*N* = 91)
TEAEs	5 (24)	2 (22)	4 (44)	2 (22)	2 (29)	2 (22)	6 (67)	1 (17)	1 (8)	3 (25)	28 (31)
Grade 3 or higher	0	0	0	0	1 (14)	0	0	0	0	0	1 (1)
Serious TEAEs	0	0	0	0	1 (14)	0	0	0	0	0	1 (1)
Treatment-related TEAEs	2 (10)	1 (11)	2 (22)	0	1 (14)	1 (11)	3 (33)	0	0	0	10 (11)
Diarrhea	0	0	0	0	0	0	1 (11)	0	0	0	1 (1)
Dry mouth	0	0	0	0	1 (14)	0	0	0	0	0	1 (1)
Nausea	0	0	0	0	0	0	1 (11)	0	0	0	1 (1)
Muscle spasms	1 (5)	0	0	0	0	0	0	0	0	0	1 (1)
Myalgia	1 (5)	0	0	0	0	0	0	0	0	0	1 (1)
Pain in extremity	0	1 (11)	0	0	0	0	0	0	0	0	1 (1)
Dizziness postural	0	0	1 (11)	0	0	0	0	0	0	0	1 (1)
Headache	0	0	0	0	0	0	1 (11)	0	0	0	1 (1)
Ventricular tachycardia	0	0	0	0	0	1 (11)	0	0	0	0	1 (1)
Fatigue	0	0	1 (11)	0	0	0	0	0	0	0	1 (1)
Heart rate increase	0	0	0	0	0	0	1 (11)	0	0	0	1 (1)
Decreased appetite	1 (5)	0	0	0	0	0	0	0	0	0	1 (1)
Hot flush	0	0	0	0	0	0	1 (11)	0	0	0	1 (1)

QD once daily, *SAD* single ascending dose, *TEAE* treatment-emergent adverse event

Supplementary Table S5 Summary of TEAEs in the MAD study

	Cohort (dose level)							
n (%)	Placebo (*n* = 20)	Cohort 1 50 mg QD (*n* = 9)	Cohort 2 100 mg QD (*n* = 9)	Cohort 3 150 mg QD (*n* = 9)	Cohort 4 200 mg QD (*n* = 9)	Cohort 5 400 mg QD (*n* = 9)	Cohort 6 300 mg BID (*n* = 14)	Total (*N* = 79)
TEAEs	5 (25)	1 (11)	5 (56)	3 (33)	4 (44)	6 (67)	8 (57)	32 (41)
Grade 3 or higher	0	0	0	0	0	0	0	0
Serious TEAEs	0	0	0	0	0	0	0	0
Treatment-related TEAEs	2 (10)	0	2 (22)	1 (11)	2 (22)	2 (22)	5 (36)	14 (18)
Palpitations	1 (5)	0	0	0	0	0	1 (7)	2 (3)
Sinus tachycardia	0	0	0	0	0	1 (11)	0	1 (1)
Tachycardia	0	0	0	0	0	0	1 (7)	1 (1)
Photophobia	0	0	0	0	0	0	1 (7)	1 (1)
Nausea	1 (5)	0	0	0	0	0	1 (7)	2 (3)
Chest discomfort	0	0	1 (11)	0	0	0	0	1 (1)
Fatigue	0	0	0	0	0	0	1 (7)	1 (1)
Hypersensitivity	0	0	0	0	0	0	1 (7)	1 (1)
Heart rate increase	0	0	0	0	1 (11)	0	0	1 (1)
Decreased appetite	0	0	0	0	0	0	1 (7)	1 (1)
Myalgia	0	0	0	1 (11)	0	0	0	1 (1)
Dizziness	0	0	0	0	0	0	1 (7)	1 (1)
Dizziness postural	1 (5)	0	0	0	0	0	1 (7)	2 (3)
Headache	2 (10)	0	0	0	0	1 (11)	3 (21)	6 (8)
Paresthesia	0	0	1 (11)	0	0	0	0	1 (1)
Alopecia	0	0	0	0	1 (11)	0	0	1 (1)
Pruritis	0	0	1 (11)	0	0	0	0	1 (1)
Rash	0	0	1 (11)	0	0	0	0	1 (1)

BID twice daily, *MAD* multiple ascending dose, *QD* once daily, *TEAE* treatment-emergent adverse event

Supplementary Table S6 Summary of zilurgisertib urine pharmacokinetic parameters following a single dose administration of zilurgisertib

Cohort	Dose (mg)	*N*	0–24 h		0–144 h		
			A_{e24} (nM)	F_{e24} (%)	A_{e144} (nM)	F_{e144} (%)	CL_R (L/h)
1	10	9	1590 ± 454	8.0 ± 2.3	2680 ± 707	13.5 ± 3.6	3.5 ± 1.6
			(1530 [33%])	(7.7 [33%])	(2590 [28%])	(13.0 [28%])	(3.3 [34%])
2	25	9	4420 ± 1030	8.9 ± 2.1	7250 ± 1390	14.6 ± 2.8	3.6 ± 1.1
			(4310 [25%])	(8.7 [25%])	(7130 [19%])	(14.3 [19%])	(3.5 [31%])
3	50	8[a]	11,200 ± 3230	11.3 ± 3.3	18,100 ± 4430	18.1 ± 4.5	4.8 ± 1.1
			(10,700 [35%])	(10.8 [35%])	(17,500 [28%])	(17.6 [28%])	(4.7 [25%])
4	100	7	17,800 ± 10,600	9.0 ± 5.4	30,300 ± 15,100	15.2 ± 7.6	3.7 ± 2.2
			(14,200 [99%])	(7.2 [99%])	(26,000 [76%])	(13.1 [76%])	(3.0 [91%])
5	175	9	37,600 ± 27,800	10.8 ± 8.0	64,800 ± 38,600	18.6 ± 11.1	3.6 ± 2.3
			(25,700 [140%])	(7.4 [140%])	(51,500 [94%])	(14.8 [94%])	(2.7 [100%])
6	250	9	64,600 ± 8230	13.0 ± 1.7	108,000 ± 9670	21.6 ± 2.0	4.4 ± 1.5
			(64,100 [13%])	(12.9 [13%])	(107,000 [10%])	(21.5 [10%])	(4.2 [32%])
7	500	6	132,000 ± 41,600	13.2 ± 4.2	213,000 ± 60,300	21.4 ± 6.1	4.3 ± 1.7
			(125,000 [40%])	(12.5 [40%])	(204,000 [35%])	(20.5 [35%])	(4.0 [46%])
Across all cohorts		57	–	10.5 ± 4.5	–	17.4 ± 6.5	4.0 ± 1.7
				(9.2 [63%])		(16.0 [49%])	(3.6 [55%])

A_{e24} amount of zilurgisertib excreted in urine in 24 h, A_{e144} amount of zilurgisertib excreted in urine in 144 h, CL_R renal clearance, *CV* coefficient of variation, F_{e24} percentage of zilurgisertib dose excreted unchanged in urine in 24 h, F_{e144} percentage of zilurgisertib dose excreted unchanged in urine in 144 h, *SD* standard deviation

Values for pharmacokinetic parameter summary are presented as mean ± SD (geometric mean [geometric %CV])

[a]One participant was excluded from cohort 3

Supplementary Table S7 Summary of zilurgisertib urine pharmacokinetic parameters on day 10 following multiple dose administrations of zilurgisertib

Cohort	Dose (mg)	*N*	A_e (nM)	F_e (%)	CL_R (L/h)
1	50 mg QD	9	29,000 ± 10,700	29.2 ± 10.8	4.8 ± 0.9
			(27,400 [36%])	(27.5 [36%])	(4.7 [19%])
2	100 mg QD	9	50,300 ± 31,600	25.3 ± 15.9	5.2 ± 1.7
			(38,300 [120%])	(19.3 [120%])	(5.0 [34%]) (*n* = 8)
3	150 mg QD	9	102,000 ± 32,100	34.3 ± 10.8	4.8 ± 1.3
			(98,700 [28%])	(33.1 [28%])	(4.7 [24%])
4	200 mg QD	9	131,000 ± 34,600	33.0 ± 8.7	4.5 ± 0.6
			(127,000 [28%])	(32.0 [28%])	(4.4 [15%])
5	400 mg QD	9	225,000 ± 43,200	28.3 ± 5.4	3.4 ± 0. 5
			(221,000 [19%])	(27.8 [19%])	(3.4 [14%])
6	300 mg BID	12	165,000 ± 68,100	27.6 ± 11.4	4.1 ± 1.1
			(144,000 [72%])	(24.1 [72%])	(3.9 [29%])
Across cohorts		57	–	29.5 ± 10.9	4.4 ± 1.2
				(26.7 [58%])	(4.3 [26%]) (*n* = 56)

A_e amount of zilurgisertib excreted in urine over the dosing interval (24 h for QD, 12 h for BID regimens), *BID* twice daily, CL_R renal clearance, *CV* coefficient of variation, F_e percentage of zilurgisertib dose excreted unchanged in urine over the dosing interval (24 h for QD, 12 h for BID regimens), *QD* once daily, *SD* standard deviation

Summary values are presented as mean ± SD (geometric mean [geometric %CV]). *N* is the number of participants. When there are fewer observations (*n*) than *N*, *n* is presented

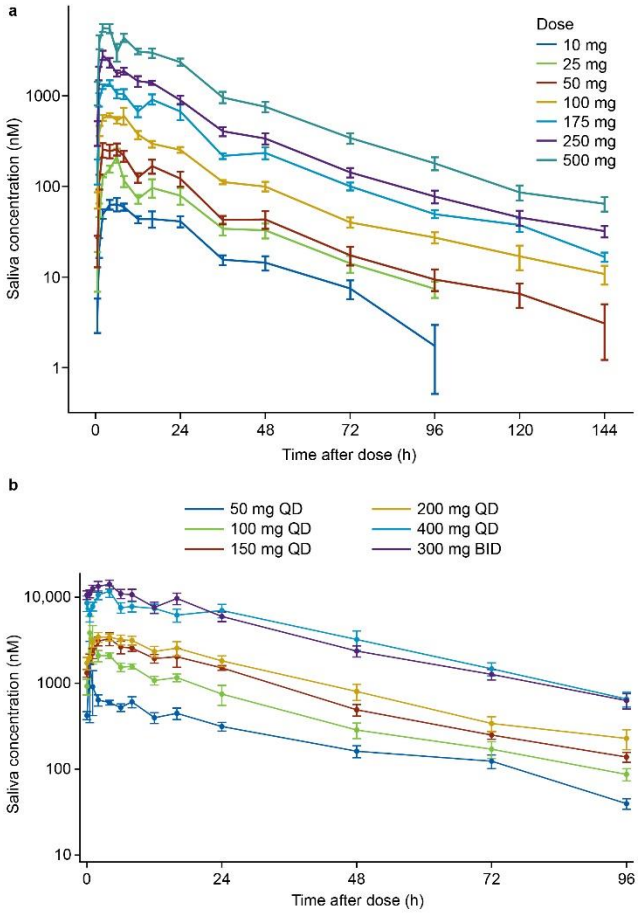
Supplementary Table S8 Summary of zilurgisertib saliva pharmacokinetic parameters on day 10 following multiple dose administrations of zilurgisertib

Cohort	Dose (mg)	*N*	C_{max} (nM)	t_{max} (h)	$AUC_{0\text{-}24}$ (h·nM)	$t_{1/2}$ (h)
1	50 mg QD	9	2040 ± 3970 (946 [135%])	4.0 (0.5–16.0)	11,900 ± 4930 (11,200 [38%])	24.5 ± 5.7 (23.9 [24%])
2	100 mg QD	8	4590 ± 5700 (3170 [92%])	3.0 (0.5–8.0)	33,200 ± 9210 (32,100 [28%])	23.4 ± 4.1 (23.1 [18%])
3	150 mg QD	9	4060 ± 1820 (3670 [52%])	6.0 (2.0–16.0)	52,500 ± 18,000 (49,700 [37%])	25.1 ± 7.3 (24.3 [27%])
4	200 mg QD	9	4180 ± 1200 (3980 [38%])	6.0 (2.0–24.0)	62,700 ± 20,000 (58,800 [44%])	22.3 ± 5.5 (21.7 [25%])
5	400 mg QD	9	12,900 ± 5950 (11,900 [43%])	4.0 (0.0–4.0)	182,000 ± 58,600 (172,000 [39%])	22.4 ± 4.6 (22.0 [18%])
6	300 mg BID	12	17,600 ± 5990 (16,700 [35%])	2.0 (0.0–8.0)	267,000 ± 93,800 (253,000 [35%])	22.6 ± 6.6 (21.8 [27%])
Across cohorts		56	–	4.0 (0.0–24.0)	–	23.3 ± 5.6 (22.7 [23%])
p values from a 1-factor ANOVA of log-transformed, dose-normalized data (factor = dose)						
Dose			0.3865	0.0351	0.0110	–
Inter-individual variability from a 1-factor ANOVA of log-transformed, dose-normalized data (factor = dose)						
Inter-individual variability (%CV)			74	–	38	–
Pairwise GMR (90% CI) from a 1-factor ANOVA of log-transformed, dose-normalized data (factor = dose)						
50 mg vs 100 mg			0.60 (0.35–1.02)	2.00 (0.00–5.48)	0.70 (0.52–0.94)	–
150 mg vs 100 mg			0.77 (0.45–1.33)	2.75 (0.00–5.48)	1.03 (0.77–1.39)	–
200 mg vs 100 mg			0.63 (0.37–1.08)	3.50 (0.00–7.48)	0.92 (0.68–1.24)	–
400 mg vs 100 mg			0.94 (0.55–1.61)	0.00 (−2.00, 2.00)	1.34 (1.00–1.81)	–

ANOVA analysis of variance, $AUC_{0\text{-}24h}$ area under the steady-state plasma concentration–time curve from 0–24 h, *BID* twice daily, *CI* confidence interval, C_{max} maximum plasma drug concentration, *CV* coefficient of variation, *GMR* geometric mean ratio, *QD* once daily, *SD* standard deviation, $t_{½}$ apparent terminal phase disposition half-life, t_{max} time to maximum plasma drug concentration

Values for pharmacokinetic parameter summary are presented as mean ± SD (geometric mean [geometric %CV]), except t_{max} values, which are median (minimum–maximum). Pairwise comparison for t_{max} is based on Hodges–Lehmann estimate with exact 90% CI. Only cohorts 1–5 with QD regimens were included in the dose-proportionality analysis using a power model. *p* value for t_{max} is from Kruskal–Wallis test

Supplementary Fig. S1 Mean ± SE zilurgisertib saliva concentration–time profile following **a** single dose administration of zilurgisertib or **b** multiple dose administrations of zilurgisertib (semi-log plot). *BID* twice daily, *QD* once daily, *SE* standard error



Supplementary Fig. S2 Correlations between zilurgisertib saliva and plasma concentrations following a single dose administration of zilurgisertib