Soticlestat, a novel cholesterol 24-hydroxylase inhibitor, reduces seizures and premature death in Dravet syndrome mice

Nicole A. Hawkins¹, Manuel Jurado¹, Tyler T. Thaxton¹, Samantha E. Duarte¹, Levi Barse¹, Tetsuya Tatsukawa², Kazuhiro Yamakawa², Toshiya Nishi³, Shinichi Kondo³, Maki Miyamoto³, Brett S. Abrahams⁴, Matthew J. During⁴, Jennifer A. Kearney¹*

¹Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL, 60611 USA

²Laboratory for Neurogenetics, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

³Neuroscience Drug Discovery Unit, Takeda Pharmaceutical Ltd., Fujisawa, Japan

⁴Ovid Therapeutics, New York, NY, 10036 USA
Supplementary Methods.

Hyperthermia-induced seizures.

\textit{Scn1a}^{RX+/} mice. To elevate core body temperature, mice were placed in a hermetically sealed plexiglass box (30×30×30 cm) with a perforated horizontal partition with a 9 x 9 grid of holes (2.5 mm diameter/each). Hot air was blown into the box using an Air-Therm (World Precision Instruments, Sarasota, FL). Rectal temperature was continually monitored at baseline and at seizure onset by an IT-18 temperature probe (Physitemp, Clifton, NJ). Before increment of air temperature, mice were acclimated to the box for at least 3 min at 37°C. Body temperature was then gradually elevated by 0.5°C every minute until seizure onset. When a seizure occurred, the mouse was promptly removed and returned to its home cage. Hyperthermia experiments occurred at 4-weeks of age from 10a.m. to 4p.m, Japan Standard Time.

\textit{Scn1a}^{+/-} mice. Core body temperature was continuously monitored with a rectal thermal probe and controlled with a feedback TCAT-2DF temperature controller (Physitemp) and heat lamp. Mice were acclimated to the testing chamber for 5 minutes. Body temperature was then slowly elevated by ~0.5°C per minute until a GTCS occurred or 42.5°C was reached. Once 42.5°C was achieved, it was maintained for 3 minutes. If no seizure occurred during the 3-minute hold, the mouse was considered seizure-free. Body temperature at the time of GTCS or maximum holding temperature was recorded for each mouse. Hyperthermia experiments were performed at P24-25 from 9a.m. to 3p.m, US Central Standard Time.

Continuous video monitoring. Spontaneous GTCS frequency was captured by continuous video monitoring using a Day/Night camera (Samsung SCB5003) equipped with an infrared lens (Tamron 13FG04IRSQ) and saved to a DVR (Samsung SRD-876D). During recording, mice had ad libitum access to food and water, and were maintained on a 14:10 light-dark cycle. \textit{Scn1a}^{+/-} mice were monitored from midnight the day of priming (12-16 hours post priming) for 14 consecutive days (336 hours) or until death occurred. Videos were scored offline by reviewers blinded to treatment to determine the frequency and severity of spontaneous GTCS using a modified Racine scale adapted for \textit{Scn1a}^{+/-} mice. To calculate seizure frequency for each subject, the total number of seizures was divided by the total hours monitored and then converted to a
daily seizure frequency (Seizures/day). The percentage of seizures with HLE was determined for each mouse based on presence or absence of HLE (Racine ≥5) for each GTCS event.

Open field and zero maze. For all experiments, mice were acclimated in the behavior suite with white noise for 1 hour prior to testing and male mice were always tested first. Females were tested separately with at least a one-hour delay after male sessions. Open field was completed first at P33-36 and then zero maze at P34-P37. After open field testing, mice were placed into a clean cage with their original littermates and vehicle or soticlestat chow until zero maze testing occurred the next day. For open field analysis, mice were placed in the center of a 56 x 56 cm arena and activity was tracked for 5 minutes using Limelight software (Actimetrics, Wilmette, IL). For zero maze analysis, mice were placed at the border of the open/closed region and activity was tracked for 5 minutes using Limelight software. Tracking files were analyzed for distance traveled and percentage of time spent in exposed parts of the maze using Limelight software (Actimetrics).

EEG Headmount Surgery. P17-18 Scn1a+/− mice were surgically implanted with prefabricated headmounts (Pinnacle Technology, Lawrence, KS) under ketamine:xylazine anesthesia. Anterior screw electrodes were 0.5-1 mm anterior to bregma and 1 mm lateral from the midline. Posterior screws were 4.5-5 mm posterior to bregma and 1 mm lateral from the midline. EEG1 channel represents recordings from right posterior to left posterior (interelectrode distance ~2 mm). EEG2 channel represents recordings from right anterior to left posterior (interelectrode distance ~5 mm). The left anterior screw served as the ground connection. Mice were treated with ketofen (5 mg/kg, SQ) perioperatively and once 24 hours post-surgery for analgesia. Scn1a+/− mice had 48-hour recovery period with ad libitum access to water and standard chow.
### Supplementary Table S1. Statistical comparisons

| Figure | Comparison | Test; Post Hoc | Value |
|--------|------------|---------------|-------|
| 1      | $Scn1a^{RX+}$ Temperature (A) | Student’s T-test | p<0.0001 |
|        | $Scn1a^{-/-}$ Temperature (B) | Welch’s T-test | p<0.04 |
| 2      | Seizure Proportion (B) | Fisher’s Exact | p<0.0001 |
|        | Seizure Frequency (B) | Mann-Whitney U test | p<0.0001 |
|        | Seizure Severity (C) | Chi-Square | p<0.0001 |
|        | HLE Frequency (D) | Mann-Whitney U test | p=0.0029 |
|        | Survival: Males (E) | Logrank Mantel-Cox | p=0.0011 |
|        | Survival: Females (E) | Logrank Mantel-Cox | p<0.0001 |
| 4      | Male Open Field (A) | One-way ANOVA; Tukey’s | F(2,85)=27.90, p<0.0001 |
|        | Female Open Field (A) | One-way ANOVA; Tukey’s | F(2,71)=8.791, p=0.0004 |
|        | Male Zero Maze (B) | One-way ANOVA | F(2,84)=2.243, p=0.1125 |
|        | Female Zero Maze (B) | One-way ANOVA | F(2,73)=0.473, p=0.6250 |
| 5      | Seizure Proportion (B) | Fisher’s Exact | p<0.03 |
|        | Seizure Frequency (B) | Mann-Whitney U test | p=0.0017 |
|        | Seizure Severity (C) | Chi-Square | p<0.0001 |
|        | HLE Frequency (D) | Mann-Whitney U test | p=0.0025 |
|        | Survival | Logrank Mantel-Cox | p<0.0037 |
Supplementary Figure S1. Seizure Diary Plots for Scn1a<sup>+/−</sup> treated with soticlestat or vehicle.

(A) Spontaneous GTCS diary plots for individual Scn1a<sup>+/−</sup> treated with vehicle control chow. Each row represents a single mouse over 14 day monitoring period or until occurrence of sudden, unexpected death. n=71. (B) Spontaneous GTCS diary plots for individual Scn1a<sup>+/−</sup> treated with 0.02% soticlestat chow. Each row represents a single mouse over 14 day monitoring period. n=48. Green circles represent subjects with no GTCS events. Purple tick mark, Severity- 1: Rearing and paddling, straub tail with no other movement. Orange tick mark, Severity- 2: Rearing and paddling, straub tail, loss of posture, short bursts of movement, often backwards. Grey tick mark, Severity- 3: Rearing and paddling with wild running and/or jumping (No loss of posture). Teal tick mark, Severity- 4: Rearing and paddling with wild running and/or jumping with loss of posture. Pink tick mark, Severity- 5: Rearing and paddling with wild running and/or jumping with loss of posture, ending in tonic hindlimb extension (HLE). Black triangle, Severity- 6: Rearing and paddling with wild running and/or jumping with loss of posture, ending in tonic hindlimb extension (HLE) and death.
Supplementary Figure S2. Seizure Diary Plots for *Scn1a*+/− treated with soticlestat or vehicle and monitored by video-EEG.

(A) Spontaneous electrographic GTCS diary plots for individual *Scn1a*+/− treated with vehicle control chow. Each row represents a single mouse over 7-day monitoring period or until occurrence of sudden, unexpected death. n=22. (B) Spontaneous electrographic GTCS diary plots for individual *Scn1a*+/− treated with 0.02% soticlestat chow. Each row represents a single mouse over 7-day monitoring period. n=20. Green circles represent subjects with no GTCS events. Purple tick mark, Severity- 1: Rearing and paddling, straub tail with no other movement. Orange tick mark, Severity- 2: Rearing and paddling, straub tail, loss of posture, short bursts of movement, often backwards. Grey tick mark, Severity- 3: Rearing and paddling with wild running and/or jumping (No loss of posture). Teal tick mark, Severity- 4: Rearing and paddling with wild running and/or jumping with loss of posture. Pink tick mark, Severity- 5: Rearing and paddling with wild running and/or jumping with loss of posture, ending in tonic hindlimb extension (HLE). Black triangle, Severity- 6: Rearing and paddling with wild running and/or jumping with loss of posture, ending in tonic hindlimb extension (HLE) and death.