Na\(_{\text{v}1.1}\) Selective Potentiators Normalize Inhibition/Excitation Imbalance and Prevent Seizures in a Mouse Model of Dravet Syndrome

**Background**

- Loss-of-function variants of **SCN1A** cause Dravet Syndrome (SMI) or EIEE1 and generalized epilepsy with febrile seizures plus (GEFS+) by decreasing Na\(_{\text{v}1.1}\) expression or conductance in inhibitory interneurons. The resulting hypo-excitability of interneurons reduces inhibitory input on excitatory neurons and leads to epilepsy and developmental delays.
- A precision medicine therapy for Dravet Syndrome should restore Na\(_{\text{v}1.1}\) activity specifically without impacting other neuronal proteins, especially ion channels.
- There are pursuing brain penetration-assisted small-molecule potentiators of Na\(_{\text{v}1.1}\) currents to allow oral dosing and titration of the Na\(_{\text{v}1.1}\) current levels in all brain areas.
- We believe that such potentiators can directly address the underlying etiology of Dravet Syndrome and thus provide a potentially disease modifying therapy for Dravet Syndrome.

**Methods**

- Voltage clamp electrophysiology was used to assess the potency and selectivity of compounds in HEK6 cells stably expressing Na\(_{\text{v}1.1}\). The expression of sodium channel was determined by measuring the increase in charge carried over 10 ms. Availability curves were generated by assessing current at test pulse following 500 ms prepulse to -120 to 0 mV in 10 mV steps. Error bars are ±SEM.
- Animals: Na\(_{\text{v}1.1}\) mix and wildtype (WT) littermates were generated as described previously.
- Brain Slice Preparation: 400 µm parasagittal cortical brain slices were prepared from >P21 mice using standard procedures.
- Electrophysiological recordings in brain slices: Whole-cell current-clamp recordings were made in cortical layer 5. Fast-spiking interneurons were identified by their characteristic fast-spiking pattern. sIPSCs and sEPSCs were recorded from layer 5 pyramidal cells in presence of NBQX/AP5 and Gabazine at HP of 20 mV and -70 mV respectively in voltage-clamp. Error bars are ±SEM.
- Na\(_{\text{v}1.1}\) 6 Hz seizure model: Seizures were induced in 20-22 days-old Na\(_{\text{v}1.1}\) mix by a 6 Hz stimulus for 3 seconds delivered through corneal electrodes and the C57Bl/6 was used to assess the potency and selectivity of compounds. Mice were placed in a chamber to monitor the presence of a seizure characterized by jaw clonus, forelimb clonus, Straub tail and loss of balance. An animal was considered “protected” if none of these 4 behaviors were observed. Binary seizure data were assessed with simple logistic regression to determine the potential of each compound to prevent seizures in vivo. The Na\(_{\text{v}1.1}\) potentiation compound XPC-8770 improved Rotarod performance supporting the potential efficacy of this mechanism in non-seizure related symptoms. The Na\(_{\text{v}1.1}\) potentiation profile provides a new, mechanistically distinct class of voltage-gated sodium channel compounds with the potential to provide an improved therapeutic profile for the overall treatment of Dravet Syndrome.

**Results**

**Potency, Selectivity and Mechanism of Action (MOA) of Na\(_{\text{v}1.1}\) Potentiator CPD's**

- XPC-8770 and XPC-7523 are representative compounds from two chemical subseries.
- The compounds selectively potentiate heterologously expressed Na\(_{\text{v}1.1}\) channels and spares potentiation and abolishing inhibition of channels Na\(_{\text{v}1.6}\) and Na\(_{\text{v}1.2}\) and Na\(_{\text{v}1.6}\) and cardiac channel Na\(_{\text{v}1.5}\).
- The compounds slow open state fast inactivation and increase sodium influx upon depolarization.
- These Na\(_{\text{v}1.1}\) potentiators destabilize steady state inactivation and increase channel availability across a range of potentials close to neuronal resting membrane potentials.
- No effects on the voltage dependence of activation are observed.
- Compounds induced ramp currents that are related to increasing neuronal excitability.

**Na\(_{\text{v}1.1}\) Potentiator Compounds Selectively Increase Firing Rate of Scn1a\(^{-/-}\) Inhibitory Neurons**

- In brain slices from Scn1a\(^{-/-}\) mice, the area under the curve (AUC) for current injections >160 pA was significantly increased in XPC-8770 indicating a higher firing frequency of fast spiking inhibitory interneurons (sIPSCs, Unpaired t-test).
- No significant effects were seen when XPC-8770 was applied to WT inhibitory interneurons.

**XPC-8770 Normalizes Spontaneous Post Synaptic Inhibitory and Excitatory Currents in Scn1a\(^{-/-}\) Neurons**

- Scn1a\(^{-/-}\) mice display lower sIPSC frequency and higher sEPSC frequency and higher than WT (unpaired t-test).
- XPC-8770 significantly increases sEPSC frequency (Two-way ANOVA, Tukey) and reduces sIPSC activity toward WT levels (unpaired t-test).

**Conclusion**

- XPC-8770 and XPC-7523 are CNS penetrant, high Na\(_{\text{v}1.1}\) selective small-molecule potentiators that impair fast inactivation, increase channel availability and increase Na\(_{\text{v}1.1}\) flux upon depolarizing inputs.
- This MOA increases impaired Na\(_{\text{v}1.1}\) interneuron excitability and normalizes excitation/inhibition imbalance in Scn1a\(^{-/-}\) mice.
- The Na\(_{\text{v}1.1}\) potentiators demonstrate target engagement in vivo by preventing seizures in a Scn1a\(^{-/-}\) 6 Hz target engagement seizure model.
- The Na\(_{\text{v}1.1}\) potentiation compound XPC-8770 improved Rotarod performance supporting the potential efficacy of this mechanism in non-seizure related symptoms.
- The Na\(_{\text{v}1.1}\) potentiation profile provides a new, mechanistically distinct class of voltage-gated sodium channel compounds with the potential to provide an improved therapeutic profile for the overall treatment of Dravet Syndrome.

**References**