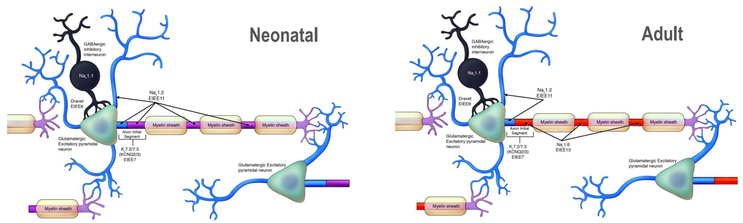
XEN393, A Novel Selective Dual Inhibitor of $Na_V1.2/Na_V1.6$ Channels Prevents Electrically-Induced Seizures in Mice and Rats

James Empfield, Parisa Karimi Tari, Girish Bankar, Nina Weishaupt, Samuel J. Goodchild, Kuldip Khakh, Michael Wilson, Thilo Focken, Alison J. Cutts, Charles J. Cohen, J.P. Johnson Jr.

Xenon Pharmaceuticals Inc., 3650 Gilmore Way, Burnaby, BC, Canada

BACKGROUND

- During early development $Na_V1.2$ is the major voltage-gated sodium channel isoform in the axons of excitatory CNS neurons. As animals, including humans, mature, the isoform distribution changes to contain a higher fraction of $Na_V1.6$ in addition to $Na_V1.2$.



- Xenon is currently developing a selective inhibitor of $Na_V1.6$ (XEN901) for epilepsy based on the hypothesis that nonselective sodium channel inhibitors such as carbamazepine/oxcarbazepine, lacosamide, and phenytoin would be better therapeutics if they inhibited action potential firing in excitatory neurons (primarily driven by $Na_V1.2$ and $Na_V1.6$ channels) without inhibiting firing of inhibitory interneurons (primarily $Na_V1.1$ channels) and also avoiding potential cardiac liabilities by not blocking $Na_V1.5$.
- Because of the dynamic nature of sodium channel expression during development, some patients, particularly very young patients in the first months of life, may benefit more from a dual acting drug that blocks both $Na_V1.2$ and $Na_V1.6$ than from a purely selective inhibitor of $Na_V1.6$. Specifically, $Na_V1.2$ gain-of-function patients (EIEE11) would likely benefit from such a compound.
- Sparing block of $Na_V1.1$ and $Na_V1.3$ is anticipated to be a benefit for patients as these channels drive firing of inhibitory interneurons in adults and neonates, respectively. Based on this theoretical framework we have created a dual $Na_V1.2/Na_V1.6$ inhibitor (XEN393) that has drug-like properties and effectively prevents electrically-induced seizures[2,3] in mice and rats.

$Na_V1.2/1.6$ dual inhibitor has opportunities in numerous "top 10" childhood genetic epilepsies[1]

Gene	Inheritance	Positive Cases	% of positive cases in dataset
SCN1A	AD	322	24.8
KCNQ2	AD	159	13.2
CDKL5	XL	99	7.6
SCN2A	AD	96	7.4
PRRT2	AD	59	7.2
PCDH19	XL	74	5.7
STXBP1	AD	61	5.1
SLC2A1	AD	47	3.6
GABRG2	AD	25	3.6
SCN8A	AD	30	3.6

METHODS

- We created a novel small molecule sodium channel inhibitor, XEN393, with selectivity for $Na_V1.2$ and $Na_V1.6$ over the other sodium channel isoforms.
- *In vitro*, Patch-clamp electrophysiology was employed to measure the potency and selectivity of XEN393 in voltage-gated sodium channel isoforms.
- *Ex vivo* brain slice electrophysiology was employed to assess the inhibition of action potentials generated through applied current to both excitatory and inhibitory neurons for a related analog.
- *In vivo*, we evaluated the ability of orally administered XEN393 to prevent electrically induced seizures in multiple electrically induced rodent models[2,3]. Wild type CF-1 mice were employed in two assays that evoked a tonic-clonic seizure with hind-limb extension in 90% of vehicle treated mice, one using a direct current stimulation and another using alternating current stimulation. Analogous experiments were repeated in rats.

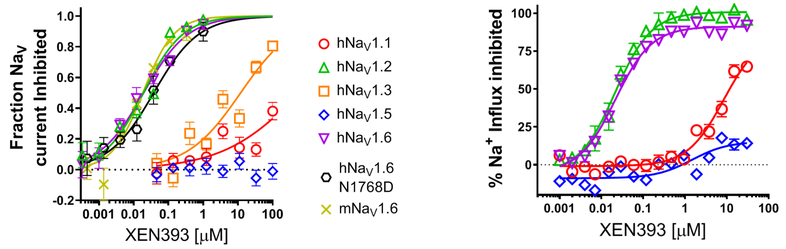
RESULTS

XEN393 has good drug-like properties, low off-target activity, and low risk of drug-drug interactions

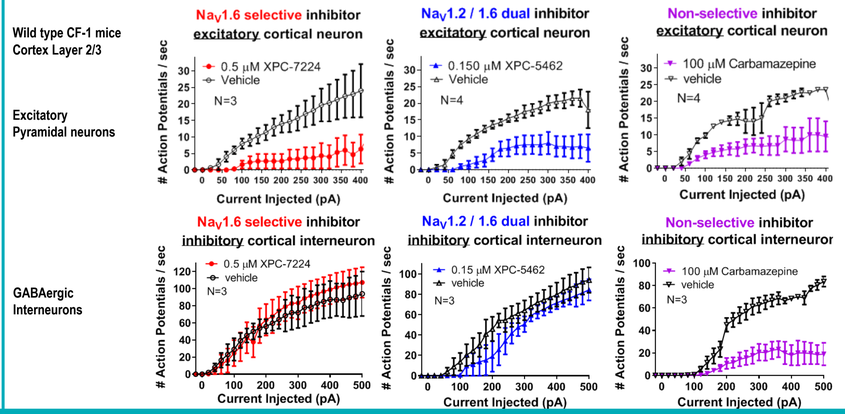
H = Human; R = Rat; M = Mouse; D = Dog; C = Cyno		XEN393
Microsomes Cl_{hep} H / R / M / D / C		**4.7 / 31 / 26 / 10 / 12**
Hepatocytes Cl_{hep} H / R / M / D / C		**9.3 / 45 / 59 / 1.1 / 12**
MDR1 Pgp: P_{app} / A to B Ratio		**9.9 / 5.2**
Plasma Protein Binding H / R / M / D (%)		**98.7 / 76.9 / 66.7 / 81.1**
Brain Homogenate Binding H / R / M / D (%)		**- / 79.3 / 82.6 / 76.9**
Safety	Gal EC_{50} (μM) / ratio	**>100 / 1**
	miniAmes	**negative**
	hERG IC_{50} (μM)	**>30**
DDI	TDI (3A4) ratio	**1.7-1.9**
	PXR EC_{50} (μM)	**>20**
PK	B/P mouse / rat / dog	**0.34 / 0.12 / 0.44**
	Rat iv $T_{1/2}$ / Cl / V_d	**6.3 / 22.1 / 12.0**
	Dog iv $T_{1/2}$ / Cl / V_d	**1.3 / 6.6 / 0.8**

- Good permeability , good CNS penetration and predicted human half life of 9.6 hours
- Measureable plasma free fraction in all species
- Good metabolic stability in human liver microsomes and hepatocytes
- AMES negative (low risk of mutagenicity)
- >30 μM on HERG
- No PXR activity (low risk of CYP enzyme induction)
- Low time dependent inhibition of CYP enzymes & Low mitochondrial tox risk (low liver tox risk)

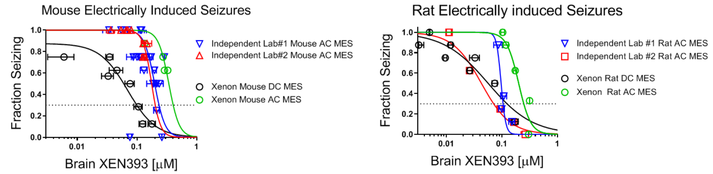
In Vitro Results: Concentration-Response Curves for XEN393 Across Sodium Channel Isoforms



Ex Vivo Results: Selective Inhibitors (Close analogs of XEN901 & XEN393) Spare Interneuron Firing While Carbamazepine Does Not



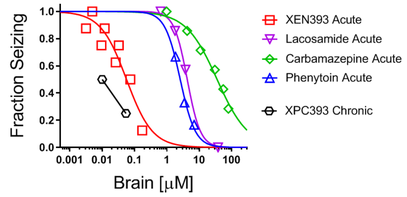
In Vivo Results: XEN393 prevented electrically-induced seizures invoked in both mice & rats with both direct or alternating current



- The brain levels required for protection in mice stimulated with alternating current (EC_{70} ~ 0.3 μM) were slightly higher than in rats or in mice stimulated with direct current (EC_{70} ~ 0.1 μM).

EC_{70} Values for XEN393 in Electrically Induced Rodent Seizure Models			
In Vivo Seizure Model	Brain EC_{70} (μM)	*In Vivo* Seizure Model	Brain EC_{70} (μM)
Mouse DC MES acute	0.1	Rat DC MES acute	0.13
Mouse DC MES chronic	0.03	Rat DC MES chronic	0.036
Mouse AC MES acute	~ 0.4*	Rat AC MES acute	0.25
Mouse AC MES acute (Independent lab#1)	0.23	Rat AC MES acute (Independent lab#1)	0.1
Mouse AC MES acute (Independent lab#2)	~ 0.20*	Rat AC MES acute (Independent lab#2)	0.07

*Approximate value based on limited data points



All data from Xenon DC MES assay in WT SD rats

XEN393 is Markedly More Potent *in vivo* than Non-Selective Sodium Channel Inhibitor Anti-Seizure Medications (ASMs)

CONCLUSIONS

- XEN393 provides a novel, mechanistically differentiated profile distinct from XEN901 and all marketed non-isoform selective sodium channel blockers.
- XEN393 selectively inhibits $Na_V1.2$ and $Na_V1.6$ channels with >600-fold selectivity over the other sodium channel isoforms including those critical for inhibitory neuron ($Na_V1.1$) and cardiac ($Na_V1.5$) function.
- Brain concentrations of XEN393 that block the target channels were well tolerated and prevented electrically induced seizures in mice and rats.
- This work enables the testing of the hypothesis that selective dual $Na_V1.2/Na_V1.6$ inhibitors, such as XEN393, that spare $Na_V1.1$, $Na_V1.3$ and $Na_V1.5$ may provide a new class of more effective and better tolerated seizure prevention ASMs in clinical practice.
- Xenon is progressing a dual, selective $Na_V1.2/1.6$ inhibitor into IND-enabling toxicology studies.

[1]Lindy, A.S. *et al*. Diagnostic outcomes for genetic testing of 70 genes in 8565 patients with epilepsy and neurodevelopmental disorders. *Epilepsia*. 2018; 59(5):1062-1071

[2] Löscher W. *et al*. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. *Epilepsy Research*. 1991; 8(2):79-94.

[3] White H.S. *et al*. The early identification of anticonvulsant activity: role of the maximal electroshock and subcutaneous pentylenetetrazol seizure models. *Italian Journal of Neurological Sciences*. 1995;16(1-2):73-77.

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