Dr. Manoj Patel is an Associate Professor in the Dept. of Anesthesiology at the University of Virginia.

A neuroscientist and electrophysiologist by training, Dr. Patel’s lab has developed isoform-specific sodium channel inhibitors for the suppression of epileptic seizures in collaboration with Dr. Mirko Rivara a medicinal chemist in the Dept. of Pharmacy at the University of Parma (Italy).
Epilepsy

- Epilepsy, characterized by unpredictable seizures, is the 4th most common neurological disorder, and affects people of all ages
  - 1 in 26 people will develop epilepsy at some time
  - >3 million adults w/ active epilepsy in US

- Epilepsy is a spectrum condition with a wide range of seizure types and control varying from person-to-person
  - No cure for epilepsy – seizures can only be treated pharmacologically
  - Unfortunately, 30% of patients are refractory to current medications
Early infantile epileptic encephalopathy 13 (EIEE13) is caused by heterozygous mutations in the SCN8A gene on chromosome 12q13. SCN8A encodes for the voltage-gated sodium channel alpha subunit Nav1.6. Nav1.6 is localized in high concentrations on excitatory pyramidal cells at the axon initial segment and at nodes of Ranvier where it mediates action potential initiation and propagation.

Sodium channel alpha subunits form the ion conduction pore. Family of sodium channels have nine (9) known members (proteins are named Nav1.1 through Nav1.9; genes are named SCN1A through SCN11A).

SCN8A mutations were first implicated in early infantile epileptic encephalopathy 13 (EIEE13) in 2012 by Michael Hammer, PhD a geneticist who found a mutation in his own daughter.

Pathogenic SCN8A mutations tend to be gain-of-function, heterozygous dominant mutations (often de novo) that lead to neuronal hyperexcitability. Seizure generally appear between birth and 18 months of age; the mean age of onset is 5 months. In addition to seizures, other neurodevelopmental disorders are associated with EIEE13, as well as an increased risk of SUDEP (severe unexplained death in epilepsy patients).
EIEE13 patients are often refractory to treatment with available drugs.

EIEE13 may make up to 1% of all epilepsies, so while rare it is not uncommon.

Figure legend: **Positions of missense mutations of SCN8A in epileptic encephalopathy.** The four homologous domains of Nav1.6 (DI to DIV) each contain six transmembrane segments (S1 to S6). The large inter-domain cytoplasmic loops 1 and 2 are evolutionarily less well-conserved than the transmembrane and linker domains. Loop 3 functions as the inactivation gate and is very highly conserved. Closed symbols, EIEE13 mutations in a single patient; open symbols, recurrent mutations.
Data on ability of early compounds (MV1062 and MV1066) to block acute seizures through the NIH National Institutes of Neurological Disorders and Stroke’s (NINDS) Epilepsy Therapy Screening Program.

The maximal electroshock seizure (MES) and subcutaneous Metrazol (sc Met) tests have become the two most widely employed preclinical seizure models for the early identification and high-throughput screening of investigational antiepileptic drugs. These tests are extremely effective in identifying new antiepileptic drugs that may be useful for the treatment of human generalized tonic-clonic seizures and generalized myoclonic seizures.

6Hz “Psychomotor” Seizure test - therapy-resistant model: psychomotor seizures induced by long-duration (3 sec), low frequency stimulus delivered through corneal electrodes.

MV1062 and MV1066 both showed promising results in the therapy-resistant model.

No toxicities were observed for MV1062 and MV1066, the numbers indicate the highest amount tested.

Data are compared to currently used medications: phenytoin (Dilantin), Lamotrigine
(Lamictal), carbamazepine (Tegretol), valproic acid and ethosuximide (Zarontin).
Bolstered by the data generated through the Epilepsy Therapy Screening Program that the family of compounds were effective in therapy-resistant seizure models they furthered their efforts to develop isoform-specific sodium channel inhibitors.

Successive rounds of design, synthesis and testing by Drs. Patel and Rivara have yielded several generations of sodium channel inhibitors and identified candidates that selectively target sodium channel isoform Na_v,1.6.

Early lead candidates include the novel compounds MV1502, MV1504, and MV1505.
Early lead candidates MV1502, MV1504, and MV1505 inhibit currents in HEK293 cells that stably express Na$_v$1.6 as measured by patch clamp electrophysiology. Figures depict dose responses and activation of time curves.

IC$_{50}$ values were 1.2µM (MV1502), 12µM (MV1504) and 5.4µM (MV1505).
Electrophysiology characterization of MV1504 (10mM) shows that it is a state-dependent blocker with an affinity for the closed/inactivated confirmation of the sodium channel.
Using a fluorescence-based high-throughput assay, a collaborator showed that several compounds (including MV1502, MV1504, and MV1505) were selective for \( \text{Na}_v 1.6 \) (both human and rat) over \( \text{Na}_v 1.1 \) and \( \text{Na}_v 1.5 \).

\( \text{Na}_v 1.1 \) is associated with inhibitory neurons. Loss-of-function mutations cause severe myoclonic epilepsy of infancy (Dravet’s Syndrome).

\( \text{Na}_v 1.5 \) is primarily found in cardiac muscle.
A clinical stage biopharmaceutical company, working to commercialize their own line of selective channel inhibitors (for other indications), offered to screen the lead compounds on their high throughput instruments. Compound were shown to be selective for Na\textsubscript{v}1.6 over Na\textsubscript{v}1.5 and Na\textsubscript{v}1.7 using two different assays, a flux-based assay and a Qube automated patch clamp instrument.

Na\textsubscript{v}1.5 is primarily found in cardiac muscle.

Na\textsubscript{v}1.7 is expressed in high levels in the nociceptive (pain) neurons and in the sympathetic ganglion neurons (involuntary nervous system).
A knock-in mouse carrying the patient mutation N1768D reproduces many features of EIEE13, including spontaneous seizures and SUDEP.
MV1505 reduces the frequency of seizures in Naᵥ1.6 N1768D knock-in mice. Seizure frequency is measured by a headset placed on each mouse; headsets can become lost during severe seizures. Each graph represents a different mouse. Seizures return when drugs are withdrawn, eventually leading to SUDEP.
MV1502 reduces the frequency of seizures in Na$_v$1.6 N1768D knock-in mice. Seizures return when drugs are withdrawn, eventually leading to SUDEP.
Summary

- Drs. Patel and Rivara have developed a series of subtype-selective sodium (Na) channel inhibitors that are selective for Na\(_{\alpha\text{1.6}}\).
- *In vitro* studies support that the compounds are effective against Na\(_{\alpha 1.6}\).
- *In vivo* studies support that the compounds have the ability to penetrate the blood-brain barrier and inhibit seizures.
The first non-provisional patent application covering these isoform-selective sodium channel inhibitors was filed earlier this year (January 2018). Patents issued claiming priority to this application could have patent life out to 2038.

A patentability search by an outside firm prior to filing revealed novel compounds. Composition of matter claims on those novel compounds are being pursued, including the current leads MV1502, MV1504 and MV1505.

Method of use claims cover a much broader family of sodium channel inhibitors.