Recent progress in sodium channel modulators for pain

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A B S T R A C T

Voltage-gated sodium channels (Na₉s) are an important family of transmembrane ion channel proteins. Structurally, they are members of the 6-TM ion channel family and are composed of a transmembrane α-subunit of approximately 260 kDa and several associated transmembrane β-subunits of lower molecular weight. The family comprises nine members Na₉.1.1-Na₉.1.9 and related Na₉. Na₉s are involved in Na⁺ ion conduction across cell membranes during cell membrane depolarization. If cell membrane depolarization reaches a threshold value Na₉ channels open to allow Na⁺ ions to flow into cells. This movement creates action potentials and nerve impulses in electrically excitable cells, for example neurons, and affects many functions for example the peripheral and central nervous system, cardiac and skeletal muscle etc. Blockade of Na₉s has been successfully accomplished in the clinic to enable control of pathological firing patterns that occur in a diverse range of conditions such as chronic pain, epilepsy, and cardiac arrhythmias. In this review we will discuss recent advances in the field of Na₉ drug discovery for the treatment of pain. In particular progress in biology, structural biology, assay technology, inhibitor development and clinical success will be discussed.

Sodium channel topology and structure: Structural studies of 6TM-topology ion channels date back to the late 1980s, when Numa et al. successfully cloned α-subunits of sodium channels.1,2 According to sequence analysis, Na₉ α-subunits are composed of approximately 2000 amino acids which assemble into four distinct domains (D1–D4), each of which consists of 6TM α-helices (S1–S6) (Fig. 1). Two helices (S5–S6) from each domain contribute towards formation of the channel pore which is responsible for Na ion conduction. The S1–S4 helices from each domain form a voltage sensor which works as a sensor of change in voltage across the cell membrane. Consequently, the pore is formed from eight helices and there are four voltage sensors surrounding the pore. Between the pore-forming helices (S5–S6) from each domain there is an extended P-loop which acts to form the Na⁺ ion selectivity filter. Other voltage-gated channels, K₆ and C₆, share common topology and mechanism of activation with the Na₉ class.3

Recently, structural understanding of this class of ion channels has been drastically improved due to the publication of site directed mutagenesis work and several crystal structures; a K₆.1.2/2.1 chimera crystal structure by MacKinnon4 and multiple Na₉Ab bacterial sodium channel apo crystal structures from Catterall.5,6 Interestingly, these structures complement each other by appearing to sample several states of the Na₉Ab channel. By comparison of the structural differences these structures provide valuable insight into gating mechanisms and conformational changes which occur to open the channel pore to ion flux.5 These studies also highlight fenestration regions within the channel pore that may constitute potential binding sites for small molecule modulators. However, to date there have been no reported co-crystal structures of any sodium channel proteins with small molecule or toxin molecules bound.
To further complement these recent advances in X-ray crystal structures, the field is also benefiting from advances in computational modeling and molecular dynamics to understand the opening/closing mechanism of voltage-gated channels. For example, a publication from Jensen et al., which describes micro second order molecular dynamics for the related KV$_{1.2/2.1}$ channels, suggests that these channels close with a hydrophobic collapse of a pore residue, which is followed by movement of the voltage-sensor domain towards the pore. Conversely, in the opening process, the voltage sensor moves outward first, which pulls the pore domain to open.

Overall, the combination of structural, mutagenesis and computational studies have been used to build an understanding of Nav channel conformational gating and function. At a simplistic level, Nav channels are believed to exist in three states characterized by conduction behavior at different voltage potentials: open, closed (resting) and inactivated. At a resting trans-membrane potential, the channels are in a non-conducting closed state. When membrane potential is decreased (depolarization) the voltage sensors (S1–S4) move outward in a rotational movement, pulling the pore open for a short period (<1 ms). Subsequently the channel then moves via a combination of fast- or slow-inactivation processes into a non-conductive inactivated state. It is currently believed that a specific loop between D3 and D4 termed the inactivation gate is responsible for fast inactivation. Finally, an increase in membrane potential (hyperpolarization) causes the Nav channels to return to the resting state.

Nav channel genetics and biology: Nav channels as pain targets gained traction with the recognition that some Na$_v$ subtypes showed preferential or exclusive expression in peripheral sensory neurons. While peripheral sensory neurons express nearly all Na$_v$ subtypes at some level, there are three subtypes, Na$_v$1.7, Na$_v$1.8, and Na$_v$1.9 which are enriched in peripheral neurons of the trigeminal and dorsal root ganglia (DRG). Of these, Na$_v$1.7 is the most broadly expressed in DRG neurons, while expression of Na$_v$1.8 and Na$_v$1.9 is largely restricted to the subset of small and medium neurons which are thought to represent the majority of nociceptors. The role of Na$_v$1.7, Na$_v$1.8, and Na$_v$1.9 in pain signaling has been well established in pharmacological, molecular and genetic studies with preclinical species. Human validation of these targets had lagged behind the animal studies until the discovery that inherited erythromelalgia (IEM) is causally linked to missense mutations in Na$_v$1.7. Since this discovery, the family of Na$_v$ channelopathies has expanded in two important areas. First, the link between Na$_v$1.7 variants and clinical pain syndromes now includes a subset of idiopathic small fiber neuropathies, a disorder which affects millions of patients. Second, additional Na$_v$ related channelopathies have been discovered which are causally linked to Na$_v$1.8 and Na$_v$1.9. These studies provide evidence for the contribution of each subtype of peripherally expressed Na$_v$ channel to pain signaling.

Na$_v$1.7: The first Na$_v$ channelopathy linked to pain to be identified was inherited erythromelalgia (IEM). IEM is characterized by extreme burning pain, typically in the distal extremities, which is triggered by moderate increases in temperature. Electrophysiological characterization of the mutant channels revealed a gain-of-function phenotype in channel activation, consistent with the pathological sensitivity to heating. To date, twenty SCN9A variants have been linked to IEM and have consistently shown a gain of function phenotype and demonstrated the ability to produce

Figure 1. (a) Na$_v$ channel structural topology. Domains D1–D4 are represented in different colors while β subunits are shown in gray. Transmembrane segments (S1–S6) labelled together with graphical representation of P-loops. (b) Side view; (c) Top view of voltage-gated sodium channel from the bacterium Arcobacter butzleri (Na$_v$Ab–PDB code 3RVY) with highlighted postulated toxin binding sites (1–6), local anesthetic binding site and significant structural features.
hyperexcitability in dorsal root ganglion (DRG) neurons. A second rare genetic disease, paroxysmal extreme pain disorder (PEPD), has also been linked to SCN9A gene mutations. PEPD also produces a burning pain accompanied by flushing of the skin but is considered clinically distinct from IEM. Electrophysiological characterization of PEPD mutations similarly revealed a gain-of-function phenotype but they disrupt fast-inactivation of Na\(_{\text{v}}\)1.7, preventing the channel from closing properly, and produce prolonged flow of sodium current and resurgent currents upon repolarization.

Following the discovery of a causal link between Na\(_{\text{v}}\)1.7 function and disorders of enhanced or aberrant pain, another rare disorder, congenital insensitivity to pain (CIP), was linked to mutations of SCN9A. These recessive mutations result in a complete loss of functional Na\(_{\text{v}}\)1.7 channels and an apparent complete loss of ability to sense painful stimuli. Affected individuals report no sensation of pain which can often lead to a significant negative impact on overall health. The most recent pain related disorder to be linked to SCN9A is idiopathic small fiber neuropathy (I-SFN), a common adult-onset pain disorder. The family of small-fiber neuropathies is extensive, potentially affecting upwards of 40 million Americans, with nearly 50% of cases classified as idiopathic. Na\(_{\text{v}}\)1.7 variants have been found in 28% of a group of patients diagnosed with idiopathic small fiber neuropathy. Electrophysiological characterization of these mutant channels also showed a gain of function affecting either activation or inactivation, resulting in increased excitability of DRG neurons.

Na\(_{\text{v}}\)1.8: The tetrodotoxin resistant Na\(_{\text{v}}\)1.8 channel has been shown to be the primary contributor of sodium flux during the upstroke of the action potential of DRG neurons. In follow-up studies of I-SFN, a group of patients negative for mutations of the SCN9A gene were subjected to exome sequencing of the SCN10A gene which encodes the Na\(_{\text{v}}\)1.8 channel. Three mutations were identified which had a high likelihood of altering Na\(_{\text{v}}\)1.8 function. Two of these mutations, L554P and A1304T, were shown to enhance recovery from fast inactivation and hyperpolarize the voltage dependence of activation, respectively. As with Na\(_{\text{v}}\)1.7, these biophysical changes were predicted to be pro-excitatory and both channels were subsequently demonstrated to produce hyperexcitability of DRG neurons.

Na\(_{\text{v}}\)1.9: The three most recently discovered Na\(_{\text{v}}\) channelopathies are associated with mutations of the Na\(_{\text{v}}\)1.9 channel. Na\(_{\text{v}}\)1.9 is biophysically distinct from other Na\(_{\text{v}}\) channels, displaying slow activation and inactivation properties. Consequently, Na\(_{\text{v}}\)1.9 does not directly contribute to the action potential waveform, but is thought to provide a variable background Na conductance that is important for determination of resting membrane potential and membrane excitability.

Two large Chinese families with episodic pain, primarily of the distal extremities, were found to harbor missense mutations within the SCN11A gene. Electrophysiological studies of the mutant channels showed no significant alteration of activation or inactivation properties but did reveal enhanced current amplitude when compared to the wild type Na\(_{\text{v}}\)1.9 channel. A different, novel Na\(_{\text{v}}\)1.9 missense mutation results in gain of channel function but gives a seemingly confusing clinical phenotype of complete insensitivity to pain. Despite the Na\(_{\text{v}}\)1.9 gain of function, the loss of pain sensitivity phenotype is presumed to arise from inactivation of Na\(_{\text{v}}\) channels responsible for action potential propagation resulting in an inability to sustain firing and conduction block. A recent study involving three hundred and forty five patients with peripheral neuropathy but without mutations in SCN9A and SCN10A identified missense mutations of Na\(_{\text{v}}\)1.9 as a cause of painful peripheral neuropathy. Eight variants of SCN11A were identified in twelve patients. Functional profiling by electrophysiological recordings showed that these Na\(_{\text{v}}\)1.9 mutations confer gain-of-function attributes to the channel, depolarize resting membrane potential of DRG neurons, enhance spontaneous firing, and increase evoked firing of these neurons.

**Sodium channel toxin-derived modulators:** Numerous natural toxins and synthetic derivatives are known to modulate sodium channels. These include both polar small molecule toxins and a variety of peptide-based venom toxins. Interestingly these toxins cover a wide range of structural classes and have been isolated from many different animal sources including fish, shellfish, spider, snail, scorpion and centipede venoms. All known sodium channels modulators are believed to act via binding to sites on the \(\alpha\)-subunits. Currently there are at least six different binding sites known for toxins, classified as Neurotoxin receptor site 1–6 (Fig. 1).

**Site 1** binds non-peptidic neurotoxins tetrodotoxin (TTX), saxitoxin and also several peptide toxins such as cone snail \(\mu\)-conotoxins (Fig. 2). The binding site for these toxins is formed by amino acids on the pore and selectivity filter loops on the extracellular end of the pore. As a result, site 1 toxins bind to directly block the pore to Na\(^+\) access. Interestingly, the sensitivity of the nine sodium channels to TTX blockade has been used to divide the family into two classes TTX-S sensitive IC\(_{50}\) <30 nM (Na\(_{\text{v}}\)1.1, 1.2, 1.3, 1.4, 1.6, 1.7) and TTX-R resistant IC\(_{50}\) >30 nM (Na\(_{\text{v}}\)1.5, 1.8). This TTX affinity difference between the Na\(_{\text{v}}\)3 is believed to be explained by the presence or absence of a key cysteine residue in the TTX-binding site.

**Site 2** binds a group of lipid-soluble toxins, including batrachotoxin, veratridine, aconitine, antillatoxin, hoiamides and grayanotoxin all of which increase activation of Na\(_{\text{v}}\) channels by binding to the open conformation of the channels leading to persistent activation (Fig. 2). A combination of mutagenesis and photoaffinity labeling experiments suggest that transmembrane segments S6 from D1 and D4 play a role in the binding site of batrachotoxin.

**Site 3** binds peptidic \(\alpha\)-scorpion and sea anemone anthopleurin toxins which work to slow the rate that the sodium channel moves from open to inactive states. These peptides are believed to bind to a site that is formed from the S3 to S4 loop at the extracellular end of the D4 voltage sensor as demonstrated by site-directed mutagenesis.

**Site 4** is known to bind \(\beta\)-scorpion toxins at the S3–S4 loop on the extracellular side of the D2 voltage sensor. These toxins act as gating modifiers that shift the activation threshold to more negative membrane potentials. Conversely, several spider toxins (e.g., ProTx-II, HwTx-IV) and a centipede toxin have been recently described to bind to this site and inhibit sodium channels by modifying the voltage dependence of channel activation towards more positive values.

**Site 5** binds the complex lipophilic polyehter marine toxins brevetoxin and ciguatoxins that have been implicated in a number of effects including inhibition of channel opening and shift of activation towards hyperpolarized potentials. Pore helices S5 from D1 and S6 from D4 have been implicated in the binding of brevetoxin as highlighted by photoaffinity labeling studies.

**Site 6** binds \(\delta\)-conotoxins which are known to slow the rate of inactivation in a similar manner to the \(\alpha\)-scorpion toxins. The location of this binding site is not well understood but is believed to be the extracellular part of the D4 voltage sensor, adjacent to site 3.

Recently there has been increased activity in the arena of synthetic toxins with the objective of generating toxin-based tools and therapeutics. Most notably, site 1 toxin tetrodotoxin (TTX) has been advanced into clinical trials by Wex Pharmaceuticals and is currently progressing in Phase III trials for cancer and chemotherapy-related pain. In addition, there have been several papers and patents outlining synthetic analogues of site 4 toxin inhibitors, most notably the synthesis of potent Na\(_{\text{v}}\)1.7 selective HwTx-IV analogs.
Screening capabilities: HTS electrophysiology has been largely developed to enable sodium and calcium channel drug discovery. In fact, the investigation of ion channels has always been challenging, with data being limited to non-functional information, such as binding and FRET based data or functional electrophysiology data where screening throughput is often at the expense of quality. Although developments in the field of automated electrophysiology now allow for its use in higher throughput screening cascades through to more in depth biophysical characterization of channel interactions, the biggest limitation remains with how to interpret this electrophysiology data. The range of platforms available and subtle differences in the protocol designs are so varied that comparing information across screening platforms is an additional significant challenge.

Current technologies: The conventional patch clamp approach using a glass pipette to patch a single cell is considered the gold standard in electrophysiological measurements and is relied upon for in depth biophysical characterization. However, planar patch clamp technologies are generally the first choice as a screening platform as they allow for electrophysiological recordings to be made from multiple cells simultaneously, with varying levels of quality and throughput. Giga seal platforms such as 16 well PatchXpress and 48 well Qpatch offer high quality, low throughput data whilst the 384 well IonWorks system deliver high throughput, lower quality data. By combining these screening technologies they allow for a comprehensive screening cascade. Recent advances with the IonWorks Barracuda, SynchroPatch and Qube provide giga seal quality data in a 384 well format, continuous voltage control and faster fluid exchanges. This permits a functional measure of compound activity at a much earlier stage in the drug discovery cascade.

Non-functional assays: One of the first methodologies employed for ion channel investigation was radioligand binding. Whilst this screen has been used for many years to triage ion channel ligands, it has some limitations, for example, the need for highly specific and selective ligands along with difficulty in functional interpretation of binding data unless there is confidence in the functional importance of the binding site. Moreover, for targets where generating stable cell lines is difficult, fluorescence based assays are sometimes the only option available for high throughput assessment.

Aims for the future: There are still many technical limitations around the quality of automated patch clamp techniques, such as access resistance and compensation, which require much improvement before automated patch clamp techniques will be able to replace conventional patch electrophysiology. Despite this, the future of screening technologies for ion channel drug discovery promises to be an exciting one. The real breakthrough will come as more clinical data becomes available to interpret which in-vitro assays or protocols are the most predictive of clinical outcomes. Recent advances in current clamp technology on the automated platforms should help with this in-vitro to clinical in-vivo translation by allowing measurement of effects on excitability.

Small molecule modulators: Small molecule binding sites: Although sodium channels have six known binding sites for neurotoxins, extensive evidence suggests most small molecule inhibitors of Na channels bind within the pore region for example local anesthetic, antiepileptic and antiarrhythmic agents (Fig. 1). Elegant site directed mutagenesis experiments suggested that these agents interact with amino acid residues within the inner cavity of the channel pore (S6 in D4), and this binding site has been named the local anesthetic (LA) binding site. The LA binding site is highly conserved across Na channels and most likely accounts for the lack of subtype selectivity for most clinically used sodium channel blockers. Clinically, non-selective modulation of sodium channels can be associated with undesirable side effects for example cardiac toxicity due to inhibition of Na1.5 channels. Strategies to identify inhibitors with improved sodium channel subtype selectivity and side effect profile include developing binding assays and site directed mutagenesis studies to elucidate and exploit novel binding sites. Several radioactively labeled toxin binding assays have been developed offering new tools in the search for novel sodium channel blockers (Fig. 2).

Figure 2. Selected toxin modulators.
mutations new classes of potent, highly selective acidic chemotypes have been reported. These compounds interact with ‘extracellular’ facing regions of the channel which appear to be the major determinants of Na\textsubscript{v} subtype selectivity (e.g., PF-04856264 (7), Na\textsubscript{v}1.7 IC\textsubscript{50} 28 nM, Na\textsubscript{v}1.5 IC\textsubscript{50} >10000 nM).

Small molecule patent literature: Over the past two years, there have been few new disclosures of Na\textsubscript{v}1.8 inhibitors in the literature since previous reviews and no new disclosures for selective Na\textsubscript{v}1.3 or Na\textsubscript{v}1.9 inhibitors. A greater number of patent applications for compounds claiming Na\textsubscript{v}1.7 inhibition or mixed Na\textsubscript{v}1.7 activity with other sodium channels for treating pain have been published. There have also been several disclosures of compounds as sodium channel inhibitors for treating pain that do not provide a description of the sodium channel tested. Representative examples from patent applications publishing over the past two years are compiled in Figures 4–14 along with biological activity, subtype selectivity and screening platform data if available. It is prudent to note that screening technologies and protocols vary significantly across the pharmaceutical industry.

Pfizer has recently disclosed two new series of potent Na\textsubscript{v}1.8 inhibitors, an arylimidazole and a benzimidazole series (Fig. 4). No selectivity data was presented in these patent applications.

In terms of Na\textsubscript{v}1.7 selective inhibition, Pfizer and Pfizer/Icagen have published patents with compounds containing arylsulfonylamides and sulfonylated amides.\textsuperscript{46–48} Pfizer has since made additional disclosures of arylsulfonylated amides and acylsulfonyl ureas and arylindazole sulfonylated amides (Fig. 5). Selectivity data is not disclosed in these patents, however it is reported that the lipophilic acid PF-04856264 (7) is highly Na\textsubscript{v}1.7 selective over Na\textsubscript{v}1.5 (Fig. 3).\textsuperscript{49} A number of other companies have recently filed patent applications for compounds occupying similar physiochemical space as the compounds described by Pfizer. These companies include Agen, Daiichi Sankyo, Merck, Xenon, DaeWoong and joint filings between Xenon and Genetech. Agen have disclosed several series of compounds with bicyclic core sulfonamides (Fig. 6). These compounds are potent Na\textsubscript{v}1.7 inhibitors with selectivity against Na\textsubscript{v}1.5. Xenon has disclosed potent zwitterionic substituted piperazine and piperazine methyleneoxysulfonylamides, and a series of arylsulfonylamides (Fig. 7). Na\textsubscript{v}1.5 data for multiple examples indicate that these compounds are Na\textsubscript{v}1.7 selective. In joint applications between Xenon and Genetech aryloxysulfonlated amides and acylsulfonyl ureas have been reported (Fig. 8). In these applications Na\textsubscript{v}1.7 membrane binding data is provided in addition to the Na\textsubscript{v}1.7 and Na\textsubscript{v}1.5 PatchXpress\textsuperscript{e} (PX) data that demonstrates selectivity for many of these compounds. Merck has claimed benzo-oxazolone core sulfonamides that are potent Na\textsubscript{v}1.7 inhibitors with good selectivity against Na\textsubscript{v}1.5 (Fig. 9). Many of the compounds are zwitterionic. The compounds from Daiichi Sankyo employ a cycloalkoxyaryl-sulfonamide to deliver potent Na\textsubscript{v}1.7 inhibitors as determined by testing on the IonWorks\textsuperscript{f} Quattro™ (IQW) platform (Fig. 9).

Purdue has continued to patent extensively on the aryloxybiaryl motifs with multiple filings over the past two years (Fig. 10). Na\textsubscript{v}1.7 activity is reported but no subtype selectivity data is provided. They employ a range of polar groups on the distal ring of the biaryl, and it will be interesting to see what effect these substitutions have on selectivity, central nervous system (CNS) penetration and solubility. Other companies reporting compounds with Na\textsubscript{v}1.7 activity (with no subtype selectivity data provided) are shown in Figure 11. The representative examples from Zalicus, Schering and Shionogi appear to be moderate Na\textsubscript{v}1.7 modulators. RaQualia have filed a number of applications with substituted heteroaryl cores that are claimed as TTX-S inhibitors. Na\textsubscript{v}1.3, Na\textsubscript{v}1.5 and Na\textsubscript{v}1.7 data were also reported (Fig. 12). The biaryl spiro-pyrrolidinone-lactams reported by Convergence are moderately potent against Na\textsubscript{v}1.7 as tested on the QPatch\textsuperscript{e} platform at the half-maximal voltage for steady state inactivation protocol. Moreover, pIC\textsubscript{50} values at ~90 mV are also provided. Two examples shown in Figure 13 are claimed in multiple filings by Convergence. The spiro-piperidine compounds published by Vertex claim activity towards a sodium channel and appear to be related to previous applications (Fig. 14).\textsuperscript{50}

Compounds in clinical development: A number of orally administered sodium channel modulators have been reported to enter clinical trials for the treatment of pain (Table 1). CNV1014802 (8) is a novel oral state-dependent sodium channel blocker being developed by Convergence (Fig. 15). CNV1014802 has completed Phase II trials for lumbar sacral radiculopathy and is in Phase II trials for trigeminal neuralgia. Furthermore, CNV1014802 was granted orphan drug designation in 2013 by the US Food and Drug Administration (FDA) for the treatment of trigeminal neuralgia.\textsuperscript{51} Pfizer has advanced several Na\textsubscript{v}1.7 and Na\textsubscript{v}1.8 selective compounds into Phase I/II clinical trials. PF-05089771 has completed Phase II clinical trials of third molar extraction and primary inherited erythromelalgia.\textsuperscript{52} Dainippon Sumitomo recently progressed a Na\textsubscript{v}1.7/Na\textsubscript{v}1.8 compound DSP-2230 into Phase I for neuropathic pain.\textsuperscript{53} NKTR-171 is a sodium channel blocker in Phase I by Nektar Therapeutics for the treatment of peripheral neuropathic pain. NKTR-171 is sodium channel unselective although it exhibits peripheral restriction together with state and use dependence leading to functional selectivity.\textsuperscript{54}

Some therapeutic agents have been reported to enter clinical trials for the treatment of pain that proceed via a non-oral administration route. AstraZeneca has reported the effects of intradermal...
administration of AZD3161 (10) in a Phase I UVIH burn study.\textsuperscript{56} Tetrodotoxin (TTX, Fig. 2), an injectable non-narcotic marine neurotoxin, is being advanced in Phase II/III clinical trials for the treatment of cancer related pain. TTX was originally developed at Wex Pharmaceuticals followed by a collaboration with Esteve.\textsuperscript{57,58} XEN402 (9) is a novel topical Na\textsubscript{v}1.7 sodium channel blocker in
Phase II clinical evaluation at Xenon Pharmaceuticals. Xenon has reported positive Phase II readouts where topical application of XEN402 reduced pain in patients suffering from primary erythromelalgia and in patients suffering from postherpetic neuralgia.\textsuperscript{58,59} Results from an oral Phase II study with XEN402 suggested positive effects in the dental model of acute inflammatory pain. XEN402 has been granted orphan drug designation by the FDA for the treatment of erythromelalgia. Moreover, the product was licensed to Teva by Xenon Pharmaceuticals in 2012 on a worldwide basis for the treatment of pain and is now called TV-45070. XEN403 has been presented as a backup candidate of XEN402/TV-45070 for the potential oral treatment of pain, although there have been no recent updates.\textsuperscript{60}

The accurate isoform selectivity and biophysical characteristics of many of the clinical compounds described above that are advancing through clinical efficacy trials have not been disclosed.
The hope is that new agents with greater subtype or functional selectivity will achieve therapeutic utility with decreased cardiovascular and CNS risks.

**Outlook and summary:** Sodium channel drug discovery is an exciting and developing field of science. It appears that pharmaceutical investment in this target class in the search for pain therapeutics is driven by two major parameters: genetic data linking Nav mutations to pain phenotypes for target validation and the ability to express stable cell lines and establish effective screen sequences. For example, recent Nav1.9 genetic data may be expected to lead to an explosive pharmaceutical effort in the same vein as Nav1.7. However, this work will be significantly hindered until stable Nav1.9 expressing cell lines are available for adequate screening. Screening methods vary greatly across the pharmaceutical sector and it is often unclear which protocol is the most predictive for efficacy in a clinical disease setting. Hopefully, as clinical data emerge this screening effort can be condensed to identify the most relevant approach for preclinical in-vitro to clinical in-vivo translation. Finally, prospective Nav drug design is complicated by the variety of available Nav binding sites across multiple
ion channel states. Perhaps the recent significant advances made in Na\textsubscript{v} structural biology coupled with the improved high quality screening capabilities can address this challenge to ultimately enhance the discovery of pharmacologically relevant Na\textsubscript{v} based painkillers.

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References and notes
