

UC Davis

UC Davis Previously Published Works

Title

Antibodies and venom peptides: new modalities for ion channels.

Permalink

<https://escholarship.org/uc/item/7bk9v2cm>

Journal

Nature reviews. Drug discovery, 18(5)

ISSN

1474-1776

Authors

Wulff, Heike
Christophersen, Palle
Colussi, Paul
et al.

Publication Date

2019-05-01

DOI

10.1038/s41573-019-0013-8

Peer reviewed

Antibodies and venom peptides: New therapeutic modalities for ion channels

Heike Wulff¹, Palle Christophersen², Paul Colussi³, K. George Chandy⁴ and Vladimir Yarov-Yarovoy⁵

¹Department of Pharmacology, University of California Davis, 451 Health Sciences Drive, GBSF Room 3502, Davis, CA 95616, USA

²Saniona A/S, Baltorpvej 154, Ballerup, DK2750, Denmark

³TetraGenetics Inc., 91 Mystic Street, Arlington, MA 02474, USA

⁴Molecular Physiology Laboratory, Infection and Immunity Theme, Lee Kong Chian School of Medicine, Nanyang Technological University, 636921 Singapore

⁵Department of Physiology & Membrane Biology, University of California Davis, 4131 Tupper Hall, Davis, CA 95616, USA

Emails: hwulff@ucdavis.edu; pc@sanonia.com; pcolussi@tetragenetics.com; gchandy@ntu.edu.sg; yarovoy@ucdavis.edu

Abstract

Ion channels play fundamental roles in both excitable and non-excitable tissues and therefore constitute attractive drug targets for a myriad of neurological, cardiovascular and metabolic diseases as well as for cancer and immunomodulation. However, achieving selectivity for specific ion channel subtypes with small molecule drugs has been challenging and there currently is a growing trend to target ion channels with biologics. One approach is to improve the pharmacokinetics of existing or novel venom derived peptides. In parallel, after initial studies with polyclonal antibodies demonstrated the technical feasibility of inhibiting channel function with antibodies, multiple preclinical programs are now using the full spectrum of available technologies to generate conventional monoclonal and engineered antibodies or nanobodies against extracellular loops of ion channels. After a summary of the current state of ion channel drug discovery, this review discusses recent developments using the purinergic receptor channel P2X7, the voltage-gated potassium channel K_v1.3 and the voltage-gated sodium channel Na_v1.7 as examples of targeting ion channels with biologics.

Introduction

Ion channels are pore-forming transmembrane proteins that allow the regulated flow of cations or anions across membranes. The IUPHAR (International Union of Basic and Clinical Pharmacology) *Guide to Pharmacology*¹ currently lists 145 genes for voltage-gated-like ion channels², 82 genes for ligand-gated ion channels³, and 52 genes for “so-called” other channels⁴ like aquaporins, connexins or store-operated channels in humans. However, since many ion channels form homo- or heteromers of two, three, four, or five subunits - which additionally may interact with auxiliary proteins - the total number of possible ion channels that can be assembled from these genes to serve very specific physiological functions is much larger. Ion channels currently constitute important drug targets for the treatment of type-2 diabetes, hypertension, epilepsy, cardiac arrhythmia, and anxiety, and many of the classical drugs on the WHO’s list of essential medicines, like nifedipine (Ca_v1.x inhibitor), amiodarone (mixed K_v channel inhibitor), phenytoin (Na_v inhibitor), or diazepam (GABA_A activator) are ion channel modulators. A recent comprehensive analysis of molecular targets⁵ estimated that 18% of small-molecule drugs exert their therapeutic effects through ion channels (see Supplementary Table 1 for a complete list of clinically used drugs targeting human ion channels). It is noteworthy that these clinically successful examples are all small molecule drugs that were developed long before their molecular targets were identified, solely by exploiting *ex vivo/in vivo* approaches. Following the initial registrations of these “classics” between the early 50s and early 80s the number of ion channel targeting drugs increased quickly, arguably often because of “me too” drug development around the original chemical structure and “incremental” property adjustments (most clearly illustrated by the dihydropyridine anti-hypertensives, the sulfonylurea type-2 antidiabetics, and the benzodiazepine anxiolytics shown in Supplementary Table 1).

Based on electrophysiological experiments, and especially with the introduction of patch-clamp technology⁶, the mode-of-actions of the most important ion channel targeting drug classes (Na_v inhibitors, K_{ATP} inhibitors etc.) were quite well established around 1990⁷. At the end of the cloning era in the late 90s, the majority of ion channels were identified revealing a complex picture with respect to subtypes, stoichiometry and pharmacology⁸. The exceptions were voltage-dependent H⁺ channels (Hv), Ca²⁺- and volume regulated Cl⁻ channels (CACL, VRAC), the stretch- and voltage activated Piezo channel, and the Ca²⁺ release activated Ca²⁺-channel (Orai), which were only cloned a decade later. It specifically became apparent that even closely related subtypes like members of the K_v1 family can have very different physiological functions, and thus can provide highly variable therapeutic and adverse effect profiles. This realization initiated an “explosion” in the number of ion channel drug discovery programs^{9, 10, 11, 12, 13}, dedicated towards development of subtype-selective small molecule drugs, tailored for improving the therapeutic index (i.e.: Ca_v2.2/3 inhibitors for neurological indications like stroke or pain without effects on Ca_v1 and thus without cardiovascular side effects; GABA_A α2/α3 selective activators for anxiety without effect on α1 containing channels and therefore without sedative properties). Hand-in-hand with this progress, cloning, molecular, and cell culturing techniques improved significantly, high-throughput automated ion channel assays were developed, and all large pharmaceutical companies upgraded (often to several millions chemical entities) and refined their

chemical libraries^{14, 15}. In the most streamlined versions a total screening campaign, including reporting on one or more subtypes, could be completed in just 2-3 months.

This tremendous and long-lasting effort across essentially the entire pharmaceutical industry should have been a leap into the future with respect to improved ion channel medicines, but very few registrations have resulted from these efforts. For example, out of the K_v channel modulator programs we reviewed in 2009¹⁰ only one compound, the K_v7 activator retigabine (known as ezogabine in the USA) for treatment of pharmacotherapy-resistant partial epilepsies made it to market in 2011 and then was withdrawn in 2017 by GlaxoSmithKline because of skin discoloration and suspected eye toxicity related to slow accumulation of a colored metabolite. It is especially sobering that even recent registrations are either based on long-known compounds (e.g. the K_v channel blocker 4-aminopyridine for multiple sclerosis¹⁶, 2010, the GABA_A activating allopregnanolone registered in 2018 for postpartum depression) or were discovered in low-throughput phenotypic screening by combining organ preparations with animal model work (the I_f (HCN) inhibitor ivabradine¹⁷, 2015). The only notable exception we could find is the CFTR potentiator ivacaftor (VX-770), which originated out of a high-throughput membrane potential assay screen and which increases the open probability of wild-type and mutant CFTR¹⁸. For a list of the ion channel targeting drugs approved in the last 10 years, see Table 1.

Inspecting the reported clinical pipelines from major pharmaceuticals and biotech companies does not change the picture significantly (Table 2): One topical polyclonal antibody, three peptides and 33 small molecule clinical programs are currently reported; most are in phase I, a handful are in phase I/II, and only two, SAGE-217, a synthetic GABA_A activating neurosteroid for major depression, and Gefapixant, a P2X₃ inhibitor for chronic cough, are in phase III. One compound, mirogabalin, an improved gabapentinoid targeting Cav channels, is under registration in Japan for peripheral neuropathic pain. To underline the case further, two of the phase II examples are “repurposed” compounds (senicapoc, a K_{Ca}3.1 inhibitor, for hereditary xerocytosis and Alzheimer’s disease and gaboxadol, a δ-subunit preferring GABA_A agonist for Angelman’s/Fragile-X syndrome), and XEN496 is retigabine now redeveloped for the orphan indication K_v7.2-mediated epileptic encephalopathy. A trend is that many of these development programs originate from small/intermediate pharmaceuticals and biotech companies, which may indicate a decline in the popularity of ion channels with the major pharmaceutical companies.

Even though small molecules will continue to be important for ion channel drug development in years to come, we are convinced that the role of ion channel targeting biologics will increase and help “revitalize” ion channels as targets for drug development. Following on the heels of the G-protein coupled receptor field¹⁹, where several dozen antibody programs have advanced into clinical trials and CCR4 and CGRP targeting antibodies are on the market, the ion channel field is presently particularly excited about antibody approaches. This article will discuss the current state of research into antibodies and peptides that target this complex and diverse group of molecules.

Challenges in targeting ion channels

Ion channels have long been regarded as difficult drug targets and the reasons that have traditionally been given for this with respect to small molecule drug discovery¹⁰ were 1) the technical difficulties in ion channel high-throughput screening; 2) the lack of crystal structures enabling “true” structure based drug design; 3) the challenges of achieving subtype selectivity, also taking into account stoichiometry of heteromeric channels and possible interactions with auxiliary subunits (a point often ignored in drug discovery), and ideally state/conformation selectivity. All these issues are also applicable to ion channel targeted biologics. The first issue has been somewhat addressed with the development of ultra-high-throughput membrane potential or flux assay systems and automated electrophysiology platforms that are currently capable of running high quality giga seal electrophysiological recordings in 48, 96 and 384 wells. However, we would like to posit that the specialized biophysical expertise that is necessary to expertly execute and analyze ion channel screens constitutes a real or sometimes perceived barrier to committing to an ion channel drug discovery program, which together with the fact that overall more resources in industry and academia have been dedicated to GPCRs than to ion channels, is probably responsible for the overall slower progress of the ion channel than the GPCR field. The 2nd point, the lack of structures, is a deficiency that is currently being addressed by advances in structural biology (see Box 1), but again somewhat more slowly than in the GPCR field. The 3rd point, obtaining relevant subtype and state/confirmation selectivity, remains a challenge for both the ion channel and the GPCR field (although voltage-clamp electrophysiology with its high temporal resolution and voltage control combined with fast application should give ions channels an advantage), and requires very detailed understanding of the exact role and context of the target protein in the chosen disease indication.

Target specificity

Since nearly all efforts within ion channel drug discovery/development previously focused on small molecules, it is worth considering the one key challenge for the field in addition to the general issues all small molecule medicinal chemistry programs face, namely optimizing pharmacokinetic properties²⁰, avoiding toxicity due to toxic metabolites²¹, and finding chemical matter that ideally allows one to obtain “strong” composition of matter patents. Obtaining selectivity within ion channel families can be difficult to achieve, since subtypes are often highly homologous. This is especially challenging for small molecule drugs due to their small sizes and therefore limited number of interaction points with the target. The field of Nav inhibitors is particularly instructive: Antiepileptics, class I antiarrhythmics, and local anesthetics target voltage-gated Nav channels at a highly conserved site within the pore lumen formed by transmembrane segments S6 in the third and fourth domains²². Thus, despite many years of drug-development by many companies, all classical Nav drugs are essentially unselective and their therapeutic value as systemically administered drugs relies solely on their strong state- or use-dependency, which favors binding to channels in pathologically depolarized or excessively firing cells.

State-dependence in this context means that a drug preferentially binds to one of the conformational states (closed, open, inactivated) that an ion channel undergoes

during its gating cycle (Fig. 1A). For voltage-gated channels like Nav, Cav and Kv channels the occupancy of these states strongly depends on the membrane potential which means that drug development programs focused on targeting neuronal ion channels must decide which state of the channel it would be most desirable to target. For example, for Nav channels that is generally assumed to be the open or inactivated state, while it might be better to target the closed state when developing a Kv channel opener. When neuronal ion channels are expressed in HEK293 or CHO cells, their voltage-sensor domains (VSD) are exposed to more depolarized resting membrane potentials (around -40 mV) instead of the more hyperpolarized resting membrane potential (around -70 mV) they would be experiencing in a neuron. This means that in drug screening assays such as flux or binding assays, that do not allow to control the membrane potential, the VSDs will tend to be in the thermodynamically favored “up” state that is present in open or inactivated states of the channel, which makes it difficult to capture the “down” state of the VSD that is present in the closed state (Fig. 1A). One of the major advantages of using manual or HTS patch-clamp electrophysiology for ion channel drug screens is that the membrane potential can be precisely controlled to capture the desired state of the channel.

The Nav channel family contains 9 functional subtypes²³, Nav1.1 to Nav1.9. Nav1.1 is expressed in fast spiking interneurons of the brain, where loss-of-function causes Dravet syndrome²³, and has recently been shown to regulate the mechanical excitability of visceral nerves in the gut^{24, 25}. Nav1.2 is the predominant brain isoform and, together with Nav1.6, is a major target for anti-epileptics. Nav1.5 is the main cardiac channel and the target for class I antiarrhythmics, whereas the analgesic effects of local anesthetics are mediated by several channels, including Nav1.7, Nav1.8, and possibly Nav1.9, which are preferentially expressed in sensory nerves²⁶. Nav1.7 and Nav1.8 have clear pain phenotypes in humans as loss-of-function and gain-of-function mutations in Nav1.7 result in congenital insensitivity to pain²⁷ and primary erythromelalgia²⁸, respectively, whereas gain-of-function mutations of Nav1.8 may cause painful peripheral neuropathy²⁹. Despite convincing preclinical effects of unselective Nav blockers in animal pain models and the effective use of the antiepileptic carbamazepine in trigeminal neuralgia pain³⁰ existing Nav channel inhibiting drugs are not optimal for general treatment of severe pain disorders due to their unselective mode-of-action. Several companies (Genentech, Biogen, Amgen, Pfizer, Bayer) are or have been developing highly subtype selective and potent small molecule inhibitors of Nav1.7 by targeting binding to a site in the domain IV voltage sensor responsible for channel inactivation rather than the classical local anesthetic site in the pore lumen^{31, 32, 33}. Based on the currently published structures, this is achievable with a series of close analogues all with a conserved aryl-sulfonamide core structure, often dramatically called the “warhead”. For example, Genentech has published an x-ray structure³¹ of the aryl-sulfonamide GX-936 bound to a receptor site within the Nav1.7 fourth voltage-sensing domain (Fig. 1B). Unfortunately, one of these small molecules, the clinical phase II compound PF-05089771 (Fig. 1B), recently failed in patients suffering from painful diabetic neuropathy³⁴, which led Pfizer to stop further development activities. Bankar et al. suggested this failure possibly reflected insufficient Nav1.7 targeting in the clinical study and recommend the evaluation of acyl-sulfonamides with better physiochemical and pharmacological properties³⁵, including a longer residence time on the target³⁶.

Since PF-05089771 is intentionally peripherally restricted, compounds with improved blood brain barrier (BBB) penetrability in humans, might better engage Nav1.7 channels expressed close to or on the bouton of dorsal root ganglion (DRG) neuron dorsal horn synapse. Another reason for the apparent failure of some Nav1.7 blockers could be interaction with β -subunits for the native channel in DRG neurons, which was not recaptured in heterologous screening systems using the “naked” α -subunit alone. Sokolov *et al.* recently demonstrated that co-expression of Nav1.7 with the glycosylated form of the sodium channel β 3, an auxiliary subunit that is upregulated in injured human sensory neurons³⁷ and in DRGs in rat pain models³⁸, makes Nav1.7 less sensitive to several state-dependent Nav blockers³⁹. Whether this would also be the case for the aryl-sulfonamides was not investigated in the study but it is interesting to speculate based on the recently solved structures of the human and electric eel Nav1.4- β 1 complexes^{40, 41}, that β -subunits could possibly prevent the binding of small molecules or antibodies to Nav1.7. Finally, it is possible that a slightly broader selectivity comprising also the other DRG channels, Nav1.8 and Nav1.9, may be needed. The Biogen compound, vixotrigine (a.k.a Raxatrigine), which is currently still active in Phase II, despite a recent failure in painful lumbosacral radiculopathy, has a different structure (Fig. 1B), passes the BBB, and is reportedly less subtype selective than the aryl-sulfonamides⁴².

An interesting and highly challenging “spin-out” of the selectivity issues with small molecules is that considerable domain homology also exists between even remotely related ion channel families, which can lead to the apparently paradoxical finding that highly subtype-selective compounds may still have off-target effects on other ion channels like the cardiac delayed rectifier Kv11.1 (hERG), inhibition of which may cause ventricular fibrillation and sudden death^{43, 44}. Unfortunately, hERG is remarkably promiscuous with respect to binding of many different small molecule chemotypes^{43, 45} and thousands of otherwise useful drug candidates have been “filtered out” due to hERG activity^{43, 45}.

Another aspect that is worth mentioning, when discussing the problem of selectivity, is species specificity. While it has been incredibly challenging to achieve subtype selectivity between closely related human channels like within the Nav or Cav family or for certain GABA_A or nACh receptor subtypes, species selective small molecules seem to be more easily attainable as demonstrated by the extremely low acute mammalian toxicity of neuroactive insecticides like the Nav channel blocking pyrethroids, the nicotinic receptor channel activating neonicotinoids and the GABA_A channel blocking polychlorocyclohexanes and fiproles⁴⁶. Examples of species specific ion channel modulators used in human medicine are the antivirals amantadine and rimantadine, which target the M2 proton channel, a member of the so-called viroporin ion channel family specific to viruses, with very little homology to prokaryotic or eukaryotic ion channels⁴⁷. However, species differences in sensitivity to drugs among mammals can also be very problematic for translational drug discovery, as exemplified by the insensitivity of the rat isoform of Nav1.7 towards inhibition by the aryl-sulfonamide inhibitor, AMG-8379³³.

Biologics versus small molecules

Even though biologics have their own well-known challenges, such as poor membrane permeability and the risk of triggering adverse immune reactions, they constitute an attractive alternative to small molecules, first-of-all because of their much higher binding selectivity (approaching true specificity), both with respect to subtypes and off-targets, than is generally possible to achieve with small molecules. Additionally, antibodies and peptides are metabolized as part of the body's normal protein dynamics and thus do not show the drug-drug interactions, and metabolism-mediated toxicity that are always a risk with small molecules. Taken together, these two advantages are often considered to be responsible for the overall higher success rate of new molecular entity biologics (13.2%) versus small molecules (7.6%) for progression from Phase-1 to gaining FDA approval⁴⁸.

The feasibility of targeting ion channels with biologics has been amply demonstrated by Nature. Venomous animals have developed a myriad of highly potent and selective peptides that can both inhibit and activate ion channels and that have been incredibly useful in probing ion channel structure-function relationships. Similarly, autoantibodies developed by a patient's own immune system can inhibit ion channel function and acutely transfer disease to experimental animals. For example, most cases of myasthenia gravis, a disease leading to skeletal muscle weakness, are caused by pathogenic antibodies that bind to nicotinic acetylcholine (nACh) receptors at the neuromuscular junction⁴⁹, while a related condition, Lambert-Eaton myasthenic syndrome is triggered by antibodies against P/Q-type voltage-gated Ca^{2+} channels on presynaptic nerve terminals⁵⁰. In both cases, some autoantibody clones have been shown to directly block the respective channels in electrophysiological experiments, while other antibodies can activate complement or induce channel internalization^{49, 50}. Another instance of a neurological disease characterized by the presence of ion channel specific autoantibodies is neuromyelitis optica, where most patients test positive for aquaporin-4 antibodies⁵¹.

Ion channel-targeted peptides as therapeutics

Since the development of exenatide⁵², a 39-amino acid glucagon-like peptide-1 agonist isolated from the venom of a lizard, and its approval in 2005 as an injectable treatment for type-2 diabetes, peptides are increasingly being considered as viable therapeutics. The FDA has approved over 60 peptide drugs (predominantly targeting GPCRs for the treatment of metabolic disease and for oncology⁵³), around 140 peptides are in clinical trials, and another 500 are in pre-clinical development⁵³. The global peptide therapeutic market is estimated to be close to US\$25 billion in 2018⁵⁴.

Venoms are a rich source of bioactive peptides with therapeutic potential^{55, 56, 57, 58}. Over eons, more than 100,000 venomous creatures – arthropods (scorpions, spiders, bees, centipedes, wasps), cnidarians (sea anemones, jellyfish), mollusks (cone snails), annelids (fire worms, parasitic worms) and vertebrates (snakes, lizards, frogs, mammals) – have used their toxic cocktails to engage important biological targets. These cocktails are deployed as weapons to immobilize or kill prey, or as defense to deter predators or microbial invaders. Over 10 million bioactive peptides and proteins are estimated to be present in animal venoms, and their immense chemical diversity is unrivalled by synthetic libraries. The ability of these animals to kill with tiny amounts of

powerful venom has inspired both fascination and fear in humans, and snakes and scorpions were deified in the ancient world⁵⁹. Traditional medicines in China, India, Greece and the Middle East have for centuries used this vast bioactive resource for medicines. In modern times, there has been an increasing interest in exploiting this extensive and relatively untapped pharmacopeia using proteomic and genomic approaches^{55, 58}. As of today, six venom derived peptides, including the Cav2.2 blocking Ziconotide, have been approved by the FDA, about a dozen are in clinical trials, and several more are in pre-clinical development^{55, 58}. Below we will discuss the ion channel targeting peptides in more detail by focusing on peptides that have entered clinical trials or started preclinical development in the last 10 years.

Ion channels are frequently targeted by peptide toxins. Starting in the 1960s, animal toxins were used as molecular tools to investigate ion channels^{60, 61, 62}. Many of these toxins have disulfide-constrained architectures that enhance their stability and protease resistance and permit tremendous variations in primary sequence without perturbation of the three-dimensional fold (Supplementary Figure 1). These characteristics make peptides with disulfide-rich scaffolds attractive as therapeutics. Figure 2A highlights the channel-modulating peptides that are discussed below. Many peptides are pore blockers^{56, 57, 58} that bind at the extracellular entrance to the channel's pore (external vestibule) and occlude the ion conduction pathway (Fig. 2B). Others bind to the voltage-sensor of the channel and impact channel-gating (Fig. 2B).

Channel-modulating peptides for the management of pain

The opioid crisis in the United States of America has spurred efforts to develop non-opioid drugs for chronic pain, but many of these efforts have failed. A notable exception is Ziconotide (Prialt®), a peptide derived from the venom of fish-hunting cone snails⁶³, which was FDA-approved in 2004. In the 1980s, ω -conotoxin GVIA from *Conus geographus* and ω -conotoxin MVIIA from *Conus magus* (Fig. 2A) were shown to block voltage-gated calcium channels in the nervous system but not muscle^{64, 65, 66}. The neuronal calcium channel was later defined as the N-type Cav2.2 channel. These peptides decrease neurotransmitter release from nociceptive afferents that terminate in the dorsal horn of the spinal cord. The peptide that advanced to the clinic was native MVIIA (Ziconotide)⁶³. It is administered intrathecally through an implanted pump because it does not cross the BBB. Ziconotide is efficacious when administered as a single therapeutic, but is increasingly combined with an intrathecal opioid for the management of refractory chronic and cancer pain. Recent guidelines also recommend Prialt® as a first-line agent for neuropathic and nociceptive pain. Prialt®'s wider clinical use is impeded by its relatively high cost, narrow therapeutic window, and by the requirement for an intrathecal pump^{63, 67}.

Another ion channel that has been suggested as a pain target based on genetic studies is the sodium channel Nav1.7^{27, 28}. μ -theraphotoxin-Pn3a, a peptide from the South American tarantula *Pamphobeteus nigricolor* (Fig. 2A) blocks Nav1.7 with picomolar affinity and selectivity over other Nav channels⁶⁸. The peptide is analgesic in rodent pain models when co-administered with sub-therapeutic doses of opioids, but displays no analgesic activity when administered on its own⁶⁸. A broad lack of analgesic activity was also found for the selective Nav1.7 inhibitors PF-04856264 and phlotoxin I⁶⁸. Amgen recently engineered AM-8145 and AM-0422, two peptides based on JzTx-V

toxin that selectively inhibit Nav1.7⁶⁹. As mentioned above, in a recent human trial the peripherally restricted, Nav1.7-selective small molecule PF-05089771 did not significantly reduce pain scores compared to placebo³⁴. Taken together, these results might suggest that selective Nav1.7 inhibitors may need to be administered with a sub-therapeutic dose of an opioid to achieve adequate analgesia^{70, 71, 72}.

The $\alpha 9\alpha 10$ nicotinic acetylcholine (nACh) receptor is also considered a therapeutic target for pain⁷³. The α -conotoxins Vc1.1 and RglA from the cone snails *Conus victoriae* and *Conus regius* respectively (Fig. 2A), antagonize $\alpha 9\alpha 10$ nACh receptors^{74, 75}. Agonism of GABA_B receptors and resulting inhibition of Cav2.2 channels via G-protein $\beta\gamma$ interaction is a second mechanism of action that has been suggested for these peptides⁷⁶. Vc1.1 was effective in rodent models of pain⁷⁷, and advanced to human phase-2 trials where it failed, possibly because it was less potent on human than rat $\alpha 9\alpha 10$ nACh receptors^{58, 63, 78}, another example of the translational challenges of mammalian species differences. RglA-4, an analogue of RglA with high affinity for both rodent and human $\alpha 9\alpha 10$ nACh receptors and no activity on GABA_B receptors, suppresses rodent cancer chemotherapy-induced neuropathic pain^{58, 63, 79} and is currently in pre-clinical development.

P2X3 receptors are being targeted with small molecules for endometriosis-associated pain (Table 2). These receptors are also inhibited by purotoxin-1 (PT1), a 35-amino acid residue peptide from the venom of the wolf spider *Geolycosa sp.*, that slows recovery from desensitization following channel activation with ATP and reduces hyperalgesia in rat models of inflammatory pain⁸⁰. Analogues of PT1 or related peptides, if optimized for specificity, potency and stability, could be advanced for pain indications.

Channel-modulating peptides for cancer therapy

Chlorotoxin (Fig. 2A), a peptide from the death stalker scorpion *Leiurus quinquestriatus hebraeus*, was initially reported to inhibit small-conductance chloride currents in epithelial cells, astrocytomas and gliomas^{81, 82, 83, 84} but later found to also bind to matrix metalloproteinase-2 (MMP2) on glioma cells⁸⁵ and not block volume-, cATP- or Ca²⁺-activated chloride channels⁸⁶. The molecular identity of the putative chloride channel blocked by chlorotoxin is currently undetermined. Human phase-1 trials a decade ago showed that a ¹³¹I-radiolabeled analog was safe following intravenous administration for high-grade glioma, but the compound was not advanced further (NCT00733798). However, there are currently efforts underway to use chlorotoxin-based fluorescent dye conjugates as so-called “tumor paint” to help visualize gliomas⁸⁷, and one analog, BLZ-100⁸⁸, was tested in a Phase-1 study (NCT02234297) in 2016, with a new Phase-2/3 study about to start (NCT03579602) in pediatric patients with CNS tumors where fluorescent-labeled chlorotoxin will be assessed with an imaging system.

TRPV6 channels are over-expressed in ovarian, breast, prostate, colon and thyroid cancers, and have been implicated in tumor progression⁸⁹. SOR-C13, a C-terminal truncation of a longer, 54-residue paralytic peptide called Soricidin (accession number POC2P6) from the short-tailed shrew (*Blarina brevicauda*), blocks TRPV6 at low nanomolar concentrations and suppresses tumors in xenograft models of ovarian and breast cancer^{58, 90}. Fluorescent-labeled and super-paramagnetic iron oxide-conjugated analogues are able to visualize ovarian tumors *in vivo* in mouse models⁹⁰. In

a phase-1 trial in 23 patients with cancers of epithelial origin, SOR-C13 stabilized disease suggesting antitumor activity⁹¹ but caused grade 2-3 dose-related hypocalcemia and atrial fibrillation in a quarter of patients⁹¹. Soricimed, the company developing SOR-C13, currently seems to be focusing on 2nd generation peptide drug conjugates.

Channel-modulating peptides for neurological diseases

The acid-sensing ion channel 1a (ASIC1a), a key mediator of acidosis-mediated neuronal damage in cerebral ischemia, is widely regarded as a potential therapeutic target for the treatment of ischemic stroke⁹². π -hexatoxin-Hi1a, a peptide from the Australian funnel-web spider *Hadronyche infensa* (Fig. 2A), delays ASIC1a channel-activation by binding to an acidic pocket critical for proton gating of the channel⁹³. In rodent stroke models, Hi1a attenuates brain damage and improves behavioral outcomes even when administered intracerebroventricularly 8 hours after stroke onset⁹³. Intravenous and intranasal routes are currently being trialed in mice.

Nav1.1 is localized in fast-spiking inhibitory neurons in the brain, and epilepsy in Dravet's syndrome, a loss of function mutation of Nav1.1, is thought to be due to reduced inhibitory neurotransmission. The peptide Hm1a from the spider *Heteroscodra maculate* (Fig. 2A), activates and slows inactivation of Nav1.1 at nanomolar concentrations²⁴. In mice carrying the human R1407X nonsense mutation, Hm1a rescued the collapse of action potentials in inhibitory interneurons without affecting excitatory neurons⁹⁴. Intracerebroventricular delivery of Hm1a reduced seizures and post-ictal mortality in Dravet syndrome mice⁹⁴. Further development of Hm1a would require microinfusion pumps like those used for Prialt® delivery or microfluidic ion pumps that electrophoretically pump ions across an ion exchange membrane and thereby deliver the peptide “dry” without fluid⁹⁵.

Channel-modulating peptides for treatment of autoimmune diseases

Potassium channels were discovered in T lymphocytes in 1984^{96, 97}. Two potassium channels, the voltage-gated K_v1.3 and the calcium-activated K_{Ca}3.1 channel, promote calcium Ca²⁺ entry into lymphocytes through store-operated CRAC (Orai/Stim) by providing a counterbalancing cation efflux^{98, 99}. Blockade of these channels therefore suppresses T lymphocyte activation. Differential expression of these channels allows preferential suppression of terminally-differentiated effector memory T cells (T_{EM}), which contribute to the pathogenesis of many different autoimmune diseases, with specific K_v1.3 inhibitors¹⁰⁰. The ShK peptide from the sea anemone *Stichodactyla helianthus* (Fig. 2A) blocks K_v1.3 with picomolar affinity but also displays high affinity for neuronal potassium channels^{101, 102}. An extensive structure-activity-relationship program led to the development of ShK-186 (Dalazatide), an analogue with picomolar affinity for K_v1.3 and 100-1000-fold selectivity over related channels^{103, 104}. Sustained high picomolar levels of ShK-186 are achieved in plasma following subcutaneous injection. Due to its long circulating-half-life, ShK-186 is effective in rodent models of multiple sclerosis, rheumatoid arthritis and atopic dermatitis when administered once every 2-3 days^{104, 105}. In phase-1 trials in healthy human volunteers, no ECG changes were observed, and no severe or life threatening adverse effects were noted. In a Phase-1b trial in patients with plaque psoriasis, Dalazatide administered twice weekly by subcutaneous injection

significantly reduced the psoriasis area and severity index in nine of ten patients¹⁰⁶. It caused temporary mild grade 1 hypoesthesia and paresthesia involving the hands, feet, or perioral area in the majority of patients¹⁰⁶, possibly because Dalazatide is cleaved into a product with decreased Kv1.3-specificity^{103, 104}. Plans for a phase-2 trial in the orphan disease inclusion body myositis¹⁰⁷ are on hold due to financial constraints.

Other efforts have focused on improving *in vivo* pharmacokinetic properties of Kv1.3-blocking peptides. PEGylation of the scorpion peptide HsTx1[R14A] prolonged plasma circulating half-life in rodents and resulted in sustained efficacy in rodent models of multiple sclerosis and rheumatoid arthritis¹⁰⁸. By screening a combinatorial ShK peptide library, novel analogues were identified that, when fused to the C-termini of IgG1-Fc, retained picomolar potency, effectively suppressed *in vivo* delayed-type hypersensitivity and exhibited a prolonged circulating half-life¹⁰⁹. Other approaches included the engineering of a scorpion toxin into a humanized antibody to achieve picomolar affinity for Kv1.3 and long plasma half-life¹¹⁰, and the development of novel formulations that achieved satisfactory blood levels of peptide inhibitors following buccal or pulmonary delivery^{111, 112}. Scientists at Amgen generated the derivative ShK[Q16K] by “brute-force” structure-activity analoging and then demonstrated sustained inhibition of plasma cytokine levels in primates with weekly administration of a PEG-conjugated version as well as efficacy in a rat model of multiple sclerosis¹¹³, while a group at Janssen created half-life-extending Fc or albumin fusion proteins¹¹⁴ with the α -KTx3 scorpion toxin OsK1 (α -KTx3.7) and tested them in minipigs. However, both companies subsequently seem to have dropped Kv1.3 as a target based on the previously made observation¹⁰⁰, that the efficacy of Kv1.3 blockers depends on the strength of T-cell stimulation and that Kv1.3 inhibition is therefore immunomodulatory rather than immunosuppressive^{115, 116}.

Other channel-modulating peptides

The μ -conotoxin CnclIIC from the cone snail *Conus consors* (Fig. 2A), a blocker of Nav1.4 sodium channels with myorelaxant and analgesic properties¹¹⁷, is marketed by Activen as XEP-018, a topical cosmetic cream to reduce periocular wrinkles^{58, 118}. SYN-Ake™, an analogue of the peptide Waglerin-1 from the Southeast Asian Temple viper *Tropidolaemus wagleri*, blocks muscle nicotinic acetylcholine receptors and modulates GABA_A receptors¹¹⁹, and is being developed as an alternative dermatological to BoTox™ for wrinkles⁵⁸.

Lastly, α CT1 (ACT1), a peptide based on the last nine amino acids of the C-terminus of connexin 43, a gap junction protein often found at the edge of wounds and associated with poor wound healing¹²⁰, has been shown in multiple Phase-2 trials to reduce mean ulcer area for chronic neuropathic foot¹²¹ and venous leg ulcers¹²². Granexin® gel is currently being tested in a Phase-3 trial (NCT02667327) for diabetic foot ulcers, which affect roughly 15% of the diabetic population globally.

Extending the plasma half-life of peptides

One weakness of peptide-based drugs is their short *in vivo* half-life due to their instability and their rapid renal elimination. The first drawback can be overcome by increasing the effective molecular mass of the peptide via conjugation to create so called “biobetters”¹²³. As discussed for several examples above, ion channel-targeting

peptides have been conjugated to polyethylene glycol (PEG), to large proteins (e.g. human serum albumin), or protein domains (e.g. antibody Fc domain) or engineered into the complementary-determining region of humanized antibodies to generate fusion proteins¹²⁴. Strategies to enhance peptide stability include peptide backbone cyclization, disulfide-bridge modification, residue substitutions, peptide stapling¹²⁵, and computational design of hyper-stable constrained peptide scaffolds¹²⁶. Backbone cyclization of the α -conotoxin RgIA enhanced its stability in human serum without perturbing the structure or function of the peptide^{127, 128, 129, 130}. However, cyclization of APETx2 decreased its activity on the ASIC3 channel while enhancing stability¹³¹. Modification of disulfide bridges is another approach to enhance stability. In the α -conotoxin Vc1.1, disulfide bridges have been replaced by non-reducible dicarba linkages, or have been eliminated and the core of the peptide stabilized by residue-substitutions near the removed bridges^{132, 133}. Both strategies improved stability and oral bioavailability of Vc1.1, but reduced activity. Stapling technologies have also been successful for stabilizing peptides that target N-methyl-D-aspartic acid receptors¹³⁴. Thus, several post-translational methods can be used to augment stability, improve circulating half-life, and even achieve oral bioavailability. The need for half-life extension, however, will depend on a number of factors, including the route of administration, nature of the disease, and whether the drug is intended for acute or chronic use. Some venom peptides are exceedingly stable in plasma, while others depot after cutaneous injection (e.g. Dalazatide¹⁰⁴). For chronic diseases such as diabetes and persistent pain, daily injectables are likely to be tolerated by patients and therefore half-life may not be as critical. Exenatide with an elimination half-life of 140 minutes requires twice daily injection in diabetic patients and has peak sales approaching \$1 billion/year.

Using peptide-channel interactions to guide the design of peptidomimetics or small molecule inhibitors

Many groups have exploited the understanding of peptide-channel interactions to design channel-modulating peptidomimetics. The earliest attempt to design a peptidomimetic of the sea anemone ShK peptide resulted in a million-fold loss in potency against Kv1.3¹³⁵. More recently developed peptidomimetic 4-arm ethylene glycol-conjugated star polymers based on scorpion toxins have achieved nanomolar potency against Kv1.3¹³⁶. Peptidomimetics have also been designed to target connexins, neuronal Cav2.2 channels^{137, 138} and the Cav β 2 subunit of cardiac L-type (Cav1.2) calcium channels¹³⁹.

In the 1990s, high-throughput screens using [¹²⁵I]-charybdotoxin competitive binding assays led to the discovery of several small molecule inhibitors (WIN-17317-3, CP-339,818) with nanomolar potency against the Kv1.3 channel^{140, 141, 142}. However, while charybdotoxin bound in the outer vestibule of the channel, these small molecules likely bound in the inner chamber below the selectivity filter. Displacement of the radiolabeled peptide by these small molecules was due to a transpore effect. Similar transpore inhibition of [¹²⁵I]-charybdotoxin competitive binding to the large-conductance K_{Ca}1.1 channel was seen with indole diterpenes¹⁴³. Radiolabeled-peptide toxin binding assays have subsequently fallen out of favor for screening and more recent high

throughput screens have used thallium flux or membrane potential- or calcium-sensing dyes.

Ion channel targeted antibodies and nanobodies

There are currently more than 60 approved therapeutic antibodies on the market, predominantly in the areas of oncology, autoimmunity and inflammatory disease, and approximately 550 antibodies are estimated to be in various stages of clinical development¹⁴⁴. However, ion channel antibody discovery and development has lagged far behind with only one antibody, a sheep polyclonal (BIL010t) targeting a non-functional form of P2X7 from Biosceptre formulated as a topical ointment for the treatment of basal cell carcinoma having reached human clinical trials thus far¹⁴⁵.

While venom peptides have long been recognized to be superior to small molecules in terms of their potency and selectivity for ion channels, targeting channels with equally selective antibodies has more recently generated intense interest, mostly driven by the highly desirable pharmacokinetic characteristics of immunoglobulins compared to peptides resulting in a much lower dosing frequency. However, antibodies also have unique properties in terms of their mode of action. Small molecules and peptides usually act by directly blocking ion flux through the open channel or by interfering with the gating processes, which can be both positive (activators) or negative (inhibitors), and therefore affect channel function on the micro- to millisecond time scale (e.g. use-dependent inhibition of Nav channels by classical small molecule anti-epileptics). Antibodies can also work by occlusion of the ion channel pore or induce allosteric-induced gating effects, but in addition they can be engineered to either lack or possess enhanced Fc-mediated functions, such as antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), and FcRn-mediated transcytosis. Antibodies can further carry toxic or radioactive payloads to target disease cells^{144, 146}. Thus, antibodies can potentially exert their modulating effect through one, or a combination of mechanistic actions, including, Fc-mediated depletion, internalization of ion channel-antibody complexes¹⁴⁷, and/or modulation of ion channel behavior with a conjugated peptide or small molecule toxin - features that may also be enhanced by bivalent avidity effects.

The above mentioned role of anti-ion channel autoantibodies in autoimmune diseases¹⁴⁸ and success in generating functional polyclonal antibodies against multiple ion channel targets¹⁴⁹ provided strong evidence for the feasibility of generating functional monoclonal antibodies (mAbs). However, overall progress in ion channel mAb discovery has been slow due to significant technical challenges that appear to be somewhat target specific. Indeed, ion channels and other multi-pass membrane proteins have recently been ranked among the “high-hanging fruit” in terms of antibody drug discovery¹⁴⁴. For example, while many ion channels share common structural motifs based around transmembrane and pore-forming domains, the surface topography of ion channels where antibodies would be predicted to bind and exert their effect (the extracellular epitope target area) can be quite different amongst ion channel families. For example, members of the acid sensing ion channel (ASIC) and the purinoceptor P2X family contain extracellular amino acids that constitute approximately 68% or 48%, respectively, of the total protein³. Conversely, voltage-gated ion channels²

such as Nav family members have far fewer amino acids exposed to the extracellular space (approximately 15%). Therefore, it is not surprising that reports of successful monoclonal antibody programs have tended to describe the discoveries of antibodies targeting the former and not the latter. Further complicating antibody discovery against these targets is the relative lack of immunogenicity of small surface loops, conservation amongst orthologs leading to tolerance in host immune animals and a relative lack of robust sources of recombinant ion channel protein to enable large scale antibody discovery programs.

Nevertheless, several strategies have been successful in generating monoclonal antibodies that inhibit ion channel function with the simplest approaches using antigenic peptides derived from ion channel extracellular (ECL) domains. A particularly popular tactic has been targeting the pore-forming E3 loop of 6-transmembrane domain Kv, Nav, or TRP channels using peptide antigens with the rationale that antibody binding to this region will inhibit ion flow. This approach has been mostly successful in generating polyclonal antibodies that block channel function¹⁴⁹ although it has also been used to generate functionally blocking mAbs targeting Kv10.1¹⁵⁰ or the T cell calcium influx channel Orai1. Using a peptide from the second extracellular loop of Orai1 a group at Amgen generated mAbs that inhibit CRAC current¹⁵¹, while a group at Novo Nordisk produced mAbs that inhibit calcium influx in T cells, suppress T cell proliferation and cytokine production, and showed efficacy in a graft-versus-host disease mouse model induced by human T cell transfer presumably by inducing Orai1 internalization¹⁵². Channel internalization also is the mechanism of action of a mAb generated against purified peptide antigens from the first ECL domain of hK_{2P}9.1 (KCNK9) that inhibits tumor growth and metastasis in mouse cancer xenograft models¹⁵³. However, there appear to be clear limitations for isolating mAbs using peptide-derived strategies presumably due to the fact, that peptide antigens, while abundant and inexpensive, typically are not representative of native structures. This challenge can be mitigated by employing immunization strategies that use ion channel antigens that contain a native fold. For example, mAbs with blocking properties have been generated by immunizing host animals with DNA (ASIC1¹⁵⁴, Kv1.3¹⁵⁵), cells expressing target ion channels (P₂X₇¹⁵⁶, Orai1¹⁵¹), ion channel containing virus like particles (P2X3¹⁵⁷), and recombinant purified ion channels (P2X3¹⁴⁷, Kv1.3¹⁵⁸). In an alternative approach, Quiang *et al.* generated a mAb that blocks ASIC1a currents with an IC₅₀ of 85 nM and reduces infarction in a rodent stroke model by panning a human scFv combinatorial antibody phage library with nanodiscs containing reconstituted truncated ASIC1a protein and then converting into a full-length IgG1 form¹⁵⁹.

The difficulty in discovering clinically relevant conventional ion channel mAbs has led academic and industry investigators to explore alternative modalities. For example, the variable domain of heavy chain only camelid antibodies, or nanobodies¹⁶⁰, are small, 12-15 kDa, modular immunoglobulins that can offer distinct advantages over conventional mAbs such as access to epitopes that otherwise would be difficult to reach with conventional immunoglobulins^{161, 162}. Additionally, the modular nature of their small singular domains allows for relatively straightforward engineering¹⁶¹ of homo- and heteromeric molecules that can increase their potency, avidity, bispecificity and lead to half-life extension (HLE). Several examples of ion channel modulating nanobodies,

including bispecific ones, have now been described including those targeting P2X7 and Kv1.3 (see below for further details).

Other modalities that are garnering considerable interest include KnotBodies, which incorporate cystine-knot proteins like venom toxins¹¹⁰ into the complementary-determining region (CDR) of the variable domain of an antibody light chain and antibodies complexed with warheads or small molecule moieties. While these fusion molecules differ from traditional antibodies where target binding is solely CDR driven, they nevertheless, represent a step-forward in the development of potential ion channel therapeutics by combining the potency of toxins with the therapeutic advantages afforded by mAbs. Another, largely unexplored group of biologics in terms of targeting and modulating ion channel function are various non-immunoglobulin protein-binding scaffolds characterized by their relatively small mass (~6-20 kDa). These include DARPins (designed ankyrin repeat proteins), affilins (ubiquitin), anticalins (lipocalin), atrimers (C-type lectin), monobodies (fibronectin type III), Kunitz domains (serine protease inhibitor) and affibodies (staphylococcal protein A domain Z)¹⁶³. As of 2015 more than 20 scaffold-derived candidates were in preclinical or clinical development with one product derived from a Kunitz domain, the kallikrein inhibitor Ecallantide (Kalbitor®), on the market for the treatment of hereditary angioedema¹⁶³. While none of the identified clinical candidates target ion channels, there is evidence suggesting that these large and diverse scaffold libraries could provide fertile ground for identifying novel ion channel binders. For example, monobodies selected from combinatorial libraries were shown to bind and block two bacterial Fluc-type fluoride channels¹⁶⁴, while high-affinity DARPins have been co-crystallized in complex with AcrB, the inner membrane pump of the *E. coli* multi-drug resistance tripartite complex AcrAB-TolC¹⁶⁵.

Case study: P2X7 as a target for cancer therapy and inflammation

The lone ion channel targeting immunoglobulin formulation that is currently in clinical development is BIL010t¹⁴⁵ from Biosceptre. BIL010t is a topical therapy for basal cell carcinoma (BCC) that contains sheep polyclonal antibodies directed against a non-functional form of the purinergic receptor channel P2X7. This form, called nfP2X7, represents a distinct conformation with a non-functional pore that is upregulated in response to high-ATP concentrations in tumor micro-environments and is required for tumor cell survival¹⁶⁶. Moreover, the P2X7 E200 peptide sequence (G²⁰⁰HNYTTRNILPGLNITC²¹⁶) whose conformation is distinct and exposed in nfP2X7 but not in WT P2X7 has enabled the generation of polyclonal and monoclonal antibodies that selectively bind nfP2X7 on the surface of tumor cells¹⁶⁶. A PEG-based topical ointment containing polyclonal antibodies purified from sheep repeatedly immunized with the E200 peptide conjugated to keyhole limpet hemocyanin caused significant reduction in B16F10 tumor growth in an orthotopic mouse model of melanoma¹⁴⁵. In a Phase-I clinical trial (NCT02587819) an ointment containing 10% BIL010t was applied to primary BCC lesions twice daily for 28 days. The treatment was well-tolerated, did not result in systemic penetration of sheep polyclonal antibodies and resulted in a decrease in lesion size in 65% of the patients with 20% showing no change and 15% showing an increase in size¹⁴⁵. In addition to BIL010t, Biosceptre is developing

BIL03s, an anti-nfP2X7 human monoclonal antibody for treatment of solid and hematological tumors.

A monoclonal antibody targeting human P2X7 was generated following immunization of mice with a mouse myeloma cell line, XS63, expressing P2X7 and screening hybridoma cell line supernatants by flow cytometry using transfected and non-transfected XS63 cells¹⁵⁶. One mAb that specifically reacted with HEK cells expressing P2X7 but not cells expressing P2X1 or P2X4 also recognized native P2X7 in human monocytes that had been differentiated into macrophages with LPS or γ -IFN. The anti-P2X7 mAb was shown to block BzATP-induced inward currents in HEK cells transfected with human P2X7 (IC_{50} ~5 nM), but not mouse or rat orthologues¹⁵⁶.

Anti-mouse P2X7 nanobodies with antagonistic (13A7, IC_{50} = 12 nM) or potentiating activity (14D5, EC_{50} = 6 nM) were isolated from phage libraries derived from llamas immunized with either HEK cells stably expressing P2X7 or cDNA¹⁶⁷. The potencies of the blocking and the enhancing nanobodies increased upon multimerization. A dimeric-HLE version of 13A7 that additionally contains the albumin-binding nanobody Alb8 to extend serum half-life was effective in ameliorating both allergic contact dermatitis and experimental glomerulonephritis in mice¹⁶⁷. Additionally, another nanobody called Dano1, that specifically recognizes human P2X7, blocked ATP-induced Ca^{2+} influx and pore formation in P2X7-expressing HEK cells with dimerization leading to increased potency (IC_{50} = 0.2 nM). Dano1 also inhibited inflammasome assembly by LPS-primed human monocytes as well as the shedding of CD62L and the externalization of phosphatidylserine by T cells. Interestingly, Dano1 was significantly more potent (20-50 fold) than the previously described conventional anti-P2X7 mAb¹⁶⁷.

Case study: Kv1.3 as a target for autoimmune disease

In addition to the validation accompanying Kv1.3 as a therapeutic target for autoimmune disease^{100, 106, 168}, its accessibility to antibodies in autoreactive effector memory T-cells makes it a particularly strong ion channel target for therapeutic intervention with immunoglobulins. This, may explain, in part, why Kv1.3 has been a popular target for the discovery and development of immunoglobulin-based molecules amongst academic and biotech researchers with examples describing polyclonal and monoclonal (conventional and nanobody) antibodies as well as KnotBodies (Fig. 3), that inhibit ion channel function and alleviate T-cell mediated autoimmune disease in animal models. For example, a polyclonal antibody (E314) purified from the sera of rabbits immunized with a 14-amino acid E3 peptide antigen directed against the hKv1.3 S5-S6 loop specifically bound to HEK cells expressing Kv1.3 but not cells expressing other Kv family members¹⁶⁹. The E314 antibody was also shown to inhibit Kv1.3 currents by approximately 90% at a concentration of 300 nM in transfected HEK cells and Jurkat T cells but showed no significant effect on Kv1.1, Kv1.2, Kv1.4, Kv1.5 or Kv11.1 (hERG)¹⁶⁹. Interestingly, a Kv1.3 vaccine was recently shown to induce high titers of anti-Kv1.3 antibodies in mice and rats that lessened clinical symptoms and decreased pathological CNS damage in a model of experimental autoimmune encephalomyelitis-presumably through the action of polyclonal anti-Kv1.3 antibodies¹⁷⁰.

Ablynx, now a Sanofi company, have described the generation of anti-Kv1.3 nanobodies isolated from llamas using a genetic prime-boost immunization strategy.

Kv1.3 nanobodies were found to be selective (10,000-fold over Kv1 family members), state-dependent and demonstrated varying functional profiles. The fast onset of functional effects strongly indicated that they were due to channel inhibition and not to internalization mechanisms¹⁵⁵. Construction of bivalent hetero- and homodimers resulted in molecules with mixed functional properties in the case of the former, namely increased potencies, target residence time, avidity effects and duration of blockade. Additionally, a trimeric nanobody displayed higher potency in T cell based assays. A nanobody comprising two identical anti-Kv1.3 nanobody monomers fused to an anti-albumin moiety to increase half-life was effective *in vivo* in reducing ear thickness in a rat delayed hypersensitivity model¹⁵⁵. More recently, conventional full-length anti-Kv1.3 mAbs have been isolated from chickens and llamas using a similar prime-boost immunization and screening strategy¹⁵⁸. Chickens were immunized and boosted with purified recombinant Kv1.3 reconstituted into liposome formulations and anti-Kv1.3 antibodies identified and cloned following B-cell screening using a gel encapsulated microenvironment (GEM) assay incorporating various Kv1.3 containing formulations including liposomes and oriented channel adhered to magnetic beads¹⁵⁸. In the case of llamas, initial immunizations were carried out with DNA and boosts with Kv1.3 liposomes. Phage libraries derived from immunized llamas were screened using Kv1.3 bound to magnetic beads. In all, 69 specific anti-Kv1.3 scFv-Fc antibodies were isolated from both host-animal platforms with 10 antibodies (9 chicken and 1 llama) demonstrating functional block of Kv1.3 current. Characterization of select scFv-Fc clones demonstrated that current block was time and concentration dependent with the most potent clone having an IC₅₀ of 6 nM. Antibodies showed no activity against related family members (Kv1.1, Kv1.2, Kv1.5), hERG or Nav1.5. Epitope binning analysis revealed that while most (7 of 10) functional antibodies segregated to one bin suggesting a dominant functional epitope, 3 antibodies segregated to different bins indicating that functional block could be achieved through distinct epitope binding events.

In an alternative approach IONTAS, a company developing a KnotBody platform based on incorporating peptide toxins with a conserved inhibitory cysteine knot (knottin) structural motif into peripheral antibody CDR loops (Fig. 3), have generated a ShK-KnotBody with a reported Kv1.3 inhibitory IC₅₀ of 8.6 nM¹⁷¹. Using a similar approach, a group at the Scripps Research Institute previously incorporated the Kv1.3 inhibitory scorpion peptide toxins Moka1 and Vm24-toxin into the CDR3H domain of a humanized anti-lysozyme antibody with high structural similarity to a bovine antibody and into the CDR2H (Moka1) and CDR3L (Moka and Vm24) domains of the humanized RSV-neutralizing antibody Syn¹¹⁰. The syn-Vm24-CDR3L antibody fusion demonstrated subnanomolar potency (IC₅₀ = 0.59 nM) in a K⁺ flux assay, inhibited activation of α CD3 stimulated T_{EM} cells with an IC₅₀ of 1 nM, displayed a long serum half-life of approximately 2 days, and showed dose-dependent inhibition of delayed type hypersensitivity reactions *in vivo* in rats.

Case study: Nav1.7 as a target for pain

A monoclonal antibody, SVmab1, with purported nanomolar potency (IC₅₀ 31 nM) was generated in mice using a peptide targeting the Nav1.7 S3-S4 extracellular loop region in the domain II (DII) voltage-sensor¹⁷². Using antibody purified from hybridoma

cultures, SVmab1 exhibited state-dependent inhibition of Nav1.7, 400-1500-fold selectivity over most Nav1.X family members, efficacy in both inflammatory and neuropathic pain models in mice and reduced scratching in both acute and chronic itch models¹⁷². However, a subsequent study using a recombinant form of the antibody (rSVmab) generated from published light and heavy chain antibody sequences was unable to bind neither the S3-S4 antigen peptide, a soluble and purified DII voltage sensor protein nor the mammalian cell expressing Nav1.7. Neither was rSVmab able to specifically block Nav1.7 current in transfected HEK293 cells¹⁷³. Interestingly, the discoverers of SVmab confirmed the apparent discrepancies between the functional hybridoma-derived and non-functional recombinant forms of the mAb and speculated that the difference may have resulted from incorrect disclosure of antibody sequences or possibly differences in post-translational modifications of antibodies produced in hybridomas compared to transfected mammalian cells¹⁷⁴. It will be of considerable interest to learn if other researchers utilizing a similar DII voltage-sensor targeted strategy can identify mAbs with similar functional properties to SVmab.

In an approach distinct from the CDR-targeted KnotBody strategy¹¹⁰, a Nav1.7 toxin-antibody conjugate was recently described that maintained the selective potency of the toxin warhead while conferring significant half-life extension and biodistribution to nerve fibers¹⁷⁵. In their study, Biswas et al. describe assembling a conjugate comprised of a nontargeting anti-2,4-dinitrophenol human IgG1 with a cysteine mutation at a surface residue (excluding CDR, effector binding domains, proline and glycine residues) attached to the tarantula venom GpTx-1 peptide toxin via a PEG₁₁ linker¹⁷⁵. In a combinatorial approach several factors including cysteine mutation sites of the carrier antibody, linker chain-length, conjugation chemistry and peptide loading were evaluated for their effect on Nav1.7 current inhibition. One antibody conjugated at substituted cysteine E384C exhibited a 30-fold loss in potency compared with naked peptide (IC₅₀ 250 nM vs 8.5 nM), but had a serum half-life of 80 h, about 130-fold longer than the naked peptide. Following intravenous administration, the GpTx-1 mAb conjugate biodistributed to mice dorsal root and sciatic nerve better than the parent mAb¹⁷⁵. Additionally, distribution of the conjugate to dorsal root and sciatic nerve was significantly more pronounced in WT mice compared to Nav1.7 knockout mice indicating that both the presence of Nav1.7 and the peptide toxin were required for distribution to peripheral nerve elements across the so-called blood nerve barrier (BNB). While the conjugate was not effective *in vivo* in a mouse histamine-induced pruritis model, most likely due to plasma concentrations that were not sufficiently greater than measured IC₅₀s, the data demonstrate the effective combination of Nav_v inhibitory properties of a toxin with the desired pharmacokinetic characteristics of an antibody.

Ion channel antibodies going forward

In many respects the ion channel antibody field is playing “catch-up” with its GPCR cousin with the latter able to boast three marketed antibodies, the anti-CCR4 antibody mogamulizumab for the treatment of Adult and Peripheral T cell lymphoma and two anti-CGRP antibodies (erenumab and fremanezumab) for migraine; there are also at least fifteen other immunoglobulins in various phases of clinical development and more projects at the preclinical stage¹⁹. The challenges associated with developing GPCR

antibodies have been described in detail elsewhere^{19, 176, 177}; suffice it to say, that in cases where functional antibodies are desirable, the GPCR and the ion channel field face the same difficulties of developing antibodies that recognize specific conformational states of the target protein (e.g. the active versus the inactive state of a GPCR or the open versus the inactivated state of an ion channel, see Fig. 1A). However, attributing the discrepancy in the current development status of GPCR and ion channel antibodies to the comparative difficulty of targeting ion channels versus GPCRs would not sufficiently acknowledge the significant advancements made in the GPCR field. These include technologies like StaR® (Stabilized Receptor), where a small number of point mutations are introduced into a GPCR to improve its thermostability without disrupting its pharmacology^{178, 179}. Heptares Therapeutics is using this technology not only to generate protein for x-ray crystallography and small molecule screening, but also to enable purification of high-quality, functional and monodisperse protein that can be incorporated into effective antigen and screening formulations for both *in vivo* immunization and *in vitro* antibody discovery platforms. Instead, the proliferation of clinical antibody candidates during the last 10 years against a difficult class of membrane proteins like GPCRs should provide a level of optimism that similar advancements in ion channels are also within reach.

Overcoming the aforementioned challenges for advancing the ion channel antibody pipeline will undoubtedly involve integrating next generation platforms into the discovery process. For example, limitations of traditional approaches such as hybridoma screening, where non-efficient fusion events and subsequent loss of rare B cells can impact immune diversity¹⁸⁰, can be mitigated by technologies that incorporate direct B-cell cloning allowing the identification of rare clones and the recovery of natively paired light and heavy chain genes¹¹⁹. Similarly, traditional display technologies such as phage and yeast-display are being further enhanced by next-generation sequencing that allows up to 10,000-fold more sequences than the Sanger method and is enabling deeper interrogation of library diversity and identification of rare clones^{181, 182}. Parallel advances in recombinant ion channel production using alternative expression hosts (e.g. *Tetrahymena thermophila*), cell-free systems and non-detergent based purification methods and formulations leading to increases in the quality (native-fold) and quantity (>mg) of purified protein or following the development of protocols enabling the production of possibly even stable, state-specific channels will likely complement newer approaches to antibody discovery and therefore increase the chances of successfully recovering antibodies with desired properties. With nanobodies it might even be feasible to target intracellular ion channel domains (see Box 2).

Overall perspective and concluding remarks

As seen from the foregoing paragraphs, ion channel drug discovery/development is approaching a cross road where future breakthroughs and new market introductions are likely to increasingly rely on biologic modalities comprising the plethora of technical opportunities based on peptides, antibodies and hybrid molecules. Given the diversity of solutions and the current speed of innovation across academia and the pharmaceutical

industry, it is difficult to pinpoint exactly which therapeutic areas will gain the most and the fastest from this development, but a conservative view is that it will largely follow the accessibility of the cellular targets. Since injected peptides and antibodies usually have quite low “volumes of distribution”, meaning that they tend to stay in the blood stream rather than penetrating deeply into tissues and cells, it is likely that hematological diseases, certain cancer forms, and immune diseases are straightforward therapeutic areas, whereas CNS diseases are much more challenging due to the tight blood brain barrier. (This view is supported by a parallel development in the field of therapeutic antibodies targeting extracellular proteins, where peripheral anti-cytokine therapy is very established and successful for treatment of autoimmune diseases, whereas attempts to treat Alzheimer’s disease with antibodies against amyloid beta are lacking behind). That said, between these opposite poles there are many opportunities defined by no or less tight endothelial barriers towards other organs (e.g. bone marrow, liver), by accessibility via local administration (e.g. lungs, skin), by administration via specific routes (e.g. intrathecal) or even by uptake across disease damaged barriers, such as the blood brain barrier in stroke and the intestinal epithelium in inflammatory bowel diseases. Considering the ion channel targets themselves it is a common view that channels with prominent extracellular loops (like GABA_A, nicotinic acetylcholine and P₂X receptors or two pore domain K⁺ channels) are more tractable by antibodies than channels with restricted extracellular exposure. However, as discussed here it is indeed possible to generate function-blocking antibodies against ion channels with limited extracellular epitopes (e.g. Kv1.3 and Orai).

While we believe that the field of ion channel targeted biologics, which at the moment can only boast the intrathecally administered Ca_v2.2 blocking peptide Ziconotide in the clinic, and a polyclonal antibody and three peptides in clinical trials, is very likely to develop and expand in the next decade, it is currently hard to predict whether venom-derived peptides or antibodies will be more successful. Peptide design is currently benefitting tremendously from the advances in ion channel structural determination and molecular modeling which together are enabling structure-based or at least structure-assisted peptide design. However, “naked” peptides typically have short circulating half-lives and, depending on whether they are intended for acute or chronic use, might require some of the half-life extending modifications or formulation approaches described here to be considered suitable for clinical development. For traditional mAbs the main challenges in our opinion will be to select the most suitable ion channel targets and then tailor the antibody characteristics to the desired therapeutic effect (e.g. inhibition or activation of channel function, channel internalization, induction of target cell apoptosis) by engineering the antibody to fit the pathophysiology of the target disease. The above discussed BIL010t antibody against a non-functional form of P₂X₇ that is required for tumor cell survival¹⁶⁶ is a perfect illustration of a very clever topical use of a polyclonal sheep antibody for basal cell carcinoma where the characteristic of the antibody seem to meet the medical need.

Text Box 1

Recent progress and challenges for ion channel structure-based drug design

In the last 5 years an increasing number of ion channel structures has become available through advances in cryoelectron microscopy (cryoEM) such as improvements in microscope design and imaging hardware, and enhanced image processing which allow the reconstruction of 3D structures from a large number of single particle 2D projection images even even in the presence of structural and conformational heterogeneity¹⁸³. While many cryo-EM structures are not high enough resolution for “true” structure-based drug design because they do not allow the possible location of hydrogen bonds or salt bridges to be seen, Rosetta computational structure refinement can be used to improve atomic details of cryoEM structures with 3-5 Å resolution¹⁸⁴. For example, in the Nav channel field both traditional x-ray and cryoEM structures now provide high-resolution structural templates for structure-based design of novel small molecules, peptides, and antibodies. However, while many of the structures contain fully resolved extracellular loops, a remaining challenge that needs to be further explored is how to stabilize the extracellular loop regions in native conformations for immunization without having to produce large quantities of full-length protein.

- An x-ray structure of a human Nav1.7– bacterial NavAb chimera³¹ with a picomolar affinity drug bound to the domain IV (DIV) voltage sensor constitutes a template for targeting the voltage sensor, which plays a key role in stabilizing Nav channels in an inactivated state, with small molecules or peptides.
- All human Nav channels have unique sequences in the extracellular loop regions within the voltage-sensing and pore-forming domains. CryoEM structures of the human Nav1.4⁴¹ and *American cockroach* NavPaS¹⁸⁵ with a small molecule and or a peptide toxin bound have all extracellular loops resolved in the pore and voltage sensor domains.
- The CryoEM structure of the *electric eel* Nav1.4⁴⁰ has all extracellular loops in the pore domain resolved.

Text Box 2

Targeting ion channel cytoplasmic epitopes with nanobodies

Immunoglobulins are not able to passively access the cell cytoplasm. Therefore, ion channel antibody discovery programs, like those directed towards other cell surface molecules, are generally designed to identify antibodies that recognize extracellular epitopes to exert their functional effect, be it steric or allosteric block, or removal of a channel from the cell surface by internalization mechanisms. Consequently, the intracellular epitope space, which is considerably larger than that displayed on the surface for many voltage-gated like ion channels (e.g. ~70% of the sodium activated K⁺ channel K_{Na}1.1), is left untargeted. In a recent review, Ingram *et. al.*¹⁶² highlighted the attributes of nanobodies (Nbs) that may allow exploiting the cytosolic side of membrane

proteins such as GPCRs or ion channels for basic research, immunodiagnostics and potentially therapeutic intervention.

Nbs are single domain antibodies comprised of the variable domain of VHH camelid IgGs. Nbs are small (~15kDa) and modular in nature, bind their targets with affinities similar to conventional antibodies¹⁸⁶ and tend to recognize epitopes in clefts of protein surfaces or at protein-interaction interfaces¹⁸⁷. Like conventional antibody light and heavy chain variable domains, Nb antigen binding is driven by three complementarity-determining regions (CDRs). However, unlike conventional antibodies that require disulfide-paired light and heavy chain variable domains to form a paratope¹⁸⁸, this is achieved in Nbs in a single domain. Therefore, for Nbs where intrachain disulfide bonds are not necessary for maintaining target specificity this attribute imparts a significant tolerance, compared to conventional antibodies, to reducing environments like the cytoplasm where Nbs can be expressed in functional form, bind cytosolic epitopes and exert a functional effect.

Cytosolic expression of Nbs has been exploited in a number of phenotypic screens to identify functional hits and further explore cellular processes. In one example, a randomized VHH-CDR3 intracellular antibody library was introduced into highly metastatic human fibrosarcoma derived cells followed by iterative rounds of cell migration assays to identify clones associated with non-migrating cells¹⁸⁹. Using this approach one nanobody clone was used to identify heterogenous nuclear ribonucleoprotein K (hnRNP-K) as having a role in metastasis¹⁸⁹. In another instance VHH coding sequences derived from alpacas immunized with inactivated Influenza A virus (IAV) or vesicular stomatitis virus (VSV) were cloned into a lentiviral vector and used in a phenotypic screen to identify intracellularly expressed Nbs that protected human A549 cells from lethal infection with either IAV or VSV¹⁹⁰. In addition to phenotypic screens, Nbs have been engineered to selectively degrade target molecules via ubiquitination by fusion to ubiquitin ligase F-box subunits^{191, 192} and have served as chaperones that bind to the intracellular surface of GPCRs to stabilize active conformations such as the β 2-adrenergic and M2 muscarinic receptors that enabled structural determination by x-ray crystallography^{193, 194}.

It remains to be determined whether targeting intracellular domains of ion channels with nanobodies will provide the same molecular insights into stabilized state-dependent structures as they have with GPCRs or whether therapeutic applications like gene therapy that result in the expression of anti-ion channel Nbs for functional disruption or degradation of targeted ion channel will move beyond speculation. Nevertheless, the propensity for Nbs to recognize epitopes in protein-protein interfaces raises the intriguing possibility of investigating the biological mechanisms of ion channel complexes. The alpha subunit comprising the ion channel pore typically represents only one of a number of regulatory and auxiliary proteins in a complex that influence channel function, trafficking, distribution and signaling. Indeed, several channelopathies are associated with mutations that disrupt ion channel complex formation and cause cardiac arrhythmias¹⁹⁵. Induced expression of Nbs raised against ion channel interaction domains may help de-convolute complex formation under varying physiological conditions, illuminate the precise temporal and spatial role of complex components and possibly identify novel targets for therapeutic intervention. New insight into the cytosolic

face of ion channel biology may simply await the design of elegant phenotypic screens utilizing intracellular nanobody libraries as described above.

Acknowledgements

We thank Aneesh Karatt Vellatt at IONTAS for providing the pdb file of the x-ray coordinates of a KnotBody Fab for Figure 3.

This work was supported by the CounterACT Program, National Institutes of Health Office of the Director (NIH OD), and the National Institute of Neurological Disorders and Stroke (NINDS), Grant Number U54NS079202 and NS100294 (to H.W.); and NMRC-CS-IRG (NMRC/CIRG/1427/2015), Singapore Ministry of Education Academic Research Fund Tier 1 (2015-T1-022-047) and Tier 2 (MOE2016-T2-2-032) (to K.G.C).

Competing interests statement

The authors declare competing financial interests: see web version for details.

Palle Christophersen is a full-time employee of Saniona A/S. Paul Colussi, is a full-time employee of TetraGenetics Inc.

Table 1: Ion channel targeting drugs approved in the last 10 years.

Table 2: Ion channel targeting drug development candidates currently in clinical trials.

Figure Legends

Figure 1. A) Cartoon representation of the Nav channel gating cycle in closed, open and inactivated states. The voltage sensing domain (VSD) segments are colored blue (S1-S3) and red (S4). The pore-forming domain segments are colored sand (S5, P1-helix, P2-helix, and N-terminal part of S6) and green (C-terminal part of S6). The gating charge carrying arginines in the S4 segment are represented by “+” signs. Key conformational changes in the Nav channel during gating between closed, open, and inactivated states are highlighted by transmembrane movement of the S4 segment (colored in red) and lateral movement of the C-terminal part of S6 segment (colored in green). Inactivation in Nav channels can be either fast and involve one of the cytoplasmic inter-repeat loops “plugging” the inner vestibule⁸ or slow and involve a rearrangement of the selectivity filter⁸. **B)** Small molecule receptor sites within Nav channels. Transmembrane view of Nav channel interaction with pore bound (lidocaine) and voltage-sensor bound (GX-936) small molecule drugs. The Nav channel structure is based on the electric eel Nav1.4 channel structure⁴⁰ (pdb: 5XSV) and is shown in ribbon representation. The pore domain is shown in beige and the voltage-sensors are shown in blue (domain I), green (domain II), yellow (domain III), and red (domain IV). Lidocaine is shown in space-filling representation and colored purple. GX-936 is shown in space-filling representation and colored light blue. The chemical structures of

vixotrigine, lidocaine, GX-936, and PF-05089771 are shown in 2D representation next to the channel.

Figure 2. A) Structures of peptide toxins targeting ion channels. Ribbon representation of peptide toxin structures colored individually and labeled. Disulfide bonds are colored in yellow. Ziconotide (pdb: 1TTK), μ -theraphotoxin-Pn3a (pdb: 5T4R_A), JZTX-V (pdb: 6CGW), Rg1A (pdb: 2JUT), chlorotoxin (pdb: 5L1C), π -hexatoxin-HI1a (pdb: 2N8F), Hm1a (pdb: 2N6O), ShK (pdb:2K9E_A), HsTx1 (pdb: 1QUZ_A), μ -CNIIIc (pdb: 2YEN). **B)** Peptide toxin receptor sites on ion channels. *Left*, Pore blocking peptide receptor site in a K_v channel illustrated by the transmembrane view of charybdotoxin in the pore of the $K_v1.2$ - $K_v2.1$ chimera structure¹⁹⁶ (pdb: 2R9R) and shown in ribbon representation. The pore is shown in beige and the voltage-sensors are shown in blue. Charybdotoxin is shown in orange. Potassium ions within the selectivity filter region are shown in sphere representation and colored purple. *Right*, Voltage sensor binding peptide receptor site in a Na_v channel illustrated by the transmembrane view of the voltage-sensor bound peptide toxin Dc1a. The Na_v structure is the American cockroach Na_vPaS structure¹⁹⁷ (pdb: 6A90) and shown in ribbon representation. The pore is shown in beige and voltage-sensors are shown in blue (domain I), green (domain II), yellow (domain III), and red (domain IV). The Dc1a toxin is shown in orange.

Figure 3. Figure illustrating the size relation of a full-length immunoglobulin G (IgG2a, pdb: 1IGT, ribbon presentation) colored in orange, an antigen-binding fragment (Fab, pdb: 1K4C) colored in orange, a KnotBody Fab colored in green, and a nanobody (pdb: 6C5W) colored in pink. The *Ecballium elaterium* Trypsin Inhibitor (EETI-II) structure within the KnotBody is shown in space-filling representation. The K_v channel structure is based on the $K_v1.2$ - $K_v2.1$ chimera¹⁹⁶ (pdb: 2R9R) and shown in ribbon representation and colored in beige. Potassium ions within the selectivity filter region are shown in sphere representation and colored in purple.

References

1. Alexander, S.P. *et al.* THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Overview. *Br. J. Pharmacol.* **174 Suppl 1**, S1-S16 (2017).
2. Alexander, S.P. *et al.* THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Voltage-gated ion channels. *Br. J. Pharmacol.* **174 Suppl 1**, S160-S194 (2017).
3. Alexander, S.P. *et al.* THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Ligand-gated ion channels. *Br. J. Pharmacol.* **174 Suppl 1**, S130-S159 (2017).
4. Alexander, S.P. *et al.* THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Other ion channels. *Br. J. Pharmacol.* **174 Suppl 1**, S195-S207 (2017).

5. Santos, R. *et al.* A comprehensive map of molecular drug targets. *Nat. Rev. Drug Discov.* **16**, 19-34 (2017).
6. Sakmann, B. & Neher, E. Patch clamp techniques for studying ionic channels in excitable membranes. *Ann. Rev. Physiol.* **46**, 455-472 (1984).
7. Hille, B. *Ionic channels of excitable membranes*, 2nd edn. Sinauer Associates: Sunderland, Mass., 1992.
8. Hille, B. *Ion channels of excitable membranes*. Sinauer Associates: Sunderland, MA, 2001.
9. Kaczorowski, G.J., McManus, O.B., Priest, B.T. & Garcia, M.L. Ion channels as drug targets: the next GPCRs. *J. Gen. Physiol.* **131**, 399-405 (2008).
10. Wulff, H., Castle, N.A. & Pardo, L.A. Voltage-gated potassium channels as therapeutic targets. *Nat. Rev. Drug Discov.* **8**, 982-1001 (2009).
11. Patapoutian, A., Tate, S. & Woolf, C.J. Transient receptor potential channels: targeting pain at the source. *Nat. Rev. Drug Discov.* **8**, 55-68 (2009).
12. Verkman, A.S. & Galletta, L.J. Chloride channels as drug targets. *Nat. Rev. Drug Discov.* **8**, 153-171 (2009).
13. Zamponi, G.W. Targeting voltage-gated calcium channels in neurological and psychiatric diseases. *Nat. Rev. Drug Discov.* **15**, 19-34 (2016).
14. Bennett, P.B. & Guthrie, H.R. Trends in ion channel drug discovery: advances in screening technologies. *Trends Biotechnol.* **21**, 563-569 (2003).
15. Dunlop, J., Bowlby, M., Peri, R., Vasilyev, D. & Arias, R. High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. *Nat. Rev. Drug Discov.* **7**, 358-368 (2008).
16. Goodman, A.D. *et al.* Sustained-release oral fampridine in multiple sclerosis: a randomised, double-blind, controlled trial. *Lancet* **373**, 732-738 (2009).
17. Vilaine, J.P. The discovery of the selective I_f current inhibitor ivabradine. A new therapeutic approach to ischemic heart disease. *Pharmacol, Res*, **53**, 424-434 (2006).
18. Van Goor, F. *et al.* Rescue of CF airway epithelial cell function *in vitro* by a CFTR potentiator, VX-770. *Proc. Natl. Acad. Sci. USA* **106**, 18825-18830 (2009).
19. Hutchings, C.J., Koglin, M., Olson, W.C. & Marshall, F.H. Opportunities for therapeutic antibodies directed at G-protein-coupled receptors. *Nat. Rev. Drug Discov.* **16**, 661 (2017).
20. Hann, M.M. & Keseru, G.M. Finding the sweet spot: the role of nature and nurture in medicinal chemistry. *Nat. Rev. Drug Discov.* **11**, 355-365 (2012).

21. Stepan, A.F. *et al.* Structural alert/reactive metabolite concept as applied in medicinal chemistry to mitigate the risk of idiosyncratic drug toxicity: a perspective based on the critical examination of trends in the top 200 drugs marketed in the United States. *Chem. Res. Toxicol.* **24**, 1345-1410 (2011).
22. Catterall, W.A. Voltage-gated sodium channels at 60: structure, function and pathophysiology. *J. Physiol.* **590**, 2577-2589 (2012).
23. Catterall, W.A. Forty Years of sodium channels: Structure, function, pharmacology, and epilepsy. *Neurochem Res.* **42**, 2495-2504 (2017).
24. Osteen, J.D. *et al.* Selective spider toxins reveal a role for the Nav1.1 channel in mechanical pain. *Nature* **534**, 494-499 (2016).
25. Salvatierra, J. *et al.* Nav1.1 inhibition can reduce visceral hypersensitivity. *JCI Insight* **3** (2018). Epub ahead of print
26. de Lera Ruiz, M. & Kraus, R.L. Voltage-gated sodium channels: Structure, function, pharmacology, and clinical indications. *J. Med. Chem.* **58**, 7093-7118 (2015).
27. Cox, J.J. *et al.* An SCN9A channelopathy causes congenital inability to experience pain. *Nature* **444**, 894-898 (2006).
28. Cummins, T.R., Dib-Hajj, S.D. & Waxman, S.G. Electrophysiological properties of mutant Nav1.7 sodium channels in a painful inherited neuropathy. *J. Neurosci.* **24**, 8232-8236 (2004).
29. Faber, C.G. *et al.* Gain-of-function Nav1.8 mutations in painful neuropathy. *Proc. Natl. Acad. Sci. USA* **109**, 19444-19449 (2012).
30. Feller, L., Khammissa, R.A.G., Fourie, J., Bouckaert, M. & Lemmer, J. Postherpetic neuralgia and trigeminal neuralgia. *Pain Res. Treat.* **2017**, 1681765 (2017).
31. Ahuja, S. *et al.* Structural basis of Nav1.7 inhibition by an isoform-selective small-molecule antagonist. *Science* **350**, aac5464 (2015).
32. Alexandrou, A.J. *et al.* Subtype-selective small molecule inhibitors reveal a fundamental role for Nav1.7 in nociceptor electrogenesis, axonal conduction and presynaptic release. *PLoS ONE* **11**, e0152405 (2016).
33. Kornecook, T.J. *et al.* Pharmacologic characterization of AMG8379, a potent and selective small molecule sulfonamide antagonist of the voltage-gated sodium channel Nav1.7. *J. Pharmacol. Exp. Ther.* **362**, 146-160 (2017).
34. McDonnell, A. *et al.* Efficacy of the Nav1.7 blocker PF-05089771 in a randomised, placebo-controlled, double-blind clinical study in subjects with painful diabetic peripheral neuropathy. *Pain* **159**, 1465-1476 (2018).
35. Focken, T. *et al.* Design of conformationally constrained acyl sulfonamide isosteres: Identification of *N*-([1,2,4]triazolo[4,3-*a*]pyridin-3-yl)methane-sulfonamides as potent and

- selective *hNav*1.7 inhibitors for the treatment of Pain. *J. Med. Chem.* **61**, 4810-4831 (2018).
36. Bankar, G. *et al.* Selective *Nav*1.7 antagonists with long residence time show improved efficacy against inflammatory and neuropathic pain. *Cell Rep.* **24**, 3133-3145 (2018).
 37. Casula, M.A. *et al.* Expression of the sodium channel β 3 subunit in injured human sensory neurons. *Neuroreport* **15**, 1629-1632 (2004).
 38. Berta, T. *et al.* Transcriptional and functional profiles of voltage-gated Na^+ channels in injured and non-injured DRG neurons in the SNI model of neuropathic pain. *Mol. Cell. Neurosci.* **37**, 196-208 (2008).
 39. Sokolov, M.V. *et al.* Co-expression of β -subunits with the voltage-gated sodium channel *Nav*1.7: the importance of subunit association and phosphorylation and their effects on channel pharmacology and biophysics. *J. Mol. Neurosci.* (2018). Epub ahead of print
 40. Yan, Z. *et al.* Structure of the *Nav*1.4- β 1 complex from electric eel. *Cell* **170**, 470-482 e411 (2017).
 41. Pan, X. *et al.* Structure of the human voltage-gated sodium channel *Nav*1.4 in complex with β 1. *Science* (2018). Epub ahead of print
 42. Deuis, J.R. *et al.* Analgesic effects of GpTx-1, PF-04856264 and CNV1014802 in a mouse model of *Nav*1.7-mediated pain. *Toxins (Basel)* **8**, E78 (2016).
 43. Sanguinetti, M.C. & Tristani-Firouzi, M. *hERG* potassium channels and cardiac arrhythmia. *Nature* **440**, 463-469 (2006).
 44. Wang, W. & MacKinnon, R. Cryo-EM Structure of the open human Ether-a-go-go-related K^+ Channel *hERG*. *Cell* **169**, 422-430 (2017).
 45. Vandenberg, J.I., Perozo, E. & Allen, T.W. Towards a structural view of drug binding to *hERG* K^+ channels. *Trends Pharmacol. Sci.* **38**, 899-907 (2017).
 46. Casida, J.E. & Durkin, K.A. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annu. Rev. Entomol.* **58**, 99-117 (2013).
 47. Nieva, J.L., Madan, V. & Carrasco, L. Viroporins: structure and biological functions. *Nat. Rev. Microbiol.* **10**, 563-574 (2012).
 48. Hay, M., Thomas, D.W., Craighead, J.L., Economides, C. & Rosenthal, J. Clinical development success rates for investigational drugs. *Nat. Biotechnol.* **32**, 40-51 (2014).
 49. Gilhus, N.E. *et al.* Myasthenia gravis - autoantibody characteristics and their implications for therapy. *Nat. Rev. Neurol.* **12**, 259-268 (2016).
 50. Titulaer, M.J., Lang, B. & Verschuuren, J.J. Lambert-Eaton myasthenic syndrome: from clinical characteristics to therapeutic strategies. *Lancet Neurol.* **10**, 1098-1107 (2011).

51. Bradl, M., Reindl, M. & Lassmann, H. Mechanisms for lesion localization in neuromyelitis optica spectrum disorders. *Curr. Opin. Neurol.* **31**, 325-333 (2018).
52. Holz, G.G. & Chepurny, O.G. Glucagon-like peptide-1 synthetic analogs: new therapeutic agents for use in the treatment of diabetes mellitus. *Cur. Med. Chem.* **10**, 2471-2483 (2003).
53. Fosgerau, K. & Hoffmann, T. Peptide therapeutics: current status and future directions. *Drug Discov. Today* **20**, 122-128 (2015).
54. Al Musaimi, O., Al Shaer, D., de la Torre, B.G. & Albericio, F. 2017 FDA peptide harvest. *Pharmaceuticals (Basel)* **11**, E42 (2018).
55. King, G.F. Venoms as a platform for human drugs: translating toxins into therapeutics. *Expert Opin. Biol. Ther.* **11**, 1469-1484 (2011).
56. Robinson, S.D., Undheim, E.A.B., Ueberheide, B. & King, G.F. Venom peptides as therapeutics: advances, challenges and the future of venom-peptide discovery. *Expert. Rev. Proteomics* **14**, 931-939 (2017).
57. Norton, R.S. & Chandy, K.G. Venom-derived peptide inhibitors of voltage-gated potassium channels. *Neuropharmacology* **127**, 124-138 (2017).
58. Pennington, M.W., Czerwinski, A. & Norton, R.S. Peptide therapeutics from venom: Current status and potential. *Bioorg. Med. Chem.* **26**, 2738-2758 (2018).
59. Beeton, C., Gutman, G.A. & Chandy, K.G. Targets and therapeutic properties of venom peptides. In: Kastin, A.J. (ed). *The Handbook of Biologically Active Peptides*. Academic Press 2006, pp 403-413.
60. Narahashi, T., Anderson, N.C. & Moore, J.W. Tetrodotoxin does not block excitation from inside the nerve membrane. *Science* **153**, 765-767 (1966).
61. McQuarrie, C., Salvaterra, P.M., De Blas, A., Routes, J. & Mahler, H.R. Studies on nicotinic acetylcholine receptors in mammalian brain. Preliminary characterization of membrane-bound alpha-bungarotoxin receptors in rat cerebral cortex. *J. Biol. Chem.* **251**, 6335-6339 (1976).
62. Ray, R., Morrow, C.S. & Catterall, W.A. Binding of scorpion toxin to receptor sites associated with voltage-sensitive sodium channels in synaptic nerve ending particles. *J. Biol. Chem.* **253**, 7307-7313 (1978).
63. Safavi-Hemami, H., Brogan, S.E. & Olivera, B.M. Pain therapeutics from cone snail venoms: From Ziconotide to novel non-opioid pathways. *J. Proteomics* **190**, 12-20. (2019).
64. Kerr, L.M. & Yoshikami, D. A venom peptide with a novel presynaptic blocking action. *Nature* **308**, 282-284 (1984).
65. Olivera, B.M. *et al.* Peptide neurotoxins from fish-hunting cone snails. *Science* **230**, 1338-1343 (1985).

66. McCleskey, E.W. *et al.* Omega-conotoxin: direct and persistent blockade of specific types of calcium channels in neurons but not muscle. *Proc. Natl. Acad. Sci. USA* **84**, 4327-4331 (1987).
67. Schmidtko, A., Lotsch, J., Freynhagen, R. & Geisslinger, G. Ziconotide for treatment of severe chronic pain. *Lancet* **375**, 1569-1577 (2010).
68. Deuis, J.R. *et al.* Pharmacological characterisation of the highly Na_v1.7 selective spider venom peptide Pn3a. *Sci. Rep.* **7**, 40883 (2017).
69. Moyer, B.D. *et al.* Pharmacological characterization of potent and selective Na_v1.7 inhibitors engineered from *Chilobrachys jingzhao* tarantula venom peptide JzTx-V. *PLoS ONE* **13**, e0196791 (2018).
70. Minett, M.S. *et al.* Endogenous opioids contribute to insensitivity to pain in humans and mice lacking sodium channel Na_v1.7. *Nat. Commun.* **6**, 8967 (2015).
71. Isensee, J. *et al.* Synergistic regulation of serotonin and opioid signaling contributes to pain insensitivity in Na_v1.7 knockout mice. *Sci. Signal.* **10**, eaah4874 (2017).
72. Pereira, V. *et al.* Analgesia linked to Na_v1.7 loss of function requires μ - and δ -opioid receptors. *Wellcome Open Res* **3**, 101 (2018).
73. Vincler, M. *et al.* Molecular mechanism for analgesia involving specific antagonism of α 9 α 10 nicotinic acetylcholine receptors. *Proc. Natl. Acad. Sci. USA* **103**, 17880-17884 (2006).
74. Clark, R.J., Fischer, H., Nevin, S.T., Adams, D.J. & Craik, D.J. The synthesis, structural characterization, and receptor specificity of the α -conotoxin Vc1.1. *J. Biol. Chem.* **281**, 23254-23263 (2006).
75. Ellison, M. *et al.* α -Rg1A: a novel conotoxin that specifically and potently blocks the α 9 α 10 nAChR. *Biochemistry* **45**, 1511-1517 (2006).
76. Callaghan, B. *et al.* Analgesic α -conotoxins Vc1.1 and Rg1A inhibit N-type calcium channels in rat sensory neurons via GABA_B receptor activation. *J. Neurosci.* **28**, 10943-10951 (2008).
77. Castro, J. *et al.* α -Conotoxin Vc1.1 inhibits human dorsal root ganglion neuroexcitability and mouse colonic nociception via GABA_B receptors. *Gut* **66**, 1083-1094 (2017).
78. Azam, L. & McIntosh, J.M. Molecular basis for the differential sensitivity of rat and human α 9 α 10 nAChRs to α -conotoxin Rg1A. *J. Neurochem.* **122**, 1137-1144 (2012).
79. Romero, H.K. *et al.* Inhibition of α 9 α 10 nicotinic acetylcholine receptors prevents chemotherapy-induced neuropathic pain. *Proc. Natl. Acad. Sci. USA* **114**, E1825-E1832 (2017).

80. Grishin, E.V. *et al.* Novel peptide from spider venom inhibits P2X3 receptors and inflammatory pain. *Ann. Neurol.* **67**, 680-683 (2010).
81. DeBin, J.A., Maggio, J.E. & Strichartz, G.R. Purification and characterization of chlorotoxin, a chloride channel ligand from the venom of the scorpion. *Am. J. Physiol.* **264**, C361-369 (1993).
82. Ullrich, N., Gillespie, G.Y. & Sontheimer, H. Human astrocytoma cells express a unique chloride current. *Neuroreport* **7**, 343-347 (1995).
83. Ullrich, N. & Sontheimer, H. Biophysical and pharmacological characterization of chloride currents in human astrocytoma cells. *Am. J. Physiol.* **270**, C1511-1521 (1996).
84. Soroceanu, L., Manning, T.J., Jr. & Sontheimer, H. Modulation of glioma cell migration and invasion using Cl⁻ and K⁺ ion channel blockers. *J. Neurosci.* **19**, 5942-5954 (1999).
85. Deshane, J., Garner, C.C. & Sontheimer, H. Chlorotoxin inhibits glioma cell invasion via matrix metalloproteinase-2. *J. Biol. Chem.* **278**, 4135-4144 (2003).
86. Maertens, C., Wei, L., Tytgat, J., Droogmans, G. & Nilius, B. Chlorotoxin does not inhibit volume-regulated, calcium-activated and cyclic AMP-activated chloride channels. *Br. J. Pharmacol.* **129**, 791-801 (2000).
87. Stroud, M.R., Hansen, S.J. & Olson, J.M. *In vivo* bio-imaging using chlorotoxin-based conjugates. *Curr. Pharm. Des.* **17**, 4362-4371 (2011).
88. Parrish-Novak, J. *et al.* Nonclinical profile of BLZ-100, a tumor-targeting fluorescent imaging agent. *Int. J. Toxicol.* **36**, 104-112 (2017).
89. Lehen'kyi, V., Raphael, M. & Prevarskaya, N. The role of the TRPV6 channel in cancer. *J. Physiol.* **590**, 1369-1376 (2012).
90. Bowen, C.V. *et al.* *In vivo* detection of human TRPV6-rich tumors with anti-cancer peptides derived from soricidin. *PLoS ONE* **8**, e58866 (2013).
91. Fu, S. *et al.* First-in-human phase I study of SOR-C13, a TRPV6 calcium channel inhibitor, in patients with advanced solid tumors. *Invest. New Drugs* **35**, 324-333 (2017).
92. Xiong, Z.G. *et al.* Neuroprotection in ischemia: blocking calcium-permeable acid-sensing ion channels. *Cell* **118**, 687-698 (2004).
93. Chassagnon, I.R. *et al.* Potent neuroprotection after stroke afforded by a double-knot spider-venom peptide that inhibits acid-sensing ion channel 1a. *Proc. Natl. Acad. Sci. USA* **114**, 3750-3755 (2017).
94. Richards, K.L. *et al.* Selective Na_v1.1 activation rescues Dravet syndrome mice from seizures and premature death. *Proc. Natl. Acad. Sci. USA* **115**, E8077-E8085. (2018).
95. Proctor, C.M. *et al.* Electrophoretic drug delivery for seizure control. *Sci. Adv.* **4**, eaau1291 (2018).

96. DeCoursey, T.E., Chandy, K.G., Gupta, S. & Cahalan, M.D. Voltage-gated K⁺ channels in human T lymphocytes: a role in mitogenesis? *Nature* **307**, 465-468 (1984).
97. Matteson, D.R. & Deutsch, C. K channels in T lymphocytes: a patch clamp study using monoclonal antibody adhesion. *Nature* **307**, 468-471 (1984).
98. Cahalan, M.D. & Chandy, K.G. The functional network of ion channels in T lymphocytes. *Immunol. Rev.* **231**, 59-87 (2009).
99. Feske, S., Wulff, H. & Skolnik, E.Y. Ion channels in innate and adaptive immunity. *Ann. Rev. Immunol.* **33**, 291-353 (2015).
100. Beeton, C. *et al.* Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases. *Proc. Natl. Acad. Sci. USA* **103**, 17414-17419 (2006).
101. Pennington, M. *et al.* Chemical synthesis and characterization of ShK toxin: a potent potassium channel inhibitor from a sea anemone. *Int. J. Pept. Protein Res.* **346**, 354-358 (1995).
102. Kalman, K. *et al.* ShK-Dap²², a potent Kv1.3-specific immunosuppressive polypeptide. *J. Biol. Chem.* **273**, 32697-32707 (1998).
103. Beeton, C. *et al.* Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channels for therapy of autoimmune diseases. *Mol. Pharmacol.* **67**, 1369-1381 (2005).
104. Tarcha, E.J. *et al.* Durable pharmacological responses from the peptide drug ShK-186, a specific Kv1.3 channel inhibitor that suppresses T cell mediators of autoimmune disease. *J. Pharmacol. Exp. Ther.* **342**, 642-653. (2012).
105. Chi, V. *et al.* Development of a sea anemone toxin as an immunomodulator for therapy of autoimmune diseases. *Toxicon* **59**, 529-546 (2012).
106. Tarcha, E.J. *et al.* Safety and pharmacodynamics of dalazatide, a Kv1.3 channel inhibitor, in the treatment of plaque psoriasis: A randomized phase 1b trial. *PLoS ONE* **12**, e0180762 (2017).
107. Mozaffar, T., Wencel, M., Goyal, N., Philips, C. & Olsen, C. Kv1.3 expression on effector memory T cells in sporadic inclusion body myositis: potential for targeted immunotherapy with dalazatide. *Neuromuscular Disorders* **27**, S158 (2017).
108. Tanner, M.R. *et al.* Prolonged immunomodulation in inflammatory arthritis using the selective Kv1.3 channel blocker HsTX1[R14A] and its PEGylated analog. *Clin. Immunol.* **180**, 45-57 (2017).
109. Zhang, H.K. *et al.* Autocrine-based selection of drugs that target ion channels from combinatorial venom peptide libraries. *Angew. Chem. Int. Edit.* **55**, 9306-9310 (2016).
110. Wang, R.S.E. *et al.* Rational design of a Kv1.3 channel-blocking antibody as a selective immunosuppressant. *Proc. Natl. Acad. Sci. USA* **113**, 11501-11506 (2016).

111. Jin, L. *et al.* Buccal mucosal delivery of a potent peptide leads to therapeutically-relevant plasma concentrations for the treatment of autoimmune diseases. *J. Control. Release* **199**, 37-44 (2015).
112. Jin, L. *et al.* Pulmonary delivery of the K_v1.3-blocking Peptide HsTX1[R14A] for the treatment of autoimmune diseases. *J. Pharm. Sci.* **105**, 650-656 (2016).
113. Murray, J.K. *et al.* Pharmaceutical optimization of peptide toxins for ion channel targets: Potent, selective, and long-lived antagonists of K_v1.3. *J. Med. Chem.* **58**, 6784-6802 (2015).
114. Edwards, W. *et al.* Targeting the ion channel K_v1.3 with scorpion venom peptides engineered for potency, selectivity, and half-life. *J. Biol. Chem.* **289**, 22704-22714 (2014).
115. Fung-Leung, W.P. *et al.* T cell subset and stimulation strength-dependent modulation of T cell activation by K_v1.3 blockers. *PLoS ONE* **12**, e0170102 (2017).
116. Chiang, E.Y. *et al.* Potassium channels K_v1.3 and K_{Ca}3.1 cooperatively and compensatorily regulate antigen-specific memory T cell functions. *Nat. Commun.* **8**, 14644 (2017).
117. Markgraf, R. *et al.* Mechanism and molecular basis for the sodium channel subtype specificity of micro-conopeptide CnIIIC. *Br. J. Pharmacol.* **167**, 576-586 (2012).
118. Del Rio-Sancho, S., Cros, C., Coutaz, B., Cuendet, M. & Kalia, Y.N. Cutaneous iontophoresis of μ -conotoxin CnIIIC. A potent Na_v1.4 antagonist with analgesic, anaesthetic and myorelaxant properties. *Int. J. Pharm.* **518**, 59-65 (2017).
119. Balaev, A.N., Okhmanovich, K.A. & Osipov, V.N. A shortened, protecting group free, synthesis of the anti-wrinkle venom analogue Syn-Ake (R) exploiting an optimized Hofmann-type rearrangement. *Tetrahedron Lett* **55**, 5745-5747 (2014).
120. Goliger, J.A. & Paul, D.L. Wounding alters epidermal connexin expression and gap junction-mediated intercellular communication. *Mol. Biol. Cell* **6**, 1491-1501 (1995).
121. Grek, C.L. *et al.* Topical administration of a connexin43-based peptide augments healing of chronic neuropathic diabetic foot ulcers: A multicenter, randomized trial. *Wound Repair Regen.* **23**, 203-212 (2015).
122. Ghatnekar, G.S., Grek, C.L., Armstrong, D.G., Desai, S.C. & Gourdie, R.G. The effect of a connexin43-based peptide on the healing of chronic venous leg ulcers: a multicenter, randomized trial. *J. Invest. Dermatol.* **135**, 289-298 (2015).
123. Strohl, W.R. Fusion proteins for half-life extension of biologics as a strategy to make biobetters. *BioDrugs* **29**, 215-239 (2015).
124. Chandy, K.G. & Norton, R.S. Peptide blockers of K_v1.3 channels in T cells as therapeutics for autoimmune disease. *Curr. Opin. Chem. Biol.* **38**, 97-107 (2017).

125. Kaspar, A.A. & Reichert, J.M. Future directions for peptide therapeutics development. *Drug Discov. Today* **18**, 807-817 (2013).
126. Bhardwaj, G. *et al.* Accurate *de novo* design of hyperstable constrained peptides. *Nature* **538**, 329-335 (2016).
127. Halai, R. *et al.* Effects of cyclization on stability, structure, and activity of α -conotoxin RgIA at the $\alpha 9\alpha 10$ nicotinic acetylcholine receptor and GABA_B receptor. *J. Med. Chem.* **54**, 6984-6992 (2011).
128. Kwon, S. *et al.* Efficient enzymatic cyclization of an inhibitory cystine knot-containing peptide. *Biotechnol. Bioeng.* **113**, 2202-2212 (2016).
129. Bergeron, Z.L. & Bingham, J.P. Scorpion toxins specific for potassium (K⁺) channels: a historical overview of peptide bioengineering. *Toxins (Basel)* **4**, 1082-1119 (2012).
130. Clark, R.J. *et al.* Engineering stable peptide toxins by means of backbone cyclization: stabilization of the α -conotoxin MII. *Proc. Natl. Acad. Sci. USA* **102**, 13767-13772 (2005).
131. Jensen, J.E. *et al.* Cyclisation increases the stability of the sea anemone peptide APETx2 but decreases its activity at acid-sensing ion channel 3. *Mar. Drugs* **10**, 1511-1527 (2012).
132. van Lierop, B.J. *et al.* Dicarba α -conotoxin Vc1.1 analogues with differential selectivity for nicotinic acetylcholine and GABA_B receptors. *ACS Chem. Biol.* **8**, 1815-1821 (2013).
133. Yu, R. *et al.* Less is More: Design of a highly stable disulfide-deleted mutant of analgesic cyclic α -conotoxin Vc1.1. *Sci. Rep.* **5**, 13264 (2015).
134. Platt, R.J. *et al.* Stapling mimics noncovalent interactions of γ -carboxyglutamates in conantokins, peptidic antagonists of *N*-methyl-*D*-aspartic acid receptors. *J. Biol. Chem.* **287**, 20727-20736 (2012).
135. Harvey, A.J., Gable, R.W. & Baell, J.B. A three-residue, continuous binding epitope peptidomimetic of ShK toxin as a K_v1.3 inhibitor. *Bioorg. Med. Chem. Lett.* **15**, 3193-3196 (2005).
136. Chen, R. *et al.* Peptidomimetic star polymers for targeting biological ion channels. *PLoS ONE* **11**, e0152169 (2016).
137. Tranberg, C.E. *et al.* ω -Conotoxin GVIA mimetics that bind and inhibit neuronal Cav2.2 ion channels. *Mar. Drugs* **10**, 2349-2368 (2012).
138. Mollica, A. *et al.* Design, synthesis and biological evaluation of two opioid agonist and Ca_v2.2 blocker multitarget ligands. *Chem. Biol. Drug Des.* **86**, 156-162 (2015).
139. Rusconi, F. *et al.* Peptidomimetic targeting of Ca_v β 2 overcomes dysregulation of the L-type calcium channel density and recovers cardiac function. *Circulation* **134**, 534-546 (2016).

140. Michne, W. *et al.* Novel inhibitors of potassium ion channels on human T lymphocytes. *J. Med. Chem.* **38**, 1877-1883 (1995).
141. Hill, R.J. *et al.* WIN 17317-3: novel nonpeptide antagonist of voltage-activated K⁺ channels in human T lymphocytes. *Mol. Pharmacol.* **48**, 98-104 (1995).
142. Nguyen, A. *et al.* Novel nonpeptide agents potently block the C-type inactivated conformation of Kv1.3 and suppress T cell activation. *Mol. Pharmacol.* **50**, 1672-1679 (1996).
143. Knaus, H.G. *et al.* Tremorgenic indole alkaloids potently inhibit smooth muscle high-conductance calcium-activated potassium channels. *Biochemistry* **33**, 5819-5828 (1994).
144. Carter, P.J. & Lazar, G.A. Next generation antibody drugs: pursuit of the 'high-hanging fruit'. *Nat. Rev. Drug Discov.* **17**, 197-223 (2018).
145. Gilbert, S.M. *et al.* A phase I clinical trial demonstrates that nP2X7 -targeted antibodies provide a novel, safe and tolerable topical therapy for basal cell carcinoma. *Br. J. Dermatol.* **177**, 117-124 (2017).
146. Kamath, A.V. Translational pharmacokinetics and pharmacodynamics of monoclonal antibodies. *Drug Discov. Today Technol.* **21-22**, 75-83 (2016).
147. Shcherbatko, A. *et al.* Modulation of P2X3 and P2X2/3 receptors by monoclonal antibodies. *J. Biol. Chem.* **291**, 12254-12270 (2016).
148. Vernino, S. Autoimmune and paraneoplastic channelopathies. *Neurotherapeutics* **4**, 305-314 (2007).
149. Sun, H. & Li, M. Antibody therapeutics targeting ion channels: are we there yet? *Acta Pharmacol. Sin.* **34**, 199-204 (2013).
150. Gomez-Varela, D. *et al.* Monoclonal antibody blockade of the human Eag1 potassium channel function exerts antitumor activity. *Cancer Res.* **67**, 7343-7349 (2007).
151. Lin, F.F. *et al.* Generation and characterization of fully human monoclonal antibodies against human Orai1 for autoimmune disease. *J. Pharmacol. Exp. Ther.* **345**, 225-238 (2013).
152. Cox, J.H. *et al.* Antibody-mediated targeting of the Orai1 calcium channel inhibits T cell function. *PLoS ONE* **8**, e82944 (2013).
153. Sun, H. *et al.* A monoclonal antibody against KCNK9 K⁺ channel extracellular domain inhibits tumour growth and metastasis. *Nat. Commun.* **7**, 10339 (2016).
154. MacDonald, L., Gao, M., Morra, M., Alessandri-Haber, N.M. & LaCroix-Fralish, M.L., inventors; Regeneron Pharmaceuticals, Inc. (Tarrytown, NY) assignee. Anti-ASIC1 Antibodies and Uses Thereof. US patent US9371383. 2016.

155. Stortelers, C., Pinto-Espinoza, C., Van Hoorick, D. & Koch-Nolte, F. Modulating ion channel function with antibodies and nanobodies. *Curr. Opin. Immunol.* **52**, 18-26 (2018).
156. Buell, G. *et al.* Blockade of human P2X7 receptor function with a monoclonal antibody. *Blood* **92**, 3521-3528 (1998).
157. Mettler Izquierdo, S. *et al.* High-efficiency antibody discovery achieved with multiplexed microscopy. *Microscopy (Oxf)* **65**, 341-352 (2016).
158. Bednenko, J. *et al.* A multiplatform strategy for the discovery of conventional monoclonal antibodies that inhibit the voltage-gated potassium channel K_v1.3. *MAbs* **10**, 636-650 (2018).
159. Qiang, M. *et al.* Selection of an ASIC1a-blocking combinatorial antibody that protects cells from ischemic death. *Proc. Natl. Acad. Sci. USA* **115**, E7469-E7477 (2018).
160. Hamers-Casterman, C. *et al.* Naturally occurring antibodies devoid of light chains. *Nature* **363**, 446-448 (1993).
161. Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annu. Rev. Biochem.* **82**, 775-797 (2013).
162. Ingram, J.R., Schmidt, F.I. & Ploegh, H.L. Exploiting nanobodies' singular traits. *Ann. Rev. Immunol.* **36**, 695-715 (2018).
163. Vazquez-Lombardi, R. *et al.* Challenges and opportunities for non-antibody scaffold drugs. *Drug Discov. Today* **20**, 1271-1283 (2015).
164. Stockbridge, R.B., Koide, A., Miller, C. & Koide, S. Proof of dual-topology architecture of Fluc F⁻ channels with monobody blockers. *Nat. Commun.* **5**, 5120 (2014).
165. Sennhauser, G., Amstutz, P., Briand, C., Storchenegger, O. & Grutter, M.G. Drug export pathway of multidrug exporter AcrB revealed by DARPin inhibitors. *PLoS Biol* **5**, e7 (2007).
166. Gilbert, S.M. *et al.* ATP in the tumour microenvironment drives expression of nfP2X7, a key mediator of cancer cell survival. *Oncogene* (2018). Epub ahead of print
167. Danquah, W. *et al.* Nanobodies that block gating of the P2X7 ion channel ameliorate inflammation. *Sci. Transl. Med.* **8**, 366ra162 (2016).
168. Matheu, M.P. *et al.* Imaging of effector memory T cells during a delayed-type hypersensitivity reaction and suppression by K_v1.3 channel block. *Immunity* **29**, 602-614 (2008).
169. Yang, X.F. *et al.* The antibody targeting the E314 peptide of human K_v1.3 pore region serves as a novel, potent and specific channel blocker. *PLoS ONE* **7**, e36379 (2012).

170. Fan, C. *et al.* A novel PADRE-K_v1.3 vaccine effectively induces therapeutic antibodies and ameliorates experimental autoimmune encephalomyelitis in rats. *Clin. Immunol.* **193**, 98-109 (2018).
171. Vellatt, A.K. KnotBodiesTM: creating ion channel blocking antibodies by fusing Knottins into peripheral CDR loops. Precision Medicine and Ion Channel Retreat 2017; 2017.
172. Lee, J.H. *et al.* A monoclonal antibody that targets a Na_v1.7 channel voltage sensor for pain and itch relief. *Cell* **157**, 1393-1404 (2014).
173. Dong, L. *et al.* Evaluation of recombinant monoclonal antibody SVMab1 binding to Na_v1.7 target sequences and block of human Na_v1.7 currents. *F1000Res* **5**, 2764 (2016).
174. Bang, S. *et al.* Differential inhibition of Na_v1.7 and neuropathic pain by hybridoma-produced and recombinant monoclonal antibodies that target Na_v1.7: Differential activities of Na_v1.7-targeting monoclonal antibodies. *Neurosci. Bull.* **34**, 22-41 (2018).
175. Biswas, K. *et al.* Engineering antibody reactivity for efficient derivatization to generate Na_v1.7 inhibitory GpTx-1 peptide-antibody conjugates. *ACS Chem. Biol.* **12**, 2427-2435 (2017).
176. Douthwaite, J.A., Finch, D.K., Mustelin, T. & Wilkinson, T.C. Development of therapeutic antibodies to G protein-coupled receptors and ion channels: Opportunities, challenges and their therapeutic potential in respiratory diseases. *Pharmacol. Ther.* **169**, 113-123 (2017).
177. Ayoub, M.A. *et al.* Antibodies targeting G protein-coupled receptors: Recent advances and therapeutic challenges. *MAbs* **9**, 735-741 (2017).
178. Magnani, F. *et al.* A mutagenesis and screening strategy to generate optimally thermostabilized membrane proteins for structural studies. *Nat. Protoc.* **11**, 1554-1571 (2016).
179. Serrano-Vega, M.J., Magnani, F., Shibata, Y. & Tate, C.G. Conformational thermostabilization of the β 1-adrenergic receptor in a detergent-resistant form. *Proc. Natl. Acad. Sci. USA* **105**, 877-882 (2008).
180. Starkie, D.O., Compson, J.E., Rapecki, S. & Lightwood, D.J. Generation of recombinant monoclonal antibodies from immunised mice and rabbits via flow cytometry and sorting of antigen-specific IgG⁺ memory B cells. *PLoS ONE* **11**, e0152282 (2016).
181. Rouet, R., Jackson, K.J.L., Langley, D.B. & Christ, D. Next-generation sequencing of antibody display repertoires. *Front. Immunol.* **9**, 118 (2018).
182. Yang, W. *et al.* Next-generation sequencing enables the discovery of more diverse positive clones from a phage-displayed antibody library. *Exp. Mol. Med.* **49**, e308 (2017).
183. Earl, L.A., Falconieri, V., Milne, J.L. & Subramaniam, S. Cryo-EM: beyond the microscope. *Curr. Opin. Struct. Biol.* **46**, 71-78 (2017).

184. Wang, R.Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *Elife* **5**, e17219 (2016).
185. Shen, H. *et al.* Structural basis for the modulation of voltage-gated sodium channels by animal toxins. *Science* **362**, 6412 (2018).
186. Gonzalez-Sapienza, G., Rossotti, M.A. & Tabares-da Rosa, S. Single-domain antibodies as versatile affinity reagents for analytical and diagnostic applications. *Front. Immunol.* **8**, 977 (2017).
187. Vincke, C. & Muyldermans, S. Introduction to heavy chain-only antibodies and derived nanobodies. In: Saerens, D. & Muyldermans, S. (eds). *Single Domain Antibodies*, vol. 911. Humana: Totowa, NJ, 2012, pp 15-26.
188. Sela-Culang, I., Kunik, V. & Ofran, Y. The structural basis of antibody-antigen recognition. *Front. Immunol.* **4**, 302 (2013).
189. Inoue, A., Sawata, S.Y., Taira, K. & Wadhwa, R. Loss-of-function screening by randomized intracellular antibodies: identification of hnRNP-K as a potential target for metastasis. *Proc. Natl. Acad. Sci. USA* **104**, 8983-8988 (2007).
190. Schmidt, F.I. *et al.* Phenotypic lentivirus screens to identify functional single domain antibodies. *Nat. Microbiol.* **1**, 16080 (2016).
191. Caussin, E., Kanca, O. & Affolter, M. Fluorescent fusion protein knockout mediated by anti-GFP nanobody. *Nat. Struct. Mol. Biol.* **19**, 117-121 (2011).
192. Kuo, C.-L., Oyler, G.A. & Shoemaker, C.B. Accelerated neuronal cell recovery from Botulinum neurotoxin intoxication by targeted ubiquitination. *PLoS ONE* **6**, e20352 (2011).
193. Rasmussen, S.G.F. *et al.* Structure of a nanobody-stabilized active state of the β 2-adrenoceptor. *Nature* **469**, 175-180 (2011).
194. Kruse, A.C. *et al.* Activation and allosteric modulation of a muscarinic acetylcholine receptor. *Nature* **504**, 101-106 (2013).
195. Heijman, J. & Dobrev, D. Ion channels as part of macromolecular multiprotein complexes: Clinical significance. *Herzschrittmacherther. Elektrophysiol.* **29**, 30-35 (2018).
196. Long, S.B., Tao, X., Campbell, E.B. & MacKinnon, R. Atomic structure of a voltage-dependent K⁺ channel in a lipid membrane-like environment. *Nature* **450**, 376-382 (2007).
197. Shen, H. *et al.* Structure of a eukaryotic voltage-gated sodium channel at near-atomic resolution. *Science* **355**, eaal4326 (2017).

Compound	Modality	Indication	Route of administration	Target/Mechanism	Approval/Withdrawal	Company
Brexanolone	Neurosteroid	Post partum depression	PO	GABA _A δ preferring PAM	2018	Sage Therapeutics
Clevidipine	Small molecule	Acute hypertension	IV	Ca _v 1.x inhibitor	2008	The Medicines Company
Lacosamide	Small molecule	Epilepsy	PO	Na _v inhibitor	2008	UCB
Amifampridine	Small molecule	Lambert–Eaton myasthenic syndrome	PO	K _v inhibitor	2009	BioMarin Pharmaceutical
Dronedarone	Small molecule	Arrhythmia	PO	Multi K channel inhibitor	2009	Sanofi-Aventis (Sanofi)
Eslicarbazepine	Small molecule	Epilepsy	PO	Na _v inhibitor	2009	Bial
Fampridine	Small molecule	Multiple sclerosis	PO	K _v inhibitor	2010	Acorda Therapeutics
Retigabine	Small molecule	Epilepsy	PO	K _v 7.2-K _v 7.5 activator	2011/2017	GSK
Clobazam	Small molecule	Anxiety, epilepsy	PO	GABA _A activator	2011	Maestretti Research Laboratories (Sanofi)
Clobazam	Small molecule	Anxiety, epilepsy	PO	GABA _A activator	2011	Maestretti Research Laboratories (Sanofi)
Perampanel	Small molecule	Epilepsy	PO	GluA1-4 inhibitor	2012	Eisai
Ivacaftor	Small molecule	Cystic fibrosis	PO	CFTR potentiator	2012	Vertex; Cystic Fibrosis Foundation
Ivabradine	Small molecule	Heart failure	PO	I _f (HCN) inhibitor	2005 EMA 2015 FDA	Servier Amgen
Lumacaftor/ ivacaftor	Small molecule	Cystic fibrosis	PO	CFTR chaperone plus potentiator	2015	Vertex

IV = intravenous; PO = per os

compound	company	indication	Target or mechanism	clinical phase
Granexin gel (αCT1)	FirstString Research	Diabetic foot ulcer, cutaneous radiation injury	Cx43 c-terminal mimetic peptide	III
Dalazatide (ShK-186)	Kv1.3 Therapeutics	Psoriasis, IBM	K _v 1.3 inhibitor	Ib/IIa
Tozuleristide (BLZ-100)	Blaze Bioscience	Imaging of glioma ('tumour paint')	Metalloprotease inhibitor, possible chloride channel inhibitor	I/II
SOR-C13	Soricimed Biopharma	Cancer	TRPV6 inhibitor	I
Antibodies				
BILO10t	Biosceptre	Cancer	Non-functional P2X7	I/II
Small molecules and neurosteroids				
Mirogabalin	Daiichi Sankyo	Peripheral neuropathic pain	Cav inhibitor (α ₂ δ subunit)	Registration phase
XEN007	Xenon	Hemiplegic migraine	Ca _v 2.1 inhibitor	I
Tetrodotoxin (Halneuron)	Wex Pharmaceuticals	Cancer and chemotherapy induced pain	Na _v inhibitor	III
Vixotrigine (BIIB074)	Biogen	Painful lumbosacral radiculopathy, trigeminal neuralgia	Na _v 1.7 inhibitor	II
BIIB095	Biogen	Pain	Na _v 1.7 inhibitor	I
CC-8464	Chromocell, Astellas Pharma	Neuropathic pain	Na _v 1.7 inhibitor	I
DSP-2230	Sumitomi Dainippon Pharma	Neuropathic pain	Na _v 1.7, Na _v 1.8 inhibitor	I
DSP-3905	Sumitomi Dainippon Pharma	Neuropathic pain	Na _v 1.7 inhibitor	I

RG-6029 (GDC-0310)	Roche	Pain	Na _v 1.7 inhibitor	I
Xen901	Xenon	Epilepsy	Na _v 1.6 inhibitor	I
Gefapixant	Merck, Roche	Chronic cough	P2X3 inhibitor	III
BAY-1902607^a	Bayer	Persistent chronic cough	P2X3 inhibitor	II
BAY-1817080^a	Bayer	Endometriosis, persistent chronic cough	P2X3 inhibitor	I
P2X4 inhibitor^a	Bayer	Endometriosis	P2X4 inhibitor	I
JNJ-55308942	Johnson & Johnson	Neuroinflammation, anhedonia	P2X7 inhibitor	I
Intravenous glibenclamide (BIIB093)	Biogen	Stroke	SUR1–TRPM4 inhibitor	III
Basmisanil (RG-1662)	Roche	Cognitive impairment associated with schizophrenia	GABA _A α5-subunit-targeting NAM	II
Sage-217^b	Sage Therapeutics	Major depression	GABA _A δ-subunit-preferring PAM	II
Gaboxadol (OV-101)	Ovid Therapeutics	Angelman syndrome, Fragile X syndrome	GABA _A δ-subunit-preferring agonist	I/II (repurposed)
RG-7816	Roche	Autism spectrum disorder	GABA _A α5-subunit-targeting NAM	I
Evt201	Evotec	Insomnia	GABA _A PAM	II
BIIB104	Biogen	Cognitive impairment associated with schizophrenia	AMPA activator	Ila
TAK-653	Takeda	Depression	AMPA activator	II

PF-04958242	Pfizer	Schizophrenia	AMPA activator	II
BAY-2253651^a	Bayer	Obstructive sleep apnoea	K _{2P} 3.1 inhibitor	II
ASP-0819	Astellas Pharma	Fibromyalgia	K _{Ca} 3.1 activator	II
Senicapoc	SpringWorks Therapeutics and University of California, Davis	Hereditary xerocytosis, Alzheimer's disease	K _{Ca} 3.1 inhibitor	II (repurposed)
AP-30663	Acesion Pharma	Atrial fibrillation	K _{Ca} 2 inhibitor	I/II
CAD-1883	Cadent Therapeutics	Ataxia or essential tremors	K _{Ca} 2 activator	I/II
XEN496 (retigabine)	Xenon	KCNQ2 epileptic encephalopathy	K _v 7 activator	II
GSK-2798745	GlaxoSmithKline	Heart failure	TRPV4 inhibitor	II
SB705498	GlaxoSmithKline	Rhinitis/Pain/Cough	TRPV1 inhibitor	II
Galicaftor (ABBV-2222)	Abbvie	Cystic fibrosis	CFTR	II
GLPG-1837	Galapagos, Abbvie	Cystic fibrosis	CFTR potentiator	II
ABBV-2451	Abbvie	Cystic fibrosis	CFTR	I
ABBV-2737	Abbvie	Cystic fibrosis	CFTR	I
ABBV-3067	Abbvie	Cystic fibrosis	CFTR	I
Sage-324^{a,b}	Sage Therapeutics	Essential tremor Parkinson's disease	GABA _A PAM	I
Sage-718^{a,b}	Sage Therapeutics	NMDA hypofunction	NMDA activator	I

TrpC4/5 inhibitor	Hydra/Boehringer Ingelheim	Anxiety disorder, depression	TRPC4, TRPC5 inhibitor	I
Xen1101	Xenon	Epilepsy	K _v 7 activator	I
HBI-3000	HUYA Bioscience International	Atrial fibrillation	Multi channel inhibitor	I
DWJ-208	Daewoong Pharmaceutical	Neuropathic and cancer pain	Nav1.7	I

Table 2: Ion channel targeting drugs currently in development

Cx43, connexin 43; IBM, inclusion body myositis; NAM, negative allosteric modulator; PAM, positive allosteric modulator.

AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor. Ca_v, voltage-gated calcium channel. CFTR, cystic fibrosis transmembrane conductance regulator. GABA, γ -aminobutyric acid. GluA, ionotropic glutamate receptor. HCN, hyperpolarization-activated cyclic nucleotide-gated channel. K_{2P}, two-pore domain potassium channel. K_v, voltage-gated potassium channel. K_{Ca}, calcium-activated potassium channel. Nav, voltage-gated sodium channel. NMDA, *N*-methyl-*D*-aspartate receptor. P2X, purinoreceptor. SUR, sulfonylurea receptor. TRPC, transient receptor potential canonical channel. TRPM, transient receptor potential melastatin channel. TRPV, transient receptor potential vanilloid channel.

^a Presumed to be small molecules. ^b Neurosteroids.

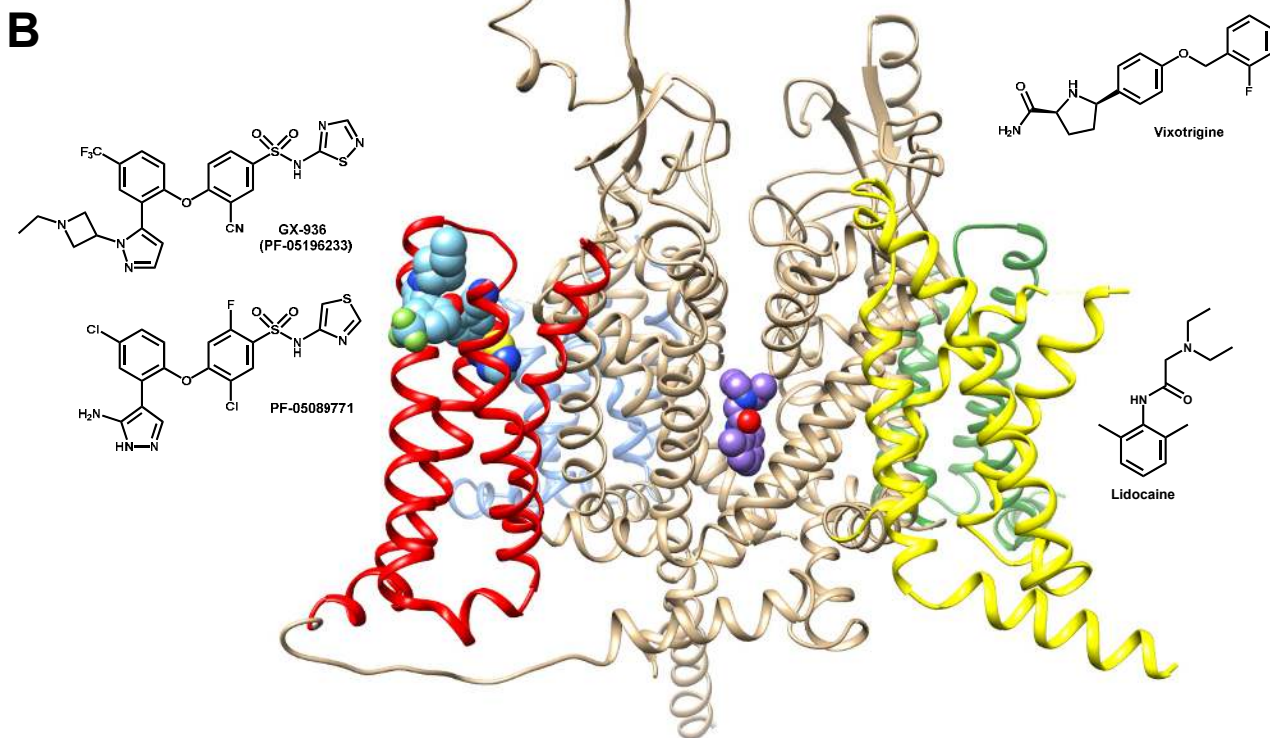
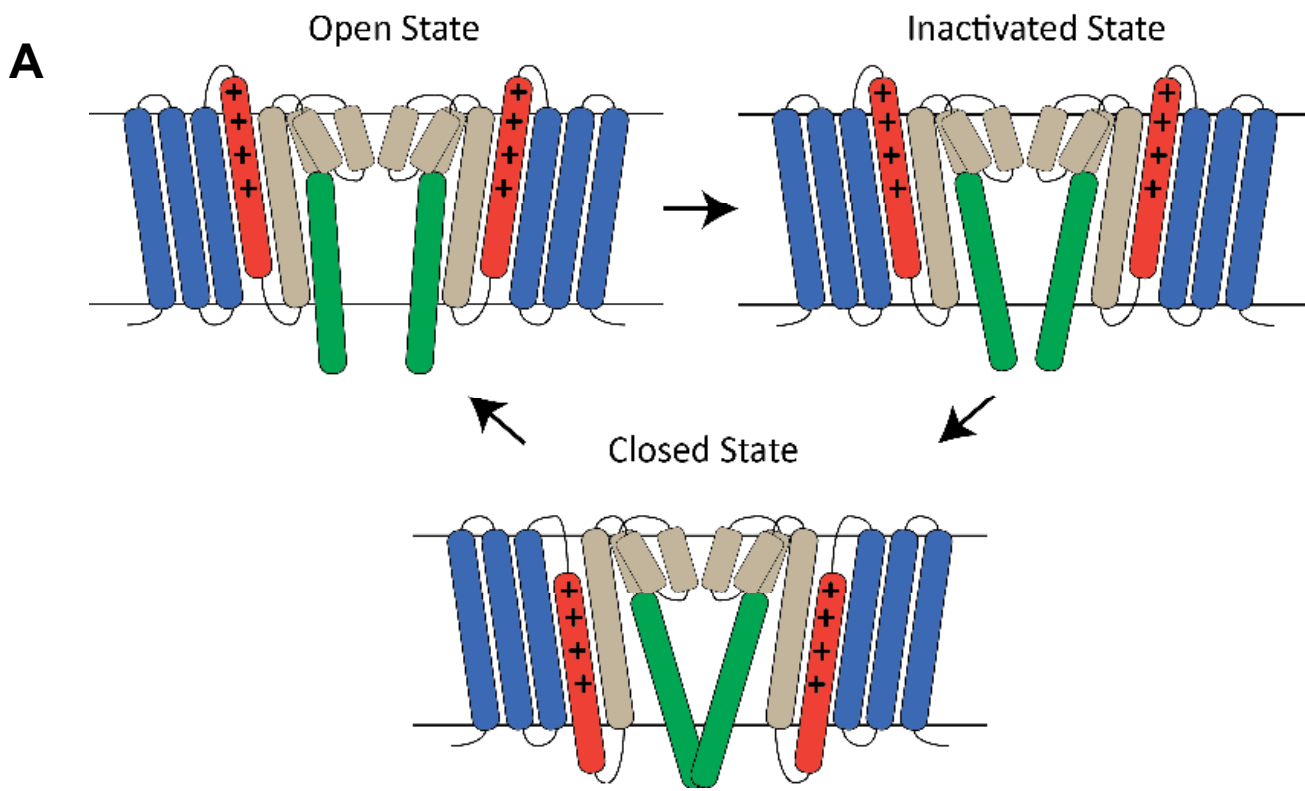


Figure 1

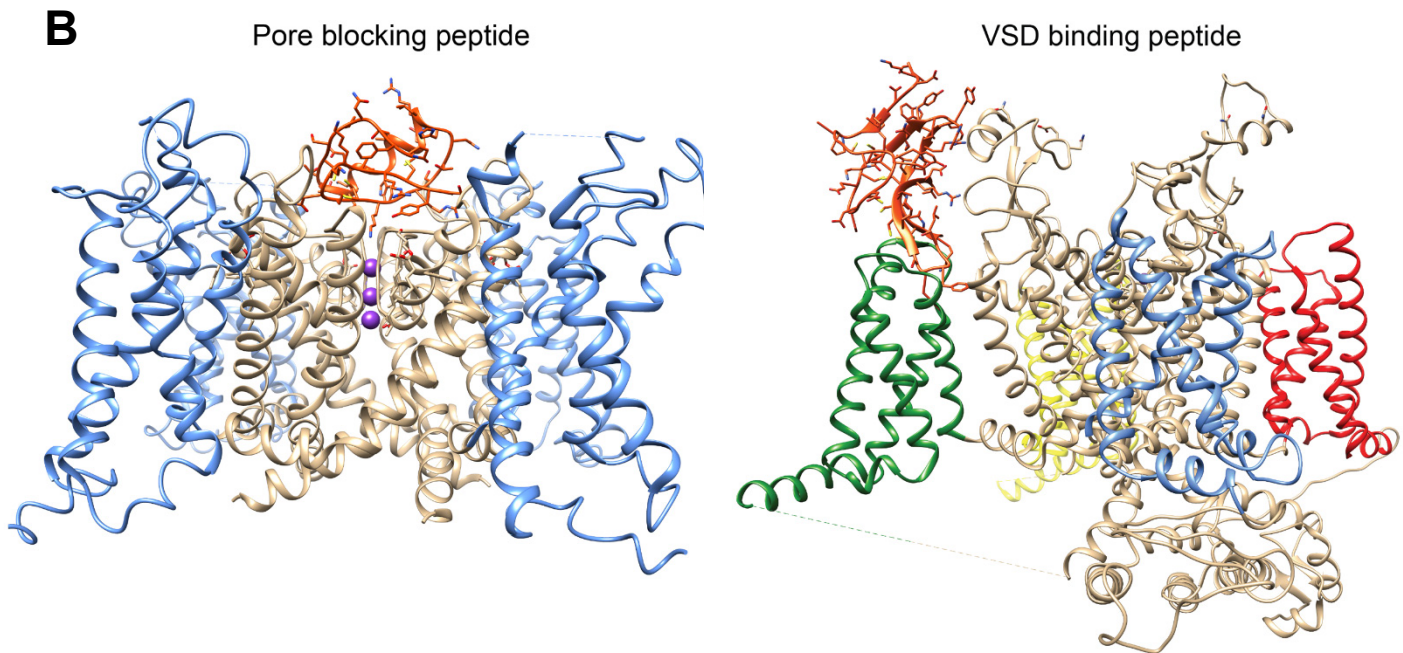
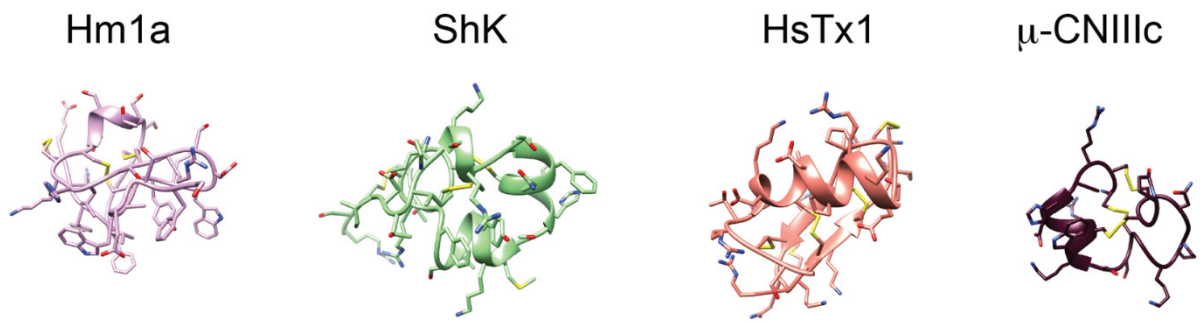
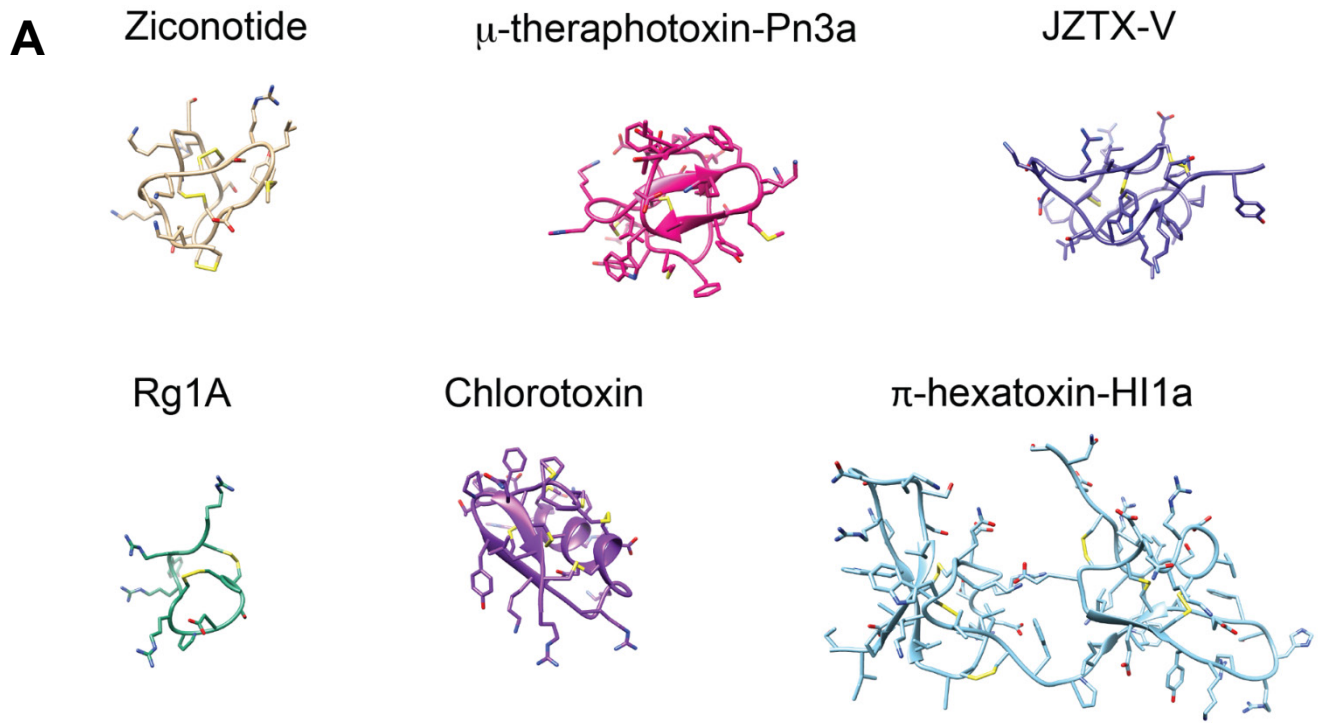


Figure 2

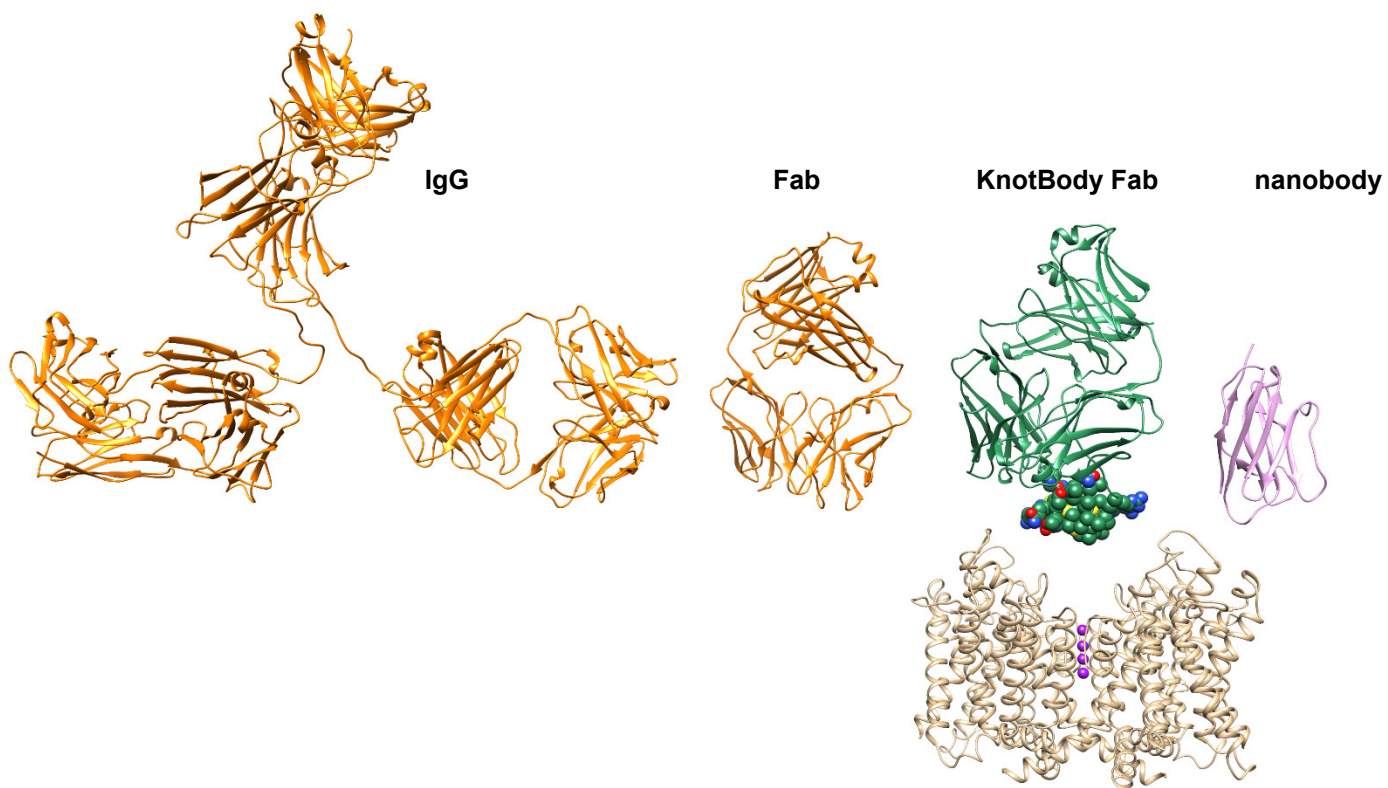


Figure 3

Supplementary Table 1: Ion Channel Drugs

	Medicine	Modality	Indication	Route of adm.	Target/Mechanism	Approval/Withdrawal	Company	Essential Medicine (WHO)
	Ziconotide	Peptide	Chronic pain	Intrathecal	Ca _v 2.2 inhibitor	2004	Elan Corporation	
	XEP-018	Peptide	Periocular wrinkles (cosmetic)	Topical	Nav1.4 inhibitor		Activen SA	
Dihydro-pyridines	Nifedipine	Small molecule	Hypertension	PO	Ca _v 1.x inhibitor	1981	Bayer	Yes
	Amlodipine	Small molecule	Hypertension	PO	Ca _v 1.x inhibitor	1990	Pfizer	Yes
	Cilnidipine	Small molecule	Hypertension	PO	Ca _v 1.x (Ca _v 2.2) inhibitor	1995	Fuji Viscera Pharmaceutical Company/Ajinomoto	
	Clevidipine	Small molecule	Acute hypertension	IV	Ca _v 1.x inhibitor	2008	The Medicines Company	
	Efonidipine	Small molecule	Hypertension	PO	Ca _v 1.x (Ca _v 3.x) inhibitor	1995	Shionogi & Co	
	Felodipine	Small molecule	Hypertension	PO	Ca _v 1.x inhibitor	1991	Hässle (AstraZeneca)	
	Isradipine	Small molecule	Hypertension	PO	Ca _v 1.x inhibitor	1990	Sandoz Pharmaceuticals (Novartis)	
	Lercanidipine	Small molecule	Hypertension	PO	Ca _v 1.x inhibitor	1997	Recordati S.p.A.	
	Nicardipine	Small molecule	Hypertension, Angina	PO	Ca _v 1.x inhibitor	1988		
	Nimodipine	Small molecule	Hypertension, vasospasm	PO/IV	Ca _v 1.x inhibitor		Bayer	
	Verapamil	Small molecule	Hypertension, angina	PO/IV	Ca _v 1.x inhibitor	1981		Yes
	Diltiazem	Small molecule	Hypertension, angina	PO	Ca _v 1.x inhibitor			

Supplementary Table 1: Ion Channel Drugs

	Gabapentin	Small molecule	Pain, Epilepsy	PO	Ca _v inhibitor (α ₂ ,δ)	1993	Parke-Davies (Pfizer)	
	Pregabalin	Small molecule	Pain, Epilepsy	PO	Ca _v inhibitor (α ₂ ,δ)	2004	Parke-Davies (Pfizer)	
	Flunarizine	Small molecule	Migraine	PO	Ca _v inhibitor	1968	Jansen Pharmaceutica (J&J)	
	Cinnarizine	Small molecule	Vertigo, motion sickness, sea sickness	IM	Ca _v inhibitor	1955	Jansen Pharmaceutica (J&J)	
	Fluspirilene	Small molecule	Schizophrenia	PO	Ca _v inhibitor (unselective)		Jansen Pharmaceutica (J&J)	
	Pinaverium bromide	Small molecule	IBS	PO	Ca _v inhibitor	1975	Solvay (Abbvie)	
	Mibefradil	Small molecule	Hypertension, angina	PO	Ca _v 3.x inhibitor	1997/1998	Roche	
	Dantrolene	Small molecule	Malignant hyperthermia	IV	RyR inhibitor	1974		
	Capsaicin	Small molecule	Muscle pain (topical)	Topical	TRPV1 activator	2009 FDA		
	Repaglinide	Small molecule	Type II diabetes	PO	Direct K _{ir} 6.2 inhibitor	1997	Novo Nordisk	
	Nateglinide	Small molecule	Type II diabetes	PO	Direct K _{ir} 6.2 inhibitor	2001	Novartis	
	Riluzole	Small molecule	ALS	PO	K _{Ca} activator Na _v inhibitor	1995	Rhone Poulenc Rorer (Sanofi)	
	Chlorzoxazone	Small molecule	Spasm	PO	K _{Ca} activator	1958	McNeilab Inc (Johnson & Johnson)	Yes
	Zoxazolamine	Small molecule	Spasm	PO	K _{Ca} activator	1955/1961	McNeilab Inc (Johnson & Johnson)	
	Ivabradine	Small molecule	Heart failure	PO	I _f (HCN) inhibitor	2005 EMA 2015 FDA	Servier Amgen	

Supplementary Table 1: Ion Channel Drugs

	Ivacaftor	Small molecule	CFTR	PO	CFTR potentiator	2012	Vertex; Cystic Fibrosis Foundation	
	Lumacaftor/ivacaftor	Small molecule	CFTR	PO	CFTR chaperone plus potentiator	2015	Vertex	
Sulfonyl-ureas	Glibenclamide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)	1966	Boehringer Mannheim/Hoechst (Novartis, Sanofi)	
	Tolbutamide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)	1956	Upjohn Company (Pfizer)	
	Acetohexamide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)			
	Carbutamide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)		Servier	
	Chlorpropamide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)	196?	Pfizer	
	Tolhexamide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)			
	Metahexamide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)			
	Tolazamide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)	196?	Pfizer	
	Glibornuride	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)		Meda Pharma	
	Gliclaside	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)	1972		Yes
	Glipizide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)	1984	Pfizer	
	Glisoxepide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)			
	Glycopyramide	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)			
	Glimepiride	Small molecule	Type II diabetes	PO	K _{Ir} 6.x inhibitor (SUR)	1995	Hoechst Marion Roussel	
	Diazoxide	Small molecule	Acute hypertension	PO	K _{Ir} 6.x activator			

Supplementary Table 1: Ion Channel Drugs

	Pinacedil	Small molecule	Hypertension	PO	K _{IR} 6.x activator	1989	Leo Pharma	
	Minoxidil	Small molecule	Hypertension, hair loss	PO, topical	K _{IR} 6.x activator	1979	Upjohn Company (Pfizer)	
	Nicorandil	Small molecule	Hypertension, angina	PO	K _{IR} 6.x activator		Upjohn Company (Pfizer)	
	Retigabine	Small molecule	Epilepsy	PO	K _V 7.2-K _V 7.5 activator	2011/2017	GSK	
	Flupirtine	Small molecule	Pain	PO	K _V 7.2-K _V 7.5 activator	1984	Asta-Medica	
	Fampridine	Small molecule	Multiple sclerosis	PO	K _V inhibitor	2010	Acorda Therapeutics	
	Amifampridine	Small molecule	Lambert–Eaton myasthenic syndrome	PO	K _V inhibitor	2009	BioMarin Pharmaceutical	
	Amiodarone	Small molecule	Arrhythmia	IV, PO	Multi K channel inhibitor	1961	Labaz company (Sanofi)	Yes
	Dronedarone	Small molecule	Arrhythmia	PO	Multi K channel inhibitor	2009	Sanofi-Aventis (Sanofi)	
	Sotalol	Small molecule	Arrhythmia	PO	Multi K channel inhibitor	1974	Mead-Johnson Pharmaceutica	
	Ibutulide	Small molecule	Arrhythmia	IV	Multi K channel inhibitor	1999	Pfizer	
	Dofetilide	Small molecule	Arrhythmia	PO	K _V 11.1 inhibitor	1999	Pfizer	
Benzo-diazepines	Clorazepate	Small molecule	Anxiety	PO	GABA _A activator	1972		
	Estazolam	Small molecule	Anxiety	PO	GABA _A activator	1990	Upjohn Company (Pfizer)	
	Flurazepam	Small molecule	Anxiety	PO	GABA _A activator	1970		
	Halazepam	Small molecule	Anxiety	PO	GABA _A activator	1981		
	Lorazepam	Small molecule	Anxiety	PO	GABA _A activator	1977	Wyeth Pharmaceuticals (Pfizer)	Yes

Supplementary Table 1: Ion Channel Drugs

	Midazolam	Small molecule	Anxiety	PO, IV ,IM	GABA _A activator	1985	Hoffman LaRoche (Roche)	Yes
	Nitrazepam	Small molecule	Anxiety	PO	GABA _A activator	1965		
	Oxazepam	Small molecule	Anxiety	PO	GABA _A activator	1965		
	Quazepam	Small molecule	Anxiety	PO	GABA _A activator	1985		
	Temazepam	Small molecule	Anxiety	PO	GABA _A activator	1981		
	Triazolam	Small molecule	Anxiety	PO	GABA _A activator	1982		
	Alprazolam	Small molecule	Anxiety	PO	GABA _A activator	1981	Upjohn Company (Pfizer)	
	Zolpidem	Small molecule	Sleep disorders	PO	GABA _A activator	1992	Synthelabo (Sanofi)	
	Flumazenil	Small molecule	Benzodiazepine antidote	IV	GABA _A binder (neutral)	1987	Hoffman LaRoche (Roche)	
	Diazepam	Small molecule	Anxiety	PO	GABA _A activator	1963	Hoffman LaRoche (Roche)	Yes
	Chlordiapoxide	Small molecule	Anxiety	PO	GABA _A activator	1960		
	Clobazam	Small molecule	Anxiety, epilepsy	PO	GABA _A activator	2011	Maestretti Research Laboratories (Sanofi)	
	Clonazepam	Small molecule	Anxiety	PO	GABA _A activator	1975	Hoffman-la-Roche (Roche)	
	Phenobarbital	Small molecule	Epilepsy	PO, IV, IM	GABA _A opener	1912	Bayer	Yes
	Alpidem	Small molecule	Anxiety	PO	GABA _A activator	1991/1995	Synthelabo (Sanofi)	
	Eszopiclone	Small molecule	Insomnia	PO	GABA _A activator		Sunovion	
	Zopiclone	Small molecule	Insomnia	PO	GABA _A activator	1986	Rhône-Poulenc (Sanofi)	
	Propofol	Small molecule	General anaesthesia	IV	GABA _A activator	1989		Yes

Supplementary Table 1: Ion Channel Drugs

	Valproate	Small molecule	Epilepsy, bipolar, migraine	PO	GABA _A modulator	1967		Yes
	Ondansetron	Small molecule	Chemotherapy associated nausea	PO	5HT ₃ antagonist	1991	GSK	Yes
	Palonosetron	Small molecule	Chemotherapy associated nausea	IV, PO	5HT _{3A} antagonist	2003 FDA 2009 EMA	Helsinn Healthcare	
	Alosetron	Small molecule	IBS associated diarrhoea	PO	5HT _{3A} antagonist	2000/2000 2002 FDA	GSK	
	Granisetron	Small molecule	Chemotherapy associated nausea	PO, IM and transdermal	5HT _{3A} antagonist	1993 FDA 2012 EMA	Beecham (GSK)	
	Phenytoin	Small molecule	Epilepsy	PO	Na _v inhibitor	1953	Parke-Davies (Pfizer)	Yes
	Phosphenytoin	Small molecule	Epilepsy	IV	Na _v inhibitor (prodrug)	1996	Parke-Davies (Pfizer)	
	Carbamazepine	Small molecule	Epilepsy	PO	Na _v inhibitor	1962	J.R. Geigy AG (Novartis)	Yes
	Eslicarbazepine	Small molecule	Epilepsy	PO	Na _v inhibitor	2009	Bial	
	Oxcarbazepine	Small molecule	Epilepsy	PO	Na _v inhibitor	1990	Geigy (Novartis)	
	Lamotrigine	Small molecule	Epilepsy, bipolar	PO	Na _v inhibitor	1994	GSK	Yes
	Lacosamide	Small molecule	Epilepsy	PO	Na _v inhibitor	2008	UCB	
	Primidone	Small molecule	Epilepsy	PO	Na _v inhibitor GABA _A modulator	1950	Imperial Chemical Industry (Astra Zeneca)	
	Ethosuximide	Small molecule	Epilepsy	PO	Ca _v inhibitor Na _v inhibitor	1960	Parke-Davis (Pfizer)	Yes
	Mesuximide	Small molecule	Epilepsy	PO	Ca _v inhibitor Na _v inhibitor		Pfizer	

Supplementary Table 1: Ion Channel Drugs


	Phensuximide	Small molecule	Epilepsy	PO	Ca _v inhibitor Na _v inhibitor		Pfizer	
	Amiloride	Small molecule	Diuretic	PO	ENaC inhibitor	1981	MSD (Merck)	Yes
	Triamterene	Small molecule	Diuretic	PO	ENaC inhibitor	1964	Smith Kline & French (GSK)	
	Tetracaine	Small molecule	Local anaesthesia	IM	Na _v inhibitor	1931		
	Procainamide	Small molecule	Arrhythmia	IV, PO, IM	Na _v inhibitor	1951	Bristol-Meyers Squibb	
	Disopyramide	Small molecule	Arrhythmia	PO	Na _v inhibitor	1977		
	Flecainide	Small molecule	Arrhythmia	PO	Na _v inhibitor	1982		
	Propafenone	Small molecule	Arrhythmia	PO	Na _v inhibitor	1994	Knoll Pharmaceuticals (Abbvie)	
	Moracizine	Small molecule	Arrhythmia	PO	Na _v inhibitor	1990/2007	DuPont (Bristol-Myers Squibb)	
	Ranolazine	Small molecule	Chronic angina	PO	Late Na _v inhibitor	2006	Gilead	
	Mexiletine	Small molecule	Arrhythmia	PO	Na _v inhibitor	Still used in US	Boehringer Ingelheim	
	Lidocaine	Small molecule	Local anaesthesia Arrhythmia	IM	Na _v inhibitor	1948		Yes
	Bupivacaine	Small molecule	Spinal anaesthesia	IM	Na _v inhibitor	1963		Yes
	Etidocaine	Small molecule	Local anaesthesia	IM	Na _v inhibitor	1972		
	Levobupivacaine	Small molecule	Local anaesthesia	IM	Na _v inhibitor	1995		
	Mepivacaine	Small molecule	Local anaesthesia	IM	Na _v inhibitor	1957		


Supplementary Table 1: Ion Channel Drugs


	Prilocaine	Small molecule	Local anaesthesia	IM	Na _v inhibitor	1960		
	Ropivacaine	Small molecule	Local anaesthesia	IM	Na _v inhibitor	1997		
	Cisatracurium	Small molecule	General anaesthesia Neuromuscular block	IV	Chrn $\alpha_1\beta_1\gamma/\epsilon\delta$ inhibitor	1995	Burroughs Wellcome (GSK)	
	Rocuronium	Small molecule	General anaesthesia Neuromuscular block	IV	Chrn $\alpha_1\beta_1\gamma/\epsilon\delta$ inhibitor	1994	Schering-Plough (Merck)	
	Succinylcholine	Small molecule	General anaesthesia Neuromuscular block	IV	Chrn $\alpha_1\beta_1\gamma/\epsilon\delta$ activator	1951		Yes
	Varenicline	Small molecule	Smoking cessation	PO	Chrn $\alpha_4\beta_2$ activator	2006	Pfizer	
	Nicotine	Small molecule	Smoking cessation	Topical (skin)	Chrn activator			
	Perampanel	Small molecule	Epilepsy	PO	GluA1-4 inhibitor	2012	Eisai	
	Felbamate	Small molecule	Severe epilepsy	PO	GluN inhibitor GABA _A modulator	1993	Carter Wallace Laboratories	
	Ketamine	Small molecule	Anaesthesia	IV	GluN inhibitor	1970		Yes
	Memantine	Small molecule	Alzheimer's	PO	GluN inhibitor	1968	Eli Lilly and Company	
	Amantidine	Small molecule	Parkinson's disease (historical for flu)	PO slow release	GluN inhibitor	2017/2018 (formulations)		



 ω-MVIIA CKGKGAKCSRLMYDCCTGSCRSKGC Ziconotide, Prialt[®]


 m-TRTX-Pn3a DCRYMFGDCEKDEDCCCKHLGCKRKMKYCAWDFTF


 α-conotoxin Vc1.1 GCCSDPRCNYDHPEIC


 α-conotoxin RgA GCCSDPRCRYRCR


 α-conotoxin RgIA4 GCCTDP-Cit-CR93-I-Tyr)QCY KCP-400


 Chlorotoxin MCMPCFTTDHQMARKDDCCGGKGRGKCYGQPQLCR Tozuleristide
Tumor Paint

SOR-C27 EGKLSSNDTEGGLCKEFLHPSKVDLPR

SOR-C13 KEFLHPSKVDLPR


 Pi-hexatoxin-H1a NECIRKWLSCVDRKNDCCGLECYKRRHSFEVCVPIPG


 Pi-hexatoxin-H1a FCLVKWKQCDGRERDCCAGLECWKRSGNKSSVCAPIT


 Kappa theraphotoxin Hm1a ECRYLFGGCSSTSDCCKHLSCRSDWKYCAWDGTF

