

ION CHANNELS

increasingly enticing targets for drug discovery

Drug discovery focused on ion-channel proteins began generating great value for pharmaceutical R&D programmes more than 30 years ago, giving rise to medicines still important in the pharmacopeia for treating human disease. Pfizer Inc's Norvasc (amlodipine besylate), for example, is one of the best-selling anti-hypertensive medications ever and acts by blocking voltage-gated calcium channels.

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Ion channels are the molecular targets of many kinds of therapeutics, from cardiovascular disorders such as angina and arrhythmias, to metabolic disorders such as Type II diabetes, to neurological disorders including pain and stroke. Well-known drugs like Novartis' Tegretol (carbamazepine) and Ortho-McNeil Pharmaceuticals Topamax (topiramate) are standard treatments for epilepsy and other seizure disorders; both block the sodium ion channel which either solely or partially contributes to the mechanism of action. Pfizer's Neurontin (gabapentin) was originally approved as an adjunctive treatment for partial seizures, and later as a treatment for postherpetic neuralgia in adults. It became a blockbuster when off-label prescribers found it effective for preventing migraines and for treating conditions ranging from mania to bipolar disorder and alcohol withdrawal.

Ion channels remain an undeniably valuable type of drug-discovery target. However in the past 10-15 years, researchers' attempts to expand the number of validated ion-channel drug targets and create new drugs against these have, for the most part, fallen flat. There is debate as to why this has occurred. Some argue that all the low-hanging fruit

has been picked, yet this seems unlikely given that only a small part of the ion channel genome has been drugged to date. Many discovery efforts were scuttled when molecules proved insufficiently selective for their intended targets, and wound up causing severe side-effects in patients. Other drug candidates simply never made it through preclinical studies they needed to pass before entering patients. Part of the problem historically may have been the degree of difficulty with the target class. Because ion channels by their very nature operate in a highly conformationally-dependent manner, designing appropriate platforms to support small-molecule drug discovery has demanded a great deal of expertise.

The advent and widespread use of higher-throughput technologies may have made the problem worse, because these methods in fact did not diminish the difficulties inherent to this target class, nor obviate the need for highly-skilled experts to design and prosecute appropriate drug-discovery programmes. Over time, the masses of data from incorrect assays muddied the field and caused an overwhelming number of failures. Additionally, while the pharmaceutical industry

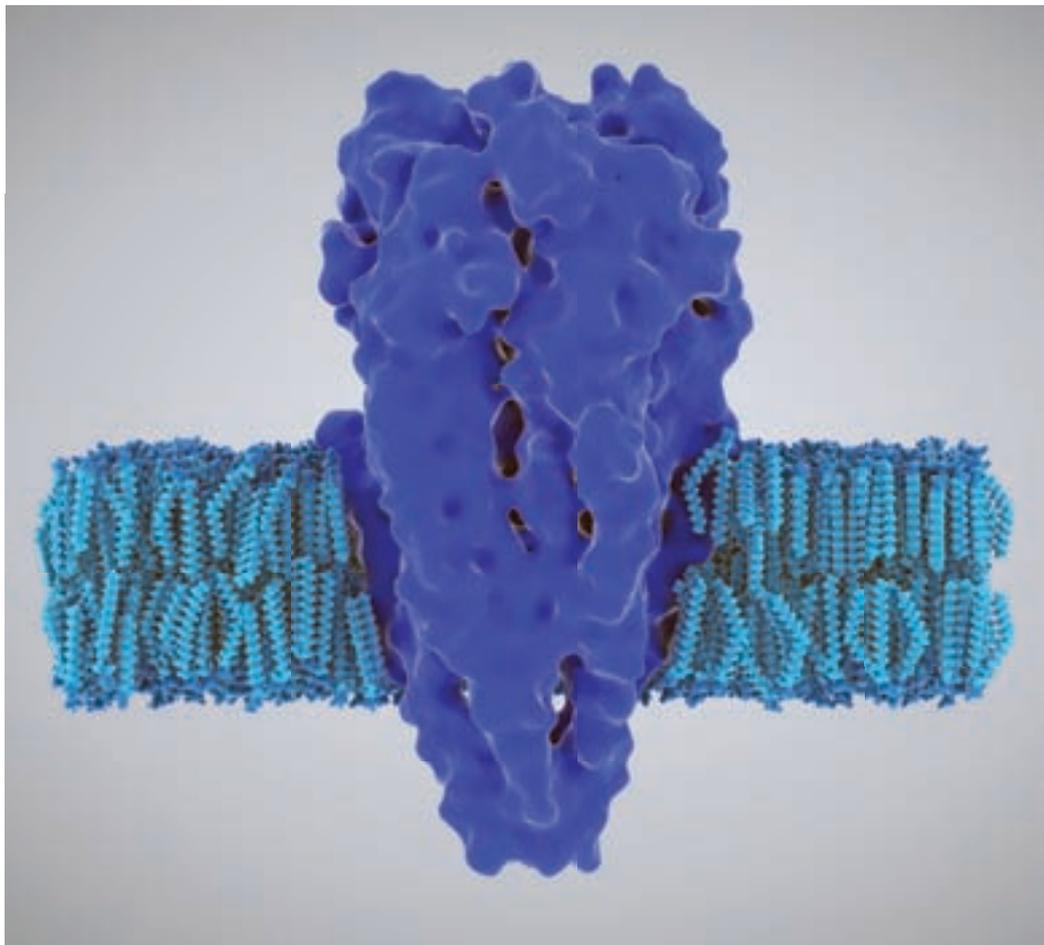


Figure 1
Artistic rendering of a transmembrane protein such as an ion channel in a lipid bilayer

has clearly embraced biologics, antibodies in particular, this enthusiasm has never extended to ion channels. The reason is understandable: scientists around the globe have had little luck identifying within or on ion channels epitopes amenable to antibody targeting.

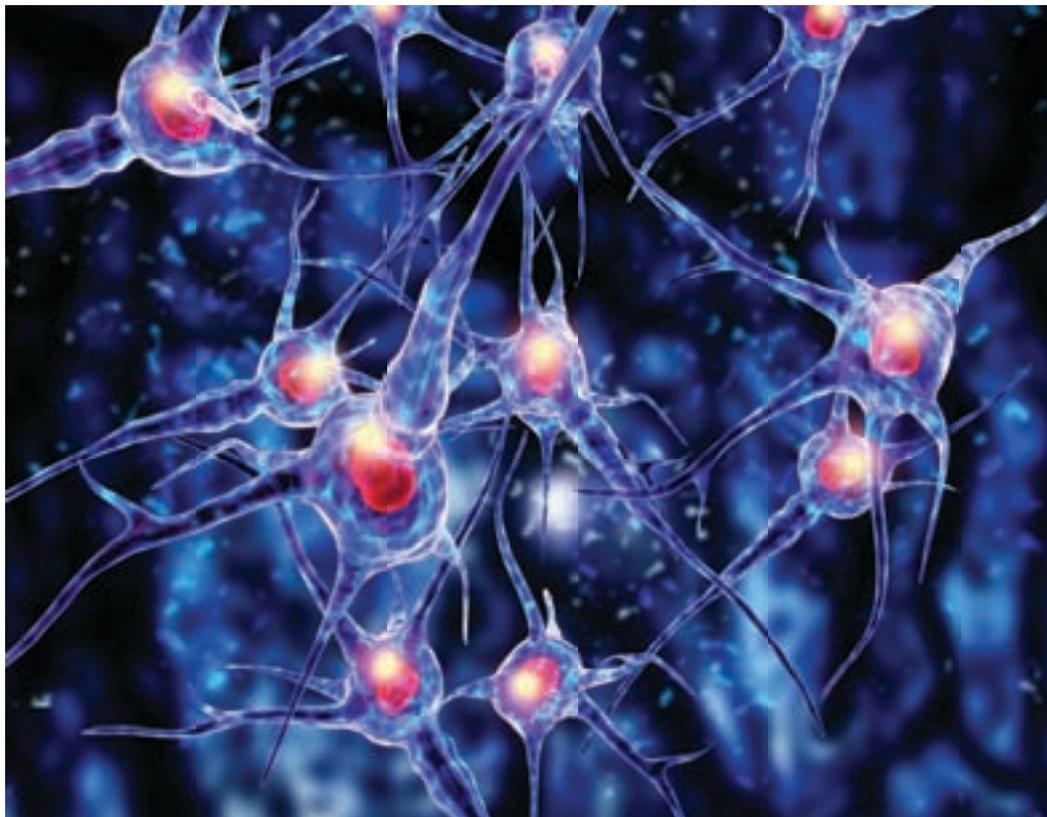
Today, many drug developers share the perception that targeting ion channels in drug discovery programmes is harder than approaching other gene-family targets such as enzymes/kinases and GPCRs (G-protein coupled receptors). In a world of finite resources, such perceptions can shift priorities and shape reality, reducing the number of new ion-channel drug-discovery projects that make it into R&D portfolios. Even a cursory review of large pharmaceutical companies' portfolios reveals that few these days are investing much in development-phase ion-channel drug discovery projects. Indeed, of late numerous studies outsourced to contract research organisations are directed toward avoiding interactions with ion-channel proteins, particular in the cardiovascular safety space.

Yet significant progress is being made and several new trends suggest that the climate and attitudes surrounding ion-channel drug discovery are becoming more welcoming. Advances in both structural and genetic studies are providing greater insight and validity in targeting ion channel proteins, while also increasing the chances of prosecuting successful ion channel programmes. The trouble spots that impeded progress in the past decade and more are not all smoothed over yet, and expert input remains vital, but drug makers now realise it is increasingly possible to design and carry out successful drug-discovery projects based on ion channels. Companies do not have to develop or maintain this expertise in-house. People deeply experienced with pertinent emerging technologies such as high-throughput electrophysiology are readily available for consult and contractual research.

This article will spotlight specific examples where advances in technology and in fundamental understanding of biology and chemistry are paving the way to better ion-channel drugs. For all

Figure 2

Artistic representation of an active network of neurons processing physiological or pathophysiological signals such as pain or epileptic seizures



the disappointments involving ion-channel drug discovery in recent years, there are also some noteworthy success stories. One of the most compelling relates to the development of drugs targeting a chloride channel known as the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). Vertex Pharmaceuticals Inc carried out the groundbreaking research to establish a technology platform, and then with the support of the Cystic Fibrosis Foundation developed the drugs Kalydeco (ivacaftor) and Orkambi (a combination of ivacaftor and lumacaftor). These came to market in January 2012 and July 2015 respectively. Kalydeco was the first drug to address the underlying cause of this disease, not just the symptoms. It is also an excellent example of the development of ‘precision’ medicines designed to treat disease caused by specific genetic mutations. Just a few such drugs exist as yet, but the list is expanding, particularly in oncology.

CFTR is a protein responsible for regulating chloride movement across membranes, and thus is vital to the movement of salt and fluids into and out of the lung and other organs. Certain genetic variants, or mutations, in the gene lead to channels that either function poorly, or never arrive at their intended location in the plasma membrane. In

Kalydeco, Vertex was able to develop a pill that helps restore proper functioning of the channel at the plasma membrane. The combination drug (ivacaftor + lumacaftor) achieves that and also corrects the so-called ‘trafficking’ defect.

We spoke to Dr Paul Negulescu, Vice-President of Research at Vertex pharmaceuticals, to ask: ‘What were the keys to the success of Vertex’s ion-channel discovery programme?’ He noted two that would likely benefit any drug-discovery effort, although they are harder to come by than to name. The first is fundamental understanding of relevant biology and chemistry. The second key is access to preclinical assays that are directly translatable to the clinical setting. Using this template, we have assessed the ion-channel drug-discovery landscape and searched for other examples where the field has progressed, or appears to be progressing, to a point where additional success stories can be expected.

One of the most significant examples we examined, and one which illustrates both the potential and the challenges in targeting ion channel proteins, revolves around industry efforts directed against the voltage-gated sodium channel Nav1.7. Almost everyone has had the experience of being treated with a local anaesthetic in the dentist’s

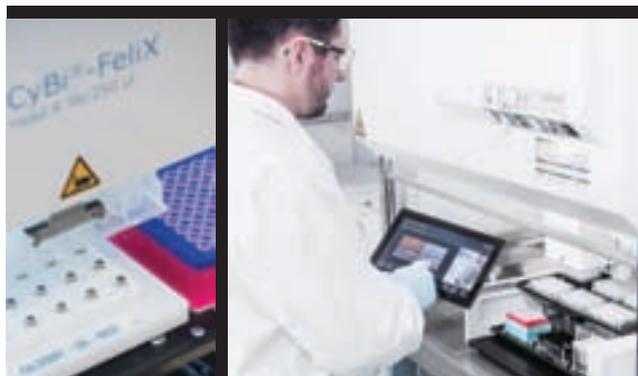
office or for minor surgical procedures, grateful for these drugs that effectively alleviate pain. The mechanism of action for local anaesthetics such as lidocaine is blockage of neuronal sodium channels, which prevents action-potential generation and conduction in nerves, and therefore prevents pain signalling. While these drugs are very efficacious when administered locally, they are not appropriate for systemic administration because of lack of selectivity among the sodium channel gene family. Systemic administration of a local anaesthetic would block signalling in the heart and brain and lead to serious side-effects.

To some extent, design of molecules that act only on rapidly firing neurons gives a measure of therapeutic index for certain conditions. This occurs even though these molecules are, like lidocaine, not selective among sodium channels. They are more efficacious when channels are very active, such as in epileptic seizures or serious ventricular arrhythmias. Historically, it was not thought possible to identify selective sodium channel blockers and so this functional selectivity was the *de facto* approach for drug discovery. This changed in 2008 when Jarvis et al reported identifying subtype-selective Nav1.8 blockers¹ and again in 2013 when McCormack et al identified and reported subtype-selective sodium channel blockers of Nav1.3 and Nav1.7². The latter was of particular interest because Nav1.7 had by then been identified in genetic studies as a channel key to regulating pain signalling (Hoeijanakers et al 2015)³. Both loss-of-function and gain-of-function mutations are impactful, leading to loss or gain of pain, respectively. Importantly, McCormack et al also identified that the binding site for these selective molecules is found in the Domain IV-voltage-sensor of the Nav1.7 channel. That site had never been associated with small-molecule sodium-channel inhibitors before.

Following this initial work by McCormack, scientists at Genentech and Xenon Pharmaceuticals published what can realistically be viewed as a breakthrough study reporting the first crystal structure of the DIV voltage sensor with a subtype-selective molecule docked in the binding site. To accomplish this researchers at these companies used an elegant approach of generating chimeric proteins combining a bacterial channel anchor and mammalian protein sequence. Bacterial channels and bacterial proteins in general are much easier to crystallise than human proteins, so combining this anchoring approach with target domains of key pharmaceutical interest, is a big step forward. This work was based on the seminal

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publication from the laboratory of Bill Catterall who in 2011 (Payandeh et al) published the first crystal structure of a voltage-gated sodium channel⁴. Ahuja et al illustrated that when inhibitors are docked in the appropriate binding site, it is possible to identify specific interactions and also a role for the phospholipid membrane in determining binding properties⁵.

Genentech and Xenon confirmed that the inhibitors act by trapping the channel in a non-conductive state. The authors closed their paper with the following statement: “We anticipate that these structures will enable drug design efforts aimed at other voltage-gated ion channels and may accelerate the development of new treatments for pain that selectively target Nav1.7.” We concur with these sentiments.

The work of these groups highlights fresh technical approaches to making better selective Nav1.7 inhibitors, while other research teams are taking a different and parallel approach – namely, elucidating which types of patients are likely to respond best if treated with these potential drugs. A glimpse of the future can be found in the work of Cao et al 2016⁶, studying a Nav1.7 inhibitor that entered clinical development. In *Science Translation Medicine*, Cao et al reported results of a sophisticated study involving peripheral neurons derived from induced pluripotent stem cells (iPSC) that originated in patients known to have gain-of-function mutations in the Nav1.7 sodium channel gene. As a result of these mutations, trial participants were suffering from primary erythromelalgia (IEM), a rare disorder characterised by intense heat and pain in the extremities including feet and hands. Importantly, the people that contributed the iPSC-derived peripheral neurons that Cao et al examined were the very same patients who had participated in a separate clinical trial investigating the effect of the first selective Nav1.7 blocker studied in man. Clinical data were reported for five subjects examining the degree of efficacy provided by the drug candidate in the treatment of pain. iPSC experiments were performed on four of these subjects.

The clinical studies were well-designed and used a well-controlled thermal stimulus to elicit pain in the subjects during two independent single-dose treatment periods. The subjects also agreed to allow iPSC-derived neurons to be generated from skin biopsies and these cells were the basis for the post-hoc studies performed and reported by Cao et al. The electrophysiological phenotype of these cells was consistent with the phenotype of the disease, in that there was a

trend toward increased spontaneous activity (ie pain signalling) in the cells that originated in IEM patients, compared to those derived from healthy controls. Additionally, heating tended to increase excitability in these cells compared to decreasing excitability in cells from the healthy volunteers. All of these findings were consistent with the pain phenotype observed in these people. Of course further study is needed, but already these data suggest that molecules able to block certain subtypes of ion channels can calm cellular signalling and so reduce pain in patients with specific genetic mutations. Cao et al’s study also provides evidence that electrophysiological testing of cells, when properly performed, predicts human biology and pharmacological effects. This was a study carried out retrospectively because the drug candidate had already been identified. We believe that these types of studies will become more common when performed prospectively. Drug hunters will be able to identify the patients who will respond most favourably to a given drug or mechanism. Just as importantly, they will be able to eliminate in advance those patients who do not respond and stop programmes that have little chance of translating to efficacious drugs. This is the future of ion channel drug discovery.

To emphasise this point we consider two more fascinating observations in the study of Cao et al that have bearing on the general direction we see ion channel drug discovery heading. The patient in whom the Nav1.7 blocker was the least effective had the phenotype most similar to those of healthy volunteers. Less sporadic, less frequent firing of neurons translates to a weaker pain phenotype. On the surface, disease-in-a-dish and pharmacological response had been recapitulated, since the most ‘normal’ patient was the least responsive to a therapy targeting severe pain while cells from patients with the most severe cellular phenotype tended to have the greatest response.

The assay was in this case a good predictor of physiological response in the patient. Perhaps even more fascinating was the fact that in the iPSC-derived neurons from healthy volunteers, there was a heterogeneous response to exposure to the channel blocker. At least one of the individuals’ cells were more likely to show spontaneous firing than the cells from other healthy normal volunteers. Does that suggest that this ‘healthy’ individual may be more prone to pain than others? We cannot make that conclusion with such a small data set, but the question clearly deserves further investigation.

These results raise the intriguing possibility that phenotypic screening of iPSC-derived neurons in people suffering painful disorders may be useful in determining whether or not a selective Nav1.7 inhibitor would be likely to bring a patient relief. Based on the results by Cao et al, it seems reasonable that a hyper-excitability phenotype would be requisite before prescribing molecules such as PF-05089771 because the drug candidates tested in this study were much less effective on the neuronal responses of the healthy volunteers. In future clinical studies, the chances of observing efficacy may be dramatically enhanced by adopting a patient-stratification approach based on assays of individuals' neuronal cells. This kind of culling and sorting of patients is a fundamental aspect of precision medicine, as it is expected to get medication to people likely to respond while sparing the cost and risks of treating others unlikely to benefit. Well before studies of iPSC-derived cells inform any additional clinical trials of ion-channel inhibitors, drug makers may find that such human translational assays can serve as lynch pins of successful drug discovery programmes.

The effort to develop a Nav1.7 drug has not yet been as successful as Vertex's CFTR-centred discovery programme ultimately proved to be. Some observers might even view Cao et al's work on Nav1.7 as a failure since it did not yet yield a marketable drug. However, we perceive the Nav1.7 story as an ongoing one, a work in progress that is contributing to understanding the biology pertinent to this sub-type and perhaps all ion channels. Vertex's Dr Negulescu described depth of scientific knowledge as a fundamental aspect of his company's – and, really, any – successful drug discovery programme. But understanding does not materialise overnight, he emphasised. The genetic defect in CFTR had been identified and studied for nearly a decade before real progress was made in a drug discovery programme. It is just now a decade since the phenotypic manifestations of Nav1.7 mutations were first described by Cox et al in 2006⁷.

Understanding of ion-channel biology continues to accelerate, thanks in large part to advances in structural studies carried out across a wide variety of ion-channel gene families ranging from sodium and potassium channels to ligand-gated ion channels such as glutamate receptors.

Studies published at the end of 2013 by Yifeng Chen, Maofu Liou, David Julius and colleagues from the University of California at San Francisco provide important evidence that chemical and temperature changes can cause structural changes

in the ion-channel protein TRPV1 (Cao et al 2013)⁸. Important in perception of pain and heat, this protein is plentiful in neural cells, where it forms pores through which ions such as calcium can pass. The recently gathered structural information confirmed several existing theories of TRP channel function, among them the idea that the ion channel changes its physical shape in response to specific stimuli. The work also provided fresh insight into binding sites that could prove relevant for pharmacological modulation, because the authors were able to obtain one structure with an inhibitor docked in a binding site that had not previously been identified. A few years later the same group published the structure of a related TRP channel, TRPA1, using the same methodology (Paulsen et al 2015)⁹.

Over the years, researchers have considered both TRPV1 and TRPA1 as targets of interest for the treatment of pain. In the context of this article, identification of the new binding site in TRPV1 provides additional information for potential design of future molecules and also suggests targeted approaches that might be applicable to other TRP channels. The TRPA1 structure, valuable in its own right, also provided scientists with deeper understanding of the similarities and differences among members of the TRP ion channel family. Although TRPV1 and TRPA1 are related gene products, the authors noted significant differences in the structures. This finding reinforces the importance of studying each protein directly, in hope of identifying specific attributes that can be leveraged in a modern drug discovery programme.

There are many other examples of ion channel proteins where examination of structural biology is dramatically expanding our knowledge base. At the time of this writing, a key-word search of the Protein Data Bank (www.rcsb.org/pdb/home/home.do) using the term 'ion channel' results in 4,267 hits. While not every hit is a *bona fide* ion channel protein, the database is replete with structures detailing both isolated domains and/or entire channels from a wide range of species including *Homo sapiens*. Clearly our structural knowledge is expanding significantly and arguably, even exponentially.

Analysis of gene variants has proved informative and of practical use too: the Vertex example cited shows how such research has enabled creation of translational assays in the sodium channel space. A broader, and equally intriguing, example of how this type of advance is being applied by people at the forefront of precision medicine is given by David Goldstein and colleagues in their article entitled 'A Roadmap for Precision Medicine in the

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Epilepsies'. This review, published in *Lancet Neurology* by the EpiPM Consortium¹⁰ includes a table of genes associated with epilepsy. Given that epilepsy is a disease of hyper-excitabile neurons, it is hardly surprising that many of the genes listed are ion channel genes. The authors make a compelling case for developing an array of *in vitro* and *in vivo* assays to enhance fundamental understanding of the biology and to simplify, at least potentially, the translation of preclinical findings to the clinical setting.

We have in this article discussed just three therapeutic areas – cystic fibrosis, pain and epilepsy – where basic understanding of ion-channel biology is advancing, and with it researchers' ability to translate this understanding to the clinic. There are certainly other examples to explore. Our conclusions from this brief analysis are encouraging; the long-predicted possibility of creating precision medicines appears at last to be manifesting into physical reality in the ion channel domain, which historically has been a rich vein for drugs in the pharmaceutical industry. Because of the commercial success of drugs like Kalydeco and Orkambi, and clinically relevant findings from world-class academic research institutions such as UCSF and Columbia University, it is likely that more and more ion channel targets will win space in drug makers' discovery portfolios in the future.

Targets supported by both structural data and genetic validation will likely be the first considered for precision medicines. Targets and compounds that can be validated by translational assays able to provide relevant data in both preclinical and human studies will have an undeniable edge in commercial decision-making. Our fundamental understanding of the biology of ion channels and our depth of technical prowess to prosecute discovery-research programmes based on these enticing targets promises only to continue to expand and grow. So too, our ability to advance with confidence into clinical development and beyond.

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