CHANNELLING DRUG DISCOVERY
current trends in ion channel drug discovery research

Ion channels are proteins that span cell membranes thus forming conduits or 'channels' through which charged ions such as sodium and potassium can pass across a normally impermeant barrier such as the plasmalemma. By so doing, ion channels can mediate a wide variety of physiological functions from the generation of action potentials in nerve cells to immune cell function and more.

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Although the pharmaceutical industry has been slow to recognise the true potential of ion channels due to a combination of disbelief that ion channel dysfunction could cause disease and a lack of screening technology to keep up with other HTS assays, ion channels are currently very much in vogue. The discovery of 'Channelopathies' and exciting emerging ion channel screening technologies herald a new era of intensive ion channel-based drug discovery. Here we review the current trends in the ion channel drug discovery business.

Why should drug companies be interested in ion channels?

We have known about ionic currents in nerve cells since 1952[1] and been able to electrically visualise single ion channels in real time using the patch-clamp technique since 1981[2]. The first sodium ion channel was cloned in 1984 (Noda et al) and the first potassium channel in 1987[3]. Since then we have realised that ion channels are physiologically important in a huge variety of functions and in all cells. In excitable cells such as nerve and muscle, ion channels generate and shape electrical signals leading to action potential propagation, neurotransmitter release and muscle contraction. Ion channels are fundamental in controlling the heart beat, sensory transduction including pain and brain function. In non-excitable cells, ion channels are involved in hormonal secretion, immune cell responsivity, cell-cycling, ion distribution and more. Why is it then that only now at the beginning of the 21st century that ion channels are suddenly creating so much interest in the pharmaceutical industry? Here we review the current trends in the ion channel drug discovery business and how a convergence of research and technology developments may provide the answer.

Ion channels are a super-family of proteins that span cell membranes and form conduits or channels through which charged ions such as sodium and potassium can pass across what is normally an impermeant barrier. We tend to think of the plasmalemma as being home to most ion channels but intracellular organelles such as mitochondria and endoplasmic reticulum also have ion channels. Broadly speaking, ion channels can be divided into those controlled by receptors and those opened and closed (gated) by changes in the voltage of the cell. By their very nature voltage-gated ion channels are particularly attractive targets, but also present a challenge for the industry particularly in

finding appropriate screening technology. This review will focus primarily on these so-called voltage-gated ion channels.

In 1984 in the first edition of his seminal book on the 'Ionic Channels of Excitable Membranes', Hille[4] recognised in his preface that ion channels went well beyond nerve cells and were likely to be important in non-excitable cells such as 'sperm, white blood cells and endocrine glands'. He also predicted that our genome would probably code for more than 50 different types of ion channel. Bearing in mind that the first ion channel had yet to be cloned (it was later that same year), this was a bold statement to make. He needn't have worried though, 18 years later the Human Genome Project predicted that there are more than 300 human genes encoding ion channels[5].

Voltage-gated ion channels are turned-on and off (or gated) by movements of so-called 'gates' created by elements of its own protein structure. In channels such as K+ channels, a change in the voltage of the cell causes a segment of the channel (known as the voltage sensor) to move within the membrane thereby opening a channel through which K+ ions and water molecules can pass one at a time. Other parts of the protein which make up the lining of the channel determine which ions can or cannot pass through the channel and also act as receptors for small molecules and toxins which can modulate these functions in various ways (**Figure 1A**). A functional voltage-gated K+ channel is composed of at least four subunits represented as cylinders, which assemble as a complex in the membrane (**Figure 1B**). Na+ and Ca2+ channels have analogous features although the four subunits found in K+ channels are contained within a single protein.

Ion channel cloning

Ever since 1984 when the first sodium channel was cloned, cloning of new ion channels has gathered pace, culminating perhaps in completion of The Human Genome Project. The combined effort of the HGP and Celera parallel project indicates that we can expect around 300 ion channel genes divided between the major ion channel families. We also know that for K+ channels, for example, which are composed of tetramers of a basic pore-forming subunit (α-subunit), it is also possible to get functional channels from permutations of α-subunits. In addition, many pore-forming subunits associate with auxiliary subunits which, while not necessarily pore-forming in themselves, can modify the properties of the ion channel, either biophysically (eg speed-up inactivation) or pharmacologically (eg increase sensitivity to drugs). While many of these α-subunits are known, many more remain to be discovered
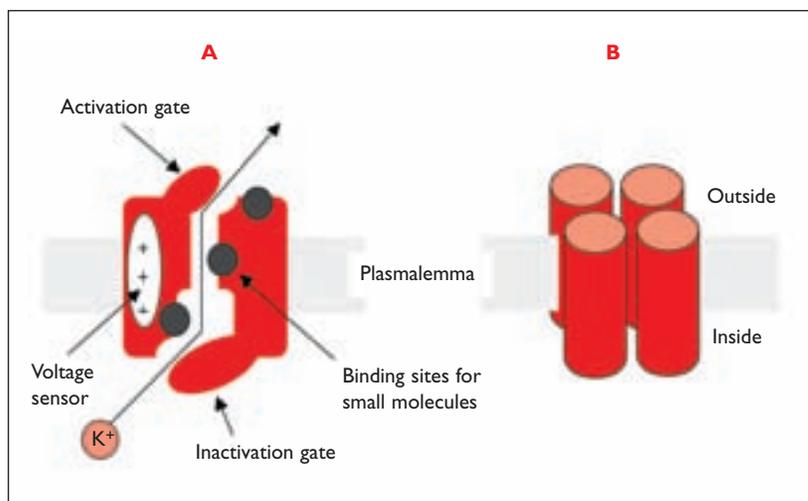
without doubt. Add to the variability of ion channel structure afforded by this heteromeric association phenomenon, variations on the basic subunit caused by 'splice variation' and it easy to imagine that the number of 300 could easily be increased by a factor of two or more. Overlayed on this variety are more or less specific tissue distributions of ion channel expression as for traditional targets such as neurotransmitter receptors. Of course some ion channels are more ubiquitous than others, but it is clear that there is real potential for selective modulation of ion channels both between tissues and within cell-types, an important consideration in any drug discovery context. At this point in time, we know that K+ currents (that is currents carried by the flux of potassium ions across a membrane) can be generated by one or more of around 70 different potassium-selective α-subunits. Na+ currents arise from around 10 different genes; there are around nine voltage-gated and another seven non-transmitter operated chloride channels and 13 voltage-gated calcium channel α-subunits. Other channel types have significant numbers of family members as well. For example, there are around 20 TRP channels, 12 Deg/ENaC channels, 13 connexins and so on.

See also: www.gene.ucl.ac.uk/nomenclature/genefamily/KCN.html,www.gene.ucl.ac.uk/nomenclature/genefamily/CACN.html and [6].

Although traditionally one thinks of sodium and potassium channels and the generation of action potentials in nerve cells, in fact all cells (as far as we know) have ion channels of some type or other. The bewildering number of different types of ion channels identified at a molecular level suggests that ion channels are important in a similarly large number of physiological processes. Sure enough, ion channel involvement ranges from action potential

Figure 1
A cartoon representation of a K+ channel showing activation and inactivation 'gates' and possible binding sites for small molecules
B Many K+ channels are composed of 4 subunits that together form the functional channel

threshold in nerve and heart to the resting potential of immune cells and sperm motility. The richness of this pool of possible therapeutic targets is surely irresistible. See also: www.neuro.wustl.edu/neuro-muscular/mother/chan.html[7,8].

Relevance of ion channels to disease (channelopathies)

For many years, ion channel modulators were seen as palliative at best. However, since the first 'channelopathy' was identified in the cystic fibrosis transmembrane regulator protein (CFTR) by Riordan et al in 1989[9], this has turned into a growth industry. There are around 30 channelopathies to date including major therapeutic areas such as diabetes, cardiac disease, deafness, blindness and epilepsy. All can be caused by ion channels that malfunction or are not expressed at all. See also [10] and www.neuro.wustl.edu/neuro-muscular/mother/chan.html.

Without doubt these links have reinforced the industry's interest in ion channels and in some cases provided the key rationale for drug discovery programmes (cystic fibrosis being the prime example).

Ion channel safety pharmacology

hERG: bête noir or best thing since sliced bread?
By the 90s, although represented by programmes in most of the big Pharma, ion channels were still not mainstream. However, that was about to change. Between 1995 and 1996 genetic linkages were established between a family of inherited cardiac disorders known as LQT and inherited mutations in the cardiac voltage-gated potassium channels, KvLQT1 and hERG and the voltage-gated cardiac sodium channel, SCN5A[11-13]. Furthermore a link was suggested between drug induced, or acquired LQT (aLQT), and block of the hERG potassium channel[14]. Next, an advisory note issued by the Committee on Proprietary Medicinal Products (CPMP)[15] sparked an enduring debate concerning the possible mechanisms by which drugs such as cisapride and terfenadine were apparently causing sudden death by cardiac arrest. The CPMP note suggested that it would be desirable to conduct a preclinical 'diagnostic' test of candidate drugs, prior to first use in humans, not only for their propensity to prolong the cardiac action potential (which can lead to dysrhythmia) but also for their potential to block cardiac ion channels. Meanwhile, the finger of blame was being pointed most emphatically at the hERG potassium channel[16].

Arguably, hERG has done more to promote ion channels to the current high level of prominence than any other single development. What has followed is a *de facto* requirement to test new drugs for their propensity to block cardiac ion channels such as hERG and the potential for drug-induced QT prolongation prior to first use in humans. If there wasn't a demand for ion channel screening resource before aLQT there certainly was afterwards. This has had a number of important consequences:

● The profile and importance of the ion channel as a target for drugs has been raised dramatically. Almost everyone in the R&D hierarchy of a drug company now knows about at least one voltage-activated ion channel: hERG.

● Since the CPMP note there has been a rapidly escalating requirement for hERG and other ion channel screens. In some cases these have been provided in house but in many companies these assays are out-sourced to other specialist organisations.

● The realisation that activity at ion channels like hERG are best eliminated early in the drug discovery process has heightened the need for screening techniques with appropriate information content and throughput that will make this possible. The issues involved in screening complicated ion channels such as hERG are now much better appreciated across the pharmaceutical industry.

As a result of the above, the specialist ion channel screeners such as Channelwork (www.cenes.com/channelwork), GENION (www.for-genion.com) and Chantest (www.chantest.com) enjoyed a booming business in LQT-related screening while the Big Pharma worked out their strategies. The established CROs have not been slow to catch on either. Now, MDS-Pharma (www.mdsps.com), Quintiles (www.quintiles.com) and Cerep (www.cerep.fr) all provide hERG screening as well as the isolated cardiac Purkinje fibre assay, recommended in the original CPMP advisory note and now being cemented in the ICH7A document (www.ifpma.org/ich1.html).

Furthermore, if any further encouragement was needed, the expanding need for 'ion channel safety screening' has fuelled the need to develop automated and high-throughput technologies for screening those channels that appear in the list of undesirables.

Ion channels as pharmaceutical targets

Existing ion channel drugs
Currently, potential for ion channel-directed drugs is relatively untapped. Only about 5% of the targets of marketed drugs are classed as ion channels[17]. Nevertheless, existing drugs that modulate ion channels already represent a valuable class of pharmaceutical agents with a total market value in excess of $8 billion in 2000.

The global pharmaceutical market is worth more than $240 billion annually with the major markets

in the US, Europe and Japan where combined sales exceed $220 billion. The therapeutic areas in which ion channel modulators are most likely to be used include the largest categories of cardiovascular (with annual sales of $48 billion) and CNS (with annual sales of $40 billion). In addition to these areas, ion channel modulators have application in a wide range of other high value areas such as pain (neuropathic pain is estimated to be worth approximately $550 million per year)[30].
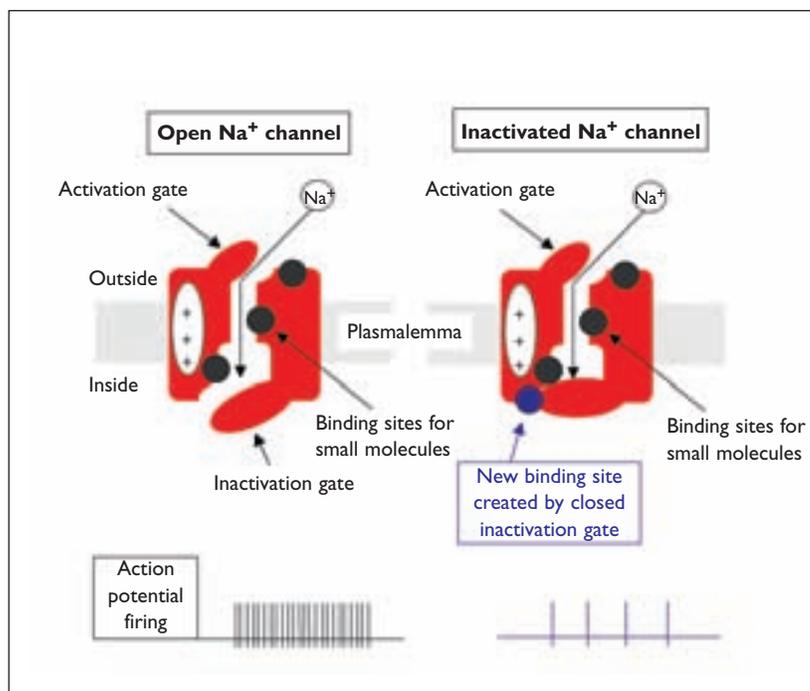
Pharma Projects lists around 100 launched ion channel modulators. The majority of these are Ca^{2+} channel blockers (60) aimed at cardiovascular sector. Around 26 Na^+ channel blockers (analgesics and anticonvulsants) and eight K^+ channel modulators (inter alia vasodialators such as minoxidil). To date no Cl^- channel modulators have been launched. Some of these are very well known and include the anticonvulsant, lamotrigine (Na^+ channel blocker); dihydropyridine antihypertensives such as nifedipine (Procardia[TM]), an L-type Ca^{2+} channel blocker; the diabetes drug, glyburide (Diabeta[TM]), which is an ATP-sensitive K^+ channel blocker and local anaesthetics such as lidocaine (Xylocaine[TM]) which is also a Na^+ channel blocker.

Many of these have been discovered serendipitously and before the real explosion in ion channel cloning, molecular genetics and screening technology development that has taken place over the last few years. Deliberate targeting of specific ion channel sub-types in drug discovery programmes promise more and better ion channel drugs.

New ion channel drugs: smart drugs?
What makes ion channels attractive targets for drug development? There are a number of features of ion channels and especially voltage-gated ion channels that makes them attractive targets for novel drugs:
● As illustrated above, ion channels are fundamental elements in physiology playing an important role in all cells and across a wide range of organs and functions from nerve cells to the immune system.
● Ion channels are under exploited and thus provide huge potential for novel drug targets.
● The huge variety of subtypes of ion channels and differential distribution within and between tissues supports the idea that selective drugs can be developed.
● The inherent gating mechanisms of ion channels offers the chance to develop state-dependent drug actions and dynamic drug therapy which responds and is dependent on activity of ion channels and behaviour of cells and tissues. For example, ion channel blockers can be engineered only to block

ion channels after they have opened and inactivated, thus not interfering with basal activity (but reducing frequency of activation – smart drugs (**Figure 2**).

Current ion channel drug discovery programmes
Pharma Projects lists around 29 active Na^+ channel projects, 28 active K^+ channel programmes, three active Cl^- programmes and 19 Ca^{2+} programmes. This appears to show a trend away from Ca^{2+} to Na^+ (consistent with the launch of a large number of Ca^{2+}-based cardiovascular-based drugs) and a sustained emphasis on K^+ channel modulators. It also appears to represent a significant effort by the industry as a whole. For comparison, Pharma Projects also lists 20 SRI, 20 tyosine kinase, nine AchE-I and five statin programmes, among many others of course.

As well as exploring new ion channel families, we can probably expect to see companies revisiting areas such Ca^{2+} modulators in the light of new information (eg cloned sub-types) and cardiac ion channels armed with additional information from molecular genetics and the molecular mechanisms underlying acquired LQT (discussed above).

Patent activity
Patent activity is often a guide to the commercial interests of the pharmaceutical industry being as it is, highly dependent on intellectual property for recouping the massive investment that goes into drug discovery and development. **Figure 3** shows

Figure 2
Smart drugs. A blocker targeted at a binding site created in the 'inactivated' state of the channel will not prevent opening of the channel *per se*, but could delay recovery from inactivation which normally occurs between action potentials. This results in fewer action potentials being generated per unit time

the growth in granted US Patents from 1980 to the end of 2001. Note that the industry did not wait for ion channels to be cloned eg ROMK1, the first K+ channel to be Patented, issued in 1994. Indeed much of the early activity reflected development of Ca^{2+} channel blockers in the dihydropyridine family using binding assays and functional cardiovascular screens and it was not until the late 90s that Ca^{2+} channel sequences were granted Patents. However, in the K+ channel area, there has been an acceleration in granted Patents since the ROMK1 Patent issued and it does seem logical that molecular cloning and Patenting therein should encourage pharmaceutical activity. Not only is the trend upward for Ca^{2+} and K+, Na+ and Cl-related Patents also show an upward trend. Although drug companies have been relatively slow to enter the ion channel arena, there is a growing recognition of the potential value in the sector. In part this seems to be driven by molecular cloning but it is also clear that a significant reason for this reticence has been the lack of suitable screening technology. However, this is about to change as can be seen in the Figure and we can expect to see the number of ion channel-related filings accelerating.

Screening technology: the key to success

A number of developments in R&D over the last 10 years have converged. A major constraint on developing new ion channel-based drugs has been the difficulty in screening ion channels (voltage-gated) at the throughputs required of the modern industry in a cost-effective way and with functionally relevant screens. Effective exploitation of the ion channel arena has been awaiting the development of new technology.

Patch-clamp: the gold standard
Undoubtedly the definitive method for studying ion channel function is that of patch-clamping. In the form that most practitioners know it, patch-clamping was developed in the late-70s and the definitive text on the subject published in 1981 by Hamill et al[2]. The technique has gone practically unaltered since then. As far as biophysically and pharmacologically-characterising ion channels, it has remained the gold standard. The method can detect signals in the pA range and even measure the current passing through a single ion channel protein in real time. The time-resolution is in the tens of microseconds range and, crucially, patch-clamps allow the experimenter to fix the membrane potential of the cell (voltage-clamp). However, with respect to the practical considerations of the phar-

maceutical industry, it has effectively been written off as a screening platform because it is a manual and laborious procedure and hence slow (~100 dp/wk). Instead a good deal of effort has been made in developing alternative methods of monitoring ion channel activity that can be integrated into industry-standard compound screening formats with corresponding high throughput.

### High throughout ion channel screening
Fluorescence
The principle methods in ion channel programmes today are: receptor binding assays, flux measurements and fluorescence detection techniques. The principle advantage of these approaches is their medium to high throughput (15-60K dp/wk) albeit at the expense of information content. In particular ligand displacement assays say nothing about functional activity of unknown compounds and and are unlikely to detect novel types of modulators by definition. Of the functional assays developed, fluorescence has been seen as the most cutting edge. This methodology exploits changes in fluorescence that occur either with changes in the concentration of ions or changes in the membrane potential of the cell. For more details see Denyer et al[18] and Xu et al[19]. Since FLIPR^{TM} (fluorometric imaging plate reader) was developed by Noveltech (now manufactured by Molecular Devices Inc) for Upjohn in the USA to screen the ROMK1 K+ channel, the art has been refined considerably. The VIPR^{TM} (voltage ion probe reader) developed by Aurora Biosciences to exploit its proprietary FRET dye systems has improved both time-resolution and sensitivity of this approach to the best yet. However, in spite of the undoubted significance of the FLIPR^{TM} in bringing the first multi-well (now

Figure 3
Ion channel-related Patents issued in the US since 1980. Source: www.delphion.com. Patents which mention voltage-gated ion channels in the title or abstract were selected. The cumulative number, which includes composition of matter as well as sequence, assay and use Patents, is plotted on the y-axis against year (x-axis)
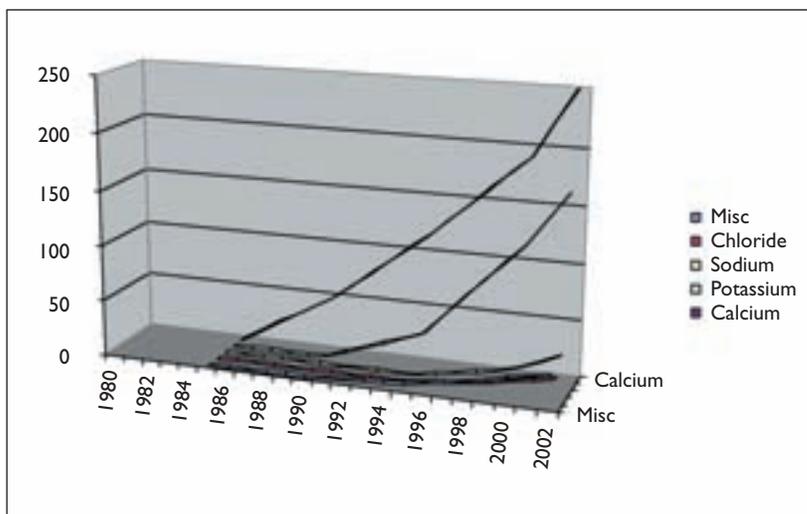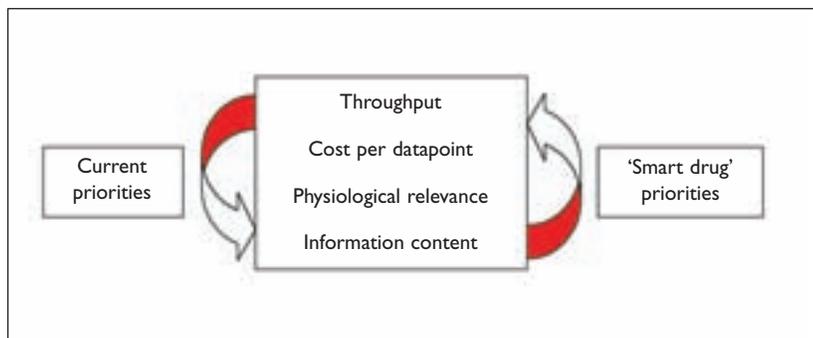
Figure 4

Current trends emphasising quantity not quality could be reversed with development of data-rich screening technology such as HTS patch-clamp

384-well based) ion channels screen to drug companies, FLIPR^{TM} is largely used for Ca^{2+} flux/mobilisation measurements and has not proved to be a generic ion channel screening tool. While VIPR II^{TM} (also 384-well based) clearly has superior time-resolution and suffers less from quenching artifacts than the non-ratiometric FLIPR^{TM}, in common with FLIPR^{TM} and other fluorescence methods it still suffers from relatively high false positive and negative rates of up to ~5%.

Flux assays

Use of radioactive surrogate channel ions was a popular early solution to side-step the need for electrophysiological assays such as patch-clamp. Although arguably a more relevant measure than the indirect measure of voltage detected by most fluorescence assays, flux assays have lost out in time resolution and the negative association with radioactivity. However, flux is back in vogue with the development of atomic absorption methods (pioneered by Bayer) which allow use of non-radioactive surrogates such as Rb^+ without the radioactivity 'headache'. Commercial systems are available from Thermo-Elemental UK (Cambridge) and Aurora Biomed (Canada) and this approach has recently been launched as a commercial service in a Quintiles plc (Scotland)/BioFocus plc(Cambridge) joint initiative. Currently, the technique is optimised for K^+ channel screening using Rb but it may also be possible to adapt the technique to read other surrogate ions for other types of voltage-gated ion channels. As with fluorescence, there is no ability to control the membrane potential of the host cell and as such is suited to primary screening in combination with hit validation and lead optimisation using medium to high-throughout patch-clamp techniques. Throughputs are claimed to be of the order of 30,000 cpds/week[8]. Without adequate patch-clamp back-up use of the technique, as with fluorescence, should be treated with caution.

Rethinking ion channel screening

The trend in the pharmaceutical industry's priorities has been to emphasise the screening of as many compounds as possible per unit time, often sacrificing informational content and physiological relevance of the screen in the process (**Figure 4**).

This is the mentality that has driven the FLIPR and VIPR approaches to ion channel screening. However, there has been a consistent clamour from the scientists running ion channel drug discovery programmes for a viable electrophysiological screens to support, and in some cases replace, the state of the art represented by FLIPR and VIPR. Some of the reasons for the dissatisfaction with fluorescence and other HTS assays are as summarised below.

Limitations of current HTS ion channel techniques:

● Many channels generate too small a signal or are too transient to record. For example the currents generated by the Nav1.3 Na^+ channel 'inactivate' within 20ms following activation with a depolarising (activating) voltage step. HTS fluorescence methods can resolve down to the 1-10sec time frame at best.

● None of the non-patch clamp methods are suited to controlling the voltage of the host cell and as such cannot properly control the gating of the ion channel in question. This precludes precise targeting of modulators to specific states of the ion channel which not only limits the scope for smart drugs that interact dynamically with ion channels but may result in significant false hit rates. An example of a target class that may require 'state-aware' screening is that of voltage-gated Na^+ channels where is it known that existing clinically-used blockers preferentially block the inactivated form of the channel rather than either closed or open states.

● In any case, increased throughputs at the primary screening level has lead to a bottleneck at the validation of hits and lead optimisation stages that do require electrophysiology techniques.

Figure 5 illustrates how automated patch-clamp systems could alleviate the bottleneck that is created where current HTS screens can be applied due the slowness of conventional patch-clamp used to validate hits and in lead optimisation. Furthermore, where fluorescence and flux assays are inappropriate, HTS patch-clamp will be able to replace existing approaches, perhaps in conjunction with smaller focused libraries following a trend exemplified by Arqule in the USA[20]. High-throughput patch-clamp systems which retain high information content will dramatically increase the quantity and quality of the data fed back into

medicinal chemistry in the lead optimisation process which should be reflected in better drugs.

Automated electrophysiology

The result is that in spite of the great technical advances made in fluorescence in particular, there has been a continuing demand for genuine electrophysiological methods that can be automated and hence accelerated. As recently as 1998, a review of HTS approaches to ion channel screening relegated patch-clamping to a low-throughput methodology and not viable for HTS, reflecting perhaps a resignation in the drug industry that this was not to be. Ingenious but limited approaches such as the Cytostar-T scintillating microplate (Amersham) were still being advanced. But things have changed. Patch-clamping is on the agenda again. Two companies have developed automated patch-clamp systems, Sophion Biosciences (Ballerup, Denmark) has robotised a traditional patch-clamp workstation in its Apatchi and CeNeS Channelwork (Cambridge) has invented a novel form of patch-clamping (the first departure from Hamill et al's protocol published 20 years ago) which utilises the so-called

Interface-Patch[TM] Clamp technique which was launched in 2000 in the AP1 AutoPatch[TM]. Possibly encouraged by these developments there is a renewed vigour attached to the quest for patch-clamp devices with high throughput. It is illustrative of the significant progress being made in this field that, in contrast to the 1998 review[18], a comparable review published in 2001[19] cites no fewer than 10 alternative and competing approaches to automating patch-clamp with the aim of developing HTS patch systems. Other electrophysiological approaches are also being developed (see below).

Automated patch clamp systems
AutoPatch (CeNeS)

● AP1: The first generation 'interface patch-clamp', this system revolutionised patch-clamping by patching on to cells held at a liquid-air interface. Reduced to a one-dimensional process without requiring optics, the whole process is automated from $G\Omega$-seal formation through to whole cell recording and drug application[21,24]. Although a single-cycle device, multiple machines can be operated by unskilled operators thereby scaling up throughout with reduced

Figure 5

Flow chart illustrating current bottleneck in current ion channel screening and future screening options using emerging automated and HTS patch-clamp technologies. HTS patch-clamp should at least keep up with a FLIPR-based screen (1% hit rate from a diverse compound library)- currently around 60K dp/wk max, (ie $\geq$ 600 dp/wk). HTS patch-clamp promises throughputs of up 30K dp/wk, providing an alternative to current FLIPR-like primary screens, particularly in conjunction with smaller focused libraries
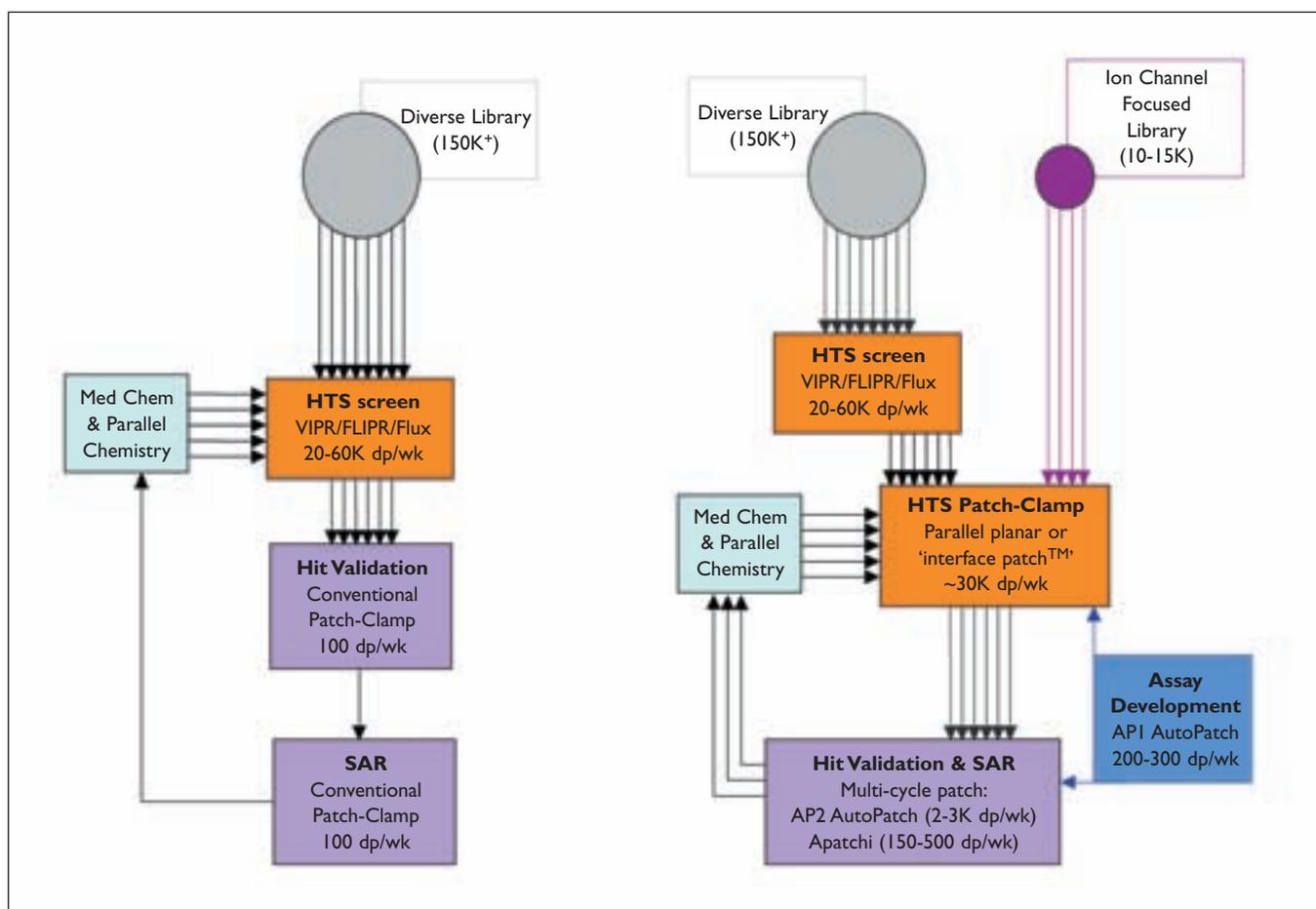
Figure 6: Graphical comparison between 'interface patch' and 'planar patch' approaches. INTERFACE-PATCH / PLANAR PATCH
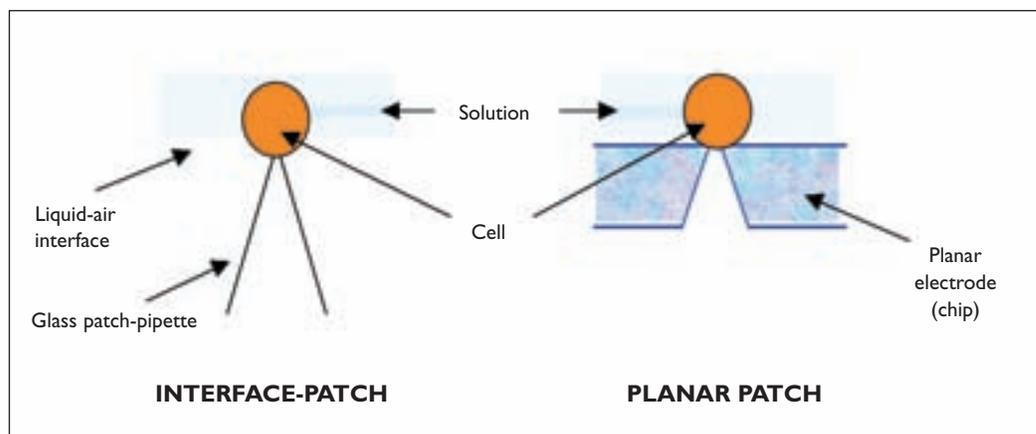
human recourse requirements. Throughput is 200-300 dp/wk. Five AP1s are installed at GSK in the UK and USA and two systems at Wyeth in the USA.

● AP2: A sequential recording device derived from AP1 but which can automatically switch between up to 48 recording sites in a 'patch plate' without reloading cells or pipettes. The system is integrated with the AP1's 96-well plate based drug delivery system providing dramatically increased capacity and at least an order of magnitude higher throughput. In late development, the system has been pre-ordered by Wyeth in the US. Throughput is expected to be ~2-3K dp/wk.

Apatchi-1^{TM} (Sophion)

● Originally developed at NeuroSearch (Denmark) in conjunction with Pfizer, the Apatchi-1 is a robotised patch-clamp workstation which utilises motorised manipulator systems and sophisticated image recognition software to automatically place patch pipettes on cells and thereby establish patch-clamp recordings. The system also incorporates a fluid handling system and carrousel of eight patch pipettes which permits up to eight recordings in sequence without reloading by an operator. The system is extremely reliant on the precision of its hardware and software to avoid pipette crashes as the cells are presented in dishes in the traditional fashion. Pfizer has one Apatchi system installed in Sandwich, UK.

Parallel patch-clamp systems

The quantum leap in ion channel screening throughout will come from parallel processing systems. As yet, no automated parallel patch systems have been developed although a number of companies and research groups are working on novel recording electrodes, electronic amplifiers and fluid handling systems that are expected to

be integrated into such devices. The challenge with parallel systems will be to retain as much of the power of conventional patch-clamp and automated systems such as the AP1 AutoPatchTM system with the required throughout at processing at an affordable price and in a practical format.

Many design issues have to be addressed which include:

● How to achieve parallel patching from a significant number of active sites without sacrificing sampling frequency and voltage-clamp integrity. Overall throughputs of ~30K dp/wk should be sufficient.

● Individual control of recording sites from GΩ-seal formation through to recording to maximise recording hit rates and longevity of individual recordings (≥ 50% hit rate).

● Facility for single or multiple drug solution applications to individual recording sites.

● Maximise stability of recordings.

● Minimise cross-talk and distributed capacitance between and across sites.

● How to handle the potentially vast amount of data acquired.

● How to fully-automate the process into a robust and deskilled process at an affordable price (1$ per data point?).

Broadly-speaking there are two approaches to the electrode design (**Figure 6**) currently being explored by different groups:-

● Interface-Patch^{TM} which uses glass patch pipettes as in conventional patch-clamp and the AutoPatch^{TM}.

● Planar electrodes ('chips'). In the planar electrode a GΩ-seal is formed between cell and a pore formed by microfabrication techniques in a chip made of silicon or other materials.

A number of companies and academic groups are working toward parallel patch-clamp devices

Table 1
Main players in the development of HTS patch-clamp technology platforms

COMPANY	ELECTRODE DESIGN	STATUS
Aviva Biosciences
San Diego, USA	Silicon; field potential cell positioning & suction control	Single pore chip recordings from mammalian cells; overall whole cell recording success rate ~15-20%; not automated[22]
Axon Instruments
Union City, CA, USA	Sylgard cast chip; suction control	Whole cell recordings established with mammalian cells on single pore chips; incidence of GΩ-seals ~50%; not automated[23]
CeNeS Channelwork
Cambridge, UK	Glass patch pipette; suction control	Interface Patch validated in automated AP1 & AP2 devices; whole cell recording rate ~50-60% in mammalian cells; parallel version (AP3) in development; control software already developed for AP1 & AP2[21, 24]
Cytion (subsidiary of Molecular Devices)
Lausanne, Switzerland	Planar silicon; control of seal formation by electrical field	GΩ-seals on single pore chips reported for vesicles; no data reported for mammalian cells[25]
Cytocentrics
Reutlingen, Germany	Glass patch pipette/silicon; suction control	Glass pipette-based with a centring technique using porated silicon structures; claim high success rates[26]
Essen Instruments
(20% owned by Molecular Devices)
Ann Arbor, MI, USA	Not disclosed	Whole cell data reported for mammalian cells from array of 20 recording sites; parallel voltage-clamp claimed; hit rate not disclosed; not automated; commercial device in development[27]
Nanion Technologies
Munich, Germany	Planar glass; suction control	Whole cell data reported on single pore chips for mammalian cells; ~50% hit rate; multi-pore arrays in development; not automated[28, 29]
Sophion Biosciences
Ballerup, Denmark	Planar silicon; suction control	No data on chip performance reported; parallel array in development

and a comparison of the approaches and progress made by the main commercial players is summarised in **Table 1.** One of the fundamental challenges of planar electrodes has been reliably forming the GΩ-seal which is critical for successful patch-clamp. This of course is a well-established phenomenon with glass fabricated patch-electrodes as used in 'interface patch' clamp.

Other electrophysiological approaches
Impedance probes
● CeNeS Channelwork, Cambridge, UK. (TΩRM^{TM}) The 'transepithelial resistance measurement technique' (TΩRM)[31] exploits single mammalian cells or confluent pseudo-epithelial layers of mammalian cells expressing the ion channel of choice. It is then possible to measure changes in the overall

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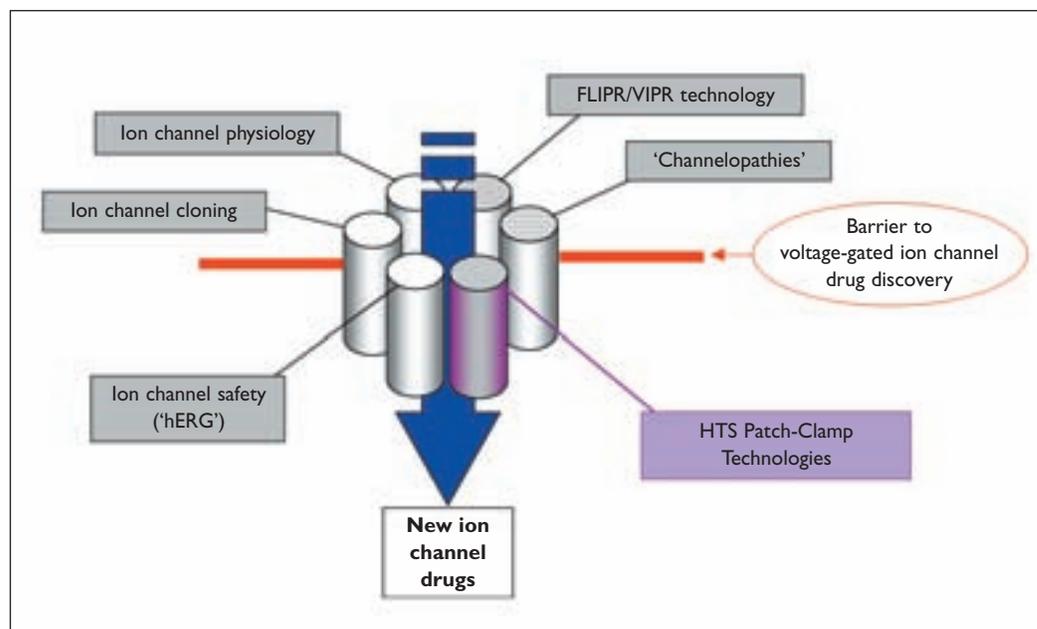**12** Curran, ME et al. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell, 1995. 80(5): p. 795-803.
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● Adaptive Screening, Cambridge, UK (CytoFlux™) Probably measures transmembrane impedance. Although details are scant, this UK company claims to have developed a 96-array parallel processing drug screening tool based around impedance measurement across membranes that may be combined with fluorescence imaging and field potential stimulation.

Frog oocyte voltage-clamp systems

A number of two-electrode voltage-clamp based systems, based around the use of Xenopus (frog) oocytes, are being developed by several companies including:

● Axon Instruments (Union City, CA, USA) OpusXpress™: eight-channel (egg), semi-automated recording system launched at the end of 2001. Increases throughput by parallel recording.

● Abbott Labs (Abbott Park, IL, USA) In-house system: parallel eight-egg screening system with integrated drug delivery and analysis systems with increased throughput and hands-off capability.

● Multichannel Systems (Reutlingen, Germany) RoboCyte™: an automated sequentially recording system based around a 96-well array. The system also automatically injects oocytes with the appropriate RNA for the required ion channel target. No increase

in throughput but long-term unattended operation provides increased capacity per working day.

● Scion Pharmaceuticals (Boston, MA, USA) HTEP™: drug application and experimental design system. This system facilitates scheduling of drug application and voltage-step protocols but does not constitute an automated recording system, is limited to oocytes and is comparable throughout to manually-operated recording setups.

Significant differences in the background experienced by cloned ion channels heterologously expressed in frog eggs can significantly affect the sensitivity of channels to modulators. This factor, combined with the requirement to inject every single egg with RNA encoding the ion channel of choice and seasonal variation in expression limit the usefulness in drug discovery. Nevertheless, as a workhorse for novel gene expression, the oocyte remains a valuable tool and these technologies will undoubtedly prove useful in this context. It is not known whether any customers have taken delivery of any of the commercial systems yet.

Ion channel drug discovery: coming of age

Wholehearted enthusiasm by the pharmaceutical industry for ion channel drug discovery has taken its time coming. Wholesale exploitation of ion channels in drug discovery has been held back by concerns in a number of areas as discussed above:
● Physiological relevance of ion channels.

● Therapeutic relevance of ion channels (channelopathies).

● Molecular correlates of physiological ionic currents and potential for specificity of ion channel modulators (cloning).

● Screening technology development (FLIPR/VIPR and HTS patch-clamp).

● Ion channel safety screening.

Ironically, an undesirable attribute of ion channel modulators, namely hERG block, has catalysed much of this progress in recent years. The final missing 'subunit' (HTS Patch-Clamp Technologies) of this particular drug discovery and development pipeline is about to be inserted (**Figure 7**). Will the ion channel modulators really start to flow now?

A number of the big pharma recognised the potential for ion channels in the late 80s and 90s but did not necessarily have the required resources. As a result a number of niche businesses were established in the late 90s to cater for the excess demand. These included Channelwork™ the contract research division of CeNeS Pharmaceuticals (Cambridge, UK), GENION (Hamburg, Germany), ChanTest (St Louis, USA) and Zenas Technologies (New Orleans, USA), all of which specialised to a greater or lesser extent in providing ion channel screening on a contract basis.

ICAgen was the first company formed expressly to exploit ion channels in drug discovery and has enjoyed an almost unique niche for a number of years, although companies like NeuroSearch (Denmark) have also had quite an ion channel emphasis. Both ICAgen and NeuroSearch have relied very heavily on traditional patch-clamping to support their programmes. However, like Big Pharma, while making use of medium to high throughout primary screening platforms such as FLIPR and flux, they also have lacked the killer ion channel screen required to really exploit ion channel targets to the full. Now, reflecting the combined progress in the field summarised above, there are likely to be some new challengers to these incumbents.

SCION (Boston, USA) and IONIX (Cambridge, UK) are both betting on ion channels in a big way. SCION has interests in the Kv1.1 channel (demyelinating disorders) among others while IONIX, ostensibly a 'pain' company, will be focusing to a major extent on ion channels (inter alia the SNS sodium channel) expressed in sensory neurones. Both were founded in 2001. Other specialist companies already working in the ion channel domain may also enter the fray. These tend to fall into one or both of two categories:

● Ion channel contract research providers (Channelwork, GENION [now EVOTEC-OAI], Chantest, Zenas).

● Ion channel technology developers (AVIVA Biosciences, Axon Instruments, Channelwork, Essen Instruments, Cytion [now Molecular Devices Inc], Nanion Technologies, Sophion Bioscience).

Many of these companies aspire to contract services/drug discovery on the back of their technology. However, whether any of these companies will succeed in making the transition from instrument developer to drug discoverer (à la Aurora via the VERTEX acquisition) remains to be seen. CeNeS' Channelwork is currently being positioned to spin-out into a new 'ion channel company' and seems uniquely well placed to make the transition, having developed both contract ion channel research and commercial automated patch-clamp technology businesses in parallel.

All of which seems to indicate that we are indeed on the verge of a golden age in ion channel drug discovery. It remains to be seen who is best prepared to reap the rewards. **DDW**

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Andrew Silverthorne has a BSc in Biological Sciences from Leicester University, UK and following post-graduate research work investigating the role of growth factors on oral cancer at Bristol University, joined Xenova Ltd where he was involved in drug discovery research, assay development, HTS and preclinical research. He then went on to join the business development consultancy Connect Pharma (now PharmaVentures) before joining CeNes where he is currently Head of Business Development with commercial responsibility for its ion channel contract research and technology development businesses.

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