Antibodies as ion channel modulators and drugs

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After academic research as a neuroscientist and 20 years of commercial ion channel drug discovery, Marc is now a freelance blogger, advisor and consultant for clients worldwide, where he shares his expertise and enthusiasm for all aspects of ion channel screening. He is particularly interested in automated patch clamp, and exploiting the potential of human iPS stem cell assays to facilitate the successful translation of new drugs into the clinic.

With current discussions about the changing dominance of small molecules vs biologics as clinical therapeutics, it seems a good time to provide an overview of the history and current status of antibody ligands as ion channel modulators. This review highlights academic and industry efforts to develop polyclonal and monoclonal antibodies, single domain nanobodies, and antibody-toxin chimeras as tool compounds and preclinical leads for immunology, cardiac, neuroscience and oncology applications. It includes case studies on K_v, K_{Ca}, K_{2P}, K_{ir}, $Na_v1.x$, Ca_v, TRPx, P2X, ASIC and Orai channel antibodies with functional activity revealed by manual and automated patch clamp (APC) electrophysiology. Over 25 years of ion channel antibody work has been reviewed in the last decade,[1-7] but this blog article updates the area with more recent publications and commercial insights.

Historical ion channel polyclonal antibodies

Some may be surprised by the diversity and efficacy of early attempts to create ion channel antibodies, where academic and industry groups generated polyclonal antibodies able to functionally inhibit various Na_v, Ca_v, K_v and TRPx channels (review1). Several groups discovered polyclonal antibodies that could partially inhibit K_v channels by targeting epitopes in voltage sensor domain (VSD) S1-S4 helices, including rapid and potent nM blockers of $K_v1.2$ and $K_v3.1$ channels with moderate selectivity,[8] and a $K_v1.3$ blocker that showed both acute and more potent and complete chronic inhibition (30-300 nM) with excellent selectivity over closely related $K_v1.x$ isoforms, $K_{Ca}3.1$, and cardiac hERG, $K_v7.1$, $Ca_v1.2$ and $Na_v1.5$ channels.[9] Similar VSD epitope targeting has produced state-dependent inhibitors of Na_v and Ca_v channels. Israeli researchers generated rabbit polyclonal antibodies against domain I VSD S4 helix of vertebrate Na_v channels that bound well when cells and brain synaptosomes were depolarized,[10] and modulated Na_v current fast inactivation in DRG sensory neurons and reduced sciatic nerve action potential amplitude. Similarly, immunization with a peptide to the DIV S6 helix loop of $Ca_v1.2$ α1D channels, associated with dihydropyridine (DHP) binding and $Ca_v1.2$ channel gating, yielded a polyclonal antibody with state-dependent binding to cardiac and neuronal $Ca_v1.x$ channel isoforms which partially (49% effect) inhibited Ca_v currents in depolarized sensory neurons.[11]

Surprisingly, early attempts to produce ion channel antibodies by immunizing animals with native tissue expressing Na_v and Ca_v channel proteins also produced state-dependent mAbs. For example, rabbit skeletal muscle T-tubule protein extracts elicited a mAb that potently (IC_{50} 60 nM), rapidly and irreversibly inhibited α1S-mediated $Ca_v1.2$ currents by retarding activation biophysics.[12] However, this block was incomplete and while it was selective over T-type $Ca_v3.x$ and outward K_v currents, mAb 1A produced similar gating effects on TTX-R Na_v currents. Similarly, Israeli researchers generated a series of mAbs against Torpedo electric eel electroplax tissue that inhibited both muscle and neuronal cell Na_v currents (IC_{50} of 133 nM) and blocked rat optic and sciatic nerve conduction.[13] Each mAb produced a complex mix of state-dependent (hyperpolarizing shift of activation and inactivation, altered current kinetics) and state-independent effects (reduction in peak conductance with no change in biophysics) reminiscent of scorpion toxins,[14] suggesting that these early Na_v channel mAbs bind to different VSD helices and control Na_v channel gating in a similar way to subtype- selective small molecules targeting $Na_v1.7$ and other $Na_v1.x$ channels discovered in later drug discovery programs.

A more common approach for ion channel antibody design used sequence comparisons and hydropathy plot analysis which identified the extracellular loop between the S5 helix and pore region (S5-S6; E3 loop or E3 turret) as the best epitope for immunization and generation of inhibitory antibodies. While this method can generate more ligands with subtype selectivity through binding to accessible and diverse epitopes, these antibodies lack the state-dependent mechanisms described above for voltage-dependent ion channel antibodies, although state-dependent mAbs have been identified for P_2X and GlyR ligand-gated ion channels (see below). Several E3-targeting polyclonal antibodies

have been found that functionally inhibit high-voltage activated $Ca_v1.x$, $Ca_v2.1$ and $Ca_v2.2$ channels. Stanford[15] and Dartmouth researchers[16] in the US generated polyclonal antibodies against $Ca_v2.1$ E3 turret epitopes to mimic Lambert-Eaton myasthenic syndrome and paraneoplastic cerebellar ataxia where human cancers trigger auto-antibodies against $Ca_v2.1$. Their antibodies could rapidly and selectively inhibit $Ca_v2.1$ (and $Ca_v2.2$) currents in heterologous cells, cancer cell lines and cerebellar neurons, inhibited cerebellar synaptic transmission by decreasing presynaptic release probability, and produced a cerebellar ataxia phenotype *in vivo*. Potency was moderate (20-250 mg/ml) and inhibition was partial (20-40%), and the antibodies occluded ω-GVIA but not ω-Aga IVA toxin binding which suggests binding to the pore rather than VSDs.

David Beech's lab in the UK gained more notice several years later for their use of E3-targeting to develop functional polyclonal ion channel antibodies against several TRPx receptors (and $Na_v1.5$ channels). Their antibodies specifically labeled and functionally inhibited TRPC1 and TRPC5 currents and store-operated Ca^{2+} entry (SOCE) in smooth muscle and heterologous cells,[17-18] and a TRPM3 antibody rapidly but incompletely inhibited Ca^{2+} entry with selectivity over TRPC5, TRPV4, TRPM2 isoforms and endogenous P_2X-mediated responses.[19] This paper made an impact in the ion channel drug discovery field as acute functional inhibition of TRPM3 ionic currents was demonstrated on the Patchliner, validating the use of APC platforms for ion channel antibody screening. Amgen were one of the first companies to publish on ion channel antibodies shortly after, generating polyclonal and monoclonal ligands against the E3 pore loop of TRPV1 receptors.[20] Intriguingly, the polyclonal but none of the 26 mAbs showed functional inhibition of Ca^{2+} influx in heterologous cells; also, the anti-rat antibody Ab-156H was more effective at inhibiting proton-activated (full block, IC_{50} of 30 - 300 nM) than capsaicin, heat, or endogenous ligand TRPV1 responses. This was significant, as small molecule drug discovery efforts at this time suggested that modality-specific TRPV1 antagonism may avoid the thermal side-effects seen with broad spectrum inhibitors in preclinical models and human patients.

These early polyclonal antibodies provided key validation data that potency and state-dependence could be achieved, but they suffered from moderate selectivity and partial inhibition and their variability and production costs precluded further therapeutic development. Later work focused on other antibody formats with increased potency, efficacy and selectivity which have been progressing preclinically with several ultimately reaching the clinic.

More recent monoclonal, single domain and antibody-toxin chimeras

Work in academia and industry over the last 10 years has shifted to more specific and consistent monoclonal antibodies (mAbs) and smaller single domain nanobodies, and chimeric scaffolds where ion channel toxins are fused to full length IgG receptors or incorporated into long CDR-3 loops of Fc light chains to enable access to smaller cryptic binding sites on ion channels in addition to the E3 turret region. Catherine Hutchings has been tracking the progress and diversity of antibody R&D and drug discovery programs targeting ion channel and GPCR membrane proteins during this period, and Figure 1 shows a summary of the ion channel targets as of 2020.[4,6] The vast majority of the ion channel antibody programs are still at the discovery or preclinical stage, albeit with a 3 fold increase in programs from 2016 to 2020, with the Biosceptre P_2X7 mAb for cancer and a Daiichi Sankyo Orai-1 mAb for atopic dermatitis the only clinical candidates.

Monoclonal antibodies dominated ion channel drug discovery efforts during 2010-2020, utilizing traditional immunization or phage display library panning methods with isolated peptide epitopes, whole proteins, purified membranes or intact cells. It is common to find 1-50 mAb 'binders' but few are functional inhibitors of ion channel, so single B-cell sorting and immunization of other species such as cows and chickens with longer CDR3 loops have

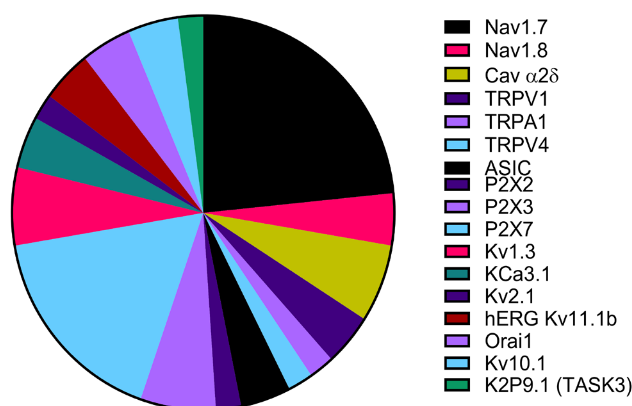

Figure 1 ***Ion channel antibody targets of academic and commercial R&D programs.*** *Adapted from data in [6] and used with permission and under Creative Commons open access license 4.0 (http://creativecommons.org/licenses/by/4.0/).*

been used to identify rare and novel antibody ion channel modulators. More recently nanobodies and other single domain VHH ion channel antibodies have been found by immunizing camelids or sharks or panning epitopes against phage display libraries, and these smaller scaffolds benefit from improved solubility, stability and tissue penetration and reduced immunogenicity compared to mAbs, whilst maintaining good potency and selectivity for specific ion channels. They may also be easier and cheaper to produce, purify and engineer for therapeutic use,[21] and nanobodies

Format	Size	Binding affinity	Functional potency	Plasma half-life	Immunogenicity	Multi-functional
Monoclonal Antibody	Large	pM - nM	1 - 10 nM	Days	High*	Bi-specific
Nanobody	Small	nM	10 - 500 nM	Hours	Low	Modular
Ab-toxin chimera	Large	nM - µM	0.5 - 3 µM	Day	Moderate	Bi-specific
Ab-toxin fusions	Large	pM - nM	0.3 - 300 nM	Day	Moderate	Bi-specific

Table 1 *Summary of key structural and biological properties of different antibody formats. * Immunogenicity is reduced in mAbs from humanised mice and human phage display libraries.*

can be linked in a modular fashion to improve potency and create complex multivalent modulatory effects. Finally, several companies have taken a alternative approach by combining traditional Ig or long CDR3 loop antibodies or single domain Fc scaffolds with ion channel peptide toxins to create hybrid chimeras with a long plasma half-life and tunable ion channel potency and selectivity. These smaller single domain nanobody formats and antibody-toxin chimeras, especially those incorporating long CDR3 loops, are thought to get privileged access to cryptic binding sites not available to classical antibodies or small molecules – and this is especially important to obtaining potent, selective and functional ion channel antibody modulators. Each of these antibody formats have their own strengths and weaknesses as potential therapeutics, and their structural and biological properties are compared in Table 1.

Antibodies for immunology, auto-immune disease and neuroinflammation

Many early mAb programs in the 2010s targeted ion channels expressed on immune cells in an effort to develop immunosuppressant and anti-inflammatory agents that were more balanced in terms of potency, selectivity and safety than existing peptides and small molecules.

In 2013 Novo Nordisk and Amgen both published on mAbs generated against Orai-1, the pore subunit of the store-operated Ca^{2+} entry (SOCE) I_{CRAC} channel complex that regulates mast and T-cell activation and is implicated in auto-immune diseases, graft-host rejection and allergies. Both groups found that epitopes from the 2nd extracellular loop of Orai-1 generated selective mAbs that could inhibit T-cell function *in vitro* and *in vivo*, but the Novo Nordisk mouse mAb 10F8 did not appear to directly inhibit I_{CRAC} currents but rather triggered protein internalization after prolonged cell incubation to reduce rheumatoid arthritis T-cell Ca^{2+} signaling, cytokine release and proliferation.[22] In contrast, the fully human Amgen mAbs (from immunization of humanized mouse with cells expressing hOrai-1) functionally inhibited recombinant I_{Crac} currents recorded on APC as well as store-operated Ca^{2+} influx, NFAT transcription and cytokine release.[23] Binding affinity for hOrai-1 was high (20 - 100 pM) and this translated into potent *in vitro* activity in the 1 - 10 nM range and 50-70% efficacy, although acute and rapid inhibition of I_{CRAC} currents required ~50 nM of 2C1.1 mAb. Daiichi Sankyo revealed their Orai-1 IgG1 antibody program a decade later, with the humanized mAb DS-2741a inhibiting I_{CRAC}-mediated effector T-cell activation and mast cell degranulation *in vitro* and *in vivo* without affecting regulatory T-cells, with the species-specificity of the mAb requiring use of human Orai1 knock-in mice.[24] Preclinically DS-2741a reduced dust mite antigen-induced dermatitis and graft-versus-host disease in mice models,[25] and Ph I and Ib clinical trials were initiated in 2020 in Japan to assess safety and efficacy after sub-cutaneous injection in healthy and atopic dermatitis patients,[6] but these were terminated in 2021.[26]

A popular immunological disease target for antibody scaffolds is $K_v1.3$, which is up-regulated on T-EM cells and implicated in various auto-immune diseases (reviews[3,4,7]). Tetragenetics have taken a broad approach in developing

"As biologics gain prominence in therapeutics, now is the time to examine the progress of antibody ligands as ion channel modulators, including polyclonal and monoclonal antibodies, nanobodies, and antibody-toxin chimeras, and their applications in immunology, cardiology, neuroscience, and oncology"

Marc Rogers, Founder and Director, Albion Drug Discovery Services Ltd

both traditional mAbs as well as single domain nanobodies against various ion channels, utilizing a novel ciliate Tetrahymena expression system to produce large quantities of intact folded protein suitable for immunization and library panning. They published on their $K_v1.3$ program in 2018 (in collaboration with Crystal Biosciences and argenx), identifying [69] $K_v1.3$-binding monoclonal and scFv antibodies from proteoliposome-immunized chickens and llamas of which 10 functionally and rapidly inhibited $K_v1.3$ currents by 40-80% (400 nM).[27] The best chicken and sole llama antibody were relatively potent blockers (IC_{50} 6 and 110 nM) with good selectivity over $K_v1.x$ gene family members and cardiac hERG and $Na_v1.5$ channels, but there is little public information on subsequent preclinical development.

Academic and industry groups have modified $K_v1.3$-targeting antibodies by incorporating toxins into full length or Ig fragments. Most companies added $K_v1.3$ toxins into the long CDR3 domain of human and cow mAbs to enable warhead access to smaller binding sites than traditional mAbs and create chimeric scaffolds with better plasma exposure and stability than native toxins. Scripps researchers fused moka1 and Vm[24] $K_v1.3$-selective scorpion toxins into the CDR3 loop of humanized cow antibody VL chains to produce effective inhibitors of $K_v1.3$ channel activity and T-cell proliferation and cytokine release that were more potent than the native toxins, with one scaffold combination showing pM inhibition.[28] The preclinical profile was good with efficacy in an auto-immune disease model *in vivo* and better plasma stability and a long t½ of hours compared to minutes for native toxins. These chimeric $K_v1.3$ channel ligands also exhibited significant commercial promise as they could be produced efficiently with good yield in HEK cells and exhibited low immunogenicity, and the project was spun-out into Sevion in 2015 (mAb SVN-00129); the company is now part of Eloxx Pharmaceuticals but the program is no longer listed in their company pipeline. Janssen researchers adopted a different strategy by linking scorpion toxins OSK1 and OdK1 to IgG Fc fragments which inhibited $K_v1.3$ currents (IC_{50} 0.3 – 15 nM) and T-cell cytokine release (1 - 30 nM), albeit with lower potency than the native toxins.[30] A synthetic toxin peptide KV261 was designed and fused to the same Fc fragment to improve potency and Kv1.3 selectivity (0.1 nM, 1000 fold), but work was halted as Fc-toxin chimeras induced pro-inflammatory cytokine release and a smaller toxin-peptide version was inactive *in vivo*. Thus, it seems that incorporating ion channel toxins more seamlessly into the long CDR3 loops of mAbs is the best approach, and has been adopted by several other companies.

More recently Minotaur Therapeutics have been developing a mAb chimera (MNT-002) with the canonical $K_v1.3$ sea anemone ShK toxin incorporated into the long CDR3 loop of an IgG antibody, and also engineered a smaller monovalent ShK toxin-Fab antibody chimera that binds to the outer vestibule of $K_v1.3$ channels and reveals open conformation gating in cryo-EM studies.[31] The small Fab-ShK chimera inhibits $K_v1.3$ currents with an IC_{50} around 300 pM, similar to the native toxin and without the loss of potency seen with larger IgG Fc-toxin scaffolds. It would be remiss not to mention the pioneering work of John McCafferty at Iontas on ion channel toxin antibody chimeras in this section, whose 'knotbody' drug discovery platform was spun-out to Maxion Therapeutics in 2020. They revealed proof-of-concept data with knotbodies including toxins against $Na_v1.7$ and ASIC1 channels, and are using APC to progress a $K_v1.3$ program where various toxins have been linked to mAb CDR3 loops, and phage display and antibody maturation are used to discover more diverse scaffolds and bring potency back closer to the original nM affinity of native toxins.[32]

Also of note was an early demonstration by Ablynx that nanobodies could produce complex inhibition of $K_v1.3$ channels, including striking APC data that nanobodies with different mechanisms of action could be combined in a modular fashion to increase potency and complexity of modulation.[21] Immunization of camelids generated single domain VHH scaffolds that rapidly inhibited $K_v1.3$ currents with a mixture of gating-dependent effects (e.g. enhanced inactivation) and reduced ex vivo T-cell function, with pM-nM potency equivalent to ShK toxin and improved >1000 fold selectivity over other $K_v1.x$ gene family and hERG channels, attributed to binding to the 1st extracellular loop of $K_v1.3$ channels. Combining a monovalent nanobody into a bivalent format increased efficacy and potency as well as effect duration through decreased washout, while combining two or three nanobodies with different gating effects produced diverse allosteric modulatory effects, illustrating the unique multivalent potential of small nanobodies. The nanobodies were moderately active in an *in vivo* model of auto-immune disease with similar potency to ShK after sub-cutaneous administration, and their half-life could be extended by conjugation to an anti-albumin nanobody.

Minotaur Therapeutics also produced one of the patented Ablynx/Sanofi $K_v1.3$ nanobodies (A0194009G09) and showed that it accelerated channel inactivation and reduced current density (1 nM – 1 μM; Figure 2), and visualized binding to the E3 turret in cryo-EM studies which enhanced the dilation of the outer selectivity filter to disrupt activation and promote entry into an inactivated conformation.[31] While electrophysiology data failed to clearly show tetravalent modulation, they did reveal that more than 1 nanobody could bind and functionally modulate each $K_v1.3$ channel, and that high occupancy accelerated and stabilized the effect on C-type inactivation, nicely illustrating the potential for modularity and polyvalency in nanobody modulation of multimeric ion channels.

$K_{Ca}3.1$ are expressed and regulated alongside $K_v1.3$ channels during the proliferation and activation of various immune T-cells, macrophages and microglia.[33] Heike Wulff and her collaborators are interested in $K_{Ca}3.1$ for immunomodulatory

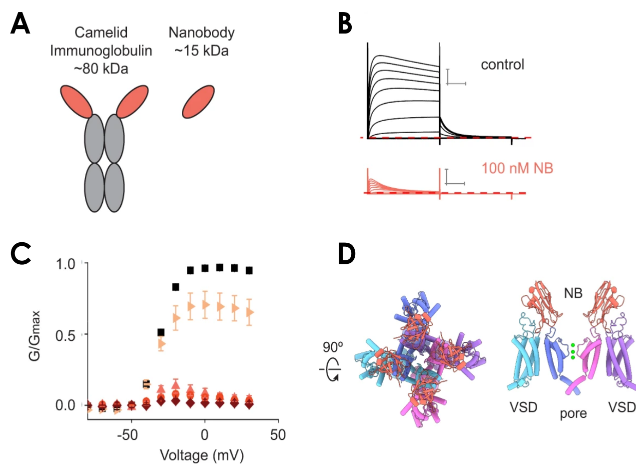

Figure 2 ***K_V1.3 nanobody inhibits current by increasing inactivation, with multiple nanobodies bound to each tetrameric K_V1.3 cryo-EM structure*** **A** *Illustration of camelid antibody and nanobody.* **B** *K_V1.3 current traces from depolarizing pulses in control solutions (black) and 100 nM nanobody (NB) A0194009G09 (orange).* **C** *G-V relations in control (black squares) and various concentrations of nanobody (1 nM, 10 nM, 100 nM or 1 µM), normalized to maximum control tail current.* **D** *Views of the K_V1.3 model show four nanobodies (orange) in complex, with a cutaway revealing two nanobodies each bound to a pore domain and a voltage-sensing domain (VSD). Adapted from data in [31] and used under Creative Commons open access license 4.0 (http://creativecommons.org/licenses/by/4.0/).*

purposes[3] and worked with Tetragenetics to find inhibitory K_{Ca}3.1 mAbs using the Tetrahymena ciliate expression system to express K_{Ca}3.1 protein which was attached to magnetic beads to pan human mAb phage display libraries.[34] From 34 specific binders they found 2 mAbs that could functionally inhibit heterologous K_{Ca}3.1 currents, with the best exhibiting an IC_{50} of 1.8 nM and selective over related K_{Ca}1.1 and K_{Ca}2.2 and cardiac hERG and Na_V1.5 channels. The mAb does not cross-react with rodent K_{Ca}3.1 protein, which will make preclinical work challenging but not insurmountable. Tetragenetics previously announced a collaboration with Dutch company ModiQuest Research in 2017 to access their phage library of human patient-derived mAbs specifically to find K_{Ca}3.1 modulators for tissue fibrosis and inflammation, but the outcome is unclear.

A number of academics and biotech & pharma companies have developed antibodies against $P2X_7$ receptors, as these are validated targets for auto-immune disease, neuroinflammation and neurodegeneration (as the large pore $P2X_7$ receptors can allow ATP and other agents to cross the cell membrane and activate downstream pathways leading to NLRP3 inflammasome activation[35]). Glaxo were the first to publish on a $P2X_7$ mAb, which they developed due to the lack of specific small molecule blockers at the time by immunizing mice with myeloma cells expressing $hP2X_7$. Their mAb exhibited species and subtype-specificity (over human $P2X_1$, $P2X_3$ and $P2X_4$ receptors) and "unexpectedly" was found to functionally inhibit $P2X_7$ currents (70% effect, IC_{50} of 5 nM) and monocyte cytokine release.[36] Over 2 decades later Australian academics confirmed that the Glaxo mAb (obtained from hybridoma clone L4) could inhibit YO-PRO dye uptake by human but not mouse $P2X_7$ receptors *in vitro* (~85% inhibition, IC_{50} 0.2 mg/mL) and reduced clinical signs of graft-host reduction in humanized mice.[37] A Japanese group working on intestinal inflammation serendipitously produced a functional rat anti-mouse $P2X_7$ mAb (1F11) by immunizing animals with ex vivo colon macrophages, finding a single IgG2 clone that bound to the ATP-binding domain of $P2X_7$ but not $P2X_1$ or $P2X_4$ receptors, and functionally inhibited $P2X_7$ dye uptake and macrophage activation *in vitro* and reduced intestinal inflammation in a model of colitis.[38]

Integral Molecular list a $P2X_7$ program for auto-immune disease in their pipeline,[39] and a case study on their website illustrates how they immunized chickens with DNA liposomes expressing native protein to generate a diverse panel of 198 mAbs from 31 antibody families with exquisite selectivity for $P2X_7$ channels.[40] Significantly, a selection of 6 specific and 1 rare mAb with pM binding affinity could functionally modulate $P2X_7$ Ca^{2+} flux in heterologous cells and native PBMCs with similar potency, including several antagonistic and one agonistic scaffold (Figure 3). This panel of mAbs were progressed preclinically for auto-immune disease as well as cancer (see section 4 below), but await partnering to move into the clinic.

Several other $P2X_7$ nanobodies have been revealed which can exhibit inhibitory or agonistic effects. Ablynx worked on $P2X_7$ alongside their K_V1.3 program,[21,41] and published a detailed paper on their preclinical mouse and human $P2X_7$ nanobodies with German and French academics.[42] Llamas were immunized with a cocktail of cDNA vectors or HEK cells expressing mouse or human $P2X_7$, and single chain VHH DNA from lymphocyte clones was incorporated into phage display libraries for panning against native and heterologous $P2X_7$-expressing cells, with the whole process designed to maximize nanobody binding to native and correctly

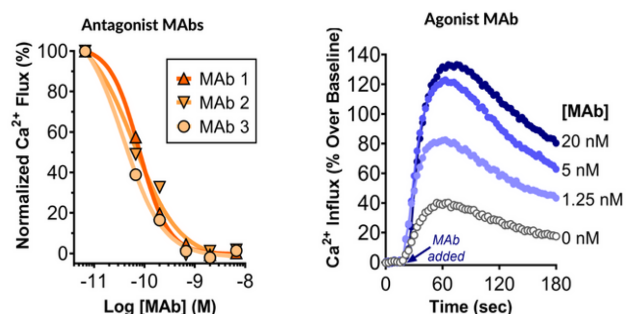

Figure 3 ***Monoclonal $P2X_7$ antibodies with inhibitory and agonistic effect developed by Integral Molecular.*** *Adapted from data in 40 and used under Creative Commons open access license 4.0 (http://creativecommons.org/licenses/by/4.0/).*

folded $P2X_7$ receptors. This yielded 31 distinct nanobody families, of which 18 specifically bound to mouse $P2X_7$ with 9 functionally modulating channel activity; 6 inhibited ATP-activated currents or Ca^{2+} influx and immune cell function *in vitro*, ex vivo and *in vivo* (exemplified by 13A7, IC_{50} of 0.2 - 12 nM), and 3 potentiated $mP2X_7$ responses (14D5, EC_{50} of 0.2 - 6 nM). Similarly, 2 of the 3 human $P2X_7$ nanobodies inhibited channel activity with greater efficacy (full block) and potency (0.2 – 0.5 nM, 10-50x) compared to Glaxo's human L4 mAb, and one was a potentiator. All of the nanobodies are selective for $P2X_7$ over closely related $P2X_1$ and $P2X_4$ isoforms and do not affect $P2X_7$-independent inflammasome activation, and the $mP2X_7$ potentiator 14D5 acted like a positive allosteric modulator as it increased ATP sensitivity but was inactive when applied alone, and exacerbated $P2X_7$ receptor-dependent immune cell responses *in vivo*. Exploiting the modularity of nanobodies (and trimeric subunit of P2X receptors), the authors constructed homo-dimers and heavy chain IgG Fc scaffolds with 2-100 fold increased potency and efficacy, and extended plasma half-life versions fused to an anti-albumin nanobody; systemic administration of a dimer-albumin construct reduced inflammation in two mouse models of human auto-immune disease. Similarly, the best anti-human $P2X_7$ nanobody blocked endotoxin-evoked cytokine release from human monocytes with sub-nM potency superior to clinical small molecule $P2X_7$ inhibitors. Later work by a PhD student in the Hamburg lab sequence-optimized these $mP2X_7$ nanobodies to improve solubility, and albumin-bound dimers could achieve high target occupancy and long exposures in the periphery and CNS after i.v or i.c.v administration.[43]

Finally, the Australian company AdAlta is worth a mention as they have a novel 'i-body' platform that fuses the asymmetric loops of shark single domain antibodies onto human proteins to produce humanized nanobodies for phage display screening, including a discovery stage TRPV4 ligand being developed for fibrosis.[6,44]

Cardiac and muscle K_v and Na_v ion channel antibodies

Peripheral ion channels are obvious choices for antibody modulators as they can be accessed through local or systemic delivery of purified biological ligands or gene expression vectors, and this section will include examples targeting cardiac $K_v7.1$, $Na_v1.5$ and Kir3/4 channels.

Let's start with a biotech story from BioMarin that I was not aware of until Q2 this year, and which seems to have had a brief and stealthy existence. The company publicly revealed the anti-$K_v7.1$ monoclonal antibody program in Q3 2023,[45] and axed it within 9 months for unknown reasons despite plans to start clinical trials in 2025.[46] BMN 355 was designed to treat cardiac LQT2 and LQT3 patients with mutations in hERG and $Na_v1.5$ channels by increasing the activity of repolarizing $K_v7.1$ currents, thereby promoting action potential repolarization and compensating for the long QTc duration and arrhythmia risk in rare disease patients. There are some $K_v7.1$ small molecule activators, but repurposing of Retigabine and other non-selective $K_v7.x$ openers is not attractive given their peripheral and CNS side-effects. Data

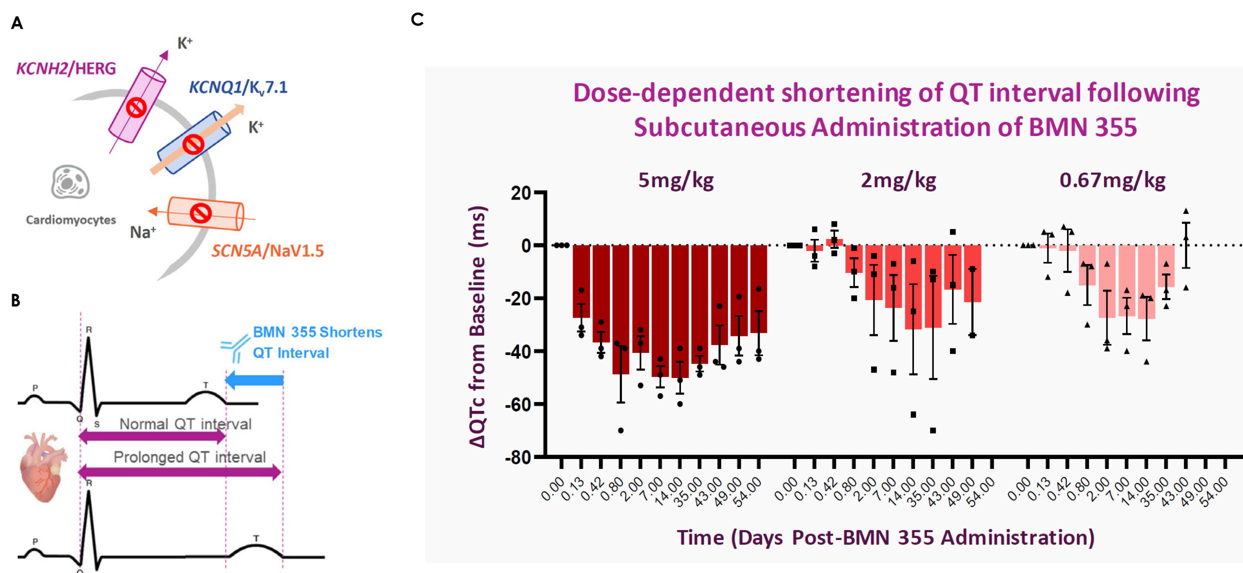

Figure 4 BioMarin $K_v7.1$ activating mAb BMN 355 shows preclinical efficacy to correct LQT cardiac channelopathies. A/B *schematic of disease mechanism, where $K_v7.1$ enhancement could compensate for LQT mutations in hERG or $Na_v1.5$ channels.* **C** *Subcutaneous application of BMN 355 dose-dependently shortens rabbit cardiac QTc duration. Images taken from public online document.*[45] *Figure used under Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).*

in a corporate website presentation show the mAb could 'correct' LQT in an *in vitro* human iPSC cardiomyocyte model, and a single sub-cutaneous delivery of 1-5 mpk of BMN 355 shortened QTc duration in a rabbit model of LQT for > 30 days (Figure 4). IND-enabling studies had completed and GMP optimization had begun, but it is unclear why the program was dropped; BMN 355 was axed along with several other programs during an asset review by new management, a result not uncommon in the drug discovery business and not limited to ion channels or mAbs.

At least two polyclonal antibodies that functionally inhibit cardiac Na_v1.5 channels have been described over the years. The Beech lab in the UK published preliminary data on a rabbit polyclonal Na_v1.5 antibody generated against the DI E3 turret S5-S6 pore loop, which produced slow and similarly incomplete 60% block of Na_v1.5 currents in HEK cells, with no effect on hNa_v1.4 channels.[18] Details on another polyclonal antibody against the neonatal splice variant of Na_v1.5 can be found in Section 4 below.

We conclude with a recent example of a cardiac chimeric antibody-toxin 'peptibody' where a human IgG1 Fc fragment is linked to tertiapin-Q, a synthetic analogue of a bee venom peptide that selectively blocks cardiac IK_{ACh} channels (encoded by KCNJ3/GIRK1/Kir3.1 and KCNJ5/GIRK4/Kir3.4 genes).[47] These potassium channels are an emerging drug discovery target for atrial fibrillation, and the designer protein was found to be 300 fold more potent than the toxin alone, completely inhibiting carbachol-activated IK_{ACh} currents in heterologous HEK cells (IC_{50} of 25 pM) and native mouse atrial, but not ventricular, cardiomyocytes. The peptibody was also active *in vivo* after i.v. administration in mice, reducing acute carbachol-induced bradycardia for up to 48 hours after a single injection, and reducing chronic age-related inducibility of atrial fibrillation. This result demonstrates some of the proposed benefits of antibody scaffolds in producing potent ion channel engagement with improved *in vivo* exposure (plasma t½ of 13 hours) and stability compared to isolated peptides or toxins. Also, the Fc-toxin peptibody could be efficiently expressed and secreted from HEK cells, easing commercial scale-up and reducing the costs of production.

Neuronal ion channel antibodies

Another obvious application of antibodies is targeting peripheral neuronal ion channels, with most programs focusing on pain therapeutics.

Na_v1.7 and Na_v1.8 channels

We have to start with Na_v1.7 as it is a popular analgesia target based on human genetics and mouse knockout models, but it did not begin well as a high profile publication from researchers at Duke University who used a DII S3-S4 VSD epitope to create a functional Na_v1.7 antibody SVmab1 with *in vivo* efficacy was later retracted due to reproducibility and data integrity issues.[48] During this period a number of biotech and pharma companies had tried and failed to replicate the Duke findings, and their lack of success triggered a paper in F1000 from Amgen and reviewed by researchers at Genentech and Janssen R&D outlining their concerns.[49] Amgen and Regeneron also filed patents on knockout and humanized Na_v1.7 mice that were used to develop Na_v1.7 monoclonal antibodies.[50,51] A number of other specialist antibody companies using various immunization and library panning techniques and have listed Na_v1.7 as a target in their pipelines and press releases and patents over the last decade (e.g. Abilita Therapeutics,[52] AbCellera, ArGEN-X,[53] Integral Molecular, Kymab,[54] MedImmune (AZ),[55] Novassay, Numab, Oblique Therapeutics, Tetragenetics,[56] Visterra/Otsuka,[57] X-Body Biosciences, Yumab) but their progress towards the clinic has been slow. While some of the 1st wave delivered functional mAb inhibitors of Na_v1.7 channels, they may have struggled when compared to the potency and claimed selectivity of many small molecule Na_v1.7 programs that held great promise at the time. This situation has switched more recently as many small molecule Na_v1.7 projects stalled due to side-effects and poor efficacy, putting wind back into the sails of a 2nd wave of Na_v1.7 antibody projects.

Shionogi represent an interesting case study in this regard, having worked on small molecule Na_v1.7 inhibitors in the 2010s,[58] but the company switched to monoclonal antibodies to achieve superior potency and selectivity and published some details in 2018 and 2024 conference presentations.[59,60] Using a similar approach to Duke, immunizing mice with a Na_v1.7 channel protein epitope and purifying mAbs from B-cell hybridomas yielded several mAbs with high affinity binding to Na_v1.7 channels on cell membranes and rat DRG tissue. Selected mAbs could functionally inhibit Na_v1.7 channels in these cells, and most significant was evidence of *in vivo* analgesia efficacy after intra-plantar and intravenous injection in a mouse model of neuropathic pain, with an expected PK-PD relationship and enhanced and prolonged analgesia after repeated administration. It looks like later work was directed at humanizing these Na_v1.7 mAbs, which now exhibit 1 nM binding affinity for Na_v1.7 protein and 1000 fold selectivity over Na_v1.x isoforms, and functionally inhibit Na_v1.7 currents in HEK cells and reduce action potential firing in rodent DRG neurons. At least one of these humanized Na_v1.7 mAbs can elicit analgesia *in vivo* and inhibit neuronal firing in the dorsal horn spinal cord, all without unwanted side-effects on locomotor activity (although the current focus is now on cardiovascular side-effects of Na_v1.7 modulators, so this data will be crucial in progressing an Na_v1.7 mAb).

Similar to approaches outlined above for K_v1.3, researchers have taken advantage of the rich repertoire of Na_v1.7-targeting animal toxins and tried to improve their efficacy and *in vivo* stability by fusing them into antibody scaffolds.

Amgen invested considerable resources into this approach, first publishing on work to conjugate previously optimized tarantula venom GpTx-1 toxin peptide analogues onto an IgG Fc single domain antibody fragment. Potency and efficacy were optimized through use of selected cysteine anchor points and linkers to the toxin peptide, resulting in candidates with functional inhibition of $Na_v1.7$ currents (IC_{50} 250 nM), a much-extended plasma half-life of 80 hours, and a bonus effect that the antibody-toxin scaffold was differentially biodistributed to nerve fibers in wild-type but not $Na_v1.7$ knockout mice.[61] However, they later revealed that this initial toxin-antibody chimera was inactive in *in vivo* pain models, so a follow-up study used the more potent $Na_v1.7$ tarantula toxin JzTx-V to develop improved analogues, and together with further optimization of linkers and Fc antibody attachment sites produced a chimera with 100-fold greater $Na_v1.7$ channel inhibition than the GpTx-1 analogues.[62] However, this came at the cost of reduced PK and target cell labeling, as well as liver accumulation. Finally, a reduction in the net charge of the JzTx-V-antibody chimera yielded a candidate with similar *in vitro* $Na_v1.7$ current block but 18 fold improvement in plasma exposure, and significantly it exhibited moderate efficacy in an *in vivo* target engagement PD model, but the authors concluded it was still sub-optimal (especially compared to preclinical small molecule $Na_v1.7$ blockers), so it looks like this program was parked.

Many companies working on $Na_v1.7$ antibodies have also listed $Na_v1.8$ as a target, and several have developed potent mAb binders that may also functionally inhibit $Na_v1.8$ currents. There is little public data on these programs, but the $Na_v1.8$ mAb TTG-108 from Tetragenetics (now part of AbCellera) is listed on several drug discovery analysis websites as having undergone preclinical pain studies in *2020*.[63,64] $Na_v1.8$ is a good example of the strengths and weaknesses of ion channel antibody approaches: it has significant sequence diversity compared to other human $Na_v1.x$ isoforms and so has the potential to elicit selective ligands, but this channel protein is also hard to express at sufficient levels for many antibody screening platforms which may have slowed progress and the number of active players. Also, recent progress by Vertex and others to develop and clinically test selective $Na_v1.8$ small molecule inhibitors for acute and chronic pain may either reignite or stifle interest in $Na_v1.8$ as a target for monoclonal and nanobody drug discovery, depending on competitor and market analysis and pipeline considerations.

It is commonly thought that ligand-gated ion channels may be more amenable for antibody drug discovery as these proteins have larger and more complex and diverse extracellular domains to which mAbs can bind and functionally interact. In terms of analgesia targets, TRPV1 and TRPA1 are the obvious choices and several programs for these targets were publicly revealed in the last decade, alongside mAbs against neuronal P2X3 and ASIC channels, while mAbs against Glycine and NMDA receptors have been developed to treat CNS diseases.

TRP channels

Oblique Therapeutics list an active TRPV1 monoclonal antibody program on their website, discovered using their protease microfluidics platform to identify novel binding epitopes.[65] TRPV1 was a popular analgesia target a decade ago but most preclinical and clinical candidates induced adverse changes in thermosensation and body temperature that precluded further development. An interesting hypothesis was developed that ligands that spared certain modalities of TRPV1 channel activation (e.g. pH vs temperature or capsaicin) may avoid such side-effects, and Oblique have discovered a mAb that exhibits stimuli-selective antagonism and is claimed to block TRPV1 activation *in vitro* and in a non-human primate preclinical *in vivo* pain model without thermal side-effects.

Oblique also listed a TRPA1 mAb project on their website in 2021, and a few years earlier X-BODY Biosciences claimed to have identified a TRPA1 mAb using their DNA-tagged human antibody library (before their acquisition by Juno Therapeutics in 2015). More transparent was a publication from Amgen who used cell, DNA and adenovirus-expressed protein to immunize mice and isolate mAbs that bound to mature TRPA1 channel proteins in cells.[66] Selected binder IgG mAbs could functionally inhibit TRPA1-mediated Ca^{2+} influx activated by cold, osmotic challenge and cognate agonists with 100-500 nM potency, with selectivity over TRPM8 and activity against rat TRPA1 channels that allowed for preclinical development. However, inhibition of TRPA1 by these mAbs was less potent than small molecule inhibitors such as AMG9090 (10-100 nM) and in many cases mAb inhibition was incomplete, and this program was not further pursued.

In the same year Amgen published on several commercial rabbit polyclonal antibodies and mAbs generated against the E3 poor loop of TRPM8 that functionally inhibited activity in heterologous cells and rat DRG sensory neurons.[67] Several of the polyclonal and mAbs could completely inhibit icilin-induced TRPM8 Ca^{2+} influx, and the Alomone polyclonal reagent ACC-049 exhibited 0.5 - 1 μM potency against hTRPM8 but higher affinity against rat and mouse isoforms (100 - 300 nM), with some modality selectivity as it was inactive against menthol activation of TRPM8 channels compared to cold and icilin, and completely inactive against cold activation of TRPA1 or heat activation of TRPV1. The Amgen researchers concluded that targeting the E3 loop of TRPx receptors could yield isoform-selective inhibitory ligands without major species differences (building on previous work with K_v and TRPC channels), but the main challenge was inconsistent evidence for full and potent channel inhibition required in a therapeutic candidate.

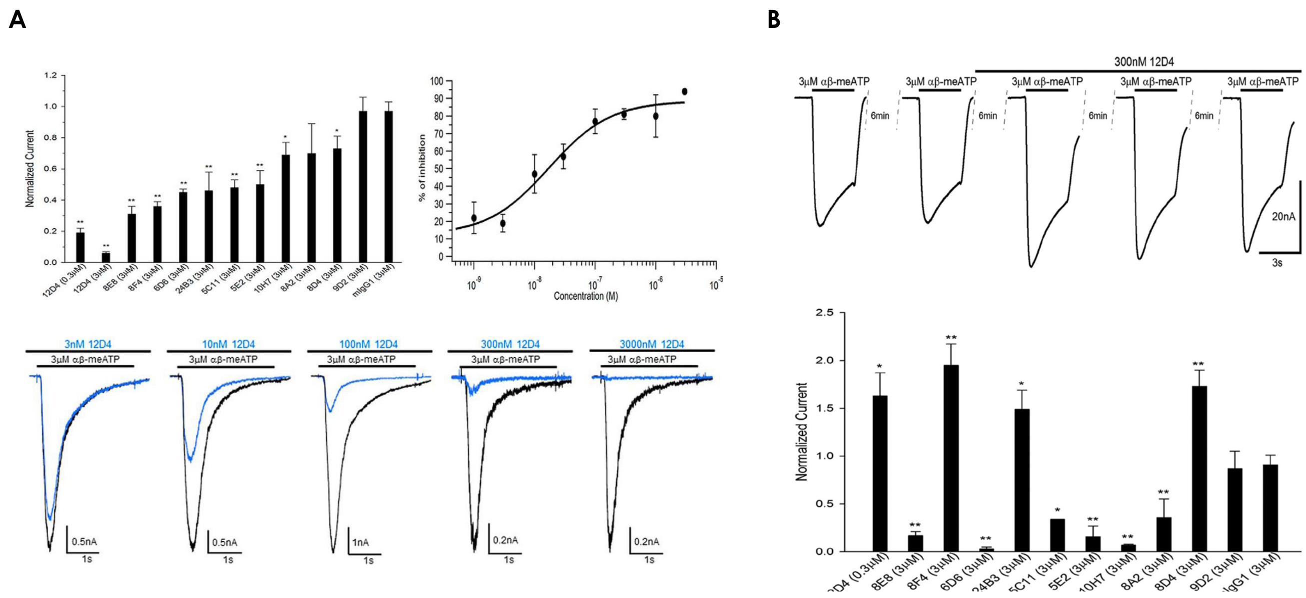

Figure 5 ***Inhibition of hP2X$_3$ (A) and potentiation (B) of hP2X$_{2/3}$ currents by a panel of mAbs developed by Rinat.*** *Adapted from data in [68] and used under Creative Commons open access license 4.0 (http://creativecommons.org/licenses/by/4.0/) from an Open Access publication.*

P2X$_3$ mAbs for pain and cough

P2X$_3$ receptors are also popular targets for antibody programs, primarily for pain indications although there has been a resurgence of interest in P2X$_3$ receptors for chronic cough after several small molecules recently reached the clinic and one received market approval.

Rinat (Pfizer) used purified hP2X$_3$ protein to immunize mice and extract mAbs that specifically bound to astrocytoma cells expressing hP2X$_3$ channels and inhibited ATP-activated Ca^{2+} influx.[68] The exemplar mAb 12D4 exhibited interesting state-dependent modulation of P2X$_3$ currents measured by manual and APC, acutely and potently stabilizing the desensitized state of P2X$_3$ homomers (IC_{50} of 16 nM) whilst potentiating the slowly inactivating current carried by P2X$_{2/3}$ heteromers, an effect seen with 3 of the other 11 mAbs; In contrast, most of the remaining mAbs acutely inhibited P2X$_{2/3}$ currents (Figure 5). Also, overnight treatment produced further inhibition of P2X$_3$ and P2X$_{2/3}$ currents, including some mAbs that were inactive in the acute patch clamp experiments. This indicates that mAbs binding to different epitopes can elicit complex and contrasting effects within and across different P2X isoform complexes to acutely modulate channel activity, and also induce longer-term internalization to chronically reduce P2X channel density and activity. This latter effect may be more therapeutically relevant during prolonged exposures after systemic delivery or genetic transfection. Most of the mAbs were less potent against rodent P2X$_3$ and P2X$_{2/3}$ isoforms (e.g. ~100 nM IC_{50} in rat DRG neurons) but sufficiently efficacious for *in vivo* testing in preclinical models of analgesia: a dose of 30 mpk delivered 3 days before each pain assay significantly reduced visceral pain, but left acute inflammatory thermal hyperalgesia and nociception unaffected.

UK company Ossianix are leveraging the small single domain shark Variable New Antigen Receptor (VNAR) antibodies to develop brain penetrant payloads, but previously revealed a R&D collaboration with Lundbeck in 2012 to develop shark nanobodies to modulate P2X3 channels to treat neuropathic pain (OSX300),[69] which was highlighted in a 2014 news article[70] but disappeared from public view in later years.

Integral Molecular developed a sub-nM P2X3 mAb in collaboration with Crystal Biosciences using their transgenic chicken platform, and this is still listed in their pipeline, for cough and chronic pain.[71]

ASIC1a

Acid-activated ASIC channels initially gained traction as analgesia drug targets, but more recent preclinical work and clinical trials with small molecules and peptide toxins indicate they may also be therapeutically useful to treat cardiac and CNS ischemia.

For example, Chinese academics used a truncated ASIC1 protein in nanodiscs to pan a ScFv phage display library and hits were fused to an IgG Fc domain to create several mAbs, with the selective ASC06 ligand producing potent, complete and sustained inhibition of ASIC1a currents and Ca^{2+} influx (IC_{50} of 85 and 3 nM) and acid-induced cell death *in vitro* (Figure 6), similar to the affinity of a PcTx1 toxin-Fc chimeric scaffold. The ASC06-IgG mAb is selective for ASIC1a channels over ASIC1b, ASIC2a, 2b and ASIC3

isoforms but retains activity against rodent ASIC1a channels which allowed for *in vivo* testing where it reduced cerebral infarct size in a mouse ischemia model 24 hours after i.c.v administration to a similar degree to 100 nM PcTx1.[72] Around the same time Regeneron revealed their work on ASIC mAbs in conference presentations and patent filings.[4,73] Humanized mice were immunized with ASIC1 channel DNA and 12 of 106 mAb binders were further profiled, with the exemplar H1M6718 showing functional inhibition of acid-evoked currents in heterologous cells but mixed *in vivo* efficacy in models of muscle hyperalgesia and chemotherapy-induced peripheral neuropathy when dosed at 10-40 mpk.

Several groups have combined antibody scaffolds with ASIC1a toxins to produce novel therapeutic ligands. Iontas (now Maxion) publicly revealed proof-of-concept studies for their knotbody approach against ASIC1a,[74] where PcTx1 toxin was fused into mAb CDR3 loops and shown by APC to moderately inhibit pH-gated currents (100 nM IC_{50}). However, their chimeric scaffolds are less potent than the native toxins measured on the same APC platform (e.g. PcTx1 IC_{50} of 1 - 10 nM),[75] but this can be reclaimed by antibody sequence optimization and affinity maturation. In contrast, a camelid nanobody generated against ASIC1a expressed in HEK cells, that binds to a site overlapping with inhibitory Mambalgin and activating MiTx toxins in cryo-EM structures, was fused to PcTx1 and produced more potent inhibition of pH-evoked currents than PcTx1 alone.[76] Intriguingly, these nanobodies did not alter any biophysical or functional parameters of ASIC1a currents when applied alone, despite high affinity binding near the thumb region of the trimeric receptor, suggesting that the nanobody-toxin chimera altered binding kinetics rather than exerting an allosteric effect.

CNS ligand-gated receptors involved in synaptic transmission and plasticity

A much older story I wasn't aware of involves a mAb (B6 B21) generated against native NMDA receptors.[77,78] US researchers immunized mice with rat dentate gyrus neurons and discovered a mAb that bound to the glycine co-agonist site and increased TCP binding and promoted NMDA receptor currents. Significantly, the mAb enhanced long-term potentiation in brain slices *ex vivo* and hippocampal-dependent learning in rabbits *in vivo*. Likely due to the difficulty in getting mAbs into the brain, a collaboration with Naurex Inc subsequently developed a tetrapeptide GLYX-13 (Rapastinel) from hypervariable regions of the mAb light chain which crossed the BBB to act as a cognition enhancer and promoted MK-801 binding and NMDA receptor-dependent synaptic plasticity, and also exerted rapid and long-lasting anti-depressant effects in behavioral models.[79] This promising profile enabled further preclinical development and Ph I safety and Ph II efficacy clinical trials in which showed promising rapid anti-depressant action after a single i.v. dose.[80]

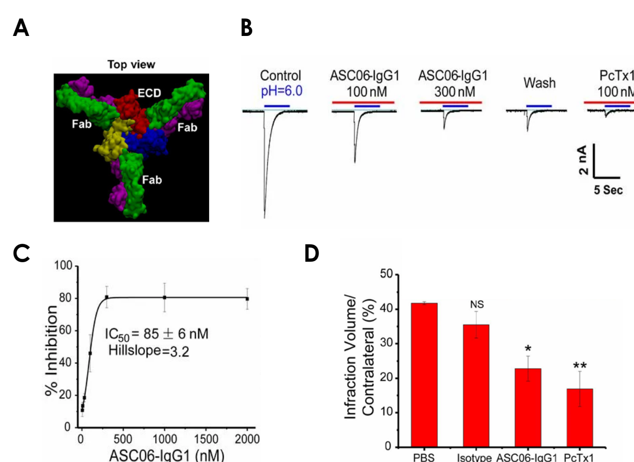

Figure 6 ***Trimeric ASIC1a mAb potently inhibits channel activity and reduces cerebral ischemia.*** **A** *Molecular dynamics simulation of the complex from top views of the ion channel.* **B** *Representative current traces from a single hASIC1a stable CHO-K1 cell with 100 or 300 nM ASC06-IgG1, PcTx1 as positive control, and recovery ("Wash") after 300 nM ASC06-IgG1 treatment.* **C** *Dose-dependent inhibition of acid-induced hASIC1a currents by ASC06-IgG1.* **D** *TTC-stained brain sections show infarct areas and volumes in PBS, isotype control, ASC06-IgG1, and PcTx1-treated mice, with significant reductions. Adapted from data in [72] and used under Creative Commons open access license 4.0 (http://creativecommons. org/licenses/by/4.0/) from an Open Access publication.*

Amgen adopted a dual strategy to develop selective small molecule and mAb positive allosteric modulators (PAMs) of glycine receptor (GlyR) α1 and α3 subunits as analgesia therapeutics, with the aim to increase inhibitory neurotransmission in the spinal cord and CNS. The mAbs were generated by immunizing mice with micelles containing human GlyR α3 protein, and filtering out hits recognizing linear peptide epitopes yielded 6 mAbs with potent activating or inhibitory effects (xC_{50} 5 - 20 nM) against GlyR responses.[81] The 3 activating mAbs elicited time-dependent increases in membrane depolarization in a FLIPR imaging assay equivalent to maximal glycine activation, and also acted as PAMs in the presence of glycine, and the 3 inhibitory mAbs blocked glycine responses by ~50%. Significantly, two of the PAMs were selective for α3 over α1 GlyR subunits, but none of the inhibitory mAbs were subtype-selective. Binding studies show that Fab fragments are 3 - 5 less potent than their corresponding mAbs but retain broadly similar selectivity. There is also some state-dependence, as PAMs favor binding to the activated GlyR and selectivity results from slower off-rates from the α3 subunit, while inhibitory Fabs greatly favor binding to the resting state of GlyRs. As the mAbs also recognize rat GlyRs *in vivo* testing of the PAMs was possible; CNS exposure was low (~0.5% of plasma) but sufficient to achieve 1-3 fold xC_{50} coverage after intravenous dosing of 10-100mpk, and the α3-selective mAb 9A11 did not

produce the side-effects seen with the non-selective mAb 19C8. These results were similar to Amgen's small molecule PAM program, and despite the complex and useful state-dependent modulatory profile of these mAbs both programs were classified as proof-of-concept and requiring further optimization of potency and selectivity to allow for preclinical PK-PD and safety testing that may lead to CNS drugs with improved therapeutic index.

Nanobodies for neuronal analgesia ion channel targets

Despite their promise of potency, selectivity and promising PK, ion channel mAbs have continued to struggle to convert binding affinity into potent and meaningful functional modulation, with significant shifts in potency and partial inhibition seen in cell-based assays. In contrast, there is growing interest in single domain nanobodies as ion channel modulators, as their small size and modularity may yield more efficacious and optimizable ligands that can bind to smaller, cryptic sites not accessible to larger mAbs. The potential of this approach was originally demonstrated by K_v1.3 nanobodies from Ablynx (see Section 1 above), and a recent publication from the National Research Council in Canada illustrates a new modality in a Na_v1.7 nanobody program they have been working on for many years.[82] The long E3 pore loop in DI of Na_v1.7 was spliced into the CDR3 loop of a single VHH chain nanobody and expressed in CHO cells to maintain proper folding and glycosylation of the epitope, and this antigen was panned against a large phage display library of naïve llama, alpaca and camel nanobodies, resulting in 6 positive clones. None of the 5 tested nanobodies had any effect on hNa_v1.7 current amplitude or voltage-dependent biophysics recorded in HEK cells on the SP384 APC platform (at 2 µM), but one (DI-D) was found to slow deactivation kinetics (Figure 7). In silico Markov state modeling and Na_v1.7 current simulation attributed this effect to a slowing in recovery from the slow inactivated to the closed state, trapping Na_v1.7 channels in a pool resistant to re-opening. Testing in rat DRG neurons showed this effect translated into reduced sensory neuron excitability through changes in action potential threshold and amplitude and repetitive firing. *In vivo* testing showed significant analgesia of the DI-D nanobody after intraplantar and intrathecal administration (0.6 – 6 nM) in various acute and chronic pain models, which matched or exceeded the efficacy of Na_v1.7 small molecule inhibitors PF-05089771 or TC-N 1752. Whole body and ex vivo organ fluorescence imaging showed that DI-D was peripherally restricted and accumulated in the spinal cord after 24 hours, and exhibited a half-life of 3.3 hours after i.v. administration (4 mpk) with minimal CNS exposure or cardiac accumulation (that might lead to on-target side effects seen with Na_v1.7 small molecules). The nanobody showed similar binding affinity to human, rat and mouse Na_v1.7 DI E3 loop constructs, and unlike many mAbs the KD values were low but similar to patch clamp potency (~1.5 µM).

Finally, the Na_v1.x gene family selectivity of DI-D was

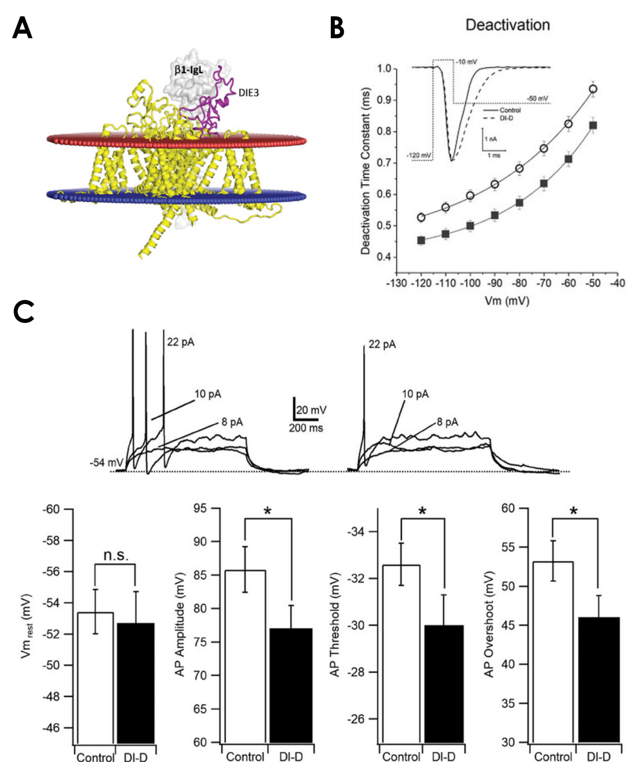

Figure 7 *Na_v1.7 DI-D nanobody binds to DI E3 pore loop adjacent to central pore and β1 subunit interaction region, and slows current deactivation and reduces DRG neuron excitability.* **A** *3D-location of DIE3 loop (purple) relative to cell membrane (red/blue planes) shown on the cryo-EM structure (PDB ID 5XSY) of the homologous electric eel Na_v1.4 channel alpha subunit (yellow) complexed with β1 subunit (gray surface)* **B** *Voltage dependence of the deactivation currents decay. The inset displays an example of deactivation tail currents with superimposed voltage-clamp protocol.* **C** *Effect of VHH DI-D on the ability of DRG neurons to elicit action potentials. Adapted from data in* [82] *and used under Creative Commons open access license 4.0 (http://creativecommons.org/licenses/by/4.0/) from an Open Access.*

predicted by sequence homology analysis of the DI E3 loop and confirmed by functional testing on the SyncroPatch 384, with Na_v1.2, Na_v1.1 and Na_v1.3 channels exhibiting highest homology but lacking key residues in the binding epitope and failing to show peak current inhibition or any kinetic changes in HEK cells. The authors conclude that the DI-D nanobody binds to a region of the E3 loop that is adjacent to the Na_v1.7 central pore and partially overlaps with the β1 subunit binding interface, so it will be interesting to discover if one or more nanobodies are required or capable of binding to each Na_v1.7 channel protein, and if additional antibody or nanobody binding sites remain accessible for more complex functional modulation.

K_{2P} channels are another peripheral analgesia target, implicated in chronic pain and migraine given their role in controlling the excitability of DRG and trigeminal neurons. Some groups have tried to develop selective small molecule

modulators (e.g. activators for pain) for members of this diverse ion channel family (e.g. LifeArc,[83] Pfizer,[84] UCSF,[85] Johns Hopkins[86]), but achieving selectivity and potency has been a challenge. In 2024 Stephen Tucker's group from Oxford and their collaborators finally published on work to identify nanobodies against TREK-2 channels which demonstrated promising potency, specificity and modularity.[87] Llamas were immunized with purified TREK-2 protein resulting in 29 binders of which 14 were tight binders and subjected to functional testing (500 nM) against TREK-2 expressed in Xenopus oocytes, revealing 1 inhibitory (Nb61), 2 activating nanobodies (Nb67 and Nb76) and one inactive binder (Nb58). Illustrating how far structure-based ion channel drug discovery has come in recent years, high resolution X-ray crystallography images were obtained of these nanobodies bound to TREK-2 protein, with the inactive binder interacting with the extracellular 'cap' domain that sits above the pore, while the active nanobodies exhibited more extensive interactions with the cap as well as extracellular loops close to the selectivity filter where the so-called 'primary gate' of K_{2P} channels is located, and the activator Nb67 also binding to residues on the intracellular face of the cap.

- Nb61 rapidly and completely inhibits TREK-2 channels with 600 nM potency but no effect on related K_{2P} isoforms, with the long CDR3 loop interacting with the M3-PH2 and P2-M4 loops. Significantly, a lysine residue appears to occlude the ion permeation pathway beneath the cap and its mutation reduces block (Figure 8D)
- Nb67 activator acts rapidly to increase current 3-4 fold with 100 nM potency and partial selectivity as it has no effect on TRAAK or TALK-2 but moderately inhibits (> 1 μM) the closely related TREK-1 channel (Figure 8C).
- Nb76 is a potent and selective activator of TREK-2 channels (400 nM) with no effect on any other K_{2P} family members, attributed to binding of nanobody CDR3 loops with TREK-2-specific residues in the inter-subunit interface and extracellular P2-M4 helix loop, with the later domain also the site of ML335 binding and activation in the 'K_{2P} Modulator Pocket'.

K_{2P} channels are tetramers and up to 4 nanobodies could bind extracellularly, although there appears to be some asymmetry in these interactions which may contribute to their functional effects. Multimeric binding makes it possible to mix-and-match different nanobodies to create more complex multivalent modulation, and this was demonstrated by linking the inactive but selective TREK-2 cap binder Nb58 with the Nb61 pore loop inhibitor, yielding a biparatropic nanobody with 250 fold higher binding affinity (250 nM) and a 16 fold increase in functional inhibition of TREK-2 currents (IC_{50} of 40 nM), attributed to dimer binding with more efficient interactions with key pore residues and occlusion of both ion exit pathways. This study is further evidence for the efficacy and utility of nanobodies as ion channel modulators, as they can completely inhibit as well as markedly enhance currents with nM potency and multimeric capabilities, and exhibit improved solubility and easier scale-up and commercial production.

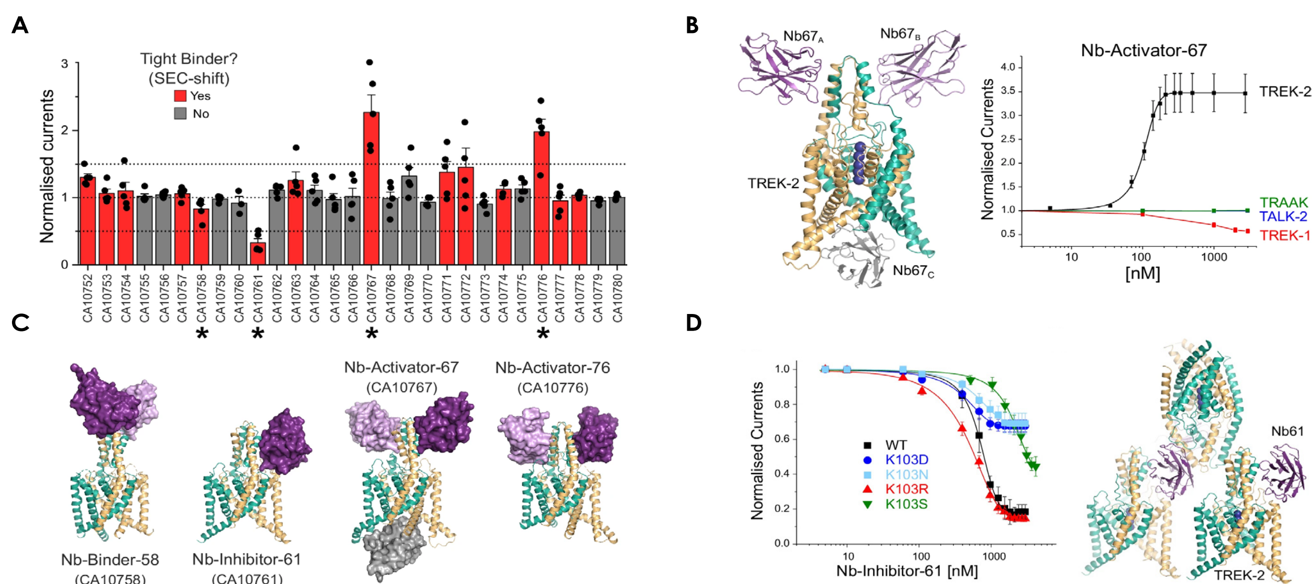

Figure 8 ***Functional activity and binding sites of K_{2P} TREK-2 nanobodies.*** **A** *Functional activity of TREK-2 nanobodies in Xenopus oocytes reveals 2 activators and 1 inhibitor amongst the 'tight binders' (red bars) of initial hits.* **B** *X-ray structures of nanobodies bound to TREK-2 protein.* **C** *Activator Nb67 is a multimeric binder with selective activation of TREK-2 compared to K_{2P} family members.* **D** *Inhibitor Nb61 interacts with the Cap and pore loops, driven by a key lysine residue on the CDR3 loop. Figures from 87 used under CC4.0 license.*

Ion channel antibodies and cancer

Ion channels are over-expressed in many cancer cells (review[88]), with antibodies developed as labeling agents more so than as functional modulators. Ion channel toxins and antibodies are useful ligands to 'light up' specific tumors and facilitate their surgical resection, and inhibition of over-expressed ion channels such as hERG, $Na_v1.5$ and TRP channels may also help to limit tumor growth, although the delivery of such ligands should be localized to avoid systemic side-effects on the heart ($Na_v1.5$, hERG) and other organs. Similarly, ion channel antibodies can be used to deliver drugs (i.e. ADC format) to the tumor site and reduce systemic exposure to these cytotoxic molecules.

A key early paper on this topic described the rational design of mAbs against the EAG1 K_v channel (encoded by the $K_v10.1$ gene), as these channels are over-expressed in many cancers and their activity can regulate cell proliferation and tumorigenesis.[89] Monoclonal antibodies were preferred to small molecule antagonists to achieve selectivity over cardiac hERG channels, with E3 turret pore loop peptide immunization producing mAbs that bound to purified and native EAG channel proteins. A mAb recognizing an EAG1-specific region further away from the pore was functionally inactive, but one that bound within the E3 cleft specifically labeled EAG1 proteins and slowly inhibited EAG currents by ~40% (IC_{50} ~70 nM), but left hERG channels unaffected and did not bind to but not EAG2-expressing cells. Inhibition of EAG1 channels by mAb[56] inhibited human cancer cell line colony formation *in vitro*, and in *in vivo* mouse xenograft models (50 mpk) it could reduce growth of breast and one pancreatic cancer but not ovarian tumors.

Antibodies against hERG channels ($K_v11.1$) have also been used as cancer-targeting payloads, including the potential to selectively bind to the hERG 1b protein that is upregulated in many cancers.[6] Whilst these antibodies may not functionally inhibit hERG currents they can be used to direct gold nanoparticles or oncology drugs to tumors, although this still carries risk of accumulating toxic ligands in the heart.

TRPV6 expression is also dysregulated in many cancers, so French researchers developed a novel strategy to create activating polyclonal antibodies by immunizing rabbits with epitopes against the S1-S2 extracellular loop and E3 turret S5-S6 pore region. These enhanced store-operated Ca^{2+} influx by 20-50% and induced cell death in human prostate cancer cell lines.[90] Interestingly, the antibodies produced biphasic effects, initially potentiating and then significantly inhibiting TRPV6 currents under manual patch clamp. It is not clear what the anti-tumor mechanism is (or if it works *in vivo*), but this case study illustrates the utility of antibodies to either potentiate or inhibit ion channel activity and elicit disease-relevant functional effects, which in the case of TRPV6 may have greater selectivity than small molecule modulators (review[91])

$Na_v1.5$ channels are also implicated in cancer cell proliferation and metastases, with a novel genetic twist as a neonatal splice variant (in DI VSD S3-S4 loop) is thought to be selectively expressed. Efforts to identify splice-variant specific small molecule antagonists were largely unsuccessful, and two tarantula toxins showed reverse selectivity for adult over neonatal variant due to the small residue differences in the VSD where they bind.[92] However, the polyclonal antibody NESOpAb previously generated against DI VSD S3-S4 loop exhibited 10 fold selectivity for neonatal over adult $Na_v1.5$ variants, rapidly inhibiting neonatal $Na_v1.5$ currents by ~70% to yield a use-dependent IC_{50} of 170 nM, and exhibiting components of closed and inactivated state-dependence, with no change in $Na_v1.5$ current kinetics and slow wash-out. Intriguingly, the polyclonal antibody could reduce $Na_v1.5$ currents in human breast cancer but not rat prostate cancer cell lines, perhaps as $Na_v1.7$ channels are upregulated in the latter tumor model.

Several mAbs have also been developed to modulate ion channels involved in cancer. Johns Hopkins researchers in the US characterized two sets of mAbs raised in mice against the M1-P1 transmembrane helix pore loop of KCNK9 TASK-3 proteins which were selective over other TASK and K_{2P} family members, unlike current small molecules.[93] Twelve of the 40 mAbs bound to HEK cells expressing TASK-3 (nM potency) and 6 of them could reduce thallium flux into these cells, but the effects were chronic and required 6-36 hour preincubation with no acute effect seen in patch clamp recordings over 20 min, likely due to mAb-induced internalization that reduced TASK-3 function. Significantly, the most potent TASK-3 mAb (Y4) reduced cell viability and increased apoptosis in various human and mouse cancer cell lines *in vitro*, and suppressed growth and metastasis *in vivo* by promoting immune cell-mediated cytotoxicity in human lung xenografts after systemic administration (4 mpk i.p.) in mice.

The Swiss company Novassay is developing mAbs to Ca_v channel $\alpha2\beta1$ accessory subunits as therapeutics to treat cancer, as well as pain and CNS diseases;[94] their most advanced candidates NVA-2001-10 and NVA-2301-10 are at the preclinical stage.[95] These mAbs are thought to work by reducing abnormal expression of functional high-voltage-activated $Ca_v1.x$ and $Ca_v2.2x$ channels in solid tumors, and downregulating the over-expressed auxiliary subunits in peripheral neurons.

Finally we can wrap-up with a program from the UK company BioSceptre who took a $P2X_7$ antibody all the way through preclinical studies in oncology to a Ph I clinical trial in 2017.[96] A significant therapeutic window was available as a neonatal non-functional variant of the human $P2X_7$ receptor ($nfP2X_7$) is present in human tumors but not healthy tissue and linked to cancer cell proliferation and

invasiveness. A polyclonal antibody (BIL010t) was developed against the unique E200 epitope by immunizing sheep and shown to reduce melanoma progression in a preclinical mouse model. This agent was the first ion channel antibody to enter clinical trials (in 2014); a topical 10% antibody ointment was tested over 28 days in an open label trial of basal carcinoma patients in UK, Australia and the US which showed significant reduction in lesion size in 65% of patients and stable disease or partial regression in a third of patients and 15% with a complete response. The company was further developing BIL010t as well as a human single domain antibody and an ADC for diagnostic and therapeutic use in leukemia and solid tumors in 2014,[97] but as with many of the commercial antibody programs outlined above, the final status and clinical outcomes and decision making remain a little opaque.

References

1. Sun, H., Li, M. (2013). Acta Pharmacol. Sin. 34, 199–204. doi: 10.1038/aps.2012.202
2. Wilkinson TCI, Gardener MJ, Williams WA. (2015). J Biomolecular Screening 20(4):454-467. doi: 10.1177/1087057114560698
3. Wulff, H., Christophersen, P., Colussi, P. et al. (2019). Nat Rev Drug Discov. 18, 339–357. doi: 10.1038/s41573-019-0013-8
4. Hutchings, C. J., Colussi, P., & Clark, T. G. (2018). mAbs, 11(2), 265–296. doi: 10.1080/19420862.2018.1548232
5. Haustrate A, Hantute-Ghesquier A, Prevarskaya N, Lehen'kyi V. (2019). Frontiers in Pharmacology 10. doi: 10.3389/fphar.2019.00606
6. Hutchings CJ (2020). Antibody Therapeutics 3 (4) 257–264. doi: 10.1093/abt/tbaa023
7. Bednenko, J., Colussi, P., Hussain, S., Zhang, Y., Clark, T. (2021). In: Pharmacology of Potassium Channels. Handbook of Experimental Pharmacology 267. Springer. doi: 10.1007/164_2021_464
8. Zhou BY, Ma W, Huang XY. (1998). J. Gen. Physiol. 111(4) 555-63. doi: 10.1085/jgp.111.4.555
9. Yang XF, Yang Y, Lian YT, Wang ZH, Li XW, Cheng LX, Liu JP, Wang YF, Gao X, Liao YH, Wang M, Zeng QT, Liu K. (2012). PLoS One 7(4):e36379. doi: 10.1371/journal.pone.0036379
10. Schwartz, A., Palti, Y. & Meiri, H. (1990). J Membrain Biol 116, 117–128. doi: 10.1007/BF01868670
11. Wyatt CN, Campbell V, Brodbeck J, Brice NL, Page KM, Berrow NS, Brickley K, Terracciano CM, Naqvi RU, MacLeod KT, Dolphin AC. (1997). J Physiol. 502 (2):307-19. doi: 10.1111/j.1469-7793.1997.307bk.x.
12. Morton ME, Caffrey JM, Brown AM, Froehner SC. (1988). J Biol Chem. 263(2):613-616. doi: 10.1016/S0021-9258(19)35395-5
13. Meiri H, Goren E, Bergmann H, Zeitoun I, Rosenthal Y, Palti Y. (1986). Proc Natl Acad Sci USA. 83(21):8385-9. doi: 10.1073/pnas.83.21.8385
14. Barhanin J, Meiri H, Romey G, Pauron D, Lazdunski M. (1985). Proc Natl Acad Sci USA. 82(6):1842-6. doi: 10.1073/pnas.82.6.1842
15. Liao, Y. J., Safa, P., Chen, Y. R., Sobel, R. A., Boyden, E. S., & Tsien, R. W. (2008). Proc Natl Acad Sci U S A 105(7), 2705-2710. doi: 10.1073/pnas.0710771105
16. Barry EL, Viglione MP, Kim YI, Froehner SC. (1995). J Neurosci. 15(1):274-83. doi: 10.1523/JNEUROSCI.15-01-00274.1995
17. Xu SZ, Beech DJ. (2001). Circ Res. 88(1):84-7. doi: 10.1161/01.res.88.1.84
18. Xu, SZ., Zeng, F., Lei, M. et al. (2005). Nat Biotechnol 23, 1289–1293. doi: 10.1038/nbt1148
19. Naylor J, Milligan CJ, Zeng F, Jones C, Beech DJ. (2008). Br J Pharmacol. 155(4):567-73. doi: 10.1038/bjp.2008.283
20. Klionsky L, Tamir R, Holzinger B, Bi X, Talvenheimo J, Kim H, Martin F, Louis JC, Treanor JJ, Gavva NR. (2006). J Pharmacol Exp Ther. 319(1):192-8. doi: 10.1124/jpet.106.108092
21. Stortelers C, Pinto-Espinoza C, Van Hoorick D, Koch-Nolte F. (2018). Curr Opin Immunol. 52:18-26. doi: 10.1016/j.coi.2018.02.003
22. Cox, J. H., Hussell, S., Søndergaard, H., Roepstorff, K., Bui, J. V., Deer, J. R., ... & Odegard, V. H. (2013). PLoS One, 8(12), e82944. doi: 10.1371/journal.pone.0082944
23. Lin FF, Elliott R, Colombero A, Gaida K, Kelley L, Moksa A, Ho SY, Bykova E, Wong M, Rathanaswami P, Hu S, Sullivan JK, Nguyen HQ, McBride HJ. (2013). J Pharmacol Exp Ther. 345(2):225-38. doi: 10.1124/jpet.112.202788
24. Aki A, Tanaka K, Nagaoka N, Kimura T, Baba D, Onodera Y, Wada T, Maeda H, Nakanishi T, Agatsuma T, Komai T. (2020). FASEB Bioadvances. 2(8):478-488. doi: 10.1096/fba.2020-00008.
25. Oh-Hora M., Aki A., Hiroaki Maeda, H., and Morimoto, K. (2020). EBMT 46th Annual Meeting, abstract A319. https://www.professionalabstracts.com/ebmt2020/Iplanner/#/presentation/5301
26. Daiichi Sankyo Ph I study of DS-2741a (2021). https://clinicaltrials.gov/study/NCT04211415
27. Bednenko, J., Harriman, R., Mariën, L., Nguyen, H. M., Agrawal, A., Papoyan, A., … Colussi, P. (2018). mAbs 10(4), 636–650. doi: 10.1080/19420862.2018.1445451
28. Wang, R.E., Wang, Y., Zhang, Y., Gabrelow, C., Zhang, Y., Chi, V., Fu, Q., Luo, X., Wang, D., Joseph, S. and Johnson, K. (2016). Proc Natl Acad Sci 113(41), pp.11501-11506. doi: 10.1073/pnas.1612803113
29. Press release: Sevion Therapeutics Presents Cow Antibody Platform at PEGS 2015 (SVN-001). https://www.businesswire.com/news/home/20150504006351/en/Sevion-Therapeutics-Presents-Cow-Antibody-Platform-at-PEGS-2015. (accessed November 2024).
30. Edwards W, Fung-Leung WP, Huang C, Chi E, Wu N, Liu Y, Maher MP, Bonesteel R, Connor J, Fellows R, Garcia E, Lee J, Lu L, Ngo K, Scott B, Zhou H, Swanson RV, Wickenden AD. (2014). J Biol Chem. 289(33):22704-22714. doi: 10.1074/jbc.M114.568642
31. Selvakumar, P., Fernández-Mariño, A.I., Khanra, N. et al. (2022). Nat Commun 13, 3854. doi: 10.1038/s41467-022-31285-5
32. Humphries, E. (2024). Talk at Biophysical Society 'Drug Discovery for Ion Channels XXIV' satellite meeting, Philadelphia USA. https://www.biophysics.org/2024meeting/program/satellite-meetings/drug-discovery-for-ion-channels-xxiv
33. Chiang, E., Li, T., Jeet, S. et al. (2016). Nat Commun 8, 14644. doi: 10.1038/ncomms14644

34. Shim, H., Nguyen, H., Yanjun Cui, Y., Colussi, P., Bednenko, J., and Wulff, H. (2020). FASEB J 34 (S1). doi: 10.1096/fasebj.2020.34.s1.03756

35. Kelley, N., Jeltema, D., Duan, Y., and He, Y. (2019). Int. J. Mol. Sci. 20, 3328. doi: 10.3390/ijms20133328

36. Buell, G., Chessell, I. P., Michel, A. D., Collo, G., Salazzo, M., Herren, S., ... & Humphrey, P. P. A. (1998). Blood, J American Soc Hematol 92(10), 3521-3528. doi: 10.1182/blood.V92.10.3521

37. Elhage A, Cuthbertson P, Sligar C, Watson D, Sluyter R. (2023). Pharmaceutics 15(9):2263. doi: 10.3390/pharmaceutics15092263

38. Kurashima, Y., Amiya, T., Nochi, T. et al. (2012). Nat Commun 3, 1034. doi: 10.1038/ncomms2023

39. Integral Molecular drug discovery pipeline: https://www.integralmolecular.com/therapeutic-pipeline/ (accessed November 2024).

40. Integral Molecular P2X7 mAb case study: https://www.integralmolecular.com/therapeutic-pipeline/ (accessed November 2024).

41. Stähler T, Danquah W, Demeules M, Gondé H, Hardet R, Haag F, Adriouch S, Koch-Nolte F, Menzel S. (2022). Methods Mol Biol. 2510:99-127. doi: 10.1007/978-1-0716-2384-8_6

42. Danquah W, Meyer-Schwesinger C, Rissiek B, Pinto C, Serracant-Prat A, Amadi M, Iacenda D, Knop JH, Hammel A, Bergmann P, Schwarz N, Assunção J, Rotthier W, Haag F, Tolosa E, Bannas P, Boué-Grabot E, Magnus T, Laeremans T, Stortelers C, Koch-Nolte F. (2016). Sci Transl Med. 8(366):366ra162. doi: 10.1126/scitranslmed.aaf8463. PMID: 27881823

43. Pinto-Espinoza, C. (2018). PhD dissertation, Staats-und Universitätsbibliothek Hamburg Carl von Ossietzky. https://ediss.sub.uni-hamburg.de/handle/ediss/8207

44. Foley, M. (2024). Talk at Discovery on Target 'Antibodies Against Membrane Protein Targets', Boston USA. https://www.discoveryontarget.com/Membrane-Characterization/16 (accessed November 2024).

45. BioMarin press release on anti-Kv7.1 monoclonal antibody program (2023). https://www.fiercebiotech.com/biotech/biomarin-rd-head-talks-2-new-pipeline-additions-strategy-sustain-best-pipeline-company

46. BioMarin press release on axing of anti-Kv7.1 monoclonal antibody program (2024). https://www.fiercebiotech.com/biotech/under-new-leadership-biomarin-axes-4-candidates-and-centers-3-assets?

47. Chidipi, B., Chang, M., Cui, M., Abou-Assali, O., Reiser, M., Pshenychnyi, S., ... & Noujaim, S. F. (2022). Proc. Natl. Acad. Sci. U.S.A. 119 (50) e2212564119. doi: 10.1073/pnas.2212564119

48. Lee JH, Park CK, Chen G, Han Q, Xie RG, Liu T, Ji RR, Lee SY. (2014). Cell 157(6):1393-1404. doi: 10.1016/j.cell.2014.03.064

49. Liu D, Tseng M, Epstein LF et al. (2016). F1000Research 5:2764 [version 1; peer review: 3 approved]. doi: 10.12688/f1000research.9918.1

50. Gingras, J., McDonough, S.I. (2012). Nav1.7 knockout mice and uses thereof. US patent US2012185956 (A1).

51. MacDonald, L., Murphy, A.J., LaCroix-Fralish, M.L. Alessandri-Haber, N.M. (2014). Mice expressing human voltage-gated sodium channels. US patent US8871996 (B2).

52. Abilita Therapeutics awarded NIH SBIR grant to develop antibodies against pain-related Nav channels. https://www.inknowvation.com/sbir/awards/nih-2018-development-highly-selective-therapeutics-targeting-nav-channel-subtypes-non

53. ArGEN-X 2013 press release detailing Nav1.7 mAbs: https://argenx.com/news/2013/argen-x-identifies-potent-antibody-antagonists-against-complex-chronic-pain-target-nav17

54. Clube, J.R. (2014). Patent US8986694 filed by Kymab for humanized Nav1.7 mice to generate mAbs. https://patents.google.com/patent/US8986694B1/en

55. Williams et al., (2015). Patent WO2015166105A2 for ion channel antibodies from MedImmune. https://patents.google.com/patent/WO2015166105A2/enIt.pdf

56. Tetragenetics press release in 2015 revealing antibody collaborations on Nav1.7, Nav1.8 and Kv1.3 channels. https://pipelinereview.com/tetragenetics-launches-partnership-program-for-discovery-and-development-of-therapeutic-monoclonal-antibodies/

57. Robinson, L. (2017). Visterra talk abstract at Discovery on Target conference in Boston, USA. https://www.discoveryontarget.com/17/Membrane-Characterization

58. Sun, S., J Cohen, C., & M Dehnhardt, C. (2014). Pharmaceutical Patent Analyst, 3(5), 509–521. https://doi.org/10.4155/ppa.14.39

59. Yoneda, S., Kasai, E., Takahashi, T., Yoshikawa, M., Onoda, J., Izumi, T., ... & Hasegawa, M. (2018). In Proceedings for Annual Meeting of The Japanese Pharmacological Society (18th World Congress of Basic and Clinical Pharmacology), PO3-2. doi: 10.1254/jpssuppl.WCP2018.0_PO3-2-25

60. Yoneda, S., Uta, D., Yasufuku, K., Yamane, T., Yoshioka, S., Takasu, K., Izumi, T., Fujita, S., Nakamori, D., Kawasaki, S., Takahashi, T., Yoshikawa, M., Ogawa, K and Kasai, E. (2024). IASP 2024 World Congress on Pain, poster TH692. https://posters.worldcongress2024.org/poster/novel-anti-nav1-7-antibodies-with-long-lasting-analgesic-effect-on-neuropathic-pain-without-sedation/

61. Biswas, K., Nixey, T. E., Murray, J. K., Falsey, J. R., Yin, L., Liu, H., ... & Miranda, L. P. (2017). ACS Chem. Biol. 12 (9), 2427–2435. doi: 10.1021/acschembio.7b00542

62. Murray, J. K., Wu, B., Tegley, C. M., Nixey, T. E., Falsey, J. R., Herberich, B., ... & Miranda, L. P. (2019). ACS Chem Biol 14(4), 806-818. doi: 10.1021/acschembio.9b00183

63. Tetragenetics Nav1.8 mAb TTG-108 PatSnap overview: https://synapse.patsnap.com/drug/42e9fb9001ed4aa781067079eda35a25 (Accessed December 2024)

64. Tetragenetics Nav1.8 mAb TTG 108 AdisInsight overview: https://adisinsight.springer.com/drugs/800061510 (Accessed December 2024)

65. Oblique Therapeutics TRPV1 pain case study. https://obliquet.com/programs/atrpv1_intended-for-treatment-of-pain/ (Accessed November 2024)

66. Lee, K. J., Wang, W., Padaki, R., Bi, V., Plewa, C. A., & Gavva, N. R. (2014). J Pharm Expt Therap 350(2), 223-231. doi: 10.1124/jpet.114.215574

67. Miller, S., Rao, S., Wang, W., Liu, H., Wang, J., & Gavva, N. R. (2014). PLoS One 9(9), e107151. doi: 10.1371/journal.pone.0107151

68. Shcherbatko, A., Foletti, D., Poulsen, K., Strop, P., Zhu, G., Hasa-Moreno, A., ... & Shelton, D. (2016). J Biol Chem 291(23), 12254-12270. doi: 10.1074/jbc.M116.722330

69. Ossianix press release on Lundbeck P2X3 collaboration (2012-2014). https://www.ossianix.co.uk/2014/12/16/ossianix-expands-and-extends-its-research-collaboration-with-lundbeck-on-cns-therapeutics-16-december-2014 (Accessed December 2024)

70. Ossianix P2X3 mAb OSX300 highlighted in BioCentury news article (2014). https://cdn.website-editor.net/a3c3cad520224a3f90107eddfe79c79a/files/uploaded/Biocentury-28-Sept-2014-pg-7.pdf (Accessed December 2024)

71. Integral Molecular P2X3 mAb collaboration with Crystal Biosciences listed on company website pipeline. https://www.integralmolecular.com/therapeutic-pipeline/ (Accessed December 2024)

72. Qiang, M., Dong, X., Zha, Z., Zuo, X. K., Song, X. L., Zhao, L., ... & Lerner, R. A. (2018). Proc Natl Acad Sci USA 115(32), E7469-E7477. doi: 10.1073/pnas.1807233115

73. Macdonald, L., Gao, M., Morra, M. R., Alessandri-Haber, N. M., & LaCroix-Fralish, M. L. (2015). U.S. Patent No. 9,150,648

74. Bell, D.C., Karratt-Vellatt, A., Surade, S., Luetkens, T., Masters, E. W., Sørensen, N. M., ... & McCafferty, J. (2018). Biophys J 114 (3) 203a. doi: 10.1016/j.bpj.2017.11.1135

75. Ridley, J., Manyweathers, S., Tang, R., Goetze, T., Becker, N., Rinke-Weiß, I., ... & Rogers, M. (2022). Front Mol Neurosci 15, 982689. doi: 10.3389/fnmol.2022.982689

76. Wu, Y., Chen, Z., Sigworth, F. J., & Canessa, C. M. (2021). Elife 10, e67115. doi: 10.7554/eLife.67115

77. Haring, R., Stanton, P. K., Scheideler, M. A., & Moskal, J. R. (1991). J Neurochem 57(1), 323-332. doi: 10.1111/j.1471-4159.1991.tb02131.x

78. Thompson, L., Moskal, J. & Disterhoft, J. (1992). Nature 359, 638–641. doi: 10.1038/359638a0

79. Moskal, J.R., Burgdorf, J.S., Stanton, J.K., Kroes, P.A., Disterhoft, R.F., Burch, J.M. and Khan, M. A. (2017). Current Neuropharm 15(1), 47-56. doi: 10.2174/1570159X14666160321122703

80. Moskal, J. R., Burch, R., Burgdorf, J. S., Kroes, R. A., Stanton, P. K., Disterhoft, J. F., & Leander, J. D. (2013). Expert Opinion Investigational Drugs 23(2), 243–254. doi: 10.1517/13543784.2014.852536

81. Simard JR, Michelsen K, Wang Y, Yang C, Youngblood B, Grubinska B, Taborn K, Gillie DJ, Cook K, Chung K, Long AM, Hall BE, Shaffer PL, Foti RS, Gingras J. (2022). J Pharmacol Exp Ther. 383(1):56-69. doi: 10.1124/jpet.121.001026.

82. Martina, M., Banderali, U., Yogi, A., Arbabi Ghahroudi, M., Liu, H., Sulea, T., ... & Stanimirovic, D. (2024). Advanced Science, 2405432. doi: 10.1002/advs.202405432

83. Wright, P. D., Veale, E. L., McCoull, D., Tickle, D. C., Large, J. M., Ococks, E., ... & Jerman, J. (2017). Biochem Biophys Res Comm 493(1), 444-450. doi: 10.1016/j.bbrc.2017.09.002

84. Loucif, A. J., Saintot, P. P., Liu, J., Antonio, B. M., Zellmer, S. G., Yoger, K., ... & Mathie, A. (2017). Br J Pharm 175(12), 2272-2283. doi: 10.1111/bph.14098

85. Bagriantsev, S. N., Ang, K. H., Gallardo-Godoy, A., Clark, K. A., Arkin, M. R., Renslo, A. R., & Minor Jr, D. L. (2013). ACS Chem Biol 8(8), 1841-1851. doi: 10.1021/cb400289x

86. Flaherty, D. P., Simpson, D. S., Miller, M., Maki, B. E., Zou, B., Shi, J., ... & Golden, J. E. (2014). Bioorganic & Med Chem Letters 24(16), 3968-3973. doi: 10.1016/j.bmcl.2014.06.032

87. Rödström, K.E.J., Cloake, A., Sörmann, J. et al. (2024). Nature Comm 15, 4173. doi: 10.1038/s41467-024-48536-2

88. Prevarskaya N, Skryma R, Shuba Y. (2018). Physiol Rev. 98(2):559-621. doi: 10.1152/physrev.00044.2016.

89. Gómez-Varela D, Zwick-Wallasch E, Knötgen H, Sánchez A, Hettmann T, Ossipov D, Weseloh R, Contreras-Jurado C, Rothe M, Stühmer W, Pardo LA. (2007). Cancer Res 67(15):7343-9. doi: 10.1158/0008-5472.CAN-07-0107

90. Haustrate, A., Shapovalov, G., Spriet, C., Cordier, C., Kondratskyi, A., Noyer, L., ... & Lehen'kyi, V. Y. (2023). Cancers, 15(6), 1825. doi: 10.3390/cancers15061825

91. Neuberger, A., & Sobolevsky, A. I. (2023). Channels 17(1), 2266669. doi: 10.1080/19336950.2023.2266669

92. Fraser, S. P., Onkal, R., Theys, M., Bosmans, F., & Djamgoz, M. B. (2022). Br J Pharmacol 179(3), 473-486. doi: 10.1111/bph.15668

93. Sun H, Luo L, Lal B, Ma X, Chen L, Hann CL, Fulton AM, Leahy DJ, Laterra J, Li M. (2016). Nature Commun. 7:10339. doi: 10.1038/ncomms10339

94. Novassay company website outline on Ca_v channel α2δ1 accessory subunit mAbs. https://www.novassay.com/ourbusiness/drug_development_overview (Accessed December 2024)

95. Novassay NVA 2301-10 Ca_v mAb overview. https://synapse.patsnap.com/drug/d0303ff24b8c4ad5a7d41e64fc0caaa8 (Accessed December 2024)

96. Gilbert SM, Gidley Baird A, Glazer S, Barden JA, Glazer A, Teh LC, King J. (2017). Br J Dermatol. 177(1):117-124. doi: 10.1111/bjd.15364

97. NHS Health Research Authority clinical research study 'Investigation of P2X7 Functionality in Cancer' (2014). https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/investigation-of-p2x7-functionality-in-cancer/ (Accessed December 2024)

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