

Ion channel gene therapies

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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.

Gene therapy includes replacement or editing of faulty genes, and methods to alter the expression (DNA transcription and RNA translation) of genes linked to human diseases (Reviews ^{1,2}). This blog will focus on methods to manipulate endogenous genes in patient cells and tissues through a variety of *in vivo* delivery vehicles, but exclude *ex vivo* cell-based therapies such as CAR-T. Ion channels are attractive targets for gene therapy for two reasons; the ability to correct mutations (channelopathies) linked to human genetic diseases such as epilepsy and cystic fibrosis, and as a long-term modulation technique that may produce more profound and selective target engagement than small molecules (e.g. $\text{Na}_v1.7$). Examples of preclinical and clinical ion channel programs utilising all of the different gene therapy techniques will be outlined, including gene replacement, DNA and RNA editing, and various antisense RNA methods to alter or correct gene and protein expression.

Diverse therapeutic indications have been tackled using gene therapy (Figure 1A), although as with other drug discovery modalities oncology is the main disease area, followed not surprisingly by rare and more common diseases driven by specific genetic anomalies. An independent database of publicly disclosed information shows that nearly 4,000 gene therapy clinical trials have been carried out from the 1990s up to 2023 ³ (Figure 1C), although this tally also includes more recent trend for cell-and-gene therapies (CGT) which combine gene editing with autologous patient cells (e.g. CAR-T immune-oncology). These trials have been carried out in over 40 different countries and include a large number of exploratory proof-of-concept studies (56% of total) carried out in small patient groups as the

various technologies, reagents and delivery vehicles were developed, validated and optimised.

This extensive clinical work has resulted in 43 CGT drugs approved by the FDA to date, 10 of which are *in vivo* gene therapies and 7 are CAR-T immunotherapies (list from the

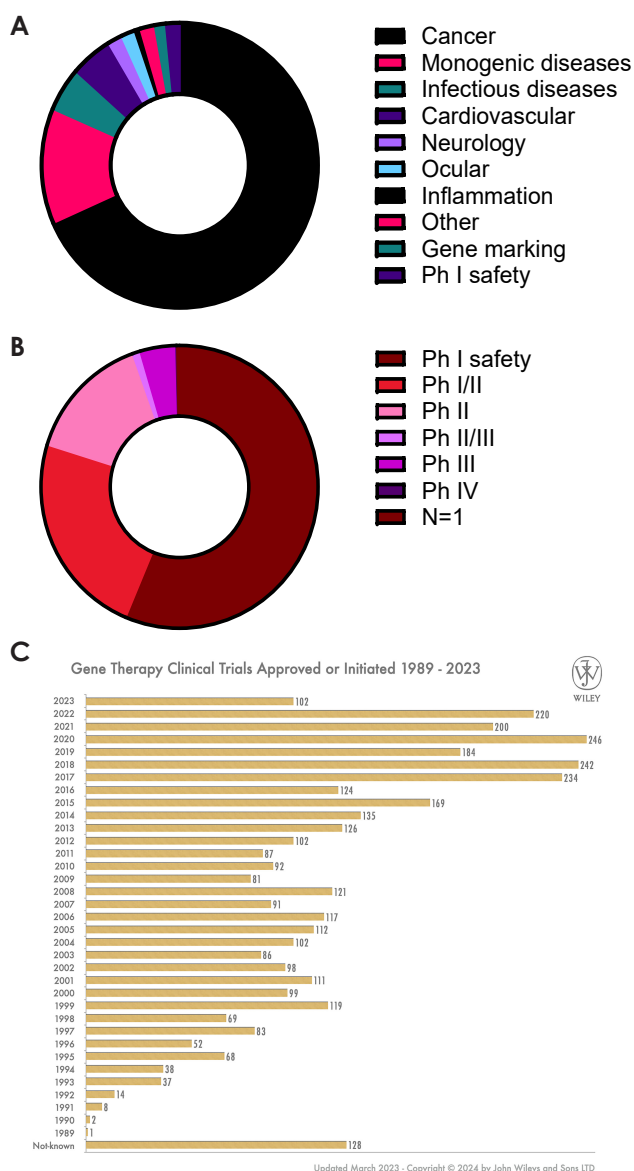


Figure 1 Gene therapies classified by disease indication and clinical trial (up to March 2023). A Gene therapy therapeutic indications. B Gene therapy clinical trials. C Clinical trials by year. Data re-plotted from Wiley Gene Therapy Clinical Trials Worldwide (<http://www.abedia.com/wiley/>).

Ion channel	Indication	ASO/RNAi	Gene editing	Gene therapy
CFTR	Respiratory	CSHL	Prime Medicine	Boehringer Ingelheim*, ReCode Tx*
ENaC	Respiratory	Ionis		
K _v 8.2	Retinal			Lions Eye Institute
K _v 7.1	Retinal		Base editing	
APOL1	Kidney	Ionis/AZ*		
Na _v 1.1	Epilepsy	Stoke TX*		Encoded Tx*
Na _v 1.2	Autism		CRISPR	
Na _v 1.2, Na _v 1.6	Epilepsy	Quiver, Ionis; Praxis*		
Na _v 1.7	Pain, cough	Quiver, OliPass, Atalanta, Olix, Regeneron, Encoded, GSK	Navega, Sangamo	
Na _v 1.8	Pain	Quiver Bioscience	Navega	
K _v 1.1, K _v 1.2	DEE, Epilepsy	Gapmer	UCL	UCL
KNa1.1	Epilepsy, DEE	Atalanta, Gapmer		
GluK2	Epilepsy	miRNA	UCL	UCL

Table 1 Examples of ion channel gene therapies under preclinical development or clinical* testing.

FDA Office of Therapeutic Products as of 03/06/2025 ⁴). Of note is the fact that the first pure *in vivo* gene therapy wasn't approved by the FDA until 2017 (Luxturna, an AAV2 encoding RPE65 gene for inherited retinal blindness) and the majority were approved after 2022, showing the slow but steady progress in getting these elegant but challenging therapies through clinical trials and into the market. Indeed, the significant maturation of this field in recent years is evidenced by several different gene therapies being approved for the same disease (sickle cell disease, beta thalassemia and hemophilia B), representing the first time that rare disease patients have such a choice ⁵. No ion channel gene therapy has yet to be approved by western regulatory agencies, but several are undergoing clinical trials and many are being explored in preclinical discovery programs (Table 1) and these will be discussed below.

Gene therapy techniques and tools

Several different techniques are being used to modify gene expression and function and it is useful to break down this section into each approach. Gene therapies can be broadly categorised into 4 strategies:

- Gene replacement therapy for monogenic diseases (correct faulty or inactive genes).
- Gene addition to improve symptoms and function in non-genetic diseases.
- Gene editing to introduce targeted changes in the genome (to correct or skip mutations, and modify expression via knock-out or promoter or repressor modulation).

- Gene (and protein) expression alteration by targeting RNA (e.g. editing, exon skipping, silencing/interference).

Expression and delivery options and challenges

A key technical issue for gene therapy is the method by which exogenous genes, RNA and expression-modifying reagents are packaged and delivered into patient cells to ensure safe and effective therapeutic results. Challenges include the size limits and immunogenicity of viral vectors and chemical delivery systems (e.g. liposomes and nanoparticles), and achieving suitable tropism for the desired cells and tissues. There is also growing interest in using exosomes (extracellular vesicles) to package and deliver gene therapy reagents, although much work remains to determine their cell specificity and safety for therapeutic applications ¹⁰⁵.

Viral vectors are a popular and proven delivery method ^{7,8}, and include a variety of non-replicating episomal adenovirus and adenovirus-associated viruses (AAV serotypes 2, 3, 4 and 9), lentiviruses for neuronal targets, and herpes simplex virus (HSV) which have already been used for several FDA-approved drugs. It is also possible to package genetic material into the protein shell of empty or inactivated viral capsids. Adenovirus and AAVs have the advantage of infecting and transducing both dividing and non-dividing cells (e.g. neurons), but their use can be limited by cargo size (Table 2), cytotoxic T-cell immune reactions (as seen in a recent Pfizer trial of an AAV gene therapy ⁹), and inactivation by neutralising antibodies in patients previously exposed to these common viruses, as well as liver toxicity from 1st pass accumulation, all of which can blunt their long-

Parameter	HSV	Adenovirus	AAV	Lentivirus
Cargo size	> 30 Kb	10 Kb	< 4 Kb	8 Kb
In vivo dosing	Yes	Yes	Yes	No
Repeat dosing	Yes	No	No	~
Tissue expression	Long-term	Short-term	Long-term	Long-term
Episomal transduction	Yes	Yes	Yes	No
Immunogenicity	No	Yes	Yes	No (ex vivo)

Table 2 Advantages and challenges of different gene therapy viral vectors. Adapted from EG 427 biotech company website (<https://eg427.com/Technology.html>)

term efficacy and safety. HSV can deliver large amounts of exogenous DNA but this can induce cytotoxicity, and the ability to maintain long-term transgene expression remains challenging. Thus, some gene therapies are so-called 'one-and-done' as they can irreversibly alter gene expression, whilst others need to be dosed repeatedly (weekly-monthly) but then risk onset of immunogenicity and toxicity. Retroviral vectors (HIV, MMLV) are now avoided as they permanently integrate into the genome.

Gene expression-modifying reagents in contrast can be added directly to cells and tissues, although they can also be packaged in delivery vehicles. Antisense oligonucleotides (ASOs) are short, single strands of DNA or RNA that bind to specific RNA sequences to either promote, modify or decrease translation of target genes into proteins (reviews ^{9,10}). In contrast, RNAi and siRNA ligands are double stranded RNA sequences that bind to and degrade specific mRNA sequences to 'silence' genes. Challenges in the use of DNA-based oligomers include high immunoreactivity whereas RNA-based scaffolds are subject to rapid degradation, and their delivery to tissues and organs beyond the liver, and especially the CNS, remain problematic ¹¹. A third category are Peptide Nucleic Acids (PNA) where the oligonucleotide backbone is composed of synthetic pseudopeptides which can bind to single or double DNA or RNA strands to prevent gene transcription or translation. They are cheaper and easier to synthesise and demonstrate higher affinity nucleotide binding than other ASOs and are resistant to cellular degradation, although their large size and poor solubility remains a challenge to

achieve cell permeability and in vivo distribution into target tissues ¹².

Liposomes and lipid nanoparticles (LNPs) are gaining traction as gene therapy vehicles as they are cheap and scalable to produce, have low immunogenicity, and can be repeatedly dosed. However, cell infection, transduction and gene expression changes and effect duration are typically lower for this technique compared to viral vectors, and some LNPs can induce clinical safety signals such as liver toxicity as seen in a recent base editor trial by Verve Therapeutics ¹³. Whilst most applications package the RNA within the empty core, it is also possible to attach ASO oligomers and other gene expression-modifying reagents to the outside of liposomes. The delivery of longer-lasting DNA by LNPs was previously avoided due to toxic immunoreactivity, but recent work has overcome this limitation by adding an anti-inflammatory fatty acid ¹⁴.

Tropism, or the selective targeting and transduction of specific cell and tissues, is another ongoing issue for gene therapy. Early i.v. gene therapy treatments (vectors, ASO and siRNA) tended to accumulate in the liver and produce toxicity, whilst limiting the passage of sufficient reagents to infect downstream organs. Different native AAV serotypes have been used as they each exhibit natural tropism for certain human cell types, and synthetic and lab-evolved variants are being developed to fine-tune this capability.

The NIH Somatic Cell Genome Editing Consortium has just published a useful atlas of AAV tropism, albeit in mouse,

"Optimizing gene therapy for cell-specific and activity-dependent expression, combined with traditional pharmacology, opens the door to precise and powerful treatments for diseases with urgent unmet needs."

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

highlighting sex differences and selective tropism of AAV4 for endothelial and pancreatic beta cells that avoids the liver where other AAVs become trapped¹⁵. Drug discovery companies are also developing novel techniques to target gene therapies to specific cells and organs. For example, Judo Bio have revealed their conjugation of siRNAs to ligands for Megalin receptors which are enriched on proximal tubules and podocytes¹⁶, allowing for regenerative binding and internalisation of siRNA molecules to knockdown kidney solute transporters. Gaining access to non-dividing neuronal cell types and the CNS remains a key challenge for gene therapies (as it is for other biologicals such as antibodies and peptides), which can be addressed through the use of lentiviruses that have a preference for neurons. Recently, Denali Therapeutics reported that their blood-brain barrier transferrin receptor antibody shuttle technology could also be used to facilitate access of ASOs into the brains of mice and non-human primates where it reduced target gene expression^{17,18}. This approach could offer a marked improvement on the limitation of intrathecal or intracerebroventricular injections of gene therapy reagents.

Gene replacement therapies

An obvious place to start with therapeutic gene therapy is to try and compensate or correct for genetic deficits in rare diseases where patients are defined by a single faulty or inactive gene. A good example of this, with notable clinical success, has been the replacement or augmentation of affected enzymes in various rare metabolic and neurodevelopmental disorders (Reviews^{19,20}). The first successful gene replacement clinical trial was carried out in 1990 using retroviral vectors expressing an adenosine deaminase minigene to transduce bone marrow cells ex vivo from 2 patients²¹. However, it took until 2003 for the first gene replacement therapy to be approved, in China.

Gendicine used injected cells expressing a wildtype p53 tumour suppressor gene to suppress head and neck cancers. The 1st gene replacement therapy approved in the west was Glybera (uniQure/Chiesi) in the EU 2012 which used AAV vectors to introduce a hyperactive copy of the LPL gene into skeletal muscle cells to normalise lipoprotein lipase levels, followed in the US by Luxturna (Spark Therapeutics) in 2017 which required retinal injection of AAV2 vectors encoding the RPE65 gene to improve vision of patients with congenital Leber Congenital Amaurosis (LCA) blindness.

No ion channel gene replacement drug therapy has been approved as of Q1 2025, but several programs are currently in mid-phase clinical trials. The choice of target is limited by the large size of many tetrameric ion channels, focusing current work on delivering smaller ion channel genes and modulating the expression of endogenous ion channel genes. At least two companies are developing CFTR gene replacement therapies using mRNA to introduce functional wildtype Cl⁻ channels into the lung to treat patients with

cystic fibrosis²². ReCode Therapeutics have an inhalable mRNA drug RCT2100 in Ph Ib trials in patients with CFTR mutations not amenable to current drugs, using a LNP mixture optimised to infect lung epithelia and produce functional copies of the wildtype CFTR channel. Boehringer Ingelheim are using an inhaled lentivirus to deliver CFTR gene DNA stably into the genome of alveolar cells in the lungs of CF patients, with Ph Ib trials of BI 3720931 starting in 2025 and Ph III studies expected to readout by 2027²³. Finally, an indirect one-time ion channel gene therapy application is being developed to treat Na_v1.1 deficiency Dravet Epilepsy. This channelopathy is driven by a mutant allele of the Na_v1.1 gene (autosomal dominant haploinsufficiency) leading to a ~50% reduction of Na_v1.1 channel protein in inhibitory neurons that leads to CNS over-excitability, seizures and neurodevelopmental deficits in young patients. Encoded Therapeutics are using an AAV vector to deliver an inhibitory neuron-specific promoter and transcription factor transgene to upregulate the expression of the unaffected SCN1A gene allele and increase Na_v1.1 current levels and normalise CNS network excitability. ETX101 is currently undergoing Ph I/II trials in the US, Australia and UK.

These 'one-and-done' gene therapies can be very effective for patients with chronic or early-onset diseases, but they are also expensive with prices of \$0.5-\$4 million due to the small rare disease target populations and single treatment dosing²⁴. Thus, some gene therapy drugs have been withdrawn from the market (e.g. Glybera) or remain inaccessible for many patient families, highlighting the disconnect between progress and innovation in basic gene therapy R&D and the commercial reality of marketing new gene therapy drugs.

Therapies to alter gene (and protein) expression

This category of gene therapies employ various nucleotide scaffolds including ASOs, PNAs and RNAi reagents to modify gene expression by binding to specific DNA or mRNA sequences (overviews^{25,26}). A significant potential advantage of these RNA gene therapies is that they have improved selectivity over small molecule ligands, and can also target regulatory domains and non-coding DNA and RNA sequences (e.g. miRNA or microRNA) that are beyond the reach of small molecules and antibodies that target the mature protein, leading to more profound and diverse modulation of target protein biogenesis. They can also be designed and validated faster and more cheaply than small molecules or antibodies. Optimisation of cell tropism and permeability of ASOs and PNAs include the addition of cell penetrating peptides, and incorporation into LNPs. However, these type of gene therapy reagents suffer from short-term effects and the need for repeat dosing which can induce immunogenicity and saturation of RNAi machinery in targeted cells.

The first ASO therapy approved by the FDA was Fomivirsen

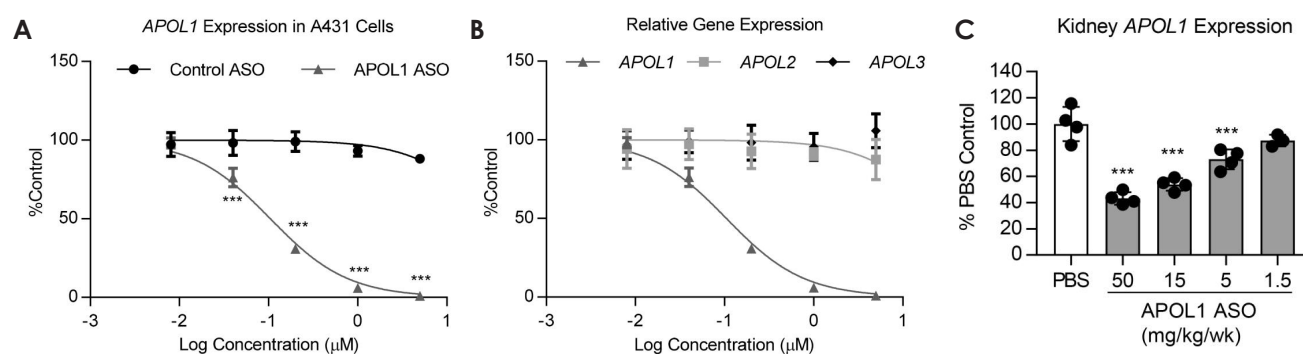


Figure 2 Ionis/AZ ASO AZD2373 reduces kidney *APOL1* channel expression and proteinuria. **A** In vitro analysis of *APOL1* levels in A431 cells ($n = 4$) was carried out using qRT-PCR following a 72-hour free uptake of either IONIS-*APOL1*Rx or a control ASO, with data normalized to GAPDH levels. IONIS-*APOL1*Rx significantly reduced *APOL1* expression relative to control. **B** A431 cells were treated with IONIS-*APOL1*Rx for 72 hours by free uptake ($n = 4$) and expression of *APOL1*, *APOL2*, and *APOL3* were measured by qRT-PCR followed by normalization to GAPDH expression. **C** Renal *APOL1* transcript was quantified by qRT-PCR and normalized to *Cyp* reference expression. Administration of IONIS-*APOL1*Rx notably lowered *APOL1* mRNA compared with PBS control.

(Novartis) in 1998, to treat cytomegalovirus retinitis in immune-compromised HIV patients by reducing viral mRNA transcription and replication. The first RNAi therapeutic approved by the FDA was Patisiran (Alnylam) in 2018, which used LNPs to deliver drug to the liver to reduce expression of transthyretin, a transport protein that misfolds and aggregates in a hereditary amyloidosis, a rare and often fatal genetic disease²⁷. There are several ion channel gene expression-modifying therapeutics currently under clinical development, and numerous others at the preclinical stage (see below). Ionis Pharmaceuticals is a company that has been a leading player in the ASO space for over a decade (e.g. gaining FDA approval for Spinraza to treat Spinal Muscular Atrophy in 2016²⁸), and an ASO they developed a few years ago to treat mutations in the kidney ion channel *APOL1* (ION532 or IONIS-*APOL1*Rx²⁹) has just concluded a successful Ph I study after being licensed to AZ in 2018³⁰. Now called AZD2373, the ASO reduces faulty *APOL1* G1 allele mRNA expression and proteinuria in preclinical animal models (Figure 2), and was progressed into the clinic to treat this form of genetic chronic kidney disease where Ph I data indicated it was safe and well tolerated and repeat dosing reduced plasma *APOL1* protein levels, paving the way for planned Ph II efficacy studies later in 2025.

Praxis Precision Medicines have taken an ASO into Ph II trials to treat gain-of-function mutations in the $\text{Na}_v1.2$ channel that underlie a class of rare childhood epilepsy (PRAX-222, Elsunsersen)³¹, with early clinical data showing it can reduce seizure frequency. The company also has a discovery phase ASO project (PRAX-100) targeting loss-of-function $\text{Na}_v1.2$ mutations. These projects exemplify the ethical and regulatory difficulties in applying precision genetic medicines to small rare disease populations and young patients, challenging agencies and companies to devise novel clinical trial design and drug approval pathways. The same is true for many of the small molecule and gene

therapies targeting $\text{Na}_v1.7$ and $\text{Na}_v1.8$ channels in small populations of painful channelopathy patients, but OliPass have tried to bypass this bottleneck by testing their $\text{Na}_v1.7$ PNA antisense drug OLP-1002 in a broader population of patients with osteoarthritis. Their PNA contains cationic lipids to increase stability and cell entry, and achieves efficacy at much lower doses than other ASOs¹². OLP-1002 binds to the pre-mRNA of the *SCN9A* gene in sensory neuron nuclei to persistently reduce the $\text{Na}_v1.7$ protein production, and due to local injections and subtype selectivity it is hoped to avoid systemic side-effects on the heart and vascular system seen recently in the clinic with small molecule $\text{Na}_v1.7$ blockers. Topline data from the Ph IIa trial released in 2023 showed both strong analgesia and a long effect duration with a 65% reduction in pain scores over 6 weeks after a single 1-2 mg sub-cutaneous injection³², although later analysis revealed a high placebo response which is an ongoing and common challenge in pain clinical trials.

Gene editing

This fourth type of gene therapy seems to get most of the attention, as it involves the ultimate power to permanently alter the human genome and irreversibly 'cure' human diseases. However, DNA editing drugs also come with a huge price tag as a one-and-done' treatment, along with significant risks such as off-target editing in the vicinity of the target gene, which may limit the approval and uptake of future DNA editing medicines. RNA editing techniques are thus being developed to avoid such complications.

I'm not a molecular biologist so this section will be a brief overview of the gene editing methods being utilised to alter ion channel gene expression, with specific details on preclinical programs outlined in section **Preclinical ion channel gene therapies**. Gene editing requires two key components; a guide to bind to specific DNA or RNA

sequences, and an editing moiety that makes changes to coding or promoter regions to alter target gene expression or correct mutations.

DNA editing uses several systems able to bind to and alter DNA sequences:

- Zinc fingers and TALENs (Transcription Activator-Like Effector Nucleases) are restriction enzyme proteins able to cleave DNA (DNases).
 - The zinc finger motif binds to short DNA sequences and operates as a dimer either side of the nuclease editing region, and the construct is small and easy to transduce into target cells and multiple motifs enables modular design and high specificity
 - TALENs comprise an array of single base pair-binding variable domains and a nuclease that also operates as a dimer, with improved specificity and efficacy especially for regions of DNA resistant to other editing molecules.
- CRISPR-Cas9 is a RNA-protein hybrid where the synthetic RNA contains a sequence that guides the Cas9 DNA endonuclease to edit a complementary DNA sequence in the genome
 - The target DNA region must be flanked by short protospacer adjacent motifs (PAMs) and the process makes double strand breaks, restricting the specificity, accuracy and utility of this method.

CRISPR gene editing has proven to be efficient and works well in vivo to make edits or introduce mutations and carry out knock-out screens faster and more cost-effectively than traditional transgenic animal or RNAi techniques. While this is useful in academia and preclinical target validation work, there are several drawbacks for clinical drug development such as off-target editing ³³ and the large size of the CRISPR-Cas9 editing plasmids which can limit viral vector and LNP delivery methods. For example, efforts are underway to identify other Cas endonucleases and novel systems such as TIGR-Tas which is 25% of the size, does not require PAMs and can edit double-stranded DNA with higher specificity ³⁴.

There are two recent variants of DNA editing techniques that bypass some of the potential drawbacks of CRISPR-Cas9 (e.g. double strand breaks, donor DNA template) but retain utility in efficiently creating genetic models of disease, as well as gene therapies to correct human diseases including those caused by ion channel mutation. Base and prime editing make specific changes to single nucleotides (producing or removing disease mutations), rather than removing or inserting larger stretches of DNA to alter disease-causing mutations or inactivate faulty alleles as most CRISPR-Cas9 therapies do (Review ³⁵).

- Base editing is a refined version of CRISPR-Cas9 that

uses a guide RNA and DNA base editor to make precise single-nucleotide changes in the genome, rather than 'cutting and pasting' a short DNA sequence.

- The lack of double strand breaks improves accuracy but off-target editing can occur.
- Prime editing can create a wider variety and larger size of sequence changes than base editing, including single nucleotide insertions, deletions, and all 12 types of point mutations.
- Even safer and more accurate than base editing with fewer off-target effects.

The first patient to undergo base editing was part of a 2022 clinical trial to deactivate the PCSK9 gene in familial hypercholesterolaemia, carried out by Verve Therapeutics. The first CRISPR gene editing therapy drug approved by the FDA was Casgevy (CRISPR Therapeutics and Vertex, Dec 2023) ³⁶, which is a combined CGT for sickle cell anemia and beta thalassemia that uses CRISPR-Cas9 to edit the regulator of fetal hemoglobin gene expression in blood stem cells. Beam Therapeutics revealed in 2025 they were using base editing in an early stage clinical trial to correct mutations in respiratory disease alpha-1 antitrypsin deficiency. Prime Medicine took their prime editor PM359 into the clinic in 2024 for chronic granulomatous disease³⁷, which is a combined cell and gene therapy (CGT) where ex vivo transduction of autologous blood stem cells corrected the NCF1 gene mutation before infusion back into patients. Over twenty other clinical trials of CRISPR-Cas9, base and prime editors were recently underway in a mixture of CGT and gene therapies ³⁸. None of these gene editing techniques have as yet yielded a clinical stage ion channel drug candidate, but there are preclinical industry programs targeting CFTR with prime editors (Prime Medicine) and Na_v1.7 and Na_v1.8 channels using CRISPR-Cas9 and zinc finger nucleases (Navega, Sangamo), and an academic consortium using base editors to correct mutations in K_{ir}7.1 and bestrophin Ca²⁺-activated Cl⁻ channels in retinal blindness (section Retinal diseases below).

Finally, a new DNA editing variant called STITCHR was recently revealed which uses a Cas9 nickase and retrotransposons to enable whole gene replacement rather than small edits, and is formulated as an RNA construct favourable to many delivery vectors and is not limited to DNA regions flanked by PAMs ³⁹. This approach could help treat genetic diseases including channelopathies with faulty ion channel subunits, once off-target specificity and in efficacy in human tissues in vivo (including the CNS) are clarified.

Epigenetic modifiers

Efforts to overcome the risk of off-target and/or permanent genome changes include epigenome editing with

nuclease-inactive enzymes (e.g. Navega dCas9), and techniques to leverage epigenetic modulation of gene expression such as methylation, transcription factors, and heterochromatin histone proteins. For example, Tune Therapeutics have screened areas around specific genes to identify sites that can enhance or repress expression and used these sequences to create specific gene therapy effectors to alter these epigenetic marks and tune transcription in a titratable and reversible manner, and have a clinical trial underway to treat Hepatitis B ⁴⁰.

Preclinical ion channel gene therapies

Not surprisingly most ion channel gene therapies are targeting peripheral tissues and pathophysiology such as pain and retinal, respiratory and kidney disease due to ease of delivering vectors or injecting reagents. However there is also notable progress in ion channel gene therapies to treat epilepsy using a variety of transduction and modulation techniques to penetrate the CNS .

Na_v1.7 gene therapies for pain and cough

It is no surprise that nearly a dozen companies have disclosed gene therapy programs targeting the Na_v1.7 channel (Table 3), given the compelling human genetic validation story around gain- and loss-of-function mutations linked to altered pain perception in rare patient populations. The interest in gene therapies is also driven by the lack of clinical efficacy for small molecule Na_v1.7 antagonists, and more recent evidence that these ligands can produce on-target side-effects on cardiovascular and hemodynamic function that are thought to be driven by inhibition of Na_v1.7 channels in autonomic ganglia. Na_v1.7 gene therapies have the potential for greater analgesia efficacy, selectivity and safety if they can produce more profound down-regulation of Na_v1.7 channel protein expression while limiting their effect to sensory neurons with specific vector tropism.

Two companies are pursuing Na_v1.7 gene editing approaches as a novel analgesia treatment. Navega Therapeutics published a study on a CRISPR-dCas9 gene editor which reduced excitability in DRG ex vivo and produced long-lasting analgesia in rodent pain assays over many weeks after a single intrathecal AAV9 delivery. Their CRISPR system uses an epigenetic repression editor designed to avoid permanent changes to the genome and is claimed to have reduced human immunogenicity ⁴¹, with the company receiving a translational grant in early 2025 to complete preclinical studies in preparation for an IND filing for NT-Z001, with plans to test the gene therapy in patients with gain-of-function mutations in the Na_v1.7 gene and those suffering from chemotherapy-induced peripheral neuropathy (CIPN) ⁴². As there remains some concern around collateral gene editing with CRISPR, both Navega and Sangamo Therapeutics are developing zinc finger editors to reduce SCN9A gene expression. Navega's zinc

Modality	Compaines and academic applications
AAV peptides	NaviPA, CRS-CRMP2 decoy
ASO	Quiver Biosciences, OliPass (PNA), Exicure
RNAi	Atalanta Tx, Regeneron (shRNA), Olix Pharma, GSK, Voyager
miRNA	Encoded Tx, miRNA-182
Zinc finger	Navega, Sangamo
CRISPR	Navega
SDRE	NIH HEAL

Table 3 Na_v1.7 gene therapies.

finger repressor is delivered by AAV and can produce long-term analgesia in rodents by profoundly reducing Na_v1.7 RNA in human DRG. Sangamo have designed separate rodent and human/NHP epigenetic SCN9A zinc finger transcription repressors in AAV9 vectors which selectively reduce Na_v1.7 RNA levels in animal DRG and human iPSC sensory neurons (Figure 3), and produce thermal and mechanical analgesia for up to 4 weeks after a single intrathecal vector injection in a chronic neuropathic pain model ^{43, 44}. As expected, AAV administration triggered some antibodies and mild necrosis and inflammation in ganglia, but these were mild and did not affect efficacy or safety readouts. Both companies have published safety data showing no cardiovascular side-effects of their Na_v1.7 gene therapies *in vivo* in rodent and monkeys, so presumably the intrathecal delivery of AAV9 vectors avoids transduction of autonomic and sympathetic ganglia that might occur after i.v. administration, and/or perhaps their AAVs lack tropism for these types of neurons, or the body can adapt to chronic Na_v1.7 down-regulation as occurs in CIP patients in contrast to reacting to sudden pharmacological inhibition. US academics also received NIH HEAL finding for an RNA editing project using endogenous ADAR enzymes and guide RNA to alter the pore permeability of Na_v1.7 channels and reduce excitability of rodent and human DRG and iPSC sensory neurons ⁴⁵.

There are a plethora of companies pursuing ASO and RNAi approaches against Na_v1.7 for pain. Quiver Biosciences (previously Q-State) in Boston have revealed a preclinical program designing ASOs that reduce Na_v1.7 RNA and protein levels and channel function in HEK cells and rodent, NHP and human DRG and iPSC sensory neurons, and demonstrate target engagement and long-term analgesia in rodent pain models after intrathecal injection ⁴⁶. Selected lead Na_v1.7 ASO candidates with low CNS side-effects will be progressed to clinical testing in patients with small fibre neuropathy (SfN) and cancer pain. More recently Atalanta revealed a Na_v1.7 RNAi project and Regeneron published on an Na_v1.7 shRNA program. Atalanta published a poster

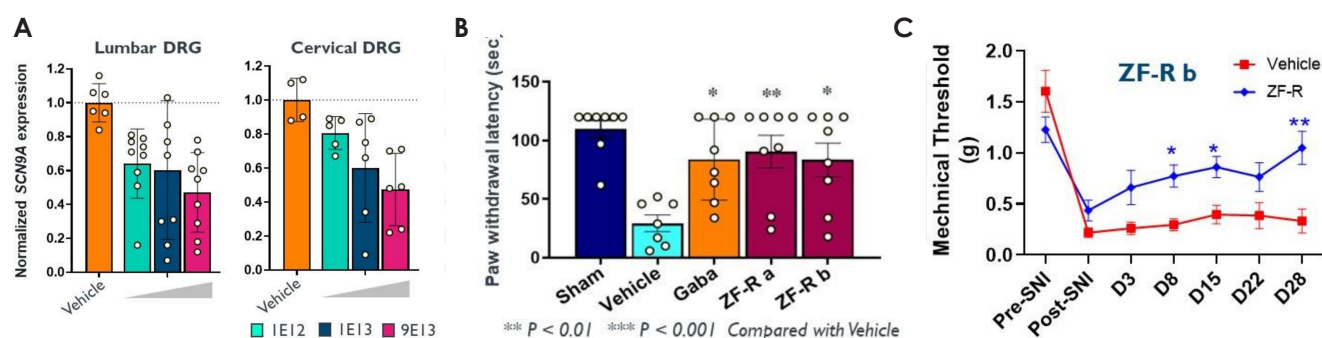


Figure 3 Nav1.7 gene editing reduces DRG expression and painful behaviour in vivo. **A** ZF-R A (clinical Candidate) reduced SCN9A in lumbar and cervical DRGs one month post-treatment. **B** ZF-Rs rescued cold-induced pain to the normal levels after one month. **C** ZF-Rs rescued mechanical induced pain over time and as early as Day 3 post-treatment. Adapted from data in ⁴³ https://www.sangamo.com/wp-content/uploads/2023/11/American-College-of-Toxicology-2023_Nav1.7_Poster_Toufan-Parman.pdf.

at IASP showing that the divalent short interfering RNA (di-siRNA) ligand ATL-301 could potently and selectively reduce mammalian Na_v1.7 expression and channel activity, and produce long-lasting analgesia (3 months) after a single intrathecal injection ⁴⁷. Regeneron published on their Na_v1.7 shRNA project at ASGCT, using a novel tropic approach of conjugating AAV9 capsids to an p75 NGF-R antibody to enhance transduction of sensory and trigeminal neurons and reduce Na_v1.7 expression after local injection and produce analgesia after intrathecal injection ⁴⁸, avoiding transduction of autonomic neurons seen with systemic administration which could lead to vascular side-effects produced by Na_v1.7 small molecules. There are two South Korean companies pursuing Na_v1.7 gene therapies. OliPass have a novel PNA antisense ligand OLP-002 which completed Ph IIa clinical trials in Australia and showed promising and long-lasting analgesia in a broad population of osteoarthritis patients ³². OliX Pharma list a Na_v1.7 RNA antisense ligand OLX401A for neuropathic on their website but it's current pipeline status is unclear ⁴⁹. The company has developed improved siRNA reagents such as asymmetric and cell-penetrating versions which are claimed to have reduced off-target effects and better cell tropism, without the need for delivery vehicles.

At least two groups have shown that small microRNA sequences can silence Na_v1.7 gene expression. A Chinese academic consortium reported that miRNA-182 and Na_v1.7 expression are coupled, with down-regulation of miR-182 after neuropathic pain injury leading to increased Na_v1.7 expression ⁵⁰. Synthetic dsRNA agomirs and antagomirs of miR-182 produced opposite effects on Na_v1.7 RNA and protein expression in DRG and mechanical sensitivity in a rat neuropathic pain assay, after ex vivo transduction or *in vivo* microinjection. Encoded Therapeutics revealed a preclinical Na_v1.7 miRNA program in 2024 ⁵¹, building on their clinical work with a Na_v1.1 ASO gene promoter for Dravet epilepsy. The AAV-delivered miRNA was selected from a library of several thousand sequences based on cross-species

homology, Na_v1.x isoform selectivity, stability and lack of off-target effects, and reduced Na_v1.7 expression in rodent DRG and human iPSC sensory neurons, so we await to see functional efficacy and safety in rodent *in vivo* assays.

There are also several novel peptide-based gene therapy approaches being developed against Na_v1.7. Building on extensive preclinical work showing that the cytosolic CRMP2 protein modulates Na_v1.7 expression and function, the laboratory of Raj Khanna designed an AAV9 vector carrying a plasmid encoding a decoy peptide of the Na_v1.7 binding domain for CRMP2 and found that it reduced Na_v1.7 function in rodent and NHP sensory neurons, and could prophylactically prevent or persistently reduce chronic pain for >4 weeks in rodent behavioural models after a single intrathecal injection ⁵². Another group of US and Chinese academics took a similar approach to discover peptide aptamers matching a region of the intracellular loop I of TTX-S Na_v1.x channels that could disrupt Na_v1.7 channel function and reduce excitability in native rat DRG and human iPSC sensory neurons. This peptide sequence was packaged into an AAV6 vector they called NaviPA to enable *in vivo* infection of DRG neurons and peripheral and central nerve terminals, which reduced spontaneous and evoked pain responses in a rat neuropathic pain model ⁵³. While promising, both of these peptide-based gene therapies lack selectivity for Na_v1.7 so further optimisation will be required to avoid side-effects and enable successful translation into the clinic.

While most Na_v1.7 gene therapy programs are targeting pain, GSK have had a long-standing interest in the role of Na_v1.3 and Na_v1.7 channels on vagal sensory neurons to control cough, and published on a program using AAV 8 vectors to microinject Na_v1.7 shRNA into the nodose ganglia of guinea pigs, which selectively reduced TTX-S currents and nodose neuron and vagal A and C-fibre excitability, and suppressed cough reflexes for several months. ⁹⁹

Finally, it is worth mentioning that several preclinical $\text{Na}_v1.7$ gene therapy programs active in the early 2020s have since been discontinued. Exicure had revealed a $\text{Na}_v1.7$ RNA knockdown program using spherical nucleic acids (SNA) where the ASO oligonucleotides are displayed across the 3D surface of LNPs, with claims that this enabled higher uptake by CNS and peripheral neurons. Data in a corporate presentation showed specific knockdown of $\text{Na}_v1.7$ expression and delivery to the rodent DRG after intrathecal injection, but later news revealed issues in translating this effect to NHPs, and the program was shelved in 2022⁵⁴. Voyager Therapeutics are another gene therapy company with several gene replacement and RNAi programs in the clinic for neurological indications, based on proprietary AAV capsids with optimised tropism for specific neuronal populations. They publicly revealed a preclinical $\text{Na}_v1.7$ siRNA program in 2019 showing that the VY-NAV01 vector efficiently transduced rodent DRG after i.v. administration⁵⁵, and shared data at the 2021 Cell & Gene Therapy conference showing selective and profound $\text{Na}_v1.7$ mRNA knockdown in DRG sensory neurons *in vitro* and *in vivo*⁵⁶, but *in vivo* pain assay efficacy data was lacking and this program disappeared from their corporate website in 2022.

$\text{Na}_v1.8$ pain gene therapies

Several of the same companies targeting pain-related $\text{Na}_v1.7$ channels with gene therapies are also looking at $\text{Na}_v1.8$ as a complimentary target, including Navega (gene editing) and Quiver Biosciences (ASOs). There is speculation as to whether such treatments may be dosed additively with their $\text{Na}_v1.7$ therapies to achieve more profound and long-lasting analgesia. In contrast, the recent clinical evidence that small molecule $\text{Na}_v1.7$ inhibitors produce on-target side-effects on heart rate and blood pressure, while recent small molecule $\text{Na}_v1.8$ blockers appear safe, may induce a pivot from $\text{Na}_v1.7$ to $\text{Na}_v1.8$.

$\text{Ca}_v3.2$ gene therapy for pain

The same academic group who published on NaviPA peptide aptamers have used the same approach to create similar ligands that bind to the intracellular loops of $\text{Ca}_v3.2$ channels to reduce current amplitude in HEK cells and excitability of DRG neurons^{57,58}. These CaviPAs can be packaged into AAV vectors and delivered to DRG *in vivo* by local injection to reduce both spontaneous and evoked pain in two rodent neuropathic pain models.

Respiratory ion channel gene therapy

While respiratory ion channels like CFTR, ENaC and TMEM16A would seem to be a natural choice for gene therapies delivered locally by inhalation to treat CF and other lung diseases, technical and biological issues have stymied their clinical development. For example, although wildtype adenoviral vectors have a natural predisposition to infecting airway tissue, replication-defective AAV vectors required for

Modality	Companies
Gene replacement	Prime Medicine, Nosis Bio, Carbon Bioscience, 4D Molecular Therapeutics, Krystal Biosciences, Arcturus Therapeutics, Spirovant
ASOs	Ref 59, Splisense
CRISPR editing	Ref 60
Prime editing	Prime Medicine Ref 61
SDRE	NIH HEAL

Table 4 Preclinical and clinical gene therapies to correct CFTR function in cystic fibrosis.

safe clinical use lack such tropism, and also induce a robust inflammatory response. Similarly, encapsulation of viral vectors in LNPs showed low efficiency of gene transfer and poor efficacy⁶.

Improved and alternative methods to deliver CFTR gene therapy are now undergoing clinical testing, including ASOs to correct specific mutations and gene replacement therapies (Table 4). Academic groups have published on novel CRISPR and prime editing methods to more efficiently correct CFTR RNA splicing defects and DNA mutations and produce remarkable recovery in Cl⁻ channel function in patient-derived airway cells^{60,61}. More recent preclinical industry efforts include a program at Prime Medicine to deliver prime editors in LNPs and AAVs to enable editing of hotspots of CFTR gene mutations to treat a large proportion of CF patients, including those living with nonsense and rare mutations and those who can't tolerate approved drugs⁶². The same company is also developing a DNA recombinase technique to insert a large artificial CFTR gene exon into lung epithelia. Another Boston biotech Carbon Biosciences revealed a novel bocavirus vector to deliver intact CFTR gene copies to lung epithelia at ASGCT⁶³, exploiting the larger cargo capacity and airway tropism of this parvovirus. The preclinical CBN-1000 capsid infects primary epithelia from CFTR patients with various CFTR mutations and increases CFTR protein levels and channel activity, and increase CFTR gene expression *in vivo* after tracheal delivery in preclinical species. The same PI who discovered Spinraza has also developed an ASO to allow read-through of nonsense mutations in the CFTR gene which promotes expression of partially functional channel proteins and Cl⁻ secretion in human bronchial cells⁵⁹.

Ionis described a series of ASOs targeting the α , β and γ subunits of the ENaC channel which reduced protein expression, channel activity and mucus accumulation and improved lung function in a mouse model of CF⁶⁴ (similar to the mechanism of Enterprise Therapeutics ENaC small molecule inhibitor ETD-001).

Epilepsy gene therapies

The numerous *de novo* and inherited mutations in many ion channels linked to various types of epilepsy have made this a rich area for gene therapies, especially as these channelopathies begin to affect CNS excitability in utero and affect neurodevelopment and quality of life in pediatric patients and into adulthood, with many patients being poorly served by traditional small molecule anti-convulsants^{65,66}. The challenge for epilepsy gene therapies is targeted delivery of reagents into the CNS, which is usually accomplished with intrathecal or intracerebroventricular injections, but can also be achieved using appropriate neuronal viral vectors and BBB shuttle systems. On the plus side, the long-lasting nature of gene therapies (but not so much ASOs) only require weekly-monthly administration.

I have already outlined two clinical programs targeting Na_v1.1 deficiency in Dravet epilepsy (Stoke Therapeutics ASO mRNA therapeutic zorevunersen/STK-001, and Encoded Therapeutics AAV vector ETX101 containing a promoter and transcription factor transgene to boost wildtype Na_v1.1 expression. Praxis Precision Medicine also have an Na_v1.2 ASO to knock-down faulty Na_v1.2 gene expression (PRAX-222/Elsunersen) which is in mid-phase clinical trials in DEE. There are also drug charity and academic preclinical programs using gene therapy to modify the expression of Na_v1.x channels involved in epilepsy. An international consortium recently published on a Na_v1.1 gene replacement AAV vector which normalised EEG signals and behavioural deficits and reduced seizures after injection into the hippocampus or thalamus of a mouse model of Dravet epilepsy¹⁰⁰. US academics recently revealed an early stage project using CRISPR prime editing to correct epilepsy-like mutations in Na_v1.2 gene in iPSC neurons with improved efficiency and minimal off-target changes¹⁰¹. The n-lorem foundation is focused on rare diseases, and lists ASOs for both Na_v1.2 and Na_v1.6 channels⁶⁷. Prof. Lori Isom has done a lot of work with Dravet Na_v1.1 ASOs in collaboration with Stoke Therapeutics, but also has a preclinical program showing that ASO gapmers can reduce the expression of SCN8A gene gain-of-function mutants and over-active Na_v1.6 channels and reduce seizures in mouse models⁶⁸, similar to data from other academic groups in collaboration with Ionis Pharmaceuticals⁶⁹.

Several different voltage-gated K⁺ channels are also promising epilepsy gene therapy targets, either through disease-linked mutations or as substrates to selectively control excitability of CNS excitatory and inhibitory neurons. For example, both gain- and loss-of-function mutations in the KCNA2 gene are linked to epilepsy although they produce opposite effects on K_v1.2 channel functions⁷⁰, and an ASO gapmer developed by Singaporean academics can selectively degrade mutant mRNA while sparing intact wildtype transcription to boost K_v1.2 currents in rat cortical neurons and normalise their action potential shape and excitability⁷¹. Similarly, mutations in the KCNA1

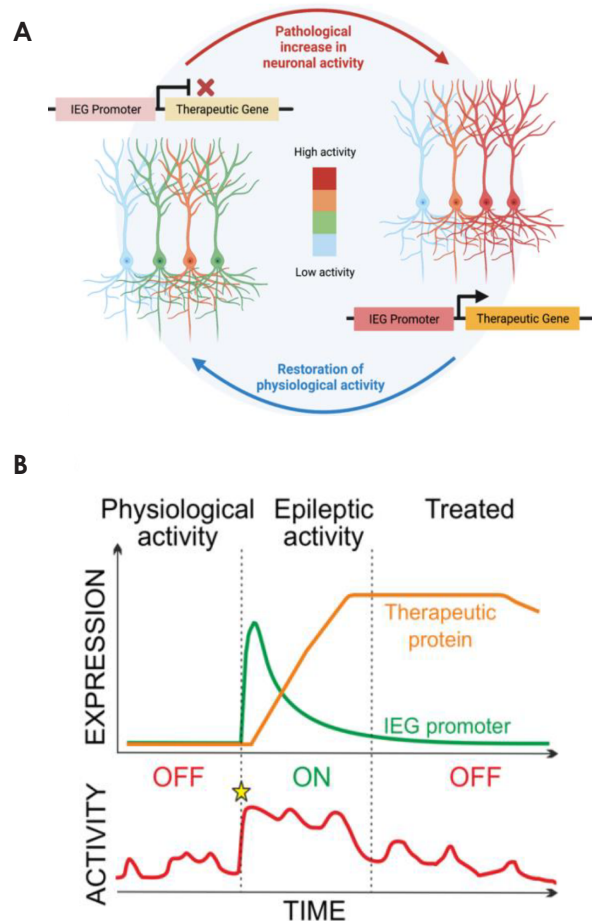


Figure 4 K_v1.1 AAV vector to induce activity-dependent reduction in seizures. **A** Diagram depicting the activity dependent strategy. **B** Representation of how an activity-regulated promoter drives transgene expression. Star indicates the onset of the pathological process. Adapted from data in⁷³ and used under Creative Commons open access license 4.0 (<http://creativecommons.org/licenses/by/4.0/>) from an Open Access publication.

gene encoding K_v1.1 channels are linked to episodic ataxia and epilepsy, and they can heteromerise with mutant and wildtype K_v1.2 channels to profoundly affect CNS excitability⁷². A group of UK academics at UCL have developed two different K⁺ gene therapies to enable more specific control of seizures. The first uses AAV vectors containing the KCNA1 gene to transduce CNS neurons and elicit activity-dependent expression after activation of the immediate early gene c-Fos promoter⁷³. Expression of inhibitory Kv1.1 channels only occurs in over-active neurons (Figure 4) and this produces persistent reduction in seizures in mice *in vivo* without affecting normal behaviours, compared to weaker effects when using the KCNJ2 K_p2.1 channel, and also works in human iPSC cortical neurons paving the way for clinical development. The second approach uses an 'engineered K⁺ channel' transgene (EKC) in a AAV9 vector containing the CaM Kinase promoter to selectively drive K_v1.1 expression in principal

excitatory neurons which reduced seizures in a focal cortical dysplasia mouse model after injection into the frontal lobe ⁷⁴. The same UCL group previously developed an AAV gene therapy using selective extra-synaptic expression of inhibitory *C. elegans* GluR ionotropic Cl⁻ channels in excitatory neurons, controlled by the same CaM Kinase promoter, to reduce epileptic firing in mice after spillover of excess glutamate ⁷⁵. This pioneering activity-dependent ion channel gene therapy was an elegant proof-of-concept, but is limited by potential immunogenicity and ethical issues with expression of non-human proteins in epilepsy patients.

Another K⁺ channel with rare epilepsy channelopathies is KNa1.1 (Slack) encoded by the KCNT1 gene, and alongside small molecule inhibitor drug discovery programs there are a number of gene therapies under development. For example, Prof. Steve Petrou's group in Melbourne published on a KNa1.1 gapmer ASO that could reduce seizures and behavioural abnormalities and increase survival in a mouse model carrying a human epilepsy gain-of-function mutation after a single intra-cerebroventricular ⁷⁶, and Atalanta Therapeutics announced an RNAi program for KCNT1 in Q1 2025 as part of their Series B funding round ⁷⁷, with promising preclinical mouse seizure suppression data for the di-siRNA ATL-201 reported in April 2025 ⁷⁸. However there are also well known risks with gene therapies, and a recent exploratory clinical trial of a KCNT1 ASO in pediatric epilepsy patients produced serious complications and one death ^{79,80}.

French researchers have recently published on a gene silencing therapy for temporal lobe epilepsy that uses AAV9 vectors carrying GRIK2 miRNA to downregulate expression of Kainate GluRs, which reduced seizures and improved working memory after injection into mouse hippocampus *in vivo* and also decreased epileptiform discharges in hippocampal slices from epilepsy patients ⁸¹. This reagent has been further optimised in a collaboration with uniQure and Regenxbio and progressed to GLP studies in NHPs where focal injection reduced GRIK2 expression in the hippocampus for up to 6 months ⁸².

Finally, academics in the EU have described a CRISPR gene editing approach to modulate the expression of low threshold T-type Ca_v3.2 channels by altering activity of the endogenous CACNA1H gene promoter ⁸³. Activation of the promoter leads to over-expression of Ca_v3.2 channels in neurons to provide another model of epileptic gain-of-function channelopathies, whereas inhibition may offer a clinical therapeutic if the gene editor can be packaged and delivered to CNS neurons *in vivo*. As well as epilepsy, both Ca_v3.2 and Ca_v1.2 channelopathies are found in autism patients, and US academics have developed an ASO to reduce alternative splicing of the Ca_v1.2 gene CACNA1C away from exon 8A that is upregulated in patients with Timothy Syndrome ⁸⁴. These ASOs correct Ca_v1.2 dysfunction in human iPSC cortical neurons *in vitro* and after transplantation into mouse brains.

Na_v1.2 gene therapy for Autism

The search for genetic drivers and risk factors for complex CNS diseases is revealing a role for many ion channels in human neurological indications, and mutations in Na_v1.2 channels have been found in autism spectrum patient populations, triggering work on gene therapies ¹⁰². Two independent groups of University California academics and their collaborators have published preprints revealing similar approaches to use CRISPR gene editing to activate wildtype alleles of Na_v1.2 and correct haploinsufficiency deficiency in patient-derived iPSC excitatory neurons and cerebral forebrain organoids ¹⁰³ and heterozygous Scn2a knock-in mice ¹⁰⁴. Gene therapy rescue increased Na_v1.2 gene and protein expression and inward Na⁺ currents and restored transcriptome gene expression profiles and iPSC excitatory neuron excitability *in vitro*, and AAV delivery to mice restored deficits in neuronal Ca²⁺ signalling, neurotransmission and excitability *in vivo*. Whilst very promising and including translational human iPSC and disease model data these papers still await peer review, and the applicability and safety of CRISPR gene editing as a human therapeutic approach for CNS diseases remains to be determined.

Retinal diseases

The eye has already been shown to be an easily accessible tissue suitable for gene therapy (see section Gene replacement therapies), and US academics are targeting several ion channels linked to retinal diseases. Mutations in K_v7.1 channels underly a monogenic form of inherited retinal degeneration (Leber congenital amaurosis type 16, LCA16), which is being corrected by genome base editing of the KCNJ13 gene using silica nanocapsules ⁸⁵. Editing of loss-of-function mutations in patient-derived fibroblasts and retinal iPSCs could produce functional channels (albeit with variable efficiency), and *in vivo* delivery of base editors to the eye restored vision in a LCA16 mouse genetic model (Figure 5). The same group has also used personalised gene therapy approaches to treat Best disease, a macular dystrophy caused by dominant negative missense mutations in the BEST1 gene that encodes a Ca²⁺-activated Cl⁻ channel. Gene augmentation, by transfection with a lentivirus vector incorporating the full-length bestrophin channel gene together with an endogenous retinal cell promoter to control transgene expression, could restore channel activity in patient-derived retinal iPSCs expressing some but not all bestrophin mutations, whereas CRISPR-Cas9 gene editing could correct all tested BEST1 mutant alleles ⁸⁶. The authors highlight the advantages in using patient-derived iPSCs to rapidly compare and optimise personalised gene therapies, but also note issues with their on-target efficiency and off-target genome effects.

Finally, researchers at the Lions Eye Institute in Western Australia have published on a KCNV2 gene therapy for cone rod dystrophy, a recessive inherited rare disease

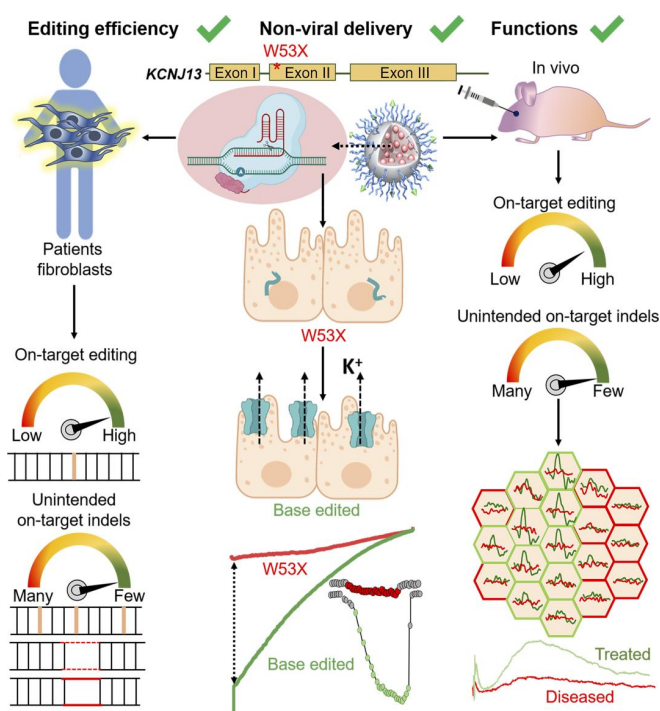


Figure 5 Base editing of $K_{ir}7.1$ channels can restore vision in genetic blindness disease. Adapted from data in ⁸⁵ and used under Creative Commons open access license 4.0 (<http://creativecommons.org/licenses/by/4.0/>) from an Open Access publication.

characterised by abnormal dark current and sight loss resulting from loss of heteromer formation between 'silent' $K_{ir}8.2$ subunits and $K_{ir}2.x$ channels ⁸⁷. While AAV8 vectors can infect retinal cells and are being used clinically, her group found that another serotype Anc80 was more effective, but constructs using either vector containing wildtype KCNV2 gene subunit could restore $K_{ir}8.2$ expression and retinal function for weeks-months after injection into the eyes of a mouse genetic model.

Optogenetic and chemogenetic applications

This topic is worth a blog on it's own, but I'll conclude with a brief overview of two other genetic techniques being used in drug discovery to leverage ion channel activity.

Optogenetics

Channelrhodopsins (ChRs) are prokaryotic light-activated ion channels with excitatory cationic or inhibitory anionic permeabilities, which have been used extensively in academia as tools to spatiotemporally manipulate excitability of many different types of mammalian cells *in vitro* and *in vivo*. The requirement for external illumination remains a clinical challenge, but is being addressed with miniaturised designs analogous to implantable cardiac pacemakers, and engineered ChRs with increased light

sensitivity, altered kinetics and longer wavelengths suitable for *in vivo* use. An obvious application is in ophthalmology, and four recent clinical trials used optogenetic systems delivered by AAVs to treat retinitis pigmentosa, choroideremia and Stargardt's disease ⁸⁸.

An early preclinical demonstration of optogenetics to treat epilepsy utilised a lentivirus containing an inhibitory halorhodopsin driven by the CaM kinase promoter to selectively transduce excitatory neurons and reduce action potential firing and seizures in cultured rodent neurons and organotypic brain slices after light stimulation ⁸⁹. Similar approaches have been extended to *in vivo* demonstrations (reviewed ^{90,91}), including a US group that used transgenic mice to selectively express a halorhodopsin in excitatory neurons or excitatory ChR in inhibitory GABAergic neurons to control seizures using an implanted EEG closed-loop optic fibre stimulation system *in vivo* ⁹², and an extension of this approach to non-human primates by UK researchers ⁹³. The UCL group mentioned in section 3.e showed that lentivirus transduction of excitatory neurons with either an engineered K_v gene therapy or a halorhodopsin optogenetic construct activated by an implanted laser light optic fibre could suppress seizures to a similar extent in mice *in vivo* ⁹⁴. Recently, US researchers provided human tissue validation of optogenetic control of seizures with AAV transfection of ChRs in hippocampal slices taken from epilepsy patients ⁹⁵.

Ongoing preclinical work and the clinical use of optogenetic reagents, neural stimulation devices and cardiac pacemakers suggests that clinical testing of optogenetic light stimulation and gene therapies to treat human diseases such as epilepsy and pain are not far away.

Chemogenetics

Chemogenetics involves the ectopic expression of engineered receptors that respond to non-cognate ligands to enable selective pharmacological activation in transduced cells and tissues. This approach of using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) has been validated preclinically for epilepsy using activation of engineered mAChR GPCRs by olanzapine ⁹⁶, but is also applicable to ligand-gated ion channels. Kriya Therapeutics are using a chimeric inhibitory $\alpha 7$ nAChR-Glycine receptor Cl^- channel edited to respond to the FDA-approved smoking cessation small molecule drug Varenicline (Figure 6), which normally activates homomeric $\alpha 7$ nAChRs, to reduce neuronal excitability after focal injection of AAVs, with a clinical stage program for trigeminal neuralgia in the clinic (KRIYA-748) and an IND-stage program for epilepsy (KRIYA-382) ⁹⁷.

In the UK a spin-out from UCL called Sania Therapeutics are using cell-specific AAVs to transduce motoneurons with a chemogenetic construct to control spasticity, but the identity of the ligand-gated ion channel and ligand remain shrouded in mystery, and their patent also mentions use of

KCC2 transporters, K_v channels and mAChRs. They have published preclinical data on their lead candidate SRx-C490 at the 2024 ASGCT conference showing that addition of ligand can reduce action potential firing in human iPSCs and neuromuscular junction organoids, and decrease spinal cord excitability and muscle spasms in mice ⁹⁸.

The examples in this section illustrate how traditional gene therapy approaches can be optimised to produce cell-

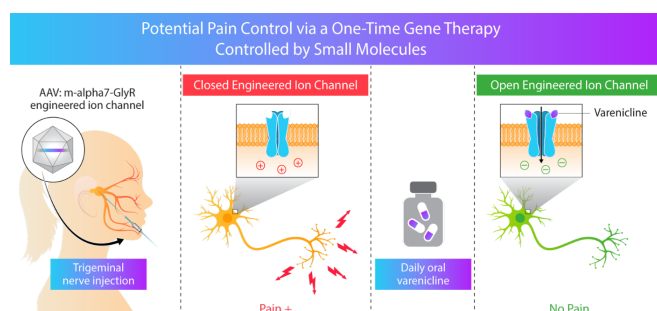


Figure 6 Schematic of KRIYA Therapeutics a7-Gly chemogenetic ionotropic platform for neurology. Adapted from from company website (<https://kriyatherapeutics.com/pipeline/neurology/>).

specific and activity-dependent changes in gene expression, and can also be combined with traditional small molecule pharmacology to create sophisticated and selective gene therapy platforms to treat a wide range of human diseases with high unmet medical need.

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