NEUROSCIENCE

NEUROSCIENCE FOREFRONT REVIEW

G. Morris, S. Schorge / Neuroscience 490 (2022) 309–314

Gene Therapy for Neurological Disease: State of the Art and Opportunities for Next-generation Approaches

Gareth Morris and Stephanie Schorge *

Department of Neuroscience, Physiology and Pharmacology, University College London, London, United Kingdom

Abstract—Gene therapy for rare monogenetic neurological disorders is reaching clinics and offering hope to families affected by these diseases. There is also potential for gene therapy to offer new and effective treatments for common, non-genetic disorders. Treatments for Parkinson's Disease are in clinical trials, and treatments for refractory epilepsies are due to enter first-in-human clinical trials in 2022. Gene therapies for these disorders are based on delivering genes that address the mechanism of the disease, not repairing a mutated gene. Similar 'mechanistic' gene therapies could offer treatments to a wide range of neurological and neuropsychiatric diseases where there is a known mechanism that could be restored using gene therapy. However, the permanent nature of most gene therapies is a serious drawback for translation of gene therapies to a wide-range of diseases because it could present risk of irreversible adverse effects. Several lines of research are aimed at developing gene therapy approaches that allow for the treatment to be turned on and off, including: using proteins activated by exogenous ligands, and promoters turned on by activators. We review these approaches and propose an overall de-risking strategy for gene therapy for common neurological and psychiatric diseases. This approach is based on using a temporary mRNA-based treatment to initially assess efficacy and safety of the planned manipulation, and only following with permanent, virally-delivered treatment if the approach appears safe and effective.

Key words: gene therapy, inducible promoters, epilepsy, microRNA, genetic therapy.

GENE THERAPY IS MOVING FROM THEORY TO CLINICS

In the last few years gene therapy treatments for rare monogenetic disorders have finally begun to reveal their promise, including Zolgensma for the severe neurological disorder SMA (NIHR, 2018; Day et al., 2021; Jensen et al., 2021). Most people, when asked about gene therapy, immediately think of this sort of gene replacement approach, where a disease caused by a known mutation is treated by delivering healthy copies of that gene.

However, the vast majority of patients affected by neurological or psychiatric diseases do not have obvious causative mutations in particular genes. Does gene therapy offer any hope for them? In the case of patients with Parkinson's Disease, there is already a clinical trial and years of slowly-accruing safety and efficacy data on the use of AAV-mediated delivery for dopamine-synthesising enzymes (Muramatsu et al., 2010; Sehara et al., 2017) and lentiviral delivery of dopamine (Palfi et al., 2014; 2018). While there is still work to be done, there is a potential treatment, and it does not rely upon patients having mutations that can be corrected in individual genes.

Similarly, gene therapy strategies are being developed to treat refractory focal epilepsies, based on findings in rodent models of acquired epilepsy that do not rely upon known mutations, but upon epileptogenic insults. Indeed, the spatially localised nature of gene therapy delivery to the brain renders it highly appropriate to focal epilepsies, where seizures arise from a relatively well-defined pathological 'epileptogenic zone', but less amenable to treatment of epilepsies with monogenic causes, where the entire brain is implicated in disease pathophysiology. These strategies, like those for Parkinson's, rely upon decades of neurophysiology showing how neurons change as epilepsy becomes established. As epilepsy develops, neuronal networks become too excitable, and gene therapy can deliver instructions to specific types of neurons in order to modulate their excitability and restore the balance between excitation and inhibition. Strategies used to date include antisense knockdown of *Adk* (Theofilas et al., 2011), and expression of *Bdnf* and *Fgf-2* (Bovolenta et al., 2010; Paradiso et al., 2011), *Gabra1* (Raol et al., 2006), *Gdnf* (Kanter-Schlifke et al., 2007),

*Corresponding author. Address: Department of Neuroscience, Physiology and Pharmacology, University College London, London WC1E 6BT, United Kingdom.
E-mail address: s.schorge@ucl.ac.uk (S. Schorge).

https://doi.org/10.1016/j.neuroscience.2022.03.010
0306-4522/

Table 1. A summary of current pre-clinical gene therapy strategies in focal refractory epilepsy

Therapeutic gene	Delivery vector	References
Adk (antisense)	AAV8	(Theofilas et al., 2011)
Fgf-2 and *Bdnf*	HSV	(Bovolenta et al., 2010; Paradiso et al., 2011)
Gabra1	AAV2	(Raol et al., 2006)
Gdnf	AAV2	(Kanter-Schlifke et al., 2007)
Kcc2	Lentivirus	(Magloire et al., 2019)
Kcna1	Lentivirus, AAV	(Wykes et al., 2012; Snowball et al., 2019)
Npy	AAV1/2, AAV1, AAV2	(Richichi et al., 2004; Sørensen et al., 2009; Noe et al., 2010)

Kcc2 (Magloire et al., 2019), *Kcna1* (Wykes et al., 2012; Snowball et al., 2019) *and Npy* (Richichi et al., 2004; Sørensen et al., 2009; Noe et al., 2010; see also Table 1). In essence, these are all different approaches to reversing the increase in network excitability that drives seizures, rather than attempts to correct a single genetic cause.

For both refractory focal epilepsy and Parkinson's disease there is justification for the risks inherent in invasive delivery of genetically modified viruses that will permanently change parts of the brain. In Parkinson's the inexorable progression of the disease, and lack of alternatives, is justification for the potential risks. In refractory epilepsy, we have justified the risks of the treatment by focussing on treating patients in our first-in-human trial (ClinicalTrials.gov Identifier: NCT04601974; EudraCT Number: 2019-000923-41) who are scheduled to have the area of the brain causing seizures surgically removed (Rosenow and Lüders, 2001; Jobst and Cascino, 2015). If adverse effects are observed, the treatment may be 'stopped' by carrying out the planned surgical removal of the treated tissue.

What is needed to offer the hope of gene therapies to people who have other neurological diseases? What are the barriers, and how might they be overcome? This review is based on our current understanding of the field and what we would wish to see in order to de-risk gene therapy for patients with common devastating neurological disorders. We focus on lessons from epilepsy where our expertise lies.

CURRENT GENE THERAPY APPROACHES IN FOCAL EPILEPSY

Epilepsy is not a rare disease. It presents a substantial global socioeconomic burden, affecting between 50 (WHO, 2019) and 70 (Ngugi et al., 2010) million people worldwide. Current frontline treatment is with anti-seizure medications (ASMs) that offer a blanket reduction in brain excitability, thereby aiming to restore the balance between excitation and inhibition. However, ASMs can be associated with severe adverse effects (Perucca and Gilliam, 2012), they do not address the non-seizure co-morbidities of epilepsy (Mula et al., 2021) and, above all, they are not adequately effective in 30% of people with epilepsy. This figure rises to roughly 75% in temporal lobe epilepsy, the most common refractory focal epilepsy (Schmidt and Loscher, 2005). Uncontrolled epilepsy can have devastating consequences including Sudden Unexpected Death in Epilepsy (SUDEP), with a crude estimated incidence of 1.16 in 1000 people with epilepsy (Thurman et al., 2014). The result is an urgent and unmet clinical need for new epilepsy therapies with superior efficacy and minimal side effect profiles.

There are several appealing characteristics of gene therapy approaches in neurological disease which make them a promising candidate for patients with refractory focal epilepsy (Kullmann et al., 2014). First, gene therapies provide very long-term expression of a therapeutic transgene, offering long-term benefit from a single intervention (Jensen et al., 2021). Transgene expression from adeno-associated virus (AAV) vectors has been documented for up to 10 years in humans (Chu et al., 2020) and 15 years in non-human primates (Sehara et al., 2017). Second, advances in promoter technology allow the control of cell-type transgene expression from viral vectors. Cell-specific expression can also be enhanced by incorporating microRNA binding sites into the transgene, to block its expression in cells expressing that microRNA. Third, viral vectors have a relatively restricted spatial spread, permitting more precise targeting of specified pathological brain networks such as the epileptogenic zone in focal epilepsies, whilst sparing distinct areas which are not implicated in disease progression (it should be noted that this property is actually a barrier to gene therapy in monogenic epilepsies where the entire brain is affected by a gene mutation, rather than a relatively restricted 'epileptogenic zone' as seen in focal epilepsies). As such, gene therapy offers the hope of a safe and permanent 'cure' for focal epilepsy and, in animal models, it is effective: several virally-delivered treatments show great promise *in vitro* and increasingly *in vivo* (Richichi et al., 2004; Raol et al., 2006; Kanter-Schlifke et al., 2007; Sørensen et al., 2009; Bovolenta et al., 2010; Noe et sl., 2010; Paradiso et al., 2011; Theofilas et al., 2011; Wykes et al., 2012; Magloire et al., 2019; Snowball et al., 2019). However, even the most enthusiastic supporters of gene therapy for epilepsy recognise that the treatments have risks, which without the fall back of surgery, would make these treatments difficult to justify.

One important limitation to current gene therapies is their permanence. Once delivered, there is no going back, as current gene therapies remain constitutively active – there is no 'on/off switch'. A related concern is that excessive expression of therapeutic transgenes may in itself be harmful. This is partially mitigated by the ability to modulate transgene expression by using stronger or weaker promoter systems (Nieuwenhuis et al., 2020) and different viral serotypes. Other obstacles remain, although experimental solutions are emerging. Concerns around oncogenesis due to viral integration are being addressed by development of nonintegrating lentiviral vectors (Yáñez-Muñoz et al., 2006) or use of AAVs which pose a low risk. Another concern is to restrict

the gene therapy only to cells participating in the pathology, thus far cell type specific promoters (e.g. CAMK2A for excitatory neurons) restrict expression to populations of neurons, but these are still not specific to the cells driving the seizures, and may also modify any local neurons that are not responsible for triggering seizures, potentially leading to off-target adverse effects. As a partial solution in the context of focal epilepsies, intraparenchymal delivery (i.e. injecting a small amount of gene therapy treatment directly into the focus) limits off target effects in distant non-epileptogenic parts of the brain and, as mentioned above, has the fall back of surgical resection (Rosenow and Lüders, 2001; Jobst and Cascino, 2015), as a rescue.

Finally, for any treatment delivered directly to the brain, there is the invasiveness of the approach, requiring (for focal delivery of gene therapies to targeted regions) stereotaxic surgery to drill a burr hole, and the insertion of an injection cannula. As transgene delivery technology improves, it may become possible to administer genetic therapies using peripheral applications, reducing the invasiveness of the approach and therefore opening up a more mainstream therapeutic application. However, the severity of neurological diseases often justifies the invasiveness of current delivery procedures. Indeed, the number of disorders for which invasive treatments, such as deep brain stimulation (DBS), are being considered suggests that if the efficacy of the treatment is sufficient and the disorder is refractory to other treatments, patients and clinicians are willing to accept invasive procedures for neurological (Camacho-Conde et al., 2022) and increasingly for psychiatric disorders (Graat et al., 2017).

Thus, if the safety of viral gene therapy continues to be established in clinics, and if invasive procedures are increasingly accepted for a growing number of neurological disorders, then the key step for gene therapy is to de-risk the approach to the point where a surgical fall back is not needed – to find a different way to rescue, or a different 'on/off switch'. Having a non-permanent option will allow gene therapy to be applied to a wider range of epilepsies and other neurological and psychiatric diseases.

For clarity, from a regulatory viewpoint, 'gene therapy' is defined as the removal, addition, or editing specifically of DNA, to treat a human disease. In the remainder of this article, we also use the term 'genetic therapy', which further incorporates approaches that specifically use or target RNAs to modulate gene expression in a therapeutic manner.

ADDING AN 'ON/OFF SWITCH': EXOGENOUSLY ACTIVATED PROTEINS

Perhaps the most obvious approach to conditional expression is conditional activation of the gene therapy product. Approaches such as optogenetics (Zemelman et al., 2002; Deisseroth et al., 2006; Häusser, 2014; Streng and Krook-Magnuson, 2019) or chemogenetics (Armbruster et al., 2007; Roth, 2017; Weston et al., 2019) allow delivery of a gene therapy agent that does nothing in the absence of an exogenous ligand, either light or a chemical respectively. Both show promise in models of epilepsy (Wykes et al., 2012; Lieb et al., 2019), but optogenetics relies upon expression of a non-human protein, and consequently presents the risk of triggering an immune response (the BBB can be compromised during seizures). Delivery of light to deep structures within the brain is also not trivial.

Chemogenetics is not as rapid as optogenetics, but does use human proteins – reducing immunogenicity – with relatively few changes and can be activated with an orally available ligand. This technology uses Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), which are engineered receptors that respond only to exogenously applied ligands. An example is hMD4i, this is a human muscarinic receptor with mutations deep within its binding pocket that change its ligand sensitivity, so that it is silent in the presence of endogenous acetylcholine, but can be activated by exogenous drugs. The initial ligand was CNO, which is not approved for use in humans, but recent studies (Weston et al., 2019) have identified a series of potential ligands that are used in patients, bind with high affinity, and in some cases have limited adverse effects. Importantly, the initial DREADD report was almost a proof of principle, and a rapidly evolving range of designer proteins is being developed (Magnus et al., 2019), including ligand gated channels (hM4 is g-protein coupled). All of these confer a degree of control over transgene expression but, if the exogenously expressed protein in the absence of ligand leads to some unexpected effect, that would remain irreversible. Moreover, a chemogenetic therapeutic approach would require patients to take daily medication to activate the system. An ideal therapeutic approach would remove the reliance on daily adherence to any kind of medication.

AN EXOGENOUS ON/OFF SWITCH TO CONTROL PROTEIN EXPRESSION

A more complete on/off switch would stop expression of the protein when not needed. Here the gene therapy remains silent and transcription is only activated when an exogenous activator is added. Various approaches are being developed in mammalian systems, and these have been recently reviewed (Kallunki et al., 2019). The general principle is to deploy simple gene regulation systems, often from bacteria, where a small molecule binding to a transcriptional activator turns on a promoter. The tetracycline dependent TetON/TetOFF systems are the best known and originators of these (Gossen and Bujard, 1992; Goverdhana et al., 2005). In many Cre-dependent mouse lines similar effects are obtained using tamoxifen to turn on expression of targeted genes (Metzger et al., 1995), and a trademarked GeneSwitch system uses low doses of the hormone mifepristone (Sirin and Park, 2003). A substantial limitation of these approaches is that these exogenous activators can have off target effects, including on regulation of other endogenous genes, so long term administration might be unac-

ceptable. What is needed is a system activated by a truly silent compound with good bioavailability.

A closely related route is to harness mechanisms within cells that regulate at the RNA level, for example building inducible synthetic RNA circuits (Wagner et al., 2018; Mc Cafferty et al., 2021). Here the regulation is not by activation of transcription but by controlling the reduction of translation by destabilising the mRNA unless an exogenous ligand (trimethoprim, an antibiotic) is added. But this system still requires expression of non-native proteins, which could trigger an immune response.

Many molecular biologists focus on concerns around leakiness or low levels of induction in the presence of the activator. For leakiness, it must be remembered that an inducible promoter with a low level of leak in the absence of the activator would be replacing current promoters that are constantly fully active, yet are already seen as promising and safe enough to progress towards clinical trials. While a leaky inducible promoter may not be perfect, it is a lot safer in pragmatic terms than a constitutively active one. For concerns about inducible promoters that produce only modest levels of expression, in epilepsy and in many other neurological and psychiatric disorders the aim is to return from pathological to normal activity – not to silence neurons altogether (indeed, if silencing were the aim, then there would be relatively little advantage over surgical resection). The aim of these gene therapies is a relatively minor modification of neuronal activity. In the case of neurons in an epileptic focus, we have found expression that drives a small but significant shift in the input–output curve of neurons is sufficient to stop seizure like activity, without noticeable effects on behaviour, even when treating the primary motor cortex (Wykes et al., 2012). In these cases, less robust induction may be an asset, whereas systems providing 1000-fold increases in expression may overwhelm and disrupt neuronal protein synthesis.

NON-PERMANENT GENETIC THERAPIES: ADVANTAGES OF RNA-BASED APPROACHES

Not all genetic therapies induce permanent changes, and there may be advantages to temporary gene therapy. A hallmark of epilepsy is that some patients after successful treatment with ASMs are able to withdraw from treatment, without recurrence of seizures. We have seen that after using over expression of Kv1.1 to reduce seizures, mice showed wide spread changes in their transcriptome, which suggested their neurons were returning from an epileptic fingerprint back towards a normal distribution of gene expression (Colasante et al., 2020; Lignani et al., 2020), providing evidence that gene therapy for epilepsy can correct dysregulation of a significant subset of genes.

The enormous investment in mRNA vaccines during the COVID pandemic has transformed mRNA synthesis, delivery and stability in clinical settings. The seminal work by Katalin Karikó and her collaborators on nucleoside modification of mRNA sequences reduces immunogenicity, and stabilises mRNA enough to provide protein expression without the need for a viral vector (Karikó et al., 2005, 2008; Sahin et al., 2014; Andries et al., 2015). The success of this approach, and its extensive safety in the vaccine rollout, raises the realistic prospect that therapeutic mRNA can be delivered directly. Gene delivery by mRNA is inherently transient and this is likely to preclude some therapeutic applications, but in others, the transient nature of the change, and the reduction of risk may be an enormous asset. An additional benefit of mRNA approaches is that, in vitro transcribed mRNA is not subject to the packaging size limitations imposed by AAV and LV vectors. Synthetic mRNAs up to 12 kb in length have been obtained (Karikó et al., 2008), raising the prospect of RNA-based genetic therapies for diseases in which the required transgene is too large to be packaged into viral vectors.

COMBINING SYNTHETIC MRNA AND VIRAL-BASED DELIVERY: A POSSIBLE ROUTE TO DE-RISK GENETIC THERAPIES FOR WIDER USE IN NEUROLOGICAL DISEASES?

The biggest regulatory concern about gene therapy is its permanence – there is no way to remove the treatment if it causes harmful adverse effects. How can we de-risk genetic therapies in order to make them a realistic prospect in diseases where physical removal of brain tissue is not an option?

We speculate that a possible solution could be a novel pre-clinical and translational strategy, combining both mRNA-based and virally-delivered approaches to take advantage of the properties of each. Broadly speaking, mRNA-based genetic therapies offer a faster, safer and, above all, transient therapeutic modality. Virally-delivered gene therapy is longer lasting but poses more risk due to its permanent nature. Therefore, a translational strategy would be to treat patients first with focal delivery of an RNA-based therapy and then, pending a successful therapeutic outcome, switch to a more permanent virally-delivered approach. The RNA-based approach is naturally transient, so if it is not beneficial or harmful, its effects will be self-limited and other treatment options can be pursued. If needed, additional synthetic degradation domains could provide an extra safety level or faster turnover. This would provide a rapid reversal if overexpression of synthetic mRNA proved to be harmful. If treatment is successful, but symptoms return as the mRNA decays, more permanent virally-delivered gene therapy can be administered. Further, some microRNA-based approaches seem to be disease-modifying in epilepsy, perhaps negating the need for permanent approaches (Jimenez-Mateos et al., 2012; Morris et al., 2019).

There are several practical considerations that would need to be addressed in order to bring this strategy to the clinic. One downside of this approach is that it would require two invasive surgeries to inject the treatments to the target region of the brain, however it is important to balance that against current clinical trials of deep brain stimulation which can involve permanent implanting of stimulating electrodes deep in the brain (recently reviewed in (Xu et al., 2020)). Another consideration is

the likely heterogeneity in transgene expression achieved by the two delivery methods, and experimental work will be required to balance the two. For example, modifications to mRNA chemistry can be made to alter their biological availability (Morris et al., 2021), and different viral serotypes and promoter systems can be used to modulate transgene expression from viruses (Nieuwenhuis et al., 2020). Finally, the two delivery methods have different cell-specificities and so this must be considered, possibly by using microRNA binding sites to confer cellular specificity via the same mechanism in each case. Using this method, it is possible that the same transgene could be delivered first by transient mRNA delivery and then, if it appears to be safe, using a longer-term viral application.

Gene therapy has the potential to transform treatment for a wide range of neurological diseases but, in its current form, has inherent risks which limit its clinical use. For situations where invasive treatments are warranted (i.e. any disorder where implanted electrodes are considered), the predominant risk of gene therapy is that, once administered, its effects are permanent and cannot be switched off. Here, we argue that the next generation of genetic therapies should target non-genetic diseases associated with discrete structures, and focus on developing strategies to minimise this risk, particularly by allowing reversibility. An intermediate step is to adopt a two-step process with a short acting mRNA-based treatment followed, where required and likely effective, by permanent virally delivered treatments.

FUNDING

GM is supported by an Emerging Leader Fellowship Award from Epilepsy Research UK (grant reference F2102 Morris). SS is supported by grants from the MRC and much of the work described here was carried out while she held a University Research Fellowship from the Royal Society.

REFERENCES

Andries O, Mc Cafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T (2015) N1-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. J Control Release 217:337–344. https://doi.org/10.1016/j.jconrel.2015.08.051.

Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL (2007) Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc Natl Acad Sci USA 104:5163–5168.

Bovolenta R, Zucchini S, Paradiso B, Rodi D, Merigo F, Mora GN, Osculati F, Berto E, Marconi P, Marzola A, Fabene PF, Simonato M (2010) Hippocampal FGF-2 and BDNF overexpression attenuates epileptogenesis-associated neuroinflammation and reduces spontaneous recurrent seizures. J Neuroinflamm 7:1–6.

Camacho-Conde JA, Gonzalez-Bermudez M del R, Carretero-Rey M, Khan ZU (2022) Brain stimulation: a therapeutic approach for the treatment of neurological disorders. CNS Neurosci Therap 28:5–18 Available at: https://onlinelibrary.wiley.com/doi/10.1111/cns.13769.

Chu Y, Bartus RT, Manfredsson FP, Warren Olanow C, Kordower JH (2020) Long-term post-mortem studies following neurturin gene therapy in patients with advanced Parkinson's disease. Brain 143:960–975.

Colasante G, Qiu Y, Massimino L, di Berardino C, Cornford JH, Snowball A, Weston M, Jones SP, Giannelli S, Lieb A, Schorge S, Kullmann DM, Broccoli V, Lignani G (2020) In vivo CRISPRa decreases seizures and rescues cognitive deficits in a rodent model of epilepsy Available at:. Brain 143:891–905. Available from: https://academic.oup.com/brain/article/143/3/891/5780426.

Day JW et al (2021) Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy in patients with two copies of SMN2 (STR1VE): an open-label, single-arm, multicentre, phase 3 trial Available at:. Lancet Neurol 20:284–293. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1474442221000016.

Deisseroth K, Feng G, Majewska AK, Miesenbock G, Ting A, Schnitzer MJ (2006) Next-generation optical technologies for illuminating genetically targeted brain circuits. J Neurosci 26:10380–10386.

Gossen M, Bujard H (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci USA 89:5547–5551.

Goverdhana S, Puntel M, Xiong W, Zirger JM, Barcia C, Curtin JF, Soffer EB, Mondkar S, King GD, Hu J, Sciascia SA, Candolfi M, Greengold DS, Lowenstein PR, Castro MG (2005) Regulatable gene expression systems for gene therapy applications: progress and future challenges. Mol Ther 12:189–211.

Graat I, Figee M, Denys D (2017) The application of deep brain stimulation in the treatment of psychiatric disorders. Int Rev Psych 29:178–190.

Häusser M (2014) Optogenetics: the age of light. Nature Publishing Group. p. 11. Available at: https://www.nature.com/nmeth/journal/v11/n10/pdf/nmeth.3111.pdf [Accessed July 5, 2017].

Jensen TL, Gøtzsche CR, Woldbye DPD (2021) Current and future prospects for gene therapy for rare genetic diseases affecting the brain and spinal cord. Front Mol Neurosci 14.

Jimenez-Mateos EM, Engel T, Merino-Serrais P, McKiernan RC, Tanaka K, Mouri G, Sano T, O'Tuathaigh C, Waddington JL, Prenter S, Delanty N, Farrell MA, O'Brien DF, Conroy RM, Stallings RL, DeFelipe J, Henshall DC (2012) Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects Available at:. Nat Med 18:1087–1094. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3438344&tool=pmcentrez&rendertype=abstract.

Jobst BC, Cascino GD (2015) Resective epilepsy surgery for drug-resistant focal epilepsy: a review. JAMA - J Am Med Assoc 313:285–293.

Kallunki T, Barisic M, Jäättelä M, Liu B (2019) How to choose the right inducible gene expression system for mammalian studies? Cells 8:1–16.

Kanter-Schlifke I, Georgievska B, Kirik D, Kokaia M (2007) Seizure suppression by GDNF gene therapy in animal models of epilepsy. Mol Ther 15:1106–1113. https://doi.org/10.1038/sj.mt.6300148.

Karikó K, Buckstein M, Ni H, Weissman D (2005) Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity 23:165–175.

Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, Weissman D (2008) Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther 16:1833–1840.

Kullmann DM, Schorge S, Walker MC, Wykes RC (2014) Gene therapy in epilepsy—is it time for clinical trials? Available at: Nat Rev Neurol 10:300–304. Available from: http://www.nature.com/articles/nrneurol.2014.43.

Lieb A, Weston M, Kullmann DM (2019) Designer receptor technology for the treatment of epilepsy. EBioMedicine 43:641–649. https://doi.org/10.1016/j.ebiom.2019.04.059.

Lignani G, Baldelli P, Marra V (2020) Homeostatic plasticity in epilepsy. Front Cell Neurosci 14.

Magloire V, Cornford J, Lieb A, Kullmann DM, Pavlov I (2019) KCC2 overexpression prevents the paradoxical seizure-promoting action of somatic inhibition. Nat Commun 10. https://doi.org/10.1038/s41467-019-08933-4.

Magnus CJ, Lee PH, Bonaventura J, Zemla R, Gomez JL, Ramirez MH, Hu X, Galvan A, Basu J, Michaelides M, Sternson SM (2019) Ultrapotent chemogenetics for research and potential clinical applications. Science 364.

Mc Cafferty S, de Temmerman J, Kitada T, Becraft JR, Weiss R, Irvine DJ, Devreese M, de Baere S, Combes F, Sanders NN (2021) In vivo validation of a reversible small molecule-based switch for synthetic self-amplifying mRNA regulation. Mol Ther 29:1164–1173. https://doi.org/10.1016/j.ymthe.2020.11.010.

Metzger D, Clifford J, Chiba H, Chambon P (1995) Conditional site-specific recombination in mammalian cells using a ligand-dependent chimeric Cre recombinase. Proc Natl Acad Sci USA 92:6991–6995.

Morris G, O'Brien D, Henshall DC (2021) Opportunities and challenges for microRNA-targeting therapeutics for epilepsy. Trends Pharmacol Sci 42:605–616. https://doi.org/10.1016/j.tips.2021.04.007.

Morris G, Reschke CR, Henshall DC (2019) Targeting microRNA-134 for seizure control and disease modification in epilepsy. EBioMedicine 45:646–654. https://doi.org/10.1016/j.ebiom.2019.07.008.

Mula M, Kanner AM, Jetté N, Sander JW (2021) Psychiatric comorbidities in people with epilepsy Available at:. Neurol Clin Practice 11:e112–e120. Available from: http://www.ncbi.nlm.nih.gov/pubmed/33842079.

Muramatsu S-I, Fujimoto KI, Kato S, Mizukami H, Asari S, Ikeguchi K, Kawakami T, Urabe M, Kume A, Sato T, Watanabe E, Ozawa K, Nakano I (2010) A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease. Mol Ther 18:1731–1735. https://doi.org/10.1038/mt.2010.135.

Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW, Newton CR (2010) Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. Epilepsia 51:883–890.

Nieuwenhuis B, Haenzi B, Hilton S, Carnicer-Lombarte A, Hobo B, Verhaagen J, Fawcett JW (2020) Optimization of adeno-associated viral vector-mediated transduction of the corticospinal tract: comparison of four promoters. Gene Therapy 28:56–74. https://doi.org/10.1038/s41434-020-0169-1.

NIHR (2018) AVXS-101 for spinal muscular atrophy. NIHR innovation observatory evidence briefing:1–8.

Noe F, Vaghi V, Balducci C, Fitzsimons H, Bland R, Zardoni D, Sperk G, Carli M, During MJ, Vezzani A (2010) Anticonvulsant effects and behavioural outcomes of rAAV serotype 1 vector-mediated neuropeptide y overexpression in rat hippocampus. Gene Ther 17:643–652.

Palfi S et al (2014) Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. Lancet 383:1138–1146.

Palfi S, Gurruchaga JM, Lepetit H, Howard K, Ralph GS, Mason S, Gouello G, Domenech P, Buttery PC, Hantraye P, Tuckwell NJ, Barker RA, Mitrophanous KA (2018) Long-term follow-up of a phase I/II study of ProSavin, a lentiviral vector gene therapy for Parkinson's disease. Hum Gene Ther Clin Dev 29:148–155.

Paradiso B, Zucchini S, Su T, Bovolenta R, Berto E, Marconi P, Marzola A, Navarro Mora G, Fabene PF, Simonato M (2011) Localized overexpression of FGF-2 and BDNF in hippocampus reduces mossy fiber sprouting and spontaneous seizures up to 4 weeks after pilocarpine-induced status epilepticus. Epilepsia 52:572–578.

Perucca P, Gilliam FG (2012) Adverse effects of antiepileptic drugs Available at:. Lancet Neurol 11:792–802. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1474442212701539.

Raol YSH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR (2006) Enhancing GABA A receptor α1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. J Neurosci 26:11342–11346.

Richichi C, Lin EJD, Stefanin D, Colella D, Ravizza T, Grignaschi G, Veglianese P, Sperk G, During MJ, Vezzani A (2004) Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. J Neurosci 24:3051–3059.

Rosenow F, Lüders H (2001) Presurgical evaluation of epilepsy.

Roth BL (2017) Use of DREADDS. Neuron 89:683–694.

Sahin U, Karikó K, Türeci Ö (2014) MRNA-based therapeutics-developing a new class of drugs. Nat Rev Drug Discovery 13:759–780.

Schmidt D, Loscher W (2005) Drug resistance in epilepsy: putative neurobiologic and clinical mechanisms. Epilepsia 46:858–877.

Sehara Y, Fujimoto K-I, Ikeguchi K, Katakai Y, Ono F, Takino N, Ito M, Ozawa K, Muramatsu S-I (2017) Persistent expression of dopamine-synthesizing enzymes 15 years after gene transfer in a primate model of Parkinson's disease. Hum Gene Ther Clin Dev 28:74–79.

Sirin O, Park F (2003) Regulating gene expression using self-inactivating lentiviral vectors containing the mifepristone-inducible system. Gene 323:67–77.

Snowball A, Chabrol E, Wykes RC, Shekh-Ahmad T, Cornford JH, Lieb A, Hughes MP, Massaro G, Rahim AA, Hashemi KS, Kullmann DM, Walker MC, Schorge S (2019) Epilepsy gene therapy using an engineered potassium channel. J Neurosci 39:3159–3169.

Sørensen AT, Nikitidou L, Ledri M, Lin E-J, During MJ, Kanter-Schlifke I, Kokaia M (2009) Hippocampal NPY gene transfer attenuates seizures without affecting epilepsy-induced impairment of LTP. Exp Neurol 215:328–333. https://doi.org/10.1016/j.expneurol.2008.10.015.

Streng ML, Krook-Magnuson E (2019) Excitation, but not inhibition of the fastigial nucleus provides powerful control over temporal lobe seizures. J Physiol. JP278747 Available at: https://onlinelibrary.wiley.com/doi/abs/10.1113/JP278747.

Theofilas P, Brar S, Stewart KA, Shen HY, Sandau US, Poulsen D, Boison D (2011) Adenosine kinase as a target for therapeutic antisense strategies in epilepsy. Epilepsia 52:589–601.

Thurman DJ, Hesdorffer DC, French JA (2014) Sudden unexpected death in epilepsy: assessing the public health burden. Epilepsia 55:1479–1485.

Wagner TE, Becraft JR, Bodner K, Teague B, Zhang X, Woo A, Porter E, Alburquerque B, Dobosh B, Andries O, Sanders NN, Beal J, Densmore D, Kitada T, Weiss R (2018) Small-molecule-based regulation of RNA-delivered circuits in mammalian cells. Nat Chem Biol 14:1043–1050. https://doi.org/10.1038/s41589-018-0146-9.

Weston M, Kaserer T, Wu A, Mouravlev A, Carpenter JC, Snowball A, Knauss S, von Schimmelmann M, During MJ, Lignani G, Schorge S, Young D, Kullmann DM, Lieb A (2019) Olanzapine: a potent agonist at the hM4D(Gi) DREADD amenable to clinical translation of chemogenetics. Sci Adv 5:4–9.

WHO (2019) Epilepsy Factsheet.

Wykes RC, Heeroma JH, Mantoan L, Zheng K, MacDonald DC, Deisseroth K, Hashemi KS, Walker MC, Schorge S, Kullmann DM (2012) Optogenetic and potassium channel gene therapy in a rodent model of focal neocortical epilepsy. Sci Transl Med 4:161ra152. Available at: https://stm.sciencemag.org/lookup/doi/10.1126/scitranslmed.3004190.

Xu L, Nan J, Lan Y (2020) The nucleus accumbens: a common target in the comorbidity of depression and addiction. Front Neural Circuits 14.

Yáñez-Muñoz RJ, Balaggan KS, MacNeil A, Howe SJ, Schmidt M, Smith AJ, Buch P, MacLaren RE, Anderson PN, Barker SE, Duran Y, Bartholomae C, von Kalle C, Heckenlively JR, Kinnon C, Ali RR, Thrasher AJ (2006) Effective gene therapy with nonintegrating lentiviral vectors. Nat Med 12:348–353.

Zemelman BV, Lee GA, Ng M, Miesenböck G (2002) Selective photostimulation of genetically chARGed neurons. Neuron 33:15–22.

(Received 8 December 2021, Accepted 9 March 2022)
(Available online 16 March 2022)