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Bringing balance to the force–regulatable gene therapy for epilepsy

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Epilepsy is a chronic disorder of the brain affecting 1% of the population, making it one of the most common neurological conditions [1]. It is characterised by recurrent seizures that may be partial or generalised to the entire brain and sometimes accompanied by loss of consciousness forming a severe handicap to a healthy lifestyle. It has no cure and an estimated 30% of patients do not respond to antiepileptic drugs, the benefit of which is often mitigated by multiple side effects and reoccurring seizures despite treatment [2, 3]. In pharmaco-resistant epilepsies, surgical resection is sometimes the only effective treatment, and although it has a high chance of success, the problem is that it might also affect eloquent cortex surrounding the epileptic focus [4].

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On the path to finding new cures, gene therapy using viral vectors to deliver therapeutic genetic material to the epileptic focus has been of interest [5, 6]. Gene delivery to the brain is challenging given the physical barriers in the form of the skull and the selective blood–brain barrier. This is further confounded by the intricate anatomy and size of arguably the most complex organ of the body. However, over the past decade steady progress has been made through improvements in vector design, discovery of vectors that cross the blood–brain barrier and access to advanced surgical techniques. Given the vast number of neurological conditions potentially amenable to gene therapy, these are welcome advancements. Life-saving, life-changing or quality of life enhancing gene therapy clinical trials for such conditions [7, 8, 9, 10] have further fuelled the appetite to develop this treatment modality.

The foundations of gene therapy for epilepsy have been laid through significant studies into the basic biological mechanisms underlying the condition, and the development of more clinically relevant animal models that are useful for pre-clinical assessment. Such rodent models are ideally based on a defined seizure focus that is not adequately treated by current anti-epileptic drugs, and are induced through either chemoconvulsant or electrically induced means. Over the past 15 years, various studies of gene therapy for epilepsy have used a range of approaches and viral vectors such as adenovirus, adeno-associated virus and lentivirus vectors to deliver the therapeutic nucleic acids coding for peptides, growth factors, neurotrophic factors, channel proteins and receptors, as previously reviewed [5]. Focal epileptic seizures are in many ways well suited to gene therapy. The defined area of the epileptogenic lesion means the therapeutic vector is not required to spread extensively from the site of injection. Containment of the vector within this relatively small area may diminish risks associated with an immune response. A further unique and significant safety advantage is that if limited or no therapeutic efficacy is measured, or an adverse event is experienced, then the transduced region could be surgically resected (dependent on distance from the eloquent cortex) offering a safety net and 'backup' therapy. However, attempting to reconfigure the modulation between excitation and inhibition of neuronal firing is a fine balancing act and one that requires careful consideration, especially, in the context of an irreversible gene therapy approach. While opto- or chemogenetic approaches have been investigated, the clinical practicalities around how the light or ligand trigger would be administered and off-targets effects in the brain remain concerns.

A recent study by Lieb et al. describes the design of a vector construct that offers the required refinement of gene therapy necessary to overcome these hurdles [11]. The authors conducted a pre-clinical study using a lentiviral vector-based approach that delivers the codon-optimised genes for the alpha and beta subunits of the glutamate-gated chloride ion channel (GluCl) derived from *Caenorhabditis elegans*. GluCl senses extrasynaptic increases in glutamate in the build-up and period of seizures and opens a channel permeable to Cl^- ions. Cl^- permeable ion channels normally cause neuronal hyperpolarisation and also shunt excitatory currents, leading to inhibition of firing. Furthermore, a previously described targeted gain-of-function mutation L9'F in the alpha-

subunit [12] further enhanced the sensitivity of the GluCl to glutamate (eGluCl). Notably, the EC50 of the enhanced GluCl channel is ~10 µM, which makes it perfectly suited for on-demand activation as a result of extracellular glutamate elevations during impending seizures. This closed-loop system that circumvents the need for opto- or chemogenetic based light or ligand control, respectively, was tested in two rat models of epilepsy: chemoconvulsant pilocarpine induced seizures, and tetanus toxin induced chronic focal neocortical epilepsy. The former is an acute model whereas the latter develops epilepsy over several weeks.

The study by Lieb et al. follows a two-decade effort by the Kullmann, Walker, Schorge groups in developing adequate models and wireless procedures for streaming and analysis of ECoGs (electroencephalocorticograms) via telemetry [13, 14, 15, 16]. Remarkably in both models, injection of eGluCl lentivector in defined cortical regions reduced the absolute number of seizures. This effect lasted over 3 weeks and was observed consistently amongst all animals tested. Furthermore, the treatment did not seem to alter basic motor function assessed in rotarod and elevated grid experiments. Control animals treated with a lentivector solely expressing green fluorescent protein were not protected against seizures. Lieb et al. have thus provided evidence that this autoregulatory gene therapy approach to epilepsy is both therapeutic and safe in rat models. However, the cellular mechanisms leading to seizure attenuation remain unclear. The sudden reduction in firing of pyramidal neurons caused by eGluCl activation implies a Cl^- conductance. As discussed in the paper the anti-seizure property is likely to involve a form of shunting inhibition, whilst the contribution of hyperpolarization is less clear. Unfortunately, the authors did not report on the reversal potential of glutamate-evoked currents in transduced neurones. Furthermore, confirmation of the localisation of eGluCl will require serial electron microscopy analysis to show that expression is mainly extrasynaptic. However, the finding that eGluCl did not affect normal circuit function is reassuring. It will also be important to assess the effect of such therapy on cognitive function and space navigation, as these are often altered in epileptic patients.

Is there room for further potential finesse and improvements? Utilising lentiviral vectors in clinical trials has a strong track record that includes successful ex vivo haematopoietic stem cell gene therapy for paediatric

neurodegenerative conditions [9, 10]. In such cases, stable integration of the transgene is required for sustained expression in rapidly dividing target cells. However, the integrating nature of lentiviral vectors does raise the spectre of possible insertional mutagenesis, as observed in clinical trials using retroviral vectors [17]. Although the risk of insertional mutagenesis in post-mitotic neurons is unknown, there is clinical precedent for direct in vivo injections into the brain of Parkinson's disease patients using the equine infectious anaemia lentiviral vector system [8]. While this supports the integrating HIV-1 based lentiviral vector used by Lieb et al., should a non-integrating vector that offers diminished risk of mutagenesis also be considered? Lentiviral vectors carrying a mutation in the integrase gene rendering them integration-deficient have been shown to efficiently transduce post-mitotic neurons in the brain and produce sustained marker gene expression through episomal expression [18, 19]. While Lieb et al. have demonstrated that the eGluCl is apparently safe in rats, do these sequences derived from invertebrate *C. elegans* pose an issue for clinical use? Furthermore, the authors report an approximate 700 μm spread of the viral vector from the site injection in the primary motor cortex. How would this translate to the larger human brain? It may be that a single injection may not provide enough spread of the vector in the human brain to transduce the target lesion area, but previous clinical trials have successfully shown that multiple injections of vector into the brain are well-tolerated [20]. Finally, there is the potential to use ivermectin (or one of the related drugs) as an adjunct to the gene therapy to increase the gain of the treatment.

In summary, Lieb et al. have provided proof-of-concept of a safe and autoregulatable gene therapy approach for epilepsy. This represents a significant and exciting step forward for the possible treatment of epilepsy through gene therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Shorvon SD. Epidemiology, classification, natural history, and genetics of epilepsy. Lancet. 1990;336:93–6.

2. Picot MC, Baldy-Moulinier M, Daures JP, Dujols P, Crespel A. The prevalence of epilepsy and pharmacoresistant epilepsy in adults: a population-based study in a Western European country. Epilepsia. 2008;49:1230–8.

3. Sisodiya SM, Lin WR, Harding BN, Squier MV, Thom M. Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. Brain. 2002;125:22–31.

4. Perry MS, Duchowny M. Surgical versus medical treatment for refractory epilepsy: outcomes beyond seizure control. Epilepsia. 2013;54:2060–70.

5. Kullmann DM, Schorge S, Walker MC, Wykes RC. Gene therapy in epilepsy-is it time for clinical trials? Nat Rev Neurol. 2014;10:300–4.

6. Bauer S, van Alphen N, Becker A, Chiocchetti A, Deichmann R, Deller T, et al. Personalized translational epilepsy research — novel approaches and future perspectives: Part II: Experimental and translational approaches. Epilepsy Behav. 2017;76:7–12.

7. Mendell JR, Al-Zaidy S, Shell R, Arnold WD, Rodino-Klapac LR, Prior TW, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. N Engl J Med. 2017;377:1713–22.

8. Palfi S, Gurruchaga JM, Ralph GS, Lepetit H, Lavisse S, Buttery PC, et al. 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. 2014;383:1138–46.

9. Biffi A, Montini E, Lorioli L, Cesani M, Fumagalli F, Plati T, et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science. 2013;341:1233158.

10. Eichler F, Duncan C, Musolino PL, Orchard PJ, De Oliveira S, Thrasher AJ, et al. Hematopoietic stem-cell gene therapy for cerebral adrenoleukodystrophy. N Engl J Med. 2017;377:1630–8.

11. Lieb A, Qiu Y, Dixon CL, Heller JP, Walker MC, Schorge S et al. Biochemical autoregulatory gene therapy for focal epilepsy. *Nat Med* 2018.
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12. Frazier SJ, Cohen BN, Lester HA. An engineered glutamate-gated chloride (GluCl) channel for sensitive, consistent neuronal silencing by ivermectin. J Biol Chem. 2013;288:21029–42.

13. Wykes RC, Heeroma JH, Mantoan L, Zheng K, MacDonald DC, Deisseroth K, et al. Optogenetic and potassium channel gene therapy in a rodent model of focal neocortical epilepsy. Sci Transl Med. 2012;4:161ra152.

14. Katzel D, Nicholson E, Schorge S, Walker MC, Kullmann DM. Chemical-genetic attenuation of focal neocortical seizures. Nat Commun. 2014;5:3847.

15. Scimemi A, Schorge S, Kullmann DM, Walker MC. Epileptogenesis is associated with enhanced glutamatergic transmission in the perforant path. J Neurophysiol. 2006;95:1213–20.

16. Chandler KE, Princivalle AP, Fabian-Fine R, Bowery NG, Kullmann DM, Walker MC. Plasticity of GABA(B) receptor-mediated heterosynaptic interactions at mossy fibers after status epilepticus. J Neurosci.

2003;23:11382–91.

17. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest. 2008;118:3143–50.

18. Yanez-Munoz RJ, Balaggan KS, MacNeil A, Howe SJ, Schmidt M, Smith AJ, et al. Effective gene therapy with nonintegrating lentiviral vectors. Nat Med. 2006;12:348–53.

19. Rahim AA, Wong AM, Howe SJ, Buckley SM, Acosta-Saltos AD, Elston KE, et al. Efficient gene delivery to the adult and fetal CNS using pseudotyped non-integrating lentiviral vectors. Gene Ther. 2009;16:509–20.

20. Souweidane MM, Fraser JF, Arkin LM, Sondhi D, Hackett NR, Kaminsky SM, et al. Gene therapy for late infantile neuronal ceroid lipofuscinosis: neurosurgical considerations. J Neurosurg Pediatr. 2010;6:115–22.