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Review Article

Recent Trends and Advancements in Experimental Epilepsy Therapeutics

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Abstract

Over the past few decades, alternative therapies for treating epilepsy have been a hot research topic. While the primary treatment option for epilepsy is anticonvulsants, one-third of patients with epilepsy do not respond to them. In such cases, the recommended alternative would be to try a surgical method, which brings about a fair share of risks and other issues, and even then not all patients are eligible for surgery. Hence, alternative therapies that are less invasive, effective, and safe must be developed to improve seizure management. This paper discusses some of the most promising strategies, which include antisense therapy, stem cell therapies, and gene therapies which include different proteomic approaches, Optogenetics, and Chemogenetics as a novel therapy that could one day be pushed into human clinical trials and if successful, into the clinic settings. The different in-vivo studies, especially those including Neuropeptide Y (NPY) and Potassium Voltage-Gated Channel (Kv1.1) have shown extremely promising results in reducing the frequency and duration of seizures. As of this date, there is only one gene therapy clinical trial for epilepsy; using a lentiviral approach to incorporate an engineered potassium (K+) channel (EKC). The other approaches though promising must be finely tuned and more in-vivo studies must be conducted to augment the need for pushing them into clinical trials to improve the quality of life for people with epilepsy. While the burden of epilepsy may have reduced over the past few decades due to improvements in the anticonvulsants, the need for these innovative treatments is imminent as they will improve the quality of life of those with refractory epilepsy.

Keywords: *Epilepsy; Refractory Epilepsy; Seizures; Antisense Therapy; Stem Cell Therapy; Gene Therapy; Optogenetics; Chemogenetics*

Introduction

Epilepsy is clinically defined as a neurological brain disease that is characterized by recurrent seizures, which are an imbalance or disruption in the homeostatic electric activity of the brain, leading to involuntary movements involving different parts of the body [1]. Depending on the area of the brain affected, seizures may manifest different symptoms. They may range from transient staring spells to severe jerking and shaking. The International League Against Epilepsy (ILAE) classified seizures depending on their site of origin and the symptoms they manifest [2]. About 40% of epilepsy cases are idiopathic; they have no known cause. Symptomatic epilepsies are genetic or acquired conditions that may have arisen due to a particular injury [3]. Under the genetic relations, chromosomal disorders are linked to Angelman Syndrome and 4P syndrome or Wolf-Hirschhorn syndrome [4]. There are also metabolic anomalies that are linked to the prevalence of seizures and epilepsy syndromes such as Creatine Transporter deficiency, Glut 1 (Glucose Transport) Deficiency Syndrome, Pyridoxine-dependent epilepsy, and Pyridoxal 5'-phosphate(P5P)-dependent epilepsy [5]. Structural abnormalities are well known to cause neurodevelopment delays and are a major cause of refractory epilepsy. Acquired epilepsies which, as the name suggests are due to acquired conditions, such as head trauma due to accidents or falls, provoked stimuli such as flashes or certain frequencies; there are a host of etiological factors that can be linked to acquired epilepsy [3]. The Quality of Life of those with epilepsy often decreases upon the reoc-

currence of seizures. It also worsens due to the side effects of the anticonvulsants, the side effects of epilepsy in itself and the unwarranted stigma that surrounds being epileptic. The mental health of those with epilepsy often deteriorates after the reoccurrence of seizures [6].

Refractory epilepsy is one of the biggest burdens of a large group of people with epilepsy. It is defined as the type of epilepsy where the seizures cannot be managed by anticonvulsants Unexpected Death in Epilepsy (SUDEP) is defined as, 'deaths in people with epilepsy that are not caused by drowning, injury, or other known cause' It was estimated that those with epilepsy have an ~35% lifetime risk of SUDEP. Despite the long-standing application of anticonvulsants, nearly 30% of epileptic patients remain unresponsive to them. In such cases, other approaches work either in conjunction with anticonvulsants as an adjunct therapy or as another focussed approach. Some of the adjunct therapies involve the ketogenic diet and neurostimulation. Surgical techniques are a common option in addressing refractory epilepsy [7].

The need for new therapeutics not just for epilepsy, but a host of other diseases is imminent. There are over thousands of clinical trials completed and in progress for these approaches for different diseases. Gene therapy has been a concept since the 1970s and it involves the use of genetic material to alter the genetic makeup and ultimately cure certain diseases, especially those that have a genetic connection to them [8]. Antisense therapy attempts to block the gene expression of specific sequences with the help of small nucleotide sequences that are complementary to the target sequence. Stem Cell Therapy has been considered as a very optimistic concept that attempts to improve the cognitive state of those with epilepsy, highlight some biomarkers common in the pathogenic state, test out new anticonvulsants, and also act as a preventative barrier against chronic epilepsy. With improvements being made in the field of molecular biology, we will soon see rapid and drastic improvements in how we can reduce the seizure frequency and duration and improve the overall quality of life. This review aims to go over some of the most promising novel therapeutics designed to reduce seizure frequency and improve the overall quality of life of those with epilepsy.

Experimental approaches

Antisense therapy

Antisense oligonucleotides (ASO) are oligomers of nucleotides that act on the mRNA transcript of the target gene, which ultimately affects the protein expression [9]. The FDA has approved 9 drugs to treat diseases such as retinitis, spinal muscular atrophy, and other genetic disorders [10]. There are ongoing projects for several neurological disorders such as Parkinson's Disease, ALS, Alzheimer's Disease, and others. ASO therapy is being considered as a promising alternative therapeutic approach for several disorders. Since its initial application in the late 1990s, ASO technology has improved its efficiency and its mode of action. Depending on the target, there are different mechanisms of action; degradation of RNAse H1, RNA Interference (RNAi), and sterically blocking the translation machinery [10].

Angelman Syndrome (AS) is a genetic neurobehavioral condition that presents with uncoordinated jerky movements, learning disabilities, language impairment and seizures 85% of patients experience seizures during the first few years, either tonic-clonic or myoclonic [4]. In AS patients, the 15q11-13 region of the maternal chromosome experiences a de novo mutation rendering it dysfunctional. This region of the chromosome bears the *UBE3A* gene that codes for ubiquitin protein ligase E3A, which is responsible for the regulation of proteostasis. AS occurs when there is at least one copy of the *UBE3A* gene that is active and expressed normally. The paternal copy of the *UBE3A* gene is imprinted with the help of a long non-coding RNA called *UBE3A-ATS*). The ASO approach in AS was designed to reduce the levels of *UBE3A-ATS*, upon which there was a significant reduction when applied to mice models and *in-vitro* models. It was also shown that upon employing this system, there was a rescue in the levels of UBE3A and improvement in the cognitive deficits. Further research is needed to understand how the paternal expression of UBE3A can be activated [11].

In terms of epilepsy, ASOs are being used to target multiple genes that are affected by this disease. The *SCN8A* gene codes for the sodium channel (NaV1.6), and mutations in this gene lead to the prevalence of aggressive and frequent seizures [12]. The medical term for this diagnosis is SCN8A encephalopathy, affecting several children in the United States. This type of epilepsy is refractory and often leads to cognitive impairment and delayed development. Nav1.6 is an important modulator of the excitatory feature of neurons. Mutations in this gene usually lead to overexpression and consequently, hyperexcitability of the neurons. ASOs designed in this approach aim to reduce the levels of *Scn8a* expression by targeting the 3'-UTR (Untranslated Region) of the gene. Indeed, the study showed that by reducing the elevated activity of SCN8A, there was a delay in the onset of seizures. In separate studies, it was shown that reduction of Nav1.6 expression would provide greater protection against spontaneous seizures *in-vivo* [12].

Another gene, *SCN1A*, when mutated causes Dravet Syndrome (DS), another developmental epileptic encephalopathy [13]. *SCN1A* codes for the sodium channel Nav1.1 and mutation in this gene lead to the insufficient expression of the channel. Specific ASOs were identified to increase the expression of the *Scn1a* transcript, and upon this expression, there was a significant reduction of tonic-clonic seizures and incidence of SUDEP [13].

Over the past few years, many technological advancements have improved the efficiency and of ASOs. The ASO treatment is now more specific, less toxic, and has better drug delivery approaches than a few years ago. ASOs differ from traditional gene therapy in essence it is non immunogenic and reversible [13]. It is also being incorporated in several other neurological disorders such as Spinal Muscular Atrophy, Huntington's Disease, and Duchenne muscular dystrophy [13].

Stem cell therapy

Stem cells can be considered as an extremely hopeful therapeutic approach due to their ability to proliferate, regenerate, and differentiate [14]. So far, the only stem cell therapy that has been FDA-approved is hematopoietic stem cell transportation or a bone-marrow transplant (Aly, 2020). All other therapies are considered experimental. There are two main objectives to using stem cells in the treatment of epilepsy. The first is to serve as a prophylactic safeguard against chronic epilepsy and the second is to enhance the cognitive state after the occurrence of the disease. One of the cell therapies being studied to improve seizure management is the transplant of neural stem cells (NSCs) to facilitate the development and differentiation of interneurons and astrocytes. in the brain [14]. Researchers, using *in-vivo* models have identified that NSCs expanded from specific sections of the brain such as the Subventricular Zone (SVZ) that contains neural stem cells, and Medial Ganglionic Eminence (MGE) that gives rise to inhibitory neurons can be cultured for a long period without being damaged, can continue to proliferate and differentiate into GABA-ergic interneurons, as well as release several neurotrophic factors; all of these entities make these cells viable candidates for donor cells in grafting therapy [14].

It is worth noting that this is only a small step towards introducing stem cell therapy to treat epilepsy in humans. These procedures still have to show substantial progress within *in-vivo* models. Focal seizures are the most common types of seizures being reported and given that it implies the ictal source is confined to a particular region of the brain, stem cell therapy is being believed to be a viable and promising treatment. The feature of it being highly selective could potentially allow us to eliminate the seizure generation capability of the affected regions. Seizures can also disrupt the circuitry of neurons in the brain, leading to a plethora of other co-morbidities. Stem cell therapy has been shown to reframe the neuronal circuit, improve the cognitive state of those with epilepsy, improve neuroprotection, and diminish the frequency and duration of seizures [15].

They bear some advantages over anticonvulsants by being more specific and focussed. It also bears some advantages over surgical approaches when the ictal source is poorly defined [15]. Within *in-vivo* studies, it was shown that while the injected stem cells from fetal rat ganglionic eminence showed the potential of survival and differentiation into the different inhibitory neurons, they were not successful in the migration. They were, however, successful in reducing the frequency of seizures by nearly 70%. Many hurdles have to be passed before

this approach can proceed to clinical trials. A better understanding of the role neuronal microenvironment in modulating migration and differentiation is critical. Astrocytes, now being considered as part of the stem cell toolbox, have a unique ability to regulate the microenvironment and could serve as an important part of the puzzle. The differentiation, networking, migration, and circuit formation can be further enhanced by certain therapeutics and behavioral conditioning. This approach must also take into consideration, the possible risk of tumorigenesis. Some additional factors that must be considered while talking about stem cell therapy are the risk of tumorigenesis, long-term effects of the stem cell graft, and its viability [15].

Researchers at the Harvard Stem Cell Institute have recently found out that upon transplanting anti-ictal hESCs into mice seizure models, there was the successful integration of the human neurons in the brain, a significant drop in seizure frequency of about half of the mice, and no seizures in the other half of the cohort [16]. This novel therapy would still require further refinement in other seizure models but would serve as a huge advancement in the field of stem cell technology and epilepsy therapeutics. Stem cells are also being looked at to screen for potential anticonvulsants and the development of personalized medicine, which could help identify other genetic markers and biomarkers.

The analysis of CSF and/or blood samples of epileptic patients have shown some potential biomarkers that may predict the occurrence of seizures. These biomarkers may also shine some light on the genetic etiologies of some epilepsy syndromes and ultimately contribute to ongoing therapeutics. Stem cell technology is being used to explain and verify the occurrence of these biomarkers. One example of such a biomarker is *SOD1*, superoxide dismutase 1, an enzyme involved in the protection against the effects of reactive oxygen species, is found to be reduced in the CSF samples of epileptic patients. In conclusion, stem cell technology has been successful in improving seizure outcomes in *in-vivo* models of epilepsy, which could one day contribute to the ongoing therapeutics as a one-time intervention for permanent seizure management and/or drastically reduced seizure frequency in people with refractory epilepsy [17].

Gene therapy

Gene therapy, which is the technology devised to rectify the altered and mutated genes, is a growing field that is showing prominent results in several diseases such as epilepsy, cancer, sickle cell anemia, and several others [8]. Gene therapy is a very broad term including several classifications are depending on the recombinant DNA technology, type of vector, mode of delivery, and source of cells. The most common and effective way to introduce the engineered gene into the body is via viral vectors, such as lentiviruses, herpes simplex virus, retroviruses, and adeno-associated viruses [18]. Within these vectors, the modified gene is loaded along with its required regulatory elements. Once these viruses are injected, they can enter the nucleus and can restore the dysfunctionality within the cell. There are non-viral vectors that have been used, such as lipids, polymer-based vectors, and in some cases, nanoparticles are being studied to deliver gene therapy packages Gene therapy is successfully being tested in human clinical trials (~200) for several neurological disorders. While as of this date, there are no FDA-approved gene therapies for epilepsy, there is constant growth and development in the basic and translational sciences in improving the efficiency of this technology [18].

NPY

NPY (Neuropeptide Y) is an extremely common and conserved polypeptide that acts as a neurotransmitter with a host of functions involving the regulation of digestion, blood pressure, metabolism, and in the case of this paper, brain activity (Li., *et al*. 2019). Within the CNS, the hippocampus has the highest expression of NPY. This can be attributed to GABAergic neurons. The other regions of the brain that also express NPY are the hypothalamus, cerebral cortex, brainstem, cerebellum, and thalamus) [19]. NPY exerts its biological functions by binding to a class of NPY receptors which are mostly G-Protein-Coupled Receptors (GPCRs). The most common ones are Y_1, Y_2, Y_3, Y_4, Y_5, and Y_6. In the case of epilepsy, the most important receptors are Y_1, Y_2, and Y_5. Granule cells are excitatory and give rise to mossy fibers that produce NPY. It was also observed in certain regions of the hippocampus, there was an increase in the Y2 receptor and that the Y2R signal-

ing is linked to the anti-epileptic properties and the Y1 receptor levels decrease leading to the unfavorable effects seen in epilepsy models [19]. The Y5 receptor, as mentioned also plays an important role in epilepsy. Activation of Y5R in certain sections of the hippocampal region can reduce the generalized seizures *in-vivo*. The hypothesis that NPY could modulate brain activity in epilepsy patients and serve as a potential therapeutic agent is based on decades worth of research conducted on *in-vitro* cultures, *in-vivo* models, and patient samples.

Studies have also shown that NPY works on the N-methyl-D-aspartate (NMDA) receptor, which is a glutamate receptor, and by down-regulating it thus suppressing neuronal excitability which is caused by the release of glutamate. Initially, it was shown that simply injecting viral vectors that code for NPY shows a decrease in spontaneous seizure frequency, as well as the progression of epilepsy [19]. Scientists then tested if they could use gene therapy to inject viral vectors encoding for NPY and Y2R in the epilepsy models. It was shown that this method prevented the worsening condition of elevated seizure frequency, much better than just NPY [19].

Potassium channel gene therapy

Potassium voltage-gated channel subfamily A member 1 or (Kv1.1) is an important factor in maintaining the electrochemical gradient of the neurons by allowing the influx of potassium ions (K^+) through the process of repolarization (NCBI, 2021). It is encoded by the KCNA1 gene, which is found on chromosome 12 (12p13.32), and mutations in the KCNA1 gene are linked to the pathogenesis of a rare neurological disorder called episodic ataxia type 1 (EA1), which is linked to poor coordination and movement. Epilepsy is a common co-morbidity of EA1. Researchers at the UCL Institute of Neurology found that the inhibition of Kv1.1 showed an increase in neuronal excitability, and the overexpression of Kv1.1 increased the threshold for action potential generation, reduces the intrinsic neuronal excitability and glutamate release, thus providing a mechanism to limit seizure activity It was shown that overexpression of Kv1.1 prevented the development of epilepsy and also reduced ictal activity after the disease was established, thus shining a light on the potential anti-convulsing and anti-epileptogenic properties of this approach [20].

To push this technology for possible use in human clinical trials, the *KCNA1* gene was modified to enhance the recovery rate of the Kv1.1 channel from inactivation and was packaged into a non-integrating lentivirus vector. This modified gene package, known as the Engineered Potassium Channel (EKC), was found to dramatically reduce the seizure frequency in rats with focal neocortical epilepsy (FNE). It was also reported that EKC was successful in reducing seizures in a rat model of Temporal Lobe Epilepsy (TLE) Upon EKC administration, there was significant suppression of electrocorticography measurements (ECoG) in the FNE models. These experiments also confirmed that this approach does not completely inhibit neuronal excitability and/or neurotransmitter release. The risk of insertional mutagenesis, which is common across all gene therapies, was minimized by using integration-deficient vectors, which have been successfully seen in preclinical trials for retinal disease and hemophilia B. As of this moment, this is the only gene therapeutic strategy that has been primed for clinical trials. The goal of this therapy is to overexpress an ion channel that reduces the increased neuronal excitability [20].

CRISPR

Ever since the concept of gene editing using CRISPR or Clustered regularly Interspaced Short Palindromic Repeats came out in the early 2000s, scientists have been very optimistic about its potential role in treating a plethora of different diseases (Simpson and Davidson, 2019). Several *in-vitro* and *in-vivo* models are being used to test the effect of this approach before administering them to humans. CRISPR has yielded very promising results in the improvement of certain conditions such as Hereditary Transthyretin Amyloidosis, Sickle Cell Anemia, and others [21]. In the field of neurological disorders, CRISPR is being used to understand the etiological aspects and treatment options of different disorders such as Frontotemporal Dementia/Amyotrophic Lateral Sclerosis (FTD/ALS), Angelman Syndrome (AS), and Pelizaeus-Merzbacher disease (PMD) Seizures are an extremely common symptom, seen in 80-90% of patients [21].

As seen in AS, where the paternal allele of *UBE3A* is silenced, CRISPR was used to reactivate the silenced paternal allele. Using Cas9, which is an exonuclease, this approach targeted genes that were responsible for the working of *UBE3A-ATS* and this reversed the silenc-

ing of the *UBE3A* paternal allele. It was also shown that this resulted in the genomic integration and the functionality of that gene being restored.

Another aspect of the CRISPR technology involves fine-tuning the expression of *Kcna1,* which codes for the previously mentioned Potassium channel (Kv1.1) A dCas9 system (dead Cas9) was used to target the gene, but unlike the other CRISPR applications, this approach did not cut the DNA. The promoter of the Kcna1 gene located upstream was targeted and the dCas9-Kcna1 system, fused to a transcriptional activator VP64 was introduced into mice models using AAV-vector technology. This approach showed a tremendous reduction in seizure burden and improvement of cognitive deficit. While the researchers are positive about the working of this therapeutic approach, they have reinforced the need for further testing on different epilepsy models to understand if the overexpression of KCNA1 is responsible for driving the anticonvulsant property of this therapy [21].

Dravet Syndrome is a rare form of refractory epilepsy and is caused by a mutation in the SCN1A gene that codes for sodium channel Nav1.1. A loss of function mutation in SCN1A prevents interneurons from suppressing excitability and. A dCas9-SCN1A gene activation system is proposed to rescue said mutation. Using this approach, they were able to see an enhanced expression of the SCN1A RNA levels, sodium channel levels, and a rescue of the working ability of interneurons.

Optogenetics

Optogenetics is the use of light to control the behavior of cells, (in this case neurons) to express photosensitive proteins that could modulate light-sensitive ion channels. This technique is also used to monitor how biochemical pathways and neuronal activity can be altered in different diseases. When stimulated by light, these specific compounds will generate a corresponding molecular response such as alterations in membrane potential. The compounds that are involved in neuronal control are called optogenetic actuators, such as channelrhodopsin, halorhodopsin, and archaerhodopsin. There are optogenetic sensors that help monitor the electrical and biological changes in the physiology of the cells. While the technology behind optogenetics has been around for decades, its potential applications in the field of neuroscience is a relatively novel approach and its results seem quite promising. The concept of optogenetics work by tagging and manipulating neurons and their activity levels. Less invasive models of optogenetics involving nanotechnology are now being developed to improve the healthcare outcomes of not only epileptic patients but those with Parkinson's disease as well [22].

Researchers at Stanford University were successful in using optogenetic-based techniques to identify a group of cells, called mossy cells that when activated, can reduce the likelihood of seizure occurrence The concept of optogenetics is being tested in conjunction with Deep Brain Stimulation (DBS) to improve its efficiency and modulation effects The use of DBS to treat epilepsy was recently approved by the FDA, and optogenetics can be used to help improve mapping the connections and areas in the brain that serve as the source of the seizures. This would allow a more precise and less-invasive intervention to improve seizure management. Scientists find this optogenetic approach insightful, as it selectively allows neurons to be activated when there is a need for it [20]. A group of neurons that have been shown to elicit seizure-like behavior, interneurons can be targeted by the optogenetic route. The inhibition of pyramidal cells in the hippocampus using optogenetic technology can be seen in several mice models of temporal lobe epilepsy, stroke-induced seizures, tetanus-induced model of neocortical epilepsy, and absence epilepsy models.

Optokindling is a technique that is used to study the course of epileptogenesis rather than just studying the occurrence of seizures. Kindling is a common model used to understand the development of epilepsy. In optokindling, cells that can be activated in epileptogenesis are genetically identified and fluorescently tagged. This allows for further specificity and helps us to understand the plasticity of the epileptic brain. The use of this technology in humans, while optimistic, still needs to overcome several hurdles in addition to those already faced by traditional gene therapy options. For this case specifically, since the brains of humans are much bigger and denser than

the rodent *in-vivo* models, a brighter and stronger light source is required. It also requires real-time seizure detection, the mechanisms of which have not been resolved yet [23].

Chemogenetics

Chemogenetics is the technique of modifying an endogenously expressed receptor or synthesizing and inserting a chimeric receptor that will respond to an exogenous ligand of choice to modulate the activity of the target cells Nucleic acid hybridizations, receptors (G-protein-coupled receptors (GPCRs) or Ligand-Gated Ion Channels (LGIC)), kinases, and other metabolic enzymes are some of the exogenous insertions used in the chemogenetic approach. Most of the recently developed chemogenetic tools are designed to disrupt the electrical activity of the cells [24]. GPCRs are a prime target of the chemogenetic technique; they are mostly involved in controlling the growth and differentiation of cells.

The Receptors Activated Solely by Synthetic Ligands (RASSLs) and Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are some examples of GPCR-based chemogenetic approaches. This technology has been around for a few years, but its possible application in treating neurological disorders yields optimism among many. RASSLs work by modulating the signaling pathways involved in neurotransmission, cell growth, and cell differentiation. DREADDs are being used by neuroscientists to recognize molecular entities involved in the transmission of neuronal signals and certain properties such as emotions, motor functions, perceptions, and behavior. Depending on the components of the GPCR signaling pathway, different types of DREADDs have been designed. The Gq, Gs, and Gi. The Gq receptor is excitatory by exhibiting an intracellular Ca^{2+} release and depolarization. The Gs receptor is also stimulatory, and it increases the cyclic AMP (cAMP) levels that propagate downstream activation of the rest of the signaling pathway. The Gi receptor is inhibitory by doing the opposite, inhibiting adenylyl cyclase and cAMP levels There have been different DREADDs designed, depending on the intended target and mode of action. For instance, hM3Dq is a common Gq-DREADD that, when activated by a specific ligand (in this case CNO - clozapine-N-oxide) caused enhanced neuronal firing, synaptogenesis, astrocyte activation, and other effects [24].

Current research has been aiming to study how DREADDs can be optimized to focus more on cell-specific modulation. hM4Di and KORD (κ-opioid-derived DREADD) are some of the common and latest applications of the Gi-coupled DREADDs. It has been shown that the use of these receptors has shown silenced terminal projection There are several areas of the DREADD technology that can be enhanced. Developing new DREADDs and identifying more efficient actuators, determining the CNS penetrability, specific cell-population activation, toxicological features, and other factors could be influential in the future of this technology [25]. In terms of their use in improving seizure management, it was shown that expression of h M4Di in hippocampal neurons reduced epileptiform activity. These neuron cultures were resistant to a commonly used anticonvulsant - valproic acid, thus raising a slim possibility of the use of this strategy in refractory seizures. This data was backed by *in-vivo* studies. Other studies have also supported the prospect of using chemogenetic approaches as a therapeutic intervention, especially by circumventing the need for an invasive approach. It was shown that soon after CNO was administered in the different seizure models of rats that were transduced with hM4Di, there was a reduction in the seizure severity. This type of chemical-genetic silencing raises the question of the application of similar administration techniques to provide on-demand administration of these actuators. However, these would only be brought about after several more years of research. GPCR-based DREADDs are not the only tool in chemogenetics [25].

Ligand-gated ion channels (LGIC) are being considered to be a viable approach. As the name suggests, these channels are activated upon the binding of a particular ligand, and they would be permeable to the corresponding cations or anions [24]. One LGIC that has been showing promising results is the Glutamate-gated chloride channel (GluCl). It was first identified in nematodes, where it is mostly inhibitory, however, it has the opposite effect in mammals. Glutamate is an excitatory neurotransmitter. GluCl allows the influx of chloride ions upon the binding of glutamate. During seizures, the level of glutamate is significantly higher.

The working theory behind this approach is that the activation of GluCl could serve as a regulatory mechanism by allowing the chloride ions to counteract the depolarization and hyperexcitability effects of glutamate [19]. But it was noticed that the GluCl receptors were not that sensitive to glutamate, even during the ictal periods. Hence, researchers induced a point mutation that would increase the sensitivity of GluCl. It was now named 'eGluCl or enhanced GluCl'. This modification was shown to significantly reduce the number of seizures in a rat model of neocortical epilepsy while having little to no effect on normal brain function [19]. There are other targets in LGICs whose role in seizure reduction has yet to be identified, such as mammalian glycine receptors, chimeric receptors, and ligands consisting of parts of acetylcholine and glycine receptors [19]. Some hurdles need to be cleared before these approaches can be pushed into clinical trials, such as immunogenicity of the exogenous factors, the safety of the activator drugs and determining how often must the receptors be activated which would determine how narrow or wide the treatment window would last for. But, considering how far chemogenetic therapies have come and the *in-vivo* results, researchers and neurologists are optimistic that a long-lasting cure for refractory epilepsy is in sight [19].

Conclusion

Ever since the first medications against epilepsy came out in the late 1900s, the scientific community has made significant progress. However, with over 30% of epileptic patients being unresponsive to traditional anticonvulsants, there is an overwhelming need for more research and clinical trials in epilepsy therapeutics. The burden of refractory epilepsy is more profound, with exacerbated seizure frequency, social stigmas, poorer quality of life, and decreased life expectancy [26]. Some of the more novel approaches aside from the standard treatments of care that were discussed in this paper were gene therapy, stem cell therapy, and antisense oligonucleotides. Viral vectors, a common mode of gene therapy, include adeno-associated viruses, lentiviruses, herpes simplex viruses, and many others are the preferred mode of delivery of gene therapy targets [18]. The selection of the vector depends on the size of the gene therapy package, type of target cell, and intended duration. Non-viral vectors include lipid-based vectors and polymer-based vectors, and in some cases, nanotechnology is being used as a prospective delivery tool [27]. Genetic engineering is being used to treat a plethora of different diseases such as cystic fibrosis, sickle cell anemia, and different cancers [8]. Within the dome of gene therapy, there are several sub classifications, depending on the target and mode of action: neuropeptides, neurotrophic factors, engineered receptors and ion channels, neurotransmitters, and others [8]. Antisense therapies are another tool in the arsenal to cure genetic abnormalities. There are several antisense therapies approved for different diseases and this is still an active area of continuous research and development. There are ongoing clinical trials for several diseases such as Huntington's Disease, Amyotrophic Lateral Sclerosis, Retinitis Pigmentosa, and many others. Stem cell therapies have shown significant improvements in clinical trials for neurodegeneration, vision impairment, and there is hope that one day we can see improvements in the case of epilepsy. This review paper provided summaries of some of the more promising alternative therapies that could be used in the treatment of refractory epilepsy. These therapies are experimental and still have to clear several benchmarks before they can be pushed to human clinical trials. Some of these issues include the safety of viral vectors, immunogenicity, minimizing off-target effects, tailoring the specificity of the treatment, and making it as less invasive as possible. For the scientific community to make these advances in epilepsy treatment, there must be continuous financial support for scientists. Milken Institute commissioned a report called 'Epilepsy Giving Smarter Guide', where it states that epilepsy research receives 10 times less funding than other brain disorders, despite having a higher prevalence [28]. The CDC estimates that 1 in 26 individuals will be diagnosed with epilepsy at some point in their lives. Epilepsy currently affects 3.4 million Americans, out of which nearly 500,000 are children. Globally, about 65 million individuals are suffering from epilepsy. However, there are several instances where the number of seizures goes underreported. This could be due to the underlying stigma associated with it, the fear of having certain privileges as a driving license revoked, or in some cases, the seizures may just go undiagnosed [29]. Hence the actual burden of epilepsy may be significantly higher. Improvements in existing treatments and the development of new therapeutics could help significantly alleviate the physical as well as mental health outcomes of those with epilepsy.

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