




# Antisense Molecules in Epilepsy—A Neuropharmacological Educational Review

Jefry Winner George Stephen<sup>1</sup>  Jesiha George Stephen<sup>2</sup> Mohammed Ali<sup>3</sup> Ashwin Kumar<sup>1</sup>  
Susanna Jose<sup>4</sup>

<sup>1</sup>Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India

<sup>2</sup>PSG Institute of Medical Science and Research, Coimbatore, India

<sup>3</sup>Department of Neurology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India

<sup>4</sup>Veeda Clinical Research Institute, Mumbai, India

**Address for correspondence** Jefry Winner George Stephen, MBBS, Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry 605006, India (e-mail: jefrywinner0@gmail.com).

Int J Ep 2022;8:44–51.

## Abstract

### Keywords

- Lafora disease
- tRNA-based therapeutic
- seizure

Epilepsy is a common neurological disorder. Epilepsy has many therapeutic options, the popular one being antiseizure medications. A good proportion of patients always responds well to the existing treatment modalities. But some patients develop resistant epilepsy, and treating them can be challenging with the current treatment; such scenarios are encountered frequently in patients, especially those under treatment for long-term as well as specific syndromes and channelopathies. Resistant epilepsy warrants the need to develop newer therapeutics for better treatment outcomes, and antisense oligonucleotides (ASOs) are one among them. Our review discusses the more recent startups called ASOs in the context of epilepsy therapeutics.

## Introduction

The disease epilepsy has been known for ages. Around 1% of the world's population is estimated to have epilepsy. The term idiopathic epilepsy is no longer used. Antiseizure medications (ASMs) are the mainstay in the treatment of epilepsy. Most patients with epilepsy respond well to the existing ASMs and remain seizure free. Many patients also attain complete remission and remain seizure free for the rest of their lifetimes. Despite all this, some patients respond poorly to the existing treatment modalities, and treating them becomes challenging. The reason for variations in response among patients with epilepsy is still unknown. So, this remains an area of research to date. Numerous agents were repurposed and tried with mixed results. And still, conventional ASMs remain the mainstay of treatment. One among the alternative agents that were newly tried is the antisense molecules. Targeting intracellular

targets that are usually not druggable is possible with these agents. This review focuses on the antisense molecules targeted for epilepsy and their progress in epilepsy therapeutics.

## What Are Antisense Molecules

The term antisense molecules comprise several classes of oligonucleotide molecules. This contains a sequence complementary to target RNA (tRNA) molecules, such as messenger RNA (mRNA), viral RNA, or other RNA (► **Fig. 1**) species that inhibit the function of their tRNA after sequence-specific binding. In the meantime, at least four classes of antisense molecules have been described: antisense oligodeoxyribonucleotide, that is, single-stranded DNA molecules, small interfering RNA (siRNA) molecules, ribozymes, and DNAzymes (► **Table 1**).

The mechanism by which the antisense molecules act, as explained above, is depicted below

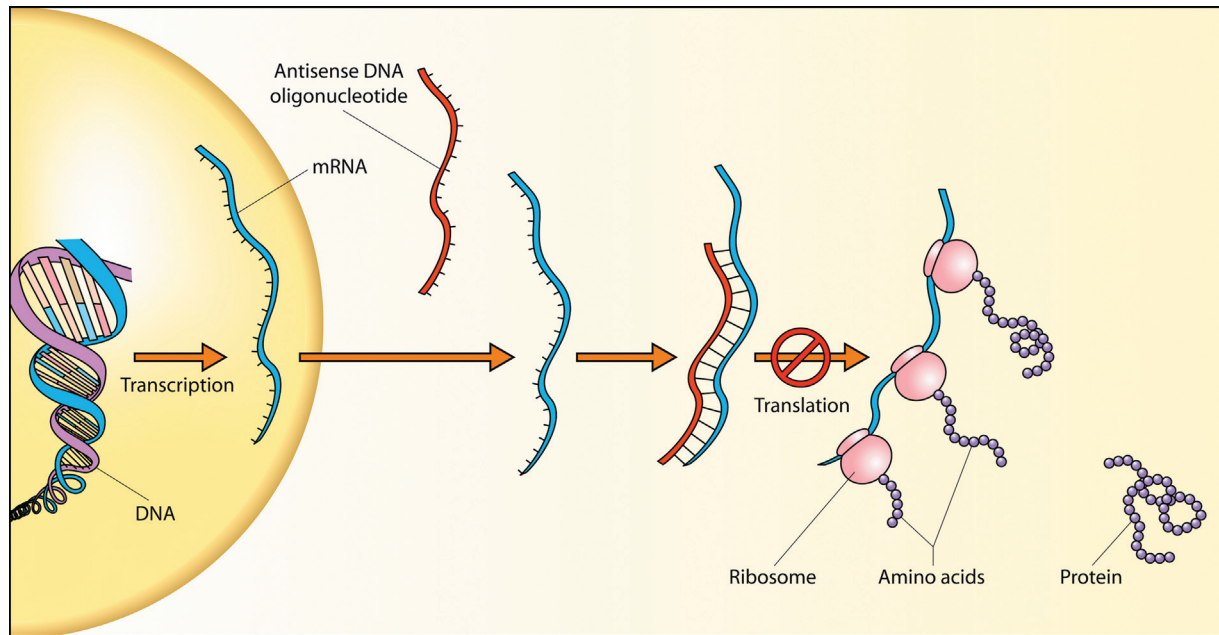
article published online  
March 4, 2024

DOI <https://doi.org/10.1055/s-0044-1779694>.  
ISSN 2213-6320.

© 2024. Indian Epilepsy Society. All rights reserved.

This is an open access article published by Thieme under the terms of the Creative Commons Attribution-NonDerivative-NonCommercial-License, permitting copying and reproduction so long as the original work is given appropriate credit. Contents may not be used for commercial purposes, or adapted, remixed, transformed or built upon. (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India



**Fig. 1** (A) Depiction of the mechanism by which the antisense molecules act. (Adapted from Robinson R. RNAi Therapeutics: how likely, how soon? PLoS Biol 2004;2(1): e28).

**Table 1** The challenges faced and the effects of various antisense molecules

	Antisense DNA (ODN)	siRNA	DNA zyme	RNA zyme
Oligo type and structure	Linear ssDNA	Linear dsRNA	ssDNA with two binding domains surrounding a central catalytic domain	Complex RNA with single and double-strand section
Size	12–25 bases	21–25 base pairs	30–35 bases	>30 bases (hammerhead RNA zymes) >50 bases (hairpin RNA zymes)
Inherent enzymatic activity	No	No	Yes	Yes
Recruited enzymatic activity	RNases H, L, or P	RISC (RNA-induced silencing complex)	No	No
Modifications	Multiple backbone modifications were established, such as PT, 2-O-methyl, 2-O-methoxyethyl, locked nucleic acids	Several changes, preferentially of terminal nucleotide overhangs found, such as 39-inverted thymidine	39-inverted thymidine to enhance stability modifications in binding arms possible	Often unmodified (mainly when expressed in vivo), several modifications are possible
Advantages	<ul style="list-style-type: none"> <li>• Easy to produce</li> <li>• Easy to modify</li> <li>• Good cell penetration, especially for modified short antisense DNAs</li> </ul>	<ul style="list-style-type: none"> <li>• Can be generated intracellularly from more significant precursors of the whole mRNA</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to produce</li> <li>• Multiple potential cleavage sites in target RNAs</li> <li>• Good in vivo cell penetration</li> <li>• No significant off-target effects</li> </ul>	<ul style="list-style-type: none"> <li>• Coding DNA sequences can express multiple potential cleavage sites in target RNAs in vivo</li> </ul>
Disadvantages	<ul style="list-style-type: none"> <li>• Off-target effects</li> <li>• Potential protein-binding (aptamer) activities</li> <li>• Activity dependent on intracellular enzymes</li> </ul>	<ul style="list-style-type: none"> <li>• Off-target effects</li> <li>• More challenging to produce limited cell penetration</li> <li>• Activity dependent on intracellular enzymes</li> </ul>	<ul style="list-style-type: none"> <li>• Empiric selection process</li> <li>• Activity depends on intracellular Mg21 levels</li> </ul>	<ul style="list-style-type: none"> <li>• Large size and complex structure</li> <li>• Empiric selection process</li> <li>• Challenging due to limited in vivo stability</li> </ul>

## Why Are They Important?

Epilepsy is a common disorder affecting many people worldwide.<sup>1</sup> Currently, more than 20 small-molecule drugs are used for epilepsy. The clinical outcomes are good for two-third of the patients, but the rest show a poor response.<sup>2</sup> In many patients with epilepsy, the response to ASMs reduces with time. Patients who respond poorly to the existing therapies need multiple ASMs to attain seizure control. One classic example of such a scenario is temporal lobe epilepsy (TLE)<sup>3,4</sup>—many patients with TLE progress to a stage where they require epilepsy surgery. Most ASMs today are designed to target ion channels. They mostly rely on modulating the conduction in neurons. Long-term use of these agents can develop resistance and poor response in those patients.<sup>5</sup> Novel approaches that can modify multiple targets up to the genetic level may be necessary to combat this problem. ASO's strategy in treating epilepsy can help us overcome our current challenges in treating drug-resistant epilepsy.<sup>6</sup>

## Epilepsy and Antisense Oligonucleotides

### Developmental Epileptic Encephalopathies

Developmental epileptic encephalopathies (DEEs) comprise a group of disorders characterized by epileptic seizures, which are difficult to treat with current-day ASMs.<sup>7</sup> The gene implicated in these conditions is SCN2A (sodium channel 2A) that encodes the  $\alpha$  subunit of the voltage-gated sodium channel (Nav1.2). Nav1.2 is involved in the initiation and conduction of action potentials. In DEE patients, Nav1.2 is the predominant isoform that leads to excessive excitation compared to people with other isoforms (Nav1.1, 1.3, and 1.6).<sup>8,9</sup> DEE is associated with de novo mutations, which constantly increase as the disease progresses.<sup>10</sup> Functional analysis has shown a gain of function mutation in the SCN2A gene that encodes for Nav1.2 associated with this disease, so gene therapy targeted to reduce SCN2A overexpression will be promising.<sup>11,12</sup> This strategy was successful in preclinical studies done in a mouse model by Li et al. Li et al administered an antisense oligonucleotide (ASO) targeting mouse Scn2a (Scn2a ASO) to a mouse model of DEE. The mice models of DEE used were engineered with human equivalent SCN2A p.R1882Q mutation. Intracerebroventricular Scn2a ASO administration into the mutated mice between postnatal days 1 to 2 significantly extended lifespan and reduced Scn2a mRNA levels by 50%.<sup>13</sup>

### Dravet Syndrome

Dravet syndrome (DS) is another disorder that belongs to the group of diseases called epileptic encephalopathies. DS is caused by de novo mutations SCN1A gene. The mutations result in the haploinsufficiency of the voltage-gated sodium channel  $\alpha$  subunit Nav1.1, which leads to abnormal channel function that manifests as epileptic seizures.<sup>14</sup> Multiple seizure types characterize DS. Patients with DS are resistant to usual ASMs. The disease is characterized by seizures, cognitive deficits, ataxia, and increased mortality.<sup>15,16</sup> There

is a risk of sudden unexpected death in epilepsy (SUDEP) in all people with epilepsy. In DS, SUDEP is increased by 20% compared to other people with epilepsy.<sup>17</sup> Recently developed and repurposed small molecules show only a partial improvement in the seizure in patients with DS. This includes stiripentol, Epidiolex, and fenfluramine.<sup>18–20</sup> Again, these drugs do not target the direct pathophysiology of DS. The haploinsufficiency of the SCN1A is essential in understanding the underlying pathology. ASOs employed in DS targets the exon sequence. Such binding helps remove the nonfunctional exon sequences interrupting with the normal exon, thereby helping produce more abundant normal functioning SCN1A mRNA. ASO approach was found to be successful in various preclinical studies. In the F1:129S-Scn1a $\pm$   $\times$  C57BL/6J mouse model of DS, a single intracerebroventricular dose of ASO at postnatal day 2 or 14 reduced the incidence of seizures. SUDEP was also reduced in the ASO group. Increased expression of the normal SCN1A transcript and Nav1.1 protein was seen in the brains of mice treated with ASO.<sup>21</sup> There was a reduction in SUDEP by 97% in mice treated with specific ASOs. Anti-SCN1A antagonists are antisense molecules against noncoding RNA, which are tried in DS.<sup>22</sup> Another mutation detected by Lenk et al is the SCN8A mutation.<sup>23</sup> The SCN8A gene encodes for Nav1.6. Mutations in SCN8A lead to encephalopathy and DS. In the experiment by Lenk et al ASOs targeted against the SCN8A exon sequence were used in mouse models of DS. The mean survival increased from 3 weeks to more than 5 months in DS.<sup>24</sup> Targeted augmentation of nuclear gene output (TANGO) introduced by Stoke therapeutics STK-001 has shown promising results in mouse models of DS. The first human trial in 18 children with DS, called the MONARCH trial, was conducted to assess the safety of STK-001 in the case of DS. Results of this phase I trial have shown an excellent tolerability profile up to doses of 30 mg in children. TANGO uses an ASO that works by binding to splice pre-mRNA to promote a process called nonsense-mediated mRNA decay exon exclusion. TANGO ASOs reduce the DNA that encodes for nonproductive proteins, restoring the protein output to near-normal or sometimes normal levels.<sup>25</sup>

Another selective gene therapy by encoded therapeutics, ETX101, aims to increase SCN1A gene expression, which has shown promising preclinical results in mouse models with DS.<sup>26</sup> CRISPR-associated protein 9 in a deactivated form increases the gene expression of SCN1A in preclinical cell line-based studies and mouse models.<sup>27</sup> Zogenix is another tRNA-based therapeutic to increase SCN1A expression. The first therapy to target tRNA, overcome nonsense mutations, and ensure the production of functional Nav1.1 is Zogenix. This tRNA-targeting molecule is currently under preclinical evaluation.<sup>28</sup>

## Channelopathies and Epilepsies

Our conduction system in the brain functions via various channels. Sodium, potassium, and calcium channels (CACN) are the most important and are integral to normal neurotransmission. When any disease affects these channels, it is

referred to as channelopathy. These channelopathies can manifest as epileptic seizures and are often difficult to treat.

### Potassium Channels in Epilepsy

Recent research has suggested that potassium channels (KCNA) play a significant role in epilepsy. The study done in preclinical animal models indicated that the KCNA mutations associated with KCNA1 are involved in SUDEP in the case of epilepsy. Kv1.1 deficiency leads to impaired neural control and cardiac rhythmicity. The pathology is assumed to be due to aberrant parasympathetic neurotransmission. This abnormality suggests KCNA1 could be a strong candidate gene for human SUDEP.<sup>29</sup> Another gene, LGI1, is found to be responsible for the proper functioning of KCNA in neurons. LGI1 is associated with autosomal dominant lateral temporal lobe epilepsy and autosomal dominant partial epilepsy with auditory features.<sup>30</sup> Patients affected by these disorders carry LGI1 mutations.<sup>31,32</sup> Since these genes were identified, antisense molecules can be developed and tried in these conditions.

### Calcium Channels in Epilepsy

Another vital channel involved in epilepsy is the brain's CACN, which is implicated in the pathogenesis of epilepsy. The CACN called CaV2.1 medium is a P/Q channel involved in epilepsy. The others include CaV3.1 and CaV3.2b and are found to be strongly associated with absence seizures in preclinical studies.<sup>33</sup> In the Genetic Absence Epilepsy Rats of Strasbourg model, the rats did not manifest absence seizure episodes with 7–9-Hz spike-wave discharges. An R1584P missense mutation in *CACNA1H*, which encodes Ca<sub>v</sub>3.2, was identified in the rats. *CACNA1H*, if expressed above a certain threshold, shows a gain of T-type channel function with the appropriate splice variant (+exon 25). This expression increases with age.<sup>34,35</sup> Again, this could be a potential target for future ASOs development.

### Lafora Disease and Antisense Molecules

Lafora disease is a severe progressive form of myoclonic epilepsy. Lafora disease manifests itself in the adolescent age group. The disease is characterized by the formation of polyglucosan inclusions called Lafora bodies. The mutations, namely EPM2A (laforin) and EPM2B (Malin) genes, are associated with this disease.<sup>36</sup> Laforin is an essential dual specificity phosphatase crucial for neurons' survival. Mutations in the EPM2A gene that encodes for laforin affect the exon sequence. Due to this, dysfunctional laforin proteins are produced that get deposited in the form of Lafora bodies.

Additionally, the EPM2B gene encodes for an E3 ubiquitin ligase involved in the polyubiquitylation of laforin and its degradation. Dysfunctional malin fails to clear laforin and leads to the accumulation of Lafora bodies within neurons. Conventional ASMs are the mainstay for treating Lafora disease. Valproate mainly controls myoclonus in Lafora condition.<sup>37</sup> The new emerging treatment for Lafora disease still in the preclinical stage is Asos. The one ASO tried is Gys1-ASO, which targets the mRNA in the brain and causes a significant reduction in Lafora body production in mouse

models. Intracerebroventricular injection of Gys1-ASO prevented Lafora body formation in young mice that had not yet formed them. In older mice that already exhibited Lafora bodies, Intracerebroventricular injection of Gys1-ASO inhibited further accumulation, markedly preventing large Lafora bodies characteristic of advanced disease.<sup>38</sup> So, hopefully, this might be promising in the case of Lafora disease.

### Temporal lobe Epilepsy and Antisense Molecules

TLE is the most common type of acquired epilepsy in adulthood.<sup>39</sup> According to seizure symptomatology, this is divided into mesial TLE and a rare variant called lateral TLE. ASMs are the mainstay in TLE, but the primary concern is their long-term use and resistance.<sup>39</sup> Our understanding of TLE is mainly based on imaging, clinical, and electrophysiological data. The most common finding in the case of TLE is hippocampal sclerosis.<sup>40</sup> TLE can occur primarily in some instances or secondary to head trauma or prolonged febrile seizures. It is suggested that hyperexcitability leads to seizure attacks secondary to gliosis.<sup>41</sup> There is a need for new drugs and treatment strategies for TLE. Understanding molecular mechanisms and the genetic basis of the disease is essential to develop ASOs for TLE.

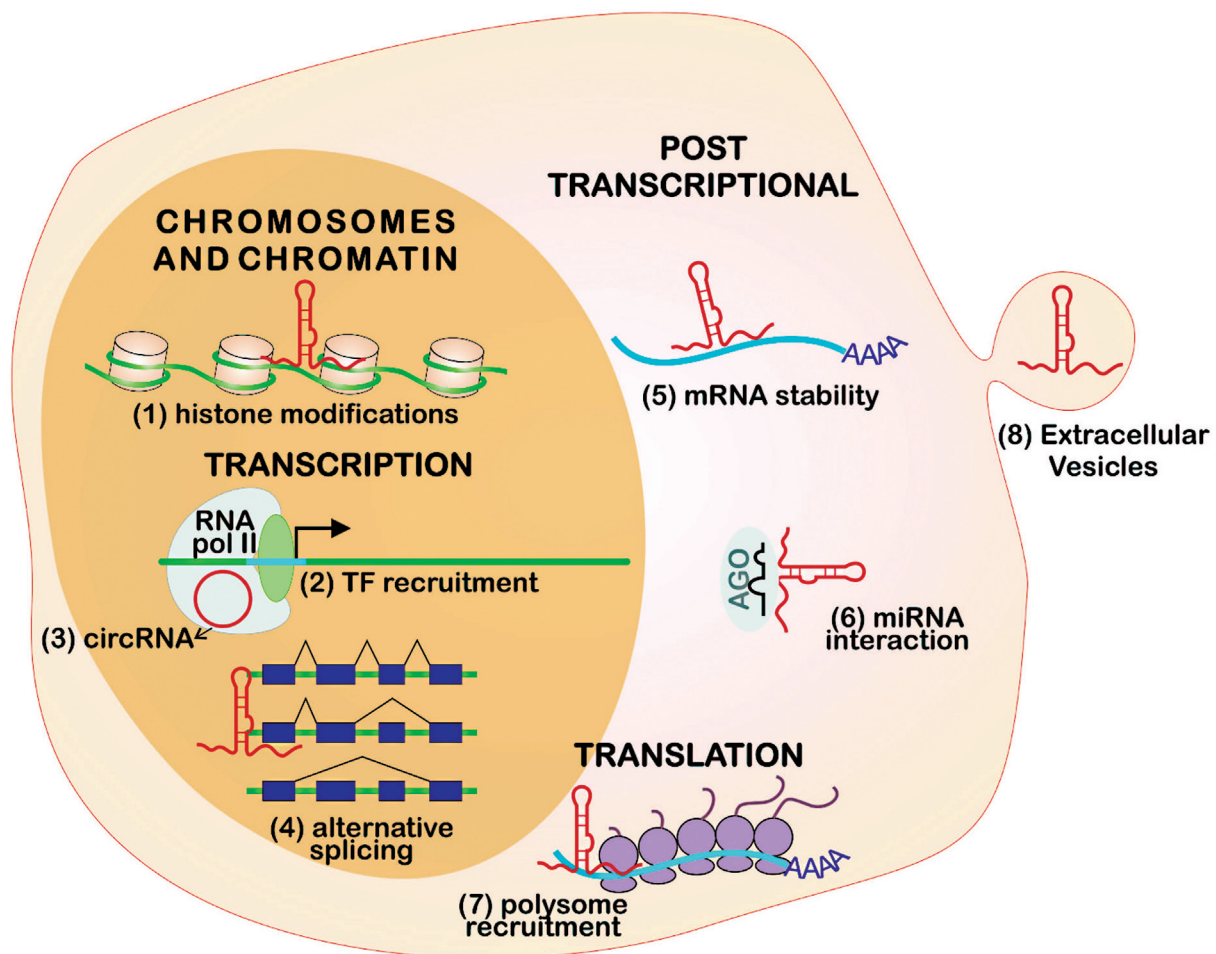
### miRNAs in TLE

It is estimated that around 60% of human proteins can be directly regulated by microRNA (miRNAs) by binding to complementary sites on mRNAs and decreasing mRNA stability and translation.<sup>42</sup> In TLE, there is altered gene expression in the hippocampal region for which the miRNAs are partly responsible, followed by short ncRNA. Recent research has subjected this to various changes, and now miRNAs are believed to be the major players in the case of TLE.<sup>43</sup> Twenty miRNAs were altered in TLE, of which 19 were upregulated, and one was downregulated. From this information, it is undoubtful that miRNAs play an essential role in the pathogenesis of TLE. But the multipathway regulation and interconnections make it a challenging target for drug development (–Fig. 2). With the knowledge from preclinical studies, it has been found that among the miRNAs, miR-134 is involved.

Studies have shown elevated levels of miRNA-134 in patients with TLE. So miR-134 antagomirs/ASOs might help in treating TLE.<sup>44</sup> This effect was successfully demonstrated in preclinical studies. Pretreatment of mice with miR-134 antagomirs reduced the proportion of animals that developed status epilepticus (SE). miR-134 antagomir was assessed in the pilocarpine-induced seizure model, which showed increased survival. In antagomir-treated mice that did develop SE, seizure onset was delayed, and total seizure power was also reduced.<sup>45</sup>

Another target of interest is miR-132, associated with hyperexcitability in the hippocampus. Silencing this via specific ASO targets has successfully reduced hippocampal damage in post-seizure animal models. The damage to the hippocampus was evaluated with the help of a biopsy.<sup>46</sup> Another molecule, miR-46a, serves as an ASO target for TLE and SE. In this study, the C57BL/6 TLE mouse model, with the help of lithium-pilocarpine protocol, was used to study the role of miR-46a. The Racine scale





**Fig. 2** Schematic representation of ncRNA mechanisms of action. ncRNAs regulate gene expression both at transcriptional and post-transcriptional levels. miRNA, lncRNA, and circRNA act in various ways, promoting or inhibiting the expression of specific targets. Mechanisms of action of ncRNAs are summarized within each box. (Adapted from Fernandes JCR, Acuña SM, Aoki JL, Floeter-Winter LM, Muxel SM. Long non-coding RNAs in the regulation of gene expression: physiology and disease. *Non-Coding RNA* 2019;5(1):17).

was used to evaluate the seizure severity after intranasal delivery of miR-146a. There was an increase in latency and reduced seizure severity. miR-146a is also found to modulate inflammatory cytokines supporting its anti-inflammatory role via the toll-like receptor pathway in the brain.<sup>47</sup>

### lncRNAs in TLE

As their name suggests, they have a nucleotide length of around 200. There is excellent preclinical evidence regarding the role of long non-coding RNAs (lncRNAs) in TLE. They are known to influence the development of seizures by different mechanisms.<sup>48</sup> In preclinical studies, 497 lncRNAs were expressed in hippocampal sclerosis, 294 lncRNAs were up-regulated, and 203 were downregulated. Furthermore, 399 differentially expressed mRNAs were identified. Among them, 236 were upregulated, and 163 were downregulated. In recent years, lncRNAs also regulate proinflammatory cytokines in the hippocampus, which is elevated in preclinical models of TLE.<sup>49</sup> The role of lncRNAs in synaptic plasticity is well known. lncRNAs were found to have a significant relationship with brain-derived neurotrophic factor (BDNF).<sup>50</sup> Preclinical studies show that BDNF levels are high in the

cerebral cortex of TLE mouse models, which can be cleared by lncRNAs called BDNF-antisense RNA.<sup>51</sup> So, targeting the latter and developing compounds analogous to it can help treat patients with TLE (→ Fig. 2).

lncRNA is ubiquitously expressed in the brain and regulates several genes involved in dendritic and synaptic development.<sup>50</sup> A study by Wan et al highlighted that lncRNA nuclear enriched abundant transcript-1 (NEAT1) is known to play a significant role in the inflammatory response in TLE models. Preclinical studies have also demonstrated markedly increased levels of NEAT1 in hippocampus sclerosis, which suggests this could be a potential target for ASOs.<sup>52</sup> Another lncRNA of interest is lncMEG3 that is highly expressed in TLE. MEG3 plays a significant role in regulating the inflammatory cytokines in our brain, and enhancers of MEG3 could help halt the progression of TLE.<sup>53</sup> In the studies by Han et al, H19 plays a significant role in casp3 expression and apoptosis. Increased lncH19 suggests that H19 plays a substantial role in inducing microglial activation through JAK/STAT pathway.<sup>54</sup> NEAT2 depletion by anti-NEAT2 siRNA resulted in the loss of hippocampal neurons. LY294002 (2-4-morphonyl-8-phenylchromone) is an inhibitor of the PI3K/Akt signaling

pathway, which could reverse such neuronal loss and is found to be successful in rat models with epilepsy.<sup>55</sup> The advent of ASOs has made lncRNAs druggable targets. In the future, this approach could hopefully become a therapeutic approach for TLE.

### **circRNAs in TLE**

Circular RNAs (circRNAs) are the newcomers in this domain and are known to regulate various aspects of gene expression and post-translational aspects. CircRNAs contain miRNA binding sites called miRNA response elements (MREs). This enables circRNAs to sequester the target miRNA, a process known as the “miRNA sponge effect.” They are called miRNA sponges, which can bind to them and inhibit their effects that play a vital role in pharmacoresistant TLE.<sup>56,57</sup> Even though the understanding regarding circRNAs is limited, studies show the regulatory part of circRNAs in the brain. They play vital roles in transmission events, synaptic plasticity, apoptosis, and other aspects of neuronal activity.<sup>58</sup> circRNA-0067835, associated with miRNA for regulation, was decreased in preclinical models of TLE. These levels correlated with increased seizure frequency, reinforcing the sponge effect.<sup>59</sup>

In the hippocampus, the overall expression profile of circRNAs showed 43 circRNAs. Among them, 26 were upregulated and 17 were downregulated. Various studies have found an inverse association between MREs and target miRNA expression.<sup>60</sup> circEFCAB2 with miR-485-5p and circ-DROSHA with miR-1252-5p were strongly associated with the expression of epilepsy-associated genes CLCN6 and ATP1A2, respectively.<sup>61</sup> Circulating circ-DROSHA might be a promising biomarker for the clinical diagnosis of TLE.<sup>62</sup> In a study by Chen et al, circ-0003170 was upregulated in animal models of TLE. Circ-0003170 was found to play a crucial role in cell viability, oxidative stress, and apoptosis. Knockout models of circ-0003170 ameliorated oxidative stress damage induced by circ-0003170, which indicates a potential ASO target.<sup>63</sup> A recent interesting experiment by Zheng et al found that increased circ-DROSHA weakened the neural injury of the TLE cell model. Circ-DROSHA could bind to miR-106b-5p to mediate the expression of myocyte-specific enhancement factor 2C (MEF2C); circ-DROSHA regulated MEF2C expression via sponging miR-106b-5p. Circ-DROSHA alleviated cytotoxicity in the TLE cell model by enhancing cell proliferation and repressing cell apoptosis.<sup>64</sup> All these can be exploited as potential ASO targets in the future. No drug targets have been developed targeting these circRNAs, which might be feasible once the knowledge regarding this strengthens.

### **Status Epilepticus and Antisense Molecules**

SE is a medical emergency characterized by prolonged seizures. This condition results from the failure of the mechanisms responsible for seizure termination. SE could also be due to excessive initiation complexes in the epileptic foci leading to abnormally prolonged seizures. According to International League Against Epilepsy, SE can be fatal in 10 to

20% of cases who experience this condition despite treatment.<sup>65</sup> So, a targeted prophylactic measure is necessary for this population. Antagomir miRNA-134 successfully prevents the emergence of seizures in preclinical studies. Pre-treatment of mice with miR-134 antagomirs reduced the proportion of animals that developed SE. miR-134 antagomir was assessed in the pilocarpine-induced seizure model, which showed increased survival. In antagomir-treated mice that did develop SE, seizure onset was delayed, and total seizure power was also reduced.<sup>45,46</sup> ASOs could help develop suitable preventive measures for seizure control in patients predisposed to developing SE.

### **Current Status of ASOs in Epilepsies**

These promising molecules could change the fate of patients with epilepsy, especially those with resistant epilepsy. Many rare epilepsy syndromes are resistant to treatment by conventional ASMs, and ASOs are being tried to combat this approach. Some other conditions ASOs are tested for include encephalopathy. Since our review is confined to epilepsy, we have just listed them.

SCN8A encephalopathy  
Huntington s' disease  
Duchenne muscular dystrophy  
CDKL5 deficiency disorder

### **Ataluren the ASO in a Clinical Trial**

Ataluren, previously known as PTC124, is a bioactive molecule that modulates the translation machinery.<sup>66</sup> The compound allows for the readthrough of PTCs during mRNA translation, facilitating the production of full-length functional proteins.<sup>67</sup>

A phase 3 study (NCT02758626) on 16 patients in a double-blind crossover manner failed to reduce the seizure frequency<sup>68</sup> effectively. The results of Ataluren seem disappointing after successful preclinical studies, but this is unavoidable in the case of drug discovery and therapeutics. Further studies and future perspectives might rectify this and aid in developing successful ASOs. So, they could be practically used in the treatment of epilepsy.

### **Conclusion**

ASOs have proven themselves to be potential agents for the treatment of epilepsy. The various ASOs discussed above have certain limitations and challenges. Further research is required to unleash their application in therapeutics. Epilepsy therapeutics have different therapeutic options but still pose a challenge in many cases. ASOs can be a better replacement for such patients in the future. Despite various pharmacological and pharmaceutical challenges, we believe ASOs will find a place in future epilepsy therapeutics. However, time and determined research will have a significant role in bringing them to reality.

**Conflict of Interest**  
None declared.

## References

- Thijs RD, Surges R, O'Brien TJ, Sander JW. Epilepsy in adults. *Lancet* 2019;393(10172):689–701
- Bialer M, White HS. Key factors in the discovery and development of new antiepileptic drugs. *Nat Rev Drug Discov* 2010;9(01):68–82
- Pitkänen A, Henshall DC, Cross JH, et al. Advancing research toward faster diagnosis, better treatment, and end of stigma in epilepsy. *Epilepsia* 2019;60(07):1281–1292
- Klein P, Dingledine R, Aronica E, et al. Commonalities in epileptogenic processes from different acute brain insults: do they translate? *Epilepsia* 2018;59(01):37–66
- Blümcke I, Thom M, Aronica E, et al. International Consensus Classification of Hippocampal Sclerosis in Temporal Lobe Epilepsy: a task force report from the ILAE Commission on Diagnostic Methods. *Epilepsia* 2013;54(07):1315–1329
- Löscher W. The holy grail of epilepsy prevention: preclinical approaches to antiepileptogenic treatments. *Neuropharmacology* 2020;167:107605
- Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017;58(04):512–521
- Liao Y, Deprez L, Maljevic S, et al. Molecular correlates of age-dependent seizures in an inherited neonatal-infantile epilepsy. *Brain* 2010;133(Pt 5):1403–1414
- Gazina EV, Leaw BT, Richards KL, et al. 'Neonatal' Nav1.2 reduces neuronal excitability and affects seizure susceptibility and behaviour. *Hum Mol Genet* 2015;24(05):1457–1468
- Maljevic S, Reid CA, Petrou S. Models for discovery of targeted therapy in genetic epileptic encephalopathies. *J Neurochem* 2017;143(01):30–48
- Wolff M, Johannesen KM, Hedrich UBS, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain* 2017;140(05):1316–1336
- Berecki G, Howell KB, Deerasooriya YH, et al. Dynamic action potential clamp predicts functional separation in mild familial and severe de novo forms of SCN2A epilepsy. *Proc Natl Acad Sci U S A* 2018;115(24):E5516–E5525
- Li M, Nikola J, Jafar-Nejad P, et al. Antisense oligonucleotide therapy for SCN2A gain-of-function epilepsy. *BioRxiv* January 1, 2020:289–900
- Dravet C, Bureau M, Dalla Bernardina B, Guerrini R. Severe myoclonic epilepsy in infancy (Dravet syndrome) 30 years later. *Epilepsia* 2011;52(Suppl 2):1–2
- Dravet C, Bureau M, Oguni H, Fukuyama Y, Cokar O. Severe myoclonic epilepsy in infancy: Dravet syndrome. *Adv Neurol* 2005;95:71–102
- Guerrini R, Aicardi J. Epileptic encephalopathies with myoclonic seizures in infants and children (severe myoclonic epilepsy and myoclonic-astatic epilepsy). *J Clin Neurophysiol* 2003;20(06):449–461
- Cooper MS, McIntosh A, Crompton DE, et al. Mortality in Dravet syndrome. *Epilepsy Res* 2016;128:43–47
- Brigo F, Striano P, Balagura G, Belcastro V. Emerging drugs for the treatment of Dravet syndrome. *Expert Opin Emerg Drugs* 2018;23(04):261–269
- O'Connell BK, Gloss D, Devinsky O. Cannabinoids in treatment-resistant epilepsy: a review. *Epilepsy Behav* 2017;70(Pt B):341–348
- Buck ML, Goodkin HP. Stiripentol: a novel antiseizure medication for the management of Dravet syndrome. *Ann Pharmacother* 2019;53(11):1136–1144
- Han Z, Chen C, Christiansen A, et al. Antisense oligonucleotides increase *Scn1a* expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome. *Sci Transl Med* 2020;12(558):eaaz6100
- Hsiao J, Yuan TY, Tsai MS, et al. Upregulation of Haploinsufficient Gene expression in the brain by targeting a long non-coding RNA improves seizure phenotype in a model of Dravet syndrome. *EBioMedicine* 2016;9:257–277
- Lenk GM, Jafar-Nejad P, Hill SF, et al. Scn8a antisense oligonucleotide is protective in mouse models of scn8a encephalopathy and dravet syndrome. *Ann Neurol* 2020;87(03):339–346
- Wagnon JL. TANGO with SCN1A: can this molecular dance defeat Dravet syndrome? *Epilepsy Curr* 2020;21(01):60–61
- Sullivan J, Wirrell EC. Dravet syndrome as an example of precision medicine in epilepsy. *Epilepsy Curr* 2022;23(01):4–7
- Isom LL, Knupp KG. Dravet syndrome: novel approaches for the most common genetic epilepsy. *Neurotherapeutics* 2021;18(03):1524–1534
- Colasante G, Lignani G, Brusco S, et al. dCas9-based Scn1a gene activation restores inhibitory interneuron excitability and attenuates seizures in Dravet syndrome mice. *Mol Ther* 2020;28(01):235–253
- Chilcott E, Díaz JA, Bertram C, Berti M, Karda R. Genetic therapeutic advancements for Dravet syndrome. *Epilepsy Behav* 2022;132:108741
- Glasscock E, Yoo JW, Chen TT, Klassen TL, Noebels JL. Kv1.1 potassium channel deficiency reveals brain-driven cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. *J Neurosci* 2010;30(15):5167–5175
- Kalachikov S, Evgrafov O, Ross B, et al. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nat Genet* 2002;30(03):335–341
- Morante-Redolat JM, Gorostidi-Pagola A, Piquer-Sirerol S, et al. Mutations in the LGI1/Epitempin gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Hum Mol Genet* 2002;11(09):1119–1128
- Nobile C, Michelucci R, Andreazza S, Pasini E, Tosatto SC, Striano P. LGI1 mutations in autosomal dominant and sporadic lateral temporal epilepsy. *Hum Mutat* 2009;30(04):530–536
- Ottman R, Winawer MR, Kalachikov S, et al. LGI1 mutations in autosomal dominant partial epilepsy with auditory features. *Neurology* 2004;62(07):1120–1126
- Rajakulendran S, Hanna MG. The role of calcium channels in epilepsy. *Cold Spring Harb Perspect Med* 2016;6(01):a022723
- Coenen AM, Van Luijtelaar EL. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. *Behav Genet* 2003;33(06):635–655
- Turnbull J, Tiberia E, Striano P, et al. Lafora disease. *Epileptic Disord* 2016;18(S2):38–62
- Nitschke F, Ahonen SJ, Nitschke S, Mitra S, Minassian BA. Lafora disease - from pathogenesis to treatment strategies. *Nat Rev Neurol* 2018;14(10):606–617
- Ahonen S, Nitschke S, Grossman TR, et al. Gys1 antisense therapy rescues neuropathological bases of murine Lafora disease. *Brain* 2021;144(10):2985–2993
- Patel DC, Tewari BP, Chaunsali L, Sontheimer H. Neuron-glia interactions in the pathophysiology of epilepsy. *Nat Rev Neurosci* 2019;20(05):282–297
- Catalanotto C, Cogoni C, Zardo G. MicroRNA in control of gene expression: an overview of nuclear functions. *Int J Mol Sci* 2016;17(10):1712
- Li MM, Li XM, Zheng XP, Yu JT, Tan L. MicroRNAs dysregulation in epilepsy. *Brain Res* 2014;1584:94–104
- Bencurova P, Baloun J, Musilova K, et al. MicroRNA and mesial temporal lobe epilepsy with hippocampal sclerosis: whole miRNome profiling of human hippocampus. *Epilepsia* 2017;58(10):1782–1793
- Jimenez-Mateos EM, Engel T, Merino-Serrais P, et al. Antagomirs targeting microRNA-134 increase hippocampal pyramidal neuron spine volume in vivo and protect against pilocarpine-induced status epilepticus. *Brain Struct Funct* 2015;220(04):2387–2399
- Wang W, Guo Y, He L, et al. Overexpression of miRNA-137 in the brain suppresses seizure activity and neuronal excitability: a new

- potential therapeutic strategy for epilepsy. *Neuropharmacology* 2018;138:170–181
- 45 Gao X, Guo M, Meng D, et al. Silencing MicroRNA-134 alleviates hippocampal damage and occurrence of spontaneous seizures after intraventricular kainic acid-induced status epilepticus in rats. *Front Cell Neurosci* 2019;13:145
  - 46 Wang J, Zhao J. MicroRNA dysregulation in epilepsy: from pathogenetic involvement to diagnostic biomarker and therapeutic agent development. *Front Mol Neurosci* 2021;14:650372
  - 47 Tao H, Zhao J, Liu T, et al. Intranasal delivery of miR-146a mimics delayed seizure onset in the lithium-pilocarpine mouse model. *Mediators Inflamm* 2017;2017:6512620
  - 48 Lee DY, Moon J, Lee ST, et al. Dysregulation of long non-coding RNAs in mouse models of localization-related epilepsy. *Biochem Biophys Res Commun* 2015;462(04):433–440
  - 49 Cui Z, Zhang X, Song H, et al. Differential long non-coding RNA (lncRNA) profiles associated with hippocampal sclerosis in human mesial temporal lobe epilepsy. *Int J Clin Exp Pathol* 2019;12(01):259–266
  - 50 Wan Y, Yang ZQ. LncRNA NEAT1 affects inflammatory response by targeting miR-129-5p and regulating Notch signaling pathway in epilepsy. *Cell Cycle* 2020;19(04):419–431
  - 51 Maag JL, Panja D, Sporild I, et al. Dynamic expression of long noncoding RNAs and repeat elements in synaptic plasticity. *Front Neurosci* 2015;9:351
  - 52 Zhang H, Tao J, Zhang S, Lv X. LncRNA MEG3 reduces hippocampal neuron apoptosis via the PI3K/AKT/mTOR pathway in a rat model of temporal lobe epilepsy. *Neuropsychiatr Dis Treat* 2020;16:2519–2528
  - 53 Han CL, Liu YP, Zhao XM, et al. Whole-transcriptome screening reveals the regulatory targets and functions of long non-coding RNA H19 in epileptic rats. *Biochem Biophys Res Commun* 2017;489(02):262–269
  - 54 Wu Q, Yi X. Down-regulation of long noncoding RNA MALAT1 protects hippocampal neurons against excessive autophagy and apoptosis via the PI3K/Akt signaling pathway in rats with epilepsy. *J Mol Neurosci* 2018;65(02):234–245
  - 55 Lipovich L, Dachet F, Cai J, et al. Activity-dependent human brain coding/noncoding gene regulatory networks. *Genetics* 2012;192(03):1133–1148
  - 56 Zhang X, Hamblin MH, Yin KJ. The long noncoding RNA Malat1: its physiological and pathophysiological functions. *RNA Biol* 2017;14(12):1705–1714
  - 57 Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. *Nature* 2013;495(7441):384–388
  - 58 Thomas LF, Sætrum P. Circular RNAs are depleted of polymorphisms at microRNA binding sites. *Bioinformatics* 2014;30(16):2243–2246
  - 59 You X, Vlatkovic I, Babic A, et al. Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat Neurosci* 2015;18(04):603–610
  - 60 Gong GH, An FM, Wang Y, Bian M, Wang D, Wei CX. Comprehensive circular RNA profiling reveals the regulatory role of the CircRNA-0067835/miR-155 pathway in temporal lobe epilepsy. *Cell Physiol Biochem* 2018;51(03):1399–1409
  - 61 Lee WJ, Moon J, Jeon D, et al. Possible epigenetic regulatory effect of dysregulated circular RNAs in epilepsy. *PLoS One* 2018;13(12):e0209829
  - 62 Chen F, Zheng H, Zhang W, et al. circ\_0003170 aggravates human hippocampal neuron injuries by regulating the miR-421/CCL2 axis in cells models of epilepsy. *Gen Physiol Biophys* 2021;40(02):115–126
  - 63 Zheng D, Li M, Li G, et al. Circular RNA circ\_DROSHA alleviates the neural damage in a cell model of temporal lobe epilepsy through regulating miR-106b-5p/MEF2C axis. *Cell Signal* 2021;80:109901
  - 64 Lemcke H, Steinhoff G, David R. Gap junctional shuttling of miRNA—a novel pathway of intercellular gene regulation and its prospects in clinical application. *Cell Signal* 2015;27(12):2506–2514
  - 65 Betjemann JP, Josephson SA, Lowenstein DH, Burke JF. Trends in status epilepticus-related hospitalizations and mortality: redefined in US practice over time. *JAMA Neurol* 2015;72(06):650–655
  - 66 Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 2007;447(7140):87–91
  - 67 Hirawat S, Welch EM, Elfring GL, et al. Safety, tolerability, and pharmacokinetics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. *J Clin Pharmacol* 2007;47(04):430–444
  - 68 Devinsky O, King L, Bluvstein J, Friedman D. Ataluren for drug-resistant epilepsy in nonsense variant-mediated Dravet syndrome and CDKL5 deficiency disorder. *Ann Clin Transl Neurol* 2021;8(03):639–644