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Perspective

Genetic therapeutic advancements for Dravet Syndrome

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ABSTRACT

Dravet Syndrome is a genetic epileptic syndrome characterized by severe and intractable seizures associated with cognitive, motor, and behavioral impairments. The disease is also linked with increased mortality mainly due to sudden unexpected death in epilepsy. Over 80% of cases are due to a *de novo* mutation in one allele of the *SCN1A* gene, which encodes the α-subunit of the voltage-gated ion channel $Na_V1.1$. Dravet Syndrome is usually refractory to antiepileptic drugs, which only alleviate seizures to a small extent. Viral, non-viral genetic therapy, and gene editing tools are rapidly enhancing and providing new platforms for more effective, alternative medicinal treatments for Dravet syndrome. These strategies include gene supplementation, CRISPR-mediated transcriptional activation, and the use of antisense oligonucleotides. In this review, we summarize our current knowledge of novel genetic therapies that are currently under development for Dravet syndrome.

1. Introduction

Dravet Syndrome (DS; also known as Severe Myoclonic Epilepsy of Infants (SMEI) [1] is an early-onset encephalopathy accounting for 1.4% of pediatric epilepsy cases [2] with a reported incidence of approximately 1 in 12,200 to 1 in 40,900 live-births [2–5]. DS typically manifests around the first year of life with prolonged, febrile & afebrile seizures, developmental delay becomes apparent from around the second year of life [6] and severe intellectual disability in most adults [6–10]. Unfortunately, patients with DS have an increased risk of death of approximately 15% after 10 years of follow-up [11] exhibiting high epilepsy mortality due to status epilepticus and sudden unexpected death in epilepsy (SUDEP) [12].

Although pharmacological and dietary treatment modalities are available for patients with DS, these are often inadequate. Therefore, developments in the field of genetic therapies have significantly progressed in the last five years. Thus, in this review, we will be discussing the genetic causes of DS and the future of genetic therapies for DS.

2. Genetic causes of Dravet Syndrome

SCN1A (located on chromosome 2q24.3), encodes the α-subunit of a voltage-gated ion channel, $Na_V1.1$ [13]. *SCN1A* mutations account for >80% of DS cases [14], of which truncating and missense mutations are most frequently found and are found to be associated with more severe phenotypes, with a smaller proportion of intronic splice site changes also seen [15]. Functional studies have shown that approximately 80% of all *SCN1A* missense mutations lead to a loss of function (LOF), resulting in haploinsufficiency; of these, LOF-causing mutations are primarily associated with the pore region of $Na_V1.1$ [2,16]. Whole-exon deletions or duplications have also been reported [17]. The vast majority of DS-causing mutations are *de-novo* [18] with only 5% of cases inherited in an autosomal dominant fashion [19,20].

SCN1A mutations have also been associated with Benign Febrile Seizures, Genetic Epilepsy with Seizures plus (GEFS+), and Intractable Childhood Epilepsy with Generalized Tonic-Clonic Seizures (ICEGTC) [21,22]. Previously, there have been difficulties in establishing a genotype-phenotype correlation, which has made clinical diagnosis and distinction of DS or other GEFS+ difficult for practitioners. However, recently Brunklaus et al. have developed a clinical-genetic prediction model which helps with early detection of whether the patient will develop DS or GEFS+ [7].

Although most cases of DS are caused by *SCN1A* variants, other genes such as *SCN1B* [23,24], *GABRG2* [25], *GABRA1* [26], *STXBP1* [26], *HCN1* [27], *CHD2* [28], and *PCDH19* [29,30] have also been implicated in clinically similar encephalopathies. However, these cases are usually characterized by atypical presentation of the disease phenotype, hence they are classified as borderline DS [31]. Furthermore, several cases of mosaicism have been reported for *SCN1A*; however, patients exhibit on average milder epilepsy phenotypes [32–35]. Similarly, mutations leading to gain of function *SCN1A* variants also do not present with classic DS manifestations, and these cases of DS will not be discussed here.

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3. Dravet Syndrome mouse models

Rodent models of DS recapitulate most aspects of the disease phenotype, including seizures, hyperactivity, anxiety-like behavior, inflexibility, low sociability, cognitive deficits [36–38], and SUDEP [39,40].

Over the last decade or so, several different mouse models for DS have been generated. The first was described in 2006 by Yu et al. where the authors established a *Scn1a*−/− mouse model which developed spontaneous seizures, ataxia, and died by postnatal day 15 (P15) [41]. Heterozygous mice developed spontaneous seizures and sporadically died after P21. Elevations in body temperature have shown to trigger myoclonic and generalized seizures, reiterating the febrile seizures present in patients with DS [42]. Furthermore, Miller et al. showed that *Scn1a*+/− mice develop spontaneous seizures and premature death occurs around 3 weeks of development [43]. Both groups demonstrated a strong correlation between mouse strain and phenotype presentation with the C57BL/6J genetic background, showing a more severe phenotype than the 129Sv background strain. Other mouse models have been generated by introducing a point mutation in *Scn1a* gene and have described less severe phenotype [44–47]. For example, $Scn1a^{A1783V}$+/− presents a 70% mortality rate at 8 weeks of age, cognitive impairment, anxiety, hyperactivity, and a reduced threshold for heat-induced seizures [48]. Recently, two independent groups have reported a sex difference in heterozygous DS mice, with females exhibiting more frequent spontaneous seizures of higher severity [49], as well as a higher degree of mortality than males [50].

Functionality studies conducted in the rodent models have highlighted the association of inhibitory interneurons to DS, where sodium currents were reduced in the GABAergic interneurons of both *Scn1a*+/− and *Scn1a*−/− DS mice [41], resulting in an enhanced neuronal excitability, followed by a reduction in action potentials [36]. Deleting *Scn1a* in somatostatin-expressing, parvalbumin-positive or GABAergic neurons led to a reduction of postsynaptic potentials [51], increased hyperthermia sensitivity and seizure propensity [41], or epilepsy and death [52], respectively. Collectively, these findings point to a seizure mechanism caused by an imbalance in the inhibition of neuronal networks due to loss of function of $Na_V1.1$, and multiple disease traits caused by functional deficits in varying interneurons [41,53,54].

4. Genetic therapies for Dravet Syndrome

Development of genetic therapies for severe neurological disorders is vastly changing. In this part of the review, we will summarize the different types of viral and non-viral genetic therapeutic technologies developed for Dravet syndrome.

4.1. Viral gene therapy

Several types of viral vectors are available for gene therapy applications, each offering their own advantages and disadvantages, examples of which can be found in Table 1. The use of lentiviral vectors for DS has been limited due to the unstable nature of the *SCN1A* coding sequence in bacteria [55], rendering amplification of this gene for viral vector packaging significantly more challenging. Therefore, here we will primarily discuss the uses of Adeno-associated virus (AAV) and adenoviral vectors, as they are the most relevant in the DS field.

AAVs are the most common gene therapy vector associated with CNS disorders [72], and have been shown to be highly efficient when treating neurological disease [73]. Particularly, AAV9 has been the main serotype used in CNS-targeted therapies as it has been shown to cross the blood-brain barrier in neonatal and adult animals following intravenous delivery [74–76]. Furthermore, Zolgensma (AAV9_*SMN1*) has been successfully implemented as a single-dose treatment in pre-clinical [77] and clinical trials for Spinal Muscular Atrophy [60], resulting in its recent FDA and EMA approval.

A range of gene therapy strategies are being investigated for the treatment of DS. These are highlighted in Table 2 and are discussed in more detail below.

Traditional gene supplementation therapies have proven challenging for DS due to the large and unstable nature of *SCN1A* cDNA, yet a recent study overcame this through codon optimization and packaging into a high-capacity adenoviral vector [78]. Administration of high capacity adenoviral *SCN1A* gene therapy to adolescent heterozygous DS mice by stereotaxic injections into multiple brain regions resulted in increases in *SCN1A* mRNA, Nav1.1 protein, survival, reduction of thermal-induced seizure, and several behavioral readouts [78]. This study provided crucial evidence into the treatment of older DS mice, improving translatability of treatments to those patients who have had a diagnosis of DS for some time.

Niibori et al. designed an AAV9 vector encoding the multifunctional β1 subunit, which combines with the α subunit of heterotrimeric Nav1.1 protein. The β1 subunit has shown to modulate ion flow through the sodium channel, emerging as a potential therapeutic target. Administration of AAV9 Nav1.1 β1 subunit vector to P2 heterozygous DS mice via bilateral intracerebroventricular (ICV) and intracisternal magna administration [50] resulted in a greater increase in survival of treated female mice than treated male mice. In contrast, a battery of behavioral tests showed increased improvement in the male mice than the treated female mice [50]. However, the treatment was not able to reduce the susceptibility of mice to thermal-induced seizures.

Encoded Therapeutics have investigated specific regulatory regions controlling *SCN1A* expression, with the aim of targeting these to increase expression and Nav1.1 protein production [79]. ETX101 is an innovative gene therapy technique that comprises an AAV vector encoding an engineered transcription factor targeted to GABAergic interneurons, which is small enough to be packaged into an AAV vector unlike the *SCN1A* gene itself (AAV9-RE^{GABA}-eTF^{SCN1A}). *In vitro* studies conducted on human-induced pluripotent stem cell-derived GABAergic interneurons led to increased *SCN1A* mRNA expression and Nav1.1 protein [79]. When administered to P1 *Scn1a*+/− DS mice via bilateral ICV, this resulted in increased survival, significant improvement in hyperthermic seizure threshold, and reduction in spontaneous seizures [80]. Furthermore, bio-distributional and safety studies were conducted in non-human primates (NHP). Encoded Therapeutics are currently recruiting patients for a non-interventional study into the natural history of DS known as ENVISION [81], which will be followed in 2022 with an interventional clinical trial testing of ETX101 called ENDEAVOR [82].

Sarepta and StrideBio are currently within the discovery phase of a potential therapy called STRX-240, but little is known about this program. It is expected that the therapy would employ the STRIVE™ platform to engineer AAVs with increased efficiency, lower immunogenicity, and improved tissue-specific tropism [83], bespoke for a DS gene therapy.

4.2. Gene editing

Gene editing allows specific modifications to be made at target regions of DNA and has been a major aspiration in precision medicine and biotechnology as a treatment modality for genetic disorders. Gene editing has previously been used to cure different diseases in mice [84–88]. CRISPR (clustered regularly interspaced short palindromic repeats) mechanisms have proven successful

Table 1
Viral gene therapy approaches. + indicates an advantage, whereas – indicates a disadvantage.

Viral vector	Vector particleand genome	Advantages and disadvantages	Example gene therapy products	Reference (s)
Adeno-associated viral (AAV)	• 25 nm, non-enveloped • Single-stranded DNA • 4.7 Kb coding capacity	+ Range of serotypes to specify cellular tropism + High titers + Strong and sustained transgene expression - Limited capacity, especially in self-complementary form - Possible pre-existing immunity - Recent safety concerns over high-dose AAV therapy	• Glybera: AAV1_*LPL*^{S447X} • Luxturna: AAV2_*RPE65* • Zolgensma: AAV9_*SMN1*	[56–62]
Adenoviral	• 100 nm, non-enveloped • Double-stranded DNA • 37 Kb capacity	+ Range of serotypes + Capsid highly amenable to protein engineering + High transduction efficiency + Large capacity to accommodate large genes - Transient transgene expression - High inflammatory response	• Gendicine: Ad_*Tp53* • Oncorine: E1B mutant • Covid-19 vaccine (AstraZeneca)	[63–65]
Lentiviral	• 100 nm, enveloped • Single-stranded RNA • 7.5–9 Kb capacity [66]	+ Low immunogenicity and host-immunologically naïve + Efficient transduction of mitotic and quiescent cells + Integrating and non-integrating forms available - Low titers - Difficult to scale up production	• Kymriah: *ex vivo* LV_anti-CD19 transduced autologous T cells • Zynteglo: *ex vivo* LV_$\beta^{A\text{-}T87Q}$-*globin* transduced autologous T cells	[67–71]

in various gene editing systems [89], with Cas9 induction of double-stranded breaks, nickase-induced single-stranded breaks, base editors, prime editors, Cas13 RNA targeting, and RNA-guided effector proteins.

Nuclease defective forms of Cas9, known as dead Cas9 (dCas9) act as a scaffold to transport transcriptional inhibitors (CRISPRi), transcriptional activators (CRISPRa), histone alternants, and epigenetic modifiers to specific target genome sequences [90–93]. Recently researchers have fused dCas9 to an engineered reverse transcriptase to insert new genetic information into a target DNA region. Prime editing guide RNA (pegRNA) sequences are utilized to encode the DNA sequence insert as well as a high specificity to a target locus [89]. Base editing without the creation of double-stranded breaks can be induced by cytosine or adenosine deaminases, allowing correction of point mutations, or gene inactivation through the generation of stop codons [94]. Both CRISPR prime editing and CRISPR base editing systems have demonstrated evidence of high efficiency with rarely detected off-target effects [89,95,96].

Gene editing techniques for neurological disorders are being developed in preclinical studies. Examples of molecular targets that have been approached so far include mutant APP in Alzheimer's disease [97,98], mutant HTT in Huntington's disease [99] and *SOD1* in amyotrophic lateral sclerosis (ALS) [100]. For an excellent reviews of neurological gene editing, see Duarte and Deglon, 2020 [94] and Lubroth et al, 2021 [101].

In the context of DS, gene editing approaches using dead Cas9 fused with the transcriptional activators VP64 or VPR in order to enhance *Scn1a* transcription have been developed ([102;103]; Table 2). Colasante et al. developed a single gRNA specific to the *Scn1a* proximal promoter and delivered this via a dual AAV9 approach to neonatal heterozygous DS mice, specifically targeting GABAergic interneurons using the Dlx5/6 promoter. This gene editing treatment sufficiently stimulated *Scn1a* gene expression and reduced, but not ameliorated, thermal-induced seizures [102]. Yamagata et al. fused dCas9 to a second-generation transcriptional activator, VPR, in which VP64 is combined with the p65 subunit of NF-κB and the viral transcription factor Rta [103]. An AAV-PHP.eB encoding four tandem gRNAs was administered to 4-week-old haploinsufficient CRISPR-ON Cre-lox DS mice via tail vein injections, which resulted in upregulation of *Scn1a* in inhibitory neurons, ameliorated behavioral defects, febrile seizures, and mortality [103].

4.3. Non-viral genetic therapies

Genetic material can be delivered to cells without the use of a viral vector. Some non-viral approaches (Table 3) include the use of antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), phosphorodiamidate morpholino oligonucleotides (PMOs), naked plasmid DNA, or bifunctional RNAs. The goal of non-viral genetic therapies is not always to simply restore a copy of a functional gene; these can be used to alter transcription, splicing, alter transcript stability, or disrupt translation initiation [107].

In recent years, the development of ASO treatments for neurological disorders has increased in popularity. ASOs are short, single-stranded oligonucleotides which are complementary to the desired mRNA target and can alter protein expression [108]. Perhaps the most successful of these, culminating in FDA and EMA approval for the treatment of Spinal Muscular Atrophy is Spinraza. This ASO binds to *SMN2* pre-mRNA at the ISS-N1 splice silencing sequence, preventing negative splice factors from binding this site, allowing the recognition of exon 7 by cellular splicing machinery and thus the inclusion of exon 7 in the mature, full-length *SMN2* mRNA transcript, achieving great therapeutic benefit [109].

Programs in various stages of preclinical and clinical development for other neurological disorders include Batten's disease caused by mutations in *MFSD8* [110], Huntington's disease (NCT02519036: [111]), ALS (NCT01041222: [112–114]) and more. For excellent reviews on the development of ASO therapeutics, please see Rinaldi and Wood, 2017 [115] and Wurster and Ludolph, 2018 [116].

The use of non-viral gene therapies has shown great promise for channelopathies. For example, pre-clinical study using a gain of function *KCNT1* mouse model demonstrated after a single intracerebroventricular injection of ASO's to neonatal and post-natal *Kcnt1*−/− mice resulted in controllable seizures and increase survival [134].

Secondly, elevated sodium channel excitability caused by gain-of-function *Scn8a* variants could be ameliorated following neonatal delivery of an ASO, leading to an increase in survival from 2 weeks to 9 weeks [135]. When the same ASO was delivered to DS model mice, the reduction in *Scn8a* expression could partially compensate for *Scn1a* haploinsufficiency, highlighting the role of disease modifiers in achieving excitatory/inhibitory balance and DS phenotype (Lenk et al., 2020).

Table 2
Genetic therapies in development for Dravet Syndrome.

Compound Company or academic group	Key aspects of technology	Pre-clinical Results	Clinical development stage (if applicable)	Ref(s)
Viral gene therapy				
AdV-CAG-*SCN1A* Mora-Jimenez et al., University of Navarra	• Adenoviral vector encoding codon optimized *SCN1A* • 5-week-old stereotaxic bilateral injection ($4x10^6$ or $2x10^7$ vg/mouse) • Basal ganglia/cerebellum dual injections or basal ganglia/ cerebellum/pre-frontal cortex triple injections	• SH-SY5Y cells and primary neuronal cultures: dose-dependent increase in *SCN1A* mRNA and Nav1.1 protein • ***Scn1a*$^{WT/A1783V}$ mice:** • Significant reduction in interictal epileptiform discharges in high-dose cohort, partial effect in low-dose • 100-day survival: 95% (dual) and 100% (triple) treated vs 65% control • Triple-site treated mice significant increase in seizure threshold temperature • Behavioral improvements in treated mice in novel object, marble burying and rotarod tests, but not Morris water maze	No clinical development as yet	[78]
AAV9-pGad1-Navβ1-myc Niibori et al., University of Toronto	• AAV9 encoding auxiliary sodium channel subunit; Navβ1 • P2 bilateral ICV and ICM injection ($8x10^{10}$ vg/mouse)	**129 Sv-*Scn1a*^{tm1Kea}/*Mmja*+/- DS mice:** • Significantly higher mortality in untreated females vs males • Treated mice more likely to survive vs control • Survival effect more robust in females • Untreated: 1–24 seizures/day in 24 h prior to death • Reduction in seizure frequency in males, but not females following treatment • No effect of treatment on susceptibility to heat-induced seizures • Behavioral correction in males, but not females, in open-field, elevated plus maze, and passive avoidance tests	No clinical development as yet	[50]
AAV9-RE^{GABA}-eTF^{SCN1A} ETX101 Encoded Therapeutics	• AAV delivery of engineered transcription factor • Single P1 bilateral ICV dose (range $1.7x10^{10}$-$5.1x10^{10}$ vg/mouse) • Increase expression of endogenous *SCN1A* specifically in GABAergic interneurons	• Upregulation of *SCN1A* in human iPSC-derived GABAergic interneurons • P1 ICV injection: 30% more *SCN1A* in GABAergic neurons containing eTF^{SCN1A} transcript than without, Nav1.1 levels in brain 61.4 ± 14.1% vs 45.7 ± 11.7% in controls • Proportion of spontaneous-seizure mice 67% vs 20% control, 88% seizure free in hyperthermic assay vs 13% control • 90-day survival: 100% vs 50% control • 470-day survival: 83.2% vs 31.4% control**Non-human Primates (NHP)** • Single unilateral ICV injection to juvenile cynomolgus macaques ($4.8x10^{13}$-$8x10^{13}$ vg/animal) • Transgene expression throughout the brain, including cortex and hippocampus • No Dorsal root ganglion related toxicity observed	• ENVISION (NCT04537832; recruiting) • Observational study • 50 patients, aged 6–60 months • Plans to launch ENDEAVOR clinical trial in 2022	[79,80,82]
Gene editing				
AAV9-*Scn1a*-dCas9A Colasante et al., San Raffaele Scientific Institute	• CRISPR-ON dCas9-VP160/64 • Single gRNA targeting proximal promoter • Activation of *Scn1a* transcription • Dual AAV system: TRE-dCas9-VP64 and U6-sgRNA-mDlx5/6-tTA-tdTomato • ICV delivery at P0	• Primary hippocampal neurons: 4-fold *Scn1a* mRNA increase, 2-fold increased Nav1.1 • Wild type cortical interneurons: increased firing rate following *Scn1a*-dCas9A • *Scn1a*+/- cortical interneurons: 3.5-fold *Scn1a* mRNA increase, 2-fold increased Nav1.1 • Electrophysiological rescue • ***Scn1a*+/- DS mice:** • Increased *Scn1a* mRNA at 2 weeks of age • Increased seizure threshold temperature by hyperthermia and infection-induced fever • Shorter seizure duration vs control	No clinical development as yet	[102]

Table 2 (*continued*)

Compound Company or academic group	Key aspects of technology	Pre-clinical Results	Clinical development stage (if applicable)	Ref(s)
AAV-PHP.eB *Scn1a*-dCas9-VPR Yamagata et al., RIKEN Brain Science Institute	• CRISPR-ON dCas9-VPR • 4 gRNA multiplex • Single AAV delivery ($1.8x10^{11}$ vg/mouse) at 4 weeks of age	**dCas9-VPR$^{VPR/+}$/Vgat-Cre$^{Cre/+}$/Scn1a$^{RX/+}$ triple mutant DS mice:** • Increase in *Scn1a* mRNA and Nav1.1 protein • 12-week survival: 100% treated vs 82% control • Increased seizure threshold temperature • Longer latency to clonic seizures • Rescue of increased exploratory activity, anxiety, and thigmotaxis seen in control mice in open-field and elevated plus maze	No clinical development as yet	[103]
Non-viral genetic therapies				
AntagoNAT Hsiao et al., OPKA Health Inc, USA,	• ASO • Inhibition of a repressive long, non-coding RNA associated with *Scn1a* • 'Unsilencing' of *Scn1a* • Intrathecal injections to 7-week-old heterozygous DS mice. Repeated injections, once a week for 4 weeks.	• 86% AntagoNATs led to increase in *SCN1A* mRNA *in vitro*: 4.2-fold vs control • Highly specific to *SCN1A* **Scn1a^{E1099}X+/- DS mice:** • Dose-dependent 10–30% increase of *Scn1a* mRNA in brain vs control • 70% decline in average seizure number (20 μg group) vs control • Seizure threshold temperature significantly increased vs control**African green monkey:** • Adult, single IT injection • Presence of AntagoNAT in parvalbumin-positive hippocampal interneurons • Upregulation of brain *Scn1a* mRNA	No clinical development as yet	[47]
STK-001 Stoke Therapeutics	• ASO – TANGO technology • Intrathecal delivery • Splice-correcting ASO to increase production of full-length *SCN1A* mRNA isoforms	**129 Sv-*Scn1a*^{tm1Kea}+/- DS mice:** • Dose-dependent increase in *Scn1a* mRNA and Nav1.1 protein • P2 ICV injection (20 μg) 90-day survival: 97% treated vs 23% control. P14 ICV injection (60 μg) 90-day survival: 85% treated vs 64% control • SUDEP: 50% control mice, 5.2% P2-treated mice, 18.2% P14-treated mice • Reduced spontaneous seizure frequency and longer latency to first seizure in P2-treated mice vs control	• Phase 1/2a, open label clinical trials: • MONARCH (NCT04442295; recruiting) • 10, 20, 30 mg SAD, 20 mg MAD. Safety, tolerability and pharmacokinetics. • SWALLOWTAIL (NCT04740476; enrolling by invitation). Open-label extension study of MONARCH participants • ADMIRAL MAD up to 70 mg • BUTTERFLY 36 patients, aged 2–18 years • Observational study	[104–106]

AdV = adenoviral vector, vg = vector genomes, AAV = adeno-associated viral vector, P1 = post-natal day 1, ICV = intracerebroventricular, ICM = intracisternal magna, DS = Dravet Syndrome, iPSC = induced pluripotent stem cell, SUDEP = sudden unexpected death in epilepsy, CRISPR = clustered regularly interspaced short palindromic repeats, gRNA = guide RNA, ASO = antisense oligonucleotide, IT = intrathecal, TANGO = targeted augmentation of nuclear gene output, SAD = single ascending dose, MAD = multiple ascending dose.

Table 3
Non-viral gene therapy vectors.

Non-viral vector	Structure	Mechanisms of action	Example gene therapy products	Reference(s)
ASO	• ~18–30 nucleotides • Singlestranded • Nuclease resistant • Diverse chemistries – 2′MOE and PMO	• RNaseH competent – degradation of target RNA; gene silencing • Steric block – bind to target with high affinity, interfere with RNA/RNA and/or RNA/protein interactions; splice correction, corruption or isoform switching	• Spinraza: *SMN2* pre-mRNA • Vitravene: CMV_*IE-2* • Kynamro: *Apolipoprotein B100*	[107,117–125]
PMO	• Singlestranded • Charge-neutral chemistry	• RNaseH-independent steric blockade; inhibit protein translation • Intron/exon targeting; modulate pre-mRNA splicing	• Eteplirsen: Exondys51 • Golodirsen: Exondys53	[107,126–129]
siRNA	• 19 + 2 (complementary + overhang) nucleotides • Doublestranded	• Guide Argonaute2 within RNA-induced silencing complex (RISC) to target; RNA cleavage and silencing	• Onpattro: *Transthyretin*	[107,130,131]
Plasmid DNA	• Circular, double-stranded DNA	• Gene augmentation	• Neovasculgen: pCMV_*VEGF165*	[132,133]

A non-viral gene therapy has been reported by Hsiao et al. where they developed synthetic AntagoNATs for the treatment of DS (Table 2). NATs, or natural antisense transcripts, are long, non-coding RNAs associated with many gene loci that influence transcriptional regulation of nearby, associated genes [47]. It is possible to target these NATs with the use of oligonucleotides, known as AntagoNATs [47]. By inhibiting the repressive long non-coding RNA specific to *Scn1a*, AntagoNATs are able to increase expression of *Scn1a* itself, and thus potentially provide therapeutic benefit to patients with DS. *In vivo* studies demonstrated significantly reduced seizure phenotype improvements in excitability hippocampal interneurons after repeated intrathecal injections to 7-week heterozygous DS mice [47].

A novel technology, Targeted Augmentation of Nuclear Gene Output (TANGO), has been developed by Stoke Therapeutics for DS. In this instance, TANGO uses splice-correcting ASOs to reduce the production of non-productive messenger RNA; naturally occurring mRNA transcripts containing premature stop codons that are degraded via nonsense-mediated decay [107]. The TANGO technology works by targeting ASOs to alternative splice sites, promoting the generation of productive, full-length isoforms, thus increasing the translation of $Na_V1.1$ protein [136]. These novel ASOs were administered to P2 DS heterozygous mice and showed a significant increase in survival and in reducing spontaneous seizures [136]. Orphan drug designation has been granted to STK-001 by the FDA. The MONARCH phase 1/2a trial (NCT04442295) enrolling patients with DS aged 2–18 years [106] is underway in the US with preliminary safety and pharmacokinetic data expected to be available in the second half of 2021. The UK MHRA has recently granted authorization to initiate the ADMIRAL phase 1/2a clinical trial [137] assessing safety and tolerability of doses up to 70 mg of STK-001 with enrolment to begin later this year.

Tevard Biosciences and Zogenix [138] are developing two tRNA-based gene therapies for DS. Read-through technology can override premature stop codons caused by nonsense mutations, thus increasing the amount of full-length *SCN1A* mRNA available. However, little information is available in the public domain regarding data from preclinical experiments.

5. Conclusion

DS is a very complex and a severe condition where antiepileptics are often inadequate in controlling seizures and are unable to improve the cognitive decline in patients. There is no definitive genotype-phenotype correlation that has been delineated, as genetic mutations often lead to different phenotypic characteristics in patients. This, therefore, impedes the early diagnosis of DS and limits the potential medicinal therapies that could effectively slow-down disease progression. However, with the recent developments of the novel prediction model for patients with DS, this hopefully will aid and assist with prognostic counseling and early decisions on therapeutics [7].

Gene therapy for CNS disorders have developed tremendously over the years with AAV vectors being the major driving force for clinical trials [84]. Clinical trials intravenously delivering AAV9 to children with SMA have demonstrated that the vectors are safe and efficient in ameliorating the disease symptoms [60], illustrating that this field is vastly growing and moving in the right direction in treating neurological disorders. Gene editing tools are showing great promise in correcting specific point mutations and thus restoring correct protein formation [139]. Gene editing and gene therapy tools are constantly developing and therefore provide opportunities for novel treatments for neurological disorders.

Recent advancements in RNA-based therapies and positive therapeutic outcomes in pre-clinical studies for neurological disorders, further highlight the great advancements being made in genetic therapies.

Encoded and Stoke therapeutic genetic therapies for DS have been approved by the regulatory bodies [79,136] and thus provide an alternative treatment, for a long-term control of symptoms and potentially improved cognitive behavioral outcomes by increasing the expression of $Na_V1.1$. Several academic groups and biotechnology companies are developing further genetic therapeutic technologies applicable to DS, providing hope for this kind of treatment modality in the future.

As more genetic therapies for DS move toward clinical trials, we must consider how data from pre-clinical studies translate to patients with DS. Importance of outcomes such as survival or reduction in SUDEP is often prioritized in pre-clinical research, whereas measures of intellectual disability, behavioral deficits, and frequency of seizures may be more informative in determining the efficacy of the treatment. Furthermore, there is a need for early identification of DS to enable recruitment into clinical trials at an early age, for example to avoid pre-existing antibodies to AAV vectors,as it has been reported that children with neurological conditions seem to have increased seroprevalence compared to healthy children [140–142]. Combining these aspects, together with data and key measures from natural history studies will be necessary to design effective DS clinical trials.

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Conflict of interest

No conflict of interest.

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