Brain delivering RNA-based therapeutic strategies by targeting mTOR pathway for axon regeneration after central nervous system injury

Ming-Xi Li1, Jing-Wen Weng2, Eric S. Ho3,*, Shing Fung Chow2,*, Chi Kwan Tsang1,*

Abstract
Injuries to the central nervous system (CNS) such as stroke, brain, and spinal cord trauma often result in permanent disabilities because adult CNS neurons only exhibit limited axon regeneration. The brain has a surprising intrinsic capability of recovering itself after injury. However, the hostile extrinsic microenvironment significantly hinders axon regeneration. Recent advances have indicated that the inactivation of intrinsic regenerative pathways plays a pivotal role in the failure of most adult CNS neuronal regeneration. Particularly, substantial evidence has convincingly demonstrated that the mechanistic target of rapamycin (mTOR) signaling is one of the most crucial intrinsic regenerative pathways that drive axonal regeneration and sprouting in various CNS injuries. In this review, we will discuss the recent findings and highlight the critical roles of mTOR pathway in axon regeneration in different types of CNS injury. Importantly, we will demonstrate that the reactivation of this regenerative pathway can be achieved by blocking the key mTOR signaling components such as phosphatase and tensin homolog (PTEN). Given that multiple mTOR signaling components are endogenous inhibitory factors of this pathway, we will discuss the promising potential of RNA-based therapeutics which are particularly suitable for this purpose, and the fact that they have attracted substantial attention recently after the success of coronavirus disease 2019 vaccination. To specifically tackle the blood-brain barrier issue, we will review the current technology to deliver these RNA therapeutics into the brain with a focus on nanoparticle technology. We will propose the clinical application of these RNA-mediated therapies in combination with the brain-targeted drug delivery approach against mTOR signaling components as an effective and feasible therapeutic strategy aiming to enhance axonal regeneration for functional recovery after CNS injury.

Key Words: axon sprouting; axon regeneration; brain targeted drug delivery; CNS injury; ischemic stroke; mTOR; nanoparticle; neural circuit reconstruction; PTEN; RNA-based therapeutics

Introduction
It is well known that the central nervous system (CNS) injury triggers spontaneous recovery. For instance, ischemic stroke alters the expression of genes that stimulate the growth of axons and dendrites, followed by circuit reconnection and synaptic reorganization, pruning, and functional recovery (Cramer, 2018). These molecular events often occur in the penielsional tissues related ipsilateral areas and functionally related contralateral areas, and spinal cord (Carmichael et al., 2017). The nervous system has a surprising plasticity, especially in the peripheral nervous system. Unfortunately, spontaneous recovery is often incomplete, leaving many patients with long-term disability.

Axon regeneration after CNS injury is very limited because of the extrinsic and intrinsic factors. Extrinsic factors consist of the glial scar, myelin debris, and glia-secreted growth-suppressive molecules such as Nogo and myelin-associated inhibitors (He and Jin, 2016). Previous works have demonstrated that blocking these extrinsic inhibitory factors is insufficient to trigger axon regeneration after axotomy in the CNS. Intriguingly, recent findings have uncovered the pivotal role of intrinsic factors in stimulating axon regeneration in various CNS injury models (Fawcett, 2020). Among them, the mechanistic target of rapamycin (mTOR) pathway has been convincingly demonstrated to be one of the most important intrinsic regenerative pathways in almost all major CNS injuries such as stroke, brain, and spinal cord damages. In this review, we will discuss the recent findings of the mTOR pathway in association with axon regeneration and identify the promising targets after CNS injury. In addition, we will discuss the potential application of RNA-mediated strategy for these targets. Finally, we will review the recent advance concerning the novel brain delivery technology to facilitate the clinical application of these strategies.

Database Search Strategy
In this narrative review, we performed the NCBI PubMed database search for identifying English-language articles that reported the role of mTOR regenerative pathway in axon regeneration after CNS injury, RNA-based therapy, and the brain-targeted drug delivery from 2001–2021. Search terms included CNS injury, mTOR, axonal regeneration, axon sprouting, RNA-mediated therapy, nanoparticles, brain-targeted delivery, ischemic stroke, spinal cord injury, retinal ganglion cell (RGC) repair, and traumatic brain injury. The authors also included primary research papers, case reports, and review articles. The titles and abstracts for inclusion were independently reviewed by the authors. Additional relevant articles were also identified from the referenced citations. For the DrugBank search, “sRNA” was the term used. For the search of clinical trials, we narrowed down to “Interventional studies” where “Intervention” is “sRNA”, and “Status” included completed and all kinds of recruiting modes. We filtered only “Interventional studies” where “Intervention” is “Antisense oligonucleotide”, and “Status” included completed and all kinds of recruiting modes.

*Correspondence to: Eric S. Ho, PhD, hoe@lafayette.edu; Shing Fung Chow, PhD, afschow@hku.hk; Chi Wkan Tsang, PhD, tsangch@jnu.edu.cn.
https://orcid.org/0000-0002-0713-9545 (Eric S. Ho); https://orcid.org/0000-0002-4588-5346 (Shing Fung Chow); https://orcid.org/0000-0002-0133-1544 (Chi Kwan Tsang)

Funding: This work was supported by the National Natural Science Foundation of China (No. 81974210), the Science and Technology Planning Project of Guangdong Province, China (No. 2020A0505100045) and the Natural Science Foundation of Guangdong Province (No. 2019A1515010671), all to CKT.

How to cite this article: Li MX, Weng JW, Ho ES, Chow SF, Tsang CK (2022) Brain delivering RNA-based therapeutic strategies by targeting mTOR pathway for axon regeneration after central nervous system injury. Neural Regen Res 17(10):2157-2165.
Axonal Regeneration after Central Nervous System Injury

CNS injury after stroke induces a rapid loss of neurons and axons which accounts for the loss of nerve connections during the acute phase and subsequently induces various degrees of plasticity during the spontaneous regenerative phase (Tsang et al., 2018). At the molecular level, stroke injury induces axon sprouting, dendritic branching, and synaptogenesis for remapping of neural circuits and re-constitution of the lost connectivity (Carmichael et al., 2017). Axonal sprouting is fundamental to the spontaneous regeneration of injured axons to establish new connections around the peri-infarct regions, or in areas in different lobes in the ipsilateral and contralateral hemispheres, depending on the size and location of the infarct. Axonal sprouting can also occur from the contralateral cortex to the peri-infarct region and the ventral spinal cord (Carmichael et al., 2017). In contrast to stroke, most injured axons resulting from axotomy or trauma in the adult brain or spinal cord do not show significant spontaneous regeneration (He and Jin, 2016). Multiple challenges exist such as the focal ischemic stroke in adults for recovery. Given that the spontaneous CST axon stump at the site of injury is polarized by the growth cones, and the requirement of axon extension over a long distance to reconnect with their targets. One of the most difficult hurdles is the synthesis of building materials such as cytoskeletal, neurofilaments, and plasma membrane in the regenerating axons (He and Jin, 2016). However, after the developmental stage, the activity of the intrinsic regenerative pathway is often diminished to avoid cellular overgrowth in the adult stage. Based on the reasoning above, the group of He examined the evolutionary conserved mTOR signaling pathways which may contribute to the diminished regenerative capacity in adult CNS neurons (Park et al., 2008). By using the genetic knockout approach in the adult retinal ganglion cells (RGCs) and optic nerve injury model, they examined the effect of knocking down the cortical mTOR expression in mice including Mtorc1, Mtorc2, and PTEN on axon regeneration. Surprisingly, they found that deletion of PTEN alone is sufficient to promote robust axon regeneration without manipulation of extrinsic factors in the lesion site (Park et al., 2008). They further confirmed that PTEN down-regulation has a similar effect of mTOR pathway which produces a similar effect. Importantly, they showed that mTOR activity is suppressed in the axotomized RGCs of wild-type adult mice (Park et al., 2008). These results convincingly demonstrate that mTOR signaling is the intrinsic axon regenerative pathway which mediates axon regeneration by targeting its negative signaling components can promote axon regeneration after adult CNS injury (Park et al., 2008). Since this pioneering work, substantial follow-up studies from different laboratories have confirmed the key role of mTOR pathway in axon regeneration in various CNS injury models (He and Al-Ali, 2017; Huang et al., 2019; Williams et al., 2020; Bhowmick and Abdul-Muneer, 2021; Ma et al., 2021).

In addition to axon regeneration, the administration of human recombinant insulin has been reported to fully restore mTOR activity in injured RGCs which is accompanied by robust regeneration of dendrites and activation of synaptogenesis (Agostinone et al., 2018). By knocking down Raptor, the specific and essential mTORC1 component, the authors further demonstrated that insulin-dependent mTOR activation is required for restoration of dendritic branching, arbor complexity, and re-establishment of the field area. These findings indicate that the activation of mTOR signaling can promote the survival of dendrites and synaptogenesis, and restores circuit function after traumatic CNS injury as well as in neurodegenerative diseases such as glaucoma. After a stroke, the peri-infarct cortex responds by triggering axonal sprouting that leads to functional recovery in various CNS injury models (Carmichael, 2017). It has been reported that the dendritic morphology changes in regions of axonal sprouting in the peri-infarct cortex (Agostinone et al., 2018). Since dendrites can be remodeled after the loss of different innervation, the remodeling dendrites and the spared or sprouting neurons could promote synaptogenesis. A series of seminal works performed by Carmichael’s group has shown that this limited capacity can be significantly increased by stimulating the intrinsic neuronal regrowth program in the motor, somatosensory, premotor cortex, and motor corticospinal projections in the cervical spinal cord (Carmichael et al., 2017). Not surprisingly, mTOR pathway is an ideal target for promoting stroke recovery as it has pleiotropic effects on multiple cellular processes involved in the neuronal repair. Physiologically, the peri-infarct region and white matter periphery can stimulate the regeneration of injured axons to produce similar axon growth-promoting effects. We and others have recently found that mTORC1 regulates MAF1 and superoxide dismutase 1 (SOD1) as two immediate downstream effectors that mediate the key cellular processes including the biogenesis of ribosomes and translation machinery, metabolism, and redox homeostasis (Tsang et al., 2018; Willis, 2018; Wang et al., 2021). Although the precise mechanism by which mTOR pathway regulates axon regeneration remains unclear, a large body of evidence has suggested that mTOR pathway is intimately linked to axon regeneration after CNS injury through the following downstream regulations.

Possible Mechanisms of mTOR-Mediated Axon Regrowth

mTOR integrates diverse upstream signals including neurotrophic factors, neurotransmitters, growth hormones, nutrients and energy levels, and stress stimuli to regulate various aspects of growth, survival, metabolism, and cellular homeostasis (Tsang et al., 2007). mTOR forms two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), with shared and distinct components. mTORC1 pathway is highly sensitive and inhibited by rapamycin and its derivatives, while mTORC2 is less sensitive to rapamycin treatment (Liu and Sabatini, 2020). Since most of the mTOR functions associated with CNS repair are mediated by mTORC1, we will focus on this pathway.

mTORC1 promotes protein synthesis, cell growth, and proliferation by activating two well-characterized downstream substrates, S6K and 4E-BP, which are the key regulators of mRNA translation and protein synthesis (Liu and Sabatini, 2020) (Figure 1). As a well-studied mTOR target, S6K1 has been suggested as a positive effector of axon outgrowth. However, a recent study has challenged this idea by showing that PF-4708671, a selective S6K1 inhibitor, stimulates corticospinal tract regeneration and increases axon density beyond the injury site, and produces significant locomotory recovery (Ali et al., 2017). Therefore, not all the positive effectors of mTOR could promote axon regeneration in vitro and in vivo (Tsang et al., 2018). Although the detailed mechanisms remain to be determined, we and others have recently found that mTORC1 regulates MAF1 and superoxide dismutase 1 (SOD1) as two immediate downstream effectors that mediate the key cellular processes including the biogenesis of ribosomes and translation machinery, metabolism, and redox homeostasis (Tsang et al., 2018; Willis, 2018; Wang et al., 2021). Notably, a neuron should generate a large amount of ribosomes to support protein production in the growth cones. In the absence of mTORC1 activity, ribosome biogenesis and growth of Kirsten rat sarcoma viral oncogene homolog (K-RAS)-driven lung cancer (Wang et al., 2021), suggesting that mTOR may fine-tune the rate of ribosome biogenesis via Sod1. As Sod1 catalyzes the conversion of superoxide to H2O2, it has been reported that Sod1 plays a role in the recovery of a mouse model of closed head injury (Beni et al., 2006). Interestingly, a recent study using unbiased mutagenesis screening in C. elegans demonstrated that regulation of the biogenesis of ribosomes and translation machinery is coupled and crucial to hypoxic sensitivity (Itani et al., 2021). Although the detailed mechanism remains to be determined, neuronal mTOR is likely to play a critical role in ribosome biogenesis which is the determining factor for new protein synthesis and axon regrowth (Twiss and Fainzilber, 2009). Notably, a neuron should generate a large amount of ribosomes and other translation machinery to meet the demand for a high protein production rate for supporting the long-distance axon regeneration. We and others have identified that MAF1 is a conserved downstream effector of mTOR for regulation of RNA polymerase III-dependent transcription which controls the synthesis of 5S ribosomal RNA and transfer RNAs which are the essential components of ribosomes and translation machinery (Li et al., 2006; Wei et al., 2006). Although mTOR controls mTORC1 pathway, we and others have further found that MAFL1 binds to the PTEN promoter to enhance PTEN promoter acetylation and increase PTEN expression (Li et al., 2016). Therefore, upregulation of mTOR pathway is likely to promote mTOR-mediated biogenesis of ribosomes and transcription machinery in support of axon regeneration.
Regulation of Local Protein Translation

mTOR has a well-known effect on cap-dependent protein translation which has been implicated in local protein synthesis in non-neuronal cells (Liu and Sabatini, 2020). Local biosynthesis is a plausible solution for the neurons to reduce energy-costly axonal transport from the cell bodies. Local protein synthesis mediated by mTOR activity in axons has been reported to affect pre-synaptic plasticity in the mature mammalian brain (Younts et al., 2016). A recent relevant study further demonstrated that mTOR activity is required for local translation, including its mRNA and the retrograde injury signaling molecules such as importin β1 and STAT3, in injured axons (Terenzi et al., 2018). Maintenance of the axonal pool of mTOR mRNA enables rapid and local upregulation of protein synthesis in injured axons. These results indicate that mTOR pathway plays a key role in the regulation of local translation and injured response of axon.

Growth cones form the axon projections that are required for establishing neural circuits during neurodevelopment and neural repair after injury. A recent study, using the growth cone sorting and subcellular RNA-proteomic mapping technique, analyzed local transcriptions and proteinomes in vivo in the developing axon projections in the cerebral cortex (Poulopoulos et al., 2019). This study found that mTOR and the mRNAs that contain mTOR-dependent motifs for their translation are accumulated in these growth cones of developing projections, suggesting that mTOR-dependent local protein translation plays a crucial role in axon growth. Intriguingly, the authors identified that approximately 80% of all significantly growth cone-enriched transcripts contain the S′ terminal oligopyrimidine (TOP) motif. The expression of TOP-transcripts, which include the ribosomal proteins and translation initiation factors, is tightly coupled to cell growth. Importantly, their expression is under direct control of mTOR (Tsang et al., 2007). The knockout approach was further used to demonstrate that mTOR signaling is necessary for trans-hemispheric axonal growth in vivo (Poulopoulos et al., 2019). These studies unveil the mTOR and its pathway, as a key player of cellular translation machinery, at the leading edge of growing long-range axon projections, suggesting that mTOR plays an important role in neural repair after injury.

As mTOR is a key sensor for monitoring various pathophysiological conditions, the stress environment after stroke or traumatic CNS injury diminishes this important intrinsic regrowth pathway. The encouraging evidence discussed above further reinforces the exciting prospect that reactivation of mTOR pathway may drive axonal regeneration following injury. As shown in Figure 1, the RNA-mediated therapeutic strategies such as antisense oligonucleotide (ODN) by knocking down the negative regulators in mTOR pathway was a promising approach. Since there are many negative inhibitory factors in the mTOR pathway, knocking down these factors can upregulate mTOR pathway activity, making the RNA-mediated knockdown approach particularly attractive. For this reason, we will focus on the use of RNA-based therapeutics and discuss the feasibility of targeting them as a promising therapeutic strategy for promoting neural repair after CNS injury.

RNA-Based Therapeutics

There are three classes of RNA-based therapeutics: antisense oligonucleotide (AS-ODN), RNA interference (RNAi-ODN), and aptamer. Here we will highlight the distinctive features of AS-ODN and RNAi-ODN. Readers who are interested in more information about them are recommended to refer to review articles dealing with each class of therapeutic strategy (Sherman and Contreras, 2018; Setten et al., 2019; Roberts et al., 2020). The overarching goal of RNA-based therapeutics is to intervene disease pathways by modulating fundamental RNA-associated processes, including splicing, polyadenylation, protein translation, and mRNA degradation, through sequence-specific binding of oligonucleotides to targeted pre- or mature messenger RNAs (pre-mRNAs or mRNAs).

Traditional small-molecule approaches achieve therapeutic outcomes by interfering the functions of disease-associated targets, usually proteins, using small organic drug compounds that interact with them. Such an approach requires the existence of viable drug binding pockets or receptors in the targets, which are hard to determine or even non-existent, leading to undruggable gene targets, e.g. oncogene KRAS (Kessler et al., 2019; Moir et al., 2020). In the targets, which are hard to determine or even non-existent, leading to undruggable gene targets, e.g. oncogene KRAS (Kessler et al., 2019; Moir et al., 2020). RNA-based therapeutics, unlike small-molecule drugs that require structural information of the binding sites, derive targets from genomic sequence information, which is readily and cheaply available due to the advance of high throughput DNA sequencing with ever-decreasing cost. Choosing the unique signature sequence of a target's mRNA requires much less effort than determining the optimal binding site of a target protein that fits the drug molecule. Sequence-based targeting also boosts RNA-based therapeutics the capability to differentiate wild-type and mutant targets. Recently, diseases have been reported to be associated with non-coding RNAs (Statello et al., 2021; Siciliano et al., 2020). In the absence of protein products, it further enhances the application of RNA-based therapeutics. Importantly, the manufacturing process and delivery platform of RNA-based therapeutics for various therapeutic areas, such as vaccines, cancer, etc., are chiefly identical, greatly streamlining effort in pharmacokinetics study. That said, it comes down to a specific drug delivery, and tissue remains the most challenging task (Didiot et al., 2016; Migiani et al., 2019; Tai, 2019). More details will be discussed below.
RNA Interference
Another class of RNA-based therapeutics is small interference RNA (siRNA) that harnesses the RNA interference (RNAi) cellular pathway. siRNA is a double-stranded RNA molecule. Each strand of the siRNA is 21 bps long, but the two strands differ at positions 1 to 19, leaving two overhanging bases at the 3′ termini. One strand of a siRNA is named the guide strand or antisense strand, which is the strand that is utterly complementary to the target transcript. The other strand is called the passenger strand or sense strand in which no active role is played in it in the RNAi pathway (Elbashir et al., 2001).

siRNAs are first processed by Argonaute2 (ARGO2) in the cytoplasm. ARGO2 will remove one strand from the double-stranded siRNA molecule, forming the RNA-induced silencing complex (RISC). Whether the removed strand by ARGO2 is the guide strand or the passenger strand is arbitrary (Gregory et al., 2005). Further studies are needed to elucidate the degree to which the discarded strand underpins the choice of strand made by ARGO2. From the therapeutic perspective, measures must be taken to safeguard no off-target effect can be induced by the passenger strand even though it does not interact with the target siRNA. RISC utilizes the sequence specificity of the guide strand to recognize the target transcript. Upon binding, enzymatic slicing activity of ARGO2 (Roberts, 2015) will be activated to cleave the target transcript at a position near the middle of the guide strand, i.e., between positions 10 and 11 (Schurmann et al., 2013), diminishing the biological functions of the transcript.

Similar to AS-ODNs, selected RNA nucleotides of the siRNA are substituted by modified RNAs such as 2′-O-methyl. Regarding the proportion and position of the modified RNAs that can achieve optimal results, it is beyond the scope of this review.

Five siRNA therapeutics are found in DrugBank, in which two have been approved by FDA (Additional Table 3). ClinicalTrials.gov indicates 20 active interventional trials (Additional Table 4). Thus, siRNA therapeutics have yet to be developed in the neurological areas.

Workflow for Bioinformatic Identification of Target Sites
Figure 2 summarizes a multistep process for identifying putative target sites of a gene target. The process begins with the gene target postulated to achieve the therapeutic goal. The next step is to identify potential binding sites on the gene target. The process begins with the gene target postulated to achieve the therapeutic goal. The next step is to identify potential binding sites on the gene target (Additional Table 4). We will use the antisense strand of the target site sequence while leaving mismatches and/or gaps at the two termini. Besides searching for homologous sites in transcripts, it is advisable to expand the radar to the whole genome as homologous sites present, for example, in introns and splice site junctions may attenuate unintended normal splicing process and lead to the desired clinical outcome. Once the specificity of candidate target sites has been warranted, their accessibility to ODNs will be assessed. Here we will focus on RNA secondary structures and RNA binding proteins (RBPs). A valuable data source for RNA secondary structures is RNA Atlas of Structure Probing or RASP (Li et al., 2018). This database has compiled an example of the number of secondary structures of ODNs generated by different techniques on various cell lines and species, including humans, mice, plants, bacteria, fungi, and viruses. Besides data browsing, the RASP portal also caters to large volume data download via their programmable interface. RASP has a particular RASP format. Further options are provided for users to download data from a specific species, experimental technique, cell line, data type (which means scoring method), etc. With the RNA secondary structure data, candidate target sites can be assessed if significant secondary structures can hinder binding in the proximity.

Moreover, the binding of RBPs may block ODNs from accessing the target site. The Encode project (www.encodeproject.org, 2018) is a valuable source of RBP binding sites (Yee et al., 2019). At the time of writing, the ENCODE project has archived results from 737 experiments on 258 RNA binding proteins produced by seven assays on five cell lines (ENCODE Project Consortium, 2012). Similar to secondary structure data, RBP binding data is formatted in BED and available for download.

At the end of the pipeline, the candidate target sites that pass the above multilevel of vetting will be handed over for experimental validation.

Antisense Oligonucleotide Targets of Phosphatase and Tensin Homolog
As previously discussed, numerous reports have demonstrated that PTEN deletion could promote robust and long-distance axon regeneration in adult CNS neurons in various injury models. Hence, antagonizing the expression of the PTEN gene is a viable therapeutic goal. Here, we will use PTEN as an example to illustrate how the pipeline produces candidate target sites for the AS-ODN platform.

The first step is to collect all wild-type isoforms of PTEN. We identified human PTEN isoforms from NCBI RefSeq and ENSEMBL databases. RefSeq database suggests three PTEN transcripts (NM_000314, NM_001304717, and NM_001304718). Starting with ENSEMBL, it is recommended to obtain isoforms from at least two databases for cross-validation purposes. More details will be discussed below.

If the target is the mutant form of the transcript, the oligonucleotide target site must include the mutated sequence instead of the wild-type counterparts. If the type of mutation is insertion, the junctions of insertion become the signature sites of the target. For deletion, the flanking regions of the deleted segment are the only possible place to bind the ODN without interfering with the wild-type transcripts.

If the target is a specific unmethylated isoform, all isoforms of the target gene must be aligned to figure out the isoform-specific regions. It is noteworthy that the presence of such region(s) is not guaranteed. Furthermore, the target regions should be free from genetic variations. Tools such as Ensembl Variant Effect Predictor (Chapman et al., 2013) and BioMart (Smedley et al., 2015) are helpful to uncover such variations.

To streamline testing and avoid confounding factors, it is ideal to have a single set of ODNs to be tested in animal models, such as in mice, and in humans. Previous studies have shown many ultra-conserved regions were discovered among diverse species, e.g., humans, rats, mice, cows (Bejerano et al., 2004, Ho and Gershon, 2011).

In the next step, the long target regions will be split into overlapping short sequences (potential target sites) with their length equal to the required length of the RNA therapeutic platform. The size of target sites ranges from 9 to 20 bases with siRNA near to the low-end and AS-ODN near to the high-end. Afterward, we will examine the biochemical properties of each potential target site, such as GC%, which affects the self-dimerization potential.

Those potential target sites that have fulfilled the above criteria will be checked further for sequence specificity. Word-based alignment tools such as BLASTN (Sayers et al., 2021) can be used to search against the genome and transcripts. It is noteworthy that as target sites are usually short, word-based alignment algorithms tend to bias perfect matches in the middle of a target site sequence while leaving mismatches and/or gaps at the two termini. Besides searching for homologous sites in transcripts, it is advisable to expand the radar to the whole genome as homologous sites present, for example, in introns and splice site junctions may attenuate unintended normal splicing process and lead to the desired clinical outcome.
The pipeline is designed to address three major concerns: accessibility, specificity, and binding affinity. Avoiding RNA secondary structures and sites being blocked by RNA-binding proteins (RBPs) are vital to ensure that the oligonucleotide (ODNs) can hybridize with the target site in the absence of interference. To eliminate the off-target effect, ODNs must specifically bind to the target site. As such, isoforms, genetic variations of the target gene must be incorporated for sequence comparison, followed by sequence homology searching. The last factor is the sequence propensities of the binding sites such as GC-content, CpG induced immunity, and self-annealing potential. In this multistep process, a large number of ODN binding sites are eliminated, alleviating effort such as GC-content, CpG induced immunity, and self-annealing potential. To eliminate the off-target effect, binding proteins (RBPs) are vital to ensure that the oligonucleotide (ODNs) can hybridize with the target site in the absence of interference. To eliminate the off-target effect, ODNs must specifically bind to the target site. As such, isoforms, genetic variations of the target gene must be incorporated for sequence comparison, followed by sequence homology searching. The last factor is the sequence propensities of the binding sites such as GC-content, CpG induced immunity, and self-annealing potential. In this multistep process, a large number of ODN binding sites are eliminated, alleviating effort for experimental validation.

There are twenty-eight antisense oligonucleotide (AS-ODN) drugs registered in DrugBank, where nine of them are approved, as shown in Additional Table 1. The majority of them belong to cancer treatment. Whereas siRNA drugs, only five are found, out of the five, two have been approved. Since 2018, twelve active AS-ODN clinical trials have been conducted, spanning phases I to III (Additional Table 2). The conditions cover from rare diseases to cancer to common diseases. Twenty-one active clinical trials have been found for siRNA since 2012, more than AS-ODN. Similar to AS-ODN, trials span all three phases.

### Challenges of Delivering RNA Therapeutics to the Brain

Despite RNA therapy having emerged as an attractive strategy for the treatment of various diseases, their clinical application is hindered by several hurdles from the viewpoints of drug delivery. They are negatively charged and hydrophilic macromolecules which are difficult to be taken by cells. Also, they are biologically unstable with a short half-life due to rapid degradation by nucleases, and subsequent renal clearance. Moreover, naked RNA therapeutics can induce undesired off-target side effects and immune responses by binding to Toll-like receptors (Bishop et al., 2015). In particular, the instability of RNA in the host would limit the effectiveness of the neural repair. While there is no clinical study currently available to evaluate this issue, several lines of evidence from preclinical studies have convincingly depicted in Figure 2. The total length of the two exons is 1009 bps, resulting in 990 putative antisense ODNs. Figure 3B displays the first five putative antisense ODNs that target the 3’-end of exon 1. These putative ODNs will be checked if their sequence propensities fulfill criteria such as the minimum GC content and melting temperature (Tm), which are essential in securing binding. To avoid self-dimerization, ODNs that share a high degree of self-complementary must be eliminated. For example, the PTEN fragment GCAGCCGCCGCGGCCGCCGC is 80% complementary to itself. ODNs forming into secondary structures is another property that should be avoided. There are open-source tools that calculate the minimum free energy of an RNA sequence, such as ViennaRNA and RNAlib (Lorenz et al., 2011). ODNs that share high homology to non-target genes or their regulatory regions may lead to the off-target effect, so the pipeline must identify and discard them. For the PTEN example, putative ODNs were aligned to the human genome and the human transcriptome using NCBI BLASTN (Camacho et al., 2000). The former reveals potential bindings to the regulatory, exonic, and intronic regions of genes, while the latter provides additional exonic-exon splicing junction information. Since these ODNs are short, BLASTN will automatically readjust or optimize search parameters that facilitate their alignments against large sequence databases. Despite that, additional adjustments are recommended as shown below:

- Choose the “Somewhat similar sequences” program (BLASTN).
- Reduce the “Max target sequences” to 10 from the default 100 as it will speed up the searching process.
- Lower the “Expected threshold” to 0.01, demanding statistically significant hits, and it will expedite searching as well.
- Choose the smallest “Word size”.
- Uncheck all the filters and masks so that the entire genome and transcriptome are searched.

The number of putative ODNs is usually large, manual screening is prohibitive for a sizable report as such. Therefore, it is recommended to download the hit-table in a readily computer-readable format, e.g., .csv, so that hit information can be extracted by a simple Python script, for example.

### RNA-Based Therapeutics Approved or Under Clinical Trials

There are twenty-eight antisense oligonucleotide (AS-ODN) drugs registered in DrugBank, where nine of them are approved, as shown in Additional Table 1. The majority of them belong to cancer treatment. Whereas siRNA drugs, only five are found, out of the five, two have been approved. Since 2018, twelve active AS-ODN clinical trials have been conducted, spanning phases I to III (Additional Table 2). The conditions cover from rare diseases to cancer to common diseases. Twenty-one active clinical trials have been found for siRNA since 2012, more than AS-ODN. Similar to AS-ODN, trials span all three phases.

### Challenges of Delivering RNA Therapeutics to the Brain

Despite RNA therapy having emerged as an attractive strategy for the treatment of various diseases, their clinical application is hindered by several hurdles from the viewpoints of drug delivery. They are negatively charged and hydrophilic macromolecules which are difficult to be taken by cells. Also, they are biologically unstable with a short half-life due to rapid degradation by nucleases, and subsequent renal clearance. Moreover, naked RNA therapeutics can induce undesired off-target side effects and immune responses by binding to Toll-like receptors (Bishop et al., 2015). In particular, the instability of RNA in the host would limit the effectiveness of the neural repair. While there is no clinical study currently available to evaluate this issue, several lines of evidence from preclinical studies have convincingly
suggested that RNA-mediated knockdown of target genes could promote significant neural repair and regeneration. For example, it has been reported that shRNA-based knockdown of CCR5 through intracranial injection induces robust axonal sprouting and regeneration in the mouse cerebellar cortex after stroke (Duan et al., 2015). Consequently, the induction of neurogenesis and cognitive decline improved (Joy et al., 2019). Axon regeneration after optic nerve injury in mice was also reported by intravitreally delivery of shRNA against PTEN (Duan et al., 2015). Additionally, the antisense oligonucleotide delivery by intracerebroventricular injection to mice has demonstrated effectiveness for the treatment of spinocerebellar ataxia type 2 (Scoles et al., 2017). To overcome the RNA instability issue, several approaches can be used, such as chemical modification of mRNA or miRNA, and relating about which more need to be studied in peripheral blood or cerebrospinal fluid. Moreover, the drug administration frequency, dosage, and carriers can be adjusted to meet the therapeutic level. Currently, there are several clinical trials using the antisense oligonucleotides and antisense oligonucleotides in patterns of targeting Huntington's disease. These results suggest that the RNA-based knockdown approach is promising to induce neural repair.

The BBB represents another major obstacle to successful RNA delivery to the brain through the systemic circulation. It is comprised of brain capillary endothelial cells, astrocytes, pericytes, microglia, neurons, and mast cells, synergically forming a dynamic interface to control the exchange of substances between the blood and the brain. The tight junctions formed between the adjacent endothelial cells restrict the entry of most of the therapeutic agents, only allowing passive diffusion of small water-soluble molecules but to a little extent. Moreover, lipid-soluble molecules with molecular weights of less than 400 Da are favorable to cross the BBB through transmembrane diffusion (Crawford et al., 2016). Even though the efflux transporter expressed on the surface of endothelial cells, such as P-glycoprotein and multidrug resistance proteins, can extrude these foreign molecules passing the BBB. Carrier-mediated transport, transporter-mediated transcytosis, and adsorptive-mediated transcytosis are known mechanisms to facilitate the BBB transportation, but are selective to a few essential compounds like glucose, insulin, and albumin. Cell-mediated transcytosis is another possible route for immune cells, and pathogens across the BBB. Recent evidence also confirms the BBB dysfunction in central nervous system (CNS) disorders including Alzheimer’s disease (AD), Parkinson’s disease (PD), stroke, etc. (Sweeney et al., 2018). Although the disease-induced BBB disruption is commonly assumed to promote the entry of therapeutic agents into the CNS, it complicates the process by altering the expression of tight junctions and transporters on the BBB.

The research of Stoicea et al. (2016) suggests that the BBB allows the exchange of miRNA between the cerebral and the blood, and the bloodstream is a promising biomarker for the diagnosis of CNS diseases. However, miRNA possesses a more diluted concentration in the blood in health conditions and is prone to leak from the brain in pathological conditions. On the other hand, siRNA with molecular weights of around 13 kDa cannot cross the BBB (Mathupala et al., 2006).

Current Strategies for RNA Therapeutics

Overcoming the Blood-Brain Barrier

One of the obvious strategies is to bypass the BBB, which can be achieved through the alteration of the administration route. The intracerebral administration is the most direct approach to delivering therapeutics into the brain, which requires the assistance of neurosurgical surgery to achieve precise delivery, implantation of drug reservoirs such as Glaidel® wafers for less frequent dosing, and convection-enhanced delivery for creating a positive hydrostatic pressure to drive the agents to the targeted site (Oh et al., 2020; Saraiva et al., 2016; Yu et al., 2017). The intrathecal injection is also practical to deliver the drug into the CNS to circumvent the BBB. However, these methods are all invasive, requiring specific techniques but also bringing a painful experience to the patients, resulting in an increased burden of healthcare professionals and patient non-compliance. Safety issues related to the risk of infection and traumatic injuries are also considerations. More recently, the intranasal administration has appeared to be a promising alternative to circumvent the BBB. It is a non-invasive route that allows the drug to enter the brain directly through the nasal passages and trigeminal nerves. Although this neuronal connection is feasible to deliver a wide variety of substances including the nucleic acids to the brain, it also possesses some limitations like small nasal volume, enzymatic degradation, and mucociliary clearance. Advanced drug delivery systems such as nanoparticles are commonly employed to overcome these problems (Rodriguez et al., 2017; Hao et al., 2020).

BBB disruption is another strategy to improve drug delivery to the brain. Both chemical and physical means are available to enhance the permeability of the BBB. However, many studies have also shown that they can also improve the delivery efficiency of RNA therapeutics. One of the most studied is to target the BBB by shrinking the endothelial cells, opening the tight junctions, and subsequently promoting the passive diffusion of large molecules, such as siRNA, across the BBB. (Park et al., 2015). Vasoadjustive agents like bradykinin, cerivastatin, or eicosatetraenoic acid (ETE) also studied for this purpose (Borlongan and Emerich, 2003; Liu et al., 2010). While they could effectively permeabilize the brain tumor capillaries, the poor effect was shown on brain endothelial cells in vitro and unknown whether it can efficiently open the BBB in vivo (Downs et al., 2015). Nowadays, it is facilitated by microbubbles with diameters of 1–10 μm so that less acoustic energy is required. Nevertheless, the disruption of BBB can not only allow for the entry of therapeutics but also increase the risk of the entry of undesired neurototoxic and micro-embolism. It may also cause the inflammation of the BBB and the disturbance of glucose uptake, leading to dysfunction of the brain.

Even if the RNA therapeutics can reach the CNS through the methods mentioned above, only restricted amounts of them may enter the targeted cells and hit their therapeutic effects. Therefore, enhancing the intracellular delivery, reducing extracellular nucleases, and hard to penetrate the lipid cell membrane, owing to their hydrophilic and negatively charged nature. After intracellular uptake, they may be degraded by intracellular enzymatic enzymes. To resolve these problems, various chemical modifications are introduced. For example, terminal modification (3’ and 5’) using polyethylene glycol (PEG) and sugar molecules are able to improve their stability and cellular uptake, while substitution of the terminal sugars like mimetics and sugar analogs. N-acetylgalactosamine can promote their cellular uptake. In addition, partial replacement of the nucleosides with pseudouridine is effective to reduce the immunogenicity of the RNA therapeutics (Miele et al., 2012). However, chemical modification is a technique as genetic engineering. The exogenous nucleic acids should be preserved, and the improvement of the stability and cellular uptake is limited.

Viral vectors represent the most efficient gene transfer vehicles since they naturally evolve to infect cells. Commonly used viral vectors are developed from adenovirus, adeno-associated virus, and retrovirus, particularly lentivirus. Notwithstanding the promising transfer capacity of the viral vectors, they also possess significant disadvantages like limited loading capacity and strong immunogenicity. Their native tropism often does not match the therapeutic need, i.e., they are unable to efficiently enter the correct cell types. Moreover, they do not infect naturally. Additionally, the production of viral vectors is of large difficulty and high cost to be scaled up (Waehler et al., 2007).

Therefore, nanoparticle-based vehicles have progressed substantially in recent years, aiming to transport RNA therapeutics to the brain. They can co-load carriers with sizes ranging from 1 to 1000 nm, and of a synthetic or natural origin. They are much more flexible than viral vectors that are easily engineered to achieve the requirements for better delivery of nucleic acids, including stability, high encapsulation efficiency and loading capacity, enhanced cellular uptake and sustained cargo release, as well as specific cell targeting. Also, with the assistance of the other strategies mentioned above, the delivery efficiency of nanoparticles can be further improved. Building on these merits, many research groups are currently working on the development of various nanoparticles for RNA therapeutics delivery to the brain (Additional Table 5).

Nanoparticles for RNA Therapeutics Delivery to the Brain

Polymeric nanoparticles

Polyethyleneimines (PEIs) are the most widely used polymer for RNA therapeutics transportation. It is a polycationic polymer rich in amine groups, and available in both linear and branched forms with different molecular weights. As a positively charged polymer, PEI can complex with nucleic acids electrostatically and efficiently enter the cells through endocytosis. Subsequently, PEI can retain a weak-base buffering property to protect nucleic acids from endosomolysis and degrade, and induce the ‘proton sponge effect’, resulting in the burst of endosomes and release of drugs to the cytoplasm (Vermeulen et al., 2018). PEI nanoparticles were found to be efficiently delivered siRNAs to different cells in the brain after intranasal administration (Rodriguez et al., 2017). However, the low biocompatibility and non-biodegradability have limited the clinical application of PEI nanoparticles. It was also known to be increased with the increase of molecular weight and positive charge density, but the transfection efficiency is diminished with low molecular weight. Hence, measures are taken to modify the PEI. After conjugation with negatively charged deoxycylic acid, the cytotoxicity of PEI (12 kDa) nanoparticles was significantly decreased while its high transfection efficiency was preserved (Oh et al., 2020). For the PEI with a low molecular weight (800 Da), cell-penetrating peptide like INGR was proposed for surface modification to increase the efficient targeting delivery (Park et al., 2015).

As an FDA-approved biomaterial, poly(lactide-co-glycolide) (PLGA) is one of the most investigated polymers for clinical usage. Despite its virtue of better biocompatibility and biodegradability than PEI, PLLGA is difficult to encapsulate with negatively charged nucleic acids electrostatically and efficiently enter the cells. Although this neuronal connection is feasible to deliver a wide variety of substances including the nucleic acids to the brain, it also possesses some limitations like small nasal volume, enzymatic degradation, and mucociliary clearance. Advanced drug delivery systems such as nanoparticles are commonly employed to overcome these problems (Rodriguez et al., 2017; Hao et al., 2020).
is noteworthy that long-term knockdown of PTEN in the non-neuronal brain cells such as astrocytes could induce gliomas (Wu et al., 2020). To avoid this potential negative effect, these nanoparticles conjugated with the neuron targeting moieties are desirable to promote neural regeneration with reduced risk of oncogenesis.

While PEI, PLGA, and PEG are all synthetic polymers, chitosan is a natural polymer commonly used for gene delivery. Chitosan is a polysaccharide derived from chitin with a highly biocompatible, biodegradable, and non-immunogenic nature. Like PEI, it can form a complex with RNA therapeutics through the electrostatic interaction between its amine groups and the anionic nucleic acids. This strong interaction, on the other hand, is one of the drawbacks of chitosan, making it hard to release the loaded cargo into the cytoplasm. Chitosan also allows for surface modification by varied materials like transferrin antibodies and bradykinin B2 antibodies for brain targeting (Gu et al., 2017).

Lipid-based nanoparticles

Liposomes are small vesicles composed of single or multiple phospholipid bilayers with an aqueous core where allows for RNA therapeutics encapsulation (Wei et al., 2016). It is widely used for gene delivery and has already been available on the market for mRNA vaccines. Also, solid lipid nanoparticles with a solid hydrophobic core coated with a phospholipid monolayer are capable of transporting nucleic acids, where siRNA were electrostatically bound to the outer surface (Erel-Akbaba et al., 2019). The lipid-based nanoparticles generally have a very stable structure to protect nucleic acids from degradation, but their half-life in vivo is restricted by their fast clearance in the liver and spleen. To overcome this limitation, PEGylation acts as a golden standard to prolong their circulation time. In addition, they can deliver nucleic acids to various target sites such as T7 promoters or IRGd (a tumor-penetrating peptide) for higher transfer efficiency to the brain and the tumor (Erel-Akbaba et al., 2019; Wei et al., 2016).

Recently, a new class of lipopolymeric nanoparticles has been investigated for RNA therapeutics delivery, thus combining the advantages of both polymers and liposomes. These nanocarriers were successfully focused on glioma cells (Yu et al., 2017) using epoxide-terminated lipids and low-molecular-weight polypeptides, exhibiting therapeutic benefits to the attenuation of brain tumor growth via the CED method.

Inorganic nanoparticles

Inorganic materials have been utilized for nanomedicine development, including iron oxide, gold, silica, etc. They are biodegradable, biocompatible, and can be made in various sizes and morphologies. Among them, inorganic-based nanoparticles have been proposed for magnetic purposes and received the most attention. With the combination of cationic polymers like PEI, high encapsulation efficiency of RNA therapeutics could be achieved. Increased brain tumor cell apoptosis was observed with PEI-coated ZnFe2O4 nanoparticles carrying lethal-7a miRNA (Yin et al., 2014).

Peptide-based nanoparticles

Peptides were commonly used to directly conjugate with RNA therapeutics or nanoparticles as a functional moiety. However, recent advances in nanotechnology have employed peptides as the backbone of the nanoparticles as a functional moiety, allowing for facile incorporation of the incorporated molecules (Liu et al., 2011). The packaging RNA (pRNA), an alternative approach, forms a thermodynamically stable and water-soluble, so PEGylation is not required.

Exosomes

Exosomes are nanosized lipid vesicles naturally secreted from almost all the cell types, carrying non-coding RNAs for intercellular communication. Nowadays they have been utilized as a unique vehicle for various therapeutic. Thanks to their endogenous origin, exosomes elicit a minimal immunogenic response and are stable in the systemic circulation. They are also believed to possess specific cell selectivity. Exosomes isolated from the blood of a medium speed facilitated the transport of the brain of zebrabfish (Yang et al., 2017). Researchers also modified the exosomes with RVG to achieve brain targeting effect (Cooper et al., 2014; Liu et al., 2015). However, critical issues associated with the choice of exosome origin and the procedure of cargo loading exist as a barrier for exosomes to reach maximum potential in clinical application.

Conclusion and Future Perspectives

Compelling evidence has demonstrated that reactivation of the regenerative mTOR pathway can substantially promote axon regeneration, neural repair, and functional recovery after CNS injury. We specifically discussed the potential of PTEN as a promising target using the antisense oligonucleotide approach. We have discussed the characteristics of RNA-based therapeutics and a bioinformatics pipeline to predict a manageable number of viable ODN binding sites, saving the effort of screening. The binding of the antisense ODN binding sites requires significantly less effort than identifying binding pockets used by small molecule drugs. As datasets from large-scale RNA-protein interactions and RNA folding studies are available, and more are in the making, an accurate genome-wide landscape of ODN binding sites can be obtained, accelerating the development of RNA therapy. While this review focuses on the RNA knockdown approach, the mRNA transient expression is an alternative approach to promote neural regeneration that can be used as a functional tool for transgene expression of AKT or other positive upstream regulators of mTOR pathway could be considered. However, the RNA-based transgene expression, such as the expression of viral spike protein for the COVID-19 vaccine, can only be achieved for a short time due to the short half-life and cytokine storm. In addition, there are other challenges and obstacles associated with mRNA therapy, including mRNA’s molecular size, charge, intrinsic instability, and targeted delivery issue. Importantly, with the success of current mRNA vaccines, it is evident that it is viable and safer for mRNA molecules into the human body via the appropriate vehicle, making this technology a promising therapeutic tool for a comprehensive application.

Pharmaceutical companies such as Moderna and BioNTech are developing an mRNA-based therapy for treating cystic fibrosis. That has demonstrated the enthusiasm of this strategy. Considering the scant examples of non-vaccine mRNA-based transient transgene expression, the antisense and mRNA approaches focused in this review are clearly not the only approach.

On the other hand, it is noteworthy that although promising, targeting mTOR pathway (e.g. by PTEN deletion) is not without potential limitations. Concerning the effect on brain development, neuron-specific PTEN mutant mice show seizures, ataxia, and brain enlargement with increased ROS and dyslipid of neural cell populations by 9 weeks and such abnormality is resembling Hmeritine-duclos disease (Backman et al., 2001). Hyperactivation of mTOR signaling by selective deletion of PTEN in the granule cells was report to form aberrant cortical connections, which correlated with the suppressive function of the dentate gyrus, resulting in epileptogenesis (Pun et al., 2012). Regarding the studies in conditional deletion of PTEN in adult mice, it has been observed that the corticospinal tract axons emanating from PTEN-deleted cortical motoneurons in adult mice have thicker axons without triggering compensatory increases in myelination. Also, unilateral deletion of the same PTEN knockout mice results in impaired motor coordination (Gallent and Steward, 2018). Similar to our colleagues reporting that the knockdown of PTEN in adult mice can induce symptoms reminiscent of human autism spectrum disorder such as seizures, macrocephaly, anxiety, and social interaction deficits (Kwon et al., 2006). A follow-up study further indicated that the maintenance of circadian rhythms may be impaired by PTEN mutation (Ogawa et al., 2007). Thus, the transient PTEN knockdown seems to control the optimal effect on the neural repair without causing undesirable effects. Compared with virus-mediated knockdown of PTEN, our ODN-mediated approach would be clinically easier to maintain the transient and dosage of PTEN downregulation. While RNA therapy appears to be effective for CNS diseases, delivery of RNA oligonucleotides to the brain remains a huge challenge. The successful development of this technology could be a promising direction for developing the next generation of RNA therapy for the treatment of CNS injury.

Author contributions: Review conception and design by CKT, SFC, ESH. All authors contributed towards literature retrieval, editing and critically revising the manuscript for important intellectual content. All authors approved the final version of this manuscript for publication.

Conflicts of interest: The authors declare that there are no conflicts of interest associated with this manuscript.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Open access statement: This is an open access journal, and articles are distributed under the terms of the Creative Commons AttributionNonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work, but only so long as they credit the author, provide a link to the license, and clearly mark their changes, such as if appropriate credit is given and the new creations are licensed under the identical terms.
References

Agostine J, Alarcon-Martinez L, Gamlin C, Yu WQ, Wong ROL, Di Polo A (2018) Intrinsic signaling promotes dendrite and synapse regeneration and restores circuit function after axonal injury. Brain 141:1963-1988.

Ali HI, Al-Shehhi Y, Yousif T, Matar Y, El-Sayed M, Samir Y, Xu XM, Lemmon VP, Bayburt IL (2017) The mTOR Substrate S6 Kinase 1 (S6K1) is a negative regulator of axon regeneration and a potential drug target for central nervous system injury. J Neurosci 37:7079-7093.

An S, Jiang X, Shi J, He X, Li J, Guo Y, Zhang Y, Ma Y, He L, Jiang C (2015) Single-component self-assembled RNA nanoparticles functionalized with tumor-targeting INGR delivering abundant siRNA for efficient glioma therapy. Biomedicines 3:217-240.

Backman SA, Stambolic V, Suzuki A, Haight J, Elia A, Pretorius J, Tsao MS, Shannon P, An S, Jiang X, Shi J, He X, Li J, Guo Y, Zhang Y, Ma H, Lu Y, Jiang C (2015) Single-component self-assembled RNA nanoparticles functionalized with tumor-targeting INGR delivering abundant siRNA for efficient glioma therapy. Biomedicines 3:217-240.

Baker BF, Lot SS, Condon TP, Cheng-Flournoy S, Lesnik EA, Sasmor HM, Bennett CF (1997) 2′-O-(2-Methoxy) ethyl-modified anti-intercellular adhesion molecule 1 (ICAM-1) oligonucleotides selectively increase the ICAM-1 mRNA level and inhibit formation of the ICAM-1 translocation initiation complex in human umbilical vein endothelial cells. J Biol Chem 272:11999-12005.

Bejerano G, Pheasant M, Makunin I, Stephen S, Kent WJ, Mattick JS, Haussler D (2004) Ultraconserved elements in the human genome. Science 304:1321-1325.

Beni SM, Tinter J, Alexandrović AG, Galton-Krool N, Baralzi A, Kohen R, Grigoradić N, Simeonidou C, Shohami E (2006) CuZn-SOD deficiency, rather than overexpression, is associated with enhanced recovery and attenuated activation of NF-κB after brain trauma in mice. J Cereb Blood Flow Metab 26:478-490.

Bhowmick SN, Abdul-Munem PM (2021) PTEN block stems corticospinal and raphaeospinal axonal regeneration and promotes functional recovery after spinal cord injury. J Neuropathol Exp Neurol 80:169-181.

Bishop CJ, Kozlowski KL, Green JI (2015) Exploring the role of polarity structure on intracellular nucleic acid delivery via polymeric nanoparticles. J Control Release 219:148-149.

Borlongan CV, Emerich DF (2003) Facilitation of drug entry into the CNS via transience permeation of blood brain barrier: laboratory and preliminary clinical evidence from radixin receptor agonist, Cereport. Brain Res Bull 60:297-306.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+ architecture and applications. BMC Bioinformatics 10:421.

Cramer SC (2018) Treatments to promote neural repair after stroke. J Stroke 20:57-70.

ClinicalTrials.gov (2021) https://clinicaltrials.gov.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+ architecture and applications. BMC Bioinformatics 10:421.

Cramer SC (2018) Treatments to promote neural repair after stroke. J Stroke 20:57-70.

Davis CA, Hitz BC, Sloan CA, Chan ET, Davidson JM, Gabdank I, Hilton JA, Jain K, Cramer SC (2018) Treatments to promote neural repair after stroke. J Stroke 20:57-70.

Geary RS, Norris DY, Lu R, Bennett CF (2015) Pharmacokinetics, biodistribution and cell uptake of antisense oligonucleotides. Adv Drug Deliv Rev 87:46-51.

Gregory RI, Chendrimada TP, Cooch N, Shekhatker R (2005) Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell 123:631-640.

Gu J, Al-Bayat K, Ho EA (2017) Development of antibody-modified chitosan nanoparticles for the targeted delivery of siRNA across the blood-brain barrier as a strategy for inhibiting HIV replication in astrocytes. Drug Deliv Transl Res 7:497-506.

Hao R, Sun B, Yang L, Ma C, Li S (2019) Radio-modified nanoparticles improve ischemic brain injury by nasal delivery. Drug Deliv 27:772-781.

He Z, Jin Y (2016) Intrinsic control of axon regeneration. Neurosci 90:437-451.

Ho ES, Gunderson SI (2011) Long conserved fragments upstream of Mammalian polysomal retention sites. Genome Biol Evol 3:654-666.

Howe KL, Achuthan P, Allen J, Aliferis J, Aronov MD, Arzate JM, Aziz AG, Bennett R, Bhai J, Bills K (2021) Ensembl 2021. Nucleic Acids Res 49:D884-891.

Huang WY, Jiang C, He HB, Iacojo TT, Cheng C, Huang J, Liu J, Zhang R, Shao JF (2019) mIR-122 regulates astroglial glutamate transporters via the Akt and mTOR signaling pathway post ischemic stroke. Brain Res 1649:231-239.

Itani OA, Zhong X, Tang X, Scott BA, Van Y, Filibote S, Lim Y, Hsieh AC, Bruce JE, Van Gist M, Crowder CM (2021) Coordinate regulation of ribosome and RNA biogenesis controls hypoxic injury and translation. Curr Biol 31:122-137.

Kessler D, Gmachl M, Mantoulidis A, Martin LJ, Zoephel A, Mayer M, Gollner A, Covini D, Fischer S, Gerstberger T, Gasiorowski T (2019) Drugging an undruggable pocket on KRAS. Proc Natl Acad Sci U S A 116:15823-15829.

Kouni FM, Hurley LA, Daniels WL, Day ES, Hua Y, Hao L, Peng CY, Merkle TK, Queisser MA, Ritter C, Zhang H (2015) miR-182 integrates apoptosis, growth, and differentiation programs in glioblastoma. Genes Dev 29:732-745.

Kwon CH, An S, Lin Y, Kim KW, Hensley LL, Baker SJ, Parada LF (2006) miRNA-specific nuclease as cre-mouse line with cre activity in specific neuronal populations. Genesis 44:130-135.

Kwon EJ, Skalak M, Lo B, Bhatia SN (2016) Neuro-targeted nanoparticle for siRNA delivery to traumatic brain injuries. ACS Nano 10:7926-7933.

Lee J, Mendell JT (2020) Antisense-mediated Transcript Knockdown Triggers Promemato transcription. Mol Cell 77:1044-1054.e3.

Lee TJ, Haque F, Shu D, Yoo JI, Liu Y, Yokek RA, Horbinski C, Kim TH, Kim SH, Kwon CH, Nah JW, Kao RB, Guo P, Guo Y (2015) RNAV nanoparticles as a vector for targeted siRNA delivery into glioblastoma model mouse. Oncotarget 6:14766-14776.

Li H, Tsang CK, Watkins M, Bertram PG, Zheng KS (2006) Nutrient regulates Tor1 nuclear localization and association with RNA polymerase. Nature 442:1058-1061.

Li HB, Zhang X, Chen PS, Wang L, Tsang CX, Lee WY (2018) Convergent synthesis and characterization of fatty acid-conjugated polyethylene glycol-block-polysiloxane-cyclodextrin nanoparticles for improved delivery into the brain. Eur Polym J 98:394-401.

Pou Zh, Xu X, Zhang QC (2021) RASP: an atlas of transcriptome-wide RNA secondary structure probing data. Nucleic Acids Res 49:D183-191.

Li S, Nian EH, Yin Y, Benwitzon LI, Tung S, Vinters VH, Bahat FR, Stenzel-Poore MP, Kawaguchi R, Coppola G, Carmichael ST (2015) GDF15 is a signal for axonal sprouting and functional recovery after stroke. Nat Neurosci 18:1737-1745.

Li Y, Tsang CK, Wang S, Li XX, Yang Y, Fu L, Huang W, Li M, Wang HY, Zheng XS (2016) MAF1 suppresses AKT-mTOR signaling and liver cancer through activation of PTEN transcription. Hepatology 63:1928-1942.

Jiang XH, Sun H, Nichols JG, Crooke ST (2017) RNAi-based APOBEC hiPSC-1 resistant antisense oligonucleotides are robustly active in directly RNA cleavage within both the cytoplasm and the nucleus. Mol Ther 25:2075-2092.

Kim LX, Han Z, Leon HY, Kach J, Jing E, Weyn-Vanheentenryck S, Downs M, Corrionero A, Chi HW, Scharrer J, Venkatesh K, Hua L, Gao S, Ticho B, Nash H, Anzarov (2020) Antisense oligonucleotide modulation of non-productive alternative splicing upregulates genes. Expression. Nature 33:1442-1449.

Uo GJ, Sabatini DM (2020) mTOR at the nexus of nutrition, growth, ageing and disease. Nat Rev Mol Cell Biol 21:183-200.

Guo J, Cai J, Zhang Y, Fu X (2019) mTORC1 directly phosphorylates and regulates human MAF1. EMBO J 38(17):2433-2448.

Jiang CH, Cai N (2017) miR-192 promotes axonal outgrowth and regeneration via mTOR activation. FASEB J 35:e21526.

Liu X, Zhao R, Guo H, Wang P, Chen Y, Liu Y, Zhang J, Zeng Y (2021) Double-strand RNase P RNA nanoparticles functionalized with tumor-targeting INGR delivery to the brain. Eur Polym J 148:1-14.

Liu G, Al-Bayati K, Ho EA (2016) BRD4 regulates BRD4-PTEN homeostasis in glioma cells. Cell 164:1544-1558.

Galea EW, Steward O (2018) Neuronal PTEN deletion in adult cortical neurons triggers progressive growth of cell bodies, dendrites, and axons. Exp Neurol 303:12-28.
| Name          | Description                                                                                                                                                                                                                                                                                                                                 | DrugBank ID | Approved |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|----------|
| Aprinocarsen  | Aprinocarsen is a specific antisense oligonucleotide inhibitor of protein kinase C-alpha.                                                                                                                                                                                                                                                   | DB06451     | False    |
| GEM-231       | GEM231 is a second-generation antisense oligonucleotide targeting the mRNA of the R1alpha regulatory subunit of cAMP-dependent protein kinase.                                                                                                                                                                                                   | DB05798     | False    |
| Nusinersen    | An antisense oligonucleotide that induces survival motor neuron (SMN) protein expression, it was approved by the U.S. Food and Drug Administration (FDA) in December 2016 as Spinraza for the treatment of children and adults with spinal muscular atrophy. It is administered as a direct intrathecal injection.                                | DB13161     | True     |
| Volanesorsen  | Volanesorsen is an antisense oligonucleotide that binds to apoC-III mRNA to prevent its translation.                                                                                                                                                                                                                                       | DB15067     | True     |
| AEG35156      | A second-generation synthetic antisense oligonucleotide with potential antineoplastic activity. AEG35156 selectively blocks the cellular expression of X-linked inhibitor of apoptosis protein (XIAP), a pivotal inhibitor of apoptosis that is overexpressed in many tumors.                                                                                | DB06184     | False    |
| Fonvirsen     | Fonvirsen is an antisense 21 mer phosphorothioate oligonucleotide. It is an antiviral agent that was used in the treatment of cytomegalovirus retinitis in immunocompromised patients, including those with acquired immunodeficiency syndrome.                                                                                                        | DB06759     | True     |
| Inotersen     | Inotersen is a transthyretin-directed antisense oligonucleotide for the treatment of polyneuropathy caused by hereditary transthyretin-mediated amyloidosis in adults.                                                                                                                                                                                    | DB14713     | True     |
| LEraAON       | NeoPharm is developing liposome-encapsulated, c-Raf antisense oligodeoxynucleotides (LEraAON) for the potential treatment of various solid tumors, including those that have become resistant to radiation or chemotherapy. Phase I/II trials commenced in March 2001 and were ongoing as of June 2003.                                                   | DB04973     | False    |
| AVI-4020      | AVI-4020 is a neogene antisense drug candidate for the treatment of patients with acute West Nile virus disease who have a serious neurological impairment (WNV neuroinvasive disease).                                                                                                          | DB05873     | False    |
| ATL1102       | ATL1102 is a second-generation antisense inhibitor of CD49d, an immune-system protein known as VLA-4, an immune cell molecule. It works by entering cells and targeting genes.                                                                                                                        | DB04997     | False    |
| GTI 2040      | GTI-2040 is a substance that is being studied as a treatment for cancer. It belongs to the family of drugs called antisense oligonucleotides.                                                                                                                                                                                                                       | DB05801     | False    |
| ISIS 113715   | ISIS 113715 is our second-generation antisense inhibitor of protein tyrosine phosphatase 1b, or PTP, in the treatment of type 2 diabetes.                                                                                                                                                                                                      | DB05506     | False    |
| AVI-4065      | AVI-4065, a phosphorodiamidate Morpholino oligomer, is an investigational antisense hepatitis C virus drug.                                                                                                                                                                                                                                  | DB05620     | False    |
| ISIS 14803    | ISIS 14803 is a 20-unit antisense phosphorothioate oligodeoxynucleotide that binds to hepatitis C virus RNA at the translation initiation region of the internal ribosome entry site (IRES) and inhibits protein expression in cell culture.                                                                                  | DB05803     | False    |
| LY2275796     | LY2275796 is a second-generation antisense anti-cancer drug candidate for clinical development. LY2275796 targets eukaryotic initiation factor 4E, a protein involved in the translation of key growth and survival factors that drive tumor progression, angiogenesis, and metastases.                                   | DB05165     | False    |
| Apatorsen     | Apatorsen is a second-generation antisense drug which in preclinical experiments, inhibits the production of heat shock protein 27 (a cell survival protein) found at elevated levels in many human cancers including prostate, lung, breast, ovarian, bladder, renal, pancreatic, multiple myeloma and liver cancer.                               | DB06094     | False    |
| AT11101       | AT11101 is a second-generation antisense drug designed to block the synthesis of the insulin-like growth factor 1 (IGF1) receptor, a protein involved in the regulation of cell growth in psoriasis. AT11101 is being developed as a cream for the topical treatment of mild to moderate cases of psoriasis.                        | DB05023     | False    |
| Viltolarsen   | DMD is an X-linked recessive allele characterized by a lack of functional dystrophin protein, which leads to progressive ambulatory, pulmonary, and cardiac function and is invariably fatal.                                                                                                           | DB15005     | True     |
| Egaptivon pegol| ARC1779 is a therapeutic aptamer antagonist of the A1 domain of von Willebrand factor (VWF). It works by entering cells and targeting genes.                                                                                                                                                                                                     | DB05202     | False    |
| GTI-2501      | GTI-2501 is a novel antisense drug that has shown a favorable safety profile in preclinical studies and a phase I clinical trial. In phase II clinical trial, it is combined with docetaxel for the treatment of hormone-refractory prostate cancer.                                                                                           | DB05406     | False    |
| Casimersen    | DMD is an X-linked recessive allele characterized by a lack of functional dystrophin protein, which leads to progressive impairment of ambulatory, pulmonary, and cardiac function and is invariably fatal.                                                                                                    | DB14984     | True     |
| AVI-4557      | AVI-4557 is an oral antisense compound that selectively inhibits the metabolic enzyme cytochrome P450 3A4 (CYP), an important liver enzyme responsible for the metabolism or breakdown of approximately half of currently marketed drugs.                                                      | DB05447     | False    |
| Golodirsen    | Golodirsen is a morpholino antisense oligomer designed to treat about 8% of patients with DMD. This is an X-linked condition leading to progressive muscle degeneration that begins in early childhood, rendering many patients wheelchair-bound by age 12.                                         | DB15593     | True     |
| Mipomersen    | Mipomersen sodium, which was known as the investigational drug, isis-301012, is the salt form of a synthetic phosphorothioate oligonucleotide. Mipomersen sodium prevents the formation of apolipoprotein B (apo B)-100, resulting in a decrease in the levels of apo B, low-density lipoprotein, and total cholesterol.                     | DB05528     | True     |
| Trioxalen     | Trioxalen (trimethylpsoralen, trioxsalen or trisoralen) is a derivative of furanocoumarin and psoralen, obtained from several plants, mainly Psoralea corylifolia. Like other psoralens, it causes photosensitization of the skin. It is administered either topically, or orally in conjunction with UV-A (the least damaging form of ultraviolet light) for phototherapy treatment of vitiligo and hand eczema. | DB04571     | True     |

DMD: Duchenne muscular dystrophy.
### Additional Table 2 | RNA-based therapeutics from ClinicalTrials.gov

| Acronym | Title | Interventions | Phases | Identifier |
|---------|-------|---------------|--------|------------|
| ILLUMINATE | A study to evaluate efficacy, safety, tolerability and exposure after a repeat-dose of sepofarsen (QR-110) in LCA10 (ILLUMINATE) | Drug: sepofarsen  
Other: Sham | II/III | NCT03913143 |
| INSIGHT | Extension study to study PQ-110-001 (NCT03140969) | Drug: QR-110  
Other: Sham | I/II | NCT03913130 |
| KIK-AS | A study of the safety and tolerability of GTX-102 in children with Angelman syndrome | Drug: GTX-102  
Drug: Sham | I/II | NCT04259281 |
| PRECISION-HD1 | Safety and tolerability of WVE-120101 in patients with Huntington’s disease | Drug: WVE-120101  
Drug: Placebo | I/II | NCT03225833 |
| PRECISION-HD2 | Safety and tolerability of WVE-120102 in patients with Huntington’s disease | Drug: WVE-120102  
Drug: Placebo | I/II | NCT03225846 |
| Unknown | 24-Month open label study of the tolerability and efficacy of inotersen in TTR amyloid cardiomyopathy patients | Drug: inotersen  
Drug: Placebo | II | NCT03702829 |
| Unknown | An open-label extension study of STK-001 for patients with Dravet syndrome | Drug: STK-001  
Drug: Placebo | II | NCT04740476 |
| Unknown | A phase 2b clinical study with a combination immunotherapy in newly diagnosed patients with glioblastoma- the ImmuneSense Study | Biological: IGV-001 Cell Immunotherapy  
Biological: Placebo  
Procedure: Standard of Care (SOC): Radiation Therapy  
Drug: SOC: Temozolomide | II | NCT04485949 |
| Unknown | Open-label extension study to evaluate the safety and tolerability of WVE-120102 in patients with Huntington’s disease | Drug: WVE-120102  
Drug: Placebo | I/II | NCT04617860 |
| Unknown | Open-label extension study to evaluate the safety and tolerability of WVE-120101 in patients with Huntington’s disease | Drug: WVE-120101  
Drug: Placebo | I/II | NCT04617847 |
| Unknown | BP1001-A in patients with advanced or recurrent solid tumors | Drug: BP1001-A (Liposomal Grb2 Antisense Oligonucleotide)  
Drug: BP1001-A (Liposomal Grb2 Antisense Oligonucleotide) with paclitaxel | I | NCT04196257 |
| Unknown | ION-682884 in patients with TTR amyloid cardiomyopathy | Drug: ION 682884  
Drug: Placebo  
Drug: IGV-001 Cell Immunotherapy  
Drug: Placebo  
Procedure: Standard of Care (SOC): Radiation Therapy  
Drug: SOC: Temozolomide | II | NCT04843020 |

### Additional Table 3 | siRNA therapeutics from DrugBank (https://go.drugbank.com/)

| Name | Description | DrugBank ID | Approved |
|------|-------------|-------------|----------|
| Sirna-027 | Sirna-027 is a chemically modified short interfering RNA (siRNA) targeting vascular endothelial growth factor receptor-1 (VEGFR-1). VEGFR-1 is a key component of the clinically validated vascular endothelial growth factor (VEGF) pathway. | DB05896 | False |
| Bevasiranib | Bevasiranib is a small interfering RNA (siRNA) targeting vascular endothelial growth factor A (VEGF-A). | DB06642 | False |
| Asvasiran | Asvasiran is a siRNA that targets the respiratory syncytial virus (RSV) N gene and inhibits viral replication. It has the potential to treat or prevent RSV infection. | DB05638 | False |
| Inclisiran | Inclisiran is a long-acting, synthetic small interfering RNA (siRNA) directed against proprotein convertase subtilisin/kexin type 9 (PCSK9), which is a serine protease that regulates plasma low-density lipoprotein cholesterol (LDL-C) levels. | DB14901 | True |
| Givosiran | Givosiran is a small interfering RNA (siRNA) directed towards 5-aminolevulinic acid synthase, a critical enzyme in the heme biosynthesis pathway. | DB15066 | True |
| Acronym | Title | Interventions | Phases | Identifier |
|---------|-------|---------------|--------|------------|
| Unknown | EphA2 siRNA in treating patients with advanced or recurrent solid tumors | Drug: EphA2-targeting 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC)-encapsulated siRNA Other: Laboratory biomarker analysis Other: Pharmacological study | I | NCT01591356 |
| Unknown | APN401 in treating patients with recurrent or metastatic pancreatic cancer, colorectal cancer, or other solid tumors that cannot be removed by surgery | Drug: APN401 Other: Laboratory biomarker analysis | I | NCT03087591 |
| Unknown | An open-label extension study of an investigational drug, fitusiran, in patients with moderate or severe hemophilia A or B | Drug: Fitusiran (SAR439774) | I/II | NCT02554773 |
| Unknown | A study of single and multiple ascending doses of LEM-S401 in healthy participants | Drug: LEM-S401 Drug: Placebo Other: Placebo saline | I | NCT04707131 |
| Unknown | A study to evaluate safety, efficacy of intralesional injection of STP705 in patients with cutaneous squamous cell carcinoma in situ skin cancer (isSCC) | Drug: STP705 | I | NCT04844983 |
| ORION-3 | An extension trial of inclisiran compared to evolocumab in participants with cardiovascular disease and high cholesterol | Drug: Inclisiran Drug: Evolocumab | II | NCT03060577 |
| ORION-5 | A study of inclisiran in participants with homozygous familial hypercholesterolemia (HoFH) Tivanisiran for dry eye in subjects with Sjogren's syndrome | Drug: Inclisiran for injection Drug: Placebo Drug: Tivanisiran sodium ophthalmic solution Drug: Vehicle ophthalmic solution | III | NCT03851705 |
| ILLUMINATE-A | A study to evaluate lumasiran in children and adults with primary hyperoxaluria type 1 | Drug: Placebo Drug: Lumasiran | III | NCT03681184 |
| ILLUMINATE-B | A study of lumasiran in infants and young children with primary hyperoxaluria type 1 | Drug: Lumasiran | III | NCT03905694 |
| PHYO2 | A study to evaluate DCR-PHXC in children and adults with primary hyperoxaluria type 1 and primary hyperoxaluria type 2 | Drug: DCR-PHXC Drug: Sterile normal saline (0.9% NaCl) | II | NCT03847909 |
| PHYO4 | Study to evaluate safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of DCR-PHXC in PH type 3 patients A study to evaluate long-term safety and clinical activity of givosiran (ALN-AS1) in patient with acute intermittent porphyria (AIP) | Drug: DCR-PHXC Drug: Sterile Normal Saline (0.9% NaCl) Drug: givosiran (ALN-AS1) | I/II | NCT04555486 |
| PHYO3 | Long term extension study in patients with primary hyperoxaluria | Drug: DCR-PHXC | III | NCT04042402 |
| ILLUMINATE-C | A study to evaluate lumasiran in patients with advanced primary hyperoxaluria type 1 | Drug: Lumasiran | III | NCT04152200 |
| Unknown | Olpasiran trials of cardiovascular events and lipoprotein(a) reduction- DOSE finding study | Drug: Olpasiran Drug: Placebo | II | NCT04270760 |
| Unknown | The study of an investigational drug, patisiran (ALN-TTR02), for the treatment of transthyretin (TTR)-mediated amyloidosis in patients who have already been treated with ALN-TTR02 (patisiran) | Drug: Patisiran (ALN-TTR02) | III | NCT02510261 |
| Unknown | A study of fitusiran (ALN-AT3SC) in severe hemophilia A and B patients without inhibitors | Drug: Fitusiran Drug: Factor concentrates | III | NCT03417245 |
| ATLAS-INH | A study of fitusiran (ALN-AT3SC) in severe hemophilia A and B patients with inhibitors | Drug: Fitusiran Drug: Bypassing agents | III | NCT03417102 |
| Unknown | ENVISION: A study to evaluate the efficacy and safety of givosiran (ALN-AS1) in patients with acute hepatic porphyrias (AHP) | Drug: Givosiran Drug: Placebo | III | NCT03338816 |
Recent studies on the nanoparticles for RNA therapeutics delivery to the brain

| API | Nanoparticle | Administration route | Disease | Major observation | Study |
|-----|--------------|----------------------|---------|------------------|-------|
| siRNA against alpha-synuclein (a-Syn) | RVG modified exosomes | Intravenously | Parkinson's disease | After 7 days of the injection of siRNA-loaded RVG exosomes, the a-Syn mRNA and protein level was significantly reduced in transgenic mice. | Cooper et al., 2014 |
| lethal-7a miRNA (let-7a) | PEI-coated ZnFe₂O₄ nanoparticles | – | Brain tumor | The combination of miRNA and magnetic hyperthermia significantly enhanced the apoptosis of brain cancer cells. | Yin et al., 2014 |
| siRNA against luciferase | iNGR modified PEI nanoparticles | Intravenously | Glioma | The administration of the nanoparticles achieved marked accumulation of siRNA in glioma sites. | An et al., 2015 |
| miRNA-182 | Spherical nucleic acid nanoparticles | Intravenously | Glioblastoma | The nanoparticles could efficiently penetrate the blood-brain barrier and selectively disseminate in the glioblastoma, leading to decreased tumor burden and prolonged animal survival with no adverse events observed. | Kouri et al., 2015 |
| siRNA against luciferase | Folic acid-conjugated pRNA-3W1 nanoparticles | Intravenously | Glioblastoma | The nanoparticles successfully targeted the brain tumor cells and glioblastoma stem cells with no accumulation in either normal brain cells or other major organs. | Lee et al., 2015 |
| siRNA against opioid receptor mu (MOR) | RVG modified exosomes | Intravenously | Morphine relapse | The RVG exosomes efficiently delivered the siRNA to the mouse brain and significantly inhibit the MOR expression. | Liu et al., 2015 |
| siRNA against beta-secretase 1 (BACE1) | Rabies virus glycoprotein (RVG)-modified poly(mannitol-co-PEI) gene transporter (R-PEG-PMT) | Intravenously | Alzheimer's disease | R-PEG-PMT significantly improved the siRNA delivery to the brain through a synergistic effect of caveolae-mediated endocytosis and receptor-mediated transcytosis, leading to BACE1 suppression in the mice brain. | Park et al., 2015 |
| siRNA against caspase 3 | RVG-transportan nanoparticles | Intravenously | Traumatic brain injuries | The nanoparticles could accumulate in neurons adjacent to the injured sites and silence the targeted gene. | Kwon et al., 2016 |
| miRNA-124 | Proamine sulfate coated poly(lactide-co-glycolide) (PLGA) nanoparticles | Intracerebrally | Parkinson's disease | The nanoparticles exhibited pro-neurogenesis potential in both physiological conditions and disease mouse models. They also alleviated the motor symptoms of diseased mice. | Saraiva et al., 2016 |
| siRNA against epidermal growth factor receptor (EGFR) | T7 peptide-liposome-proamine-chondroitin sulfate nanoparticles (T7-LPC/siRNA NPs) | Intravenously | Brain tumor | In the in vivo tumor therapy experiment, compared to the non-targeted nanoparticles, a larger amount of T7-LPC/siRNA NPs accumulated specifically in brain tumor tissue, resulting in a significant downregulation of EGFR expression and a longer survival time. | Wei et al., 2016 |
| siRNAs targeting human spliceosome associated factor 3 and cyclin T1 | Transferrin antibody and bradykinin B2 antibody modified chitosan nanoparticles | - | Human immunodeficiency virus infection | The nanoparticles significantly improved the cellular uptake and gene silencing in astrocytes. | Gu et al., 2017 |
| siRNA against Beclin1 | PEI nanoparticles | Intranasally | Human immunodeficiency virus infection | The PEI-siRNA nanoparticles could suppress the target protein expression with no major adverse reactions observed in the brain tissue. | Rodriguez et al., 2017 |
| siRNA against vascular endothelial growth factor | Exosomes from bEnd.3 cells | Intravenously | Brain tumor | The exosomes enhanced the amount of siRNA delivered to the brain of zebrabird and inhibited the growth of cancer cells. | Yang et al., 2017 |
| siRNA against (sex-determining region Y)-box 2, oligodendrocyte transcription factor 2, spalt like transcription factor 2, and POU class 3 homeobox 2 | 7Cl1 lipopolymeric nanoparticles | Intratumoral convection-enhanced delivery | Glioblastoma | The delivery of multiple siRNA through nanoparticles could attenuate the malignant tumor growth in the patient-derived xenograft mouse model of glioblastoma. | Yu et al., 2017 |
| siRNA against glioma-associated oncogene homolog 1 | PEI- spherical nucleic acid nanoparticles | - | Glioblastoma | The nanoparticles could significantly suppress the proliferation of glioblastoma cells and sensitize neurospheres to temozolomide. | Melamed et al., 2018 |
| siRNA against EGFR and programmed death-ligand 1 (PD-L1) | iRGD-conjugated solid lipid nanoparticles | Intravenously | Glioblastoma | Together with radiation therapy, the nanoparticles could accumulate in the glioblastoma region and downregulate the expression of EGFR and PD-L1, leading to significantly decreased tumor size and increased mouse survival. | Erel-Akbaba et al., 2019 |
| miRNA-124 | RVG29 modified polyethylene glycol-poly(lactide-co-glycolide) (PEG-PGLA) nanoparticles | Intranasally | Ischemic brain injury | The modification of RVG29 significantly improved the targeted delivery of miRNA to the brain and ameliorated the symptoms of ischemic brain injury. | Hao et al., 2020 |
| Heme oxygenase-1 (HO1)-mRNA | Polyethyleneimine (PEI) nanoparticles | Stereotaxically | Ischemic brain injury | Among all the polymer tested, deoxycylic acid conjugated PEI2k nanoparticles possessed the highest delivery efficiency and tolerable toxicity. Compared with plasmid DNA, mRNA showed higher gene expression, thus more efficiently reducing the infarct size. | Oh et al., 2020 |