Oligonucleotides as a Novel Therapeutic Approach: An Innovative Area for Drug Delivery in Neurological Disorders

Yasir Qasim Almajidi*, Rana Kadum Muslim*, Yaseen T. khalf **
***Department of pharmacy, Baghdad College of Medical Sciences, Baghdad, Iraq.**
****Department of pharmacy, Alfarabi University College.**

Article Info:

Received Aug 2023
Revised Nov 2023
Accepted Dec 2023
Published Jan 2025
Corresponding Author email:
yasie.qasim.mohammed@bcms.edu.iq
Orcid: https://orcid.org/0000-0001-9632-0444

DOI: https://doi.org/10.32947/ajps.v25i1.1065

Abstract:

RNA-based therapeutics have emerged as one of the most potent therapeutic options used for the modulation of gene/protein expression and gene editing with the potential to treat neurodegenerative diseases. However, the delivery of nucleic acids to the central nervous system (CNS) by the systemic route, remains a major hurdle.

To overcome this pitfall, this review focuses on oligonucleotide-based novel strategies including liposomes, carbon nanotubes, quantum dots, solid lipid nanoparticles, nano lipid carriers, polymeric nanoparticles, mesoporous silica, dendrimers, aptamers, nanobodies etc. These strategies are designed to overcome these barriers by different pathways and mechanisms of transport across the blood–brain barrier. Ongoing preclinical and clinical studies are assessing the safety and efficacy of Antisense Oligonucleotide) ASOs) in multiple genetic and acquired neurological conditions. The current review provides an update on novel approaches, preclinical, clinical evidence, and delivery route of ASOs. The administration of FDA-approved ASOs in neurological disorders is also described. The current evidence on the safety and efficacy of ASOs in brain diseases will help identify opportunities for a broader range of nucleic acids and accelerate the clinical translation of these innovative therapies.

Keywords: Antisense Oligonucleotide, Neurodegenerative, Small interfering RNA, Micro RNA, Blood–brain barrier, Therapeutic responses**.**

النهج العلاجي القائم على اوليغونيكليوتيدات: منطقة متقدمة لتوصيل الأدوية في الاضطرابات العصبية

ياسر قاسم الماجدي *, رنا كاظم مسلم *, ياسين طه خلف **
***قسم الصيدلة، كلية بغداد للعلوم الطبية، بغداد، العراق.*
****قسم الصيدلة، كلية الفارابي الجامعة، بغداد، العراق.*

الخلاصة:

ظهرت العلاجات القائمة على الحمض النووي الريبي (RNA) كواحدة من أقوى الخيارات العلاجية المستخدمة لتعديل التعبير الجيني / البروتيني وتحرير الجينات مع إمكانية علاج الأمراض التنكسية العصبية. ومع ذلك، فإن توصيل الأحماض النووية إلى الجهاز العصبي المركزي (CNS) عن طريق المسار الجهازي، لا يزال يمثل عقبة رئيسية. للتغلب على هذا المأزق، تركز هذه المراجعة على الاستراتيجيات الجديدة القائمة على قليل النوكليوتيد بما في ذلك الجسيمات الشحمية، والأنابيب النانوية الكربونية، والنقاط الكمومية، والجسيمات النانوية الدهنية الصلبة، وحاملات الدهون النانوية، والجسيمات النانوية البوليمرية، والسيليكا ميسوبوروس، والمتشعبات، والأبتامير، والأجسام النانوية، إلخ. تم تصميم هذه الاستراتيجيات للتغلب على هذه الحواجز من خلال مسارات وآليات مختلفة للنقل عبر الحاجز الدموي الدماغي. تقوم الدراسات قبل السريرية والسريرية الجارية بتقييم سلامة وفعالية متعدد نيوكليوتيدات معاكس في العديد من الحالات العصبية الوراثية والمكتسبة. . تقدم المراجعة الحالية تحديثًا للطرق الجديدة والأدلة قبل السريرية والأدلة السريرية وطريق تسليم متعدد نيوكليوتيدات. يوصف أيضًا إدارة متعدد نيوكليوتيدات المعتمدة من قِبل إدارة الأغذية والعقاقير في الاضطرابات العصبية. ستساعد الأدلة

الحالية على سلامة وفعالية متعدد نيوكليوتيدات في امراض الدماغ في تحديد الفرص لمجموعة أوسع من الأحماض النووية وتسريع الترجمة السريرية لهذه العلاجات المبتكرة.

الكلمات المفتاحية: قليل النوكليوتيد المضاد للاتجاه، التنكس العصبي، الحمض النووي الريبي المتداخل الصغير، الحمض النووي الريبي الصغير، الحاجز الدموي الدماغي، الاستجابات العلاجية

INTRODUCTION:

Neurological disorders (NDs) are the leading cause of disability and the second leading cause of death worldwide. In the past 30 years, the absolute numbers of deaths and people with disabilities owing to neurological diseases have risen substantially, particularly in low-income and middle-income countries, and further increases are expected globally as a result of population growth and ageing [1]. Disorders like huntington, parkinson, amyotrophic lateral sclerosis (ALS), glioblastoma, epilepsy, alzheimer's disease (AD), duchenne muscular dystrophy (DMD), stroke, spinal muscular atrophy (SMA), spinal and bulbar muscular atrophy (SMBA) etc, are the severe disorders affecting quality of lifen[2].

Alzheimer's disease (AD) is the most common neurodegenerative disorder characterised by impairment in cognitive behaviour followed by an accumulation of Aβ-42 plaques and neurofibrillary tangles (hyperphosphorylated tau protein) extracellularly, hippocampus shows episodic memory in AD patients [3]. Dementia is the main pathological indication is accumulation of Aβ-42 amino acid isoform [4]. The pathophysiology of Parkinson's disease (PD) associates with accumulation and transmission of α-synuclein aggregates i.e., misfolding of presynaptic neuronal protein followed by preferential loss of dopamine in substantia nigra associated neurons in midbrain [5]. The patient shows symptoms of rigidity, tremor, bradykinesia, and postural discomfort [6]. Huntington's disease (HD) is an autosomal ND commonly faced by mid-aged adults. It is characterized by decline in motor activities, cognition, shows psychiatric symptoms followed by brain atrophy. The principle cause is cytosine-adenine-guanine (CAG) trinucleotide repeats in the first exon of huntingtins gene [7]. ALS is referred to as Lou Gehrig's disease and is characterised by progressive degeneration of lower and upper motor neurons in the brainstem and cerebral cortex. Superoxide dismutase 1 (SOD 1) and TARDBP gene are pathogenic mutants associated with ALS [6]. SMA is an autosomal recessive neuromuscular disorder featured with point mutation or deletion of telomeric motor neurons; it shows hypotonia and generalised muscle weakness [7]. It is a common reason for mortality in infants. DMD is associated with a rare progressive neuromuscular disorder which shows muscle wasting and weakness in middle adolescent age; main indication is mutation in dystrophin encoded DMD gene [8]. Stroke is classified into 2 types. Ischemic stroke: artery which efficiently carries blood to the brain gets blocked. Haemorrhagic: cerebral aneurysm gets burst and bleed in brain and forms haematoma, which causes spasms in cerebral vessels [9]. Glioblastoma is a form of malignant primary brain tumour, β tumour growth factor is responsible for glioblastoma. Epilepsy is one of the common chronic neurological disorders caused due to abnormal electrical conduction within the brain, which leads to unpredictable seizures [10]. It is caused due to alteration in expression of genes which controls neurotransmitter release and signalling, ion channels and synaptic transmission [11]. SMBA is a polyglutamine disease family member that indicates onset in adulthood and is an x-linked neurodegenerative disorder. It occurs due to expansion in cytosine-adenine-guanine trinucleotide which encodes the androgen receptor gene. Disease

progression is manifested by motor and skeletal muscle weakness and atrophy [12]. Hence, the review article mainly focuses on therapeutic strategies to treat neurodegenerative disorders by combining oligonucleotides with that of Novel drug delivery system (NDDS).

METHODOLOGY:

Five electronic databases including Medline, PubMed, Cochrane Library, Scopus and Web of Science were electronically searched to identify related articles using the combination of following keywords: Oligonucleotide, Neurodegenerative, Huntington, Parkinson, Amyotrophic Lateral Sclerosis, Glioblastoma, Epilepsy, Alzheimer's disease, Duchenne Muscular Dystrophy, Stroke, Spinal Muscular Atrophy, Spinal and Bulbar Muscular Atrophy, Novel approaches, Preclinical, Clinical, etc.

ABBREVIATION:

AD: Alzheimer's disease, **ALS**: Amyotrophic lateral sclerosis, **ASO**: Antisence oligonucleotide, **ASGPR**: Asialoglycoprotein receptor, **ApoE:** ApolipoproteinE, **Aβ-42**: Amyloid β 42, **BA**: Bioavailability, **BBB**: Blood brain barrier, **BCECs**: Brain capillary endothelial cells, **CNS**: Central nervous system, **CNT**: Carbon nanotubes, **CRISPR**: Clustered regularly interspaced short palindromic repeat, **CMV**: Cytomegalovirus, **CMC**: Critical micelle concentration, **DMD**: Duchene muscular dystrophy**, DOX:** Doxorubicin**, DP:** Deoxycholic acid conjugate polyethylenimine**, DP-Cur:** Curcumin loaded DP micelle, **EpCAM:** Epithelial cell adhesion molecule**, Fv:** Variable fragment, **Glu-PIC/Ms:** Glucosylated polyion complex micelles, **GLUT1**: Glicose transporter1, **GSC:** Glioma stem like cells, **HD**: Huntington disease, **IV:** Intravenous, **LNP**: Lipid nanoparticle, **miRNA**: MicroRNA, **mRNA**: Messenger RNA, **MNP**: Magnetic nanoparticle, **MOE:** Methoxyethyl, **MWCNT:** Multiwalled carbon nanotubes, **NDs:** Neurological disorders, **NDDS:** Novel drug delivery system, **NPs**: Nanoparticles, **PD:** Parkinson,s disease, **PEI:** Polyethyleneimine, **PEG:** Polyethylene glycol**, PDCD4:** Programmed cell death 4, **PTEN**: Phosphate and tensin homologue, **QDs**: Quantum dots, **RA**: Rheumatoid arthritis, **RES**: Reticulo endothelial system, **RSV**: Respiratory syncytial virus, **SMA**: Spinal muscular atrophy**, SBMA**: Spinal and bulbular muscular atrophy**, SELEX:** Systematic evaluation of ligands by exponential enrichment, **SLN**: Solid lipid nanoparticle, **SLE**: Systemic lupus erythematosus, **siRNA**: Small interfering RNA, **SWCNT**: Small walled carbon nanotubes, **SeQDs**: Selenium quantum dots, **SINPs**: Mesoporous silica nanoparticle, **TALENs**: Transcription activator like effector nucleases, **TDDS**: Transdermal drug delivery system, **TMZ**: Temozoloamide**, TJ:** Tight junction**, TTP:** Thrombotic thrombocytopenia purpura**, VEGF**: Vascular endothelial growth factor, **IT:** Intrathecal, **SC**: Subcutaneous, **IV**: Intravenous, **URI:** Upper respiratory tract infection.

NEED OF NOVEL DRUG DELIVERY SYSTEMS (NDDS) FOR TREATMENT OF NDs:

The main obstacle to delivering therapeutics to the brain is the blood-brain barrier (BBB), a diffusion passage between the brain and circulating blood; hence, a defined delivery strategy is required to overcome this obstacle. Several nanotechnology approaches are taking birth for brain-specific targeting [13]. The NDDS includes carbon nanotubes, magnetic nanoparticles, quantum dots, liposomes, solid lipid nanoparticles (SLN) etc, [14] are widely used as they have nano dimensions and therefore can easily penetrate brain cells. They also offer vast advantages including:

1- Drug is protected from biological or chemical degradation because of nanoencapsulation

2- Retention time of the drug is increased at site of absorption
3- Perform cellular internalization
4- Encapsulated drug is monitored for release kinetics
5- Specific ligands can be surface modified for targeted drug delivery
6- Systemic side effects can be reduced by minimizing drug distribution at non-target sites [15].
7- Due to their advanced features like composition, size, surface morphology, and easy preparation, magnetic nanoparticles are preferred for targeted therapies and diagnostics [13].
8- Carbon nanotubes composed of carbon as a structural backbone offer strength, high electronic and thermal conductivity, similar to the neuronal network of ion channels and signaling proteins [16].
9- Liposomal nanoparticles can quickly accumulate in tumor growth. Hence, offers biocompatibility, biodegradability and low toxicity [17].
10- Quantum dots shows fluorescent property, making them a selective candidate for visualising tumors; if they are coated with graphene, they can be used in PD.
11- 1µm size of SLN makes them attractive for tumour targeting as they avoid blood clotting, leading to embolism, limit the use of organic solvents, ensure biocompatibility and safety, and involve physiologic lipids[18].

ROLE OF OLIGONUCLEOTIDES IN NEUROLOGICAL DISORDERS:

Antisense oligonucleotides (ASOs) are single-stranded DNA (ssDNA) molecules aimed to target RNA [19]. ASOs can be naturally or chemically modified that bind to mRNA or microRNA. This binding restricts functionality of complementary RNAs. ASOs show sequence-specific in vitro antiviral activity therefore and can be assumed as a drug carrier for specific targeting in different NDs. The most versatile chemically modified ASO backbone is phosphonothioate, which improves poor nuclease resistance, binding affinity and imparts better pharmacokinetic properties to ASOs. mRNA is the most targeted site for ASOs as they transfer genetic data from DNA to protein and are therefore used as genetic modulating tool and therapeutic drug target [20]. ASOs comprise different backbones and chemistries that work by various mechanisms [21]. ASOs consist of 12 –30 nucleotides making them liable for targeting RNA by Watson rick pairing, and they are discovered to bind mRNA for protein-coding and microRNA for noncoding. ASOs perform various functions:

1- Performs sugar modification to provide binding affinity for target RNA
2- Enhance metabolic stability
3- Enhance cellular uptake and distribution in tissue by potentiating protein binding [22].
4- Inhibiting pre-mRNA splicing by blocking splicing factors
5- Inhibit mRNA translation by blocking ribosomal subunits
6- ASOs are short, single-stranded, and can restore and modulate protein sequence [23].

In NDs treatment BBB remains the challenge that conventional dosage forms cannot overcome; oligonucleotides serve as primary carriers in brain therapeutic due to above mentioned properties, they serve specific targeting, avoid toxicity etc [24].

SMALL INTERFERING RNA (siRNA) IN NDs: siRNA is double-stranded (dsRNA), chemically modified, 22-25 base pair long molecules, gene splicing by siRNA is efficient and validates gene expression [25]. Like ASOs, siRNA has sense strand complementary to mRNA [26]. They are chemically conjugated with hydrophobic ligands like cholesterol or a

combination of docosahexaenoic acid with phosphocholine head, the siRNA ASO are equipped with phosphonothioates backbone which provides efficacy and minimal toxic effects. Functional siRNA targets apolipoprotein E (apo E) implicated in NDs like AD and ALS. For sustained silencing divalent siRNA is used in NDs like HD [27], divalent siRNA inhibits messenger RNA (mRNA) and protein synthesis by silencing the Huntington gene. siRNA mechanism initiates by cleavage of dsRNA molecule into small RNA duplexes of 21 nucleotides by an enzyme endonuclease named dicer, this siRNA molecules are taken up into a matrix of enzyme known as Ribonucleotide Induced Silencing Complex (RISC) that block mRNA in targeted gene splicing [28] as shown in (Figure 1).

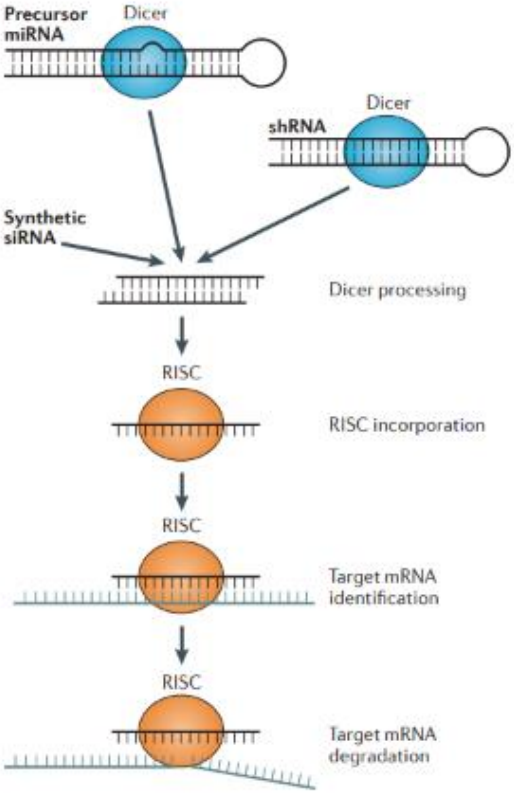

Figure 1: Demonstration of siRNA [29]

MICRO RNA (miRNA) IN NDs: miRNA serves as biomarkers and therapeutics in AD as they can invade CSF and blood. In AD 22-23 nucleotide containing small noncoding miRNA which shows affinity to 3' untranslated region (UTR) in mRNA for controlling gene expression, this miRNA performs mRNA degradation by specific targeting. Transcription of miRNA is performed by RNA polymerases 2/3 into RNA precursors called pri-miRNA, this mechanism commences in nucleus. Drosha is a nuclear RNase 3 enzyme that further processes formed pri-miRNA into 70 nucleotides and moulds them in a hairpin-like structure. Exportin 5 exports miRNA in cytoplasm. Dicer is also an RNase 3 enzyme that converts pri-miRNA into small mature RNA duplex that combines with RNA induced silencing complex (miRISC), which is then carried to Argonuate (AGO), miRNA complex with AGO and forms (miRNA-AGO) complex which is capable of targeting mRNA and serves as a biomarker in the pathogenesis of AD [30], as shown in Figure 2.

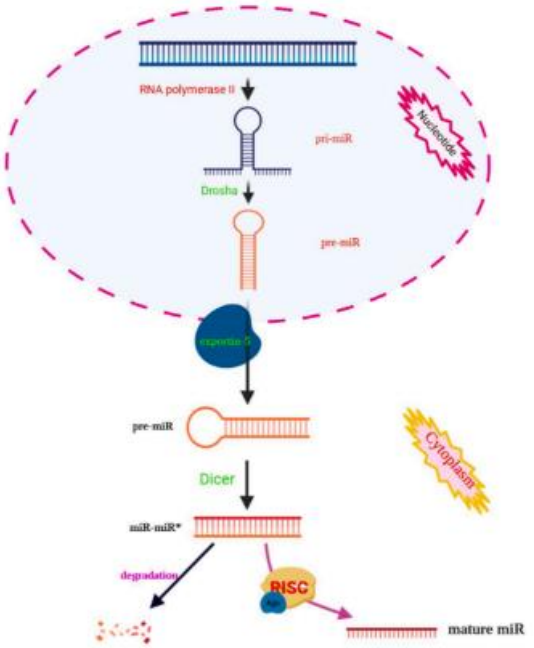

Figure 2: Demonstration of miRNA in Alzheimer's disease [31]

In epilepsy, miRNAs serve various pathological targets for upregulation and downregulation, Transcription and Translation, apoptosis, excitatory and inhibitory neurotransmission. Upregulation markers miR-146 act on Interleukin Associated Kinase Enzyme (IRAK) and TNF Receptor Associated Factor (TRAF) pathway, and downregulation marker miR-128 acts for extracellular regulatory kinase enzyme signalling (ERK) pathway [32] as shown in (Figure 3).

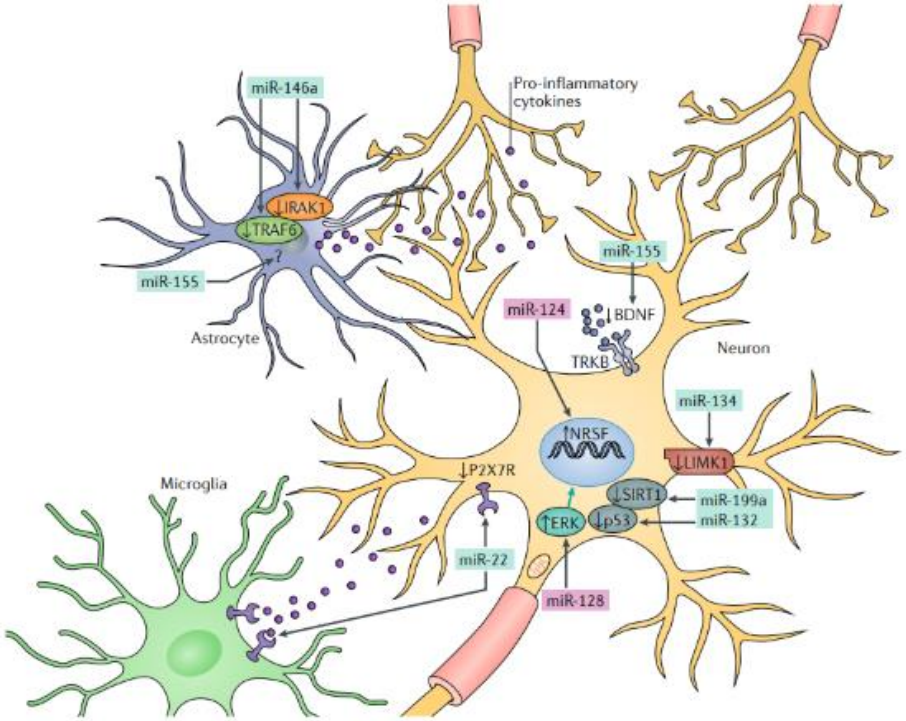

Figure 3: Demonstration of miRNA in seizures [33]

DMD shows muscle regeneration which can be modulated by miRNA targeting muscle stem cells to repair or regenerate lost ability. Many miRs are discovered for DMD therapeutics with various mechanism like miR-1 targets histone deacetylase 4 and promotes myoblast differentiation, in vivo experiments found miR-1 and miR-206 restricts proliferation of myogenic progenitor cells and downregulate protein α-1gap junctions, miR-486 targets platelet-derived-growth-factor-receptor β, tensin homolog and phosphatase pathway and many splicing factors like arginine/serine for differentiating myoblasts [24]. Glioma stem-like cells (GSC) are initiators of tumour growth specifically malignancies, they can be classical, mesenchymal, or proneural. miR-93 in autophagy in brain tumors is an essential oncogenic miRNA. miR-93 actuates with mechanism of TNF-β pathway suppression and suppresses autophagic modulators SQSTMI, ATG4B, ATG5, and beclin 1[34].

DRUG DELIVERY APPROACHES FOR OLIGONUCLEOTIDE:

With the emerging development in nanotechnology, nanocarriers are made multifunctional for diagnostic and therapeutic applications. These nanocarriers protect the encapsulated drug from rapid elimination; they can be complexed with targeting species such as folic acid, peptides, antibodies, ASOs, aptamers etc.,[35]. ASOs are carried to the brain via NDDS like liposomes, SLN, magnetic nanoparticles, and carbon nanotubes. ASOs are promising candidates for the delivery system in the brain but clinically face hurdles like rapid renal clearance, tendency for nucleases disposition, lesser incorporation in tumour cells, initiation of immunological response etc. NDDS is designed in a way to fill this void. Safety of the delivery system is assured before being implicated in clinical trials, therefore, safety studies are done in rat models to evaluate nanotoxicological concerns [36].

1. LIPOSOMES:

Gold nanoparticle liposomes (AuNPs) are recently discovered with features of biological inertness, ease of synthesis, and commercial feasibility. AuNPs possess a large surface area and spherical structure, making them capable of conjugating CNS ligand-specific oligonucleotides [37]. ApoE, a significant indicator in various NDs, is modified using multifunctional ionisable proton liposomes for carrying genetic materials in brain endothelial cells[38]. Liposomal oligomers are bifunctional with a peptide obtained from ApoE to bind on the domain to cross BBB and addition of phosphatidic acid for Aβ-42 plaques in AD [39]. For improving pharmacokinetics and biodistribution, liposomes were PEGylated and developed for transferrin-modified osthole-loaded liposomes, this osthole-loaded stealth liposome shows prolonged blood circulation time, it also targets SDF1 which is a ligand present on CXCR 4 an indicator of AD. Stealth liposomes improve cell viability and provide a protective effect when evaluated after administrating in mice brains. Aβ-42 aggregation can be prevented by using Apoptrotein E2 bioactive molecule which gives a significant outcome by targeting BBB via gene therapy [40]. To make liposomes cross BBB, different transport systems are used, such as Carrier-Mediated transcytosis (CMT), Absorptive-Mediated Transcytosis (AMT), and Receptor-Mediated Transcytosis (RMT). CMT relay on glucose transporter (GLUT 1) and large neutral amino acid transporters carriers, CMT when gets triggered with GLUT causes a change in conformation of receptor, small molecules are transported via CMT. RMT offers selective transport of large molecules via ligand binding like lactoferrin, transferrin, and insulin. AMT operates through electrostatic charges, positive charge surface with negative charge over plasma membrane [41], as shown in Figure 4.

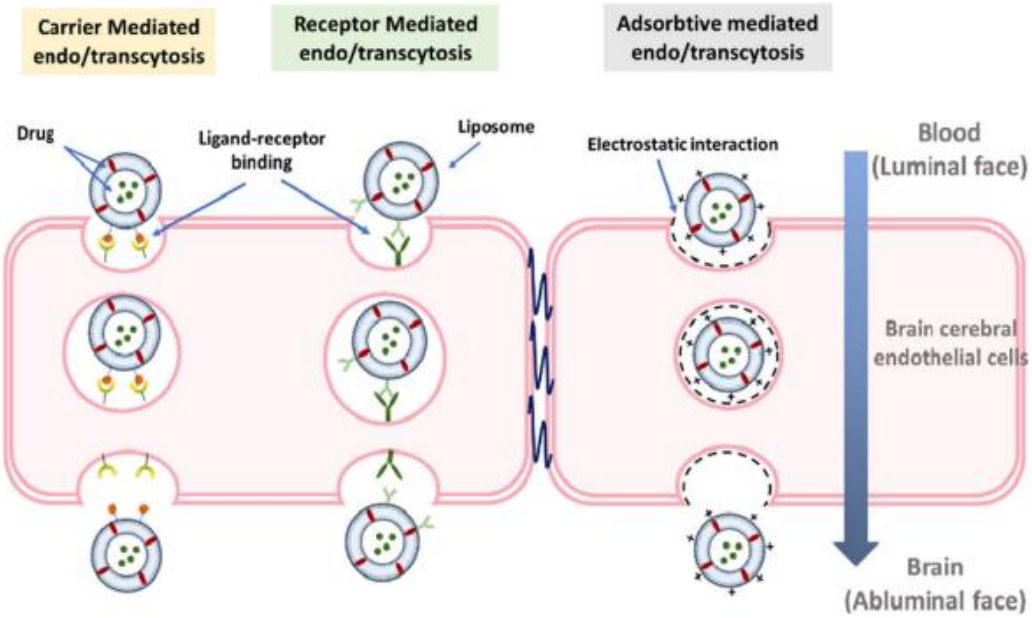

Figure 4: Drug transport mechanism of liposomes [42]

2. SOLID LIPID NANOPARTICLES:

SLNs are used as a vehiculating carrier for neoplastic brain delivery, they preferred over other carriers due to their specificity in targeting, acceptable release profile, avoidance from degradation, better tolerability and is physically stable. Vascular Endothelial Growth Factor (VEGF) is a pathway regulating tumour neo-angiogenesis; therefore, Anti VEGF ASOs are prepared. This Anti VEGF ASO shows significant downregulations of VEGF, shows potential reduction in both central and peripheral VEGF expression, thus inhibiting neoangiogenesis [43]. When loaded in SLN, Nicotinamide inhibits histone deacetylase in AD, quercetin loaded SLN shows reverse in neurodegeneration when examined in vivo. Bromocriptine drug used in PD shows an increase in plasma half-life, concentration in CNS and stabilized plasma level. Curcumin-loaded SLN performs mitochondrial dysfunction, reduces oxidative stress, and recovers neuronal loss. Antiepileptic drugs like amiloride and muscimol, when loaded in SLN, show anticonvulsant effects by sustained release compared to conventional dosage forms by restriction in focal seizures in in-vivo rat models. SLN carries drugs via Passive diffusion and Paracellular transport. CMT and efflux transport systems are protein-based transport system. RMT involves 3 consecutive steps i.e., endocytosis, intracellular vesicular transport, and exocytosis. AMT though has low affinity of binding but has high ability of binding like RMT. It works on electrostatic charges present on molecule and plasma membrane of BBB [44], as depicted in Figure 5.

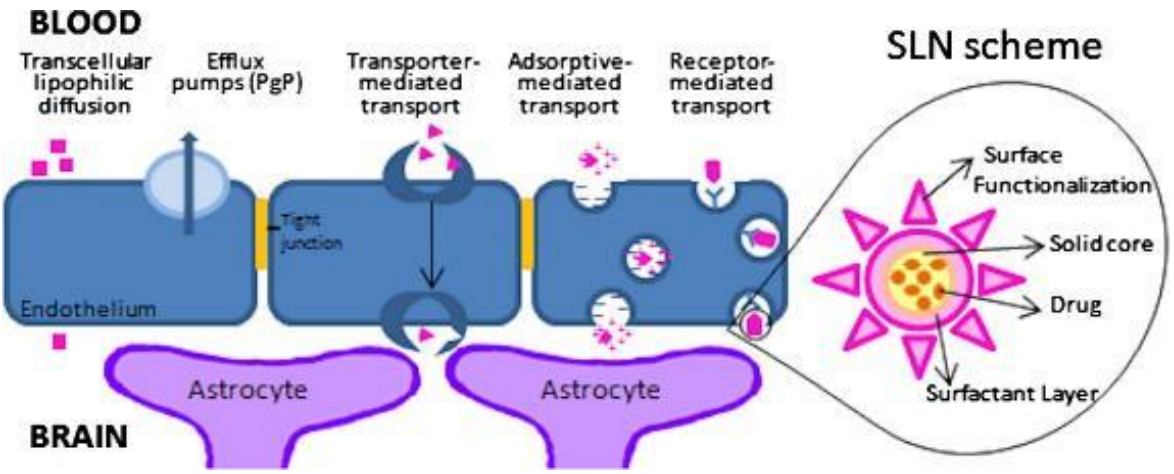

Figure 5: Drug transport mechanism of SLN [45]

3. NANOSTRUCTURED LIPID CARRIERS:

NLC is a composition of lipids from solid liquid origin which is crystalline in nature, it offers many advantages over SLN like improves the volume of drug load, flexibility in release kinetics of drug and shelf life of drug, they are biocompatible, nontoxic, site specific [46]. NLCs are fabricated with cytarabine to target meningeal leukaemia. Temozolomide (TMZ), a well-known drug for brain cancer was encapsulated with DNA as NLC, this loaded NLC is made functionalised by adding arginine–aspartic acid-glycine peptides. This (TMZ-NLC) complex was effective in reducing tumour growth. Studies show Vinpocetine (VPT) is an short half-life drug and is used in cerebral vascular ischemia, to overcome its short half-life it is encapsulated as (VPT-NLC) complex [47]. To improve therapeutic efficacy of NLC they are PEGylated. R-Flurbiprofen was developed as an ester prodrug with PEGylation to treat AD in rats' models, showing a decrease in Aβ accumulation. The glial cell-derived neurotropic factor is an alternative neuroprotective to treat PD, as it protests dopaminergic nerves from toxins and promotes nerve differentiation. But due to the hydrophilic charge's inability to cross BBB, short half-life, molecular charge, and rapid degradation are other associated problems. Therefore, NLCs are prepared showing acceptable drug concentration in the brain and improve locomotor activity in male albino Sprague Dawley rats. Recombinant tissue plasminogen activator is the most recently used bioactive for ischemic stroke, which is FDA-approved. Idebenone is an antioxidant that shows relative enhancement in bioavailability and increased brain distribution when evaluated in Wistar albino rats when formulated in NLC [48]. Drug transport mechanism of NLC is shown in Figure 6.

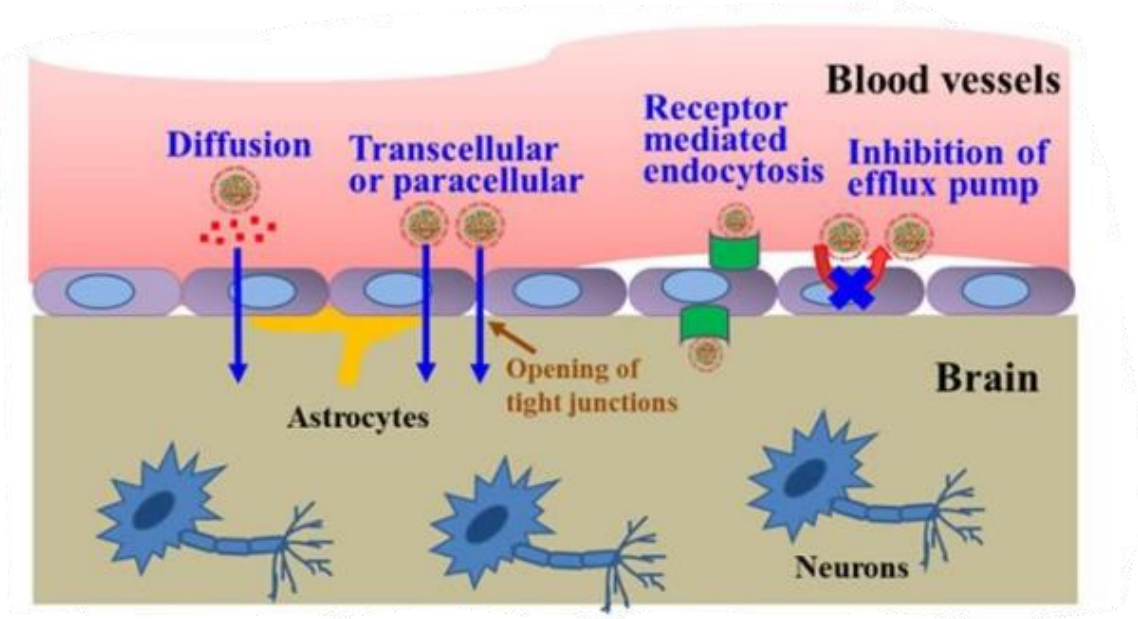

Figure 6: The drug transport mechanism of NLC [49]

4. MAGNETIC NANOPARTICLES (MNP):

MNPs are considered as applicable techniques for targeting brain tumours, as they possess a magnetic core, layer for recognition, and capacity for therapeutic load, the recognition layer provides a platform for site-specific targeting [50]. Inorganic MNP's are used to visualize Aβ-42 plaques in AD. The heat produced by MNPs alters the magnetic field which can be used to deaggregate Aβ-42 plaques in AD. In PD, α-synuclein expression was downregulated by functionalising MNP with nerve growth factor as it mediates endocytosis at site-specific receptor targeting. MNPs are used in tracking epileptogenic cells in epileptic brain [51]. Magnetite PEGylated nanoparticles are given through siRNA in AD. Modifying at 3' and 5'end with thiol group was done at siRNA to target BACE1 and prevent Aβ accumulation. The modified siRNA was co-immobilized by translocation protein OmpA which improves endosomal property of siRNA and encourages cellular internalization. The cell viability of PEGylated siRNA MNP was 82% and OmpA was 89% in the human fibroblast cell line (HFCL). Thus, it can be concluded that multifunctional MNP can deliver siRNA at targeted sites in AD [52]. MNP crosses BBB via RMT, AMT or by providing an external magnetic field [53] as shown in Figure 7.

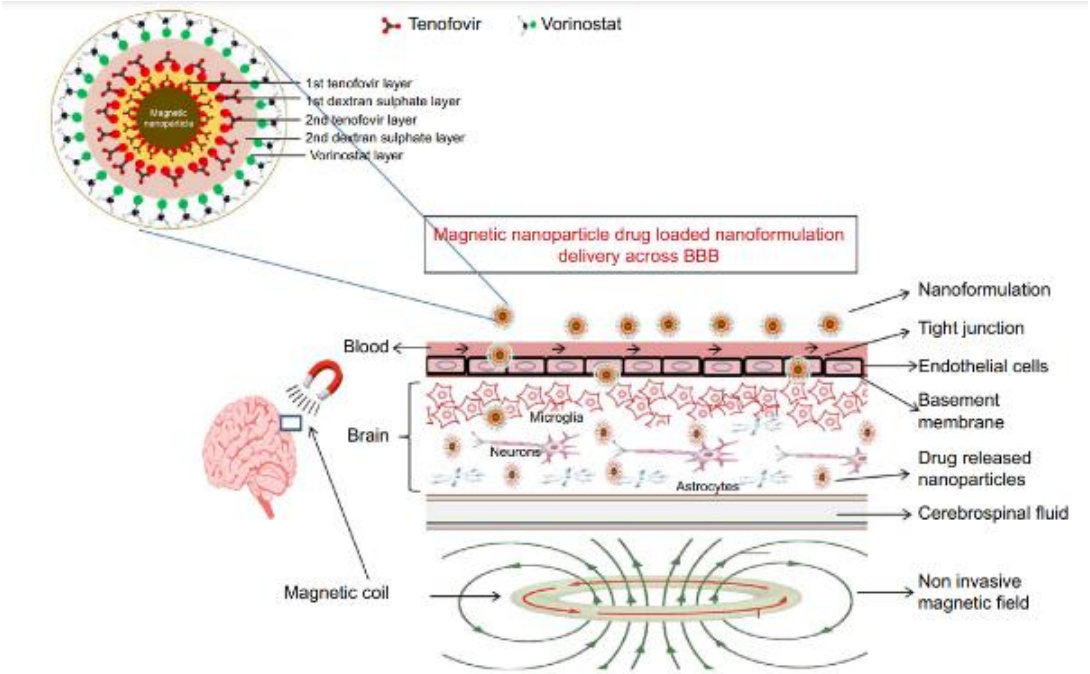

Figure 7: Drug transport mechanism of MNP [54]

5. CARBON NANOTUBES (CNT):

CNTs are featured with intrinsic chemical, physical, mechanical, and electrical properties which make them attractive in treating NDs, they are of 2 types single-walled (SWCNT) and multiwalled (MWCNT). CNTs made functional with targeted ligands show immunogenic properties and improved payload capacity. MWCNT when modified with DNA and siRNA in combination with oligodeoxynucleotides shows cytotoxic effect on glioma cells. SWCNTs are used in stroke and brain tumours. Due to SWCNT's feasibility and efficacy, they deliver dopamine at target site in PD [55]. Amine-coated SWCNT provides neuroprotection in ischemic stroke followed by MCAO with change in behaviour in rats models with addition CNT can be used in nanofibers for peripheral growth and restoration [35]. CNT was coated with PEG-SWCNT showing neuroprotection of anti-thyroxine hydroxylase positive neurons [56]. Delivery of levodopa in PD is made sustained by carboxylation CNT. In orthotopic brain glioma MWCNT serves as drug delivery of oxaliplatin. Peptide transcriptional activator, biotin, polyethyleneimine and oxaliplatin forms complex, in vivo evaluation in mice found that complex is more cytotoxic than oxaliplatin. MWCNT were made surface functionalized with an amino group and caspase 3 siRNA was examined in an endothelin stroke rat model. It was found that neuronal death was prevented by gene silencing and inhibiting caspase 3[57]. CNT transports drugs via RMT and passive diffusion. Compared to conventional dosage form CNT shows facilitated diffusion and can easily invade tumour cells as shown in Figure 8.

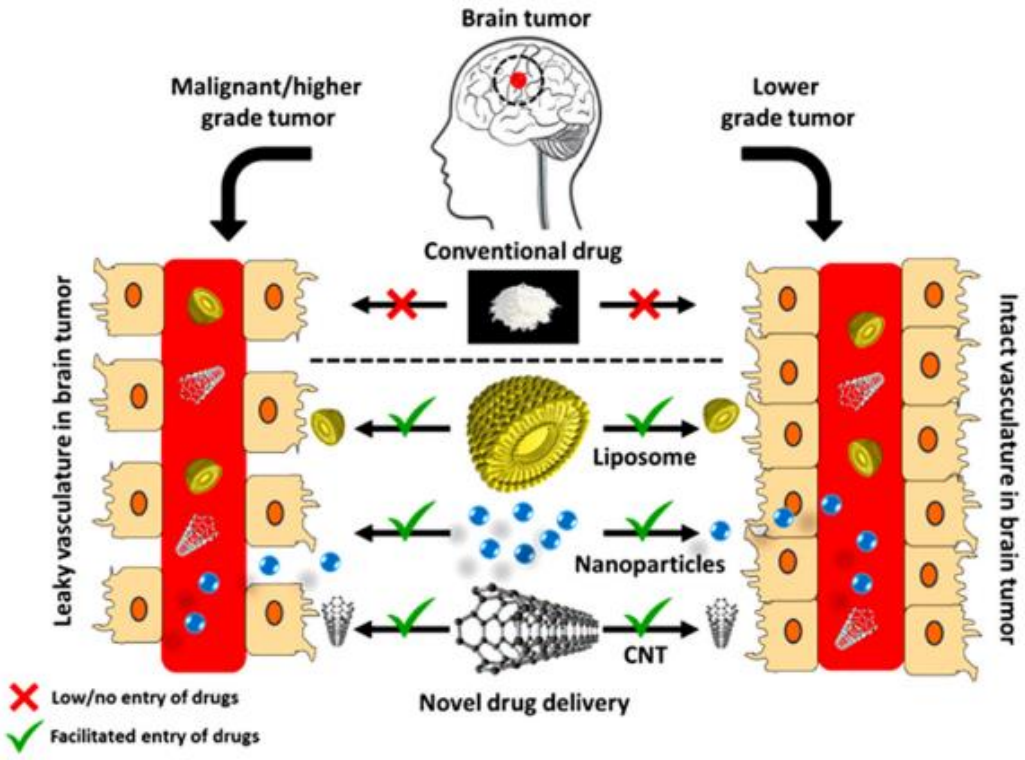

Figure 8: Drug transport mechanism of CNTs [58]

6. QUANTUM DOTS (QDs):

QDs are equipped with large surface area, biocompatibility, fluorescence character and small size, making them liable for crossing BBB. QDs can serve as siRNA transporters as they provide fluorescence imaging and specific targeting. Mitomycin C was formulated for anticancer purposes with chitosan nanocarrier coated with manganese-Doped zinc sulphide Quantum Dots (Mn ZnS QDs) which showed sustained action [59]. QDs also provide support in transporting miRNA. miR-21 and miR-29a are standard tumor markers of miRNA which induce apoptosis and remove tumor cells [46]. Zinc oxide quantum dots (ZnO QDs) are loaded with NGF (nerve-growth-factor) for treating PD as NGF belongs to neurotrophin and aids in neuronal growth and provide strength to weaker neurons. For improving solubility, QDs are coated with polymethacrylate and co-polymethacrylic acid. Glutathione is complex with QDs as glutathione receptors are present over BBB to deliver drugs via RMT. In vivo studies in rat models conclude ZnO. QDs provide better hemocompatibility and negligible cytotoxicity [47]. A proteolytic enzyme metalloproteinase activates inflammatory leucocytes and performs neuronal apoptosis by degrading extracellular matrix. To reduce expression of metalloproteinases siRNA was complexed with QDs which showed an increase in production of collagen, an improved BBB integrity was observed by measuring increased in electrical resistance in cells treated with siRNA-QDs complex [60] as shown in Figure 9.

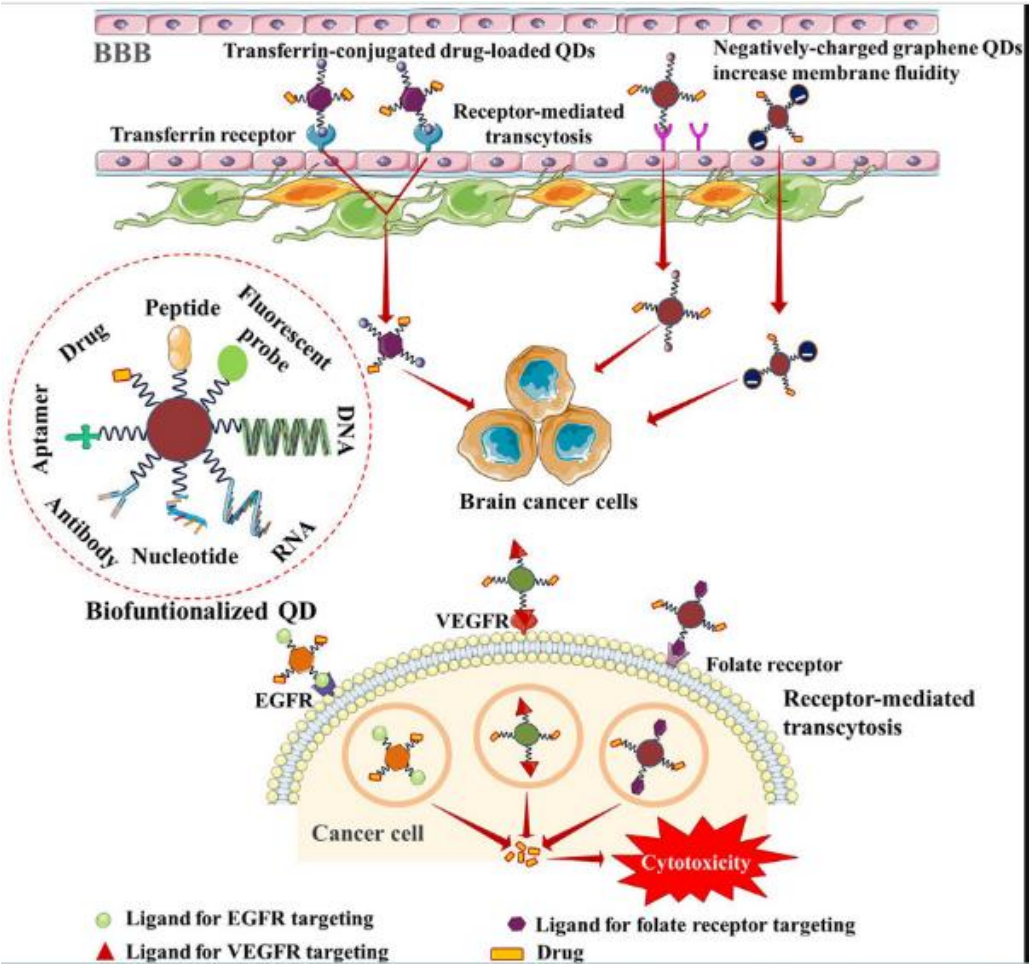

Figure 9: Drug transport mechanism of quantum dots [61]

7. POLYMERIC NANOPARTICLES:

NPs show unique features like biological safety i.e. lack of mutagenesis, less immunogenicity, chemical flexibility, ease of synthesis, and low production costs [62]. Widely used polymers for brain gene delivery are polyethyleneimines (PEI), poly(beta-amino ester)s (PBAE), and Poly(lactic-co-glycolic acid)(PLGA) [63]. It exhibits favourable characteristics such as entering BBB and averting nuclease degradation of target genes, avert renal excretion and reticulo endothelial system (RES) clearance, & shows target specificity [64]. K. Miyata et al. [65]constructed GLUT1-targeting glucosylated-polyion complex micelles (Glu(X)-PIC/Ms) and showed ASOs collection in cerebrum is effective by not involving invasive IV method because of multiple glucose-installed PIC/Ms, because they show polyvalent bonding to GLUT1 present onto the plasmalemma of brain capillary endothelial cells (BCECs). Translocation at apex region of the brain fasting mouse injected with glucose activates recycling. Concurrently BCECs basal region were distributed with Glu(X)-PIC/Ms. PIC/Ms are gathered from ~100 block copolymer strands & ~35 ASO molecules, nevertheless of glucose units, fulfilling these parameters such as **1**.A size of less than 50 nm, **2**. 80–100 min half-life property of blood circulation, & **3.** Optimization of glucose units & its density on glucose surface (strands of block copolymer (50/100). K. Miyata et al. showed a distinctive design of delivering ASOs (non-invasively) to BBB viz glycaemic controlled i.e. provoked externally, that shows quick & good impact to treat neurological diseases like HD &

ALS diseases found in cerebral cortex & hippocampus [65].

8. POLYMERIC MICELLES:

A structure having a shell of core & block copolymer (having both lipophilic & hydrophilic properties). These are obtained by molecules which are amphiphilic in nature and get self-assembled at critical micelle concentration (CMC). C6 glioblastoma cells were studied to see anti-proliferative activities. Its efficacy was like polyethyleneimine (PEI) 125k (25 kDa) & deoxycholic acid conjugate polyethylenimine (DP). This complex was more effective than curcumin (cur) or antisense oligonucleotides against miR-21 (miR21ASO) as only delivery agents studied in animal models for intracranial glioblastoma. DP carries ASO and hydrophobic drugs, therefore curcumin efficiency is increased in C6 cells as compared to plain curcumin. MiR21-ASO complex stability increases due to DP-cur. Micelles core hydrophobicity and stability increase due to insertion of curcumin. miR21ASO/DP is less stable than miR21ASO/DP-cur as examined in heparin assay. PE125K has low transfection efficiency than DP-cur. DP micelles are more efficient to transfection than complex of DP-cur; found through MIT assay the toxic nature of DP is less than PEI25k. Hence such favourable features make micelles a great vehicle for miR21 to treat glioblastoma [66].

9. MESOPOROUS SILICA:

AS1411 (AGRO100) is a nucleic acid sequence i.e. oligonucleotide in G quadruplex the first drug that targets nucleoprotein [67]. It shows better results in clinical trial phases with anti-tumor activity in every line of cancerous cells [68]. The Fahimeh Charbgoo group [69] targeted this NP(mesoporous silica) using bi-functional components with trapezoid shape using a divalent aptamer consisting of ATP and AS1411 aptamer. Here the drug is delivered to cells because mesoporous particles have properties of targeting tumor with doxorubicin (DOX). In vitro study showed improved uptake of NP by cells due to the coating & shapes of NP on permeability of BBB. But due to large size of NP (50-240nm) which cannot pass BBB proved in in-vivo studies in mice[70]. “Another study on Zebrafish embryos demonstrated that blood-brain barrier penetration is dependent on the surface charge and the size of the nanoparticles, with enhanced transport capacity related to negative charges and reduced sizes. Negatively charged mesoporous silica nanoparticles penetrate through the Zebrafish larval blood-brain barrier [71]. Resveratrol, a drug to treat removal of excessive reactive molecules of oxygen & nitrogen can be penetrated to BBB by transcytosis mechanism with receptor of lipoprotein of low-density type peptide ligand conjugated with NP coated with polylactic-polylactic acid with enhanced potential. An antioxidant-based treatment for Neurological disorders & injuries to neurons shows promising results with NP with size of 200 nm as optimum in vitro model [72] as shown in Figure 10.

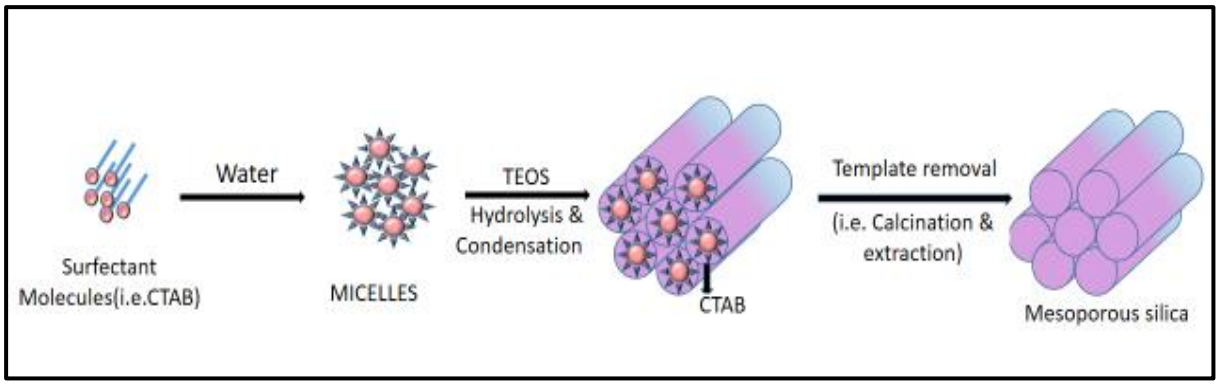

Figure 10: Preparation of Mesoporous Silica [73]

10. APTAMERS:

"Aptamers are cell type-specific oligonucleic acids or peptides that bind to a specific target molecule" [74]. Just like monoclonal antibodies, aptamers are also highly specific with good affinity & procedure to produce aptamers is systematic evaluation of ligands by exponential enrichmen (SELEX)[75]. It has advantages in clinical application with low systemic toxicity [76]. Aptamers internalization happens inside the cells using two mechanisms i.e., macropinocytosis & endocytosis mediated viz receptor [77]. The nucleus of drug DOX is aromatic and can get incorporated in DNA, this way drugs are connected to aptamers by method of physical intercalation [78]."Noncoding RNA such as siRNA, miRNA, and ASOs are structurally akin to aptamers, so their synthesis is enabled with systemic administration. For example, therapeutic RNA can be directly linked with aptamers for cancer therapy. The linkage is constructed through hybridization or a covalent bond" [79]. Affinito et al. found a new aptamer A40s that can deliver stem cells with miR-34c (tumor suppressor miRNA) and anti-miR10b (Targeting metastasis-promoting anti-miRNAs) by internalization in glioblastoma (GBM) viz differential cell-SELEX. It can be able to pass the BBB to target tumor and preventing its growth & also its relapse by binding specifically to a 130-kDa, 976-amino acid transmembrane glycoprotein (EphA2) receptor [80,81]. An example of bifunctional aptamers is the Anti-Epcam Aptamer (SYL3C) conjugated to the human transferrin receptor (TfR) aptamer. The SYL3C aptamer, against Epithelial Cell Adhesion Molecule (EpCAM), was truncated in a sequence of 17-mer and fused to the TfR aptamer, an anti-transferrin DNA aptamer of 14 nucleotides. These aptamers were bound together to build a bifunctional system that could cross the BBB via transcytosis through the TfR aptamer and inhibit metastases through the anti-EpCAM aptamer" [82].

11. NANOBODIES:

A fragment of antibody which is active within cell is called Nanobodies. Variable fragment (Fv) sites determine the specificity of antibody. Genes that encode antibody binding sites within cells such as antibody fragments are more considered, showing good engineering capacity and specificity that recognizes after translational changes. To improve solubility within cells concurrently with pharmacodynamic property, antigen-antibody complexes are retargeted by fusing peptides in multifunctional constructs, where potency is improved by fusing with PEST dregon which are proteolytic targeting signals. Nanobodies transfected stably into stem cell transplants protect them from misfolded foreign proteins. Good stability, high binding properties to target site, small size in nanometers, and minimum immunogenicity are good advantages of nanobodies [83].

12. DENDRIMERS:

Dendrimers are the most flexible, with proper composition & structurally regulated NDDS, as shown in Figure 11. The various biological membranes through endocytosis pathway viz internalizing cell. It gets absorbed intracellularly due to the regulation of proteins like occludin and actin (close junction), & can be reversible that depends on concentration of dendrimers & charge on the surface [84]. Many scientists have shown that these nanocarriers can cross BBB which is impaired after administration in blood, in case of neuroinflammation related to brain or retina by targeting microglia & astrocytes [85]. For treatment in neurological diseases and improving memory of brain, Gothwal et al. formed dendrimers of PAMAM lactoferrin modified & loaded with rivastigmine and showed improved BA due to high absorption about 8 times in brain and 9,8 times less cytotoxicity; it indicated the increasing memory function and the locomotive changes in rat brain [86].

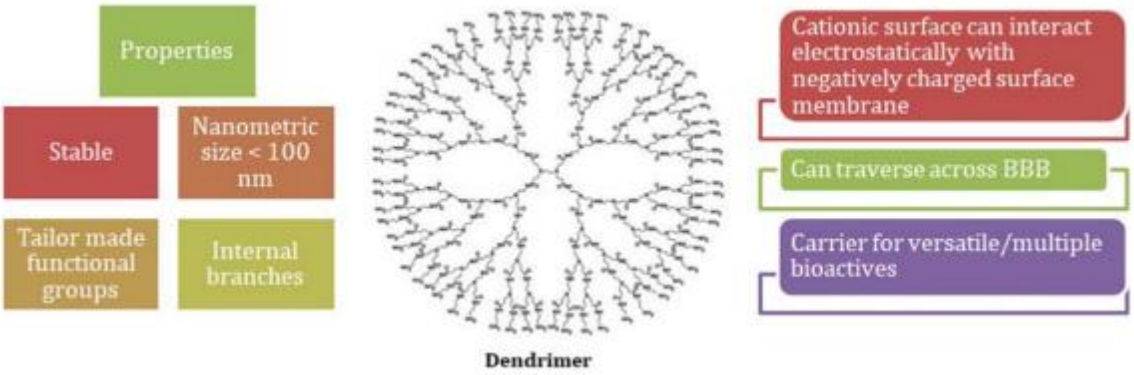

Figure 11: Schematic presentation of properties of dendrimers [87]

THERAPEUTIC RESPONSE OF PRE-CLINICALLY TESTED STRATEGIC GENES:

In preclinical trials Aβ cDNA shows depletion in amount of Aβ deposition when examined in transgenic mice (Tg2576) for AD. For ALS, ASO was used as complementary strand for C90RF72 repeats to inhibit RNA expression. In transgenic R6/2 mouse model siRNA restrict HTT mutant gene expression thereby increasing survival. HTT gene-specific silencing was performed on N171-82Q mouse model using miRNA which shows improved motor functioning and survival. ASO infusion was targeted at HTT gene in HD mouse model which shows positive response in motor functions in HD. Rats model were induced with IL-1which show reduced infarct volume in stroke [88]. In PD GBA1, pathologic risk factor is expressed for glucocerebrosidase enzyme which performs catalysis of glucocerebroside to cerebroside and glucose. Mutation of GBA1 causes accumulation of α-synuclein neurofibrillary tangles, therefore ASO is used to normalise the functioning of GBA1[89]. AR-ASO was administered in AR97Q mice model which shows inhibition in expression of mutant AR in CNS. This inhibition delays onset and progression of motor dysfunction [90]. Muscle regeneration is the symptomatic cause of DMD, miRNA serve as therapeutic target in DMD, overexpression of downregulated miR-29 promotes restoration of muscle regeneration, its intravenous and intramuscular administration in mdx mice which is popularly used in experimental work on DMD shows restriction on fibrogenesis, inhibit collagen and microfibrillar associated protein 5 thereby restores muscles function. In vivo, another target miR-431 shows muscle regeneration in dystrophic mdx mice [24]. Studies found that cytotoxic treatment for brain tumor when combined with autophagy suppressor miR-93 shows inhibition in tumour growth

of GSC [90]. A sequence of peptide RVG in coat protein of rabies virus is complexed with siRNA to treat brain injury, the complex shows probably 80% of neuronal specificity and also downregulates action of caspase-3, an apoptotic protein [91]. Table 1 shown summary of the main recommended predictive assays during nonclinical testing of individualized ASOs drug products for severely debilitating or life-threatening diseases.

Table 1. Summary of the main recommended predictive assays during nonclinical testing of individualized ASOs drug products for severely debilitating or life-threatening diseases [92]

ASOs toxicity	Predictive assays	In vitro/in vivo
Off-target effects	In silico evaluation/in vitro validation	In silico and in vitro
Immunostimulatory effects	• Quantification of cytokines/chemokines release in human PBMC or WBA by ELISA • Quantification of cytokines as well as CCL22 mRNA levels in BJAB cells by qRT-PCR	In vitro
Toxicities in high exposure organs	• Cytotoxicity by caspase assay Predictive assays for hepatotoxicity: • Quantification of LDH and ATP in primary hepatocytes • Caspase assay in transfected mouse 3T3 fibroblasts or human HepG2 cells • Evaluation of liver enzymes in mice/NHP Predictive assays for nephrotoxicity: • Quantification of EGF in human kidney tubule epithelial cells • Quantification of kidney injury biomarkers using chip-cultured HRPTEC • Quantification of urinary biomarkers (eg, b2-microglobulin and KIM-1) by ELISA	• In vitro • In vitro • In vitro • In vivo • In vitro • In vitro • In vitro
Thrombocytopenia	Evaluation of platelet activation in human or NHP platelet-rich plasma or whole blood by flow cytometry (activation of CD62P and PAC-1)	In vitro and in vivo
Inhibition of coagulation Complement activation	• Quantification of PT and aPTT in vitro in human/mouse/NHP citrated plasma • Quantification of split products of the APC (C3a, Bb, and C5a) in vitro in human/NHP/mouse serum	• In vitro • In vitro
CNS-specific toxicities	• Prediction of neurotoxicity from sequence features • Quantification of spontaneous calcium oscillations in primary cortical neuronal cultures	• In silico • In vitro

CLINICAL STUDIES

Currently, many preclinical studies are focusing on the delivery of siRNA-based therapeutics into the brain. Although these drugs have not yet reached clinical trials, the successful delivery of siRNA to non-CNS tumour tissue using nanoparticle-based delivery systems following systemic administration has been demonstrated in multiple trials, providing proof-of-concept for RNAi-based therapeutics in humans [93]. The current FDA-approved ASOs in neurological disorders include nusinersen for treatment of SMA, inotersen for polyneuropathy caused by hereditary transthyretin amyloidosis, and eteplirsen, golodirsen, viltolarsen, and casimersen for DMD [94] (Table 2). Also, Table 3 ASO-based therapeutics for the treatment of brain disease currently in clinical trials.

Table 2. FDA-approved antisense oligonucleotides in neurological disorders [95, 97]

Drug	Disease	Target mRNA	Suggested Dose	Adverse Events
Nusinersen (Spinraza)	SMA	SMN2 to increase exon 7 inclusion	12 mg: four loading doses. The first 3 doses are injected at 14-day intervals and the fourth dose at 30 days after the third dose. IT route	Nephrotoxicity, coagulation defects, thrombocytopenia, and IT-related adverse events including headaches, fever, and meningitis
Inotersen (Tegsedi)	Polyneuro pathy	TTR to prevent protein synthesis	300 mg Once per week by SC	Injection site reaction, headaches, fever, nausea, decreased appetite, liver inflammation, and nephrotoxicity. One patient who received inotersen in a clinical trial died after intracranial haemorrhage due to thrombocytopenia.
Eteplirsen (Exondys 51)	DMD	DMD to skip exon 51	30 mg/kg Once per week as 35- to 60-min infusion. IV route.	Hypersensitivity reaction headaches, nausea, vomiting, UTI, nasopharyngitis, balance disorder, contact dermatitis, and arthralgia.
Golodirsen (Vyondys 53)				Hypersensitivity reaction, headaches, nausea, vomiting, cough, fever, abdominal pain, nasopharyngitis, falls, and nephrotoxicity.
Viltolarsen (Viltepso)				UTI, injection site reaction, cough, fever, contusion, arthralgia, diarrhoea, nausea, vomiting, abdominal pain, reduced ejection fraction, urticarial, and nephrotoxicity.
Casimersen (Amondys 45)				UTI, cough, fever, headaches, arthralgia, pain in mouth and throat, and nephrotoxicity.

Table 3. ASO-Based Therapeutics for the Treatment of Brain Disease Currently in Clinical Trials[98]–[100]

Identifier	Disease	Drug	Phase	Delivery Route

NCT04849741	Alexander disease	ASO inhibiting GFAP synthesis (ION373)	I–III	IT
NCT03186989	Alzheimer's disease and frontotemporal dementia	ASO inhibiting tau protein synthesis (IONIS-MAPTRx)	I/II	IT
NCT04768972	Amyotrophic lateral sclerosis with FUS mutation	ASO inhibiting FUS protein synthesis (ION363)	III	IT
NCT04931862	Amyotrophic lateral sclerosis or frontotemporal dementia with documented mutation of G4C2 repeat expansion in the first intronic region of the C9orf72 gene	ASO targeting C9orf72 mRNA to selectively reduce C9orf72-repeat-containing transcripts (WVE-004)	I/II	IT
NCT04494256	Amyotrophic lateral sclerosis with no mutation in SOD1 or FUS gene	ASO inhibiting ataxin-2 synthesis (BIIB105)	I	IT
NCT04856982	Amyotrophic lateral sclerosis with SOD1 mutation	ASO inhibiting SOD1 protein synthesis (Tofersen)	III	IT
NCT04259281	Angelman Syndrome	ASO inhibiting UBE3A antisense transcript to increase paternal UBE3A expression (GTX-102	I/II	IT
NCT04428281	Angelman Syndrome	ASO inhibiting UBE3A antisense transcript to increase paternal UBE3A expression (RO7248824)	I	IT
NCT05127226	Angelman Syndrome	ASO inhibiting UBE3A antisense transcript to increase paternal UBE3A expression (ION582)	I/II	IT
NCT04123626	Autosomal dominant retinitis pigmentosa due to the P23H mutation in the RHO gen	ASO inhibits P23H protein expression while preserving expression of the wild-type rhodopsin protein (QR-1123)	I/II	IVT
NCT04442295	Dravet syndrome	ASO targeting nonproductive alternative splicing in SCN1A mRNA to upregulate Nav1.1 (STK001)	I/II	IT
NCT04906460	Duchenne muscular dystrophy	Exon-skipping ASO to promote skipping over exon 53 in DMD pre-mRNA (WVE-N531)	I/II	IV
NCT04004065	Duchenne muscular dystrophy	Peptide-conjugated eteplirsen to promote skipping over exon 51 in DMD pre-mRNA (vesleteplirsen or SRP-5051)	II	IV
NCT05524883	Duchenne muscular dystrophy	Exon-skipping ASO to promote skipping over exon 51 in DMD pre-mRNA (DYNE-251)	I/II	IV

CONCLUSION:

BBB protects the brain from foreign materials and is a big obstacle in brain-targeted drug delivery. To enhance therapeutic efficacy, new techniques that can enhance BBB crossing should be developed. Recently, nanotech techniques such as NP, liposomes, dendrimers,

micelles, and carbon nanotubes as nanocarriers are being explored extensively to bypass the BBB and transfer the proper amount of medicine to the specified brain region. The size of the nanoparticles is crucial in influencing the efficiency of endothelial transcytosis clearance from the plasma and intrinsic pathways. The safety and rate of degradation of the oligonucleotide are influenced by its structure, but the chemical formulation of the nanoparticles principally controls the site and rate of drug delivery. Effective oligonucleotide gene therapy in humans has needed intrathecal injection because of the significant structural differences between human and livestock illness models. In animal models, intraventricular or intravenous injection of oligonucleotides on nanostructures has achieved some success. By preserving the nucleic acid, boosting mobility all over the BBB, and promoting overexpression of target cells, NDDS offers various advantages for delivering therapeutic oligonucleotides to the CNS. Since the previous two decades, ASO/RNA-based therapeutics have developed. RNAi has yet to reach its full therapeutic effects, even though several clinical trials have been approved, are underway, or have been finished with successful attempts worldwide.

References

1- W. Grabowska-Pyrzewicz, A. Want, J. Leszek, and U. Wojda, “Antisense oligonucleotides for Alzheimer’s disease therapy: from the mRNA to miRNA paradigm,” *eBioMedicine*, vol. 74, p. 103691, Dec. 2021, doi: 10.1016/j.ebiom.2021.103691.

2- D. Kim *et al.*, “Graphene quantum dots prevent α -synucleinopathy in Parkinson’ s disease,” *Nat. Nanotechnol.*, 2018, doi: 10.1038/s41565-018-0179-y.

3- S. Swarbrick, N. Wragg, S. Ghosh, and A. Stolzing, “Systematic Review of miRNA as Biomarkers in Alzheimer’s Disease,” *Mol. Neurobiol.*, vol. 56, no. 9, pp. 6156–6167, Sep. 2019, doi: 10.1007/s12035-019-1500-y.

4- C.-J. Choong, K. Baba, and H. Mochizuki, “Gene therapy for neurological disorders,” *Expert Opin. Biol. Ther.*, vol. 16, no. 2, pp. 143–159, Feb. 2016, doi: 10.1517/14712598.2016.1114096.

5- A. Tomitaka *et al.*, “Surface-engineered multimodal magnetic nanoparticles to manage CNS diseases,” *Drug Discov. Today*, vol. 24, no. 3, pp. 873–882, Mar. 2019, doi: 10.1016/j.drudis.2019.01.006.

6- C. F. Bennett, A. R. Krainer, and D. W. Cleveland, “Antisense Oligonucleotide Therapies for Neurodegenerative Diseases,” *Annu. Rev. Neurosci.*, vol. 42, no. 1, pp. 385–406, Jul. 2019, doi: 10.1146/annurev-neuro-070918-050501.

7- V. Sardone, H. Zhou, F. Muntoni, A. Ferlini, and M. Falzarano, “Antisense Oligonucleotide-Based Therapy for Neuromuscular Disease,” *Molecules*, vol. 22, no. 4, p. 563, Apr. 2017, doi: 10.3390/molecules22040563.

8- D. C. Henshall *et al.*, “MicroRNAs in epilepsy: pathophysiology and clinical utility,” *Lancet Neurol.*, vol. 15, no. 13, pp. 1368–1376, Dec. 2016, doi: 10.1016/S1474-4422(16)30246-0.

9- T. Hirunagi, K. Sahashi, K. G. Meilleur, and M. Katsuno, “Nucleic Acid-Based Therapeutic Approach for Spinal and Bulbar Muscular Atrophy and Related Neurological Disorders,” *Genes*, vol. 13, no. 1, p. 109, Jan. 2022, doi: 10.3390/genes13010109.

10- H. Gandhi, A. K. Sharma, S. Mahant, and D. N. Kapoor, “Recent advancements in brain tumor targeting using magnetic nanoparticles,” *Ther. Deliv.*, vol. 11,

no. 2, pp. 97–112, Feb. 2020, doi: 10.4155/tde-2019-0077.

11- F. Sonvico *et al.*, "Surface-Modified Nanocarriers for Nose-to-Brain Delivery: From Bioadhesion to Targeting," *Pharmaceutics*, vol. 10, no. 1, p. 34, Mar. 2018, doi: 10.3390/pharmaceutics10010034.

12- A. Yu. Gerasimenko *et al.*, "Interfaces Based on Laser-Structured Arrays of Carbon Nanotubes with Albumin for Electrical Stimulation of Heart Cell Growth," *Polymers*, vol. 14, no. 9, p. 1866, May 2022, doi: 10.3390/polym14091866.

13- S. Grafals-ruiz, N.; Rios-vicil, C.I.; Lozada-delgado, E.L.; Quiñones-díaz, B.I.; Noriega-rivera, R.A.; Martínez-zayas, G.; Y., "Brain Targeted Gold Liposomes Improve RNAi Delivery for Glioblastoma," pp. 2809–2828, 2023.

14- H. Nsairat *et al.*, "Lipid nanostructures for targeting brain cancer," *Heliyon*, vol. 7, no. 9, p. e07994, Sep. 2021, doi: 10.1016/j.heliyon.2021.e07994.

15- E. Manek, F. Darvas, and G. A. Petroianu, "Use of Biodegradable, Chitosan-Based Nanoparticles in the Treatment of Alzheimer's Disease," *Molecules*, vol. 25, no. 20, p. 4866, Oct. 2020, doi: 10.3390/molecules25204866.

16- K. Morihiro, Y. Kasahara, and S. Obika, "Biological applications of xeno nucleic acids," *Mol. Biosyst.*, vol. 13, no. 2, pp. 235–245, 2017, doi: 10.1039/C6MB00538A.

17- A. Bortolozzi, S. Manashirov, A. Chen, and F. Artigas, "Oligonucleotides as therapeutic tools for brain disorders: Focus on major depressive disorder and Parkinson's disease," *Pharmacol. Ther.*, vol. 227, p. 107873, Nov. 2021, doi: 10.1016/j.pharmthera.2021.107873.

18- H. S. Min *et al.*, "Systemic Brain Delivery of Antisense Oligonucleotides across the Blood–Brain Barrier with a Glucose-Coated Polymeric Nanocarrier," *Angew. Chem. Int. Ed.*, vol. 59, no. 21, pp. 8173–8180, May 2020, doi: 10.1002/anie.201914751.

19- C. Rinaldi and M. J. A. Wood, "Antisense oligonucleotides: the next frontier for treatment of neurological disorders," *Nat. Rev. Neurol.*, vol. 14, no. 1, pp. 9–21, Jan. 2018, doi: 10.1038/nrneurol.2017.148.

20- H. Li, Y. Yang, W. Hong, M. Huang, M. Wu, and X. Zhao, "Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects," *Signal Transduct. Target. Ther.*, vol. 5, no. 1, p. 1, Jan. 2020, doi: 10.1038/s41392-019-0089-y.

21- D. Brenner, A. C. Ludolph, and J. H. Weishaupt, "Gene specific therapies – the next therapeutic milestone in neurology," *Neurol. Res. Pract.*, vol. 2, no. 1, p. 25, Dec. 2020, doi: 10.1186/s42466-020-00075-z.

22- J. F. Alterman *et al.*, "A divalent siRNA chemical scaffold for potent and sustained modulation of gene expression throughout the central nervous system," *Nat. Biotechnol.*, vol. 37, no. 8, pp. 884–894, Aug. 2019, doi: 10.1038/s41587-019-0205-0.

23- M. J. Gomes, S. Martins, and B. Sarmento, "siRNA as a tool to improve the treatment of brain diseases: Mechanism, targets and delivery," *Ageing Res. Rev.*, vol. 21, pp. 43–54, May 2015, doi: 10.1016/j.arr.2015.03.001.

24- A. E. Aránega, E. Lozano-velasco, L. Rodriguez-outeiriño, F. Ramírez de Acuña, D. Franco, and F. Hernández-torres, "Mirnas and muscle regeneration: Therapeutic

targets in duchenne muscular dystrophy," *Int. J. Mol. Sci.*, vol. 22, no. 8, 2021, doi: 10.3390/ijms22084236.

25- M. H. Pourhanifeh *et al.*, "Autophagy in cancers including brain tumors: role of MicroRNAs," *Cell Commun. Signal.*, vol. 18, no. 1, p. 88, Dec. 2020, doi: 10.1186/s12964-020-00587-w.

26- M. Fathi, S. Majidi, P. S. Zangabad, J. Barar, H. Erfan-Niya, and Y. Omidi, "Chitosan-based multifunctional nanomedicines and theranostics for targeted therapy of cancer," *Med. Res. Rev.*, vol. 38, no. 6, pp. 2110–2136, Sep. 2018, doi: 10.1002/med.21506.

27- M. Barani, M. Mukhtar, A. Rahdar, G. Sargazi, A. Thysiadou, and G. Z. Kyzas, "Progress in the Application of Nanoparticles and Graphene as Drug Carriers and on the Diagnosis of Brain Infections," *Molecules*, vol. 26, no. 1, p. 186, Jan. 2021, doi: 10.3390/molecules26010186.

28- ALTERMAN, Julia F., et al. A divalent siRNA chemical scaffold for potent and sustained modulation of gene expression throughout the central nervous system. Nature biotechnology , 2019, 37.8: 884-894.

29- Wittrup A, Lieberman J. Knocking down disease: a progress report on siRNA therapeutics. Nature Reviews Genetics , 2015, 16.9: 543-552.

30- X. Niu, J. Chen, and J. Gao, "Nanocarriers as a powerful vehicle to overcome blood-brain barrier in treating neurodegenerative diseases: Focus on recent advances," *Asian J. Pharm. Sci.*, vol. 14, no. 5, pp. 480–496, Sep. 2019, doi: 10.1016/j.ajps.2018.09.005.

31- SARVESTANI, Fatemeh Sabet, et al. microRNAs in liver and kidney ischemia reperfusion injury: Insight to improve transplantation outcome. Biomedicine & Pharmacotherapy, 2021, 133: 110944.

32- M. Agrawal *et al.*, "Biomaterials in treatment of Alzheimer's disease," *Neurochem. Int.*, vol. 145, p. 105008, May 2021, doi: 10.1016/j.neuint.2021.105008.

33- BRENNAN, Gary P.; HENSHALL, David C. MicroRNAs as regulators of brain function and targets for treatment of epilepsy. Nature Reviews Neurology , 2020, 16.9: 506-519.

34- M.-W. Seo and T.-E. Park, "Recent advances with liposomes as drug carriers for treatment of neurodegenerative diseases," *Biomed. Eng. Lett.*, vol. 11, no. 3, pp. 211–216, Aug. 2021, doi: 10.1007/s13534-021-00198-5.

35- X. Tian *et al.*, "Bioactive Materials Recent advances in the development of nanomedicines for the treatment of ischemic stroke," *Bioact. Mater.*, vol. 6, no. 9, pp. 2854–2869, 2021, doi: 10.1016/j.bioactmat.2021.01.023.

36- M. K. Satapathy *et al.*, "Solid Lipid Nanoparticles (SLNs): An Advanced Drug Delivery System Targeting Brain through BBB," *Pharmaceutics*, vol. 13, no. 8, p. 1183, Jul. 2021, doi: 10.3390/pharmaceutics13081183.

37- A. Khosa, S. Reddi, and R. N. Saha, "Nanostructured lipid carriers for site-specific drug delivery," *Biomed. Pharmacother.*, vol. 103, pp. 598–613, Jul. 2018, doi: 10.1016/j.biopha.2018.04.055.

38- C. Tapeinos, M. Battaglini, and G. Ciofani, "Advances in the design of solid lipid nanoparticles and nanostructured lipid carriers for targeting brain diseases," *J. Controlled Release*, vol. 264, pp. 306–332, Oct. 2017, doi: 10.1016/j.jconrel.2017.08.033.

39- A. Ayub and S. Wettig, "An Overview of Nanotechnologies for

Drug Delivery to the Brain," *Pharmaceutics*, vol. 14, no. 2, p. 224, Jan. 2022, doi: 10.3390/pharmaceutics14020224.

40- C. Xiang, Y. Zhang, W. Guo, and X.-J. Liang, "Biomimetic carbon nanotubes for neurological disease therapeutics as inherent medication," *Acta Pharm. Sin. B*, vol. 10, no. 2, pp. 239–248, Feb. 2020, doi: 10.1016/j.apsb.2019.11.003.

41- Henna, T.K.; Raphey, V.R.; Sankar, R.; Shirin, V.K.A.; Gangadharappa, H. V; Pramod, K, "Carbon nanostructures: the drug and the delivery system for brain disorders," 2020.

42- JUHAIRIYAH, Firda; DE LANGE, Elizabeth CM. Understanding drug delivery to the brain using liposome-based strategies: Studies that provide mechanistic insights are essential. The AAPS Journal , 2021, 23: 1-16.

43- X. Guo *et al.*, "Multifunctional Selenium Quantum Dots for the Treatment of Alzheimer's Disease by Reducing Aβ-Neurotoxicity and Oxidative Stress and Alleviate Neuroinflammation," *ACS Appl. Mater. Interfaces*, vol. 13, no. 26, pp. 30261–30273, Jul. 2021, doi: 10.1021/acsami.1c00690.

44- S. Devi *et al.*, "Quantum Dots: An Emerging Approach for Cancer Therapy," *Front. Mater.*, vol. 8, p. 798440, Jan. 2022, doi: 10.3389/fmats.2021.798440.

45- GASTALDI, Lucia, et al. Solid lipid nanoparticles as vehicles of drugs to the brain: current state of the art. European Journal of Pharmaceutics and Biopharmaceutics, 2014, 87.3: 433-444.

46- M. Sadat, S. Gharbi, S. Jafarinejad-farsangi, and Z. Ansari-asl, "Toxicology in Vitro Secondary toxic e ff ect of graphene oxide and graphene quantum dots alters the expression of miR-21 and miR-29a in human cell lines," *Toxicol. In Vitro*, vol. 65, no. February, p. 104796, 2020, doi: 10.1016/j.tiv.2020.104796.

47- Almajidi, Yasir Q.; Albadri, Ahmed A.; Jwaid, Mazen M.; and khalaf, Yaseen T. "Utilization of Semiconductor Nanocrystals In Cancer Diagnose And Treatment," Maaen Journal for Medical Sciences. 2023; 2 (4), 137-146. https://doi.org/10.55810/2789-9136.1034

48- G. Perini, V. Palmieri, G. Ciasca, M. De Spirito, and M. Papi, "Unravelling the Potential of Graphene Quantum Dots in Biomedicine and Neuroscience," *Int. J. Mol. Sci.*, vol. 21, no. 10, p. 3712, May 2020, doi: 10.3390/ijms21103712.

49- KHAN, Namrah, et al. Nanostructured lipid carriers-mediated brain delivery of carbamazepine for improved in vivo anticonvulsant and anxiolytic activity. International Journal of Pharmaceutics, 2020, 577: 119033.

50- W. Chen, Y. Hu, and D. Ju, "Gene therapy for neurodegenerative disorders: advances, insights and prospects," *Acta Pharm. Sin. B*, vol. 10, no. 8, pp. 1347–1359, Aug. 2020, doi: 10.1016/j.apsb.2020.01.015.

51- R. M. Lane *et al.*, "Translating Antisense Technology into a Treatment for Huntington's Disease," in *Huntington's Disease*, S. V. Precious, A. E. Rosser, and S. B. Dunnett, Eds., in Methods in Molecular Biology, vol. 1780. New York, NY: Springer New York, 2018, pp. 497–523. doi: 10.1007/978-1-4939-7825-0_23.

52- S. Naqvi, A. Panghal, and S. J. S. Flora, "Nanotechnology: A Promising Approach for Delivery of Neuroprotective Drugs," *Front.*

Neurosci., vol. 14, p. 494, Jun. 2020, doi: 10.3389/fnins.2020.00494.

53- L. K. C. Lee, L. I. Leong, Y. Liu, M. Luo, H. Y. E. Chan, and C. H. J. Choi, "Preclinical Nanomedicines for Polyglutamine-Based Neurodegenerative Diseases," *Mol. Pharm.*, vol. 18, no. 2, pp. 610–626, Feb. 2021, doi: 10.1021/acs.molpharmaceut.0c00506.

54- Jayant, Rahul Dev, et al. Sustained-release nanoART formulation for the treatment of neuroAIDS. International journal of nanomedicine , 2015, 1077-1093.

55- R. M. Kandell, L. E. Waggoner, and E. J. Kwon, "Nanomedicine for Acute Brain Injuries: Insight from Decades of Cancer Nanomedicine," *Mol. Pharm.*, vol. 18, no. 2, pp. 522–538, Feb. 2021, doi: 10.1021/acs.molpharmaceut.0c00287.

56- C. A. Stein and D. Castanotto, "FDA-Approved Oligonucleotide Therapies in 2017," *Mol. Ther.*, vol. 25, no. 5, pp. 1069–1075, May 2017, doi: 10.1016/j.ymthe.2017.03.023.

57- A. Salazar-ramiro *et al.*, "Cytotoxicity induced by carbon nanotubes in experimental malignant glioma," pp. 6005–6026, 2017.

58- MEHER, Jaya G., et al. Carbon nanotubes (CNTs): a novel drug delivery tool in brain tumor treatment. In: Nanotechnology-Based Targeted Drug Delivery Systems for Brain Tumors. Academic Press, 2018. p. 375-396.

59- A. Kont, M. R. Aburto, J. F. Cryan, and C. M. O. Driscoll, "Advances in the Design of (Nano) Formulations for Delivery of Antisense Oligonucleotides and Small Interfering RNA: Focus on the Central Nervous System," 2021, doi: 10.1021/acs.molpharmaceut.0c01238.

60- J. Yang, K. M. Luly, and J. J. Green, "Nonviral nanoparticle gene delivery into the CNS for neurological disorders and brain cancer applications," *WIREs Nanomedicine Nanobiotechnology*, vol. 15, no. 2, Mar. 2023, doi: 10.1002/wnan.1853.

61- CHAKRABORTY, Pratik, et al. Quantum dots: The cutting-edge nanotheranostics in brain cancer management. Journal of Controlled Release, 2022, 350: 698-715.

62- B. N. Singh, V. K. Gupta, J. Chen, and A. G. Atanasov, "Organic Combinatory Approaches for Gene Therapy," *Trends Biotechnol.*, vol. xx, pp. 1–3, doi: 10.1016/j.tibtech.2017.07.010.

63- ALBADRI, Ahmed A.; ABDULBAQI, Mustafa R.; ALMAJIDI, Yasir Q. Recent Trends in Chronopharmaceutics, Pulsatile Drug Delivery System. *Al Mustansiriyah Journal of Pharmaceutical Sciences* , 2019, 19.4: 41-49.

64- Z. Sezgin-bayindir, A. D. Ergin, M. Parmaksiz, A. E. Elcin, Y. M. Elcin, and N. Yuksel, "Evaluation of various block copolymers for micelle formation and brain drug delivery: In vitro characterization and cellular uptake studies," *J. Drug Deliv. Sci. Technol.*, vol. 36, pp. 120–129, Dec. 2016, doi: 10.1016/j.jddst.2016.10.003.

65- K. Sydow, H. Nikolenko, D. Lorenz, R. H. Müller, and M. Dathe, "Lipopeptide-based micellar and liposomal carriers: Influence of surface charge and particle size on cellular uptake into blood brain barrier cells," *Eur. J. Pharm. Biopharm.*, vol. 109, pp. 130–139, Dec. 2016, doi: 10.1016/j.ejpb.2016.09.019.

66- A. R. Rama *et al.*, "Circular Sponge against miR-21 Enhances the Antitumor Activity of Doxorubicin against Breast Cancer Cells," *Int. J. Mol. Sci.*, vol. 23, no. 23, p. 14803, Nov. 2022, doi: 10.3390/ijms232314803.
67- X. Tan *et al.*, "A curcumin-loaded polymeric micelle as a carrier of a microRNA-21 antisense-oligonucleotide for enhanced anti-tumor effects in a glioblastoma animal model," *Biomater. Sci.*, vol. 6, no. 2, pp. 407–417, 2018, doi: 10.1039/C7BM01088E.
68- L. Han *et al.*, "Redox-responsive theranostic nanoplatforms based on inorganic nanomaterials," *J. Controlled Release*, vol. 259, pp. 40–52, Aug. 2017, doi: 10.1016/j.jconrel.2017.03.018.
69- Q. Ma, Y. Li, and X. Su, "Silica-nanobead-based sensors for analytical and bioanalytical applications," *TrAC Trends Anal. Chem.*, vol. 74, pp. 130–145, Dec. 2015, doi: 10.1016/j.trac.2015.06.006.
70- X. Tong, L. Ga, J. Ai, and Y. Wang, "Progress in cancer drug delivery based on AS1411 oriented nanomaterials," pp. 1–36, 2022, doi: 10.1186/s12951-022-01240-z.
71- P. J. Bates, D. A. Laber, D. M. Miller, S. D. Thomas, and J. O. Trent, "Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer," *Exp. Mol. Pathol.*, vol. 86, no. 3, pp. 151–164, Jun. 2009, doi: 10.1016/j.yexmp.2009.01.004.
72- Y. Shen, B. Cao, N. R. Snyder, K. M. Woeppel, J. R. Eles, and X. T. Cui, "ROS responsive resveratrol delivery from LDLR peptide conjugated PLA - coated mesoporous silica nanoparticles across the blood – brain barrier," *J. Nanobiotechnology*, pp. 1–17, 2018, doi: 10.1186/s12951-018-0340-7.
73- ZAKARIA, Zulfirdaus, et al. A review of progressive advanced polymer nanohybrid membrane in fuel cell application. International Journal of Energy Research, 2020, 44.11: 8255-8295.
74- J. Wang, Z. Lu, M. G. Wientjes, and J. L. Au, "Delivery of siRNA Therapeutics : Barriers and Carriers," vol. 12, no. 4, pp. 492–503, 2010, doi: 10.1208/s12248-010-9210-4.
75- J. Zhou and J. Rossi, "Aptamers as targeted therapeutics: current potential and challenges," *Nat. Publ. Group*, 2016, doi: 10.1038/nrd.2016.199.
76- M. R. Dunn, R. M. Jimenez, and J. C. Chaput, "Analysis of aptamer discovery and technology", doi: 10.1038/s41570017-0076.
77- L. Wan *et al.*, "ce pt us cr t," *Expert Opin. Drug Deliv.*, vol. 0, no. 0, p. 1, 2019, doi: 10.1080/17425247.2019.1575808.
78- C. Lines, "Supporting Information".
79- P. Amero, S. Khatua, and C. Rodriguez-aguayo, "Aptamers : Novel Therapeutics and Potential Role," 2020.
80- A. Af *et al.*, "The Discovery of RNA Aptamers that Selectively Bind Glioblastoma Stem Cells," vol. 18, no. December, pp. 99–109, 2019, doi: 10.1016/j.omtn.2019.08.015.
81- MARAIE, Nidhal K.; ALMAJIDI, Yasser Qasim. Effect of different mucoadhesive polymers on release of ondansetron HCl from intranasal mucoadhesive in situ gel. *Al Mustansiriyah Journal of Pharmaceutical Sciences* , 2017, 17.2: 10-10. .
82- F. Rahbarizadeh and D. Ahmadvand, "Nanobody ; an Old Concept," pp. 299–338, 2011, doi: 10.3109/08820139.2010.542228.
83- S. Sadekar and H. Ghandehari, "Transepithelial transport and toxicity of PAMAM dendrimers:

Implications for oral drug delivery ☆," *Adv. Drug Deliv. Rev.*, vol. 64, no. 6, pp. 571–588, 2012, doi: 10.1016/j.addr.2011.09.010.

84- A. Sharma, J. E. Porterfield, E. Smith, S. Kannan, and R. M. Kannan, "PT," *J. Controlled Release*, p. #pagerange#, 2018, doi: 10.1016/j.jconrel.2018.06.003.

85- A. Gothwal, K. N. Nakhate, A. Alexander, and U. Gupta, "Boosted Memory and Improved Brain Bio-availability of Rivastigmine: Targeting Effort to Brain using Covalently Tethered Lower Generation PAMAM Dendrimers with Lactoferrin," 2018, doi: 10.1021/acs.molpharmaceut.8b00537.

86- GAURO, Rahul, et al. Advances in dendrimer-mediated targeted drug delivery to the brain. Journal of Nanoparticle Research, 2021, 23: 1-20.

87- C. J. Choong, K. Baba, and H. Mochizuki, "Gene therapy for neurological disorders," *Expert Opin. Biol. Ther.*, vol. 16, no. 2, pp. 143–159, 2016, doi: 10.1517/14712598.2016.1114096.

88- A. Bortolozzi, S. Manashirov, A. Chen, and F. Artigas, "Oligonucleotides as therapeutic tools for brain disorders: Focus on major depressive disorder and Parkinson's disease," *Pharmacol. Ther.*, vol. 227, p. 107873, 2021, doi: 10.1016/j.pharmthera.2021.107873.

89- T. Hirunagi, K. Sahashi, K. G. Meilleur, and M. Katsuno, "Nucleic Acid-Based Therapeutic Approach for Spinal and Bulbar Muscular Atrophy and Related Neurological Disorders," 2022.

90- M. H. Pourhanifeh, M. Mahjoubin-tehran, and M. R. Karimzadeh, "Autophagy in cancers including brain tumors: role of MicroRNAs," pp. 1–22, 2020.

91- R. M. Kandell, L. E. Waggoner, and E. J. Kwon, "Nanomedicine for Acute Brain Injuries: Insight from Decades of Cancer Nanomedicine," *Mol. Pharm.*, vol. 18, no. 2, pp. 522–538, 2021, doi: 10.1021/acs.molpharmaceut.0c00287.

92- GOYENVALLE, Aurélie, et al. Considerations in the preclinical assessment of the safety of antisense oligonucleotides. Nucleic acid therapeutics, 2023, 33.1: 1-16.

93- J. E. Zuckerman and M. E. Davis, "Clinical experiences with systemically administered siRNA-based therapeutics in cancer," *Nat. Rev. Drug Discov.*, vol. 14, no. 12, pp. 843–856, Dec. 2015, doi: 10.1038/nrd4685.

94- F. Alhamadani *et al.*, "Adverse Drug Reactions and Toxicity of the Food and Drug Administration–Approved Antisense Oligonucleotide Drugs," *Drug Metab. Dispos.*, vol. 50, no. 6, pp. 879–887, Jun. 2022, doi: 10.1124/dmd.121.000418.

95- F. Alhakmani, S. A. Khan, and A. Ahmad, "Determination of total phenol, in-vitro antioxidant and anti-inflammatory activity of seeds and fruits of Zizyphus spina-christi grown in Oman," *Asian Pac. J. Trop. Biomed.*, vol. 4, pp. S656–S660, Jul. 2014, doi: 10.12980/APJTB.4.2014APJTB-2014-0273.

96- Y. Weng and Y. Huang, "The Advances of Biomacromolecule-based Nanomedicine in Brain Disease," in *Nanomedicine in Brain Diseases*, X. Xue, Ed., Singapore: Springer Singapore, 2019, pp. 181–208. doi: 10.1007/978-981-13-8731-9_7.

97- M. C. P. Mendonça, A. Kont, M. R. Aburto, J. F. Cryan, and C. M. O'Driscoll, "Advances in the Design of (Nano)Formulations for

Delivery of Antisense Oligonucleotides and Small Interfering RNA: Focus on the Central Nervous System," *Mol. Pharm.*, vol. 18, no. 4, pp. 1491–1506, Apr. 2021, doi: 10.1021/acs.molpharmaceut.0c01238.

98- Astra Zeneca (last), "Eplontersen Met Co-Primary and Secondary Endpoints in Interim Analysis of the NEURO-TTRansform Phase III Trial for Hereditary Transthyretin-Mediated Amyloid Polyneuropathy (ATTRv-PN)," 2022, [Online]. Available: https://www.astrazeneca.com/media-centre/press-releases/2022/eplontersen-phase-iii-trial-met-co-primary-endpoints.html

99- J. Kim *et al.*, "Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease," *N. Engl. J. Med.*, vol. 381, no. 17, pp. 1644–1652, Oct. 2019, doi: 10.1056/NEJMoa1813279.

100- M. Amanat, C. L. Nemeth, A. S. Fine, D. G. Leung, and A. Fatemi, "Antisense Oligonucleotide Therapy for the Nervous System: From Bench to Bedside with Emphasis on Pediatric Neurology," *Pharmaceutics*, vol. 14, no. 11, p. 2389, Nov. 2022, doi: 10.3390/pharmaceutics14112389.