CNS-Targeting Therapies for Lysosomal Storage Diseases: Current Advances and Challenges

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During the past decades, several therapeutic approaches have been developed and made rapidly available for many patients afflicted with lysosomal storage disorders (LSDs), inborn organelle disorders with broad clinical manifestations secondary to the progressive accumulation of undegraded macromolecules within lysosomes. These conditions are individually rare, but, collectively, their incidence ranges from 1 in 2,315 to 7,700 live-births. Most LSDs are manifested by neurological symptoms or signs, including developmental delay, seizures, acroparesthesia, motor weakness, and extrapyramidal signs. The chronic and later-onset clinical forms are at one end of the continuum spectrum and are characterized by a subtle and slow progression of neurological symptoms. Due to its inherent physiological properties, unfortunately, the blood-brain barrier (BBB) constitutes a significant obstacle for current and upcoming therapies to achieve the central nervous system (CNS) and treat neurological problems so prevalent in these conditions. To circumvent this limitation, several strategies have been developed to make the therapeutic agent achieve the CNS. This narrative will provide an overview of current therapeutic strategies under development to permeate the BBB, and address and unmet need for treatment of the progressive neurological manifestations, which are so prevalent in these inherited lysosomal disorders.

Keywords: lysosomes, small molecules, therapy, enzyme replacement therapy, extracellular vesicles, exosomes, liposomes, gene therapy

LYSOSOMAL STORAGE DISEASES: INBORN ORGANELLE DISORDERS PREDOMINANTLY AFFECTING THE CNS

Lysosomal storage diseases (LSDs) are inborn organelle disorders characterized by multisystemic and progressive manifestations, being most of them neurological in nature (Table 1) (Patil and Maegawa, 2013; Maegawa, 2019). Intravenous enzyme replacement therapy (ERT), the mainstay treatment for several LSDs, does not address the neurological problems, as these recombinant proteins, large molecular-weight molecules, are unable to permeate through the blood-brain barrier (BBB) effectively. Several novel therapeutic agents such as intrathecal or intracerebroventricular delivery of enzymes, fusion proteins that cross the BBB, substrate reduction therapy (SRT), pharmacological chaperones (PCs), and gene therapy, are currently being developed to treat the neurological manifestations of LSDs (Figure 1).
TABLE 1 | Enzyme-deficiency LSDs, classified according to primary substrate storage.

| Disease                          | Deficient Enzyme       | Primary storage metabolite | Gene, locus (inheritance) | Neurological Symptomatology                                                                 | Non-neurological Symptoms                                      |
|----------------------------------|------------------------|-----------------------------|---------------------------|---------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| **COMMON NEUROLOGICAL LSDs**     |                        |                             |                           |                                                                                              |                                                                  |
| Gaucher disease (Types I, II, III) | Glucosylceramidase     | glucosylceramide            | GBA 1q21 (AR)             | Type I—(adult) parkinsonism, peripheral neuropathy                                         | Hematological—anemia, leucopenia, thrombocytopenia               |
|                                  |                        |                             |                           | Type II—(fetus—12-months) stridor, ocular motor apraxia, dysphagia, dystonia, pyramidal tract signs, and sometimes opisthotonus | Visceromegaly—hepatosplenomegaly                                |
|                                  |                        |                             |                           | Type III—(>1-year-old) myoclonic and tonic-clonic seizures, horizontal supranuclear gaze palsy, ataxia[1-4] | General—low energy                                              |
|                                  |                        |                             |                           | Hematological—anemia, leucopenia, thrombocytopenia                                         | Cardiac—pulmonary hypertension                                  |
|                                  |                        |                             |                           | Visceromegaly—hepatosplenomegaly                                                            | Dermatological—neonatal ichthyosis                               |
|                                  |                        |                             |                           | General—low energy                                                                         |                                                                  |
|                                  |                        |                             |                           | Cardiac—pulmonary hypertension                                                             |                                                                  |
| Fabry disease                    | α-Galactosidase A      | globotriasy/llceramide      | GLA Xq22 (X-linked)       | Acroparesthesia, dysesthesia, recurrent acute and chronic pain, hearing impairment, tinnitus, recurrent cerebrovascular disease | Renal—chronic renal disease                                     |
| Pompe Disease                    | α1,4-glucosidase (acid maltase) | glycogen                   | GAA 17q25.3 (AR)          | Lower limb-girdle and truncal muscular weakness combined with exercise intolerance, migraines, dysphagia | Hypertrophic cardiomyopathy                                     |
| MPS I (Hurler, Scheie, Hurler/Scheie) | α-Iduronidase           | Dermatan sulfate, heparan sulfate | IDUA 4p16.3 (AR)          | Global developmental delay, carpal tunnel syndrome, myelopathies, spinal cord compression   | Skeletal—dysostosis multiplex and multiple joint contractures Organomegaly |
| MPS II (Hunter)                  | Iduronate sulphatase   | Dermatan sulfate, heparan sulfate | IDS Xq28 (X-linked)       | Global developmental delay, carpal tunnel syndrome, myelopathies, spinal cord compression   | Cardiac valvulopathies and hypertrophic cardiomyopathy            |
| MPS III Sanfilippo syndrome      | MPS-IIIA—heparan sulphamidase | Heparan sulfate             | SGSH 17q25.3 (AR)         | Global neurodevelopmental delay (1st stage), behavior problems characterized by temper tantrum, aggressive behavior, and extreme restlessness (2nd stage), Severe dementia, decline motor functions (3rd stage) | Coarse facies, obstructive air way, dysostosis multiplex (thoracolumbar kyphosis, abnormally shaped vertebrae and ribs, spina!e ribS, hypoplastic epiphyses, thickened diaphyses, and bullet-shaped metacarpals), Brain MRI with ventricular dilatation and enlargement of subarachnoid spaces, thin corpus callosum, enlarged perivascular spaces |
|                                  | MPS-IIIB—acetyl α-glucosaminidase | Heparan sulfate             | NAGLU 17q21.2 (AR)        |                                                                                              |                                                                  |
|                                  | MPS-IIIC—acetyl CoA: α-glucosaminide | Heparan sulfate             | HGSNAT 8p11.21 (AR)       |                                                                                              |                                                                  |
|                                  | MPS-IIID N-acetyl glucosamine-6-sulphatase | Heparan sulfate             | GNS 12q14.3 (AR)          |                                                                                              |                                                                  |
TABLE 1 | Continued

| Disease | Deficient Enzyme | Primary storage metabolite | Gene, locus (inheritance) | Neurological Symptomatology | Non-neurological Symptoms |
|---------|------------------|---------------------------|--------------------------|-----------------------------|---------------------------|
| MPS IVA (Morquio A) | Acetyl galactosamine-6-sulphatase | Keratan sulfate, chondroitin 6-sulfate | GALNS 16q24.3 (AR) | Normal neurodevelopmental, carpal tunnel syndrome, myelopathies, spinal cord compression | Skeletal–dysostosis multiplex and multiple joint contractures, Organomegaly, Obstructive Sleep Apnea, Cardiac valvulopathies and hypertrophic cardiomyopathy |
| MPS IVB (Morquio B) | β-Galactosidase | Keratan sulfate | GLB1 3p22.3 (AR) | Global developmental delay, carpal tunnel syndrome, myelopathies, spinal cord compression | Skeletal–dysostosis multiplex and multiple joint contractures, Organomegaly, Obstructive Sleep Apnea, Cardiac valvulopathies and hypertrophic cardiomyopathy |
| MPS VI (Maroteaux-Lamy) | Acetyl galactosamine 4-sulfatase (aryl sulfatase B) | Dermatan sulfate | ARSB 5q14.1 (AR) | Normal neurodevelopmental, carpal tunnel syndrome, myelopathies, spinal cord compression | Skeletal–dysostosis multiplex and multiple joint contractures, Organomegaly, Obstructive Sleep Apnea, Cardiac valvulopathies and hypertrophic cardiomyopathy |
| MPS VII (Sly) | β-Glucuronidase | Dermatan sulfate, heparan sulfate, chondroitin 6-sulfate | GUSB 7q11.21 (AR) | Global developmental delay, carpal tunnel syndrome, myelopathies, spinal cord compression | Skeletal–dysostosis multiplex and multiple joint contractures, Organomegaly, Obstructive Sleep Apnea, Cardiac valvulopathies and hypertrophic cardiomyopathy |

GLYCOSPHINGOLIPIDOSIS

| Disease | Deficient Enzyme | Primary storage metabolite | Gene, locus (inheritance) | Neurological Symptomatology | Non-neurological Symptoms |
|---------|------------------|---------------------------|--------------------------|-----------------------------|---------------------------|
| Niemann-Pick (type A, type B) | Acid sphingomyelinase | Sphingomyelin | SMPD1 11p15.4 (AR) | Psychomotor development progresses no further than the 12-months level, after which neurologic deterioration is relentless | NP-A: hepatosplenomegaly with progressive hypersplenism and stable liver dysfunction, interstitial pulmonary disease, osteopenia, atherogenic lipid profile |
| Niemann-Pick C Disease | NPC1 and NPC2 | Unesterified cholesterol and several glycosphingolipids | NPC1 and NPC2 (AR) | Psychomotor development progresses no further than the 12-month level, after which neurologic deterioration is relentless | NP-A: hepatosplenomegaly with progressive hypersplenism and stable liver dysfunction, interstitial pulmonary disease, osteopenia, atherogenic lipid profile |
| Farber Disease | Acid ceramidase | Ceramide | ASAH1 8p22 (AR) | Severe progressive impairment of psychomotor development and neurologic deterioration with epilepsy | Progressively deformed joints, subcutaneous nodules, and progressive hoarseness (laryngeal involvement) |
| Gangliosidosis | Gm1-β-galactosidase | Gm1 ganglioside, Keratan sulfate, oligos, glycolipids | PSAP 10q22.1 (AR) | Type I: rapidly progressive with hypotonia, severe Type II: neurodegeneration, extrapyramidal signs, gait disturbance | Short stature, kyphosis, and scoliosis of varying severity Cardiomyopathy |
| Gangliosidosis | Gm1-β-Hexosaminidase A (Tay-Sachs) | Gm1 ganglioside, oligos, glycolipids | HEXA 15q23 (AR) | Progressive weakness, loss of motor skills, decreased attentiveness, and increased startle response at 3–6 months with seizures, blindness, spasticity. In late-onset forms: progressive dystonia, spinocerebellar degeneration, motor neuron disease, and, in some individuals with adult-onset disease, a bipolar form of psychosis | Hepatosplenomegaly, coarse facial features, cardiac involvement, cherry red spot and dysostosis multiplex |
| Gangliosidosis | Gm1-β-Hexosaminidase B (Sandhoff) | Gm1 ganglioside, oligos, glycolipids | HEXB 5q13 (AR) | | |

(Continued)
| Disease | Deficient Enzyme | Primary storage metabolite | Gene, locus (inheritance) | Neurological Symptomatology | Non-neurological Symptoms |
|---------|------------------|----------------------------|--------------------------|----------------------------|---------------------------|
| **LEUKODYSTROPHIES** | | | | | |
| Krabbe | β-Galactosylceramidase (GALC) | Galactosylceramide, galactosylsphingosine (psychosine) | GALC, 14q31.3 (AR) | Infantile onset: progressive leukodystrophy with a classical infantile-onset associate with severe neurologic impairment and deterioration and ultimately death by 2 years of age | Most symptoms are related to the neurological complications including dysphagia, recurrent pneumonias and multiple joint contractures |
| Metachromatic Leukodystrophy | Arylsulfatase A (ASA) | Sulfatides | ARSA, 22q13.33 | Late-infantile: motor weakness, hypotonia, clumsiness, frequent falls, toe walking and slurred speech generalized or partial seizures, hearing and visual loss and peripheral neuropathy | Gallbladder abnormalities (polyposis, wall thickening, cholelithiasis, sludge) |
| Multiple Sulfatase Deficiency | Multiple sulfatase | Sulfatides, glycolipids, GAGs | SUF1M1, 3p26.1 (AR) | Hypotonia, developmental regression and progressive neurodegeneration, nystagmus, dysmyelinating motor sensory neuropathy | Coarse facial features, visceromegaly, corneal clouding, upper airway obstruction, dysostosis multiplex |
| **OLYGOSACCHARIDOSES (GLYCOPROTEINOSES)** | | | | | |
| Aspartylglycosaminuria | Aspartylglucosamine | AGA, 4q34.3 (AR) | Speech delay, behavioral disturbances, extra-pyramidal signs with incoordination and ataxic gait, seizures | Growth spurt in infancy, gingival hypertrophy, angiookeratomas, recurrent respiratory infections are |
| Fucosidosis | α-Fucosidase | Glycoproteins, glycolipids, Fucoside-rich oligosaccharides | FUCA1, 1p36.11 (AR) | Seizures, cognitive impairment, seizures, spasticity and motor weakness | Coarse facial features, short stature, dysostosis multiplex, angiookeratoma corporis diffusum, hepatosplenomegaly, upper airway obstruction, recurrent pneumonias |
| α-Mannosidosis | α-Mannosidase | Mannose-rich oligosaccharides | MANSA, 19p13.2 (AR) | Incoordination, ataxic gait, metabolic myopathy, and incoordination. Spastic paraplegia spasticity, rigidity, and dyskinesia slight strabismus, hydrocephalus sensorineural deafness | Facial coarseness, lumbar gibbous, hepatomegaly, and dysostosis multiplex |
| Schindler disease | N-acetylgalactosaminidase | Sialylated asialoglycopeptides, glycolipids | NAGA, 22q13.2 (AR) | Neuroaxonal dystrophy, moderate psychomotor retardation, autistic features | Profuse angiokeratoma corporis diffusum, hepatomegaly and cardiomyopathy |

(Continued)
TABLE 1 | Continued

| Disease         | Deficient Enzyme | Primary storage metabolite | Gene, locus (inheritance) | Neurological Symptomatology                                                                 | Non-neurological Symptoms                                                  |
|-----------------|------------------|---------------------------|---------------------------|------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Sialidosis      | Neuraminidase    | Oligos, glycopeptides     | NEU1 6p21.33 (AR)         | Developmental delay, myoclonic epilepsy, visual impairment and ataxia, generalized tonic-clonic or myoclonic seizures, progressive visual impairment along with night blindness, nystagmus | hydroptic fetalis, retinal cherry-red spot, coarse facial features, hepatomegaly, dysostosis multiplex, corneal opacities |
| Mucolipidosis IIa/β, IIIα/β | GlcNAc-1-P transferase | Oligos, GAGs, lipids | GNPTAB 12q23.2 (AR) | Developmental delay in motor milestones with preservation of receptive and expressive speech, stuttering, seizures, motor weakness | dysostosis multiplex, corneal cloudiness, cardiac valvulopathies (aortic and mitral valve), obstructive sleep apnea |
| Mucolipidosis IV | Mucolipin        | Sulphatides, glycolipids, GAGs | TRIMPL1 19p13.2 (AR) | Neuropsychomotor delay and subsequent visual impairment, speech delay (receptive language is better than expressive language) progression to severe dysarthria or anarthria, slow chewing, slow eating and swallowing, and spastic diplegia or quadriplegia. Hyptonia, hyperreflexia and spasticity | corneal clouding, retinal degeneration |
| Wolman/CESD     | Acid lipase      | Cholesterol esters        | LIPA 10q23.31 (AR)        | Wolman disease form; Cognitive impairment hypotonia bilateral ptosis and external ophthalmoplegia | Hepatosplenomegaly Jaundice, steatosis, fibrosis, cirrhosis and/or liver failure) atherosclerosis (coronary artery disease, stroke) hypersplenism (i.e., anemia and/or thrombocytopenia) malabsorption |
| Galactosialidosis | Protective protein cathepsin A (PPCA) | Sialyloligosaccharides | CTSA 20q13.12 (AR) | Hypotonia, spasticity and seizures, cognitive decline | hydroptic fetalis, angokeratomas, hepatosplenomegaly, coarse facial features, cardiomyopathy, ‘cherry red spot’ and dysostosis multiplex |
| Danon Disease   | Lysosome-associated membrane protein 2 (LAMP-2) | cytoplasmatic debris and glycogen | LAMP2A Xq24 | Motor weakness and muscle atrophy are predominantly noted in proximal musculature as shoulder girdle muscles, however, some patients may present distal muscles. Elevated serum levels of creatine kinase (CK) are elevated as well as in 50–60% of female patients | Cardiomyopathy |

AR, autosomal recessive.
The unmet need for more efficacious drug delivery to the central nervous system (CNS) is currently the primary problem and often attribute to the properties of the BBB, considered the “problem behind the problem” when developing therapies for neurological conditions (Pardridge, 2005). The BBB is a term used to describe the unique properties of the microvasculature of the CNS, constituted by continuous non-fenestrated vessels, containing a series of additional “gatekeepers” tightly regulating the movement of molecules, ions, and cells between the blood and its neural cells (Figure 1) (Daneman and Prat, 2015). Neurodegenerative disorders such as Alzheimer’s, Parkinson’s, and multiple sclerosis, among others, constitute ~17% of total deaths globally (GBD 2016 Neurology Collaborators Group, 2017). The majority of the promising drugs with the potential to treat CNS disorders, including mostly small molecules, fall in the late pre-preclinical phase due to their failure to achieve therapeutic levels in the brain. In fact, among the current small molecule drugs approved, only 2% or less can produce significant trough levels in CNS and used therapeutically to treat different neurobehavioral manifestations. Those are mostly antipsychotics, mood stabilizers, anti-depressants, and antiepileptics (Ghose et al., 2012). Many orphan disorders also manifest with significant progressive neurodegeneration and have served as models for the development of novel strategies to address the BBB drug delivery challenge (Begley et al., 2008). The efficacy of therapeutic options depends on the bioactivity of the therapeutic agent, but equally important is its ability to reach critical targets within tissues and cells affected by the disease processes. Intravenous administration is the simplest and least invasive method for systemic delivery of drugs by infusing molecules directly into the circulatory system. The blood supplies all cells and tissues with vital elements and nutrients.
However, the delivery of macromolecules such as proteins from the circulation into adjacent cell layers is limited by a variety of biological barriers and selectivity of cell uptake mechanisms. The macromolecular transport across plasma and intracellular membranes are regulated by integral transmembrane channels or carriers. For any particular drug, the specific components required for uptake might be limited or lacking, especially in specialized tissues such as the endothelium of the CNS. Exogenous molecules interface with at least three distinct barriers that modulate transport into the brain: BBB, the blood-cerebrospinal fluid (CSF) barrier, and the arachnoid barrier (Begley et al., 2008; Abbott et al., 2010). The BBB is formed by vascular endothelial cells and other neural cells making a specialized network of capillaries with an extensive interface for blood-brain exchange with a total surface area of 12–18 m² in humans (Pardridge, 2001, 2007a; Begley et al., 2008). Molecules in the blood gain access to the brain by exploiting selective cell surface transporters or by free diffusion if the molecule is lipophilic and has a molecular weight <400 Da (Pardridge, 2006; Sweeney et al., 2018). In response to the challenges imposed by BBB to treat neurological manifestations which are prevalent in several LSDs, a number of strategies to deliver small molecules and biological agents to the CNS include neurosurgical-based interventions, chemical-based strategies (e.g., promoting lipid solubility of the molecules) (Corraliza-Gomez et al., 2019); and biology-based strategies (e.g., vesicular mechanism regulated by endogenous BBB transporters) (Sweeney et al., 2018).

**PERSPECTIVES ON THE CNS-TARGETING THERAPIES AND THEIR LIMITATIONS**

Unfortunately, for the vast majority of the neurological forms of LSDs, the core of patient management is based on multidisciplinary supportive care, which includes addressing the disease-related neurological complications with neurosurgical procedures. The classical example is the decompression of the cervical spine and the placement of ventricular-peritoneal shunt for hydrocephalus (Muenzer et al., 2009b). In addition, the use of anticonvulsants for seizures and assistance for patients with learning disabilities, orthopedic interventions to alleviate spinal deformities, joint limitations, and retractions; nutritional support; cardiac surgery when involvement of cardiac valves or coronaries are part of the complex care required in management of patients diagnosed with LSDs.

Over recent years, intensive and continual efforts have been made to develop therapies to tackle the neuro-pathogenesis of these inborn organelle disorders (Alderson et al., 2019). The approaches developed to treat LSDs are based on different strategies, each directed to manipulate a specific event in the downstream pathogenic cascades. The majority of these approaches target the deficiency of lysosomal enzyme/protein and/or the accumulated substrate. The former can be achieved by administering the recombinant human lysosomal enzyme, or by taking advantage of the ability of cells to uptake the agent through the mannose 6-phosphate receptor (Ghosh et al., 2003), delivering it to the lysosomal compartment. This approach is the enzyme replacement therapy (ERT) and is currently the core therapeutic modality used in the majority of LSDs, but limited to treat non-neurological, or somatic, manifestations of these conditions (Platt, 2018).

More recently, to address the poor biodistribution of the agent to certain organs, including CNS studies on modifying the ERT agent have emerged (Condori et al., 2016) and skeletal muscle (Koeberl et al., 2011; Peng et al., 2017; Han et al., 2019). To investigate the potential of large-molecular weight compounds to treat neurological disorders, novel approaches are required to surmount the BBB. Based on the ability of the adsorptive-mediated endocytosis of plant RTB-lectin, plant-based bioproduction of a fusion enzyme of ERT agent and lectin were accomplished, preserving the lysosomal enzymatic activity (Acosta and Cramer, 2020). Two murine LSD models, IDUA−/− (MPS I), GM1 gangliosidosidosis mice, treated with the ERT agent-RTB-lectin showed significant normalization of substrate levels in CNS, and correction of learning and memory deficits of the mouse model (Condori et al., 2016; Ou et al., 2018). Other studies have shown that the cellular uptake of α-iduronidase (IDUA), the enzyme deficient in MPS I, is enhanced by a fusion of IgG-IDUA fusion protein, where the IgG domain is a genetically engineered monoclonal antibody (Mab) against the human insulin receptor (HIR) (Boado et al., 2008, 2013). Based on these studies on murine IDUA−/− (MPS I), a phase 1/2 trial was performed in which patients affected by MPS I received the HIRMAb–IDUA fusion protein by intravenous infusion for 52-weeks DQ, and the cortical gray matter volume of the brain were stabilized by valanafusp alpha treatment. Somatic manifestations were stabilized, or improved, based on urinary glycosaminoglycan levels, hepatic and spleen volumes, and shoulder range of motion (Giugliani et al., 2018). The adverse events were dominated by transient hypoglycemia in 6.4% of subjects enrolled (Giugliani et al., 2018). Further studies will be necessary in earlier symptomatic or presymptomatic patients with the neuronopathic predictive genotype of MPS-I, as well as, studies in other neurological lysosomal storage disorders are needed. In preclinical studies, two fusion candidates, IDUAe1 and IDUAe2, were identified and shown to be desirable receptor-mediated binding, endocytosis, and transendothelial transport as well as appropriate lysosomal enzyme trafficking and biological function (Wang et al., 2013). The increased peripheral IDUAe1 or IDUAe2 through the hepatic expression resulted in BBB capillary-depleted endothelial cells and protein delivery into non-endothelium perivascular cells, neurons, and astrocytes in the murine MPS-I model, achieving levels of 2–3% of normal brain IDUA activities (Wang et al., 2013). Using the mucopolysaccharidosis type IIIA (MPS-III) murine model, a neurological MPS caused by the N-sulfoglucosamine sulfohydrolase deficiency (Table 1), studies on a chimeric sulphamidase, containing a signal peptide from Apolipoprotein B (ApoB-BD), showed a highly secreted iduronate-2-sulphatase (IDS) and efficient BBB transcytosis and restoration of sulphamidase activity in the brain of treated mice (Sorrentino et al., 2013).

Alternatively, the recombinant enzyme can be secreted by donor stem-cell-derived cells or genetically manipulated cells from the patient. This strategy is the hematopoietic stem-cell
In a recent study, a brain-targeted hematopoietic stem cell gene therapy approach using lentiviral IDS fused to ApoEII (IDS.ApoEII) compared to a lentivirus expressing wild-type (normal) IDS to standard bone marrow transplant. In mucopolysaccharidosis II mice, only IDS.ApoEII mediated complete normalization of brain pathology and behavior, providing significantly enhanced correction compared to IDS. Gleitz et al. (2018) In addition, the corrected macrophages traffic to the brain, secreting IDS/IDS.ApoEII enzyme for cross-correction. The IDS.ApoEII enzyme showed to be more active in plasma and was taken up by endothelial cells, and transcytosed across the BBB at a significantly higher rate than the native IDS via both heparan sulfate/ApoE-dependent receptors and mannose-6-phosphate receptors (Gleitz et al., 2018). Recently, lentiviral vector-mediated expression of the chimeric GALC enzymes was shown to be safe and leads to supranormal enzymatic activity in both neural and hematopoietic cells. The IDSpGALC showed enhanced expression and secretion in comparison to the unmodified GALC. The chimeric GALC enzymes produced by LV-transduced cells reduce intracellular galactosylceramide (GalCer) storage and effectively correct GLD murine neurons and glial cells. These findings suggest that ERT of chimeric GALC can be used by the affected CNS cells and tissues, supporting the development of novel and more effective GT approaches for GLD (Ricca et al., 2020).

The gene encoding the deficient lysosomal enzyme can be delivered to the cells through the use of AAV systems. Subsequently, patients cells can synthesize the normal lysosomal enzymatic cells (Biffi, 2017a,b). This approach is known as ex-vivo gene therapy. Another approach is to restore the equilibrium between the synthesis of the accumulated, or “stored,” substrates, and their limited degradation due to the lysosomal enzymatic deficiency (Platt and Jeyakumar, 2008; Marshall et al., 2016). This strategy is called substrate reduction therapy (SRT), which is achieved by modulating the primary substrate biosynthesis with small molecule inhibitors of critical limiting steps. The “reduction of the accumulated substrate” can also be achieved by promoting the clearance of primary and secondary accumulated products from the affected cells (Gatto et al., 2017; Torra et al., 2018).

In this review, we focus on current treatment strategies for LSDs targeting the CNS, including those that hold promise for future advancements in the treatment of the progressive neurological manifestations of these devastating disorders.

**THERAPEUTIC STRATEGIES POTENTIALLY TARGETING CNS MANIFESTATIONS OF LSDs**

**Small Molecule Therapies**

Small molecules are most frequently used experimentally to modulate the function of a protein target and assess the consequences. The small molecules are low-molecular-weight (<900 Da) synthetic organic compounds that have antagonistic or agonistic effects on intra and extracellular targets. By being small molecules, these therapeutic agents are more likely to cross the BBB and achieve the CNS, and ultimately the neural cells with endolysosomal network dysfunction due to the lysosomal enzyme/protein deficiency. Examples of small molecules that have been used to treat LSDs are pharmacological chaperones (PCs), proteostasis regulators, substrate reduction therapy (SRT) agents, and drugs that increase gene expression by suppressing translation termination, also known as premature termination codon (PTC) suppressing agents. Here, we describe the current classes of small molecules that have shown evidence of BBB penetration and potentially treating some neurological manifestations of some LSDs (Table 2).

**Pharmacological Chaperones (PC)**

A number of missense pathogenic gene variants encode mutant misfolded proteins, resulting in interactions with elements of the endoplasmic reticulum (ER)-associated degradation (ERAD) pathway (Bhattacharya and Qi, 2019), and subsequent premature degradation by the endoplasmic reticulum quality control (ERQC) machinery (Maegawa et al., 2007; Balch et al., 2008; Mu et al., 2008b). Pharmacological chaperones (PCs) interact earlier with the target misfolded mutant protein in the endoplasmic reticulum, in the case of lysosomal enzymes, assisting its folding, attenuating its high-free energy ($\Delta G_{\text{f}}$), toward a lower $\Delta G_{0}$ state (Powers and Balch, 2013), passing the ERQC, and evading the ERAD (Wiseman et al., 2007). Subsequently, the properly folded mutant protein reaches the cis-Golgi network and, where specific biochemical modifications allow the interactions with mannose-6-phosphate receptors and, eventually, landing in the lysosomal compartment (Sawkar et al., 2006). Once at the lysosomes, due to its acidic environment (pH 4–5) and abundance of the degraded and accumulated substrate, optimal PCs lose their affinity to the target mutant, leaving their “site-of-binding,” often the active-site, free for interactions with the natural enzymatic substrate (Tropak and Mahuran, 2007). Several compounds have been tested as potential PCs for the treatment of neurological symptoms of lysosomal disorders. These compounds include ambroxol (Maegawa et al., 2009b; Klionsky et al., 2016), and most recently progranulin (Jian et al., 2016) and NCGC607 for the treatment of Gaucher disease (Aflaki et al., 2016); pyrimethamine for late-onset Tay-Sachs disease (Maegawa et al., 2007; Clarke et al., 2011; Oscher et al., 2011); N-octyl-epi-β-valienamine (Aflaki et al., 2016) and, recently, derivatives of 4-epi isoagomine for β-galactosidase deficiency, betaine for aspartylglucosaminuria (Lebl et al., 2017) and the current PC molecule approved for Fabry disease, migalastat (Germain et al., 2016; Hughes et al., 2017). Protein homeostasis, or proteostasis, consists of a network of interacting activities, or “interactome,” in charge of maintaining the “health” of the proteome and the organism (Balch et al., 2008; Song et al., 2013; Wang and Segatori, 2013). This network comprises several ER-resident chaperones and degradation elements as well as stress-responsive signaling pathways that detect the misfolding and/or aggregation of proteins in specific subcellular compartments using stress sensors, which respond by generating an active transcription factor (Roth and Balch, 2011; Klionsky et al., 2016; Kelly, 2020).
### TABLE 2 | Overview of approved, orphan drug designations, off-labeled therapies for treating lysosomal storage disorders, and examples of some products under development with an orphan drug designation.

| LSDs          | Therapeutic agent                  | Type/Current status                        | Evidence of CNS penetration |
|---------------|------------------------------------|--------------------------------------------|-----------------------------|
| Gaucher disease (GD) | Imiglucerase (Cerezyme) | ERT-IV (produced in CHO cells)/approved* | None                        |
|               | Velaglucerase (VPRIV)            | ERT-IV (produced in human cells)/approved* | None                        |
|               | Taliglucerase (Elelyso)          | ERT-IV (produced in plant cells)/approved** | None                        |
|               | Miglustat (Zavesca)             | SRT/approved*                              | None                        |
|               | Eliglustat (Cordelga)            | SRT/approved                               | None                        |
|               | Ambroxol                         | PC/off-labeled use neuronopathic forms of GD | Myoclonic epilepsy and cognition improvement; decreased CSF glucoSPG (Narita et al., 2016; Pawlinski et al., 2016; Charkhand et al., 2019; Kim et al., 2020) |
| Fabry Disease | Agalsidase beta (Fabrazyme)      | ERT-IV (produced in CHO cells)/approved*  | None                        |
|               | Agalsidase alfa (Replagal)       | ERT-IV (produced in human cells)/approved** | None                        |
| MPS-I         | Migalastat (Galafold)            | PC/approved*                               | None                        |
|               | Laronidase (Aidurazyme)          | ERT-IV (produced in CHO cells)/approved    | None                        |
|               | HSCT                               | Cell therapy/approved                      | Efficacious on neuronopathic MPS-I (Prasad and Kurtzberg, 2010a,b; De Ru et al., 2011) |
| Fusion-ERT    |                                     | HIRMAb-IDUA-IV#/ODD                       | Preliminary evidence in small and short clinical studies (Giugliari et al., 2019)/ODD |
| MPS-II        | Idursulfase (Elaprase)            | ERT-IV (produced in human cells)/approved* | None                        |
|               | Idursulfase (Elaprase)            | ERT-IT (produced in human cells)/ODD       | Mild improvement ODD (Muenzer et al., 2016) |
|               | Idursulfase (Elaprase)            | ERT/ODD                                   | None                        |
| MPS-IIIA      | Sulfamidase                       | ERT/ODD                                   | Decline in HS in CSF/ no change in neuro endpoints/ODD |
|               | LYS-SAF302 (Lysogene/Sarepta Therap.) | IV-GT (systemic)                          | No results from clinical trials NCT03812869 |
|               | scAAV9.U1a.hSGSH (Abeona Therap) | IV-GT (systemic)                          | No results from clinical trials NCT02716246 |
|               | Ex vivo HSCT                      | CD34+ -Lenti-transduced(SGSH)              | No results from clinical trials NCT02041405 |
| MPS-IIIB      | SBC-103(rhNAGLU) (Alexion Pharm.) | ERT-IV                                    | Negative results (Whitley et al., 2019) |
|               | NAGLU-IGF2 fusion protein         | NAAGLU-IGF2 fusion protein -IV#/ODD        | No results from clinical trials NCT03315182 |
|               | rAAV9.CMV.hNAGLU                  | IV-GT (systemic)                          | No results from clinical trials NCT0330453 |
|               | rAAV2/5-hNAGLU (JumQure Biopharma B.V.) | IT-GT (local)                          | No results from clinical trials NCT0330453 |
|               | Elosulfase (Vimizim)              | ERT-IV (produced in CHO cells)/approved*  | None                        |
|               | Galsulfase (Naglazyme)            | ERT-IV (produced in CHO cells)/approved*  | None                        |
|               | Vestronidase alfa (Mepsevi)       | ERT-IV (produced in CHO cells)/approved*  | None                        |
| Pompe Disease | Alglucosidase alfa (Lumizyme)     | ERT-IV (produced in CHO cells)/approved*  | None                        |
| GSD-II        | Alglucosidase alfa (Myozyme)      | ERT-IV (produced in CHO cells)/approved** | None                        |

(Continued)
TABLE 2 | Continued

| LSDs                                                                 | Therapeutic agent                                      | Type/Current status                                      | Evidence of CNS penetration                  |
|----------------------------------------------------------------------|--------------------------------------------------------|----------------------------------------------------------|--------------------------------------------|
| Metachromatic Leukodystrophy                                         | avalglucosidase alfa, (neo-GAA; ATB200, Amicus)+AT2221 (miglustat) | ERT-IV (rhGAA-ATB200)/PC (AT2221)/ODD                  | None (Xu et al., 2019)                      |
| Globoid-cell leukodystrophy (GLD), Krabbe disease                     | Ex vivo HSCT                                            | GT-Lenti-transduced (ARSA)/ODD                          | Stabilization of neurocognition and white-matter signal in brain MRI studies (Sessa et al., 2016) |
| Lysosomal acid lipase deficiency (Wolman disease/cholesterol ester storage disease) | HSCT                                                   | Cell therapy/approved                                   | HSCAT at < 30 days of age, improvements in mobility, speech, oropharyngeal function (Altevweit et al., 2018) |
| Neuronal ceroid lipofuscinosis type 2                                | Sebelipase (Karuma)                                     | ERT-IV (produced in egg white-genetically modified chicken)/Approved* | None                                      |
| Niemann–Pick disease type B (Acid sphingomyelinase deficiency)       | Cerliponase (Brineura)                                  | ERT-IT (produced in recombinant CHO cells)/Approved*   | Reduce progression of neuro-cognitive decline (Markham, 2017; Schulz et al., 2018) |
| Niemann–Pick disease type C                                           | miglustat (Zavesca)                                    | SRT (oral)                                              | HSEM velocity; improvement in swallowing capacity, auditory acuity, and a slower deterioration |
| Niemann–Pick disease type C                                           | Vorinostat                                              | HDAC inhibitor/off-labeled use neuronopathic forms of GQ | None                                      |
| Niemann–Pick disease type C                                           | 2-Hydroxypropyl-β-Cyclodextrin (VTS-270)                | IT-VTS-270                                              | Stable but slower-than-average Cognitive Scales (Farmer et al., 2019) |
| α-Mannosidosis                                                        | velmanase alfa/(Lamzede)                                | ERT-IV (produced in CHO cells/approved)                 | None (Borgwardt et al., 2018)               |

*Approved in Europe, USA, and other countries; †Approved in USA, Brazil and Canada; ‡Approved in Europe. CHO, Chinese Hamster Ovary; CHO cells; enzyme replacement therapy; ERT, enzyme replacement therapy; glucosylceramide; GSD, glycogen storage disease; GT, gene therapy; HIRMAb, human insulin receptor monoclonal antibody; HSEM, horizontal saccadic eye movement; HSCs, hematopoietic stem cells; HSCT, hematopoietic stem cell therapy; HSP, heat shock protein; IV, intravenous (systemic); IT, intra-thecal (local); MPS, mucopolysaccharidosis; NAGLU, alpha-N-acetylglucosaminidase; ODD, orphan drug designation; SRT, substrate reduction therapy; PC, pharmacological chaperones.

Proteostasis Regulators

Proteostasis regulators are defined as small molecules able to increase the residual function of lysosomal mutant enzymes by manipulating components of several folding and protein degradation signaling pathways (Kelly, 2020). By affecting and interacting with components of the interactome network, the proteostasis regulators have a broader and more pervasive outcome effect on multiple misfolded mutant proteins, which implies in a broader clinical application for several “misfolding protein disorders.” (Mu et al., 2008a; Kelly, 2020). One of the examples of proteostasis regulators is bortezomib, an approved-proteasome inhibitor approved for myeloma, which showed to decrease the levels of intracellular accumulated cholesterol in cultured cells with NPC1 deficiency from individuals with the Niemann-Pick disease type C (Macias-Vidal et al., 2014).

In addition, the same proteostasis regulator showed to restore glucocerebrosidase (GCase) activity from Gaucher disease by inhibiting histone deacetylases and preventing mutant GCase degradation (Fan et al., 2011). Other histone deacetylase inhibitors, vorinostat, and panobinostat showed to decrease the cholesterol typically accumulated in human Niemann-Pick C1 fibroblasts (Pipalia et al., 2017). Also, the manipulation of ER-calcium concentrations in the ER has been investigated as a potential therapeutic approach with promising results in Gaucher disease, Niemann-Pick disease type C, and mucolipidosis type IV (Matellonga et al., 2017). However, the long-term effects of the regulation of the proteostasis cannot be predicted. The link between autophagy impairment and lysosomal disorders has led to novel approaches to modulate cellular clearance, which has been investigated in preclinical studies (Palmieri et al., 2017), but the translation to clinical trials is still premature.

Substrate Reduction Therapy

The substrate reduction therapy (SRT) agent consists of a small molecule that partially inhibits the biosynthesis of the natural substrate accumulated secondary to the enzymatic deficiency. It is an attractive approach to mitigate the load of the primary and eventually secondary substrate of the deficient lysosomal enzyme (Platt et al., 1994a,b). The first substrate reduction therapy agent developed for a LSD was miglustat, an imino-sugar deoxynojirimycin, and its alkylated...
derivatives, known inhibitors of the N-linked oligosaccharide processing enzymes (Platt et al., 1994b). In preclinical studies, the imino sugar N-butyldeoxynojirimycin showed also to interact with glucosyltransferases, especially inhibiting ceramide-specific glucosyltransferase that catalyzes the first step in glycosphingolipid biosynthesis (Platt et al., 1997). Miglustat was later approved for the treatment of Gaucher disease type I (Cox et al., 2000), and Niemann-Pick C disease (Patterson et al., 2007). Despite the reduction of the increasing levels of the glycosphingolipids in the CNS of murine models of gangliosidoses, in early human clinical trials in GM2 and GM1 gangliosidoses, the small molecule failed to achieve sufficient CNS levels to result in measurable clinical benefits (Maegawa et al., 2009a,c; Shapiro et al., 2009).

Studies using miglustat in combination with ERT for neuropathic Gaucher disease (types II and III) showed no significant alterations of clinical endpoints (Schiffmann et al., 2008). Despite the anecdotal reports on neurological improvement in some cases (Capablo et al., 2007), no SRT agents, a standard of care has been used with ERT. In this setting, and given the significant side effects of miglustat (Pastores et al., 2007; Giraldo et al., 2009), another ceramide analog, eliglustat, was developed by Dr. Shayman’s group (Mceachern et al., 2007; Shayman, 2010, 2013), and later approved for the treatment of Gaucher disease type I (Cox et al., 2015; Mistry et al., 2015). However, eliglustat fails to permeate the BBB, limiting the therapeutic use to non-neuropathic symptoms of Gaucher disease. Small molecules that function as cholesterol-lowering agents have been identified for Niemann-Pick disease type C disease (Camargo et al., 2001; Davidson et al., 2009).

The intrathecal 2-hydroxypropyl-β-cyclodextrin has been shown to have clinical benefits or stabilization of disease progression in most of the 14 patients reported (Ory et al., 2017; Farmer et al., 2019). Further clinical trials with a larger number of patients with NPC studies are either being analyzed (NCT01747135) or ongoing (NCT03887533, NCT03893071) using the intrathecal and intravenous route, respectively. In terms of the mucopolysaccharidoses (MPS) I, II, and III (all subtypes), and their neurological manifestations, the genistein, an isoflavone, and the protein tyrosine kinase and epidermal growth factor inhibitor resulted in the reduction of glycosaminoglycan (GAG) accumulation (Malinowska et al., 2009). The early phase clinical studies showed a reduction of urinary excretion of GAGs and plasma heparan sulfate concentration in patients with MPS III (De Ruijter et al., 2012). However, the absolute reduction of these metabolites was small after a 12-months treatment period coinciding within the range as observed in untreated patients. Also, genistein showed no efficacy in the clinical endpoints when examining in 30 affected MPS-III enrolled in the study (De Ruijter et al., 2012). Evidence of another potential mechanism of action of the 2-hydroxypropyl-β-cyclodextrin has been described against cytotoxic psychosine, which is a sphingolipid found at high and cytotoxic levels in Krabbe disease due to the GALC deficiency in the CNS (Katabuchi et al., 2018).

Small Molecules Targeting Nonsense Mutations in LSDs

By definition, nonsense pathogenic genetic variants result in the premature termination codon (PTC) and subsequent translation of a truncated protein that is typically degraded by nonsense-mediated decay. An extensive meta-analysis on the Human Gene Mutation Database has revealed that approximately 11% of all described pathogenic variants resulting in human inherited disorders are caused by nonsense mutations (Mort et al., 2008; Bidou et al., 2012). Nonsense pathogenic variants are identified in several LSDs that may potentially benefit from small molecules that function as PTC suppression-stimulating agents by promoting “read-through” the premature termination or stop codon. In LSDs, chloramphenicol showed to increase the IDUA gene expression and, subsequent, α-L iduronidase (IDUA) activity in cell lines derived from patients with MPS-I (Mayer et al., 2013). Also, B84 suppressed the IDUA-W392X nonsense mutation much more efficiently than any of the other compounds tested. NB84 treatment restored enough functional α-L-iduronidase activity to partially reverse abnormal GAG accumulation and lysosomal abundance in mouse embryonic fibroblasts derived from the Idua-W392X mouse (Wang et al., 2012). In cultured oligodendrocytes from Twitcher mice (galc<sup>twi/twi</sup>), naturally occurring murine model for Krabbe disease, and patient fibroblasts with nonsense mutations in GALC gene, the nonsense-mediated mRNA decay (NMD) inhibitor 1 (NMDI1) (Durand et al., 2007) increased the levels of mRNA and rescued galactocerebrosidase (GALC) activity in a dose-dependent manner, and improve the morphology of the differentiated oligodendrocytes (Luddi et al., 2016). In infantile neuronal ceroid lipofuscinoses, using the novel Cln1(R151X) mouse model, mice receiving PTC124 (ataluren) showed enhancements of the palmitoyl-protein thioesterase 1 activity were noted, but limited to the liver and muscle tissues, alluding the challenges of biodistribution of this specific class of small molecules (Thada et al., 2016).

Cell Therapy

The basis and rationale of using hematopoietic stem cell transplantation (HSCT) to treat LSDs originated from elegant and pioneering experiments of Neufeld and cols. who demonstrated in cultured cells of patients with different mucopolysaccharidoses, cross-correction with reductions of the accumulated GAGs were observed (Fratantoni et al., 1968; Neufeld, 1989). The HSCT potential resides in the permanent biological source (donor cells) of steady functional lysosomal enzymes secretion and their internalization through the mannose-6 phosphate receptor system by surrounding “recipient” affected cells (Platt et al., 2018). The mannose 6-phosphate receptor allows the exogenous lysosomal enzyme to directly targeting the dysfunctional and enlarged lysosomes. The hematopoietic stem cells (HSCs) can also cross the BBB and differentiate into microglial cells, and populate the CNS, becoming active sources of lysosomal enzymes (Tan et al., 2019). Numerous factors may impact the efficacy of the HSCT in LSDs and other inherited metabolic disorders. These factors can be clinical and HSCT-related, including drug regimens.
of conditioning pre-HSCT, presence and degree of graft-vs.-host disease, time of neutropenic recovery, and, ultimately, clinical status and, especially, the disease stage of the affected patient undergoing the procedure (Biffi, 2017b). From a biological standpoint, several factors can affect the level of “cross-correction” of the deficient lysosomal enzyme, including the number of donor HSCs administered, level of differentiation once in CNS, the degree of secretion of the lysosomal enzyme by the donor HSCs, and also the properties of the lysosomal enzyme to be uptaken by the recipient neural cells. The sum of these factors will determine the outcome, which is the arrest and/or improvement of neurological manifestations of LSDs. In addition, in the setting of a generalized lysosomal dysfunction, the extracellular microenvironment is altered by the presence of a number of cytokines and inflammatory response cells, which also contribute substantially to the pathogenesis of the neurodegeneration observed in LSDs (Walkley, 2009; Platt et al., 2018). If successful, with full engraftment, the HSCT can single-intervention, offering a lifelong source of secretion of “deficient” enzyme for the recipient patient. However, the physiological demands of lysosomal levels may change during the natural history of the LSDs. The donor HSCs are allogeneic donors either from family-related members, usually siblings. Given the setting of early diagnosis and also with the newborn screen for MPS-I and Krabbe disease, the Umbilical cord blood (UCB) as an alternative stem cell source has been used in HSCT (Lund, 2018). The UCB is a safe and effective source of HSCs.

In terms of limitations, only in MPS-I (iduronate deficiency), the HSCT procedure has shown to be efficacious to prevent and attenuate the neurological manifestations of the neuronopathic forms (Ballen et al., 2013). The numbers of umbilical HSCs are usually lower than those from bone marrow sources. In pediatric patients, the UCB source can provide desired doses of HSCs number/kilogram, whereas, adults undergoing HCT may suffer from inadequate cell numbers (Ballen et al., 2013). As in an adult setting, the UBC as a source of HSCs becomes limited, several approaches have been proposed, including combined infusion of 2 UCB units (Barker et al., 2001), increasing the homing efficiency of UCB cells (Cutler et al., 2013), and expanding the UCB in vitro (Klionsky et al., 2016). Recently, small molecule, carlecortemcel-L, a copper chelator known for its ability to expand UCB HSCs in vitro (Peled et al., 2004), allowed a selected CD133+ cell expansion post-selection from UCB units, followed by a 21-days expansion with cytokines and carlecortemcel-L in vitro (Stiff et al., 2018).

In sum, the significant advantages of the HSCT are dependent upon a number of factors, and a higher concentration of enzymes delivered to the CNS will result in better clinical outcomes. Therefore, it is essential that HSCs source, either the UCT- or BM-derived, to be screened for the carrier status of the specific lysosomal enzyme deficiency diagnosed in the recipient patient.

Since 1980, several patients with LSDs have been treated and a wide spectrum of outcomes reported. At present, HSCT is highly recommended for patients with MPS-I if they are younger than 24 months, as per the European Union (Clarke et al., 2017), and 36-months, as per US guidelines (De Ru et al., 2011). The MPS-I is the only LSD in which full prevention of the neuropathogenic processes is observed if the HSCT is done before the neurocognitive impairment arises (Aldenhoven et al., 2015). In addition, HSCT has been beneficial for patients with metachromatic leukodystrophy (MLD) and Krabbe disease if the transplantation is performed at the presymptomatic stage (Escolar et al., 2005; Mcgraw et al., 2005; Cartier and Aubourg, 2008; Duffner et al., 2009; Krageloh-Mann et al., 2013; Tejera et al., 2013; Groeschel et al., 2016; Van Rappard et al., 2016; Allewelt et al., 2018; Kwon et al., 2018). However, cognitive decline and neurodegenerative disease are observed in a number with early-onset Tay-Sachs disease and Sandhoff disease (Boelens et al., 2014), MPS-III and MPS-II (McKinnis et al., 1996; Guffon et al., 2009; Muenzer et al., 2009a; Scarpa et al., 2011), who underwent HSCT. Recently, in preclinical studies, encouraging results of human neural cells in several LSD murine models, including Niemann-Pick A disease (spingomyelinase deficiency) (Shihabuddin et al., 2004), infantile neuronal ceroid lipofuscinosis (Tamaki et al., 2009) and MLD (Biffi et al., 2004; Givogri et al., 2006). The substantial attenuation of the neuropathology in the CNS of murine models should be reproduced in a larger brain before moving this therapeutic strategy into the clinic. In addition, a well-characterized and non-tumorigenic human-derived source of stem cells will be needed.

In general, several factors, including age at diagnosis, and HSCT, the clinical status, levels of chimerism, and specific genotype can also be determinants of the final clinical outcome for each patient undergoing this therapeutic modality (Biffi, 2017b). However, mortality has been reduced to approximately 10%, with variable rates for different lysosomal disorders (Prasad and Kurtzberg, 2008; Biffi, 2017b).

**CSF-Delivery of ERT Agents**

Currently, ERT is considered an effective treatment for non-neurological manifestations of LSDs. It is conceivable that, to circumvent the BBB, ERT agents directly delivered into the cerebrospinal fluid (CSF) can achieve the neural cells. Several routes can be used, including intracerebroventricular (ICV) injection into the lateral ventricle (through a catheter/reservoir) or intrathecal (IT) injection into the lumbar spine or subarachnoid space at the cisterna magna lumbar puncture or an IT drug-delivery device. The first ERT agents to be CSF injected were laronidase (Aldurazyme) in a patient with MPS-I- with cervical spinal cord compression (SCC), and unable to undergo necessary neurosurgery procedures (Munoz-Rojas et al., 2008). Another patient received the galsulfase ERT agent for MPS-VI (Bernal-Bayard et al., 2010). The IT-administered ERT was well-tolerated and safe, and some improvement in symptoms of SCC was observed (Klionsky et al., 2016). Later on, three-phase 1/2 studies investigating the safety (as primary outcome) and efficacy of CSF-delivery through IT injections for MPS-I, MPS-II, and MPS-III were performed (Klionsky et al., 2016; Muenzer et al., 2016; Nestrail et al., 2017). One patient with attenuated MPS-I patient after receiving IT ERT showed signs of neurological benefits, improving brain structure, and reversing cognitive decline (Nestrail et al., 2017). In addition, IT ERT was also assessed in conjunction with HSCT. Twenty-four patients received over 1–2 min in 4 mL of Elliott’s B® solution.
containing recombinant ERT agent iduronidase (0.05 mg/kg) at four discrete time points: 8–12 weeks before HSCT, 2 weeks before HSCT, 100 days after HSCT, and 6 months after HSCT (Eisengart et al., 2019). All of the patients who demonstrated increases in IQ 2 years after treatment had at least a 50% reduction in CSF glycosaminoglycan non-reducing ends across the four-timepoints (Eisengart et al., 2019). The most notable limitation of this study is a lack of a comparison CSF from a group that received only intravenous ERT and HCT. In 2017, cerliponase alfa (Brineura®) was approved-based on the clinical trials showing to slow loss of ability to walk or crawl (ambulation) in symptomatic pediatric patients 3 years of age and older with late infantile neuronal ceroid lipofuscinosis type 2 (CLN2), also known as tripeptidyl peptidase 1 (TPP1) deficiency. In a clinical study of 24 patients with CLN2 receiving an intraventricular infusion of cerliponase alfa, the slope of decline in motor and language function was less than the one observed in the historical controls. Serious adverse events included the failure of the intraventricular device and device-related infections (Schulz et al., 2018).

In terms of immunogenicity, in the majority of patients who had intravenous ERT at least 6 months preceding the IT administration, no immune response or antibody was present in the CSF (Klionsky et al., 2016)]. The patients who developed antibodies against the ERT agents in the CSF did before the IT-ERT administration, and no safety issues were a concern. It is important to point out that significant ERT agent leakage occurs to the systemic circulation during IT-ERT administrations (Chung et al., 2017). For this reason, CSF-administered ERT agents can eventually result in systemic effects in non-CNS areas of patients receiving only this route as ERT administration.

Gene-Therapy
The LSDs, as single-gene disorders with well-characterized biochemical and clinical phenotypes, are ideal candidates for gene therapy approaches. Also, the critical threshold of 10–15% of residual enzymatic activity for most lysosomal enzymes, below which no disease is manifested (Conzelmann and Sandhoff, 1983; Sandhoff and Conzelmann, 1984), has shown that only a small percentage of correction is required to reinstate the physiological turnover of the primary substrate and shift the cell homeostasis (Conzelmann and Sandhoff, 1991; Schueler et al., 2004). The gene therapy approach can be accomplished in two general ways, in vivo and ex vivo, which are both discussed below.

In vivo Gene Therapy
The gene therapy (GT) approach has made significant advances in several animal models of LSDs (Platt, 2018). Based on the encouraging pre-clinical studies in animal models, early phase clinical trials have started and shown positive results. A number of approaches have been developed to administer GT agents to target the CNS. Most of them are based on the direct administration of recombinant adeno-associated vectors (rAAVs) administered systemically (mostly intravenously) or locally injected (CNS). The rAAVs are derived from small, non-enveloped, and non-integrating AAVs. The transduction efficiency and safety characteristics make the rAAVs the ideal candidate for gene delivery (Bey et al., 2017). The rAAV serotypes traditionally used in gene-transferring into CNS are the 1, 2, 5, 8, 9, and the recombinant human (rh)10. Usually, rAAV-mediated gene transfer in the brain efficiently targets neurons and scarcely astrocytes, oligodendrocytes, and microglia (Hocquemiller et al., 2016). Specific rAAV serotypes, including AAV9 and 10, can cross the BBB, allowing transduction of the CNS after systemic administration (Foust et al., 2009; Hocquemiller et al., 2016). As issues raised on the CSF-injected ERT, because of leakage, local rAAV administration into the CNS results in transduction of non-target tissues and peripheral transgene expression, particularly in the liver (Hocquemiller et al., 2016). The leakage may trigger immune responses and can mitigate the efficacy of the in vivo GT. Transient immunosuppression as induction of tolerance has been developed (Colella et al., 2018; Keeler et al., 2019). In particular, relevant results have been obtained with strategies inducing liver-mediated tolerance or neonatal AAV-mediated systemic expression of a therapeutic protein before CNS-directed in vivo GT (Hinderer et al., 2015). Based on this and other preclinical studies (Fraldi et al., 2007), a phase I/II clinical trial was initiated in patients affected by MPS type IIIA. In this study, four children aged between 2 years 8 months and 6 years received intracerebral injections of an adeno-associated virus (AAV) vector carrying the complementary DNA encoding sulfamidase and the gene for the sulfatase-modifying factor SUMF1 (Tardieu et al., 2014). The therapeutic vector was administered to the brain by stereotoxic surgery. The immunosuppressive regimen given 2 weeks before the procedure was generally well-tolerated. In two patients, the brain magnetic resonance imaging (MRI) showed brain size and neuropsychological tests were performed. The brain atrophy seemed to be stable, but tended to increase in the other two individuals. In three other patients, moderate improvements in neurocognitive areas, including behavior, attention, and sleep, were noted in neuropsychological evaluations (Tardieu et al., 2014; Marco et al., 2019). Intracerebral injections of adeno-associated viruses carrying a therapeutic gene have also been performed or are under evaluation in other LSDs and neurodegenerative diseases (Hocquemiller et al., 2016).

Ex vivo Therapy
As we described earlier, the BBB prevents circulating molecules, either small or macromolecules, from entering the CNS (Pardridge, 2007b). Hematopoietic cells permeate the BBB, where they subsequently differentiate into microglia. The HSC in neurological LSDs may be beneficial but is associated with significant risks of GVHD host and other complications that range from 8 to 10%, even in the most advanced centers (Graf, 2017; Tan et al., 2019). In this setting, to circumvent the risks associated with the allogenic HSCT, autologous HSCs becomes attractive as it opens the opportunity to genetically modify the patient’s HSCs to express the deficient lysosomal protein. The lentiviral vectors (LVs) showed strong safety and the most efficient tool to deliver genes to HSCs (Magrin et al., 2019). The lentiviral genome is stably integrated into the host cell genome after transduction, allowing a long-term and stable transgene expression over time and in the differentiated progeny of the
original transduced HSPCs (Naldini, 2019). In general, to inject the lenti-transduced HSCs, a partial or full myeloablative bone marrow will be required to allow “space” and promote the novel HSCs engraftment (Biffi, 2017b; Naldini, 2019). One successful ex vivo gene therapy targeting CNS has been reported in MLD, an LSD caused by arylsulfatase A (ASA) deficiency and manifested by a severely progressive demyelination of CNS and peripheral nervous system. Based on previous studies (Lattanzi et al., 2010; Meneghini et al., 2016), nine children asymptptomatically diagnosed with MLD underwent haemopoietic stem-cell gene therapy (HSC-GT) in a non-randomized, open-label, single-arm phase 1/2 trial with 3-years follow-up (Sessa et al., 2016). Initial data of some of the first subjects enrolled were published earlier (Biffi et al., 2013). After reinfusion of the genetically modified cells, high enzyme activity was found throughout haemato poetic lineages and in the cerebrospinal fluid. Eight patients, seven of whom received treatment when presymptomatic, had prevention of disease onset or halted disease progression as per clinical and instrumental assessment, compared with historically untreated control patients with early-onset disease. Gross Motor Function Measures scores for six patients up to the last follow-up showed that gross motor performance was similar to that of normally developing children. The extent of benefit appeared to be influenced by the interval between HSC-GT and the expected time of disease onset (Sessa et al., 2016). The ad-hoc results showed evidence of the safety and therapeutic benefit of ex vivo gene therapy for a neurological LSD when administered at presymptomatic or very early-symptomatic disease stages. Despite these promising results, the long-term safety and efficacy of the approach are needed to assure options for patients affected MLD and other LSDs (Sessa et al., 2016).

Nano-Vesicle-Based Delivery of Therapies for LSDs

Since the BBB forbids the translocation of large proteins into the CNS, only certain small low-molecular-weight or lipophilic molecules can pass through this natural barrier. Several types of nanovesicle-based delivery technologies have been designed to transport therapeutic agents to CNS to allow BBB delivery and membrane permeability. Amongst these approaches, polymeric nanoparticles (NPs), lipid NPs, liposomes, as well as extracellular vesicles (EVs), such as exosomes, produced by cells can be used to transport bioactive enzymes and other types of therapeutic molecules to CNS (Figure 2, Table 3). The specific characteristics that allow delivery through BBB are the size of vesicles/ NPs, their lipophilic composition, and the ability to carry a high payload of the chosen therapeutic agent. The modifications of these nanomaterials with specific ligands can open the possibility to target the nanocarriers to a specific tissue, cell, and even an organelle. In addition, these nanosystems offer a protective niche for the delivered cargo, due to increased physicochemical and biological stability of encapsulated and carried products. Intravenously injected enzymes avoid being exposed to blood and reach the body organs more efficiently if these molecules can be protected by the nanocarriers. Moreover, the delivery of therapeutic molecules to organs of interest in a more targeted manner can make the delivery more effective and control the half-life of the delivered enzymes. The qualities of these nanosystems also provide improved bioavailability, and many of these vehicles of delivery are non-toxic, biocompatible, and biodegradable (Onaca-Fischer et al., 2012; Pirooznia et al., 2012; Del Grosso et al., 2019).

The EV-Mediated Delivery of Therapied to Target CNS

The heterogeneity of EVs

All eukaryotic cells secrete extracellular vesicles (EVs), which vary in function and characteristics, but they all carry specific molecules from the cell of origin to the target cells, thus performing essential roles in the cell-to-cell communication and homeostasis. EVs are a heterogeneous population of vesicles that vary in size, biogenesis, and protein markers (Raposo and Stoorvogel, 2013). The subpopulation of EVs include exosomes, microvesicles (MVs), apoptotic bodies (APOs), oncosomes (Di Vizio et al., 2009; Minicacci et al., 2015), and exomers (Nolte’T Hoen et al., 2012). In particular, exosomes are small EVS, which have a size range of 30–120 nm (Thery et al., 2002), contain tetraspanin-based biomarkers CD9, CD63, or CD81 (Raposo and Stoorvogel, 2013), and have unique biogenesis originating in the endosomal pathway (Raposo and Stoorvogel, 2013). Exosomes are capable of carrying and transmitting protein and RNA cargo, but also specific lipids and small molecules (Rajendran et al., 2006; Alvarez-Erviti et al., 2011a; Noerholm et al., 2012). This exosomal cargo can be subsequently transmitted to other cells. Because exosomes travel in physiological fluids, these vesicles can reach cells in distant organs to transmit their contents from the cells of origin (Alvarez-Erviti et al., 2011b). Since exosomes are formed by all known cell types, not surprisingly, they are also present in the CNS. Within the brain, exosomes have specific functions regulating the interactions between cells, such as neurons and glial cells, for instance by mediating and facilitating the delivery of bioactive molecules from one cell to another (Frühbeis et al., 2012; Zhang and Yang, 2018).

Brain-homing signals for exosomes

Although exosomes contain some molecules on their surface that allow them to reach specific organs, engineered exosomes containing modified membrane decorations that result in improved targeted delivery. One of the ways by which exosomes can be engineered to possess increased targeting capabilities is by a fusion of targeting peptides to the extracellular region of proteins displayed on naïve exosomes. One of such proteins with an extracellular N-terminus is Lamp2b (Alvarez-Erviti et al., 2011b), which is abundantly present on exosomal membranes. Lamp2b molecule can then fused with a peptide utilized for the targeting of specific organs (Alvarez-Erviti et al., 2011b). For the CNS-specific deliveries, rabies viral glycoprotein (RVG) peptide (YTIWMPENPRGTPCDIFTNSRGKRASNG) can be used, which specifically binds to the acetylcholine receptor 3. Dendritic cells-derived exosomes engineered to express an exosomal membrane protein, Lamp2b, which was fused to the neuron-specific RVG (rabies virus glycoprotein) peptide were used to deliver exosomes to the brain by intravenous injections. These exosomes containing this siRNA cargo were introduced
by electroporation. By using this model, exosomes were able to deliver the cargo to neurons, microglia, and oligodendrocytes in the brain, where the carried siRNA resulted in a gene knockdown (Alvarez-Erviti et al., 2011b). The RVG peptide was also used by other groups for exosome homing to the brain (Yang et al., 2017). Another type of peptide that can be fused with Lamp2b to target exosomes to the brain is T7 peptide. Antisense miRNA oligonucleotides against miR-21 (AMO-21) with potential for the treatment of glioblastoma were delivered by exosomes decorated with Lamp2b-fused T7 peptide since the transferrin receptor is overexpressed on the surface of glioblastoma cells. T7-exosomes had a higher delivery efficiency to glioblastoma cells in comparison to the unmodified exosomes or RVG-decorated exosomes in vitro. Similar observations were obtained in vivo, where the delivery of AMO-21 by exosomes via an intravenous injection led to a reduction in the miR-21 levels, followed by the reduction of tumor size (Kim et al., 2020). Finally, muscle-specific peptide (MSP) identified by in vivo phage display (ASSLNIA) can also be fused with Lamp2b for muscle-specific delivery of exosomes (Alvarez-Erviti et al., 2011b).

Exploring Therapeutic EVs to Treat Disorders Affecting CNS

Although exosomes were evaluated in terms of the therapeutic function in such CNS conditions as glioblastoma (Kim et al., 2020), these and other natural nanovesicles have not been extensively characterized as delivery vehicles of therapeutic agents for LSD. A single study focused on exosome-based delivery of a lysosomal enzyme for LSD treatment, in which lysosomal β-glucocerebrosidase (GBA) was fused to a protein that anchored it to the exosomes, termed vesicular stomatitis virus glycoprotein (VSVG), without altering the protein function, but significantly increasing the levels of β-glucocerebrosidase. This is an evidence that the lysosomal the enzyme payloads within exosomes are active. The β-glucocerebrosidase enclosed within these HEK293-derived exosomes was taken up by HEK293 recipient cells by the endocytosis pathway. Finally, such obtained exosomes were explicitly targeted to endocytic compartments and increased GBA activity in the recipient cells (Do et al., 2019).

Macrophage-derived EVs were also used for brain delivery of a soluble lysosomal enzyme tripeptidyl peptidase-1, TPP1, for the treatment of Neuronal Cereoid Lipofuscinoses 2 (CLN2) or Batten disease (Haney et al., 2019). Because macrophages are often associated with inflamed tissue, macrophage derived EVs were hypothesized to interact with inflamed tissues for enhanced targeted delivery of the therapeutic protein. In this study, TPP1-encoding plasmid DNA was transfected into macrophages, or TPP1 protein was packed into the empty EVs by sonication or permeabilization with saponin, and all these methods yielded proper incorporation of functional TPP1 into the vesicles. EVs also increased the stability of TPP1 and were able to deliver the protein to the recipient cells, where over 70% of the protein was localized to lysosomes. TPP1-containing EVs were also administered intraperitoneally and led to the accumulation of TPP1-EVs in brain tissue, improving the lifespan of the late-infantile neuronal ceroid lipofuscinosis mouse (Haney et al., 2019).

Finally, companies such as Evox Therapeutics have an interest in applying exosome-based deliveries for the improved treatment of Niemann-Pick disease type C (Zipkin, 2019). The exosomes produced by Evox Therapeutics carry a copy of the NPC protein to the neural cells by using surface molecules that bind certain, yet undisclosed, ligands (Hean et al., 2019). The EV-based therapies are promising delivery systems for the proteins, which carry large payloads of cargo, and also vectors which are used for gene therapy-based therapeutic approaches.

Liposome-Based Delivery of LSD Therapeutics

Liposomal drug delivery systems composed of biocompatible nanomaterials enhance the efficacy of ERT since these nanostructures encapsulate and protect the enzymes in biodegradable micelles. Liposomes are spherical vesicles that contain one or more lipid bilayers, and their hydrophilic character is ideal for efficient drug delivery. The bilayer
components affect the properties of liposomes, including the rigidity, charge, and fluidity of the bilayer. Unsaturated phosphatidylcholine lipids obtained from natural sources can be used to manufacture liposomes, but these species of lipids increase the permeability and negatively affect the stability of these vesicles. On the other hand, saturated phospholipids containing longer acyl chains are more rigid and impermeable (Akbarzadeh et al., 2013). ERT utilizing liposomes that contain enzymes which are deficient in LSDs were already proposed in the 1970s and 1980s (Patel and Ryman, 1974, Gregoriadis et al., 1974, Steger and Desnick, 1977, Gregoriadis et al., 1978, Umezawa et al., 1985). However, in these early studies, liposomes often failed to specifically localize the enzyme of interest to the brain (Umezawa et al., 1985). Since then, considerable effort has been made to improve the targeted delivery of therapeutic proteins to CNS by liposomes.

### Table 3: Vesicle-based delivery systems of therapeutic molecules for the treatment of LSDs.

| Vesicle type       | Modification                                                                 | Carried enzymatic or other cargo | Condition associated with enzyme deficiency | Type of study | References                  |
|--------------------|------------------------------------------------------------------------------|----------------------------------|---------------------------------------------|---------------|-----------------------------|
| **EXOSOMES**       |                                                                              |                                  |                                              |               |                             |
| HEK293-derived exosomes | Vesicular stomatitis virus glycoprotein for the improved le of carried cargo protein | β-glucocerebrosidase            | Gaucher disease                             | In vitro study | Do et al., 2019           |
| macrophage-derived EVs | tripeptidyl peptidase-1                                                   | Neuronal Ceroid Lipofuscinosis (NCL)-2, Batten disease | In vitro and in vivo study                  | Haney et al., 2019 |
| **LIPOSOMES**      |                                                                              |                                  |                                              |               |                             |
| liposome           | Functionalized with Arginine-Glycine-Aspartic acid (RGD) peptides for improved cellular uptake | α-glucosidase                   | GM1 gangliosidosis                           | In vivo study | Gregoriadis, 1978          |
| liposome           |                                                                              | α-mannosidase                   | Mannosidosis                                | In vivo study | Patel and Ryman, 1974      |
| liposome           |                                                                              | neuraminidase                   | Mucolipidosis type I                        | In vivo study | Gregoriadis et al., 1974   |
| liposome           |                                                                              | β-glucosidase                   | Fabry disease                               | In vivo study | Umezawa et al., 1985       |
| nanoliposomes      | Functionalized with Arginine-Glycine-Aspartic acid (RGD) peptides for improved cellular uptake | α-glucosidase                   | Fabry disease                               | In vivo study | Cabrera et al., 2016       |
| VNeo-liposomes     | Guanidinylated neomycin (GNeo) transporter for enhanced delivery to lysosomes | α-L-iduronidase                 | Mucopolysaccharidosis type I                | In vitro study | Hamil et al., 2017         |
| liposomes          | Lysosomotropic octadecylrhodamine B (Rt) for enhanced delivery to lysosomes | Glucocerebroside                 | Gaucher disease                             | In vitro study | Koshkaryev et al., 2011    |
| **NANOPARTICLES** |                                                                              |                                  |                                              |               |                             |
| PLGA acidic NPs    | Acidic NPs for re-acidification of the defective lysosomes                  | glucocerebrosidase-mutant cells, PD | In vitro and in vivo study                  | Bourdenx et al., 2016 |
| NPs                | saposin C for the protection of biologically active cargo                   | acid b-glucosidase              | Pompe disease                               | In vivo study | Sun et al., 2020           |
| PLGA NPs           | Ang2- (Angiopep-2), g7, and T12 (transferrin binding)-functionalized NPs to improve the delivery to brain | galactosylceramidase             | Krabbe disease                              | In vivo study | Del Grosso et al., 2019    |
| polystyrene particle | Coupled to anti-ICAM-1 antibody for an improved targeting to organs         | α-glucosidase                   | Fabry disease                               | In vivo and in vitro study | Hsu et al., 2014, Hsu et al., 2011 |
| polystyrene particle | Coupled to anti-ICAM-1 antibody for an improved targeting to organs        | acid α-glucosidase              | Pompe disease                               | In vivo study | Hsu et al., 2012           |
| NPs                | Coupled to anti-ICAM-1 antibody for an improved targeting to organs         | acid sphingomyelinase           | Acid sphingomyelinase deficiency            | In vivo study | Muro et al., 2006          |
| PLGA NPs           | 7-aminoacid glycopeptide for increased targeting of NPs                    | Albumin was used as a model     | NPs were tested in MPS-I and II models       | In vivo and in vitro study | Salvalao et al., 2016    |
| Protein-based NPs  | Human serum albumin (HSA) and 30 KDa silkworm proteins were used to make the NPs to enhance cellular uptake | β-glucosidase                   | Fabry disease                               | In vivo study | Lee et al., 2016           |
| trimethyl chitosan-based polyelectrolyte complex-based nanocarriers | respond to low pH by the triggered release of the loaded protein for controlled release, Atto 647 N modification | α-galactosidase                   | Fabry disease                               | In vivo study | Giannotti et al., 2011    |
| quantum dots       | Guanidinylated neomycin (GNeo) for improved cell binding                    | β-glucosidase or α-iduronidase   | MPS-VII and MPS-I                           | In vitro study | Sarrazin et al., 2010      |

ICAM-1, intercellular Adhesion Molecule 1; MPS, mucopolysaccharidosis; PD, Parkinson disease.
First, liposomes can be modified to contain homing signals to enhance liposome-mediated transport to specific organs. Immunoliposomes—or antibody-directed liposomes—are sterically stabilized with PEG, which is conjugated to a linker lipid, allowing for conjugation of thiolated antibody on the surface of the vesicle. The monoclonal antibodies are used as modifiers attached to the surface of such liposomes. Examples of antibodies that were previously used to direct the liposomes to the brain are the OX26 antibody recognizing the transferrin receptor, which increased the uptake of liposomes to rat brain and specifically to the microvascular endothelium that builds the blood-brain barrier (Huwyler et al., 1996).

Second, the liposome modification can increase the uptake of liposomes by cells. Here, modification of the liposomal surface with a peptide has enhanced the intracellular penetration of liposomes. Also, nanoliposomes functionalized with Arginine-Glycine-Aspartic acid (RGD) peptides containing α-galactosidase-a, had an enhanced enzymatic activity and improved intracellular penetration (Cabrera et al., 2016). Proteins such as apolipoprotein E can be added on the surface of liposomes for more efficient delivery of receptor-mediated uptake of the liposomes to the cells (Ansari et al., 1997).

To further improve the delivery of liposomes to lysosomes, guanidinylated neomycin (GNeo) transporter can be used. GNeo is capable of transport of bioactive cargo inside the cells, which can also be of high molecular weight. GNeo works by a process utilizing heparan sulfate proteoglycans in the cell surface. The synthesis of GNeo-lipids, which were then incorporated into the liposomes, termed GNeo-liposomes, or GNeosomes, was able to enhance the specific delivery of these particles to lysosomes, which encapsulated α-L-iduronidase (IDUA). These GNeosomes successfully restored the function in fibroblasts isolated from a patient that lacked this enzyme due to the lysosomal storage disorder mucopolysaccharidosis type I (MPS I) (Hamill et al., 2017). Finally, lysosomotropic octadecylrhodamine B (Rh) (Koshkaryev et al., 2011) was used to increase the delivery of the encapsulated galactosephosphodiesterase vagaclascerase-α to the lysosomes in the Gaucher’s fibroblasts, where a 68% increase in the targeted delivery was observed in contrast to liposomes that were not modified with the Rh (Thekkedath et al., 2013).

NP-Based Delivery of Therapeutic Molecules for the Treatment of LDS

Another particle type that can be used for the delivery is polymeric NP. Poly-(lactide-co-glycolide), PLGA is one of the preferred materials used for the production of NPs, which is a non-toxic material and thus offers increased biocompatibility (Patel et al., 2012). PLGA has been approved by the Food and Drug Administration for administration in humans. As an example, the catalytic activity of lipase was improved by immobilization of this enzyme onto a porous super magnetic polymeric microsphere carrier. The glycerol-oleic-acid-esterification activity of the immobilized lipase increased seven fold in comparison with the naked enzyme. Whereas, the activity normalized to the total protein content of the immobilized enzyme was improved by 200-fold, and the stability of the lipase was also improved by the immobilization of this enzyme to the NPs (Meng et al., 2013).

The targeting function of NPs can be enhanced by the NP modifications, where peptides, antibodies, and aptamers can be added to the surface of these structures. As an example, Ang2- (Angiopep-2) (Demeule et al., 2008), g7 (Tosi et al., 2011), and Tf2 (transferrin-binding)-functionalized (Santi et al., 2017). PLGA NPs containing cross-linked enzyme aggregates were tested in the encapsulation efficiency, activity yield of encapsulated enzymes, and targeting to the brain if delivered intraperitoneally to a TWI murine model of Krabbe disease (Del Grosso et al., 2019). These NPs were able to promote recovery in the brain up to the control mice level. Specifically, the enzyme-delivering NPs lead to significant GALC activity in the organs, where both targeted and control NPs accumulated in the liver and kidneys 4h after injection. However, only the targeted NPs were detected in the brain, displaying a GALC activity that was comparable to the activity of the heterozygous mice for GALC, which is identical to the wild-type mouse phenotype.

In contrast, the non-targeted NPs did not show any significant GALC activity increase in the brain tissues (Del Grosso et al., 2019).

Another modification of NPs is antibody coupling. One of the commonly targeted molecules is ICAM-1, which is an endothelial surface protein upregulated in many LSD pathologies, such as inflammation or metabolic imbalance (Springer, 1994). Polystyrene and PLGA polymer nanocarriers targeting ICAM-1, delivered by intravenous injection to mice, were reported not to cause lung injury or have other adverse effects, such as abnormal vascular permeability. These NPs were rapidly removed from the circulation and accumulated in various organs such as the kidney, heart, liver, spleen, or lung (Garnacho et al., 2008). Polystyrene particles coupled to an antibody specific to ICAM-1 were then used to improve targeting of α-galactosidase (αGal), which is an enzyme affected in the Fabry disease. While naked αGal intravenously delivered to animals preferentially remained in circulation and not up taken by tissues and organs, αGal coupled to nanocarriers accumulated in the brain, kidneys, heart, liver, lungs, and spleen (Hsu et al., 2014). The nanocarriers also specifically were targeted to lysosomes and led to an enhanced globotriaosylceramide degradation, thus constituting possible improvement of drug delivery for Fabry disease (Hsu et al., 2011). The same ICAM-1 molecule was targeted for the delivery of ERT to treat Pompe disease caused by a deficiency of acid α-glucosidase (GAA). GAA was coupled to ~180 nm polystyrene bead-based nanocarriers coated with anti-ICAM-1, and this enhanced targeting of anti-ICAM/GAA NCs to organs in comparison to the naked enzyme delivery (Hsu et al., 2012). Anti-ICAM-1-coated nanocarriers have also been used to target recombinant proteins, such as human acid sphingomyelinase to ICAM-1-positive cells, including activated endothelial cells and Niemann–Pick disease patient fibroblasts. The sphingomyelinase delivery by nanocarriers was enabled by...
the clathrin-mediated endocytosis pathway, which is distinct from the clathrin-dependent endocytosis. As a result, the bioactive proteins were successfully delivered to lysosomes, leading to a decreased accumulation of lysosomal lipids (Muro et al., 2006).

Peptides are another class of modifications that can be added to NPs to enhance their targeting to brain regions. A 7-aminoacid glycopeptide (g7) was used to decorate PLGA NPs, which were then tested in MPS I and MPS II models. The g7-modified PLGA NPs successfully delivered high molecular weight molecules across the BBB in a murine model, where a model molecule FITC-albumin was used as an example (Salvalao et al., 2016).

Human serum albumin (HSA) and 30Kc19 silkworm protein were tested as an enhancement strategy for the delivery of α-galactosidase. HSA was used because this protein is an FDA-approved molecule, and it binds to receptors within the caveolae while the 30Kc19 silkworm protein enhances cellular uptake and stabilizes the cargo (Park et al., 2012, 2014). Using primary cultured fibroblast, 30Kc19-HSA NPs led to enhanced cellular uptake of the carried protein. The α-galactosidase carried by these NPs had improved globotriaosylceramide degradation in the fibroblasts (Lee et al., 2016).

Controlled release of proteins to the lysosomes can also be achieved by functional polyelectrolyte-based NPs, which respond to low pH by the triggered release of the loaded protein. In one study, trimethyl chitosan (TMC)-based polyelectrolyte complexes (PECs) nanocarriers were used to deliver α-Gal. These NPs released the enzyme at acidic pH, and upon their further functionalization with fluorescent group Atto 647N, PECs were shown to be further internalized by human endothelial cells and enriched in the lysosomes (Giannotti et al., 2011).

Apart from transferring molecules, NPs can be used to lower the pH within lysosomes. PLGA acidic NPs, which were transported to lysosomes, were able to restore impaired lysosomal function glucocerebrosidase-mutant cells by inducing re-acidification of the defective lysosomes. The same PLGA-acidic NP was localized to neurons following the intracerebral injection, where they were able to attenuate the neurodegeneration in vivo in the murine model of PD by rescuing the lysosomal dysfunction (Bourdenx et al., 2016).

Other modifications to the nanovesicles include the addition of stabilization agents. Saposin C (SapC) is a lysosomal glycoprotein which naturally protects GCase from the degradation (Sun et al., 2003). The CNS-selective delivery system based on nanovesicles of saposin C (SapC) and dioleoylphosphatidylserine (DOPS) was used to deliver acid b-glucosidase (GCase) via the BBB. The enclosure of the enzyme within the nanovesicles improved the stability and activity of the GCase and mediated the uptake of the enzyme by a mannose receptor-independent pathway. SapC-DOPS-GCase-containing nanovesicles were able to penetrate through BBB via the surface phosphatidylserine. These vesicles also led to a reduction of GCase substrate levels, attenuating brain inflammation (Sun et al., 2020).

Quantum Dots

One more category of the nanocarriers worth mentioning are quantum dots, also known as “artificial atoms,” which are semiconductorbased nanocrystals in a size range of 2–10 nm. Guanidinylated neomycin (GNeo), which targets liposomes to lysosomes (Hamill et al., 2017), as described above, was also used to modify quantum dots, which were able to undergo endocytosis and translocate the lysosomes. The activated ester of GNeo was conjugated to β-glucuronidase or α-iduronidase enzymes, which did not affect enzymatic activity. This GNeo modification enabled efficient binding of the heparan sulfate on primary human fibroblast cells, where the GNeo quantum dots containing the enzyme led to a restoration of glycosaminoglycan turnover in the cells (Sarrazin et al., 2010).

CONCLUSIONS AND FUTURE DIRECTIONS

The mainstay therapy for LSDs, ERT, and current small molecules functioning as SRT and PC agents have a very limited biodistribution to CNS, PNS, and even other organs including bone, skeletal and cardiac muscle and connective tissues (Hollak and Wijburg, 2014). A summary of the current therapies targeting the CNS are described in Table 2. In the majority of LSDs, the predominant clinical manifestations are neurological in origin and associated with progressive morbidity. Therefore, there is an urgent and unmet need for therapies to the CNS, tackling the primary defect (enzyme deficiency/substrate build-up), and the multiple secondary pathogenic cascades (Pastores and Maegawa, 2013; Giugliani et al., 2018; Platt, 2018; Platt et al., 2018). Despite remarkable advances in the understanding of neuropathogenesis of these disorders, the CNS impairment remains the major cause of disabilities, hospitalizations, and the requirement of prolonged and complex care for the affected patients. Cognizance of this unmet therapeutic need and challenges imposed by the BBB, several therapeutic modalities have been investigated, including modified ERT agents, novel small molecules, HSCT, gene therapy and innovative therapeutic-CNS delivery strategies. The advances in combinatorial chemistry, nanomolecule and chemical synthesis allowed the expansion of the chemical space, resulting in novel and unique molecules capable of permeating the BBB and tackling the neurodegenerative processes in the CNS. A better understanding of exosomes and other nanovesicles as carriers of signaling metabolites will allow us to explore the therapeutic aspect of these nanovesicles (O’loughlin et al., 2012; Van Niel et al., 2018). The advances in, now “micro,” robotic technologies, high-resolution microscopy will offer multiple and concomitant readouts in high-through screening (HTS) assays, generating more disease-relevant and precise and “hits.” (O’loughlin et al., 2012; Van Niel et al., 2018). In addition, the “tissue-chips” or “microphysiological systems,” bioengineered microsystems capable of recreating aspects of human organ physiology and function, will accelerate the validation and prioritization of the small molecule “hits” against a specific target and its mutant variants (Low et al., 2020). With the advances of mass spectrometry and next-generation sequencing (NGS), allowing both newborn screen programs and early diagnosis,
respectively, an increasing number of individuals are being diagnosed at early or presymptomatic disease stages. In this setting, diverse modalities and more tailored and individualized therapeutics will be required to treat and, eventually prevent, the lysosomal, cellular, and organ damages, often irreversible by the time of diagnosis. In sum, applying the basic understanding of the neuropathogenesis to develop safer and target therapies, the prevention of the LSD manifestations throughout the life span of early diagnosed individuals, who maybe “apparently healthy,” will be achieved.

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The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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