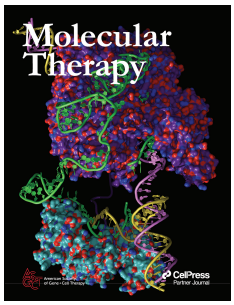

Targeting the Central Nervous System in Lysosomal Storage Diseases: Strategies to Deliver Therapeutics Across the Blood-Brain Barrier

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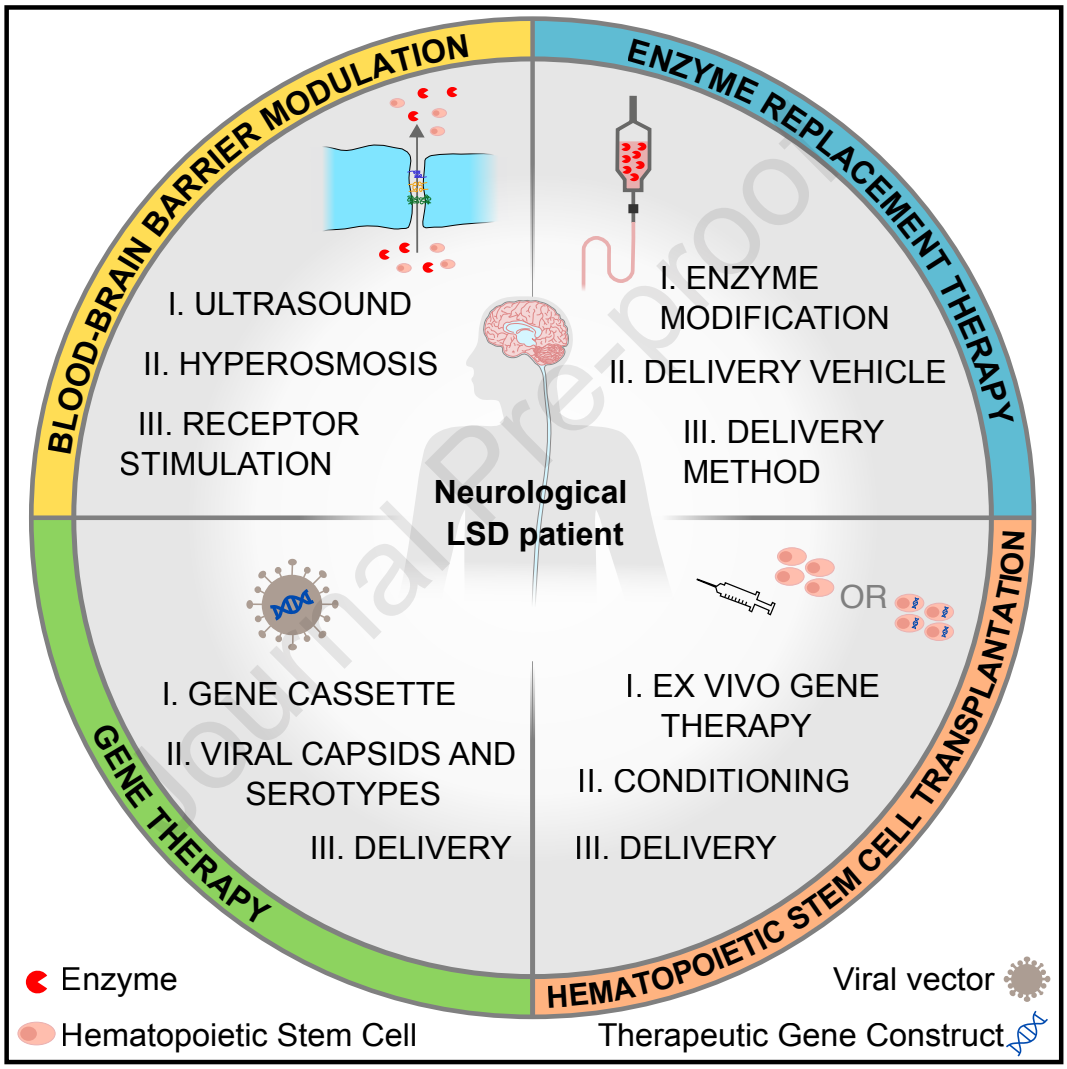
BLOOD-BRAIN BARRIER MODULATION
I. ULTRASOUND
II. HYPEROSMOSIS
III. RECEPTOR STIMULATION
ENZYME REPLACEMENT THERAPY
I. ENZYME MODIFICATION
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Neurological LSD patient
GENE THERAPY
I. GENE CASSETTE
II. VIRAL CAPSIDS AND SEROTYPES
III. DELIVERY
HEMATOPOIETIC STEM CELL TRANSPLANTATION
OR
I. EX VIVO GENE THERAPY
II. CONDITIONING
III. DELIVERY
Enzyme
Hematopoietic Stem Cell
Viral vector
Therapeutic Gene Construct

Targeting the Central Nervous System in Lysosomal Storage Diseases: Strategies to Deliver Therapeutics Across the Blood-Brain Barrier

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1. Abstract

Lysosomal storage diseases (LSDs) are multisystem inherited metabolic disorders caused by dysfunctional lysosomal activity, resulting in the accumulation of undegraded macromolecules in a variety of organs/tissues, including the central nervous system (CNS). Treatments include enzyme replacement therapy, stem/progenitor cell transplantation and in vivo gene therapy. However, these treatments are not fully effective in treating the CNS as neither enzymes, stem cells nor viral vectors efficiently cross the blood-brain barrier (BBB). Here we will review the latest advancements in improving delivery of different therapeutic agents to the CNS and comment upon outstanding questions in the field of neurological LSDs.

2. Introduction

Lysosomal storage diseases (LSDs) are a group of more than 70 inherited metabolic disorders characterised by deficient function of lysosomes, organelles whose function is to catabolise macromolecules. The lysosome contains an array of hydrolytic enzymes that together with transporters, lysosomal membrane proteins and targeting motifs are accountable for the proper functioning of the cell recycling apparatus. Defects in any of these components result in the aberrant accumulation of undegraded macromolecules, or “storage products”, disruption of cell homeostasis, cell dysfunction and, in some cases, cell death[1]. Prevalence of each LSD is very low, however, when considered as a group they affect a significant minority of live births (12.1 – 25 per 100,000[2]). LSDs are genetically heterogeneous, and can be classified into subcategories depending upon the type of macromolecule involved (reviewed by Platt

et al[1]). They are multisystem diseases which affect different tissues and organs to a variable degree depending on lysosome/substrate distribution, and expression profile of the causative gene(s). Clinical symptoms range in severity depending upon the extent to which a specific LSD affects each cell type, tissue, or organ; however, 50-70%[3,4] significantly affect the central nervous system (CNS), resulting in severe and progressive neurodegeneration. Brain damage commonly begins in early infancy but can also occur during adulthood in late onset forms. Neurological LSDs (summarised in Table 1) are often fast-progressing fatal diseases, therefore substantial effort has been made to develop effective treatments.

Currently, there are several experimental and clinical treatments available for specific LSDs with the collective aim of restoring enzyme function. Standards of care include i) enzyme replacement therapy (ERT) to deliver exogenous enzyme directly to the patient[5]; ii) hematopoietic stem/progenitor cell (HSC) transplantation, in which patients receive either allogeneic or autologous HSCs which are genetically modified ex vivo (HSC gene therapy) and are able to engraft the CNS, providing a source of functional enzyme[6,7]; and iii) substrate reduction therapy which utilises small molecules to attenuate accumulation of specific macromolecules[8]. Ongoing clinical trials are evaluating improved standard of care approaches, especially for HSC gene therapy, whilst also testing alternative approaches. These include in vivo gene therapy, which delivers a healthy copy of the defective gene directly to patients' cells[9]; and chaperone therapy to guide correct protein folding of patients' aberrant enzymes to improve their catalytic function[10].

Despite some of these treatment strategies being successful for specific forms of LSDs[11], there are still a number of drawbacks. Each treatment has different limitations: ERT is immunogenic, must be administered regularly and has limited efficacy in some organs[5]; HSC transplantation (HSCT) necessitates chemotherapeutic pre-conditioning and has a risk of transplant-associated morbidity and mortality[6,7]; substrate reduction therapy, like ERT, does not correct the primary defect and some molecules are associated with undesirable secondary side effects[8]; and a range of gene therapy vectors can be immunogenic[12].

One limitation which is common to all these strategies is the inability, or limited ability, of ~~all~~ therapeutic agents to cross the blood-brain barrier (BBB) and reach the CNS or,

in the specific case of HSCT therapy, to engraft rapidly enough and in optimal numbers to prevent the rapid neurological deterioration that occurs in some LSDs. Consequently, in recent years there has been a strong focus on increasing delivery of therapeutic agents to the CNS. Innovations in CNS delivery have been recently discussed from the perspective of nanoparticles[13] and small molecules[14]. However, methods to improve delivery of enzymes, stem cells and viral vectors to the CNS have not been reviewed in recent years. This review will focus upon methods to increase delivery across the BBB, with emphasis on the latest advancements in targeting HSCT, ERT and viral vectors to the CNS, and discuss the future of CNS-directed LSD therapy.

3. The blood-brain barrier

The BBB is a selectively permeable barrier between the CNS and the systemic circulation which controls exchange of solutes and protects the brain from toxins and potential pathogens circulating in the bloodstream[15]. It is comprised of neurovascular units, in which brain cells closely interact with the vasculature. The neurovascular unit involves multiple cell types: endothelial cells, pericytes, astrocytes, neurons, and microglia. Endothelial cells are the primary component and are supported by pericytes, perivascular cells that embrace the vessels and provide them with stability. Astrocytic end feet ensheath almost the entire abluminal surface of microvessels[16], and neurons and perivascular microglia interact with these cells to establish the neurovascular unit (Figure 1). Brain endothelial cells are especially vital for restricting BBB permeability, and have particular properties which enable them to perform this function including i) reduced transcellular flux, ii) lack of fenestrations, iii) greater mitochondrial density to assist rapid metabolism, iv) specialised transport systems and v) high electrical resistance as a result of an increased number of tight junctions between endothelial cells compared to other tissues and organs[17] (Figure 1). Multiple proteins are involved in tight junctions, namely junctional adhesion molecules (JAMs), claudins, zonular occludens and occludin[15]. Under normal conditions, they prevent molecules from leaking across the BBB through the paracellular transport pathway, which represents one of the two main transport routes across the BBB (Figure 1). Alternatively,

molecules can move transcellularly with some crossing the BBB by passive diffusion, while most require assistance from carrier proteins (carrier-mediated transcytosis; CMT), receptors (receptor-mediated transcytosis; RMT) or vesicles (adsorptive mediated transcytosis; AMT)[17].

However, in pathological conditions BBB integrity can be disrupted, allowing passage of substances which would normally not be able to cross. In the case of some CNS diseases (including Alzheimer's, Parkinson's), systemic diseases (e.g., diabetes mellitus and chronic cerebrovascular disease), and viral infections (e.g., viral encephalitis), disruptive remodelling of tight junctions results in reduced BBB integrity, leading to neuroinflammation that further contributes to increased BBB permeability[18-23]. A greater understanding of the role of neurovascular units and tight junctions in the transport of therapeutic agents across the BBB, and being able to manipulate transport to increase delivery, may be vital for the effective delivery of therapeutic agents to treat the neurological component of LSDs. In the following sections, we will explore how current treatments have been modified to improve stem cell, enzyme, and viral vector delivery across the BBB, including methods which exploit aspects of BBB transport pathways or bypass the barrier altogether.

4. Brain Blood Barrier Manipulation

Several methods have been employed to disrupt the BBB with the aim of temporarily increasing permeability for LSD therapeutic agents (reviewed by Hersh[24]).

Here we will briefly explore both non-selective and selective methods that assist delivery of only specific cells and/or enzymes.

4.1 Focused ultrasound

The use of magnetic resonance thermometry to guide focused ultrasound pulses in the presence of microbubbles, allows to briefly compromise BBB permeability. Ultrasound pulses cause the microbubbles to expand and contract, temporarily separating endothelial tight junctions, which facilitates passage of therapeutics without allowing pathological events to occur[25]. In relation to neurological LSDs, the method has been used to deliver GFP-labelled neural stem cells to wild type rat brains[26], and to transport enzyme across the BBB in an MPS I murine model, restoring 75% of

normal enzyme activity in the treated brain hemisphere[27]. Investigation of this method's safety and feasibility is underway in a number of neurological diseases and is employed in a phase I trial delivering Cerezyme® (an analogue of the β-glucocerebrosidase enzyme, which is also defective in the LSD Gaucher disease) across the BBB in Parkinson's disease patients (NCT04370665).

4.2 Hyperosmotic agents

Intravenously delivered hyperosmotic agents increase BBB permeability by shrinkage of brain endothelial cells and consequent tight junctions widening[28]. This temporarily augmented permeability allows a generalised increase in migration of substances from the bloodstream. The hyperosmotic agent mannitol has been used in murine models to deliver adeno-associated viral (AAV) vectors to the CNS in Sandhoff disease[29], MPS IIIB[30,31] and CLN2 deficiency[32], showing enhanced delivery and greater therapeutic effect. However, the potential for toxic substances to cross the BBB during the period of non-selectively enhanced permeability, or for cerebral oedema to occur if mannitol enters the brain, has limited its use in patients despite its clinical safety profile[33].

4.3 Receptor stimulation

Receptor stimulation can increase delivery of enzymes across the BBB by relocalising receptors to the luminal surface of brain endothelial cells. Studies in the LSD field have predominantly focused on the mannose 6-phosphate (M6P) receptor, a transport mechanism in the brain present during early post-natal development but lost during maturation[34]. Murine studies have shown that administration of epinephrine[35] or retinoic acid[34] stimulates M6P receptors and significantly elevates M6P-mediated transport of the lysosomal enzyme β-glucuronidase (P-GUS, defective in MPS VII) across the BBB. Further work involving direct stimulation of specific adrenoreceptors with α1/2 agonists suggested that increased enzyme uptake was likely due to redistribution of M6P receptors from an intracellular pool to the intra-luminal surface of brain microvascular endothelial cells[34,36]. These studies suggest that manipulation of receptor-mediated transport is a viable method for increasing selective delivery of enzymes across the BBB.

5. Enzyme Replacement Therapy

The concept of enzyme replacement therapy (ERT) as a potential treatment for LSDs (reviewed in Solomon and Muro, 2017[5]) first arose in the mid-1960s, however a further three decades of development were required to generate the first effective, clinically approved ERT. ERT entails administration of fully functional exogenous enzyme to the patient, mainly via intravenous injection. The enzyme is taken up by patients' cells via endocytosis and trafficked to lysosomes, where it compensates for endogenous enzyme dysfunction. ERT's limitations have been extensively reviewed elsewhere[5]; the major one of relevance to neurological LSDs is the inability to treat organs which are difficult to access – particularly the musculoskeletal, cardiovascular, ocular and central nervous systems[1]. In the following sections, we will review strategies employed to circumvent this limitation.

5.1 Enzyme Modification

5.1.1 Fusion proteins

Modification of the therapeutic enzyme with an exogenous protein subunit might enable interaction with a specific receptor to increase CNS uptake. Multiple fusion proteins have been tested for efficacy in augmenting CNS delivery in LSD murine and/or primate models, including an acidic amino acid tag[37], the fat-binding apolipoprotein E (ApoE)[38-40] and importantly antibody conjugates targeting endogenous BBB transport receptors including the insulin receptor[41-43] and the transferrin receptor[44]. Antibody-conjugated enzymes harness the receptor-mediated transport pathway to cross the BBB into the CNS. Results from in vivo studies demonstrated reduction of substrates and neuroinflammation in MPS II murine and primate models[43,44], and highlighted a safety propfile[41,42]. A number of clinical trials (ClinicalTrials.gov NCT03128593, NCT03568175, NCT04573023) have pursued this further; following a successful phase I/II trial of iduronate-2-sulfatase fused with an anti-human transferrin receptor antibody in MPS II patients[45], results of a phase II/III study showed significantly reduced substrate accumulation both in the CNS and peripheral tissue, in addition to positive neurocognitive changes, whilst demonstrating a clinical safety profile consistent with current standards of care[46]. This strategy has now been approved for clinical use in Japan[47].

5.1.2 Chemical Modification

An alternative to fusion proteins is the chemical modification of lysosomal enzymes to alter receptors' affinity, allowing an elevated blood concentration of the therapeutic enzyme in order to maintain a high concentration at the BBB for prolonged periods. This approach has been tested in MPS VII[48,49] and MPS IIIA[50-52] murine models, showing significant reduction in CNS lysosomal storage biomarkers[48-52].

5.2 Delivery

5.2.1 Injection Routes

A range of different injection routes have been tested for ERT to improve enzyme delivery (Figure 2). Beyond traditional intravenous (IV) injection, intracerebroventricular (ICV) injection[53,54] has been reported most extensively in recent literature in comparison to intrathecal lumbar, cisternal[53] or intravenous injection[55], or to control conditions[54,56-63]. A number of studies have reported that ICV is effective for ERT in multiple neurological LSD animal models[53-55,57-62]. However, these studies raise an important issue as sufficient enzyme delivery for therapeutic effect[59] remains a challenge. Treleaven et al observed that less than 1% of the total ERT dose reached the CNS of a Niemann-Pick Type A (NPA) murine model. Higher doses did not increase this percentage, suggesting that the enzyme uptake mechanism is saturated. However, whilst this is an extremely small proportion, it was distributed widely throughout the CNS, and previous work in the same murine model[61] demonstrated significant reduction of storage product levels and partial alleviation of motor abnormalities. The study proposed ERT scaling by CNS weight to maintain this therapeutic level in larger rodents[59]. Work in the NPA model raised a second potential limitation with ICV delivery; despite therapeutic effect on the CNS as a whole, they observed a steep gradient in therapeutic enzyme from outer to inner brain regions, raising the possibility that therapeutic correction may be less successful in deeper tissue[60]. However, elevating the concentration of therapeutic enzyme may trigger an immune response against the exogenous enzyme, as was observed in a few MPS I mice given high-dose intravenous ERT[64]. Thorough investigation of toxic effects of high-dose ERT, and the impact upon CNS therapeutic correction, are required.

Other CNS-targeted ERT injection routes, including intrathecal (IT), intranasal (IN) and intracisternal (IC), have been tested to varying degrees. IT and IC methods have been trialled in a similar range of animal models to ICV[56,65-76], with IT being shown to have

greater benefit over IV in a single MPS I A patient[65]. IN delivery has only been tested in a murine model of MPS I[77,78], and, similar to ICV injection, only a very minimal percentage of the total dose of therapeutic enzyme (estimated 0.001%) reached the brain[78]. Despite studies reporting a predominantly positive effect on the neurological pathology, these injection routes entail reduced quantity of administered enzyme and consequently a reduced effect in deep brain tissues[56,66,68,70,73,77,78].

Few studies have directly compared the effect of different ERT injection routes on LSD CNS pathology. ICV proved more therapeutically effective than IC injection in two studies[53,55] despite being the most invasive[53]. Comparison between ICV and IT has shown mixed results; in a canine model of MPS II, ICV injection of ERT was superior, with correction of deep brain tissues[55]; however, in wild type non-human primates and canines IT delivery gave better results[67], which was supported by a small-scale trial of IT injection in MPS II model mice by the same group; however, no mice were injected using the ICV route, limiting direct comparison in the disease model setting[67]. Altogether, these studies suggest that ICV injection is the most effective for delivering ERT to the CNS in LSD models, however the concerns regarding non-homogenous delivery throughout the brain and the limited percentage of treatment delivered to the CNS (which, albeit small is sufficient to exert a therapeutic effect) suggest that other strategies may need to be employed.

5.2.2 Delivery Vehicles

Delivery vehicles such as nanoparticles[13], extracellular vesicles[79], polymersomes[80,81] and quantum dots[82] have been explored to improve enzyme delivery to the CNS in LSDs. Whilst quantum dots have only been investigated in an in vitro setting[82], successful in vivo studies have been conducted with polymersomes[81], extracellular vesicles[79] and nanoparticles; of these, nanoparticles have been researched most extensively. Multiple studies have employed nanoparticles to successfully deliver therapeutic enzymes to the CNS of Gaucher disease[83], Krabbe disease[84] and MPS II murine models, reporting reduction of storage products to non-pathological levels[85] (for a thorough review of the role of nanoparticles in LSD treatment up to 2016, please refer to Martin-Banderas et al[13]). However, it is important to note that not all CNS LSDs are amenable to treatment using nanoparticles; three different nanoparticle formulations tested in a MLD murine model showed no increase in CNS enzyme levels, perhaps due to the therapeutic enzyme itself interfering with the targeting of the nanoparticles to the CNS[86]. The authors speculate that this could be due to

interference of the enzyme's charge or side chain oligosaccharides with surfactant coating or apolipoprotein recruitment, which are reported to be key mechanisms in BBB transport of nanoparticles. Therefore, it is possible that other delivery vehicles may also be limited by this issue.

5.2.3 Delivery Devices

Initially, subcutaneous delivery devices were designed to enable continuous delivery of therapeutic enzymes to the CNS[87]. Devices which deliver therapeutic enzymes via the ICV[88-91] or IT route[92,93] were tested in the past decade and proved effective in MPS[88,89,93], MLD[90] and NCL[91] murine models. In 2017, an infusion pump which delivers into the cerebrospinal fluid was tested in a canine model[87], but the study concluded that repeated IC or intra-spinal delivery was more effective. Furthermore, continuous delivery of therapeutics via these devices necessitates storage of the enzyme at body temperature for prolonged periods, which is likely to compromise enzyme stability and therefore limit utility. Consequently, research focus has now shifted towards devices with no indwelling enzyme reservoir. An IT drug delivery device utilised for monthly dosing was tested in a clinical trial for MPS II patients[92] (ClinicalTrials.gov Identifier NCT02055118); early results indicated a promising 80% reduction in storage substrate, however over 50% of the trial participants had their device removed because of significant adverse events[92], either due to the device breaking or the infusion cannula migrating away from the delivery site. Recent trials of a new ICV device (Ommaya reservoir) in MPS IIIB patients have proven more effective (EudraCT 2017-003083-13; ClinicalTrials.gov NCT02754076 and NCT03784287), and the device has been applied to delivery of an ERT approved for ICV dosing in MPS II patients[94].

5.2.4 Convection Enhanced Delivery

One alternative strategy which has predominantly been applied to augment ICV delivery for brain tumour treatment, is convection enhanced delivery[95], where catheters are stereotactically inserted and, using image guidance, directed into the interstitial spaces before an infusion pump is used to drive delivery, therefore not requiring a high concentration of the therapeutic agent[95]. The only in vivo application of this strategy for Gaucher disease[96] ERT showed progressive and complete filling of the CNS target regions with therapeutic enzyme, while a trial in a single patient with type 2 Gaucher disease demonstrated safety[96]. Another clinical study evaluated safety of convection enhanced delivery for gene therapy agents in late infantile NCL patients,

reporting no adverse effects of the procedure and enzyme infusion rates between 50 and 90%[97]. However, there has been very limited further testing of this method in neurological LSDs, perhaps due to the range of risks associated with this procedure, primarily backflow, air bubbles and flow within brain tissue[95].

6. Stem and Progenitor Cell Transplantation

The requirement for a permanent, long-term fix which delivers lysosomal enzyme to all affected tissues in LSD patients has pushed scientists to look at other treatments beyond ERT. One promising alternative is stem and progenitor cell transplantation, which can generate lifelong tissue-resident sources of functional lysosomal enzyme that can relieve both somatic and neurological pathology[6]. Stem and progenitor cells are injected into the patient, where they engraft in affected tissues, contribute to the patients' resident cell populations, and secrete functional enzyme. The ability of stem and progenitor cells to potentially cross the BBB and provide cross-correction in the CNS has led to this strategy being trialled for a range of neurological LSDs. To date, hematopoietic stem and progenitor cells (HSCs) have been most commonly trialled in LSD animal models, and also human patients[6,7]. Other stem cells have been used for transplantation specifically targeting the CNS in LSDs, including neural stem cells and, to a lesser extent, mesenchymal stem cells.

Analysis of neural and mesenchymal stem cell transplants for CNS LSDs can be found in a number of recent reviews[98-100]; herein we will focus on HSCT as the most promising strategy. HSCs can either be isolated from a healthy donor (allogeneic transplantation), or in an autologous manner using the patients' own genetically modified cells to provide a healthy copy of the mutated/non-functional gene[6].

A yet-to-be-identified subpopulation of transplanted HSCs is able to cross the BBB following the use of specific chemotherapy or irradiation based conditioning regimes and replace tissue resident microglia in the CNS[101,102]. The newly generated microglia secrete functional lysosomal enzyme which can be taken up by neighbouring enzyme-deficient brain cells in a process called cross-correction (Figure 3, "cross correction in the brain" panel)[6,7,98,99,103]. At the same time, differentiation of HSCs (that do not engraft the CNS) reconstitutes the entire hematopoietic system, thereby providing a peripheral source of therapeutic enzyme (Figure 3, "reconstituting hematopoietic

lineages" panel). However, treatment of the CNS remains a challenge. In HSCT, cellular engraftment is not instantaneous, and gradual expansion of the transplanted cell population is required before lysosomal enzyme activity can be restored[6,99]. During this period, neurological symptoms often progress, which significantly reduces the impact of HSCT[6]. Furthermore, efficacy of HSCT in the CNS can be limited by (i) insufficient quantity of transplanted cells being trafficked to the CNS or (ii) not enough functional lysosomal enzyme from engrafted cells being expressed in the CNS[99]. At present, most studies in this field are designed to improve the ability of stem cells to secrete functional enzyme once they have engrafted the CNS, rather than increasing the absolute number which cross the BBB, because this aim is more achievable with current knowledge and technologies. In the coming sections, we will explore innovative strategies targeted to the CNS pathology of LSDs.

6.1 Pre-conditioning agent

In bone marrow transplants, a pre-transplantation chemotherapeutic conditioning regime is essential to deplete patients' resident HSCs and, possibly, resident microglia[102,104], which in the CNS facilitates engraftment of a HSC subpopulation upon transplantation[102] (Figure 3). The most widely used pre-conditioning agent, busulfan, has been demonstrated to deplete resident microglia more effectively than alternative conditioning regimes (irradiation or treosulfan) in mice[102], specifically by causing microglial senescence and exhaustion of their regenerative ability[105]. Some studies suggest that busulfan could also be responsible for vascular injury and BBB disruption[106,107], hypothesizing that a perturbed BBB could be accountable for the increased HSC engraftment. However, recent work by Cartier and colleagues suggests a non-inflammation or non-BBB-disruption-induced permissive engraftment following busulfan conditioning[105]. Busulfan associated with significant systemic toxicity in patients[108]; in addition, in mice it has been shown to cause a permanent inhibition of adult neurogenesis, suggesting a potential cognitive deficit for patients undergoing this regime[105] and emphasising need for the future development of alternative pre-conditioning strategies with lower toxicity.

In this direction, antibody-based pre-conditioning regimes with reduced toxicity have been tested in mice[109-111], and regimes which specifically target the hematopoietic lineages have successfully been used in severe combined immunodeficiency patients[112], or immunocompetent mice and dogs[113-115]. However, the ability of

antibody-based regimes to deplete resident microglia in the CNS and allow neurological engraftment of transplanted HSCs has not been determined.

Another option might be brain-targeted conditioning; this could potentially improve treatment efficacy in the CNS of neurological LSD patients. A new synthetised and highly selective brain penetrant CSF1R inhibitor, PLX5622, has been used for extensive and specific microglial elimination in a murine model of Alzheimer's disease[116]. Moreover, in a recent study wild-type mice were pre-treated with PLX5622, lethally irradiated, and then received a bone marrow transplant. Mice receiving the CSF1R inhibitor showed a depletion of microglia and subsequent microglia replacement at the CNS-wide scale (around 90%) compared to non-treated mice, which show a minimal engraftment only in specific regions[117].

6.2 Ex vivo Stem Cell Gene Therapy Enhancement

When considering the two sources of therapeutic enzyme generated by HSCT, namely the peripheral cells of the reconstituted hematopoietic system and the tissue-resident macrophages, ex vivo gene therapy of autologous HSCs can be utilised in two ways to deliver a greater level of therapeutic benefit to the CNS. Firstly, by engineering vectors so that each genetically corrected cell secretes a supraphysiological level of enzyme, therapeutic benefit might be achieved in the CNS even with a limited number of engrafted cells. Moreover, studies have demonstrated that even a modest increase in enzyme activity in the CNS can provide therapeutic benefit; for example, restoring enzyme expression to 3.7% of wild-type levels in MPS II mice following HSCT was sufficient to correct CNS disease phenotype[118]. Secondly, modifying the enzyme sequence in the gene therapy construct so that therapeutic enzyme produced by peripheral hematopoietic cells can cross the BBB more easily also potentially enhances therapeutic effect in the CNS. Many of the methods of HSCT gene construct modification overlap with previously discussed enzyme modifications. Additionally, HSC gene therapy for LSDs has been reviewed in depth by Biffi and colleagues[6], therefore we will only briefly discuss it here.

Enzyme modification has been utilised in the HSC gene therapy setting by improving, prior to HSC transduction, the characteristics of the viral vectors used, or the therapeutic genes they contained. Similar to in ERT, fusion proteins have been included in the gene therapy constructs in order to increase uptake by the CNS[38,118]. Other modifications of the gene construct have focused on careful choice of promoters

in order to promote gene expression. Appropriate choice and manipulation of the promoter could increase production, and secretion, of supraphysiological levels of functional enzyme and potentially increase uptake by enzyme-deficient brain cells[119,120]. For example, utilisation of the myeloid promoter CD11b to promote expression of the codon-optimised therapeutic enzyme specifically in myeloid cells (including microglia) and not in progenitors or other hematopoietic cells (to avoid potential toxicity) has proven beneficial in the CNS of MPS IIIA[121] and MPS IIIB[122] murine models, and has been taken further for MPS IIIA treatment with completed pre-clinical safety studies[123].

An alternative approach to construct modification, which has been applied to a gene therapy construct but not yet combined with HSC gene therapy, focuses on promoting enzyme secretion and increase post-translational activation speed in MPS IIIA[124,125] and MPS VII[126] mice. Further investigation is required to ascertain whether these concepts could perhaps be applied to HSC gene therapy for neurological LSDs. Both modification of the enzyme or promoting its expression via editing of the gene therapy construct have resulted in improved pathology correction in neurological murine LSD models and represent a valid approach to targeting the CNS in LSDs. However, neither of these methods assist infiltration of the CNS by stem and progenitor cells.

Overall HSC gene therapy has shown to be effective in targeting the neurological pathology in LSDs[127-132] (and reviewed in[6]). HSC gene therapy clinical trials in MLD[127-129] and adrenoleukodystrophy (ALD)[130-132] patients showed high levels of therapeutic enzyme expression, reduction of storage products and improvement of the clinical phenotype. Based on the efficacy and safety profile, the European Commission (EC) granted approval for marketing of HSC gene therapies for MLD and ALD at the end of 2020 and 2021, respectively[133,134].

6.3 Injection routes

There has been less extensive investigation of transplantation injection routes in HSCT than in ERT for neurological LSDs, however similar injection sites have been tested for stem and progenitor cell delivery to the CNS (Figure 2). Studies in MPS VII[135] and MPS I[136] murine models support the use of ICV delivery to increase therapeutic effect in the CNS. Work by Capotondo and colleagues provided fundamental insight into the success of engraftment and fate of transplanted cells, demonstrating that HSCs do engraft the CNS, and give rise to microglia-like cells with

biochemical characteristics matching bona fide microglia[101]. Comparison to conventional intravenous delivery provided evidence for ICV injection leading to more rapid engraftment of the CNS and a greater abundance of therapeutic enzyme in a murine model of MLD[102]. Combined, these studies support ICV delivery to improve therapeutic benefit in the CNS of LSD patients.

7. In vivo gene therapy

Whilst we have already discussed using viral vectors for ex vivo gene therapy ~~(GT)~~, we have not yet considered them as an independent treatment option. In vivo gene therapy involves delivering the therapeutic gene directly to patients' cells using a viral vector. In LSDs, gene therapy facilitates expression of therapeutic concentrations of functional lysosomal enzyme by directly modifying a subset of patients' own cells[9]. A large range of viral vectors have been trialled for this purpose. In the last decade or so, AAVs emerged as the most useful vectors for CNS-directed gene therapy due to their transduction efficiency, wide tropism, and relative safety profile. In particular, direct administration of small, non-enveloped, and non-integrating AAVs, named recombinant adeno-associated viral vectors (rAAVs), has been trialled both systemically and locally. A comprehensive overview of retroviral, lentiviral, and adenoviral-based vectors together with a discussion of their pros and cons for in vivo gene therapy and CNS-targeting has been provided in recent reviews[9,137,138]. Here we will focus on the most relevant pre-clinical and clinical data, specifically discussing how to increase AAV-mediated CNS-targeted expression.

7.1. Use of different AAV serotypes and capsids

One of the greatest advantages of rAAVs over other viral vectors is the possibility to choose different serotypes – for example, those with CNS-tropism can be utilised with the aim of improving in vivo gene therapy outcome for neurological LSD patients. Several in vivo studies showed that serotypes 5, 8, 9, and the recombinant human (rh)10 can cross the BBB, each to a different extent, allowing transduction of the CNS following systemic administration[139-142]. For example, AAV9 was shown to be able to cross the BBB and improve neurological symptoms post-systemic administration in LSD animal models[143,144]. Two open-label, dose-escalation, Phase 1/2 global clinical trials assessing AAV9 technology via a single-dose intravenous infusion are currently

underway for young (2 years old or less) and asymptomatic (development quotient > 60) MPS IIIA (NCT02716246) and MPS IIIB (NCT03315182) patients, called ABO-102 and ABO-101 respectively. For the MPS IIIA trial, data collected at different time points (6, 12, and 24 months post-treatment) from the three dose-escalating groups, highlighted a provisional safety profile in all patients with time- and dose-dependent statistically significant reductions in cerebrospinal fluid and plasma heparan sulfate levels, and stabilisation or improvement of adaptive behaviour and/or cognitive function[145,146]. Another trial on MPS IIIA patients in middle and advanced phases of the disease receiving the highest dose of ABO-102 (3×10e13 vg/kg) has recently terminated due to lack of efficacy (NCT04088734)[147]. Preliminary results from the MPS IIIB trial were also promising, with multiple disease biomarkers providing clear evidence of a biological effect in patients[148].

Indeed, use of serotypes able to naturally target the CNS, such as AAV9, has been pivotal in providing access to the CNS. However, the low efficiency and lack of target specificity mean that high vector load needs to be used, potentially leading to toxicity. Generation of novel capsids would be important in increasing AAVs' specificity and efficiency. Years of capsid engineering efforts using different platforms have now yielded a number of improved CNS capsids for rodents, which are undergoing pre-clinical testing[149-151]. In one recently published study, Chen et al evolved a family of AAV capsid variants that can efficiently transduce both the central and peripheral nervous system in rodents. Both vectors also enable efficient targeting in non-human primates[152].

7.2 Increased AAV dosing

Historically, serotypes AAV8 and AAV9 have preferential tropism for liver and muscle[153], but when used at higher doses, these serotypes might achieve more widespread tissue expression, including in the CNS. However, dose-related neurotoxicity has been reported in large animal models treated with high doses of AAV9[154]. Severe adverse events have been described in at least three clinical trials for other genetic disorders where high doses of the vector were administered, including increased serum transaminase (NCT03306277), complement activation and acute kidney injury (NCT03362502), and sepsis-induced deaths (NCT03199469)[155]. These observations highlight the need to gather further safety data and, even when this has been obtained, these findings must be considered carefully because the

severe immune response observed in these patients was not seen previously in animal models, making the outcome of this strategy to increase widespread tissue targeting unpredictable[156].

7.3. Local delivery of AAVs

Local AAV delivery may allow BBB circumvention and enhanced delivery of therapeutics to the CNS. As described before for ERT and HSCT, there are several routes of administration to exploit (Figure 2) and the choice of one over another takes into account several factors such as injection route difficulty and its prime therapeutic sites, the type of enzyme to express, cell type(s) to target and their localisation and distribution within the CNS. As direct CNS administration routes were discussed previously (sections 5.2.1 and 6.3) and have been extensively reviewed elsewhere[157], only a few relevant examples will be discussed here.

Preliminary results of clinical trials for CLN2-deficiency (Batten disease) paediatric patients based on intracerebral injection of AAV2 (NCT00151216)[158] or AAV2/rh10 (NCT01161576, NCT01035424 and NCT01414985)[159] have demonstrated a slower rate of grey matter loss and a significantly reduced rate of neurological decline including motor and language function. Intracerebral administration of AAV2/rh10 and AAV2/5 has also been trialled for MPS IIIA (NCT01474343, NCT03612869)[160] and MPS IIIB (EudraCT 2012–000856-33)[161] respectively, showing moderate improvement in neuropsychological evaluations of behaviour, attention, and sleep. Furthermore, a phase I/II clinical trial for intracerebral delivery of AAV2/rh10 for early onset MLD has reached completion and results should be available soon (NCT01801709). In several of the children treated in these clinical trials, a mild systemic immune response was observed[159], while others presented with abnormal MRI results and experienced seizures[159], or AAV vector was present in urine[160]. These observations perhaps suggest leakage from the CNS injection site into the periphery, triggering the immune response and hampering overall in vivo gene therapy efficacy. Transient immunosuppression by neonatal AAV-mediated systemic expression of a therapeutic gene prior to CNS-targeted in vivo gene therapy, and induction of liver-mediated tolerance[142,156,162] have been trialled in MPS IIIA patients to address these concerns, with promising results[160,163].

In terms of other injection routes, a small number of clinical trials based on intrathecal/intracisternal administration of AAV9 serotype for MPS IIIA (EudraCT 2015–000359-26), MPS I (NCT03580083) and MPS II (NCT03566043) are currently underway. Only a small number of pre-clinical studies of intracerebroventricular injection have been conducted to date; pre-clinical studies in CLN2-deficient dogs with AAV2[164,165] showed delay of neurological progression and prolonged lifespan[165], however one animal experienced impaired cardiac function, likely due to augmented storage deposition in the heart[164]. In other pre-clinical studies performed in Niemann-Pick C[166], MPS IIIA[167] and MPS I mice[166], animals treated intracerebroventricularly with AAV2/9 showed reduced neurodegeneration, increased motor function and extended lifespan.

7.4 Optimisation of gene therapy cassette and AAV engineering

An indirect method to target the CNS is to engineer systemically delivered AAVs to produce enzymes which have an enhanced ability to cross the BBB. This can be achieved by including fusion proteins in the therapeutic construct (as discussed extensively in section 5.1). Alternatively, the use of tissue-specific promoters, secreting peptides and optimised gene sequences can increase expression, secretion, and uptake of the therapeutic enzyme respectively[168]. This strategy might also overcome the limitation of using serotypes that have restricted CNS tropism. In addition, bioinformatics-guided design of lysosomal enzymes may not only improve enzyme production/secretion/uptake, but also reduce immunogenicity[169].

8. Conclusions and future perspectives

Here we have explored strategies to increase the ability of enzymes, stem cells or viral particles to engraft the damaged CNS of neurological LSD' patients (Figure 4).

Whilst choosing the appropriate therapy for each LSD is of vital importance, timing of the intervention is almost as critical. Treatments administered ~~or~~ when patients are still asymptomatic, have proven to be more effective in both animal models[170-172] and patients[173-177], highlighting the need for early intervention and implementation of newborn screening (NBS) for more LSDs. In this direction, *in utero* intervention may circumvent the BBB selectivity issue, as at this developmental stage the BBB is not yet functional; moreover, transplanted cells can engraft and occupy the microglial

niche during the same developmental timeframe as resident cells[178], removing the need for pre-conditioning. To this end, a pre-clinical study in MPS VII mice showed that *in utero* delivery of ERT or HSCT improved neurological symptoms[178]. In utero HSCT has been successfully applied in small scale clinical trials for severe combined immunodeficiency patients, and less successfully for thalassemia patients[179]. However, a careful benefit/risk assessment of in utero procedures must be performed and further pre-clinical and clinical studies, would be required to support routine application.

Among all the strategies described here, BBB manipulation techniques are relatively easy and cheap compared to others, however they provide non-selective permeability, posing the risk of toxicity[24]. For clinical application to be a realistic prospect, toxicity must be limited, and patients would require strict monitoring for adverse events.

Immunogenicity of therapeutics needs to be considered carefully too, as this can trigger the immune system and subsequently reduce treatment efficacy. In the case of ERT, the repeated infusion of enzyme often results in immune reaction against the enzyme itself[64] and furthermore negatively affects therapeutic impact in the CNS; following ERT in an MPS I canine model, animals with a high titre of antibody against the therapeutic enzyme showed less significant reduction of storage accumulation in the brain than those with lower antibody titres[180]. Similarly, despite rAAVs for in vivo gene therapy having several advantages over other viral vectors, including relatively low immunogenicity[181], long-term gene expression[182,183] and wider tissue tropism, they still trigger the immune system; T-cell responses to the transgene might appear after AAV-based vector administration[184,185]. Moreover, as the majority of humans have already been exposed to several wild-type AAV serotypes, neutralizing anti-capsid antibodies might be either present in patients prior to treatment[186-188] or arise quickly following the first administration, rendering vector re-administration not a viable option[189]. Continued efforts to minimise the immunogenicity of all therapeutics is vital to the success of ERT and gene therapy, especially in the CNS where prolonged inflammation can have particularly severe negative consequences, as shown for example in the case of viral encephalitis, in Alzheimer's patients and in association with diabetes[18,19,21-23].

Another important point is the need for efficient targeting. A notable disadvantage of rAAVs for CNS-targeted gene therapy is that they can only efficiently transduce neurons, and no other disease-relevant brain cells such as microglia,

oligodendrocytes, or astrocytes[140,190]. However, not all cells must be corrected in order for treatment to be able to exert therapeutic effect due to so called “cross-correction”, especially if therapeutics have been modified to deliver supraphysiological levels of enzyme.

Among the most successful and safe strategies for neurological LSDs is HSCT gene therapy. In the last two years, two medicinal products based on HSC gene therapy strategies have been approved in Europe; Libmeldy®[129] for MLD and Skysona™[191] for ALD (NCT01896102, NCT03852498, NCT02698579). Skysona™ has also received FDA accelerated approval on September 2022[192].This has brought great enthusiasm and renewed hope to neurological LSD patients.

A further consideration for wide adoption of these single administration gene therapies in healthcare systems is pricing and reimbursement. Current models of payment for chronic therapies such as ERT accept regular costs year on year for the lifetime of an individual; the cumulative costs of which can be considerable with a recent cost-analysis estimate between €9.3-9.7 million (£8.1-8.5 million) for LSD treatment[193]. This needs to be balanced against a once off payment for single administration cell and gene therapies, where although the initial price may be considerable, this is deemed appropriate given the long term overall clinical benefit[194,195].

Even though HSC gene therapy holds ~~a~~ great potential, one main issue remains for its clinical suitability, namely the suboptimal, and in some case minimal, engraftment of HSCs to the CNS. The goal is to engraft a sufficient number of HSC-derived cells able to differentiate into microglia and act as a constant and never-ending source of enzyme secretion. A crucial role for a successful CNS engraftment is played by the conditioning regime chosen to clear the niche (by depletion of the native microglia) for donor HSCs. Engraftment to the CNS is significantly improved by busulfan conditioning compared to irradiation[102], with busulfan being the regime of choice for neurological LSD patients[196] prior to transplantation. However, busulfan is associated with a substantial systemic toxicity[108], and alternative strategies based on CNS-targeted microglial depletion may represent less toxic and safer pre-conditioning alternatives for neurological LSD patients in the longer term.

Another way to increase CNS engraftment would be to focus on improving stem cells’ crossing of the BBB; studies aiming to understand what HSC subpopulation engraft

the CNS and the mechanisms they use to cross the BBB would be helpful in devising new strategies to increase BBB cell permeability.

At the moment, no single therapeutic approach discussed here provides the perfect solution for every neurological LSD[197], supporting the idea that for these neurometabolic disorders, the CNS component remains a significant challenge. However, in these monogenic severe disorders, where there is a clear genetic component and pathway to be addressed, there is a unique opportunity to develop therapeutics that can have significant impact and which, if successful, may have wider application to more common forms of neurodegeneration.

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10. Author contributions

B.J.C wrote the manuscript's draft under the supervision of H.B.G and S.B. S.B coordinated the work, contributed to the draft, finalised the manuscript and acquired funding.

11. Conflict of Interests

H.B.G. is the CEO of Orchard Therapeutics. The other authors declare that they have no conflict of interest.

12. Keywords

Lysosomal storage diseases (LSDs); central nervous system (CNS); blood-brain barrier (BBB); enzyme replacement therapy (ERT); hematopoietic stem/progenitor cell (HSC) transplantation; gene therapy.

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Figure 1. The neurovascular unit and blood-brain barrier transport pathways. Graphical depiction of the neurovascular unit (NVU), the fundamental anatomical and functional unit of the blood-brain barrier (BBB), including the key protein components of tight junctions (TJs) between brain endothelial cells which control the paracellular transport pathway. Alternatively, molecules may be transported transcellularly via passive diffusion (a), carrier-mediated transcytosis (CMT) (b), receptor-mediated transcytosis (RMT) (c) or adsorptive mediated transcytosis (AMT) (d).

Figure *2*. Clinically relevant delivery routes for LSD therapeutic agents. Graphical summary of the injection routes used to deliver enzyme replacement therapy (ERT), stem cells and viral vectors in neurological LSDs.

Figure 3. Overview of hematopoietic stem and progenitor cell transplantation (HSCT) approaches. Graphical summary of HSCT using either allogenic donor cells, or genetically modified patient cells in order to secrete a supraphysiological level of the defective enzyme. HSCT mediates therapeutic effect in the central nervous system by a HSC subpopulation crossing the BBB, engrafting the CNS, and generating genetically modified microglia, which provides a source of therapeutic enzyme to cross-correct neighbouring enzyme-deficient brain cells.

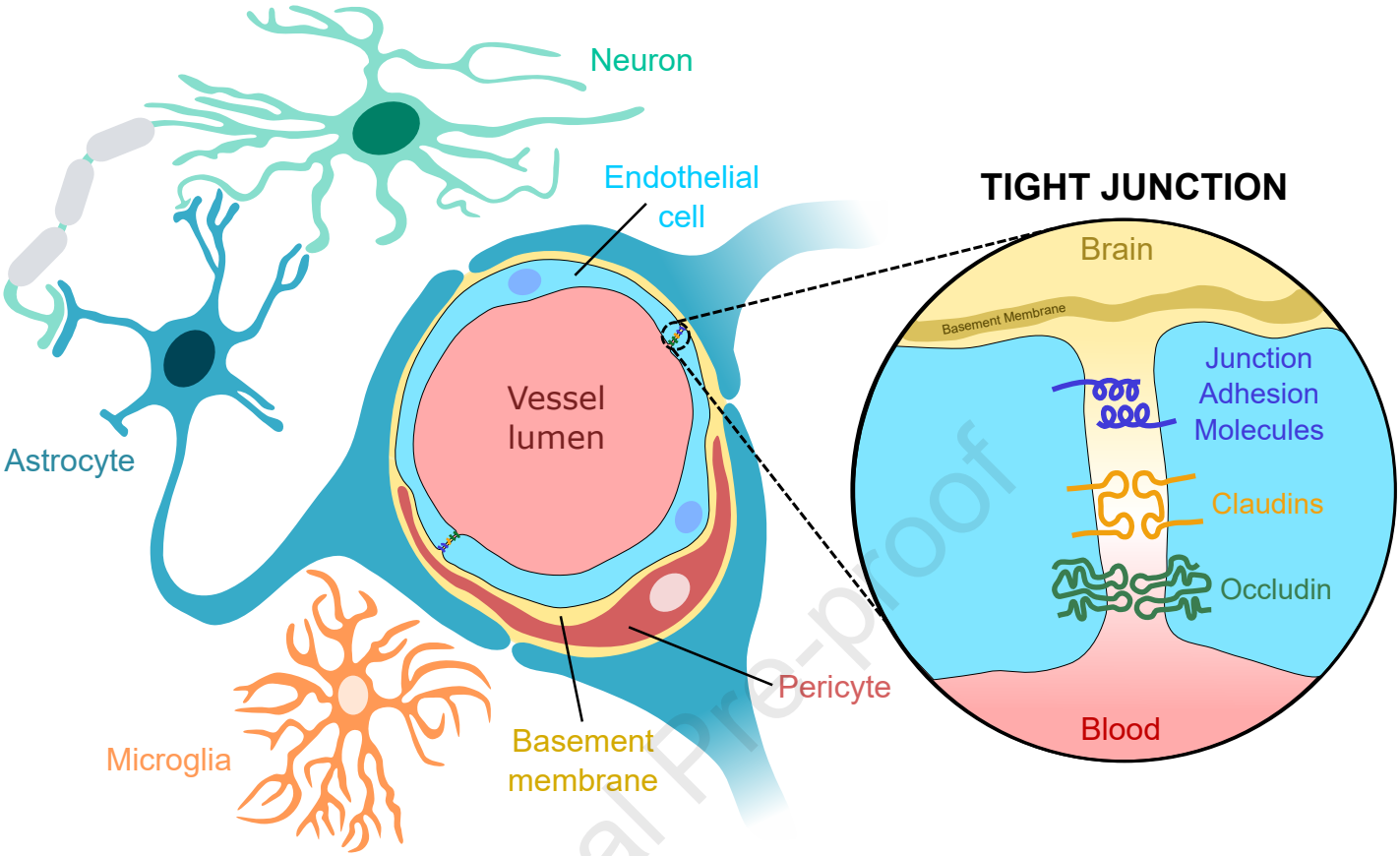
Figure 4. Summary of the different strategies used to improve delivery of therapeutic agents to the central nervous system (CNS) in the treatment of lysosomal storage diseases (LSDs). (A) BBB disruption strategies: (i) ultrasound or (ii) hyperosmotic agents can be used to disrupt the integrity of the blood-brain barrier (BBB); (iii) stimulation of receptors can increase passage of enzymes and/or stem cells across the BBB. **(B)** Enzyme replacement therapy can be targeted to the CNS by modifying enzymes directly (i) or using delivery vehicles to facilitate easier passage across the BBB (ii). A range of delivery methods (iii) including convection enhanced delivery, direct injection routes and delivery vehicles can be used to target the CNS**.**

(C) Ex vivo genetic modification of stem cells using gene therapy (i), different pre-conditioning regimes/agents (ii) and different injection routes (iii) have been trialled to improve CNS targeting in stem and progenitor cell transplantation. **(D)** Modifications of gene therapy constructs, including optimisation of the gene cassette (i) and selection of viral serotype with CNS tropism (ii), and specific injection routes (iii) can be utilised to target the CNS with in vivo gene therapy approaches

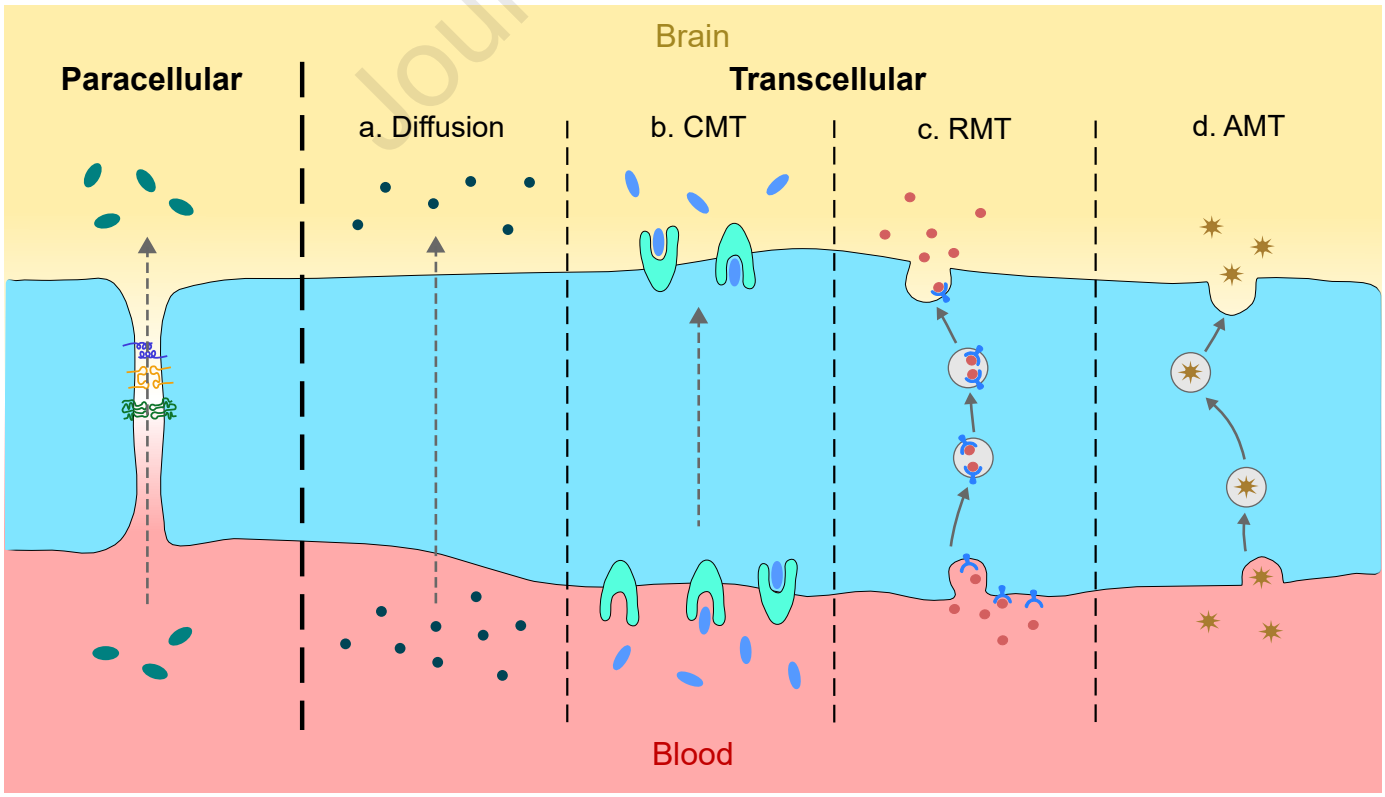
Table 1. Neurological LSDs. Summary of neurological LSDs including details of defective gene, primary protein involved and associated lysosomal storage product. Adapted from Platt et al[1].

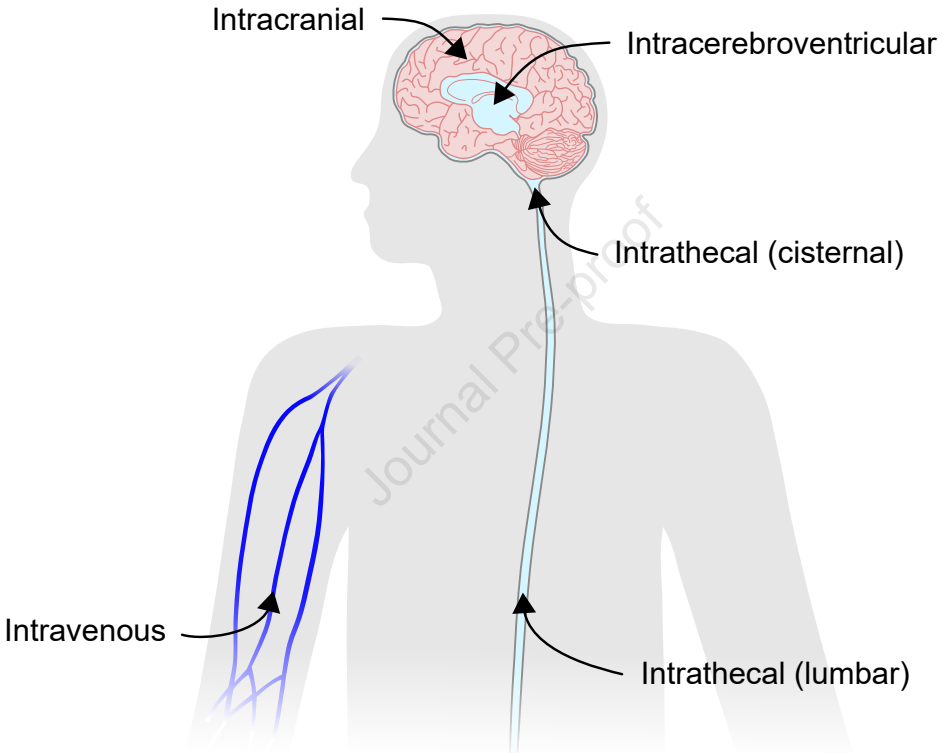
Neurological LSD (*gene*)	**Primary defective protein (Substrate/storage product)**
Mucopolysaccharidoses (MPS)	
MPS I – Hurler Syndrome (*IDUA*)	α-L-Iduronidase (Dermatan sulphate, heparan sulphate)
MPS II (*IDS*)	Iduronate 2-sulphatase (Dermatan sulphate, heparan sulphate)
MPS III Type A (*SGSH)*	N-Sulphoglucosamine sulphohydrolase (Heparan sulphate)
Type B (*NAGLU*)	N-Acetyl-α-glucosaminidase (Heparan sulphate)
Type C (*HGSNAT*)	Heparan-α-glucosaminide-N-acetyltransferase (Heparan sulphate)
Type D (*GNS*)	N-acetylglucosamine-6-sulphatase (Heparan sulphate)
MPS VII (*GUSB*)	β-Glucuronidase (Dermatan sulphate, heparan sulphate, chondroitin 6-sulfate)
Sphingolipidoses	
Fabry disease (*GLA*)	α-Galactosidase A (Globotriaosylceramide)
Gaucher disease – Type II, III and perinatal lethal form (*GBA*)	β Glucocerebrosidase, (Glucocerebroside and glucosylsphingosine)
GM1 gangliosidosis – Type I, II and III (*GLB1*)	β-Galactosidase (GM1 ganglioside, keratan sulphate and oligosaccharides)
GM2 gangliosidosis Tay-Sachs (*HEXA*)	β- Hexosaminidase (GM2 ganglioside, glycosphingolipids and oligosaccharides)
Sandhoff (*HEXB*)	β- Hexosaminidase (GM2 ganglioside, GA2 glycolipid and oligosaccharides)
GM2 activator deficiency (*GM2A*)	GM2 ganglioside activator (GM2 ganglioside and glycosphingolipids)
Krabbe disease, also known as Globoid cell leukodystrophy (*GALC*)	Galactosylceramidase (Galactocerebroside and psychosine)
Metachromatic leukodystrophy (*ARSA* and *PSAP*)	Arylsulfatase A and prosaposin (Sulfatides)
Niemann–Pick disease type A (*SMPD1*)	Sphingomyelin phosphodiesterase (Sphingomyelin)
Glycoproteinoses	
α-Mannosidosis Type I, II and III (*MAN2B1*)	Lysosomal α- mannosidase (Mannose- rich oligosaccharides)
β-Mannosidosis (*MANBA*)	β- Mannosidase (Man(β1>4) N-acetylglucosamine)
Fucosidosis (*FUCA1)*	α-L-Fucosidase (Fucose-rich oligosaccharides, glycoproteins and glycolipids)
Aspartylglucosaminuria (*AGA*)	Aspartoglucosaminidase (Aspartylglucosamine)
Schindler disease – Types I-III (*NAGA*)	α- N-Acetylgalactosaminidase (Sialylated or asialo glycopeptides and glycosphingolipids)
Sialidosis type II (*NEU1*)	Neuraminidase-1 (Sialylated oligosaccharides and glycopeptides, LAMP1 and amyloid precursor protein)
Glycogen storage diseases (GSD)	
GSD II, also known as Pompe disease (GAA)	Lysosomal α-glucosidase, also known as acid maltase (Glycogen)
Lipid storage diseases	

Acid lipase deficiency – Wolman disease (*LIPA*)	Lysosomal acid lipase/cholesteryl ester hydrolase (Cholesteryl esters, triglycerides and other lipids)
Post-translational modification defects	
Mucolipidosis II – I-cell disease (*GNPTAB*)	N- Acetylglucosamine-1-phosphotransferase subunits α/β (Oligosaccharides, glycosaminoglycans and glycosphingolipids)
Integral membrane protein disorders	
Danon disease (*LAMP2*)	L AMP2 (Cytoplasmic debris and glycogen)
Action myoclonus-renal failure syndrome (*SCARB2*)	Lysosomal integral membrane protein (Unknown)
Sialic acid storage disease (*SLC17A5*)	Sialin (Sialic Acids)
Niemann-Pick disease – Type C (*NPC1* and *NPC2*)	NPC intracellular cholesterol transporter 1 and 2 (Cholesterol and sphingolipids)
Mucolipidosis IV (*MCOLN1*)	Mucolipin 1 (Lipids and mucopolysaccharides)
Neuronal ceroid lipofuscinoses (largely unknown heterogeneous mix of substrates)	
CLN1 (*PPT1*)	Palmitoyl- protein thioesterase 1 (Lipidated thioesters and saposins A and D)
CLN2 (*TPP1*)	Tripeptidyl peptidase 1 (Subunit c of mitochondrial ATP synthase)
CLN3 (*CLN3*)	Battenin (Subunit c of mitochondrial ATP synthase)
CLN4 (*DNAJC5*)	Cysteine string protein (Subunit c of mitochondrial ATP synthase)
CLN5 (*CLN5*)	Ceroid- lipofuscinosis neuronal protein 5 (Subunit c of mitochondrial ATP synthase)
CLN6 (*CLN6*)	Transmembrane ER protein (Subunit c of mitochondrial ATP synthase)
CLN7 (*MFSD8*)	Major facilitator superfamily domain containing 8 (Subunit c of mitochondrial ATP synthase)
CLN8 (*CLN8*)	Protein CLN8 (Subunit c of mitochondrial ATP synthase)
CLN9 (*N/A*)	N/A
CLN10 (*CTSD*)	Cathepsin D (Saposins A and D)
CLN11 (*GRN*)	Granulin (Unknown)
CLN12 (*ATP13A2*)	Cation- transporting ATPase 13A2 (Inorganic cations)
CLN13 (*CTSF*)	Cathepsin F (Unknown)
CLN14 (*KCTD7*)	Potassium channel tetramerization domain containing 7 (Unknown)
Lysosome-related organelle disorders	
Chédiak–Higashi disease (*LYST*)	Lysosomal trafficking regulator (Size and movement of lysosomes)

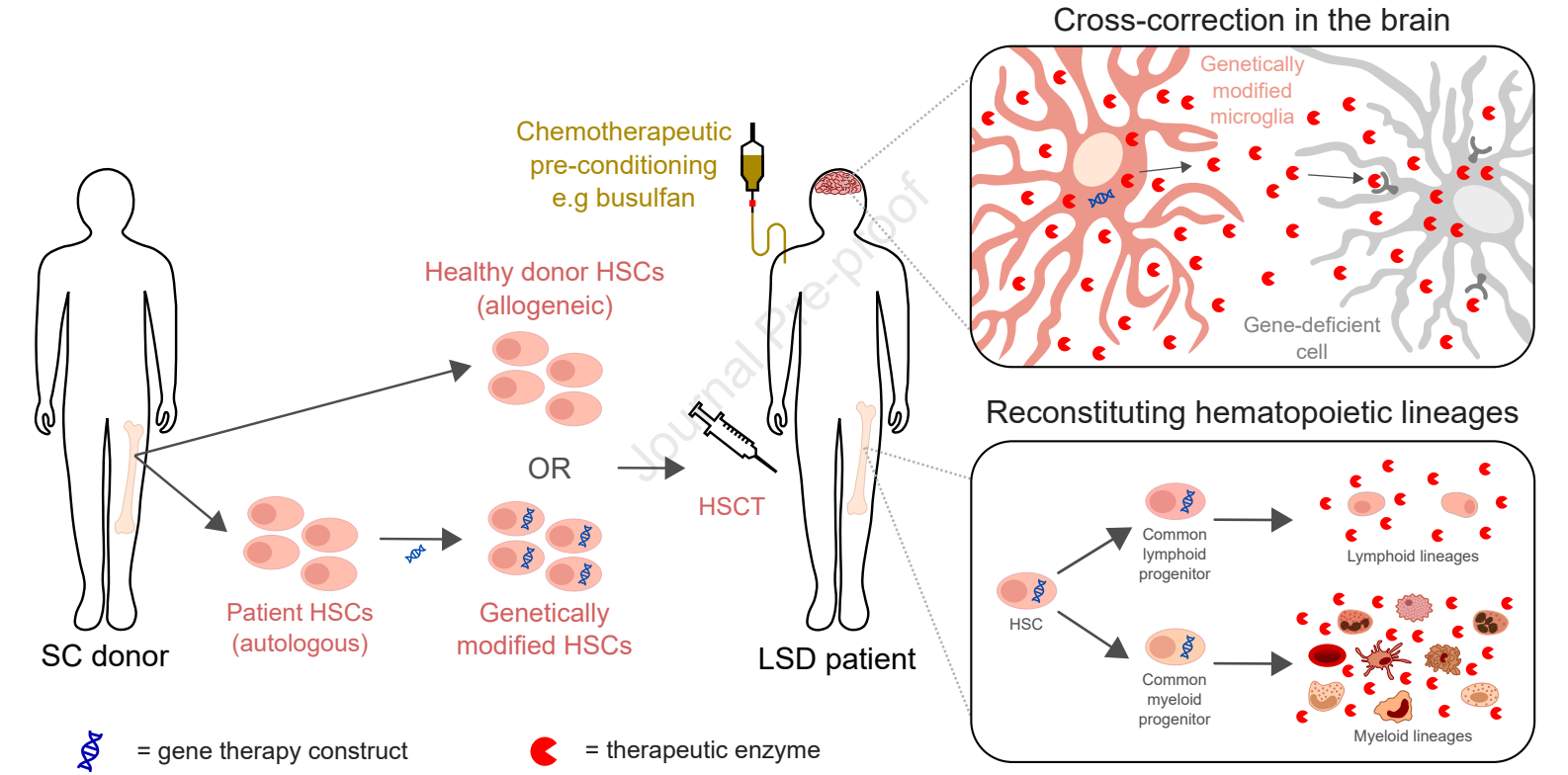


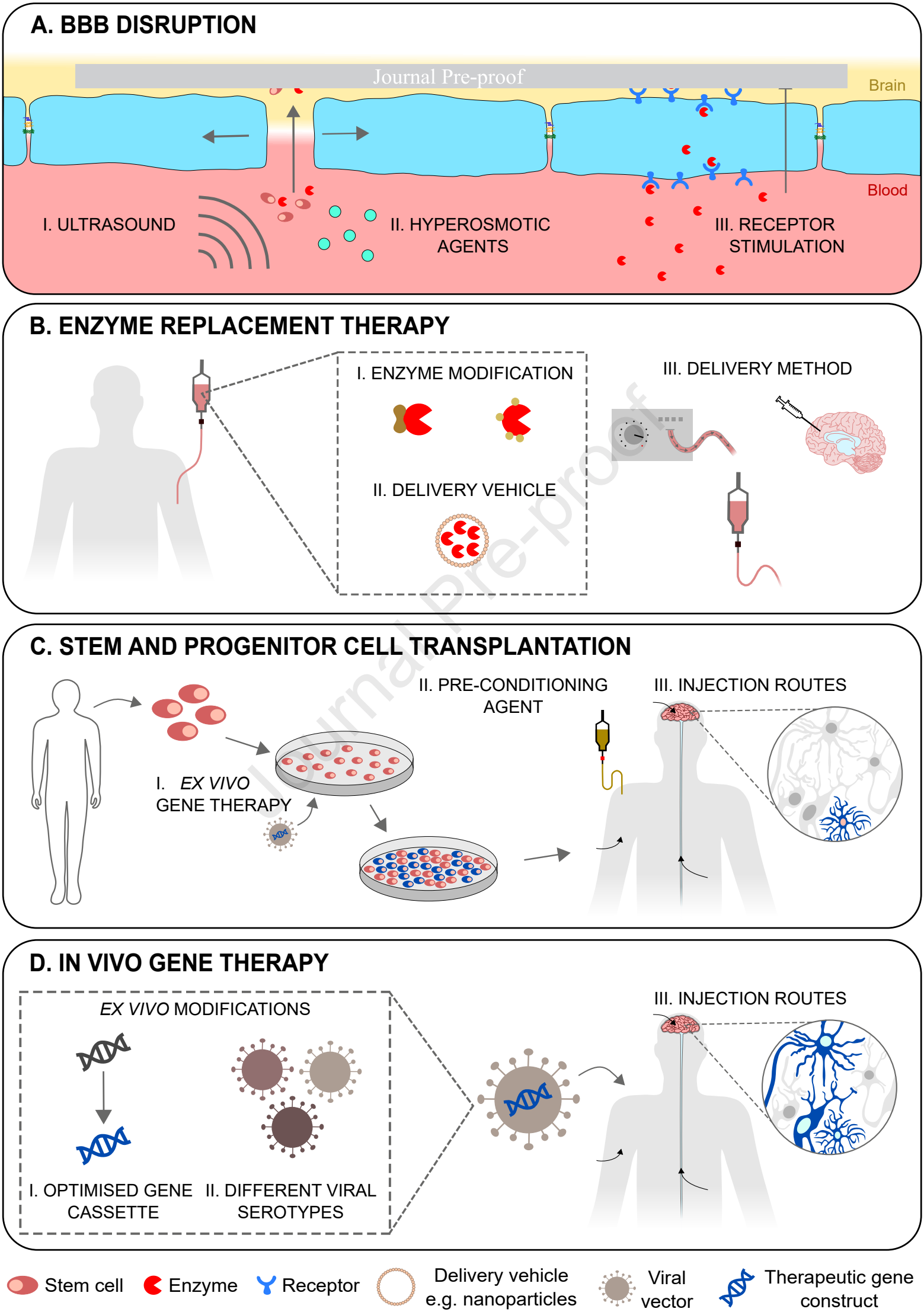
BLOOD-BRAIN BARRIER TRANSPORT PATHWAYS





HEMATOPOIETIC STEM AND PROGENITOR CELL TRANSPLANTATION




A. BBB DISRUPTION
Journal Pre-proof
Brain
Blood
I. ULTRASOUND
II. HYPEROSMOTIC AGENTS
III. RECEPTOR STIMULATION
B. ENZYME REPLACEMENT THERAPY
I. ENZYME MODIFICATION
II. DELIVERY VEHICLE
III. DELIVERY METHOD
C. STEM AND PROGENITOR CELL TRANSPLANTATION
I. EX VIVO GENE THERAPY
II. PRE-CONDITIONING AGENT
III. INJECTION ROUTES
D. IN VIVO GENE THERAPY
EX VIVO MODIFICATIONS
I. OPTIMISED GENE CASSETTE
II. DIFFERENT VIRAL SEROTYPES
III. INJECTION ROUTES
Stem cell
Enzyme
Receptor
Delivery vehicle e.g. nanoparticles
Viral vector
Therapeutic gene construct

Benedetti and colleagues provide a comprehensive overview of therapeutic strategies targeting the CNS for the treatment of neurological Lysosomal Storage Disorders, a group of severe metabolic diseases. The review focuses on the latest advancements in improving therapeutics' delivery across the blood-brain barrier and comment upon outstanding questions in the field.