REVIEW ARTICLE

Targeting Neurological Aspects of Mucopolysaccharidosis Type II: Enzyme Replacement Therapy and Beyond

Alessandra Zanetti[1,2] · Rosella Tomanin[1,2]

Abstract

Mucopolysaccharidosis type II (MPS II) is a rare, pediatric, neurometabolic disorder due to the lack of activity of the lysosomal hydrolase iduronate 2-sulfatase (IDS), normally degrading heparan sulfate and dermatan sulfate within cell lysosomes. The deficit of activity is caused by mutations affecting the *IDS* gene, leading to the pathological accumulation of both glycosaminoglycans in the lysosomal compartment and in the extracellular matrix of most body districts. Although a continuum of clinical phenotypes is described, two main forms are commonly recognized—attenuated and severe—the latter being characterized by an earlier and faster clinical progression and by a progressive impairment of central nervous system (CNS) functions. However, attenuated forms have also been recently described as presenting some neurological involvement, although less deep, such as deficits of attention and hearing loss. The main treatment for the disease is represented by enzyme replacement therapy (ERT), applied in several countries since 2006, which, albeit showing partial efficacy on some peripheral organs, exhibited a very poor efficacy on bones and heart, and a total inefficacy on CNS impairment, due to the inability of the recombinant enzyme to cross the blood–brain barrier (BBB). Together with ERT, whose design enhancements, performed in the last few years, allowed a possible brain penetration of the drug through the BBB, other therapeutic approaches aimed at targeting CNS involvement in MPS II were proposed and evaluated in the last decades, such as intrathecal ERT, intracerebroventricular ERT, ex vivo gene therapy, or adeno-associated viral vector (AAV) gene therapy. The aim of this review is to summarize the main clinical aspects of MPS II in addition to current therapeutic options, with particular emphasis on the neurological ones and on the main CNS-targeted therapeutic approaches explored through the years.

1 Introduction

Mucopolysaccharidosis type II, also known as Hunter disease, first described more than 100 years ago, is a rare genetic condition, transmitted as an X-linked trait, and belonging to the wide group of the lysosomal storage disorders (LSD) [1]. Together with a general somatic involvement affecting most organ systems, a distinctive trait of about two-thirds of the patients is heavy neurological impairment, mainly characterized by a significant developmental delay

Key Points

About two-thirds of MPS II patients present with the severe form of the disease, which affects all organs including the CNS, with a significant impairment of cognitive and behavioral functions.

Up until now, several therapeutic strategies targeting the CNS have been developed, including cell-based therapies, gene therapy, and genome-editing therapies, as well as brain-targeting ERT approaches. Some of them are still under clinical trial, while some have been approved by the regulatory agencies for CNS treatment of MPS II.

All approaches so far evaluated for MPS II present both advantages and limitations, and thus far no ideal therapeutic solution is available for the neurological disease, although among the therapies under development, the most promising appear to be fusion proteins.

✉ Rosella Tomanin
   rosella.tomanin@unipd.it

1   Laboratory of Diagnosis and Therapy of Lysosomal
    Disorders, Department of Women's and Children's Health
    SDB, University of Padova, Via Giustiniani, 3, 35128 Padua,
    Italy

2   Istituto di Ricerca Pediatrica Città della Speranza,
    35127 Padua, Italy

and behavioral difficulties, with hyperactivity and impulsivity, once defined as aggression [2]. From the retrospective clinical data collected during the last decades by the international registry known as the Hunter Outcome Survey (HOS) [3, 4], we know that early signs of the disease may appear around 1–1.5 years of age, especially in the severe forms. However, children are not commonly diagnosed precociously, due to the overlapping signs and symptoms with other pediatric neurological disorders, misleading the correct diagnosis [5]. This causes a significant delay also in the application of the available therapeutic options, reducing their potential efficacy.

Although it remains a therapeutic option in some countries, hematopoietic stem cell transplantation (HSCT) has not shown great efficacy in MPS II [6, 7]. For a long time the high risk/benefit ratio has discouraged HSCT application in the disease. However, the development of a new preconditioning regimen prior to HSCT in the last 2 decades has reduced the mortality rate, as well as risks of infection and graft-versus-host disease [8]. A lowering of the age at transplant has led to a better outcome in some more recently described cases [9, 10].

Together with symptomatic therapy, the most applied therapeutic strategy since 2006 is enzyme replacement therapy (ERT), consisting of weekly intravenous (i.v.) injection of the recombinant functional enzyme. However, ERT, showing some efficacy at the somatic level, has been revealed to have poor efficacy or be totally ineffective in all central nervous system (CNS) manifestations [2, 11].

Hence, the challenge to find an efficient treatment for the brain impairment in MPS II, as well as for other neuronopathic pediatric disorders sharing similar problems of drug impenetrability in the brain, remained mostly unanswered, albeit several brain-targeted therapeutic strategies have been proposed and tested, both preclinically and clinically, throughout the years. Finding a brain-targeted path to reach and treat the neurological implications of the syndrome would represent a hope for many other pediatric diseases where the CNS is involved, leading the way to other drugs that could be delivered using the same or similar pathways or strategies.

In the present review, we report the main clinical features of the disease, including the neurological ones, and summarize the most significant brain-targeted approaches evaluated in recent years for MPS II, their technical advantages/limitations, and the positive outcomes or difficulties encountered in their preclinical or clinical application.

2 MPS II: The Disease and its Clinical Features

Mucopolysaccharidosis type II (MPS II), or Hunter disease, is a rare pediatric metabolic disorder caused by the deficit of the lysosomal hydrolase iduronate 2-sulfatase (IDS). Hunter disease is inherited as an X-linked trait; therefore, only male subjects are affected, though a few cases of female patients have also been reported in literature, mostly due to an unbalanced inactivation of the X chromosome during the lyonization process in the female carriers [12–14].

IDS is an enzyme required to degrade the glycosaminoglycans (GAG) heparan sulfate and dermatan sulfate; hence, its deficit causes the accumulation of these two macromolecules, both within the endolysosomal compartment and in the extracellular matrix. Such pathological deposits progressively impair cellular functioning, leading to cell death in most tissues and organs.

MPS II belongs to the cluster of mucopolysaccharidoses (MPSs), a subgroup of the lysosomal storage disorders (LSD) that overall includes 12 different syndromes so far (including the recently discovered MPS-plus [15] and Usher syndrome type IV [16, 17]). The disease has a reported incidence that varies in different geographic areas and from one country to another, ranging from 0.38 per 100,000 live newborns in Brazil to 1.09 per 100,000 live newborns in Portugal, being in general much lower in European countries compared with East Asian ones [1]. It is also likely that the aforementioned incidence rates may be underestimated at the moment, since the diagnosis of MPS II remains difficult and in some countries underconsidered [18]. Except for the very few suspected cases highlighted by newborn screenings, which sometimes include MPS II [19–22], diagnosis of Hunter commonly starts with clinical evidence of early signs or symptoms known to be associated to the disorder or in general to mucopolysaccharidoses. However, this may be challenging if symptoms are subtly expressed [23], thus contributing to a delayed diagnosis. Next, evaluation of urinary GAG content is performed, followed by the analysis of some lysosomal enzyme activities, enabling a differential diagnosis between MPS II and the other MPSs. The detected enzyme deficit finally drives the molecular identification of the causal gene variant [24]. In recent years, the application of the next generation sequencing approach has allowed the process to be reversed and shortened by applying a genome-targeted (gene panels) [25, 26] or untargeted (whole genome or whole exome sequencing) [27] analysis to the DNA of the subjects with suspected MPS II. This eliminates the intermediate steps, reducing costs and accelerating a patient's diagnosis.

Although MPS II generally occurs on a continuum of clinical phenotypes, commonly two main forms are recognized—attenuated and severe—albeit an intermediate form has also been reported in some cases. The severe phenotype, present in about two-thirds of the diagnosed patients [28], has an earlier onset than the other two forms, before 2 years of age [29], and also on average an earlier *exitus*, commonly around the second decade of life. The attenuated form presents with a later onset, around 3.5–4 years of age, and a longer life expectancy, commonly mid-adulthood, with patients possibly reaching their 50s or 60s in some cases [30–32]. Clinical peripheral involvement is equally compromised in all forms, with almost all of the organs progressively involved [33], although in the attenuated cases the progression is described as slower. Atypical as well as late-onset presentations have been rarely described [34, 35]. IDS enzyme activity is rather defective in all subjects, commonly undetectable at biochemical evaluation, and this does not help the differential diagnosis and the following prognosis of the neurological impairment, which characterizes the severe form and which will be discussed in detail in the next section. The disease has different signs and symptoms, although the most common traits are coarse facial features, skeletal deformities and joint stiffness, growth retardation, cardiorespiratory impairment [36, 37] including a diffuse valvulopathy [38], hearing loss, and organomegaly. Clinical diagnosis is mainly based on the typical facial features, liver and spleen enlargement, dysostosis multiplex, growth retardation, and joint stiffness often observed by orthopedists at the time of the first evaluation [39]. Neurological involvement is also observed in about two-thirds of the cases [2, 40, 41], commonly in the severe forms of the disease. In MPS II patients, death commonly occurs because of cardiorespiratory failure [1, 42].

From 2005 to 2022, an international survey collecting data on patients with a confirmed diagnosis of MPS II was carried out, known as the Hunter Outcome Survey (HOS). Aims of the project were to better describe the natural history of the disease and to monitor the long-term ERT efficacy, as most of the enrolled subjects were under enzyme treatment [3].

3 Neurological Aspects of MPS II

3.1 Pathogenesis of Neurological Disease

CNS disease comprehension has always been particularly difficult in MPS II. Since in the vast majority of the patients, no or extremely low levels of IDS enzyme activity can be detected, independently from the clinical form of the disease, it remains unclear which other primary or secondary molecular alterations, apart from the common enzyme deficit, could drive the neuronopathic prognosis in severe patients. Focusing our attention on the primary effect of the IDS deficit, i.e., the GAG accumulation progressively involving cells, tissues, and organs and causing their malfunctioning, may provide some pathogenetic information. However, it does not seem to be sufficient to explain the entire pathological scenario [43]. For a few years, we have known that elevated levels of brain heparan sulfate (HS) and partially catabolized HS fragments are commonly identified in MPS patients with significant central nervous system involvement [44]. However, several questions remain open. What determines the higher HS level in the brain of neuronopathic patients considering the poor genotype–phenotype correlation? When does this pathological accumulation start? What is the primary metabolic alteration? Hence, there is a connection between CNS involvement and HS accumulation, which, however, cannot explain the neuronopathic drift and its origin. Other secondary deposits, as cholesterol, as well as alterations involving basic processes such as autophagy and mitophagy, essential for correct cell functioning, together with morphogen signaling, have been identified as altered in MPS II and in other LSD [43]. Bhalla et al. [45] in 2020 reported high levels of the marker of neuronal degeneration neurofilament light chain (Nf-L) in cerebrospinal fluid (CSF) and serum of MPS II neuronopathic patients; moreover, Nf-L levels correlated with CSF HS levels. The same authors identified high levels of lysosomal lipids such as gangliosides GM3 and GD3, bis(monoacylglycerol)BMP, and glucosylceramides in CSF. In addition, we know that the neurodevelopment of the children appears mostly normal in their first year of life, but it remains unclear when the neurological drift begins. To help the understanding of the origin of CNS impairment, several in vivo and in vitro models have been proposed and analyzed. Two brain areas of the MPS II mouse model were analyzed a few years ago from our group by an RNA-Seq approach [46], which identified in the model versus the wild type, differentially expressed genes involved in mitochondrial impairment and oxidative stress and some possible connections with chronic neurodegenerative diseases, as Alzheimer and Parkinson's diseases. Differential alterations between the severe and the attenuated forms, which could be extremely informative on the primary metabolic alterations driving toward a neuronopathic prognosis, are currently mainly conducted in reprogrammed human-induced pluripotent stem cells. They mostly derived from primary somatic cells of the patients [47, 48], which can be subsequently differentiated neurogenically [49–51]. This strategy could help delve deeper MPS II CNS pathogenesis,

being at the same time an easy model for the testing of new CNS-targeted therapeutic molecules.

3.2 Neurological Signs and Symptoms

Together with a wide range of somatic symptoms, at least two-thirds of MPS II patients present a deep progressive neurological impairment, mainly identified with a developmental delay of cognitive skills and significant behavioral problems [28]. In patients with Hunter syndrome many genomic variants have been identified, most of which are private, being half of them present only in a single family. Thus, although each patient is hemizygote, carrying only one variant, the analysis so far conducted have highlighted a limited genotype–phenotype correlation. In the experience of our laboratory, even in cases where the molecular diagnosis was successfully conducted in the early phases of the disorder, it is very difficult to understand a possible prognosis for the patients. This is different in the cases of large deletions/insertions or recombinational events, for the vast majority associated with a severe clinical phenotype, with rapid neuronopathic evolution (Zanetti et al.; personal communication). A high level of CSF GAG has been reported in MPS II patients with neurocognitive impairment [52], while in the attenuated forms the level was reported as intermediate between severe MPS II patients and surrogate-normal children.

Thus, to better define how the disease will evolve, it is important to strictly monitor the patients with suspected Hunter disorder using a multidisciplinary approach, which, for the neurological form, will be extremely difficult to conduct due to important differences in the timing of its development and progression [53] and considering that attenuated forms may also present some neurological involvement, although less intense, such as deficits of attention and hearing loss [54]. According to a recent paper, among all mucopolysaccharidoses, "MPS II has the greatest uncertainty about the neurocognitive progression of the disease" [53]. As in other neuronopathic MPSs, in MPS II, neurologic development is also initially normal, and then it declines toward devastating impairment.

Together with neurocognitive decline, the main features that are most commonly described are the following: behavioral difficulties with hyperactivity/impulsivity, seizures [2, 41], communicating hydrocephalus, sleep apnea, and spinal cord compression. A study conducted in 2011 on 36 Italian patients described a very complex scenario of magnetic resonance imaging (MRI) features, where perivascular spaces enlargement (89%), white matter abnormalities (WMAs; 97%), subarachnoid space enlargement (83%), and IIIrd-ventricle dilatation (100%) were very often associated [55]. In the same study, about half of the patients showed spinal stenosis. Subarachnoid spaces

and ventricle enlargement, WMAs, and spinal stenosis were seen to progress despite ERT and might represent potential disease severity biomarkers. Spinal cord compression is often described in these children, commonly treated by surgical decompression [56]. Seizures are a less common sign (about 40% of the patients); they are usually of tonic–clonic type, but can also be focal and absence seizures, and respond to standard anticonvulsant treatments [2, 57]. Hyperactive/impulsive behavior is reported, in the absence of therapy, in about 35–40% of the cases [58], and it may be worsened by sleep disturbances [59, 60]. A recent paper underlined that behavioral and sleep manifestations are early clinical markers of CNS involvement in the neuronopathic forms of the disease [58].

The treatment of this devastating set of neurological manifestations has always represented a challenge for the scientific and medical community as well as for the pharmaceutical companies. Hunter disease is one of the many neurological and neurometabolic disorders for which difficulties in reaching the brain region impede effective use of drugs that have already been developed. The blood–brain barrier (BBB), a selectively permeable barrier between the CNS and the systemic circulation [61], works as an efficient shield to protect the brain from most forms of attack, such as toxins and pathogens. It also represents an insurmountable barrier for most drugs, which are unable to reach the correct brain target unless they are small, highly lipophilic molecules [57, 62]. Specifically for the enzymes, because of their size, molecular instability and chemical-physical properties, the delivery of these molecules represents a particularly difficult challenge [63].

4 Therapeutic Approaches, an Overview

Until the introduction of hematopoietic stem cell transplantation (HSCT) and then of enzyme replacement therapy (ERT), the management of MPS II was mainly focused on relieving the signs and symptoms of the disease. Current symptomatic therapeutic approaches are aimed at treating symptoms that have remained unresolved following the advent of ERT, such as those related to the neurological system (seizure, communicating hydrocephalus, spinal cord compression, and carpal tunnel syndrome), to the skeletal system (dysplasia and limited range of motion), and to the cardiac apparatus (mainly valvulopathies) [59].

Attempts have been made to use bone marrow transplantation (BMT) and hematopoietic stem cell transplantation (HSCT) in these patients; in most cases a poor efficacy in these children was observed, principally on the neurological side. This is very different from what happened with a related disease, MPS I or Hurler disease, where transplant is considered to be a standard of care [56]. This represented

a strong step back in the treatment of Hunter disease, since allogeneic HSCT might provide healthy cells able to cross the blood–brain barrier, thus supplying functional enzymes to treat the CNS disease through the mechanism of cross-correction [64], and in most cases, this needs to be performed only once in life [56]. It is supposed that this significantly different outcome between the two disorders may be due to the significant diagnostic delay of MPS II versus MPS I, allowing for irreversible damage in the brain, which therefore cannot benefit much from the transplant [56]. The inclusion of MPS II in a newborn screening program will potentially change this landscape, as the sooner any therapies can be applied, the better the patients' outcome will be. An early application of HSCT may also result in a better outcome [9, 10], although the procedure remains quite risky in these patients.

Since it is due to the deficit of a lysosomal hydrolase, iduronate 2-sulfatase, MPS II was one of the first disorders for which a protocol of enzyme substitution, able to provide the functional hydrolase, was developed. Enzyme replacement therapy consists of the intravenous administration of the functional recombinant version of IDS enzyme and has been available since 2006. In 2012, an additional recombinant enzyme, idursulfase beta, was approved for MPS II treatment [65]. This followed previous preclinical and clinical evaluations conducted in two other LSD, where strategies of enzyme substitution were proposed starting several years before, in 1973 for Fabry disease [66] and in 1974 for Gaucher disease [67], in both cases using enzymes isolated from the human placenta.

Clinical data collected in these 17 years of application of ERT protocol have clearly shown, since the analysis of the first data, that the administration of the recombinant enzyme exerts some benefits at the somatic level, whereas it has no efficacy in all CNS manifestations [68]. Indeed, the effectiveness of ERT for MPS II patients is significantly limited by its inability to address the neurological manifestations of the disease, as the recombinant enzyme does not cross the blood–brain barrier (BBB) at therapeutic concentrations. This is due to its large molecular size, 76 kDa, as only small hydrophobic molecules (< 500 daltons) can cross the BBB and reach an effective concentration in the brain [69], and to its chemical-physical properties. Moreover, a general inability of the lysosomal enzymes to reach the brain region due to a progressive decrease of cation-independent mannose 6-phosphate receptor (CI-M6PRs) expression, at BBB level, during brain maturation has been described [70, 71]. This represents a great unmet need for suitable treatment options [72]. Such evidence, compounded with the elevated costs of ERT, has somehow put up for discussion the opportunity to treat Hunter patients presenting with a severe form

[2, 11, 73], together with the criteria for initiating or ending the treatment with ERT [74].

To tackle this issue, different approaches based on cell therapies, gene therapies, and genome-editing technologies, as well as on brain-targeting ERT approaches, have been studied in the last 10 years (Fig. 1). All of them are based on a biological process called cross-correction, by which the therapeutic enzyme is released into extracellular fluid and endocytosed by neighboring and distant cells through CI-M6PRs binding, thus correcting the enzyme deficit [64, 75, 76].

5 Enzyme Replacement Therapy: Advantages and Limitations

As cited above, ERT has long been the most promising and applied therapeutic strategy for Hunter disease, and still is. The enzyme substitution was developed and then approved by the US FDA in 2006 (FDA Biologic License Application: 125151), while in 2007 the European Medicines Agency (EMA) authorized its use in the European Union (EMA product number: EMEA/H/C/000700). Disease-specific treatment is available in the form of the recombinant enzyme idursulfase (Elaprase®, Shire Human Genetic Therapies, USA, now Takeda Pharmaceuticals USA, Inc., Lexington, MA, USA), administered intravenously weekly. Starting 2005, a registry called the Hunter Outcome Survey (HOS), a multinational long-term survey monitoring the natural history of the disease, as well as safety/efficacy of ERT with Elaprase® [3], was established, and it was completed at the end of 2022 (https://mpssociety.org.uk/files/hos-patient-report-2023.pdf). The study progressively enrolled 1405 patients from 34 countries and provided a huge amount of data, related to the disease and the outcomes of the treatment.

In addition, in 2013 a study described the Phase I/II clinical trial conducted with a second drug called idursulfase beta (Hunterase®, Green Cross Corp.,Yongin, Korea), a recombinant IDS that is produced using a genetic engineering approach in Chinese hamster ovary (CHO) cell line [77]. Hunterase® received its first approval by the Korean FDA in 2012 and afterwards from several other drug agencies, including the US FDA and EMA, and is presently marketed in 12 countries.

Analyses of ERT safety/efficacy in MPS II has been widely conducted. Both drugs are generally well tolerated and have shown an acceptable safety profile, although infusion-associated reactions have been quite often registered, most commonly controlled by an antihistaminic, antipyretic, or corticosteroids premedication [78–80]. Some cases of anaphylactoid reactions were also reported, representing a very serious complication of the treatment, and requiring

Fig. 1 Major therapeutic approaches evaluated to target the CNS disease in lysosomal storage disorders. BBB: blood-brain barrier; CNS: central nervous system; ERT: enzyme replacement therapy; HSCT: hematopoietic stem cell transplantation; HSCGT: hematopoietic stem cell gene therapy. Created with BioRender.com

an immediate stop of the infusion, and resuscitation [81]. Furthermore, the development of anti-idursulfase antibodies, likely due to the necessary long-term, continuous administration of the drug, is registered in about half of the treated patients [79, 80], and this may reduce ERT efficacy. Nevertheless, most of the infusion-associated reactions were mild to moderate, and in most cases, patients could receive subsequent infusions [11].

An efficacy evaluation evidenced the ability of the enzyme to improve somatic manifestations of Hunter disease, although the therapeutic benefit may vary in different patients, according to several variables such as clinical phenotype or age at start of treatment, and it also varies in the different regions of the body [11]. A reduction of urinary glycosaminoglycan levels together with a decrease of organomegaly (mainly liver and spleen) is commonly observed, from the start of treatment [41, 82]. Significant improvement of endurance (for example, 6 min walking test) was reported [77, 83], and in some cases an amelioration of joint stiffness [11, 84]. The frequency of respiratory infections was also significantly reduced [84]. However, heart hypertrophy and cardiac valves, heavily involved in the disease, did not benefit much from ERT [82, 84], and neither did adeno-tonsils hypertrophy, hearing disabilities, and corneal clouding [11].

Main limitations of the recombinant enzymes are represented by the high costs of ERT, which have caused difficulties in its application in developing countries [42, 85], and difficulties in compliance in patients and families due to the need for weekly hospitalization, since the enzyme is most commonly administered under medical control. Importantly, it was clear since the beginning that idursulfase administered

via i.v. injection was not able to cross the blood–brain barrier and therefore could not have any effects on the cognitive and behavioral problems [3]. A paper published in 2020 analyzing fluid biomarkers in neuronopathic MPS II patients evidenced elevated levels of GAG (up to 30-fold) both in the CSF and in the serum of MPS II patients, compared with non-MPS patients, in whom it did not reduce following treatment with ERT [45]. In the same analysis, significantly elevated NfL levels were also detected in the CSF and serum, regardless of the treatment regimen to which patients were subjected.

From here, the idea to explore other delivery systems or strategies, to favor the enzyme crossing of the BBB, has long been considered.

6 Brain-Targeted ERT, an Update

For the brain-targeted therapeutic perspective by using ERT, there are two the main issues to take into consideration. The first one is the design of a delivery system able to transport high-molecular-weight proteins, such as enzymes, safely and efficiently across the barrier, no harming the BBB and the brain. The second issue to consider is the ability to maintain the enzyme activity all along the way, from the bloodstream to the correct target site within the cell; in this specific case the enzyme needs to maintain its degradative ability right inside the cell lysosomes. In addition, a positive effect on the improvement of the cognitive abilities of the patients, or at least in the delay of their progression, needs to be demonstrated. In fact, since many of the strategies evaluated

are somehow risky or require an invasive procedure, the achievement of an optimal risk/benefit ratio is highly recommended and ethically required.

6.1 Intrathecal ERT (IT-ERT) and Intracerebroventricular ERT (ICV-ERT)

Despite the fact that ERT cannot efficiently cross BBB following intravenous administration [86], it has been known for many years that, if administered directly into the brain, it can readily enter neural cells through their CI-M6PRs [62, 87]. Indeed, these receptors are expressed in all areas of the brain [88], in most neuronal cells, including neurons, astrocytes, or still immature oligodendrocytes [89].

There are two main routes for a direct administration of ERT to the CNS, intrathecal (IT) and the intracerebroventricular (ICV). Both of these ways present advantages and limitations; they both deliver the enzyme into the CSF—in ICV into the lateral ventricle, while in the IT procedure, the enzyme is injected into the lumbar spine or subarachnoid space at the cisterna magna [90].

For IT administration, an intrathecal drug delivery device (IDDD) is commonly used, while for ICV injections, patients need to be implanted with a reservoir as a prerequisite to allow periodical ICV administrations [91]. Both devices require a surgical procedure, often posing technical difficulties especially in very young children and especially for ICV [91], to avoid risks of hemorrhagic complications. Despite being potentially promising in treating CNS problems in neuronopathic MPS II patients, these technical difficulties had already been reported a few years ago by Okuyama and colleagues, who described them as "significant hurdles to overcome" [92].

Both techniques were known since they had been previously used to treat other conditions or diseases, such as for anti-pain drugs in cancer [93] or in Parkinson's disease [94].

Preclinical studies on IT and ICV administrations of ERT were widely conducted in different animal models of MPS II. Calias et al. in 2012 evaluated the IT and ICV injection of Elaprase® in monkey, dog, and mouse models [95]. Both ICV and lumbar IT administration to the monkeys and dogs determined a significant enzyme distribution in the brain parenchyma, together with an important cellular uptake; the spinal cord could also benefit from the lumbar IT injection, while a very little amount of idursulfase was seen following ICV administration. In the mouse model, injections of the enzyme through lumbar IT could reduce the brain cellular vacuolation, commonly seen in the histopathological analysis of the diseased samples.

ICV-ERT has recently received drug approval in Japan [96], where it is administered together with intravenous ERT. A recent study [97] reported the final results of a long-term Phase I/II clinical trial in six patients administered ICV idursulfase for 5 years, at progressive doses from 1 to 30 mg/ml every 4 weeks, together with intravenous weekly injections at the usual dosage of 0.5 mg/ml. The study evidenced good tolerability with no higher safety concerns with respect to the common i.v. treatment, and some positive results with a significant progressive decrease of CSF HS concentrations, which presenting a mean baseline value of 7.75 µg/ml, after 5 years of treatment were reduced to a mean value of 2.15 µg/ml (72.3% reduction). They also registered an increase in age in the developmental decline or at least a stabilization of the decline. As in most clinical studies, best results were obtained in patients where treatment was started before 3 years of age.

As for IT-ERT, starting in the Phase I/II clinical trial, the IDDD created numerous complications [98]; that is why, in Phase II/III of the trial, a smaller device was adopted [99]. Idursulfase-IT formulation was supplied as an isotonic solution at 10 mg/ml [99] every 4 weeks for overall 52 weeks, together with i.v. weekly injections of 0.5 mg/kg in 34 subjects (32 of whom were able to complete the study), while in 15 control patients no IT administration was performed. IT-ERT was generally well tolerated by the patients. Best results were obtained in patients younger than 6 years of age and carrying missense variants, in whom a significant GAG decrease was measured in CSF. However, analysis of the primary and secondary endpoints related to an improvement in overall intellectual ability and adaptive behavior ability, respectively, could not show a statistically significant result in the patients treated with IT-ERT versus non-treated patients [99]. Due to this insufficient efficacy outcome, authors concluded that the data obtained in the clinical study did not meet the "evidentiary standard to support regulatory filings," although a trend toward a possible positive effect was observed.

All studies conducted so far underlined the need to initiate the treatment in very young patients, where the best efficacy was registered [97, 99]. Some of the borderline effects measured in these studies may in fact be due to the elevated age of the children at start of treatment. This is particularly important for brain-targeted therapies, due to the progressive and irreversible damage affecting CNS.

6.2 Nanoparticles as Delivery Vehicles

Nanoparticles are very small delivery vehicles, 200–300 nm in diameter [63], able to safely transport therapeutic molecules to specific target sites as well as to protect the delivered molecule. Polymeric nanoparticles can offer chemical-physical and biological stability, a long circulation timing, and a great bioavailability [100]. In addition, they can target specific organs or body districts, as well as

cellular organelles, while still keeping the delivered compound active [100].

Their development has increased in the last few years, and their ability to deliver difficult macromolecules as proteins and enzymes across the blood–brain barrier has been demonstrated [63]. Specifically, the role of nanotechnology for the delivery of the enzymes needed for the treatment of lysosomal storage disorders has been highlighted in the last decade [61, 100]. In fact, nanoparticles not only can provide a delivery system but can also solve some enzyme difficulties related to their size, risk of degradability, and solubility requirements [63].

Therapeutic enzymes have been delivered to the CNS system in Gaucher disease [101] and Krabbe disease [102]. As for Hunter disease, the only preclinical in vivo study published so far was from our team in 2019 [103]. We used polylactide-*co*-glycolide (PLGA) biocompatible and biodegradable nanoparticles, approved by the FDA for human use, functionalized with a glycopeptide of seven amino acids (g7-NPs) [104, 105] for CNS targeting [103]. g7-NPs were loaded with the therapeutic IDS and, following their biochemical analysis and in vitro evaluation, they were administered via weekly i.v. injections for short-term evaluation in a mouse model of the disease. Mice were treated for 6 weeks, after which we observed a significant decrease of glycosaminoglycan deposits, both in the liver and in the brain parenchyma, together with a reduction of some neuro-inflammatory biomarkers such as GFAP, CD68, and LAMP-2 [103].

6.3 Brain-Targeted Enzyme Fusion Proteins

Several approaches based on receptor-mediated transcytosis such as BBB crossing strategy have been implemented in the last years and have revealed to be successful after more than 25 years of failed attempts [106]. To this aim, fusion proteins consisting of the functional enzyme fused with an antibody that binds endogenous protein receptors on the luminal side of the brain capillary endothelial cell are designed and delivered i.v.. In this way, the fusion proteins are endocytosed into the endothelial cell and afterwards released into brain parenchyma [107]. Pabinafusp alfa (JR141; JCR Pharmaceuticals) is a recombinant form of idursulfase where the IDS enzyme is fused with an anti-human transferrin receptor antibody. The fusion drug, administered through i.v. injection, binds to the transferrin receptor and, through receptor-mediated transcytosis, reaches the brain parenchyma [106]. At the moment, pabinafusp alfa is the only drug of this type that has completed the Phase III clinical trial, and in 2021 was authorized in Japan for use in mucopolysaccharidosis type II.

Preclinical experiments were conducted on the drug pabinafusp alfa in mice and monkeys, where a reduction of glycosaminoglycan levels both in the peripheral tissues and in the brain of a mouse model of MPS II were recorded [108], with a significant improvement of neurocognitive deficit [109]. The first Phase I/II open-label, multicenter, randomized clinical trial was published in February 2019 [92]. In the study, 14 patients were enrolled, and the effect of the drug on neurodegeneration was assessed with positive outcome by measuring heparan sulfate and dermatan sulfate levels in cerebrospinal fluid as biomarkers. A Phase II/III clinical trial was afterwards conducted, whose results were published in 2021 [110], while in the same year, a Phase II trial was conducted in Brazil [111]. Overall, 62 patients were involved in these clinical trials; almost 90% of them experienced stabilization or improvement in their neurocognitive impairment, and no drug-related severe adverse events [111].

In Japan, where the drug was first developed, the use of JR141 was authorized in 2021 (international patent WO 2016/208695 A1) [112], where it is currently available to MPS II patients of all phenotypic forms [113]. Very recently a paper reported the caregivers' observations recorded for seven MPS II patients, under JR141 treatment for 3.3–3.5 years [114]; five of them were affected by the neuronopathic form, and two were classified as being more similar to the non-neuronopathic form. Data collected in the interviews suggest a trend toward improvement of multiple clinical aspects, including some cognitive signs, such as language skills, self-control, abilities to follow instructions, and expressing personal needs, among others, together with some somatic signs, mainly related to organ involvement, musculoskeletal apparatus impairment, and joint stiffness, generally improving the quality of life of patients and families [114]. In January 2023 the drug was granted rare disease designation from the US FDA.

Another two similar fusion proteins have been developed and tested in clinical trial: AGT-182 (ArmaGen) where idursulfase is fused to an anti-insulin receptor antibody, and DNL-310 (Denali Therapeutics), an enzyme fusion protein that contains a low-affinity transferrin-binding peptide [106, 115]. As for AGT-182, according to ClinicalTrials.gov, a Phase I clinical trial was completed on March 17, 2017; however, no outcomes were reported. Concerning DNL-310, a potential association of the drug with anemia in two of the five patients enrolled in the clinical trial in relation to the transferrin receptors suggested the requirement of a further evaluation [106].

7 BBB Disruption Strategies

Some strategies have been proposed to increase BBB permeability, thus allowing a temporary passage of drugs from the bloodstream to the CNS. Such strategies, generally applying invasive techniques, were based on neurosurgery or

chemical/physical disruption of the barrier or via osmotic shift [116]. These techniques present, however, numerous drawbacks, mainly the invasiveness, the elevated costs of neurosurgery, and mainly high risks of infection and damages from toxins, because of the temporary opening of the BBB [117]. They were tested in some LSD, such as in MPS I, where in the mouse model of the disease, the application of the focus ultrasound, using magnetic resonance thermometry, allowed the delivery of the enzyme α-iduronidase across the BBB [118]. Osmotic shift using a co-injection of mannitol and adenovirus or adeno-associated virus were evaluated in Sandhoff disease [119], MPS IIIB [120], and CLN2 deficiency [121]. To the best of our knowledge, these strategies have not been evaluated for the treatment of mucopolysaccharidosis type II brain disease.

8 Brain-Targeted Cell and Gene Therapies

Cell and gene therapy approaches for MPS treatment involve delivering an exogenous gene or cells with the aim of expressing and secreting supraphysiologic levels of the functional enzyme able to elicit a therapeutic effect. To target and cure the brain disease in MPS II, several strategies have been implemented via both cell- and gene-based approaches or via combined ones.

8.1 Allogeneic HSCT

Allogeneic HSCT consists of transplantation of HLA-compatible bone marrow or umbilical cord blood stem cells, which can become long-term sources of functional therapeutic enzymes. Moreover, monocytic/phagocytic cells can reach the brain, by crossing the BBB, and being hosted as microglia, which in turn would be able to secrete the functional enzyme, thus correcting neuronal enzyme deficiency (Fig. 2). This was first demonstrated in 2009 by Araya, who, in a 6-year-old patient with severe MPS II, documented the distribution of donor-derived cells in the brain 10 months after cord blood stem cell transplantation [122]. To date, several authors have reported slight neurological improvements after HSCT. Ameliorations of brain magnetic resonance imaging (MRI) atrophy and category I and II brain lesions as well as of activity of daily living (ADL) were reported by Tanaka and colleagues in a nationwide follow-up in Japan [123]. Analogously stable or improved MRI brain lesions were observed by Kubaski and colleagues [124]. Improvements in cognitive, language, and motor skills were observed in a severe patient submitted to HSCT at 70 days old and followed for 7 years [9].

A recent retrospective study in 109 Japanese Hunter patients has shown that HSCT might improve the ADL scores in both mild and severe patients [125]. However, the efficacy of HSCT on the neurological system for MPS II remains controversial, due to the limited information regarding the long-term outcomes. Moreover, HSCT remains a risky procedure, although with protocol refinement, a progressive reduction of risks of life-threatening graft-versus-host reactions and/or infections have been obtained [126, 127].

8.2 Gene Therapy

As MPS II is a monogenic disease with a well-known pathophysiology, it is a good candidate for gene therapy. Indeed, through the mechanism of cross-correction, the therapeutic enzyme produced after genetic modification by a depot organ can be captured by other cells and organs, and a healthy status can be obtained with at least 5–15% of the normal enzyme level [128]. Moreover, gene therapy might overcome many ERT limitations, such as the need for repeated administrations, the inability to cross the BBB, and the risk of developing neutralizing antibodies against the therapeutic enzyme [129]. In recent years, several gene therapy approaches have been attempted for MPS II to specifically target the brain; to this aim different vectors and administration routes, as well as in vivo and ex vivo procedures, were studied [130].

8.2.1 Hematopoietic Stem Cell Gene Therapy (HSCGT)

HSCGT has several advantages over allogeneic HSCT. It relies on the patient's own cells, thus bypassing the risk of graft-versus-host disease (GVHD); moreover, as with other gene therapy approaches, it has the potential to achieve supra-physiological levels of the therapeutic enzyme. Finally, HSCGT might provide a sustained life-long source of the enzyme, without the need for frequent enzyme infusions, thus potentially improving patients' quality of life [72].

Hematopoietic stem cell gene therapy using lentiviral vectors was successfully applied in the MPS II mouse model by Wakabayashi et al. [131]. A correction of neuronal manifestations via reduction of lysosomal storage and autophagic dysfunction in brain was observed together with an overall stabilization of neuronal functions. A recent study from the same group confirmed previously reported results and evidenced that strong preconditioning is required to obtain an efficient engraftment of the genetically modified stem cells and thus an amelioration of CNS involvement in MPS II mice [132]. However, lentiviruses' random integration into
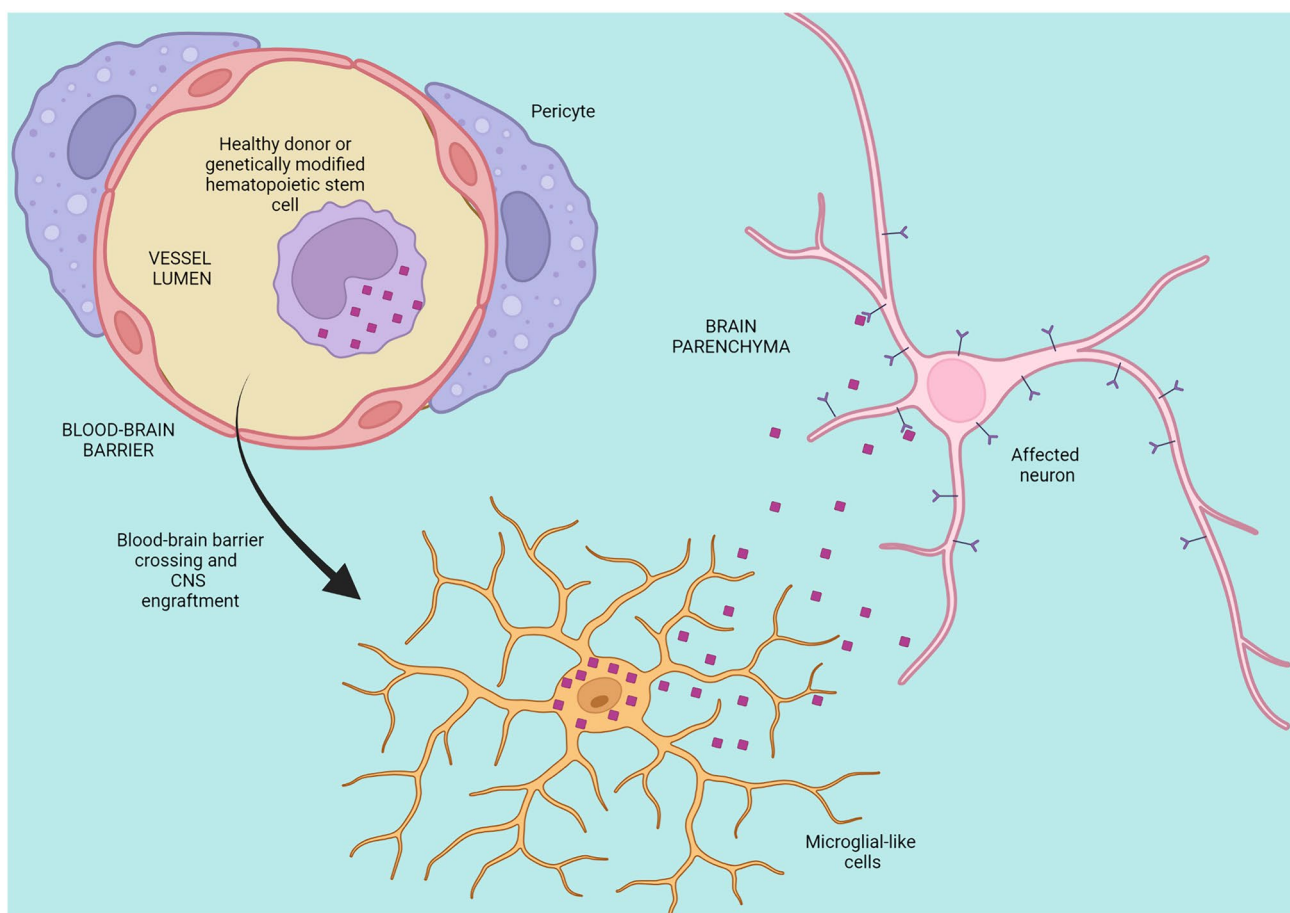
Fig. 2 BBB crossing by monocytic/phagocytic cells and cross-correction mechanism. Healthy donor or genetically modified hematopoietic stem cells can reach the brain by crossing the BBB; then, cells can be hosted as microglia-like cells, which in turn will secrete the func-tional enzyme that will correct the neuronal enzyme deficit through the cross-correction mechanism. BBB: blood–brain barrier; CNS: central nervous system. Created with BioRender.com

the genome poses potential risks such as mutagenesis and neoplastic transformation.

Recently, Das and colleagues employed lentiviral vectors encoding an optimized human *IDS* cDNA in an ex vivo hematopoietic stem cell gene therapy approach, in both young pre-symptomatic and symptomatic MPS II mice, to assess the efficacy, feasibility, and safety of the protocol [127]. The study evidenced a robust and sustained engraftment of the infused cells 17–18 months post-trans-plantation, leading to long-term supraphysiological IDS enzymatic activity in bone marrow (BM) mononuclear cells, in serum as well as in brain. Normalization of the behavioral and neurocognitive disease manifestations of both pre-symptomatic and symptomatic treated MPS II mice was reported; benefit for the skeletal phenotype was also evidenced [127].

To date, only a clinical trial using lentiviral vectors is active. The study aims to evaluate safety and efficacy of ex vivo gene therapy, via autologous CD34+ hemat-opoietic stem cells transduced with a lentiviral vec-tor containing the human *IDS* gene tagged with ApoEII (NCT05665166; https://clinicaltrials.gov/).

8.2.2 Adeno-Associated Viral Vectors

Adeno-associated viral vectors (AAVs) have emerged as a leading choice for gene therapy due to their ability to provide stable gene expression and deliver genes to both dividing and non-dividing cells. Major limitations of these viral vec-tors are the risk of immunogenicity and the potential for generating neutralizing antibodies [1]. Initial gene therapy attempts based on AAV used type 2/8 vectors administered intravenously in MPS II adult mice; both studies evidenced IDS activity restoration and full clearance of GAG storage in plasma and in several tissues including the brain [133, 134]. In 2016, Motas and colleagues administered AAV-9 vectors encoding IDS to the cerebrospinal fluid of MPS II

mice through intracisternal injections and after 4 months evidenced a significant increase in IDS activity throughout the encephalon and full resolution of lysosomal storage lesions, and disappearance of neuroinflammation [135]. Hinderer and Laoharawee obtained the same results in two subsequent studies, using a similar strategy, but administering AAV-9 vectors via intracerebroventricular injections [136, 137].

The use of a less invasive route of administration to address CNS disease was tested by the Laoharawee's group, who evidenced that a single dose of intravenously administered AAV9-h*IDS* may determine a global normalization of GAG on both sides of the BBB and prevention of neurocognitive decline in the treated MPS II mice [138].

Currently, two clinical trials (NCT03566043, NCT04571970; https://clinicaltrials.gov/) are ongoing using the AAV9.CB7.h*IDS* (RGX-121) produced by Regenxbio Inc., which is administered intracisternally or intracerebroventricularly with the aim of evaluating both safety and efficacy in improving CNS disease manifestations.

Recently, Smith and colleagues compared different doses of RGX-121 administered intravenously and intrathecally to determine whether intrathecal delivery alone can ameliorate both CNS and systemic manifestations. The relevant release of the vector into the periphery, after CSF-mediated delivery of AAV9 at a specific dose, suggests that this approach could be able to address both neurological and systemic disease manifestations in a murine model of MPS II and thus would be promising also for treating the human disease [139].

8.3 Brain-Targeted Genome Editing

Genome editing is a promising method whose aim is to repair the damage in a specific site of the genome through either non-homologous end joining or homologous recombination. To date, different editing platforms are available: zinc finger nucleases (ZNFs), transcription activator-like effector nucleases (TALENs), and, based on the CRISPR/Cas9 system, the CRISPR/Cas9- based editors and CRISPR/Cas9-prime editing tools [129].

So far, only the ZFN platforms have been studied for the treatment of MPS II, using the same strategy previously studied for the insertion in the albumin locus of human coagulation factor VIII (hF8) and factor IX (hF9) transgenes and subsequent correction of clotting defects in mouse models of hemophilia A and B, respectively [140–142]. Analogously, AAV8 vectors with albumin locus-targeting ZFN in hepatocytes were injected intravenously in MPS II mice at three different doses; treatment caused an increase of the IDS enzyme in blood and other peripheral tissues, as well as reduction of GAG in visceral organs and brain. Moreover, the high vector dose prevented the development

of neurocognitive deficit in young MPS II mice, thus resulting in a promising treatment also for the CNS [143]. These results lead to the development of a Phase I/II clinical study (NCT03041324; https://clinicaltrials.gov/) with the first patients ever being treated with in vivo gene editing therapy. The trial results evidenced a favorable safety profile of the ZNF-based platform used; however, in vivo genome editing occurred at a very low level in the liver of the evaluated subjects with no long-term sustained enzyme expression in blood [144].

9 Small Molecules

Pharmacological chaperones (PCs) are low-molecular-weight molecules able to reach the endoplasmic reticulum (ER) and modify misfolded proteins to correct them, at least partially, and render them more active in their specific function. As for lysosomal storage disorders, PCs are mainly used to increase the enzyme activity of a mutated protein. It has, in fact, been suggested that aminoacidic changes, caused by sequence mutations, may render proteins less stable due to alterations of one or more of the several steps contributing to a correctly folded or glycosylated protein. This may lead to ER or Golgi apparatus retention or degradation of the protein, which renders the protein unsuitable for transport to the lysosomes, where it exerts its hydrolytic function [145]. PCs present some important advantages since they can be orally administered; they are able to reach most body regions, and they are less immunogenic than human recombinant proteins. In addition, if administered in combined protocols with ERT, they apparently can also stabilize the co-administered recombinant protein [146], which possibly suggests that they could enable a reduction of the ERT dosage.

Chaperones have been used for almost 25 years for the treatment of other lysosomal disorders such as Gaucher and Fabry diseases [145].

Recently, D2S0, a sulfated disaccharide derived from heparin with a similar structure to the natural substrate of IDS was evaluated in vitro as a PC-based potential treatment for MPS II patients [145]. In this study, MPS II fibroblasts, carrying the p.P231L mutation, treated with 10 M D2S0 for 8 days showed a 1.97-fold increase in IDS activity and a significant reduction in Alcian blue-positive granules. The same evaluation, conducted on HEK293T cells expressing p.N63D, p.L67P, p.A85T, p.R88H, p.Y108S, and p.P231L, showed an increased IDS activity between 1.6- and 39.6-fold but no effects in cells expressing p.L314P. These promising results showed that D2S0 may function in a mutation-dependent manner, but further optimization will be necessary to increase the efficacy of the molecule. This strategy could represent an interesting therapeutic approach since about 41% of mutations affecting

the *IDS* gene are missense mutations and involve buried amino acids that can likely induce protein destabilization and misfolding [147].

The substrate reduction therapy (SRT) approach aims to reduce the bioavailability of the compound that cannot be fully metabolized by the defective enzyme ("substrate reduction"), thereby reducing the burden of the accumulating substrate [148]. This treatment can be administered orally, and it is potentially able to cross the BBB due to the size and charge of the molecules used. The evaluated strategies for MPS focus on indirect inhibition of GAG synthesis, as a direct approach would imply the use of toxic inhibitors of the enzymes related to the anabolic GAG synthetic pathway [128]. In 2008, an open-label pilot study evaluating the effectiveness of genistein, an isoflavone that reduces the synthesis of GAG, was completed on 10 patients with MPS IIIA or MPS IIIB. Patients evidenced improvements in urinary GAG and cognitive function and no adverse effects after 12 months of treatment [149]. However, these results were not replicated by the following studies [150]. Thus, to date, no effective SRT drugs for MPS II as well as for the other MPSs are available. Therefore, this approach still remains a potential therapeutic approach.

Another strategy could be the development of stop codon readthrough-inducing drugs. This approach could be applicable when the disease-causing mutation is a nonsense mutation and the patient could potentially benefit from molecules that promote the ribosome to read through the premature stop codon; this would allow protein translation to continue and, as a result, the restoration of the biosynthesis of the full-length protein [151]. To date, no studies are ongoing on candidate stop codon readthrough-inducing drugs for MPS II treatment.

10 Conclusions, Open Issues, and Future Perspectives

During the last 2 decades, several strategies have been evaluated to allow the crossing of therapeutics through the BBB, thus targeting them to the brain. In fact, the BBB, which positively protects the brain from most infective agents and circulating toxins, represents a difficult shield to cross or bypass for most therapeutic molecules. An important exception is via approaches providing healthy or genetically corrected cells, such as transplant or gene therapy approaches, or viral vectors, such as AAV, with both of these systems being able to cross the barrier. BBB crossing is particularly hard for high-molecular-weight drugs, such as recombinant proteins or enzymes such as those needed for the treatment of most lysosomal storage disorders affected by neurological impairment. Therefore, treatment of these diseases, including MPS II, remains challenging, given the fact that the mechanisms of the neuropathology are also not yet fully

elucidated, thus making the identification of new therapeutic targets and biomarkers of diagnosis/efficacy difficult.

All approaches so far evaluated for MPS II present both advantages and limitations, and to date, no ideal therapeutic solution is available for the neurological disease. All of them are quite expensive, and this represents a significant problem for developing countries, for example, with i.v. ERT.

The timing of treatment is also a critical point: treatment of patients in their early stage of life could be more effective on the neurological disorder for most approaches—"the earlier the better." However, this requires early clinical observation and suspicion, and good tools for differential diagnosis, as well as clear disease biomarkers, rapidly measurable through non-invasive procedures. The low number of patients who can be enrolled in the clinical trials, as MPS II is a rare disease, represents a further critical point. This entails rather a long time for an adequate safety/efficacy evaluation of each new therapeutic strategy, delaying its possible authorized release on the market.

Viral vectors as well as ERT approaches may lead to immunogenic responses that may subsequently reduce the treatment efficacy. Moreover, ERT-based approaches require repeated intravenous administration, and for intrathecal and intracerebroventricular routes, a continuous administration can be obtained via specific devices or reservoirs, which require a surgical procedure, which is often technically difficult. In contrast, cell and gene therapy approaches, which present the advantage of requiring a one-time administration and also look promising, are mostly still in the preclinical phase. Regarding costs, cell and gene therapies have relatively high costs related to technology development, which however could be counterbalanced by the single-administration needed and by the fact that the same technology could be applied to other disorders.

So far, the most promising approaches for MPS II appear to be fusion proteins, such as pabinafusp alfa, HSCGT approaches, and AAV vectors, all administered i.v., thus through the least invasive procedure. Nowadays, some of these approaches are available or will be available soon; therefore, the possibility of using only one therapy able to target both the somatic and the CNS disease is closer to being real or real in some cases. Specifically for the fusion proteins, in the future continuous i.v. administration might be proposed, at lower doses, mimicking the physiological production of the enzyme by the cells.

In recent years, the evaluation of several therapeutic strategies approaching the brain disease in MPS II from different sides has been revealed to be extremely positive, allowing dissection of their potential efficacy together with their limitations, thus suggesting possible improvements. These studies will also be useful in a wider scenario, developing similar approaches for other lysosomal storage disorders, with patients still suffering for the lack of therapy.

Declarations

Conflict of interest Authors declare no conflicts of interest directly or indirectly related to the work presented here.

Availability of data and material Not applicable.

Ethics approval Not applicable.

Patient consent to participate/publish Not applicable.

Code availability Not applicable.

Author contributions Both authors contributed equally to this work. Both authors read and approved the final manuscript.

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