RESEARCH ARTICLE

A Cure for Sanfilippo Syndrome? A Summary of Current Therapeutic Approaches and their Promise

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Abstract

Mucopolysaccharidoses III (MPS III, Sanfilippo syndrome) is a subtype of the Mucopolysaccharidoses (MPS), a group of inherited lysosomal disorders caused by a deficiency of lysosomal enzymes responsible for catabolizing glycosaminoglycans (GAGs). Although MPS III is rare, MPS diseases as a group are relatively frequent with an overall incidence of approximately 1 in 20,000 – 25,000 births. MPS III are paediatric diseases, which cause learning difficulties, behavioural disorders and dementia, as well as skeletal deformities and ultimately result in premature death. There are currently no approved treatments for MPS III, but a number of therapeutic approaches are under development. In the past 30 years, research using cellular and animal models have led to clinical trials involving enzyme replacement therapy (ERT), substrate reduction therapy (SRT) and gene therapy, while stem cells approaches remain at the pre-clinical stage. Although safety and clinical efficacy in animal models have shown promise, the results of clinical trials have proved costly and shown limited therapeutic effects. In this review, we describe the most recent results from clinical trials. While ERT and gene therapy are the most developed therapies for MPS III, we highlight the work that needs to be done to bring us closer to a real treatment for these devastating diseases.

Key words: Mucopolysaccharidoses III, Sanfilippo syndrome, lysosomal storage disease, neurodegeneration, enzyme replacement therapy, gene therapy, substrate reduction therapy, stem cell therapy, clinical trial

1.Introduction

Mucopolysaccheridosis type III (MPS III or Sanfilipo Syndrome) refers to one of five (MPS IIIA-E) autosomal recessive lysosomal storage diseases. Each form of MPS III is caused by a mutation in both alleles of a gene which codes for an enzyme involved in the degradation of the glycosaminoglycan (GAG) heparin sulfate (HS). As a result, partially degraded HS accumulates in lysosomes leading to lysosomal malfunction and disease [1-3]. The subtypes of MPS III are caused by deficiencies in the enzymes; sulfaminase (MPS IIIA, OMIM no. 252900) [4], α-*N*-acetylglucosaminidase (NAGLU, MPS IIIB, OMIM no. 252920)[5], heparin acetyle CoA: α-glucosaminide *N*-acetyltransferase (HGSNAT, MPS IIIC, OMIM 252930)[6], *N*-acetylglucosamine 6-sulfatase (GNS, MPS IIID, OMIM 252940) [4]; and *N*-glucosamine 3-*O*-sulfatase (arylsulfatase G or ARSG, MPS IIIE) [7]. MPS III is the most common form of MPS, with a prevalence of between approximately 0.3 and 4.1 cases for every 100, 000 births depending on the subtype [8]. MPS III manifests in a similar way regardless of subtype, although the age of onset and rate of disease progression may differ between individuals. The most striking feature of MPS III, compared to other forms of MPS is that it primarily affects the brain, distinguishing them as neurological diseases. Although other forms of MPS are linked to severe somatic symptoms and may also have neurological characteristics, only MPS III appears to heavily involve the central nervous system. Pre-natal and early stages of post-natal development appear to be normal in MPS III, with symptoms occurring during the first few years of life (1-3 years of age) and including developmental delay such as difficulty forming language, cognitive decline, hyperactivity, sleep disturbances, aggressive behaviour and seizures, especially in older children. Towards the later stages of the disease, hyperactivity and anxiety subside but patients succumb to motor impairment, eventually reaching a vegetative state, become less responsive to external stimuli and die prematurely before their third decade of life [9-11]. Unsurprisingly, these symptoms have a tremendous impact not just on the children with MPS III, but parents and primary carers of children with MPS III. Currently, there are no available treatments to effectively reverse or slow down disease progression in MPS III. Instead, most efforts are palliative, focusing more on regulating behaviour and sleep disturbances. This is because the clinical symptoms that characterize MPS III result from neuronal dysfunction, making them particularly difficult to treat. MPS types I, II, IVA, and VI for which clinical symptoms are predominantly somatic, can all be treated using Enzyme Replacement Therapy (ERT) or bone marrow and hematopoietic stem cell (HSCT) transplantation [12]. Attempts have been made to deliver missing enzyme to MPS III patients via the blood stream, but the results have been poor due to the inability of the enzyme to cross the blood-brain-barrier. Notwithstanding, a number of promising therapies are currently being tested in cell and animal models and several clinical trials are underway. In this review, we will focus on the progress that has been made for ERT, small molecules and stem cell approaches in the

past few years, which obstacles still remain and how they may be overcome.

2.Therapeutic Scope

There are two broad ways in which monogenetic diseases like MPS III can be treated. One, targeting the gene defect directly through gene therapy or two, targeting the deficiency of the protein that the gene codes for using modified ERT, the use of small molecules or stem cells. All four treatment approaches have been tested in cellular and animal models, and clinical trial. An excellent review has been written by Gaffke et al., recently [13]. Building on these reported findings, this review will concentrate on the work that has been done toward obtaining therapeutic options for MPS III in the last two years and their promise.

2.1. Cellular Models.

Cellular models serve as a useful tool for testing the molecular mechanisms, which drive MPS III, or the efficacy of various therapies. For obvious reasons, cellular models can only provide a preliminary indication of the effects of compounds on an organism. Nonetheless, ERT, substrate reduction therapy and other forms of small molecule therapies have offered promising results, which warrant further investigation.

The advent of three-dimensional (3D) organoid culture systems, generated from induced pluripotent stem cells (iPSCs) provide unprecedented potential for modelling the human brain, mimicking various developmental features at the molecular and cellular level [14-19] These scaled-down complex cellular models not only offer a way to better study the mechanism of disease in MPS III, but in addition, better test small molecules. For the first time, two stem cell lines derived from the skin of a patient with MPS IIIA and MPS IIIB, have been generated and characterized [20,21]. Although iPSC-derived organoids are yet to be generated from these lines, they represent the first step towards MPS III brain organoids. Much progress has been made using 2D-culture systems as described above, but the greater complexity achieved using 3D-organoids may bridge the gap between *in vitro* and clinical studies.

2.2 Animal Models

Animal models of genetic diseases provide a biological system to test potential therapies that have been previously investigated *in vitro*. The therapies described above have all proved relatively efficacious in cell models. However, preclinical studies in both cell (ideally human) and whole organisms are necessary in order to validate potential therapies with real clinical application. This is particularly important for MPS III, which unlike most forms of MPS, displays primarily neurological symptoms. Therefore, testing these therapies in animal models addresses the issue of a blood-brain-barrier, a structure conserved in both rodents for example, and humans.

2.3.Clinical Trials

Cellular and animal models have proved useful in identifying potential therapeutic

strategies to reduce the primary cause of the disease, GAG accumulation in the brain and its downstream effects. These studies have paved the way for 26 clinical trials for MPS III in the past 20 years, 9 of which have been completed. These include stem cell transplantation, ERT, substrate reduction therapy and gene therapy. One of the main limitations of assessing the effects of treatments in MPS III is that it is a rare disease, meaning that sample sizes tend to be small and fail to completely represent the different stage and severity of symptoms. To overcome this issue, future studies should take heed of recent recommendations on clinical trial design for the treatment of MPS III [22]. In this review, we will focus on recent developments for each therapy from their development in cellular and animal models, to the clinic.

3. The Road to the Clinic

3.1. Small molecules

3.1.2. Substrate reduction therapy

Substrate reduction therapy (SRT) aims to reduce the synthesis of GAGs, which cannot be degraded in MPS III. Unlike for other LSDs such as Gaucher type I, SRT is not approved for the treatment of any of the MPS disorders but several approaches to reducing GAG synthesis are under investigation [23]. One approach is to silence the expression of genes which code for proteins involved in GAG synthesis using siRNA and shRNA as has been shown in MPS IIIA fibroblasts [24,25] and MPS IIIC [26]. An alternative approach to reducing the synthesis of GAG is the inhibition of follicle-stimulating hormone (FSH) or epidermal growth factor (EGF), which have been previously shown to maximise the synthesis of some GAGs [27,28]. Using a technique called gene expression-targeted isoflavone therapy (GET IT), Jakóbkiewicz-Banecka et al. showed that treatment of MPS IIIA and MPS IIIB patient-derived fibroblasts with the natural tyrosine kinase inhibitor isoflavone 4', 5, 7-trihydroxyisoflavone (genistein) inhibited GAG synthesis and prevented lysosomal accumulation. This effect was eliminated in the presence of excess EGF and partially restored following an increased concentration of genistein [29]. In 2006, Malinowska et al. showed that continuous administration of genistein, given to MPSIIIB mice at a high dose for 9 months, significantly reduced lysosomal storage, heparan sulphate substrate and neuroinflammation in the cerebral cortex and hippocampus, resulting in correction of the behavioural defects observed, as well as improved synaptic vesicle protein expression and secondary storage in the cerebral cortex [30]. As a small molecule, genistein can cross the blood-brain barrier and reach the brain, making it an attractive candidate for treating MPS IIIB.

An open-label clinical study including 19 MPS III patients (aged 2.8 – 19 years) was launched in 2014 to assess the safety and effectiveness of low dose genistein (5mg/kg/day) for one year and published by Delgadillo et al. Although no serious adverse effects were observed, the study showed that there was no improvement in the disability scale, as determined using the Questionaire on Development and Behavior. Most patients had an increased disability score, or it remained the same despite a reduction in

urinary GAG levels [31]. As described above, mice treated with a high dose of genistein showed a significant reduction of HS accumulation and neuroinflammation in the brain and displayed an improvement in behaviour [30]. Following these promising results, a phase III clinical trial was launched in 2013 using a higher-dose of genistein (160 mg/kg/day) or a placebo for a year, followed by a year of open-label genistein was completed recently (July 2018). In this double-blinded, randomized placebo-controlled study, high-dose (150 mg/kg/day) genistein was orally administered to nineteen MPS III patients (age 1.25 – 18.5, mean average age of 8) for a year (EudraCT number 2013–001479-18) [32]. Safety labs, GAG levels, clinical status and history of adverse events were obtained every 3 months, a physical examination was performed every 12 months, a 9 point disability scale (FPSS) was recorded after each visit, and an annual neurocognitive test was carried out where possible [31,32]. After 12 months of treatment, no serious adverse events linked to high-dose genistein were identified and CSF HS was moderately reduced. However, the reduction in CSF was not substantial and scores on neuropsychological tests got worse or remained the same indicating no attenuation of cognitive decline [31].

3.1.3. Other small molecules

Another way in which small molecules can be utilized to treat MPS III is by adopting strategies, which target the splicing process. 20% of MPS IIIC linked mutations reside within splice sites and affect mRNA processing, making this form of MPS an eligible candidate for this particular therapy [33]. In order to rescue the normal splicing process, Matos et al. used modified U1 snRNAs, which recognise mutations in donor splice sites in MPS IIIC patients' fibroblasts [33]. Another approach, which targets mutations in an acceptor site and results in misfolded acetyl-CoA:α-glucosaminide acetyltransferase, tested a competitive inhibitor of the HGSNAT protein, glucosamine as a pharmacological chaperone to correct misfolded protein and restore normal trafficking to the lysosome [33]. While partial correction of acetyl-CoA:α-glucosaminide acetyltransferase activity was achieved, the obstacle to fulfilling the full therapeutic potential of such a strategy is the efficient delivery of RNA molecules to the brain.

3.2. Enzyme Replacement Therapy

3.2.1. *In Vitro* studies

The blood-brain barrier has in the past been a major obstacle to effective intravenous treatment using ERT. This has been further confounded in MPS IIIB by inadequate mannose-6-phosphorylation (M6P) of human α-N-acetylglucosaminidase (rhNAGLU) recombinant enzyme, resulting in poor uptake. To address this problem, Kan et al. tested the use of a modified human recombinant NAGLU enzyme by fusing the human NAGLU fragment (rhNAGLU) to a fragment of insulin-like growth factor 2 (IGF-II) that would allow trafficking into the lysosome via IGFII binding site on the Mannose 6-phosphate/IGFII receptor. They successfully demonstrated that the fusion protein was able to gain entry to MPS IIIB cells via IGF-II binding to the mannose 6-

phosphate/IGFII receptor. The enzyme remained functional and reduced the amount of Heparan Sulphate (HS) in MPS IIIB fibroblasts to the same level as found in control cells [34,35].

This insulin-like growth factor II (IGFII)-tagged NAGLU molecule has been modified to create Tralesinidase alpha (rhNAGLU-IGFII; BMN 250), which will be further discussed in **section 3.2.3**. Yogalingam et al. (2019) used this fusion protein to distinguish two cellular uptake mechanisms by which BMN 250 is targeted to lysosomes in brain cells [36]. Neurons, microglia and astrocytes are all critical cell types in MPS IIIB and therefore a better understanding of the cellular uptake mechanism(s) by which enzyme is delivered is important for developing efficient targeting mechanisms that will optimize ERT approaches. By systematically assessing the competitive cellular uptake of BMN 250 in human MPS IIIB patient fibroblasts and normal rodent-derived neurons, astrocytes and microglia, Yogalingam and colleagues found that BMN 250 is targeted to lysosomes in neurons, astrocytes and fibroblasts via MPR-mediated cellular uptake, whereas receptor-independent cellular uptake in microglia contributes to substantial lysosomal delivery of both BMN 250 and unfused rhNAGLU [36].

3.2.2. *In Vivo* studies

Intravenous delivery of ERT is already an available treatment for forms of MPS with less neurological involvement (MPS I [37], II [38], IVA [39], VI [40] and VII [41]. Adapting this classically systemic approach to gain entry to the CNS, has been the focus of recent studies for MPS IIIA and MPS IIIB. One way to achieve this is by hijacking proteins, which have no trouble crossing the blood-brain barrier. One approach is to use a molecular Trojan horse, as has been achieved by fusing recombinant protein N-Sulfoglucosamine sulfohydrolase (SGSH) with a monoclonal antibody against the human insulin receptor (HIR Mab) (HIR Mab-SGSH) that can be taken up by MPS IIIA fibroblasts and trafficked to the lysosome resulting in reduced GAG levels (72 – 83%) [42]. Following intravenous administration of this fusion protein in Rhesus monkey, HIR Mab-SGSH 0.81% of the injected dose was detected in the brain [43]. Similarly, Boado et al. have also created a fusion protein for MPS IIIB by fusing rhNAGLU to HIRMAb (HIRMAb-LL-NAGLU) [44]. As shown for HIR Mab-SGSH in MPS IIIA, MPS IIIB fibroblasts displayed efficient cellular uptake of this fusion protein, trafficking to the lysosome, and a 74% reduction in the incorporation of sulfate into intracellular GAGs [44]. When HIRMAb-LL-NAGLU was intravenously injected into a Rhesus monkey, 1% was detected in the brain [45].

A new promising in vivo study has recently been published, exploring the use of an IgG against mouse Transferrin Trojan Horse-Sulfamidase Fusion Protein in MPS IIIA mice. Large recombinant SGSH cannot cross the BBB, limiting intravenous administration a therapeutic option. To overcome this issue, Boado et al. have created a fusion protein consisting of an SGSH and IgG, where the IgG domain is a chimeric monoclonal antibody (MAb) against mouse transferrin receptor (TfR) [46]. By acting as a Trojan horse, IgG can deliver SHSG to the CNS. The resulting fusion protein (cTfRMAb-SGSH)

successfully bound the mouse TfR with high affinity and maintained the same SGSH enzyme activity to the human recombinant SGSH. After 6 weeks of treatment, 3 times a week via intraperitoneal injection starting at 2 weeks of age, significant biochemical and behavioural improvements were observed. SGSH levels were elevated (36-fold compared to 30-fold in mice treated with just recombinant SGSH) and resulted in an 85% reduction in brain and liver HS (compared to 70% in mice treated with recombinant SGSH alone). Encouragingly, the reduction in brain HS was associated with a 28% increase in latency on the rotarod test of motor activity suggesting that an IgG-SGSH fusion protein engineered to penetrate the BBB via receptor-mediated transport, may be effective in treating MPS IIIA.

Another strategy that has been adopted for MPS IIIA and MPS IIIB involves direct administration of recombinant enzyme to the CNS. This was done in MPS IIIB by using recombinant human N-acetyl-α-glucosaminidase (rhNAGLU) fused with a fragment of the insulin-like growth factor II (IGF-II) as described in **section 3.2.1** and infusing it into the brain via intracerebroventricular injection [34,35]. Following intracerebroventricular administration to MPS IIIB mice, rhNAGLU-IGFII was taken up primarily by neurons and HS levels in the CSF were significantly reduced.

Similarly, when recombinant heparin N-sulfatase was infused to CSF of MPS IIIA dogs via the cisterna magna, HS levels were decreased in the CSF and cerebral cortex, but biomarkers linked to disease were only normalized following high dose of enzyme, which as discussed previously, may have clinical implications in terms of such a treatment mounting an immune response [47]. Beard et al, have shown that the route of administration influences the success of ERT, as shown by infusing heparin N-sulfatase via Direct administration of recombinant enzyme to the brain can also be achieved via intrathecal lumbar, cisternal and ventricular administration. Lumbar infusion resulted in poor enzyme delivery and no significant reduction in GAG level, while infusion via the ventricular route proved more efficacious in decreasing GAG levels and dampening microglial activation [48]. Building on the relative success of this approach, the same group implanted an intraventricular cannula connected to a subcutaneous mini osmotic pump, allowing for a continuous low-dose infusion of recombinant human heparin N-sulfatase into the brain via the CSF. However, the therapeutic effects of this approach were not initially overwhelming, with only partial reduction of HS and GAG, and only moderate reductions in microglial activation but not astrogliosis [49]. By subsequently tweaking this method to improve implantation of the pumps in MPS IIIA, HS levels were normalized and GAG storage decreased significantly [50].

Combinatorial approaches offer another way to improve the therapeutic potential of individual strategies. By combining the creation of fusion proteins with direct administration of enzyme directly into the brain, it is possible to further improve the impact on pathological biomarkers of either one therapy alone. This was demonstrated recently by Aoyagi-Scharber et al. in MPSIIIB mice. Human α-N-

acetylglucosaminidase fused with insulin-like growth factor II (described in **section 3.3.1**) and administered intracerebroventricularly resulted in widespread distribution of the fusion protein within the CNS and was accompanied by normalization of HS levels and significant reduction of secondary storage [51]. Although this combinatorial approach has yielded promising results, it is important to note the translational limitations surrounding frequent and direct administration of fusion proteins to patients.

3.2.3 Phase I/II Clinical Trials

Given the success of ERT in cellular and animal models, Shire Human Genetic Therapies (Shire HGT) developed an enzyme replacement therapy (ERT) recombinant human heparan-N-sulfatase (rhHNS) for patients with MPS IIIA. The open-label, phase I/II dose-escalation clinical study was carried out in twelve MPS IIIA patients to assess the safety of monthly intrathecal delivery of recombinant human heparin-N-sulfatase (rhHNS) using a surgically implanted intrathecal drug delivery device (IDDD) for the duration of 6 months (NCT01155778). In terms of safety, mild-to-moderate adverse effects were reported in all twelve patients, but none appeared to be related to the recombinant enzyme directly. However, despite a reduction in HS levels in the CSF, four of twelve patients showed a decline in the developmental quotient, six were stable and no dose group showed a clearly different response pattern. Overall, rhHNS administration via IDDD was generally safe and well-tolerated but required further investigation to determine efficacy [52]. Recently, an update on this study was published by Wijburg et al. This phase IIb trial included twenty-one patients on a regimen of intrathecal rhHNS every two weeks, every 4 weeks or no treatment. Encouragingly, a clinical response to intrathecal rhHNS was observed in three of the treated patients. HS and GAG levels in the CSF were reduced in all treated patients. However, although treatment-emergent negative effects to intrathecal rhHNS were largely mild, no clear differences were detected between treated patients (age 17.8 – 47.8 months) and untreated controls (age 12.6 – 45.0 months) in terms of efficacy. Again, early intrathecal delivery of rhHNS is safe and effective at reducing HS and GAG levels in treated patients but the treatment has no neurocognitive effects (NCT02060526) [53].

The final results from a phase I/II, open-label, clinical study of intravenous recombinant human N-acetyl-α-d-glucosaminidase in children with mucopolysaccharidosis IIIB has recently been published. The study, sponsored by Alexicon Pharmaceuticals, included 11 participants age between 1 – 10 years of age and set out to evaluate the safety, tolerability, pharmacokinetics, and efficacy of intravenous administration of a SBC-103, a recombinant human NAGLU enzyme capable of crossing the blood-brain barrier [54] (NCT02324049). In this three-part study, participants were sequentially divided into three dose-escalating groups and received intravenous injections every two weeks for 24 weeks, after which patients received no treatment for a month (Part I). Patients then received a higher dose every two weeks (Part II) starting at 28 weeks, and a final dose escalation of SBC-103 every two weeks for two years in total [55]. Despite the initial results in NAGLU-deficient

mice [54], SBC-103 (rhNAGLU) was well-tolerated by MPS IIIB patients, and resulted in the reduction of HS in the CSF but had no effect on preventing brain atrophy or preventing neurocognitive decline for at any dose. Interestingly, SBC-103 was not detected in the CSF suggesting that it may not have reached the CNS[55].

3.3. Gene therapy

3.3.1. Adeno-associated virus (AAV)

To overcome the need for periodic administration of ERT or therapies based on small molecules like SRT, one-shot gene therapies are being developed for MPS III in order to provide constant production of the deficient enzyme. One of the main advantages of gene therapy is that only a proportion of cells in an organ, in this case, the brain, need to be corrected, as these corrected cells can produce a sufficient amount of the active enzyme to cross-correct neighbouring cells [56-59]. Arguably, gene therapy approaches currently stand out as the most promising therapeutic approach for treating MPS III, with the publication of various studies demonstrating the potential use of gene therapy to treat not just MPS III, but numerous other monogenic neurological diseases [60]. However, despite this promise, there are still hurdles to therapeutic efficacy with this type of therapy, relating mainly to vector type and route of administration. For example, are there differences in transduction efficiency between adeno-associated virus (AAV) and lentivirus? Can intravenously administered viruses infect neurons in the brain and is the direct administration of vector to the brain is safe?

Many AAV-based strategies involve the delivery of genes to the CNS. To achieve widespread distribution, initial experiments adopting AAV used multiple direct injections to the brain parenchyma. As the cell and tissue tropism of different AAV serotypes (AAV1 – AAV13) became better understood [61], subsequent experiments began to take advantage of the ability of certain AAV serotypes to gain access to the CNS. For example, AAV9 can cross the BBB after intravenous administration, resulting in widespread transduction of the CNS and the peripheral organs through a non-invasive procedure. Alternatively, various other AAV vectors have been administered directly to the CSF, hijacking the ventricular system to achieve global CNS gene transfer, as well as delivery to the peripheral nervous system and liver. In this section, we will focus on various studies, which together demonstrate the efficacy of different vectors and routes of administration for the treatment of MPS III.

Most gene therapy studies on MPS III use the vector adeno-associated virus (AAV), which achieve high transduction *in vivo* and have proven safe in clinical studies [62,63]. Furthermore, preclinical and clinical data provide evidence for long-term AAV-mediated gene expression in the brain, without producing any significant adverse effect [56,58,64-69]. However, in terms of efficacy, the selection of AAV virus serotype is a crucial consideration. Gilkes et al. have used AAV5, AAV8, AAV9 and AAVrh10 to deliver NAGLU to MPS IIIB mice via direct administration of the virus to the CNS. Although they found that AAV8 showed the greatest efficacy in terms of bio-distribution and transduction of NAGLU [70], other studies

using AA9-mediated NAGLU gene transfer via the CSF or systemic delivery, have also proved efficacious. A study by Ribera et al. administered AAV9 vectors carrying NAGLU to the CSF of MPS IIIB mice at 2 months of age when the disease has already become established and observed a restoration of gene expression and enzymatic activity in the CNS, which resulted in normalization of GAGs and lysosomal physiology and reduced neuroinflammation. The systemic hallmarks of the disease were also corrected, behavioural deficits improved and lifespan was extended [58]. More recently, the metabolomics profiles of MPS IIIB mice was specifically measured in MPS IIIB mice to assess the impact of systemic gene delivery [71]. Following intravenous administration of rAAV9-hNAGLU, near-complete correction of systemic metabolomic impairments was observed [71]. Ahead of a clinical gene therapy treatment, an AAV9-mediated vector carrying human NAGLU (rAAV9-CMV-hNAGLU) was tested on cynomolgus monkeys via intravenous injection [72]. Over the course of 6 months, no adverse effects were apparent and the treatment resulted in long-lasting global CNS and somatic transduction, with relatively high NAGLU activity compared to wildtype levels, both in the brain and body [72]. This study demonstrated an effective and safe profile for systemic rAAV9-hNAGLU vector delivery in nonhuman primates, providing evidence for its clinical potential in humans. Safety of intravenous administration of AAV9-mediated NAGLU gene transfer via the CMV promotor (rAAV9-CMV-h*NAGLU*) has also been tested. Meadows et al. performed an IND-enabling good laboratory practice (GLP) toxicology study in healthy and MPS IIIB mice. rNAGLU expression was rapid and persistent in the majority of CNS without any adverse clinical signs of toxicology during the 6-month study, but a dosing range for safe and effective rAAV9-CMV-hNAGLU systemic gene delivery in MPS IIIB was also identified [73].

Different AAV serotypes have also been tested in other forms of MPS III. AAVrh10 has been used to deliver SGSH in MPS IIIA mice via intraparenchymal administration [74]. AAVrh10-derived SGSH enzyme improved the breakdown of heparan sulfate and reduced microglial activation. With time, GM3 ganglioside accumulation was ameliorated and the formation of ubiquitin-positive lesions near the injection site or in regions connected to the injection site was prevented. However, these positive changes were restricted to the site of injection, and no such changes were observed in regions of the brain distant from or lacking connections with, the administration site. Therefore, to obtain adequate therapeutic efficacy, it may be necessary to administer the gene vector to multiple intraparenchymal regions in order to ensure widespread distribution of enzyme and correction of disease pathology, increasing the likelihood of infection [74]. Recently, AAV9 was used to test gene therapy in the MPS IIID mouse model for the first time [59]. Treatment of the GNS-deficient animals with GNS-expressing AAV9-derived vector delivered to the cerebrospinal fluid normalized GAG storage, improved lysosomal functionality in the CNS and somatic tissues, reduced neuroinflammation, restored normal behaviour and extended the lifespan of treated mice relative to untreated MPS IIID mice [59].

3.3.2. Autoantibodies to Adeno-associated virus

One commonly cited drawback of the use of AAV-mediated gene therapy is that individuals with pre-existing host humoral and cellular immunity to AAV capsids may be subject to limited target tissue transduction and long-term expression of the genes they carry, making them less likely to benefit from AAV-mediated gene transfer [75]. Even low levels of neutralizing antibodies against AAV capsids can result in impaired transduction of the incorporated gene following intravenous delivery [76,77]. However, Murrey et al. showed that low levels of preexisting anti-AAV9 antibodies did not affect vector transduction of rAAV9-CMV-hNAGLU in cynomolgus monkeys. Even at high levels, preexisting anti-AAV9 Abs led to reduced transduction in the liver and other somatic tissues but did not diminish transgene expression in the brain [72]. Similarly, Ribera et al. showed in their study that enzymatic activity in the CSF of dogs after administration of canine NAGLU-coding vectors to animals that were either naïve or had pre-existing immunity against AAV9, displayed similar levels of enzyme activity, suggesting that CNS efficacy would not be impaired in patients that are seropositive for AAV9 [58]. These studies demonstrate that at least for AAV9, an effective and safe profile for systemic vector delivery in nonhuman primates can be achieved.

3.3.3. Lentiviral/Adeno-associated virus combinatorial approach

Like AAV-mediated gene therapy, lentivirus has proved effective in treating MPS III. Lentiviral vectors carrying genes coding for murine heparin N-sulfatase and sulfatase modifying factor-1 have been tested in MPS IIIA. After administration via the cerebral lateral ventricles, enzyme activity was found to be between 0.5- and 4-fold greater than in normal mouse brain and ganglioside and lysosomal β-hexosaminidase levels, both of which are characteristically elevated in MPS IIIA, were significantly reduced, or were normalised [78]. Furthermore, combining different vector types via alternative routes can prove more effective at achieving disease correction than either one alone. In one study, AAV2/5-mediated and lentivirus-mediated NAGLU expression was more efficient than either one therapy alone in treating MPS IIIB [79]. MPS IIIB neonatal mice were treated with intracranial AAV2/5-NAGLU, intravenous lentiviral-NAGLU or both. All treatment groups resulted in significant biochemical and histological improvements compared with untreated MPS IIIB animals, but the animals treated with both AAV2/5 and lentivirus lived significantly longer (612 days) than animals treated with just AAV2/5-mediated gene therapy (463) or lentiviral gene therapy (358) suggesting that although MPS III disease is primarily neurological, targeting both the systemic and central nervous system early in life appears to be the most efficacious approach for treating MPS IIIB [79].

3.3.4. Phase I/II Clinical trials

A number of gene therapy clinical trials have been sponsored to test the safety and efficacy of gene therapy for the treatment of MPS III. In this review, we will focus on studies which have available data but a complete overview is described in detail by Marco et al. [60]. These

studies include phase I/II trials for MPS IIIA, MPS IIIB and MPS IIIC. To date, only one clinical trial has been completed, a phase I/II trial testing intracerebral administration of AAV10 carrying the human SGSH and SUMF1 cDNAs (SAF-301; rh.10-SGSH-IRES-SUMF1) for the treatment of MPS IIIA (NCT01474343 and NCT02053064). In this study, Lysogene recruited four children (three aged 5.5 – 6 years old, and one aged 2 years 8 months) to test the tolerance and safety of SAF-301, and assess disease biomarkers in blood, urine and CSF and brain function during one year of follow up. The results were published by Tardieu et al. [80] and reported that the therapy was safe and well-tolerated and improved brain atrophy and behaviour. All children showed a decline in cognitive ability and three patients presented with brain atrophy. After 8 weeks of treatment, MRI showed that brain atrophy has stabilized in two patients but increased in the other two, and there was a moderate improvement in behaviour, attention and sleep in three of the patients [80]. An open-label long term study was initiated five years after treatment to follow up on patients with MPS IIIA who had previously been treated with SAF-301 which ended in 2017, but no results were available. The aim was to collect additional safety and tolerability data on the treatment, and further collect data to assess the effects of SAF-301 on neurological and psychological status and biomarkers (NCT02053064).

A number of clinical studies are still underway, Esteve has developed EGT-101, a compound consisting of AAV9 containing h*SGSH* (AAV9-h*SGSH*). In this phase I/II clinical trial, EGT-101 has been administered via intra-CSF administration to MPS IIIA patients (2015-000359-26). An uncontrolled phase I/II clinical trial sponsored by UniQure Biopharma is also currently investigating the intraparenchymal administration of a recombinant AAV2/5 vector encoding human NAGLU AAV5-h*NAGLU* in four MPS IIIB patients (NCT03300453), the results of which have recently been published [81]. 30 months after injection, the treatment appeared to be safe and well-tolerated with sustained NAGLU production (15-20% of that in unaffected children) in the CSF. Compared with the natural history of MPS III syndromes, neurocognitive progression was improved in all patients, with the youngest patient having function comparable to that in healthy children.

Abeona Therapeutics is currently recruiting for two phase I/II clinical trials for both MPS IIIA and MPS IIIB. To treat MPS IIIA, a self-complementary AAV9 vector carrying the human SGSH gene under the control of a U1a promoter (scAAV9.U1a.hSGSH) called ABO-102 was delivered intravenously to participants two years of age or older in an open-label, dose-escalation phase I/II clinical trial (NCT02716246). The estimated number of participants for this study is 22 and the primary aim is to assess safety and neurocognitive function (developmental score) after 24 months and secondarily, to assess SGSH activity, liver and spleen volume, cognitive ability and urinary GAG levels. So far, no adverse events relating to scAAV9.U1a.hSGSH have been reported. Although efficacy data is yet to be published, some preliminary data is available, showing a dose-dependent and sustained reduction in CSF HS all three cohorts after 30 days. Following dosing at 14-26 months of age,

participants showed normal development 12 – 18 months post-treatment. To treat MPS IIIB, Abeona Therapeutics have also sponsored a new phase I/II trial using one-time intravenous administration of AAV9 carrying the human NAGLU gene under the control of a CMV enhancer/promoter (rAAV9.CMV.hNAGLU) called ABO-101 (NCT03315182). This two-year open-labelled, dose-escalation clinical trial will include an estimated 9 MPS IIIB patients aged between 6 – 2 years or older with a minimum Developmental Quotient of 60 or above. Two doses (2 x 10^{13} vg/kg and 5 x 10^{13} vg/kg) are being tested across two cohorts to primarily assess safety and neurodevelopment, and other secondary endpoints including neurocognitive and behavior evaluations, quality of life, enzyme activity in cerebrospinal fluid (CSF) and plasma, biomarkers in CSF, plasma and urine, and brain and liver volume. As described above, the preliminary data from the MPS IIIA study are encouraging, but no data is yet available for MPS IIIB [60] (www.clinicaltrial.org). While gene therapy has, and continues to show tremendous therapeutic promise for the treatment of MPS III disease in animal models, leading to a number of clinical trials, these trials have revealed that the ideal method of delivery of viral vectors is yet to be elucidated.

3.4. Cellular Therapies

3.4.1. Hematopoietic Stem Cells

Hematopoietic Stem Cells treatment (HSCTs) can be obtained from the bone marrow or peripheral blood of a healthy donor and transplanted into a patient. To avoid the rejection of donor cells by the patient's immune system, they must first be immunosuppressed according to a conditioning regimen [82]. Healthy, matched enzyme-secreting donor cells can then be transplanted into the patient, providing a permanent and continuous supply of protein. For a more detailed description of the history and application of HSCs in MPS, Taylor et al. have written a thorough review [83]. HSCTs is already an effective therapy for a number of inborn errors of metabolism, a good example of which is in the treatment of Hurler's syndrome if administered early. However, what has been learnt from the clinical application of HSCTs for the treatment of Hurler Syndrome, has not translated well to the treatment of MPS III [84]. Unfortunately, HSCT have not proved successful in preventing the progression of neurological disease in MPS III patients.

Furthermore, even when HSCT is administered to MPS IIIB patients before the onset of neurological symptoms, studies have shown that neurocognitive decline still ensues [85,86]. A clinical study was performed in 62 MPS patients, only 2 of which had been diagnosed with MPS III, and found that although the treatment was safe and effective overall in MPS, it is difficult to conclude the efficacy to MPS III specifically [87].

3.4.2. Umbilical Cord Mononuclear Cells

A large study on unrelated donor umbilical cord blood transplantation for inherited metabolic disorders in 159 patients, showed more promise than previously described in **section 3.4.1**. Of the 19 MPS III patients enrolled in the study, 12 survived and 9

showed disease stabilization with lesser neurological symptoms. Overall, children who received HSCT appeared to have fewer behavioral problems and better sleeping patterns as compared with children who did not receive transplants. One MPS IIIB patients received HSCT just before their second birthday and appeared to respond best to the treatment. At age 15, the patient had normal blood levels of O sulphated HS and N-sulfated HS and disease symptoms appeared to be better than MPS IIIB patients who had not received treatment [88]. However, in another study, umbilical cord blood-derived hematopoietic stem cells (UCBT) were transplanted into two MPS III patients (MPS IIIA and MPS IIIB) before the onset of neurological symptoms and monitored for 5-years. Despite uncomplicated transplantation, with full engraftment of donor cells, both patients showed progressive neurological deterioration, regression of cognitive skills, and behavioural disturbances, which was comparable to untreated patients with the same mutations [89]. In addition, the HS concentration in CSF in the MPS IIIB patient was just as high as in untreated MPS IIIB patients. Given the outcome of this clinical study, it can be concluded that like BMT, early UCBT does not prevent neurological deterioration in MPS III.

Although attempts at using HSC and UCBT transplantations in MPS III have yielded disappointing results in recent years, leading to the assumption that these approaches have little potential for being effective treatments [3,12,13], animal studies have continued to raise hope. In the mouse models of MPS IIIB, monthly intravenous administration of human umbilical cord mononuclear cells over a period of six months proved effective in reducing ganglioside accumulation, microglial activation, corrected anxiety-like behaviour and restored hippocampal cytoarchitechture [90].

3.4.4. Ex-vivo gene modification

In vivo gene therapy strategies involve the administration of viral vector particles directly to patients to provide affected cells with normal complementary DNA, *ex vivo* gene therapy approaches are based on the *ex vivo* transduction of patient cells that are subsequently infused back, potentially circumventing an immune response for foreign cells [91]. For a more extensive comparison between *in vivo* and *ex vivo* approaches to date, Fraldi et al. have recently published a review. In brief, gene therapy approaches have been developed for MPS IIIA and MPS IIIB using autologous transplantation of HSCs genetically modified using lentiviral vectors to express SGSH or NAGLU [92-95]. After transplantation, gene-corrected cells proliferate and travel to the brain where they cross the BBB to become resident cells of the CNS. Here, they secrete the deficient protein, and subsequently cross-correct other endogenous cells. These studies have shown normalization of HS, secondary storage and neuroinflammation as well as improvements in behavioural read-outs.

One way to overcome the blood-brain barrier is to target therapy directly to the CNS via parenchymal injection or via the cerebrospinal fluid. In recent years, Clarke et al. have shown that NSCs derived from reprogrammed MPS IIIB mouse embryonic fibroblasts to create iPSCs and corrected using lentiviral-

mediated human NAGLU overexpression, alleviated neuropathology [96]. It is important to underline the fact that a modified NAGLU enzyme was not necessary, as described in **section 3.2.1** above. Furthermore, very little enzyme was needed to obtain correction. These findings suggest that cell therapies represent an important line of investigation, despite current dogma. The use of neural progenitor cells to provide the missing enzyme also has regenerative potential since they can differentiate *in vivo* into neurons and astrocytes [97-101]. This is currently an underdeveloped field, given the fact that attempts to promote regeneration in the spinal cord injuries have been challenging. Nevertheless, several reports from studies on neurodegenerative animal models show that neural progenitor cells can differentiate into functional neurons, which are capable of restoring neuronal networks to a degree that impacts neurocognition behaviour [97,99,101]. For Parkinson disease, the use of neural progenitor cells is further along in the developmental process and several clinical trials are underway addressing safety and efficacy to treat Parkinson disease (NCT03128450, NCT03128450, NCT03309514, NCT03815071, NCT02452723, NCT02452723). Finally it important to underline that despite the obstacles posed by stem cell therapies compared to others, this line of research should be further pursued as it offers a unique opportunity to address neuronal loss, which other therapies do not.

CONCLUSION

The genetic cause of MPS III and the biochemistry of their gene products are well known and methods for genetic and biochemical diagnosis have been established. Therapeutic approaches have been developed, which target key aspects of the disease from its root cause to its downstream effects. With the same knowledge, HSCTs and ERT have been developed for the treatment of other forms of MPS and now both have been approved for MPS I, II, IVA, VI and VII. However, for MPS III, the road to therapy has been and continues to be a challenge given its neurological nature. Fantastic progress has been made in adapting therapies for other forms of MPS, offering up important lessons. Cellular and animal models have paved the way for several clinical trials. Among them the most advanced in development are ERT, involving either direct administration to the brain or the use of BBB-compliant fusion protein, and gene therapy using vectors administered either direct to the brain or via the bloodstream like ERT. Both approaches have presented new challenges at clinical trials, but new and modified approaches are currently under investigation. Although these approaches stand out as the most advanced, it is important to recognise their limitations now and in the future, and remain open to overcoming the barriers to other forms of therapy such as stem cell therapies. While stem cell therapies have proved disappointing to date, research into stem cell treatment for other neurodegenerative disease continue to show promise and in the process reveal areas for improvement that may be applicable to MPS III. Another important consideration, when asking how close we are to a therapy for MPS III is whether one therapy alone will ever be enough. Even if ERT, substrate reduction therapy, gene therapy or stem cell

therapies are optimised, it may be necessary to combine different approaches in order to further improve pathological outcome measures and behavioural phenotypes, but most importantly, extend the life span of patients.

A frustration in translational research is why promising results in non-human models often lead to disappointing results in clinical trials. It is worth pointing out that MPS III is a rare disorder making it difficult to normalise studies for treatment groups while maintaining a high enough number of participants to generate meaningful data. It is also important to point out that there is a disproportionate number of studies conducted on MPS IIIA and MPS IIIB. This means that some therapies, such as ERT and gene therapy, may become available for these subtypes more quickly than others. Nonetheless, despite its low prevalence, MPS III is a severely debilitating disease affecting not only the patient, parents and caregivers but society, justifying further attention and research. While researchers continue to develop therapies for MPS III, multidisciplinary teams who consider the age, clinical stage, severity and socioeconomic status of patients are essential for the proper management of those suffering with MPSIII. Non-profit organization have a pivotal role in promoting initial studies for therapy development, but this effort should be further supported by governments and pharmaceutical companies.

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