

A Phase 2/3 Trial of Pabinafusp Alfa, IDS Fused with Anti-Human Transferrin Receptor Antibody, Targeting Neurodegeneration in MPS-II

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Pabinafusp alfa (JR-141) is a novel enzyme drug that crosses the blood-brain barrier by transcytosis via transferrin receptors. In order to establish its efficacy and safety, a multicenter, single-arm, open-label phase 2/3 clinical trial was conducted in 28 Japanese patients with mucopolysaccharidosis II (MPS-II, Hunter syndrome) by intravenous administrations of 2.0 mg/kg of pabinafusp alfa for 52 weeks. The primary efficacy endpoint was changes in heparan sulfate (HS) concentrations in the cerebrospinal fluid (CSF). Secondary endpoints included assessments of neurocognitive development for central efficacy, and changes in plasma HS and dermatan sulfate (DS) concentrations for peripheral efficacy. HS concentrations in the CSF significantly decreased from baseline to week 52 ($p < 0.001$), suggesting continuous inhibition of substrate accumulations in the CNS, i.e., hitherto unaddressed progressive neurodegeneration. Evaluations of neurocognitive developments showed positive changes in 21 of the 28 patients. Serum HS and DS concentrations, liver and spleen volumes, and other assessments suggested the peripheral efficacy of pabinafusp alfa was comparable to that of idursulfase. Drug-related adverse events were mild or moderate in severity, transient, and manageable. The results establish delivery across the BBB of pabinafusp alfa as an effective therapeutic for treating both the CNS and peripheral symptoms of patients with MPS-II.

INTRODUCTION

The blood-brain barrier (BBB) is a special structure formed by capillary endothelial cells, the basement membrane, and astrocytes to selectively prevent undesirable substances from entering the central nervous system (CNS). The BBB can, however, be an impediment to the delivery of diagnostic and therapeutic large molecules (e.g., peptides, proteins, and oligonucleotides) into the brain, and presents obstacles to pharmacotherapy for CNS disorders generally.¹ Efforts have been made to enable brain delivery of these drugs by transcellular and paracellular pathways,² as well as by transient disruption of the BBB by ultrasound³ and hyperthermia,⁴ but tangible clinical benefits have so far been limited.

Hunter syndrome (mucopolysaccharidosis II [MPS II]) is an X-linked recessive lysosomal storage disease caused by a deficiency of iduronate-2-sulfatase (IDS).⁵ The catabolism of glycosaminoglycans (GAGs), such as heparan sulfate (HS) and dermatan sulfate (DS), is dependent on IDS, whose genetic mutations lead to pathological accumulation of GAGs in the lysosomes throughout the body, resulting in a broad spectrum of peripheral symptoms, including coarse facies, hepatosplenomegaly, upper airway obstruction, and cardiac dysfunction.^{5,6} Furthermore, progressive deterioration of the CNS is common in MPS-I, II, III, and VII,⁷ although the details of the neurodegenerative progression in MPS have not been fully elucidated.⁸

Intravenous enzyme replacement therapy (ERT) with recombinant human IDS (idursulfase, Elaprase) for MPS II is effective for most of the aforementioned peripheral symptoms⁹⁻¹¹ but not, because of the BBB, for the CNS disorders. As a result, progressive neurocognitive deterioration in MPS II remains a critical issue for patients and researchers alike.⁷

Efforts have been made to deliver enzymes across the BBB by utilizing endogenous protein receptors (e.g., insulin^{12,13} and transferrin¹⁴), which mediate innate transcytosis to facilitate enzyme delivery into the brain. Positive results have been reported in preclinical and translational studies.¹⁵ Intrathecal¹⁶ and intracerebroventricular¹⁷ routes of administration have also been attempted, but invasive procedures inevitably limit their general long-term use.

A BBB-penetrating fusion protein, pabinafusp alfa (JR-141),¹⁸ which consists of intact human IDS and an anti-human transferrin receptor antibody, utilizes transferrin receptor-mediated transcytosis. Its penetration across the BBB into the brain and resultant efficacy for the CNS have been demonstrated in preclinical studies,¹⁹ and a first-in-human translational phase 1/2 study²⁰ has shown marked

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Table 1. Patients' Demographics and Clinical Characteristics

Characteristics	Prior Enzyme Replacement Therapy with Idursulfase						
	None		Administered		All		
	N	(%)	N	(%)	N	(%)	
Number of subjects	3		25		28		
Age (years)	0 to 3 years old	2	(66.7)	3	(12.0)	5	(17.9)
	4 to 7 years old	1	(33.3)	8	(32.0)	9	(32.1)
	8 to 19 years old	0	(0.0)	13	(52.0)	13	(46.4)
	20 years and older	0	(0.0)	1	(4.0)	1	(3.6)
	all	3.0 ± 2.0		9.2 ± 5.5		8.6 ± 5.6	
Weight (kg)	17.33 ± 4.12		32.41 ± 14.51		30.79 ± 14.53		
Ethnicity	Asian	3	(100.0)	25	(100.0)	28	(100.0)
Duration of ERT (days)		–		2,077.2 ± 1,476.1		2,077.2 ± 1,476.1	
Idursulfase-related infusion associated reaction	no	–	(–)	11	(44.0)	11	(44.0)
	yes	–	(–)	14	(56.0)	14	(56.0)
Complications	no	2	(66.7)	9	(36.0)	11	(39.3)
	yes	1	(33.3)	16	(64.0)	17	(60.7)
MPS II-related medical history	no	1	(33.3)	6	(24.0)	7	(25.0)
	yes	2	(66.7)	19	(76.0)	21	(75.0)
MPS II-related neurocognitive impairment	no	0	(0.0)	8	(32.0)	8	(28.6)
	yes	3	(100.0)	17	(68.0)	20	(71.4)
Disease phenotype	severe	3	(100.0)	17	(68.0)	20	(71.4)
	attenuated	0	(0.0)	8	(32.0)	8	(28.6)

reduction of GAG accumulation in the cerebrospinal fluid (CSF) of patients with MPS-II, indicating successful delivery of pabinafusp alfa with favorable clinical responses. This report presents the results of a phase 2/3 multicenter, single-arm, open-label trial involving 28 patients with MPS II to establish the efficacy of pabinafusp alfa delivered across the BBB.

RESULTS

A total of 28 male patients with MPS II were recruited from 19 investigational sites in Japan to participate in the trial between August 10, 2018 and February 5, 2020. Their baseline demographics and clinical characteristics are shown in Table 1. Of these 28 patients, only 3 had not previously received standard treatment with idursulfase. All patients received 2.0 mg/kg/week of pabinafusp alfa intravenously for 52 weeks.

HS concentrations in the CSF significantly decreased from $5,856 \pm 2,614$ ng/mL before the initial administration of pabinafusp alpha to $2,124 \pm 882.6$ ng/mL at week 52 ($p < 0.001$; Figure 1). Before the initial dose, the HS concentrations in the patients with the attenuated ($n = 8$) and severe ($n = 20$) subtypes were $4,028 \pm 1,098$ ng/mL and $6,626 \pm 2,700$ ng/mL, respectively, and at week 52, they had decreased significantly to $1,721 \pm 707.2$ ng/mL and $2,294 \pm 909.9$ ng/mL.

HS concentrations in the CSF were classified according to the patients' individual subtypes (Figure 2) so that the relationships (partic-

ularly those associated with the severity and progression of neurodegeneration) between the phenotypic heterogeneity of MPS-II and the substrates in the CSF used as biomarkers of the neurodegenerative process were clear. The HS levels in the CSF at the initial dosing in the patients with the attenuated ($n = 8$, excluding one patient who deceased) and severe ($n = 19$) subtypes were $4,028 \pm 1,098$ ng/mL ($2,530$ – $5,500$ ng/mL) and $6,626 \pm 2,700$ ng/mL ($4,150$ – $15,100$ ng/mL), respectively. Despite these marked phenotypic differences between the subtypes, the HS levels had decreased significantly by 52 weeks post dosing, irrespective of the subtype, to $1,721 \pm 707.2$ ng/mL ($1,100$ – $3,340$ ng/mL) in the attenuated group and to $2,294 \pm 909.9$ ng/mL ($1,460$ – $5,450$ ng/mL) in the severe group ($p < 0.001$). Notably, HS levels in 18 of the 19 patients with the severe subtype fell below 4,000 ng/mL, which is considered to be the baseline HS level in the attenuated cases.

To ensure proper developmental assessment in the face of neurodevelopmental heterogeneity in MPS-II and the subsequent variability in treatment response among a limited number of suitable patients available for the trial, we made further evaluations to take account of both phenotypic subtypes and the clinical stages of disease progression (Table S1). The two subtypes are severe and attenuated, and patients with the former show far more marked severity and progression of neurodegeneration and associated clinical manifestations than those with the latter, who manifest fewer CNS symptoms. Patients with the severe subtype are further classified into three phases (initial,

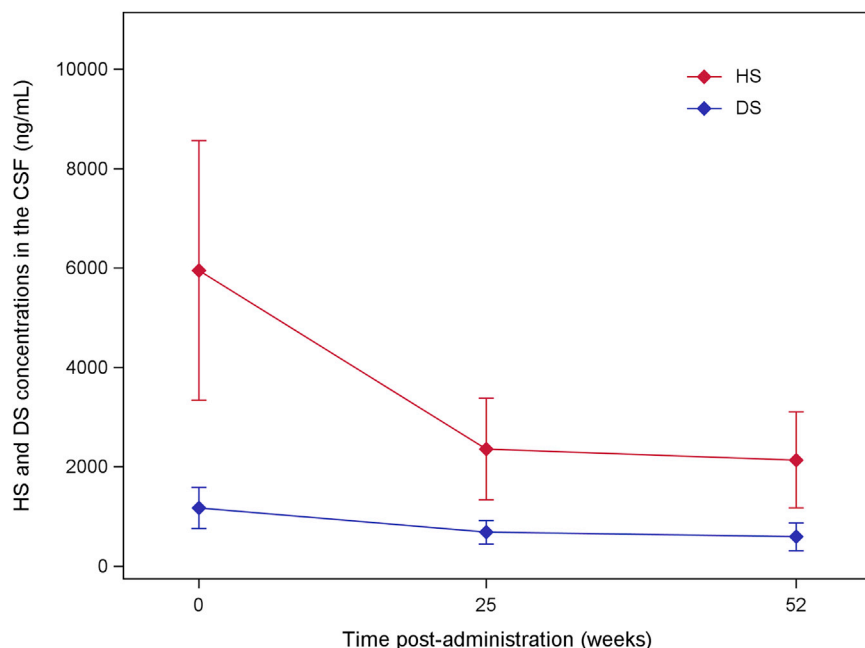


Figure 1. Time Courses of the HS and DS Concentrations in the CSF

The data are represented as means \pm SD.

middle, and late) according to their age and the stage of disease progression. Working criteria for judging treatment response are then created to reflect individualized treatment objectives and reflect heterogeneity in severity and clinical stage (Table 2).

Table 3 shows the developmental changes evaluated according to the Kyoto Scale of Psychological Development (KSPD),²¹ which are classified according to the three treatment responses defined in Table 2. Treatment response as reflected on the KPSD was based on the developmental quotient (DQ) in the attenuated group, and on age equivalent (AE) scores in the severe group, because AE scores are generally maintained in patients with attenuated MPS-II and do not, therefore, provide meaningful assessments of the effects of drugs on neurocognitive development; in patients with severe MPS-II, on the other hand, DQ invariably falls due to severe and progressive neurodegeneration, and only AE scores appropriately reflect the effects of drugs on this degeneration.

All 8 patients with the attenuated subtype showed either maintenance ($n = 7$, DQ changes ± 0.5 SD) or improvement ($n = 1$, DQ changes > 0.5 SD). Of the 20 patients with the severe subtype, 11 showed maintained AE scores ± 3 months, while 2 showed improvement in AE scores of >3 months (Table 3).

Figure 3 shows the gradients of the developmental trajectories formed by the chronological versus developmental ages. The gradient thus defined in the patients with attenuated MPS-II was 0.9543 months/months from the initial administration of the test drug through 23 and 52 weeks, which is close to the normal gradient of 1 months/months. The gradient of 0.6705 months/months in the patients with severe MPS-II in the initial phase shows an increase in develop-

mental age following the early introduction of treatment in the initial phase before disease progression. Since developmental age in such patients may increase without any treatment, however, this finding is not necessarily related to the treatment. On the other hand, the gradients of -0.0802 months/months and -0.0904 months/months in the patients in the middle and late phases, respectively, may indicate maintenance of the developmental ages of these patients at treatment initiation, although the changes may be due to the natural course of the disease. These observations show that a 52-week observation period is too short to clearly evaluate the effects of the drug on the psychological development of patients with MPS-II.

The developmental evaluation with the KSPD at 52 weeks suggests maintenance or improvement of AE scores or DQ in 21 of 25 patients (neurocognitive impairment in two of the eligible 27 patients was too severe to allow testing).

Investigators' observations of the qualitative behavioral changes over 52 weeks of treatment with pabinafusp alfa were collected from 26 patients in order to register the kinds of neuropsychiatric and behavioral changes that are difficult to evaluate by the standardized methods. The observed changes were in three major areas: speech, facial expression/liveliness, and physical movement. Positive findings were noted in 1 out of the 8 attenuated patients, and in 11 out of the 18 severe patients. Table S2 summarizes the reports in 6 patients with the severe subtype in the late phase; all 6 patients had previously received ERT with idursulfase. Positive changes in speech include increased utterances, improved verbal responsiveness, and resumed singing. In facial expression/liveliness, stable mood, decreased agitation, and increased smiling were reported.

As secondary endpoints to evaluate peripheral efficacy, serum HS concentrations showed a gradual decrease from the baseline to weeks 26 and 52 in all 24 patients (excluding 1 patient who deceased) who had previously received ERT: -320.7 ± 497.0 and -352.5 ± 484.3 ng/mL, respectively. In the 3 patients who had not received ERT, the serum HS concentrations decreased markedly: $-3,895 \pm 1,923$ and $-4,032 \pm 2,053$ ng/mL, respectively. Similar tendencies were observed regarding serum DS concentrations (Figure 4).

Liver and spleen volumes, both adjusted by body weight to take account of natural organ growth, remained fairly stable through the treatment period in the patients with prior ERT, while they decreased

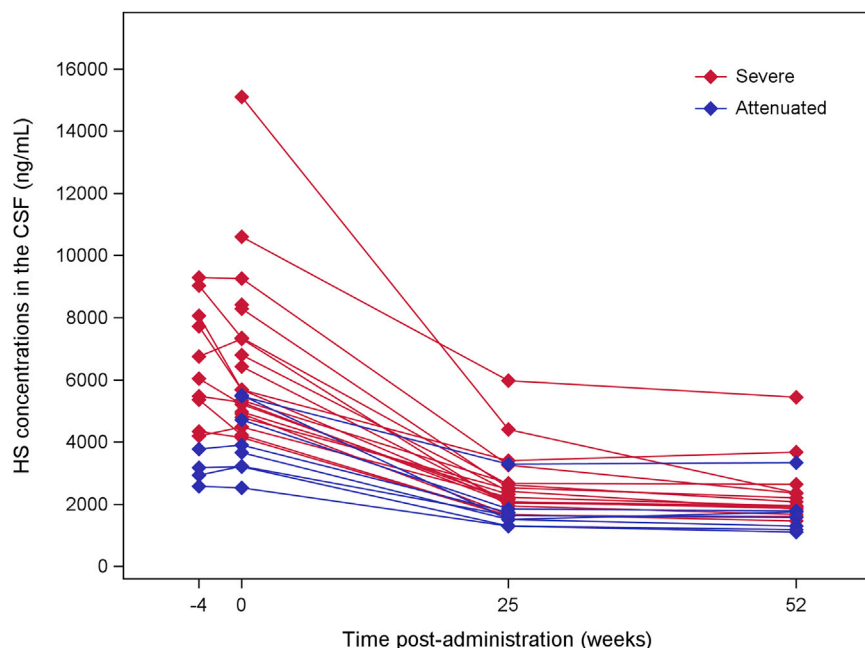


Figure 2. Time Courses of the HS Concentrations in the CSF According to the Subtypes of MPS-II

after initiation of pabinafusp alfa treatment in those without (Table S3). Cardiac function showed no significant changes during the treatment period in either group (Table S4). Changes in walk distance at the 6-minute walk test suggested stabilization and improvement in the patients treated with idursulfase for longer than 12 months (Table S5), as opposed to no consistent changes in the joint range of motion (Table S6).

Serious adverse events were reported in 5 patients, but they were judged to be unrelated to the test drug. There was one death due to respiratory failure and resultant hypoxic encephalopathy, both of which conditions are associated with MPS-II. Other adverse drug reactions were seen in 15 patients, 14 of whom presented infusion-associated reactions that were transient and clinically manageable without reduced dosage or cessation of test drug administration. The remaining one adverse event was QT prolongation. Further details of safety information about the study are summarized in Table 4.

Over the 52-week administration, 14 patients developed anti-pabinafusp alfa antibodies, but none were among the 14 who had infusion-associated reactions. We do not believe that the presence of anti-drug antibodies directly causes infusion-associated reactions or affects drug efficacy, since there was no relationship between the presence of anti-drug antibodies and the serum HS concentrations.

DISCUSSION

This trial demonstrated successful delivery of a therapeutic enzyme, pabinafusp alfa, across the BBB into the brain via transferrin receptor-mediated transcytosis, as shown by marked reduction in substrate accumulation in the CSF. Previous early clinical trials of methods to deliver drugs across the BBB^{3,4,22,23} have focused on temporary drug

delivery via a transient opening of the BBB in specific brain regions (e.g., the dorsolateral prefrontal cortex³), which is not conducive to the long-term drug administration required to treat neurodegeneration, in particular when dealing with such diffuse lesions as those found in neuropathic MPS. Attempts at drug delivery through the ventricles and subarachnoid spaces directly into the brain via intracerebroventricular^{17,24} and interthecal¹⁶ routes have shown some positive results, but these methods invariably involve invasive procedures that preclude long-term repeated administrations. In contrast, intravenous administrations utilizing the endogenous transcytosis mechanism offer sustainable and repeatable drug delivery appropriate for long-term therapy for chronic CNS disorders, and promising results have been obtained in an early clinical trial targeting insulin receptors.¹³ The present study is, to our knowledge, the first late-phase trial to establish successful delivery via transferrin receptor-mediated transcytosis across the BBB of a drug with findings suggestive of positive clinical outcomes.

In neuropathic MPS, genetic deficits of enzymes give rise to substrate accumulations, followed by a cascade of neurodegenerative events that are multifaceted, involve numerous functional and structural abnormalities, and culminate in irreversible neuronal death and brain atrophy.⁸ The CNS damage is not localized in the specific pathognomonic fashion seen in other neurodegenerative diseases, in which the resultant clinical manifestations vary depending on the given lesions. The marked phenotypic heterogeneity of MPS prevents the assessment of CNS symptoms that is essential for appropriate quantitative efficacy evaluation of new treatments in clinical trials. Along with the lack of suitable patients available to study, this constitutes a major methodological difficulty in designing clinical trials for neuropathic MPS.

This study used HS concentrations in the CSF as an endpoint, since they are considered to reflect both the fundamental pathogenesis and overall severity of neurodegeneration in each patient.²⁵ All patients in this trial showed marked reductions in CSF HS concentrations over the 52-week study period, suggesting inhibition of neurodegeneration in the CNS and reinforcing the findings of the phase 1/2 trial.²⁰

These positive findings regarding the endpoint need to be buttressed by corresponding improvements in the clinical manifestations of neurodegeneration, in particular the progressive neurodevelopmental

Table 2. Criteria for Judgement of Treatment Response at 52 Weeks According to the Kyoto Scale of Psychological Development

Treatment Response/Disease Severity and Clinical Stages	Improvement	Stabilization	Exacerbation
Attenuated	DQ changes > +0.5 SD	DQ changes \pm 0.5 SD	DQ changes < -0.5 SD
Severe: initial phase			
Severe: middle phase	AE changes > +3 months	AE changes \pm 3 months	AE changes < -3 months
Severe: late phase			

Maintenance of a developmental quotient after administration of pabinafusp alfa for 52 weeks is defined as changes in DQ within 0.5 SD, taking account of minimally important differences and potential variability in the developmental assessment results due to measurement errors. AE, age equivalent; DQ, developmental quotient.

disorders characteristic of MPS-II.²⁶ Neurocognitive development evolves over a considerable length of time, and long study periods are essential to follow the developmental trajectories. This creates great difficulties in conducting clinical trials in a timely fashion. The favorable neurocognitive changes seen in the previous 12-week trial of pabinafusp alfa²⁰ made it reasonable to assume that quantifiable improvements might be observed over a 52-week trial, although we recognize that this period is still too short to acquire conclusive neurodevelopmental findings. Over the 52 weeks, the AE scores of the patients in the initial phase of the severe subtype seemed to be stabilized, although this may simply have been a reflection of the natural course of the disease. Notably, the HS concentration in the CSF of one patient in the initial phase of the severe subtype who started treatment with pabinafusp alfa aged 22 months had dropped from 5,280 ng/mL to 1,920 ng/mL by week 52, along with an increase in AE of 13 months; another patient in the initial phase aged 21 months had an HS concentration of 10,600 ng/mL at week 0 and 5,450 ng/mL at week 52, with an increase in AE of 3 months. Taken together, it is clear that administration of pabinafusp alfa over 52 weeks led to a decrease in substrate accumulation in the CSF. Although a study period of 52 weeks is too short to evaluate the therapeutic effects of pabinafusp alfa on neurocognitive development, the significant decrease in HS concentrations in the CSF may suggest its neurocognitive efficacy.

The peripheral efficacy of pabinafusp alfa was demonstrated in terms of serum substrate reduction and liver and spleen volumes. It was less evident in terms of cardiac function, which, however, showed no substantial changes within the study period and may be interpreted as being stabilized without exacerbation due to the treatment. Administration of pabinafusp alfa led to no significant drug-related adverse events, so it can be consid-

ered comparable to idursulfase in terms of its safety profile. It has thus been shown to possess both CNS and peripheral efficacy, with its efficacy against CNS defects making it superior to the current standard ERT.

The present study has several limitations, the first of which is that it was an open study with no comparator arm. Although a double-blind comparative study with idursulfase may be most rigorous and ideal, an open study was selected as the best feasible option at the moment, through discussions with the regulatory authority in Japan, because (1) the preceding phase 1/2 study results²⁰ suggest significant reductions of the HS levels in the CSF by pabinafusp alfa even over 4 weeks, an effect not attainable by idursulfase, hence comparison between the two drugs was considered not sufficiently conducive for the primary endpoint; (2) investigators anticipated that limited availability of the feasible patients would be further undermined if the study required randomization for the conventional ERT known to be ineffective for the CNS disorders; and (3) ethical concerns were raised as the allocation of the patients suffering from progressive neurodegeneration to idursulfase for 52 weeks may imply depriving them of an opportunity to benefit from the expected CNS effects of the test drug.

The second limitation concerns the relatively short study duration of 52 weeks. The efficacy of pabinafusp alfa against neurodegeneration can only be established by longer observation, which will also clarify the associations between individual genotypic and phenotypic information, HS levels in the CSF, and long-term prognosis, so that accurate predictions of the treatment responses of individual patients are possible. This will help develop a treatment regimen to maximize the therapeutic effects of pabinafusp alfa in curbing, and hopefully preventing, the progressive deterioration of CNS functions. Long-term developmental data will therefore need to be collected duly that also enable comparison with the available natural history data²⁷ to allow detailed analysis of the drug's effects on developmental trajectories.

Third, as with the current ERT, pabinafusp alfa appears to have limited efficacy for skeletal, respiratory, and eye lesions in MPS-II. Mortality in MPS-II is reported to depend mainly on its skeletal, respiratory, and cardiac manifestations, which are caused by substrate accumulation and are not generally responsive to current ERT.⁶ Improvements in enzyme delivery to these lesions should be the next step toward better pharmacotherapy.

In conclusion, a novel technology has been established and successfully translated it into a clinical application that enables delivery of

Table 3. The Results of KPSD According to the Four Phenotypes

Classification of Disease Phenotype	Total N	Improved		Stabilized		Worsened	
		Number of Subjects	Proportion (%)	Number of Subjects	Proportion (%)	Number of Subjects	Proportion (%)
Attenuated	8	1	12.5	7	87.5	0	0.0
Severe: initial phase	2	1	50.0	1	50.0	0	0.0
Severe: middle phase	11	1	9.1	7	63.6	3	27.3
Severe: late phase	4	0	0.0	3	75.0	1	25.0

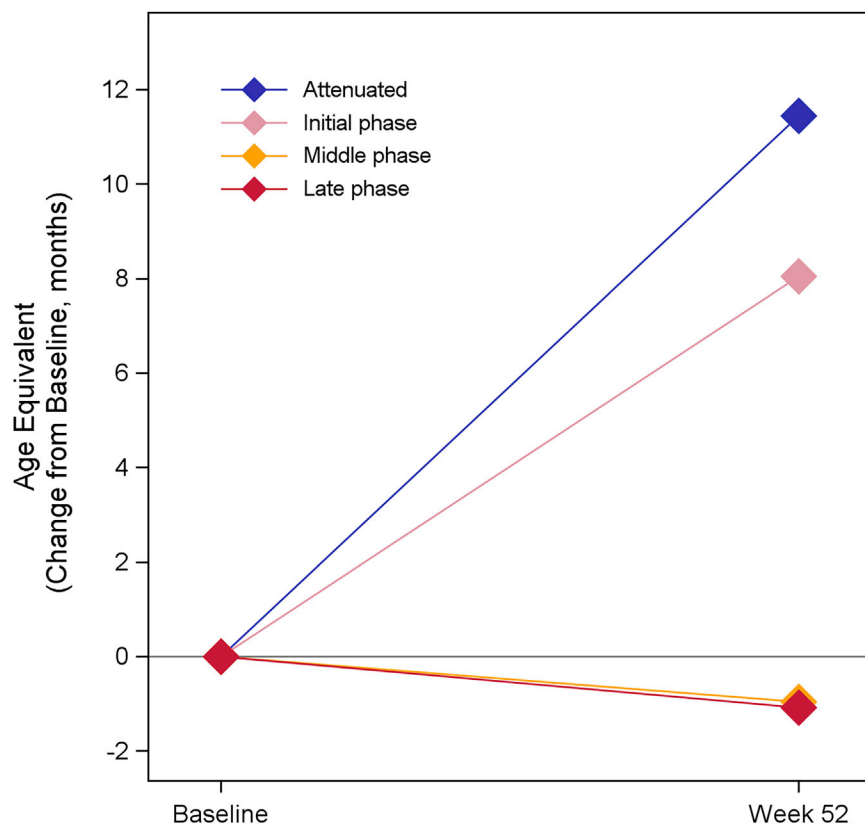


Figure 3. Changes of Gradients by Chronological and Developmental Ages According to Phenotypic Subtypes

Participants and Procedures

A total of 28 male patients were enrolled in the study. They received pabinafusp alfa intravenously at a dose of 2.0 mg/kg/week. The initial dose was started at an infusion rate of approximately 8 mL/h, which was then gradually increased. After the patients' safety had been established with the initial dose, the second and subsequent doses were administered at a constant infusion rate below 33 mL/h. Pretreatment with antihistamines, steroids, and other appropriate medications was allowed during the treatment if infusion-associated reactions were suspected.

Randomization and Masking

This was an open-label study with no masking.

Outcomes

The protocol is available online (<https://clinicaltrials.gov/ct2/show/NCT03568175>). A list of all efficacy and safety endpoints of the study is shown in Table S7. The primary efficacy

endpoint was the changes in HS concentrations in the CSF as measured at the time of the initial dose and at week 52, which were used to evaluate reductions in substrate accumulations in the CNS as indicators of neurodegeneration. Measurement of HS was conducted by high-sensitivity liquid chromatography-tandem mass spectrometry (LC-MS/MS).²⁵ The secondary endpoints for efficacy included developmental assessments with the KSPD²¹ and Vineland Adaptive Behavior Scales.²⁸ Assessments were made at the time of the initial dose, and at weeks 25 and 52 to evaluate the effects of pabinafusp alfa on neurocognitive development. Investigators' observations of the qualitative behavioral changes in the patients were also collected to register the kinds of neuropsychiatric and behavioral changes that can be subtle but potentially important, yet are difficult to capture by the aforementioned standardized methods. To evaluate peripheral efficacy, we compared changes in serum HS and DS concentrations by LC/MS/MS, liver and spleen volumes by CT, cardiac function by echocardiography, and other parameters as measured at baseline and at weeks 25 and 52. Prespecified safety endpoints were adverse events, adverse drug reactions, anti-drug antibodies, and infusion-associated reactions, as well as data from laboratory tests and electrocardiography.

large molecules across the BBB to address CNS disorders hitherto unamenable to pharmacotherapy. The positive results of this study need to be consolidated through long-term observations of neurodevelopment, which will be duly made through the ongoing extension studies of this trial and the phase 1/2 trial in Brazil, followed by post-marketing surveys, as well as through a multinational phase 3 comparative study to be initiated shortly. We hope these efforts will lead to further improvement and application of this technology to address CNS disorders at large, for which the inability to deliver drugs across the BBB has been a critical obstacle.

MATERIALS AND METHODS

Study Design

We conducted a multicenter, open-label clinical trial to evaluate the efficacy of pabinafusp alfa in treating CNS symptoms in patients with MPS II based on changes in HS concentrations in the CSF.

The study was conducted in 19 hospitals in Japan and complied with the Declaration of Helsinki. The protocol and procedures regarding informed consent were reviewed and approved by the Institutional Review Board at each participating institution.

The study consists of a treatment period of 52 weeks, which was preceded by an observation period of 4 weeks for the patients who switched from previous ERT with idursulfase or of 2 weeks for those with no prior history of ERT.

Statistical Analysis

The minimum sample size needed to evaluate the primary efficacy endpoint was calculated as in the preceding phase 1/2 study.²⁰ The effect size of pabinafusp alfa versus the standard idursulfase treatment

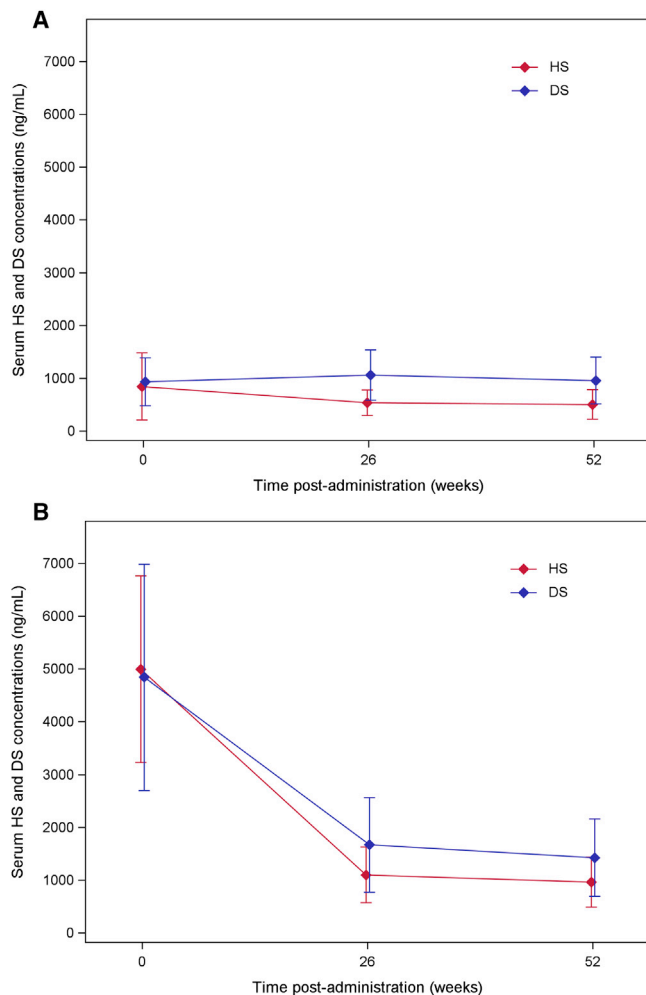


Figure 4. Serum HS and DS Concentrations in Patients with Prior ERT (Left) and Those Without (Right) at 0, 26, and 52 Weeks of Treatment with Pabinafusp Alfa

The data are represented as means ± SD.

in reducing HS concentrations in the CSF was conservatively estimated to be about 1,100 ng/mL. Detection of this effect with 80% power using a 2-sided paired t test at 5% significance level would require 5 patients for the study, but we actually enrolled a much larger number to collect sufficient data to evaluate both central and peripheral clinical efficacy.

All data analyses followed the intention-to-treat principle, whereby all patients who had received at least one dose of pabinafusp alfa were included in the analysis. One patient for whom no data on HS concentrations in the CSF at baseline or after the first dose of pabinafusp alfa were available was excluded from the primary endpoint analysis.

The paired t test was used to analyze the differences between HS concentrations in the CSF at baseline and at week 52. Summary statistics at each time point were also calculated.

Table 4. Summary of Adverse Events and Adverse Drug Reactions

Adverse Drug Reactions			
Number of subjects	28		
Number of subjects experiencing adverse events/reactions	15		
Proportion of subjects experiencing adverse events/reactions (%)	53.6		
Number of adverse events/reactions	59		
Adverse events	Number of Subjects	Proportion (%)	Number of Events
Preferred Terms			
Nervous system disorders	1	3.6	9
Dizziness	1	3.6	1
Headache	1	3.6	7
Syncope	1	3.6	1
Skin and subcutaneous tissue disorders	4	14.3	11
Urticaria	3	10.7	3
Rash	1	3.6	4
Erythema	1	3.6	4
General disorders and injection site reactions	11	39.3	38
Pyrexia	11	39.3	31
Chills	2	7.1	2
Fatigue	1	3.6	5
Investigations	1	3.6	1
Electrocardiogram QT prolonged	1	3.6	1

All statistical analyses were performed with the SAS version 9.4 statistical software package (SAS Institute, Cary, NC, USA).

Role of the Funder

The funder participated in the design of the trial, the collection, analysis, and interpretation of the data, and in the writing of the report; it is also the sole intellectual property holder and the manufacturer of pabinafusp alfa. All authors had full access to the data used in the study, and the corresponding author had final responsibility for the completion of the manuscript and the decision to submit it for publication.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.ymthe.2020.09.039>.

AUTHOR CONTRIBUTIONS

T.Y., S.S., and K.T. conceived and designed the study, and all other authors assisted in its design. T.O., Y.E., N.S., and K.N. led the trial's steering committee. Y.S. wrote the first draft of the manuscript. M.Y.

and T.I. designed and conducted all statistical analyses. All authors were involved in the interpretation and critical review of the data, and in drafting and revising the manuscript for important intellectual content; all approved the final version proposed by Y.S.

CONFLICTS OF INTEREST

T.O. has conducted consultancy for JCR Pharmaceuticals and reports research grants from BioMarin Pharmaceutical, Green Cross, Sanofi, Takeda, and JCR Pharmaceuticals. Y.E. has conducted consultancy for JCR Pharmaceuticals, and he has been awarded grants and research support from Actelion, BioMarin Pharmaceutical, and Sanofi; he has also received honoraria from Actelion, BioMarin Pharmaceutical, Sanofi, Takeda, and Dainippon Sumitomo Pharma. N.S. has conducted consultancy for JCR Pharmaceuticals, and he has been awarded grant/research support from Sanofi and Dainippon Sumitomo Pharma and honoraria from Actelion, BioMarin Pharmaceutical, Sanofi, Shire, and Dainippon Sumitomo Pharma. K.N. has conducted consultancy for JCR Pharmaceuticals.

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