Molecular Genetics and Metabolism Reports 33 (2022) 100930

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Case Report

Treatment of CLN1 disease with a blood-brain barrier penetrating lysosomal enzyme

Andreas Hahn [a], Yuji Sato [b,*], Toshiaki Ikeda [b], Hiroyuki Sonoda [b], Mathias Schmidt [b], Charlotte Pfrimmer [a], Ruben J. Boado [c], William M. Pardridge [c]

[a] Department of Child Neurology, Justus-Liebig University Gießen, Germany
[b] JCR Pharmaceuticals, Hyogo, Japan
[c] University of California, Los Angeles, USA.

ARTICLE INFO

Keywords:
Neuronal ceroid lipofuscinosis type 1
CLN1 disease
Enzyme replacement therapy
PPT1
Blood-brain barrier

ABSTRACT

Neuronal ceroid lipofuscinosis type 1(CLN1 disease) is a rare autosomal recessive lysosomal storage disease caused by genetic defects of palmitoyl protein thioesterase-1(*PPT1*), leading to accumulation of lipofuscin granules in brain and progressive neurodegeneration. Psychomotor regression, seizures, loss of vision, and movement disorder begin in infancy and result in early death. Currently, no disease-modifying therapy is available.

We report a 68-month-old boy with CLN1 treated on a compassionate use basis weekly for 26 months with a PPT1 enzyme fused to an anti-insulin receptor antibody (AGT-194), thereby enabling penetration of the blood-brain barrier (BBB). During treatment, no side effects were observed, while seizure frequency decreased, life quality improved, and the boy's general condition remained stable.

This case documents for the first time that treatment of CLN1 is principally feasible by an intravenous BBB penetrating enzyme replacement therapy using PPT1 fused with the human insulin receptor. Monitoring of side effects raised no unacceptable or unexpected safety concerns.Observed improvement of life quality related to ameliorated epilepsy control raises hope that further robust clinical trials including patients in earlier stages of disease will show positive results.

1. Introduction

Neuronal ceroid lipofuscinosis type 1 (CLN1 disease) is a rare autosomal recessive neurodegenerative disorder caused by mutations in the *PPT1* gene that encodes the lysosomal enzyme palmitoyl protein thioesterase-1 [1]. Accumulation of lipofuscin granules in the brain and peripheral nerves causes regression of psychomotor development, epilepsy, movement disorder, visual impairment, and premature death [2]. Although atypical forms with later onset occur, first clinical symptoms are usually noticed during infancy [3]. The *PPT1* gene was cloned nearly 30 years ago [4], enabling production of recombinant human PPT1 [5]. But since the PPT1 protein cannot cross the blood-brain barrier (BBB) [6], enzyme replacement therapy (ERT) is currently not available for CLN1.

Enzymes such as PPT1 can be re-engineered as IgG-enzyme fusion proteins that cross the BBB and retain enzyme activity. The IgG domain of the fusion protein is a monoclonal antibody (MAb) against an endogenous receptor transporter at the BBB, such as the insulin receptor (IR) or the transferrin receptor type 1 (TfR1) [7]. Binding of the fusion protein to the BBB receptor triggers receptor-mediated transcytosis, and results in biologic distribution to brain tissue [8].

Positive results have been shown by clinical trials with two BBB-crossing enzymes, valanafusp alfa, α-L-iduronidase fused to a MAb directed against the human IR (HIR), for treatment of mucopolysaccharidosis (MPS) I)) [9], and pabinafusp alfa, iduronate 2-sulfatase fused to a MAb against the human TfR1, for treatment of MPS II [10,11]. Recently, pabinafusp alfa has been approved in Japan.

For CLN1, a fusion protein of the HIR MAb and PPT1, AGT-194 (ArmaGen, San Diego, USA), was engineered by fusion of the human PPT1 to the carboxyl terminus of each heavy chain of the HIR MAb with a 31-amino acid linker (Fig. 1**A**). The fusion protein was expressed in a bioreactor and purified by affinity and ion exchange chromatography to homogeneity (Fig. 1**B**). The fusion protein retained high affinity binding to the HIR and high PPT1 enzyme activity [12], which was measured

* Corresponding author at: Research and Development, JCR Pharmaceuticals, 3-19 Kasuga-cho, Ashiya, Hyogo 659-0021, Japan.
E-mail address: sato-yuji@jp.jcrpharm.com (Y. Sato).

https://doi.org/10.1016/j.ymgmr.2022.100930
Received 17 August 2022; Received in revised form 19 October 2022; Accepted 21 October 2022

with a fluorometric enzyme assay [13]. The general and clinical safety and efficacy of enzymes fused to the human insulin receptor-targeting antibody has been extensively demonstrated in preclinical studies and a phase I/II trial in pediatric subjects with MPS I [9]. The in vitro characterization of AGT-194 is shown in Fig. 2. Preclinical data of the molecule will be subject to a separate publication (in preparation).

Here, we describe our experience with the first CLN1 patient receiving AGT-194 on a compassionate use basis. ERT was commenced after an explicit request and a written consent of his parents, and an approval by the ethics board of the producing pharmaceutical company based on the rapidly progressive nature, the dismal prognosis, and the lack of effective treatment for this disease.

2. Case presentation

The male patient is the only child of healthy non-consanguineous parents. He was born spontaneously after uneventful pregnancy without complications. Neonatal period and early infancy were normal. At 6 months of age, slowing of psychomotor development and deceleration of head circumference were noticed. The boy started crawling at 12 months, was able to speak 3 words and to stand with minor support at 15 months but lost these milestones before his second birthday. First seizures occurred at age 18 months, when laboratory diagnostics revealed no measurable PPT1 activity while whole exome sequencing disclosed a frameshift (c.346_347del; p. Gln116Glyfs*45) and a stop mutation (c.451C > T; p.Arg151*) in *PPT1*. Each parent was heterozygous for one mutation. Neurological examination showed a spastic-dystonic tetraparesis. Acoustically evoked potentials were normal, whereas flash-light evoked potentials could not be elicited. The EEG was of very low amplitudes without apparent background activity and sleep patterns. His epilepsy worsened subsequently with numerous absences, complex partial seizures, and myoclonic seizures as well as generalized tonic-clonic seizures per day, prompting treatment with valproate, clobazam, gabapentin, and cannabidiol.

At age 42 months, ERT with weekly intravenous AGT-194 via an implanted port system was started. AGT-194 (1 ml = 3.87 mg) was dissolved in 290 ml of 10% glucose solution and infused over 4 h. Blood glucose levels (at the start, after 1 ½ hours, and at the end of infusion), heart rate, and blood pressures were regularly monitored. Following a skin rash during one of his first infusions, the patient was pre-medicated with 40 mg prednisolone. AGT-194 was started at a dosage of 0.4 mg/kg body weight, which was slowly increased up to 2.6 mg/kg body weight weekly.

At the start of ERT, the boy could not sit, had no active speech, did not fixate or grasp, but reacted on tactile and acoustic stimuli. A cranial MRI demonstrated severe cortical and cerebellar atrophy. The boy suffered from up to 100 myoclonic and complex partial seizures per day, and 2–3 generalized tonic-clonic seizures per week, sometimes necessitating interruption by chloral hydrate.

During the treatment period, the patient had suffered from pneumonia twice and a port infection requiring device replacement. Except inflammatory characteristics in blood related to these infections, no other laboratory abnormalities were noted during regular controls. The boy's general condition slowly improved over time. No side effects or allergic reactions related to AGT-194 occurred, and blood glucose values were always in the normal range. His epilepsy became distinctly better controlled by substituting gabapentin by phenobarbitone at night, while retaining the same dosage of cannabidiol, levetiracetam, and clobazam. Occasional episodes of agitation had to be treated with single doses of chloral hydrate but resolved after switching from valproate to phenobarbitone. The patient appears emotionally more stable and calmer than before ERT, is more responsive to stimuli, and sometimes shows social smiling in response to acoustic stimuli from his family members.

At the last examination at age 68 months, no generalized tonic-clonic seizures have occurred for >12 months, although he still has some occasional complex partial and myoclonic seizures. A recent MRI remained largely unchanged compared to that at the start of ERT. Neurofilament heavy chains (Nf-H) in the CSF, a marker of neuroaxonal damage, were increased at start of ERT (785 pg/ml; normal range < 62.5 pg/ml) and somewhat lower at the last examination (570 pg/ml). The boy is blind, but his hearing appears normal. He is capable of swallowing and fed completely orally, reflecting a preserved gag reflex. The respiratory situation has also improved since repetitive daily mucus removal from the airway, constituting a large problem before the start of ERT, is no longer necessary. These positive changes have substantially improved the psychosocial situation of the family. Life quality assessed by the parental version of the 36-Item Short Form Survey (SF-36) improved substantially in several domains (Fig. 3). Seizure frequency as chronicled by the parents is summarized in Fig. 4.

3. Discussion

Here we report the first patient with CLN1 treated with recombinant human PPT1 fused with a monoclonal antibody against the human insulin receptor (AGT-194) on a compassionate use basis over a period of 26 months. Under pre-medication with steroids no allergic reactions, no hypoglycemia, and no other treatment related adverse events have been observed, suggesting no substantial side effects related to this therapy. Statements about efficacy are difficult to make since this single patient was in an advanced state of disease at the start of ERT. Moreover, no assay was available allowing measurement of PPT1 activity in the CSF, although the BBB-crossing enzyme is believed to be all but consumed within neuronal cells in the brain parenchyma, leaving few or no remnants to be secreted into the CSF [14]. However, along with the AGT-194 administration, epilepsy markedly improved over time. Most notably, generalized tonic-clonic seizures abated completely, hence

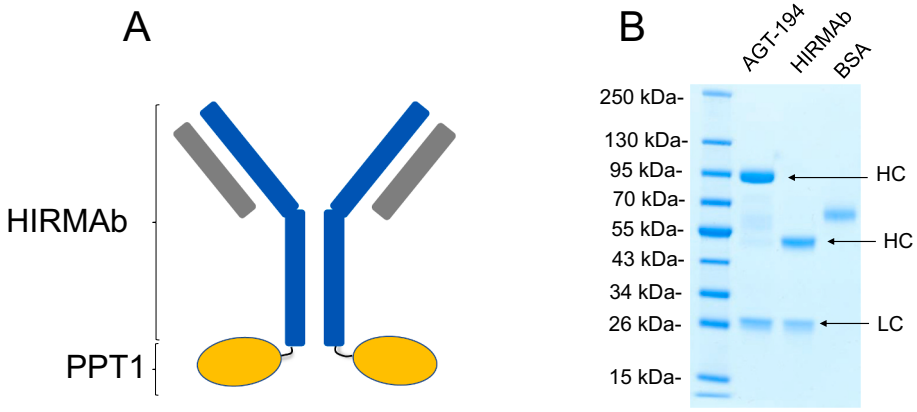
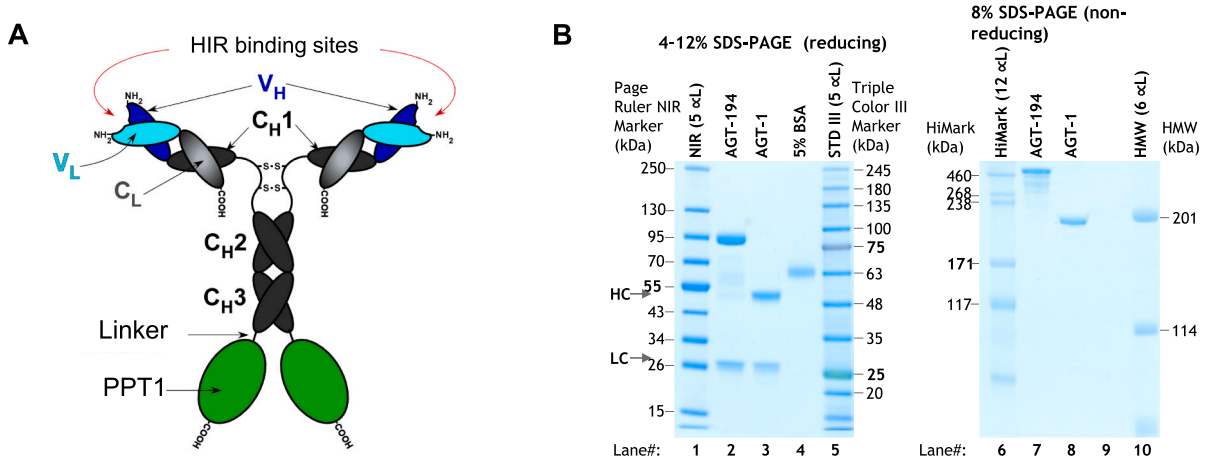


Fig. 1. A: AGT-194. The mature human PPT1 enzyme (NP_000301) was fused via a 31-amino acid linker to the carboxyl terminus of each heavy chain of the HIRMAb. Fig. 1B: SDS-Polyachrylamide gelelectrophoresis comparing AGT-194, HIRMAb and BSA. Reducing gradient SDS-PAGE gel shows the separate heavy chain (HC) and light chain (LC) of AGT-194 and the HIRMAb, in comparison to 5% BSA. AGT-194 and the HIRMAb share the same LC; the HC of AGT-194 is ~40 kDa larger than the HC of the HIRMAb owing to fusion of the PPT1 enzyme.



C

Molecule	Affinity InsR (nM)	Enzymatic activity (pmol/min/ug)
AGT-194	0.3	1,741±57
AGT-1	0.3	NA

Fig. 2. Biochemical characterization of AGT-194. A, schematic depiction of the fusion protein consisting of an anti-human insulin receptor antibody (AGT-1) and PPT 1, fused by a linker peptide. B, SDS-PAGE of purified AGT-194. Endotoxin content was <0.07 EU/mg and CHO host cell content 1 ppm. C, Affinity to human insulin receptor as measured by ELISA and enzymatic activity of AGT-194.

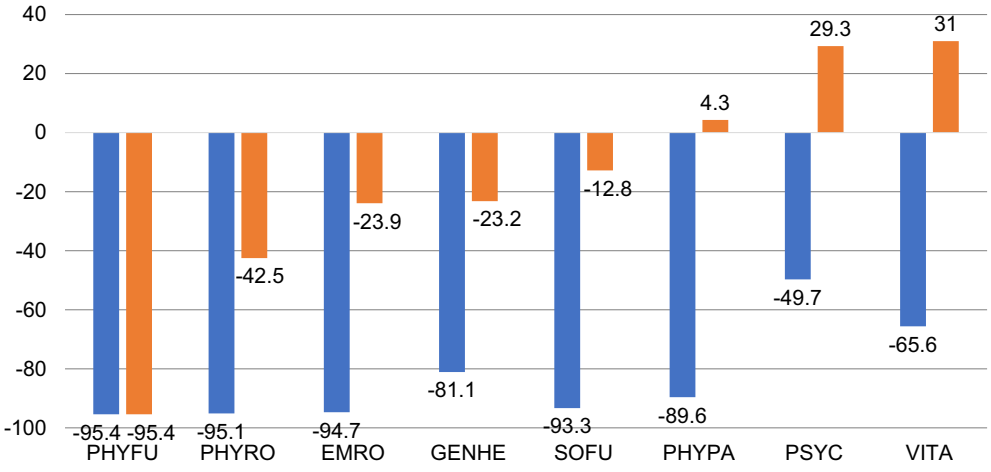


Fig. 3. The 36-Item Short Form Survey (SF-36).
Deviations from the normal mean for different domains of the SF3–6 by using the German version before (blue columns) and after 26 months of treatment with AGT-194 (orange columns), demonstrating substantial improvement in the majority of categories (PHYFU = Physical functioning; PHYRO = Physical Role Function; EMRO = Emotional Role Function; GENHE = General Health Perception; SOFU = Social Functioning; PHYPA = Physical Pain; PSYC = Psychological Well-Being; VITA = Vitality).

quality of life much improved. Although these improvements have to be interpreted cautiously since paradoxical reduction of seizure frequency in CLN1 during its course of disease has been described [15], it can be speculated that ERT with AGT-194 had positive effects in this patient despite its start at an advanced stage of CLN1.

Intravenous ERT with recombinant human enzymes is widely performed in an increasing, albeit still limited, number of LSD at large. While ERT has been shown to significantly improve organ functions, increase quality of life and prolong survival in several types of LSD, those with additional or exclusive CNS involvement (e.g. MPS I, II, III, CLN1, CLN2) receive little or no benefit from conventional ERT since the recombinant enzymes do not cross the BBB. The problem of passing the BBB can be circumvented by direct intracerebroventricular administration of enzymes via a Rickham device as with cerliponase alfa in CLN2 [16,17]. However, this technique requires repetitive strictly aseptic punctures of the implanted device preferably by an experienced team at a specialist hospital to avoid CNS infections. The Trojan horse strategy [8], i.e. the coupling of a recombinant human enzyme with an antibody directed against a BBB transporter as already approved for MPS II [11], is a least invasive and highly convenient ERT that can also be performed as home infusion therapy.

The advanced stage of disease in our patient has markedly limited clinical and laboratory evaluations to examine efficacy of the drug. However, this first experience with AGT-194 treatment suggests its potential as a feasible, safe, and effective therapy for CLN1, which is hoped to be added to the list of treatable LSD in near future. Further studies will be worthwhile to assess safety and efficacy in larger cohorts of patients with CLN1 of younger age by applying robust clinical and paraclinical endpoints.

Details of ethics approval

No specific ethics approval was necessary according to the local ethics committee (medical faculty of the Justus-Liebig-University at

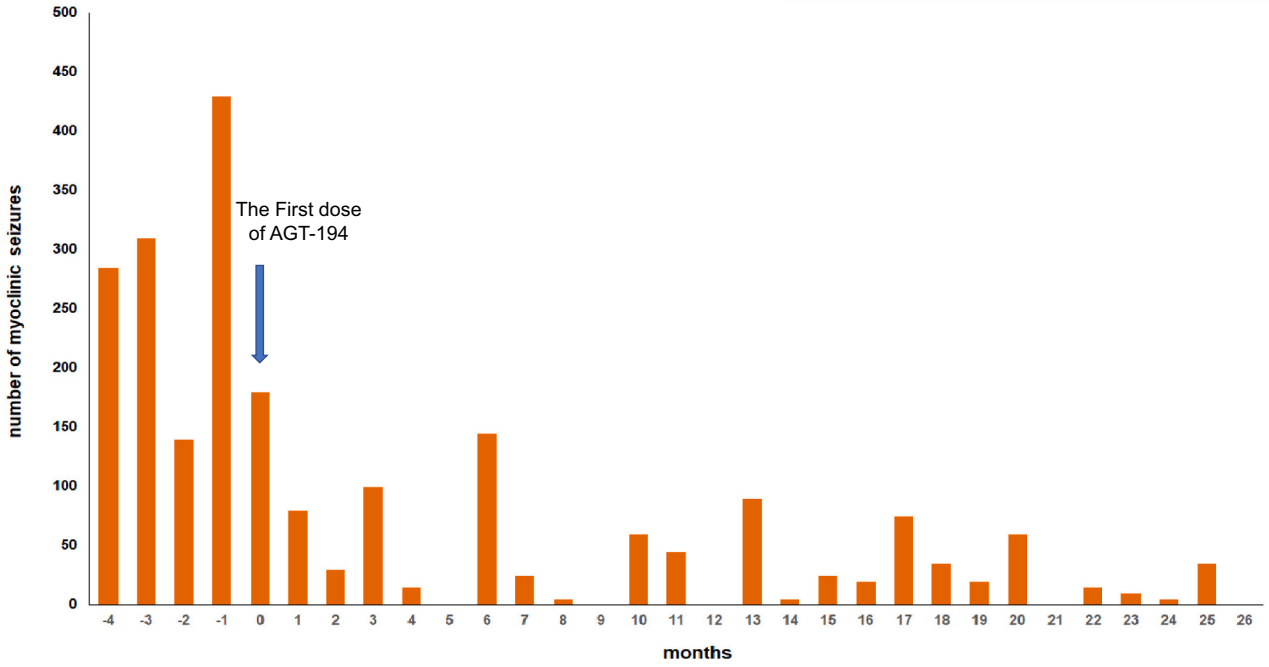


Fig. 4. Time course of myoclonic seizure frequency after the treatment with AGT-194.

Giessen, Germany, since a single patient was treated on a compassionate use basis).

A patient consent statement

Written consent was obtained from the parents of the patient regarding the enzyme replacement therapy with AGT-194.

Data availability statement

data supporting the results reported in the article can be provided on request from the authors.

Funding

The drug AGT-194 has been provided pro bono by ArmaGen (a part of JCR Pharmaceuticals) for its compassionate use for the patient. No external funding has been applied for the study.

Declaration of Competing Interest

AH and CP declare no competing interests. RB and WP are inventors of patents on brain delivery of biologic drugs. YS, HS and MS are employees of JCR Pharmaceuticals, the patent holder of AGT-194.

Data availability

Data will be made available on request.

Acknowledgement

We thank the parents of the patient who chronicled meticulously seizure frequencies in their child.

References

[1] J.A. Hawkins-Salsbury, J.D. Cooper, M.S. Sands, Pathogenesis and therapies for infantile neuronal ceroid lipofuscinosis (infantile CLN1 disease), Biochim. Biophys. Acta 2013 (1832) 1906–1909, https://doi.org/10.1016/j.bbadis.2013.05.026.

[2] A. Kohlschutter, A. Schulz, Towards understanding the neuronal ceroid lipofuscinoses, Brain Dev. 31 (2009) 499–502, https://doi.org/10.1016/j.braindev.2008.12.008.

[3] E.F. Augustine, H.R. Adams, E. de Los Reyes, K. Drago, M. Frazier, N. Guelbert, M. Laine, T. Levin, J.W. Mink, M. Nickel, D. Peifer, A. Schulz, A. Simonati, M. Topcu, J.A. Turunen, R. Williams, E.C. Wirrell, S. King, Management of CLN1 disease: international clinical consensus, Pediatr. Neurol. 120 (2021) 38–51, https://doi.org/10.1016/j.pediatrneurol.2021.04.002.

[4] L.A. Camp, L.A. Verkruyse, S.J. Afendis, C.A. Slaughter, S.L. Hofmann, Molecular cloning and expression of palmitoyl-protein thioesterase, J. Biol. Chem. 269 (1994) 23212–23219 (Epub 1994/09/16).

[5] J.Y. Lu, J. Hu, S.L. Hofmann, Human recombinant palmitoyl-protein thioesterase-1 (PPT1) for preclinical evaluation of enzyme replacement therapy for infantile neuronal ceroid lipofuscinosis, Mol. Genet. Metab. 99 (2010) 374–378, https://doi.org/10.1016/j.ymgme.2009.12.002.

[6] J.Y. Lu, H.R. Nelvagal, L. Wang, S.G. Birnbaum, J.D. Cooper, S.L. Hofmann, Intrathecal enzyme replacement therapy improves motor function and survival in a preclinical mouse model of infantile neuronal ceroid lipofuscinosis, Mol. Genet. Metab. 116 (2015) 98–105, https://doi.org/10.1016/j.ymgme.2015.05.005.

[7] W.M. Pardridge, R.J. Boado, Reengineering biopharmaceuticals for targeted delivery across the blood-brain barrier, Methods Enzymol. 503 (2012) 269–292, https://doi.org/10.1016/B978-0-12-396962-0.00011-2.

[8] W.M. Pardridge, Drug and gene targeting to the brain with molecular Trojan horses, Nat. Rev. Drug Discov. 1 (2002) 131–139, https://doi.org/10.1038/nrd725.

[9] R. Giugliani, L. Giugliani, F. de Oliveira Poswar, K.C. Donis, A.D. Corte, M. Schmidt, R.J. Boado, I. Nestrasil, C. Nguyen, S. Chen, W.M. Pardridge, Neurocognitive and somatic stabilization in pediatric patients with severe Mucopolysaccharidosis type I after 52 weeks of intravenous brain-penetrating insulin receptor antibody-iduronidase fusion protein (valanafusp alpha): an open label phase 1-2 trial, Orphanet J. Rare Dis. 13 (2018) 110, https://doi.org/10.1186/s13023-018-0849-8.

[10] H. Sonoda, H. Morimoto, E. Yoden, Y. Koshimura, M. Kinoshita, G. Golovina, H. Takagi, R. Yamamoto, K. Minami, A. Mizoguchi, K. Tachibana, T. Hirato, K. Takahashi, A blood-brain-barrier-penetrating anti-human transferrin receptor antibody fusion protein for neuronopathic mucopolysaccharidosis II, Mol. Ther. 26 (2018) 1366–1374, https://doi.org/10.1016/j.ymthe.2018.02.032.

[11] R. Giugliani, A.M. Martins, T. Okuyama, Y. Eto, N. Sakai, K. Nakamura, H. Morimoto, K. Minami, T. Yamamoto, M. Yamaoka, T. Ikeda, S. So, K. Tanizawa, H. Sonoda, M. Schmidt, Y. Sato, Enzyme replacement therapy with pabinafusp alfa for neuronopathic mucopolysaccharidosis II: an integrated analysis of preclinical and clinical data, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms222010938.

[12] R.J. Boado, J.Z. Lu, E.K. Hui, H. Lin, W.M. Pardridge, Bi-functional IgG-lysosomal enzyme fusion proteins for brain drug delivery, Sci. Rep. 9 (2019) 18632, https://doi.org/10.1038/s41598-019-55136-4.

[13] O.P. van Diggelen, J.L. Keulemans, B. Winchester, I.L. Hofman, S.L. Vanhanen, P. Santavuori, Y.V. Voznyi, A rapid fluorogenic palmitoyl-protein thioesterase assay: pre- and postnatal diagnosis of INCL, Mol. Genet. Metab. 66 (1999) 240–244, https://doi.org/10.1006/mgme.1999.

[14] Y. Sato, K. Minami, T. Hirato, K. Tanizawa, H. Sonoda, M. Schmidt, Drug delivery for neuronopathic lysosomal storage diseases: evolving roles of the blood brain barrier and cerebrospinal fluid Metab, Brain Dis. (2022), https://doi.org/10.1007/s11011-021-00893-3.

[15] S.E. Mole, G. Anderson, H.A. Band, S.F. Berkovic, J.D. Cooper, S.M. Kleine Holthaus, T.R. McKay, D.L. Medina, A.A. Rahim, A. Schulz, A.J. Smith, Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis, Lancet Neurol. 18 (2019) 107–116, https://doi.org/10.1016/S1474-4422(18)30368-5.

[16] A. Schulz, T. Ajayi, N. Specchio, E. de Los Reyes, P. Gissen, D. Ballon, J.P. Dyke, H. Cahan, P. Slasor, D. Jacoby, A. Kohlschutter, C.L.N.S. Group, Study of intraventricular cerliponase alfa for CLN2 disease, N. Engl. J. Med. 378 (2018) 1898–1907, https://doi.org/10.1056/NEJMoa1712649.

[17] E. de Los Reyes, L. Lehwald, E.F. Augustine, E. Berry-Kravis, K. Butler, N. Cormier, S. Demarest, S. Lu, J. Madden, J. Olaya, S. See, A. Vierhile, J.W. Wheless, A. Yang, J. Cohen-Pfeffer, D. Chu, F. Leal-Pardinas, R.Y. Wang, Intracerebroventricular cerliponase alfa for neuronal ceroid Lipofuscinosis type 2 disease: clinical practice considerations from US, Clin. Pediatr. Neurol. 110 (2020) 64–70, https://doi.org/10.1016/j.pediatrneurol.2020.04.018.