Neurofilament light is a treatment-responsive biomarker in CLN2 disease

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Objective

Neuronal ceroid lipofuscinosis type 2 (CLN2 disease) is a rare, progressive, fatal neurodegenerative pediatric disorder resulting from deficiencies of the lysosomal enzyme tripeptidyl peptidase 1 that are caused by mutations in TPP1. Identifying biomarkers of CLN2 disease progression will be important in assessing the efficacy of therapeutic interventions for this disorder. Neurofilament light is an intrinsic component of healthy neurons; elevated circulating extracellular neurofilament light is a biomarker of neuropathology in several adult-onset neurological diseases. Our objective was to assess whether circulating neurofilament light is a biomarker that is responsive to enzyme replacement therapy (ERT) in CLN2 disease.

Methods:

Using an ultrasensitive immunoassay, we assessed plasma neurofilament light changes during disease progression in a canine model of CLN2 disease and in ERT clinical trial CLN2 disease patients. Results: In tripeptidyl peptidase 1 (TPP1)-null dogs (N = 11), but not in control dogs [N = 6 (TPP1+/−) and N = 27 (WT)], neurofilament light levels increased more than tenfold above initial low baseline levels during disease progression. Before treatment in 21 human subjects with CLN2 disease (age range: 1.72–6.85 years), neurofilament light levels were 48-fold higher (P < 0.001) than in 7 pediatric controls (age range: 8–11 years). Pretreatment neurofilament light did not significantly correlate with disease severity or age. In CLN2 disease subjects receiving ERT, neurofilament light levels decreased by 50% each year over more than 3 years of treatment.

Interpretation: Our data indicate that circulating neurofilament light is a treatment-responsive biomarker in CLN2 disease and could contribute to understanding of the pathophysiology of this devastating pediatric disorder.

Introduction

Neuronal ceroid lipofuscinosis type 2, also known as CLN2 disease, is a rare, autosomal recessive neurodegenerative condition in the neuronal ceroid lipofuscinoses (NCL) family of lysosomal storage disorders, affecting fewer than 1 in 100,000 live births in most studied populations.1–3 A number of sequence variants in TPP1 cause a deficiency of the lysosomal enzyme tripeptidyl peptidase 1 (TPP1) leading to intracellular accumulation of autofluorescent storage material, progressive neuronal dysfunction, brain atrophy, and retinal degeneration.1,2,4 In most cases, the onset of clinical signs occurs between 2 and 4 years of age. Typically, seizures are the first signs, followed by rapidly progressive language delay, motor dysfunction, ataxia, dementia, vision loss, and early death, usually by mid-adolescence.1 In clinical trials, intracerebroventricular (ICV) infusions of the enzyme replacement
therapy (ERT) cerliponase alfa (recombinant TPP1) led to over 80% attenuation in the rate of decline in motor and language scores of the CLN2 Clinical Rating Scale over a 96-week treatment period. A canine model of CLN2 disease, the TPP1-null Dachshund, also displays progressive neurological dysfunction and brain atrophy that can be ameliorated by infusions of recombinant human TPP1.

While the CLN2 Clinical Rating Scale is a standardized, quantitative scoring system, it does not include any measures of biochemical changes. Molecular markers of CLN2 disease pathophysiology would provide a quantitative measure of disease pathology to complement the CLN2 score, enabling clinicians to track physiological changes. Studies addressing gaps in NCL biomarkers have been limited by sample number and tissue accessibility, and often focused on biochemical consequences of the lysosomal defect. N-acetylaspartate:creatinine ratios and brain and peripheral dolichol and lipid peroxidation products vary with disease state, as do urinary concentrations of mitochondrial ATP synthase subunit c, and plasma trimethyl-L-lysine and carnitine. In cerebrospinal fluid (CSF) from CLN2 disease patients, proteomic profiling identified 34 proteins that were altered compared to controls, and an untargeted metabolite profiling study found 29 features that correlated with disease severity.

Neurofilament light (NF-L, gene: NEFL), the most abundant of the four subunits comprising axonal and dendritic neurofilaments, acts in axonal growth and maintenance. While NEFL gene expression is limited to nervous system tissues, NF-L protein is detectable in CSF, serum, and plasma from healthy individuals, and is elevated in patients with neurological diseases including Alzheimer’s disease, mild cognitive impairment, Guillain–Barré syndrome, amyotrophic lateral sclerosis, Huntington’s disease, multiple sclerosis, and HIV-associated dementia. Elevated NF-L has been linked to faster brain atrophy and disease progression in Alzheimer’s disease, mild cognitive impairment, amyotrophic lateral sclerosis, Huntington’s disease, frontotemporal dementia, and progressive supranuclear palsy. Decreases in NF-L were observed following therapeutic interventions in multiple sclerosis, spinal muscular atrophy, and HIV, and following recovery from head injury. In several neurological conditions, NF-L levels in CSF and serum or plasma were correlated. Elevated levels of NF-L in animal models of neurodegeneration, and decreases following treatment in a model of HIV, demonstrate cross-species translatability of NF-L as a biomarker of active neurological disease or injury. These studies led us to evaluate whether NF-L is a disease-associated biomarker in CLN2 disease.

Methods

Canine model, cerliponase alfa treatment, and samples

Studies utilizing miniature long-haired Dachshunds were reviewed and approved by the University of Missouri Animal Care and Use Committee. All studies were conducted in accordance with the United States Public Health Service’s Policy on Humane Care and Use of Laboratory Animals. A total of 11 Dachshund dogs homozygous for the TPP1 null mutation and 6 Dachshund dogs heterozygous for the TPP1 null mutation were evaluated. TPP1-deficient dogs included in this study were treated with intravitreal cerliponase alfa by intravitreal injection, and plasma samples were collected at four timepoints between 89 and 251 days. TPP1 activity is not expected outside of the eye based on negligible systemic distribution after intravitreal injection, which is typical for large molecule protein therapeutics. Control plasma samples from 27 Beagles aged 3–24 months were purchased from BioIVT (NY, USA); control samples were collected at an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility that has Institutional Animal Care and Use Committee oversight.

Clinical trial subjects and samples

Plasma samples from human subjects with CLN2 disease were available from international clinical trials on the safety and efficacy of cerliponase alfa: BMN 190-201 (NCT01907087), BMN 190-202 (extension study of BMN 109-201; NCT02485899), and BMN 190-203 (NCT02678689), all performed in accordance with the Declaration of Helsinki. Written informed consent was provided by parents or legal guardians and approved by the relevant ethics boards. Details of the subject eligibility criteria have been published elsewhere. A single pretreatment (i.e., baseline) sample was available from 4 subjects; baseline and longitudinally collected samples (from patients with more than 3 months of treatment) were available from 15 subjects; baseline and longitudinally collected samples (from patients with fewer than 3 months of treatment) were available from 2 subjects; longitudinal samples without a baseline measurement were available from 3 subjects. Control plasma samples were obtained from seven unrelated individuals not known to have neurological diseases using the BioIVT resource (https://www.bioivt.com/).
NF-L measurements

Human and cohort 2 samples (dogs 12–17) were stored at −60 to −80°C, centrifuged to remove debris, and analyzed at Quanterix Corporation using a commercially available two-step digital immunoassay based on single molecule array (Simoa) technology – the Simoa NF-light® Advantage assay and Simoa HD-1 Analyzer (Uman Diagnostics, Umeå, Sweden and Quanterix Corporation, Lexington, MA, USA). Cohort 1 dog samples (dogs 1–11) were tested at BioMarin Pharmaceutical Inc. with the same method that used the Simoa NF-light® Advantage assay and an HD-1 Analyzer per manufacturers’ instructions. Longitudinal samples and sample groups used for comparison were tested in the same analytical run and were analyzed in duplicate at a four-fold dilution. Of 80 clinical trial samples available for analysis, data were lost from one replicate in four cases and run in singlicate due to low sample volume in two cases. Data from one control replicate was also lost.

Data processing and analysis

Some of the patient plasma samples were collected on dates without measurements of CLN2 Clinical Rating scores. To assign CLN2 Clinical Rating scores to each plasma sample, we used scores measured within 7 days from the date of sample collection (first preference) or at the latest date preceding sample collection (second preference). All statistical analyses were performed using R (version 3.4.1; https://www.r-project.org/). Differences between two groups of samples (patients vs. controls for age and baselines vs. controls for NF-L) were tested by Wilcoxon rank sum tests. Association of baseline NF-L levels with age was measured by both Spearman’s rank and Pearson correlation coefficients. Association of baseline NF-L levels with CLN2 Clinical Rating scores was measured by Spearman’s rank correlation coefficient. Association of patient NF-L levels in log scale with treatment time was examined by a linear model implemented in the limma package in R after adjusting for age at baseline and CLN2 Clinical Rating scores, and treating patients as a random effect.44,45 In the canine model of CLN2 disease, association of NF-L expression in log scale with age, and association of plasma NF-L and CSF NF-L levels in log scale were evaluated using the limma package by a linear model adjusted for differences between dogs. A P-value less than 0.05 was considered statistically significant.

Results

NF-L in a canine model of CLN2 disease increases with disease progression

In a cohort of five affected (TPP1+/−) Dachshunds and six age-matched carrier (TPP1+/+−) Dachshunds (cohort 1), NF-L levels started to increase around 4 months of age (Fig. 1A). In a second, slightly older cohort of six TPP1+/− Dachshunds progressing through the sym- tomatic stages of neurodegeneration (cohort 2), increases in plasma NF-L levels were also observed in each animal, rising to more than ten times initial levels as the animals approached end stage disease (Fig. 1B). In cohort 1, the rate of increase in NF-L levels was significantly more rapid in affected dogs (doubling time of 52 days) than in carrier dogs (doubling time of 204 days) (P < 0.001); the rate of increase in affected dogs in cohort 2 (doubling time of 48 days) was similar to cohort 1. In contrast, almost all plasma samples from control dogs (Beagles, N = 27) at 3–24 months of age showed minimal change in NF-L levels throughout this period, similar to levels seen in TPP1+/− Dachshunds and TPP1+/− Dachshunds at the very start of neuropathology (approximately 2 months of age when ventricular volume increases have been noted) (Fig. 1C).6 We note that the carrier Dachs- hunds display a larger age-dependent increase in NF-L than is seen in the TPP1+/+ Beagles; however, we cannot unequivocally ascribe that difference to the different TPP1 genotype versus breed, housing, or other variables. TPP1+/− Dachshunds are phenotypically normal, showing no signs of neurological disease and having lifespans that are typical for healthy dogs of this breed.46

At a subset of the plasma collection time points, CSF was also collected from the first cohort of Dachshunds; NF-L levels were measured in those paired CSF samples. While the absolute levels of NF-L were consistently higher in CSF compared to plasma at any given age in affected samples and carriers, the levels in plasma correlated to the levels in CSF (P = 0.021) (Fig. 2).

Patient characteristics

Plasma samples were obtained from subjects with CLN2 disease who were enrolled in clinical trials of cerliponase alfa (TPP1 ERT) and from individuals not known to have neurological diseases. Patient characteristics are shown in Tables 1 and 2. The median age of the control individuals was older than the median age of CLN2 disease subjects by 4.7 years at baseline (Wilcoxon rank sum test, P < 0.001). ERT was administered to all CLN2 disease subjects via ICV device, which had been placed 3–15 days (median = 8 days and mean = 10 days) before baseline5.

NF-L is increased in CLN2 disease subjects at baseline

Plasma samples were analyzed for NF-L at baseline and throughout the course of cerliponase alfa treatment. At baseline, NF-L levels in subjects (N = 21) were 48-fold
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Figure 1. NF-L levels increase in plasma samples from a canine model of CLN2 disease. (A) NF-L levels are shown using a log_{10} scale on the Y-axis and age on the X-axis. NF-L levels increase more rapidly in affected animals (TPP1^{-/-}; Dachshunds, N = 5, orange lines) than in carriers (TPP1^{+/+}; Dachshunds, N = 6, blue lines). Animal IDs are annotated. Four of the affected dogs (IDs 1, 2, 3, and 4) were treated with intraocular cerliponase alfa by intravitreal injection. Color bars on top represent different disease stages: no symptoms (0–60 days, green), ventricular volume change (61–210 days, yellow), failure to complete T-maze (211–280 days, orange), and death (281–329 days, red). (B) NF-L levels are shown using a log_{10} scale on the Y-axis and age on the X-axis. NF-L levels increase with progressive symptoms in a second group of TPP1^{+/+} animals treated with intraocular cerliponase alfa by intravitreal injection (Dachshunds, N = 6). Color bars on top represent disease stages as in (A). (C) NF-L levels are shown using a log_{10} scale on the Y-axis and age on the X-axis. In healthy animals (TPP1^{+/+} Beagles, N = 27), NF-L levels are not associated with age (P = 0.18). However, if the two outliers with the highest NF-L levels are excluded, the best fit regression line of NF-L levels increased by only 63% from 90 days to 720 days (P = 0.001). Boxes indicate the interquartile range (IQR), and the median is shown using an additional horizontal line. Whiskers extend up to the furthest data point within 1.5 times the IQR.

Figure 2. Positive correlation between plasma NF-L and CSF NF-L levels in a canine model of CLN2 disease. NF-L levels in plasma are shown using a log_{10} scale on the Y-axis and NF-L levels in matched CSF are shown using a log_{2} scale on the X-axis. A subset of the time points from Figure 1 that had matched plasma and CSF NF-L measurements are plotted. Animal IDs are annotated. In the affected animals (orange lines), plasma NF-L level increased by 56% (P = 0.021) if CSF NF-L level increased by 100%. Carriers are shown as blue lines or triangles.

Table 1. Patient characteristics – all subjects and controls.

| Population | CLN2 disease (N = 24) | Controls (N = 7) |
|------------|-----------------------|-----------------|
| Median age at baseline (years) | 4.28 | 9 |
| Baseline age range (years) | 1.72–6.85 | 8–11 |
| Sex | 15 F, 9 M | 7 M |
| Median CLN2 disease rating scale score (combined motor and language) | 3 | NA |

*Three patients did not provide baseline samples.

higher than those in control samples (N = 7); median (interquartile range [IQR]) = 153.2 pg/mL (118.7–183.4) in subjects versus 3.21 pg/mL (2.75–4.23) in controls (Wilcoxon rank sum test, P < 0.001) (Fig. 3). All CLN2 disease subjects had higher NF-L concentrations than any of the controls, with the lowest baseline value in a CLN2 disease subject (79.83 pg/mL) being over 14-fold higher than the highest control value (5.52 pg/mL).

NF-L is not associated with age or symptom severity at baseline

To determine if, within the patient population, baseline NF-L levels are associated with severity of neuropathology and symptoms, or with age, we examined associations between baseline NF-L, age, and CLN2 Clinical Rating scores (Fig. 4A and B). No associations were found with either age (N = 21, Pearson’s correlation coefficient = 0.08, P = 0.73; Spearman’s correlation coefficient = 0.22, P = 0.33) or combined baseline motor and language scores (N = 21, Spearman’s correlation coefficient = −0.02, P = 0.95). NF-L levels were variable across both ages and CLN2 Clinical Rating scores, with the highest levels found in subjects in the middle of the age group studied and subjects scoring in the middle of the rating scale. Subjects with low scores, and therefore more severe disease, did not consistently have the highest NF-L levels.

NF-L decreases in CLN2 disease patients following cerliponase alfa treatment

To examine the effect of cerliponase alfa on neuropathology, NF-L levels were measured in longitudinal plasma samples from 18 clinical trial subjects who had samples collected after at least 3 months of treatment. These patients provided between two and seven samples, with a median time on treatment of 33 months (range = 11–43 months). NF-L levels decreased during treatment by about 50% each year compared with the previous year (95% CI: 46.1%–53.8%; P < 0.001) (Fig. 5). While NF-L did temporarily increase at some time points for five subjects, overall NF-L levels continued to decrease over the course of treatment for all subjects.
Five subjects experienced a total of eight episodes of increased NF-L above previous levels during the first 6 months (numbers 23 and 27) and later in the course of treatment with cerliponase alfa (numbers 10, 11, 19, and 23). In four of these eight episodes, seizure activity was reported in the 2 weeks prior to collection of the sample with increased NF-L. However, in the same set of subjects, there were four other seizure activity periods reported, after which NF-L levels were decreased compared with the previous sampling. None of the episodes of increased NF-L level above previous levels was associated with concurrent cerliponase alfa dose interruption.

### Table 2. CLN2 disease patient subsets.

| Population                                      | Baseline sample only (n = 4) | Baseline and longitudinal samples, ≥3 months of treatment (n = 15) | Baseline and longitudinal samples, <3 months of treatment (n = 2) | Longitudinal samples with no baseline measurement (n = 3) |
|-------------------------------------------------|------------------------------|-------------------------------------------------------------------|------------------------------------------------------------------|----------------------------------------------------------|
| Median age at baseline (years)                  | 3.89                         | 4.35                                                              | 3.68                                                              | 3.03                                                     |
| Baseline age range (years)                      | 1.72–4.81                    | 3.10–6.85                                                         | 2.88–4.48                                                        | 2.98–4.28                                                |
| Sex                                             | 2 F, 2 M                     | 9 F, 6 M                                                          | 2 F                                                              | 2 F, 1 M                                                 |
| Baseline median CLN2 disease rating scale score | 4                            | 3                                                                 | 4.5                                                              | 6                                                        |

#### Figure 3.
NF-L levels in plasma samples from CLN2 disease patients at baseline and control individuals. Box plots of NF-L levels in two groups are displayed using a log_{10} scale on the Y-axis. NF-L levels are significantly increased in patients (N = 21, range 79.83–388.09) compared with controls (N = 7, range 2.08–5.52) (Wilcoxon rank sum test, P = 1.7 × 10^{-9}). NF-L levels are shown using a log_{10} scale, with boxes indicating the interquartile range (IQR), and the median shown using an additional horizontal line. Whiskers extend up to the furthest datapoint within 1.5 times the IQR.

### Discussion

This study is the first to examine a biochemical biomarker of treatment response in patients with CLN2 disease and is the first to examine NF-L in a pediatric population with a lysosomal storage disorder. The **TPP1**^-/-^ dogs provided an opportunity to follow NF-L during the natural course of disease development. In addition, the absence of allelic heterogeneity of the **TPP1** locus (as found in humans), the comparatively homogeneous genetic background of the animal model, and the consistent environment reduce variability in the course of the canine disease compared with the range seen in children. We demonstrated post-disease onset increases in NF-L in the canine disease model, and while there are species-specific differences in absolute levels of plasma NF-L (<5 pg/mL in control human samples versus >50 pg/mL in CLN2 disease patients, versus <20 pg/mL in control dogs versus >100 pg/mL after disease progression in **TPP1**^-/-^ dogs), markedly elevated circulating NF-L is a common feature in CLN2 disease across the two species. The pre-treatment levels observed in CLN2 disease patients is at the high end of neurological disease levels – similar to that seen in amyotrophic lateral sclerosis, and higher than many other neurodegenerative diseases.16,19,21,23,26,28

The canine **TPP1**^-/-^ model shows lysosomal storage body accumulation and follows a predictable and well-characterized disease progression, with clinical signs similar to those in children with CLN2 disease, including seizures, ataxia, and visual deficits.6,42,47 The animals reach end-stage disease status at approximately 44 weeks of age and disease progression can be ameliorated with CNS ERT,6 recapitulating the CLN2 disease patient phenotype. In this predictable genetic disease setting, mild NF-L increases were observed beginning a few weeks after ventricular volume changes are usually observed, then progressing to 100 pg/mL or higher during or before the time at which overt cognitive defects usually occurred. While successive samples from the same animal almost always showed an increase in NF-L, there was some
variability (greater than twofold) across affected animals of the same age. Baseline samples from CLN2 disease individuals in the cerliponase alfa study (age range: 1.7–6.9 years) all showed NF-L to be elevated by at least 14-fold relative to the highest level in a control individual; again, NF-L levels also showed considerable differences between individuals.

It should be noted that while no significant relationships between NF-L and age, nor NF-L and disease severity, were detected in the CLN2 disease patients studied, those relationships may not be evident in this study because the available human samples were from subjects concentrated in limited age and clinical score ranges. It is possible that investigation of more samples from younger, presymptomatic patients would reveal a progressive increase similar to that seen in the canine model. The observation of elevated NF-L in those subjects with CLN2 Clinical Rating Scale motor-language scores of 6, who have not yet suffered major declines in either motor or language function, suggests neuropathological activity similar to that in patients beginning to show decreases in CLN2 motor-language scores. Though longitudinal samples from untreated CLN2 disease patients were unavailable, the range of elevated NF-L levels in these samples is consistent with the results from the canine model of the disease where NF-L consistently rises during disease progression. It should be noted that CLN2 subjects all had an ICV device surgically placed; device placement surgery has previously been shown to result in a small and temporary increase in NF-L levels. While some of the elevated levels may be the result of the device placement, the absolute level of NF-L at baseline, the kinetics of NF-L decline, and the similar observation of elevated NF-L in the canine model (which were not catheterized) support the conclusion that NF-L is a marker of CLN2 disease and treatment response.

Importantly, NF-L levels decreased by approximately 50% annually during treatment with cerliponase alfa, with all subjects displaying decreases over the time period examined. In several subjects, levels decreased almost to those found in our control population. The repeated measurement of NF-L levels over a 1- to 4-year treatment period provides insight into the time course and consistency of the NF-L response. The observation that NF-L levels displayed a yearly, first-order logarithmic decline (approximately 50% decline compared with the
previous year), leads us to speculate that the underlying neuropathological disease progression is continuing to decrease over time with ongoing treatment.

In 5 out of 18 subjects, small and rare increases in NF-L were noted at one or two time points, totaling eight NF-L elevations out of the 57 total intervals tested across all individuals (Fig. 5). The rare instances where NF-L increased during a sampling interval were anomalies for which we were unable to identify a causal event or any association with disease activity. The frequency of sample collection does not permit discrimination between rises occurring on a time course of days or weeks. Neurological adverse events reported prior to increased NF-L were seizures, which are a hallmark of CLN2 disease. Although seizures sometimes occurred prior to NF-L increases (four out of eight episodes), there were just as many instances where seizures did not result in NF-L increase; while we cannot rule out that some seizures did result in NF-L increases, these observations suggest that seizures are unlikely to be the principal event inciting NF-L increase. The few instances of increases in NF-L levels were not explained by concurrent interruptions in dosing with cerliponase alfa.

In addition to its structural role in axons, NF-L plays a role in neuronal repair. How its role in repair is reflected in the levels of NF-L in blood is not well understood. Interestingly, Lang et al. found NF-L protein levels declined dramatically immediately after injury to mouse sciatic nerves, followed by increased NF-L protein synthesis by Day 7 postinjury. In a similar rat experiment, Nefl mRNA levels declined immediately following injury but rebounded during the late stage of neuronal recovery. In people who experienced traumatic head injury, the damage-associated molecular pattern factor S100B was elevated almost immediately after injury, while serum NF-L levels rose more gradually over time with a more extended recovery timeline than S100B to baseline levels. In our canine data, NF-L levels began to rise after the time when initial ventricular volume increases were noted. These observations are consistent with NF-L release being associated with active neuronal degeneration as well as possible repair mechanisms. Similarly, high baseline levels of NF-L in CLN2 disease subjects could be the result of NF-L being “leaked” from damaged neurons or released as a result of contemporaneous repair mechanisms. The kinetics of NF-L turnover are likely to be complex and may include both damage-induced release and repair mechanisms. Furthermore, how NF-L moves from CSF to the blood, and how it is turned over in the blood, are not fully understood, which adds another layer of complexity in interpreting individual-to-individual variation in plasma NF-L levels, as well as imperfect tracking of the concentration of NF-L in plasma and CSF. Finally, TPP1 deficiency may also result in NF-L release from peripheral neurons, which

Figure 4. Continued.
may contribute to the elevated levels observed in the blood.

To our knowledge, this study is the first to describe NF-L as a treatment-responsive biomarker of neuronal pathology in a pediatric lysosomal storage disease. NF-L may provide clinicians with a tool to objectively follow the effect of treatment with cerliponase alfa in CLN2 disease and disease-modifying therapy in other lysosomal storage diseases with neuropathology. Further study is warranted to confirm NF-L as a disease biomarker that responds to treatment and to evaluate the role of NF-L as a biomarker that may inform the timing of treatment initiation.

Acknowledgments

This study was funded by BioMarin Pharmaceutical Inc. A. Nguyen coordinated shipping of canine samples. F. Hong performed review of informed consent for human samples. P. Slasor and K. Gregg provided patient clinical data. S. Maricich and A. Shaywitz provided input on clinical meaning of the results. Editing and submission support was provided by E. Conran, Porterhouse Medical, Reading, UK.

Author Contributions

YR, CC, NP, MLK, TA, DJ, CBR and SC were involved in conception and study design. YR, CC, RKS, ACM, AH, GKY, NP, JRS, MLK, TA, DJ, CBR and SC analyzed and interpreted data. All authors contributed to drafting and reviewing the manuscript, and have approved the final version of the manuscript.

Conflicts of Interest

YR, CC, RKS, ACM, AH, GKY, JRS, TA, DJ, CBR and SC are employees and stockholders of BioMarin Pharmaceutical Inc., which manufactures the drug used as a treatment in this study. NP is a former employee and stockholder of BioMarin Pharmaceutical Inc. MLK has received grants from BioMarin Pharmaceutical Inc.

References

1. Chang M, Cooper JD, Davidson BL, et al. CLN2. In: S. Mole, R. Williams, H. Goebel, eds. The neuronal ceroid lipofuscinoses (Batten Disease) pp. 80–109. Oxford: Oxford University Press, 2011.
2. Bennett MJ, Rakheja D. The neuronal ceroid-lipofuscinoses. Dev Disabil Res Rev 2013;17:254–259.
3. Williams RE, Adams HR, Blohm M, et al. Management strategies for CLN2 disease. Pediatr Neurol 2017;69:102–112.
4. Haltia M, Goebel HH. The neuronal ceroid-lipofuscinoses: a historical introduction. Biochim Biophys Acta 2013;1832:1795–1800.
5. Schulz A, Ajayi T, Specchio N, et al. Study of intraventricular cerliponase alfa for CLN2 disease. N Engl J Med 2018;378:1898–1907.
6. Katz ML, Coates JR, Sibigtroth CM, et al. Enzyme replacement therapy attenuates disease progression in a canine model of late-infantile neuronal ceroid lipofuscinosis (CLN2 disease). J Neurosci Res 2014;92:1591–1598.
7. Stehr F, van der Putten H. Bridging NCL research gaps. Biochim Biophys Acta 2015;1852:2324–2328.
8. Dyke JP, Sondhi D, Voss HU, et al. Assessment of disease severity in late infantile neuronal ceroid lipofuscinosis using multiparametric MR imaging. AJNR Am J Neuroradiol 2013;34:884–889.
9. Wolfe LS, Ng Ying Kin NM, Palo J, Haltia M. Dolichols in neurodegeneration in Huntington’s disease. Neurology 1983;33:103–106.
10. Jokelainen K, Salmela KS, Humaloja K, et al. Blood dolichol in lysosomal diseases. Biochem Cell Biol 1992;70:481–485.
11. Siakotos AN, Schnippel K, Lin RC, Van Kuijk FJ. Biosynthesis and metabolism of 4-hydroxynonenal in canine ceroid-lipofuscinosis. Am J Med Genet 1995;57:290–293.
12. Wisniewski KE, Golabek AA, Kida E. Increased urine concentration of subunit c of mitochondrial ATP synthase in neuronal ceroid lipofuscinoses patients. J Inherit Metab Dis 1994;17:205–210.
13. Katz ML. Decreased plasma carnitine and trimethyl-L-lysine levels associated with lysosomal accumulation of a trimethyl-L-lysine containing protein in Batten disease. Biochim Biophys Acta 1996;1317:192–198.
14. Sleet DE, Tannous A, Sohar I, et al. Proteomic analysis of brain and cerebrospinal fluid from the three major forms of neuronal ceroid lipofuscinosis reveals potential biomarkers. J Proteome Res 2017;16:3787–3804.
15. Sindelar M, Dyke JP, Deeb RS, et al. Untargeted metabolite profiling of cerebrospinal fluid uncovers biomarkers for severity of late infantile neuronal ceroid lipofuscinosis (CLN2, Batten Disease). Sci Rep 2018;8:15229.
16. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol 2018;14:577–589.
17. Lee MK, Cleveland DW. Neuronal intermediate filaments. Annu Rev Neurosci 1996;19:187–217.
18. The Broad Institute of MIT and Harvard. Gene expression for NEFL (ENSG00000104725.9). Available at https://gencode.xportal.org/home/gene/NEFL. Accessed February 19, 2019.
19. Gaiottino J, Norgren N, Dobson R, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS ONE 2013;8:e75091.
20. Mattsson N, Andreasson U, Zetterberg H, Blennow K; Alzheimer’s Disease Neuroimaging I. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. JAMA Neurol 2017;74:557–566.
21. Gisslen M, Price RW, Andreasson U, et al. Plasma concentration of the neurofilament light protein (NFL) is a biomarker of CNS injury in HIV infection: a cross-sectional study. EBioMedicine 2015;3:135–140.
22. Zetterberg H, Skillback T, Mattsson N, et al. Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. JAMA Neurol 2016;73:60–67.
23. Lu C-H, MacDonald-Wallis C, Gray E, et al. Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. Neurology 2015;84:2247–2257.
24. Byrne LM, Rodrigues FB, Blennow K, et al. Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington’s disease: a retrospective cohort analysis. Lancet Neurol 2017;16:601–609.
25. Johnson EB, Byrne LM, Gregory S, et al. Neurofilament light protein in blood predicts regional atrophy in Huntington disease. Neurology 2018;90:e717–e723.
26. Meeter LH, Dopper EG, Jiskoot LC, et al. Neurofilament light chain: a biomarker for genetic frontotemporal dementia. Ann Clin Transl Neurol 2016;3:623–636.
27. Rohrer JD, Woollacott IOC, Dick KM, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology 2016;87:1329–1336.
28. Rojas JC, Bang J, Lobach IV, et al. CSF neurofilament light chain and phosphorylated tau 181 predict disease progression in PSP. Neurology 2018;90:e273–e281.
29. Zetterberg H, Hietala MA, Jonsson M, et al. Neurochemical aftermath of amateur boxing. Arch Neurol 2006;63:1277–1280.
30. Komori M, Kosa P, Stein J, et al. Pharmacodynamic effects of daclizumab in the intrathecal compartment. Ann Clin Transl Neurol 2017;4:478–490.
31. Piehl F, Kockum I, Khademi M, et al. Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. Mult Scler 2018;24:1046–1054.
32. Gunnarsson M, Malmstrom C, Axelsson M, et al. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Ann Neurol 2011;69:83–89.
33. Disanto G, Barro C, Benkert P, et al. Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol 2017;81:857–870.
34. Olsson B, Alberg L, Cullen NC, et al. NFL is a marker of treatment response in children with SMA treated with nusinersen. J Neurol 2019;266:2129–2136.
35. Wilke C, Preische O, Deuschle C, et al. Neurofilament light chain in FTD is elevated not only in cerebrospinal fluid, but also in serum. J Neurol Neurosurg Psychiatry 2016;87:1270–1272.
36. Novakova L, Zetterberg H, Sundstrom P, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology 2017;89:2230–2237.
37. Weydt P, Oeckl P, Huss A, et al. Neurofilament levels as biomarkers in asymptomatic and symptomatic familial amyotrophic lateral sclerosis. Ann Neurol 2016;79:152–158.
38. Kuhle J, Barro C, Disanto G, et al. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler 2016;22:1550–1559.
39. Clement A, Mitchelmore C, Andersson DR, Asuni AA. Cerebrospinal fluid neurofilament light chain as a biomarker of neurodegeneration in the Tg4510 and MitoPark mouse models. Neuroscience 2017;354:101–109.
40. Bacioglu M, Maia LF, Preische O, et al. Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. Neuron 2016;91:56–66.
41. Meulendyke KA, Queen SE, Engle EL, et al. Combination fluconazole/paroxetine treatment is neuroprotective despite ongoing neuroinflammation and viral replication in an SIV model of HIV neurological disease. J Neurovirol 2014;20:591–602.
42. Awano T, Katz ML, O’Brien DP, et al. A frame shift mutation in canine TPP1 (the ortholog of human CLN2) in a juvenile Dachshund with neuronal ceroid lipofuscinosis. Mol Genet Metab 2006;89:254–260.
43. Del Amo EM, Rimpelä AK, Heikkinen E, et al. Pharmacokinetic aspects of retinal drug delivery. Prog Retin Eye Res 2017;57:134–185.
44. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.
45. Smyth GK, Michaud J, Scott HS. Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics 2005;21:2067–2075.
46. Sanders DN, Kanazono S, Wininger FA, et al. A reversal learning task detects cognitive deficits in a Dachshund model of late-infantile neuronal ceroid lipofuscinosis. Genes Brain Behav 2011;10:798–804.
47. Katz ML, Johnson GC, Leach SB, et al. Extraneuronal pathology in a canine model of CLN2 neuronal ceroid lipofuscinosis after intracerebroventricular gene therapy that delays neurological disease progression. Gene Ther 2017;24:215–223.
48. Bergman J, Dring A, Zetterberg H, et al. Neurofilament light in CSF and serum is a sensitive marker for axonal white matter injury in MS. Neuroul Neuroimmunol Neuroinflamm 2016;3:e271.
49. Zhu Q, Couillard-Després S, Julien JP. Delayed maturation of regenerating myelinated axons in mice lacking neurofilaments. Exp Neurol 1997;148:299–316.
50. Lang F, Aravamudhan S, Nolte H, et al. Dynamic changes in the mouse skeletal muscle proteome during denervation-induced atrophy. Dis Model Mech 2017;10:881–896.
51. Hoffman PN, Cleveland DW. Neurofilament and tubulin expression recapitulates the developmental program during axonal regeneration: induction of a specific beta-tubulin isotype. Proc Natl Acad Sci USA 1988;85:4530–4533.
52. Shahim P, Gren M, Liman V, et al. Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. Sci Rep 2016;6:36791.