Effects of atidarsagene autotemcel gene therapy on peripheral nerves in late-infantile metachromatic leukodystrophy

Alberto A. Zambon,[1,2,†] Paola M.V. Rancoita,[3,†] Angelo Quattrini,[2] Calogera Butera,[1] Francesco Gentile,[2] Marcella Facchini,[4] Sara Mazza,[4] Salvatore Recupero,[4,5] Vera Gallo,[4,5] Andrew Shenker,[6] Nicholas D. Gollop,[7] Ubaldo Del Carro,[1] Valeria Calbi,[4,5] Clelia Di Serio,[3] Maria Grazia Natali Sora,[1] Massimo Filippi,[1,8] Alessandro Aiuti[4,5,8] and Francesca Fumagalli[1,4,5]

[†]**These authors contributed equally to this work.**

Abstract

This study evaluates the efficacy of arsa-cel gene therapy (GT) in mitigating the severity and progression of peripheral neuropathy as assessed by nerve conduction velocity (NCV) in individuals affected by late-infantile metachromatic leukodystrophy (LI-MLD).

This is a post-hoc analysis conducted on pre-symptomatic patients affected by LI-MLD treated with *ex vivo* autologous hematopoietic stem cell GT (atidarsagene autotemcel, "arsa-cel") in the context of prospective open-label, single-arm, interventional trials and expanded access programs. All patients were followed longitudinally with nerve conduction studies (NCSs) of peripheral motor (ulnar - UN, deep peroneal - DPN) and sensory (median - MN, sural - SN) nerves. These results were compared with those from a control group of untreated patients (NHx) studied with the same standardized protocol. We then analyzed the effects of baseline characteristics (age at treatment, severity of neuropathy pre-treatment expressed as age-matched NCV Z-scores) and arylsulfatase A (ARSA) enzyme activity (measured in peripheral blood myeloid CD15+ cells post treatment) on NCVs of treated patients. The primary endpoint of this post-hoc analysis was NCV, reflecting severity of demyelinating neuropathy. Changes in dermal nerve histopathology in skin biopsies were used as an exploratory outcome.

Fifteen treated and 16 NHx patients were included in the analyses, with a median age (IQR) at treatment of 13 (9.1;14.5) months. At 36 months of age, treated patients showed higher estimated NCVs in all nerves compared to age-matched controls (~15 m/s difference in motor nerves). Peripheral neuropathy was observed in the majority of treated patients at their pre-treatment examination (age range 7.3-17.4 months). Severity of pre-treatment neuropathy in treated patients did not have an effect on NCV values at 2 years post-GT, or on the rate of NCV slowing afterwards. A younger age at treatment was associated with higher NCVs of motor UN and sensory MN 2 years post-GT. Overall, ARSA levels in CD15+ cells correlated with NCVs of motor DPN at 2 years post-GT, and ARSA levels were associated with a slower decrease or a slight increase in NCVs of the DPN, UN and MN nerves afterwards.

In summary, peripheral neuropathy assessed by NCV is significantly ameliorated in LI patients treated with arsa-cel compared to untreated patients of similar age. In addition to the potential role of early age at treatment in the preservation of myelin, supraphysiological ARSA levels may slow demyelination of the DPN and other peripheral nerves. Arsa-cel may exert a stronger effect on NCV than allogeneic hematopoietic stem cell transplantation due to its greater ARSA expression.

Author affiliations:

1 IRCCS San Raffaele Scientific Institute, Milan, Italy, Unit of Neurology and Neurophysiology, Milan, 20132, Italy

2 IRCCS San Raffaele Scientific Institute, Milan, Italy, Institute of Experimental Neurology (InSpe), Division of Neuroscience, Milan, 20132, Italy

3 University Centre of Statistics in the Biomedical Sciences (CUSSB), Vita-Salute San Raffaele University, Milan, 20132, Italy

4 San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Milan, 20132, Italy

5 IRCCS San Raffaele Scientific Institute, Milan, Italy, Pediatric Immunohematology Unit and BMT Program, Milan, 20132, Italy

6 Clinical Consultant, Pennington, 08534 NJ, USA

7 Orchard Therapeutics (Europe) Limited, London W6 8PW, UK

8 Università Vita Salute San Raffaele, Milan, 20132, Italy

Correspondence to: Alberto A. Zambon

IRCCS San Raffaele Scientific Institute, Via Olgettina 60, Milan 20132, Italy

E-mail: zambon.alberto@hsr.it

Running title: Arsa-cel and peripheral neuropathy in MLD

Keywords: MLD; arsa-cel; gene therapy; peripheral neuropathy; myelin; Schwann cells

Introduction

Metachromatic leukodystrophy (MLD) is a neurodegenerative lysosomal storage disorder caused by the toxic accumulation of galactosyl-sulfatides due to a deficit of arylsulfatase A (ARSA), an enzyme located in lysosomes that catalyzes the first step in the pathway that degrades sulfatides.[1] Based on the age of symptom onset, MLD is commonly categorized into four different groups: late-infantile (LI; 0-2.5 years), early-juvenile (EJ; 2.6-6 years), late-juvenile (LJ; 7-16 years) and adult (AD; >16 years). Sulfatides are membrane lipids whose expression is restricted to certain cell types. They are present in bile duct epithelia, distal tubules of the kidney, and most importantly are abundant in both central (CNS) and peripheral nervous system (PNS). Here they play a crucial role in the maintenance of myelin (constituting 4% of all myelin lipids), axonal membrane, paranodal structure and membrane protein organization.[2,3] Accumulation of sulfatides in Schwann cells results in segmental demyelination with focal distortion and fragmentation of the myelin sheath both at the nodes of Ranvier and in the internodal region.[4-6] A sensorimotor, primarily demyelinating, peripheral neuropathy is a predominant feature of MLD.[7,8] The neuropathy is usually severe, progressive, and more consistently observed in patients with earlier onset, in particular in LI individuals. [4,8-11] While axonal loss is commonly attributed to secondary effects of myelin damage, it is possible that sulfatide accumulation may also directly induce primary neuronal degeneration.[12,13]

At present, there are a limited number of therapeutic strategies that can aim at slowing disease progression, mainly through the provision of the ARSA enzyme by heterologous or genetically-modified autologous myeloid cells.[14-17] Moreover, the extent to which the reconstitution of the

enzymatic activity can specifically arrest, prevent, or reverse peripheral myelin damage is still to be fully elucidated. In particular, the restricted delivery to the PNS, relative to the CNS, observed in allogeneic hematopoietic stem cell therapy (allo-HSCT) remains a concern,[18,19] as prolonged survival of treated patients could give rise to severe 'neuropathic' phenotypes.

In contrast to what was observed with allo-HSCT, preliminary data suggested that the ARSA over-expression achieved by autologous hematopoietic stem cell gene therapy (HSC-GT) (atidarsagene autotemcel, "arsa-cel") may be more effective in PNS than allo-HSCT, although published analyses were limited to the short-term aggregate outcomes of LI individuals (See Supplementary Appendices in Fumagalli et al[17] and Figure 2 in Sessa et al[20], where MLD03 corresponds to Pt3 of this paper).

In this study we conducted a post-hoc analysis focusing on pre-symptomatic LI (PSLI) patients who underwent arsa-cel treatment between 2010 and 2020. The primary objective was to elucidate the impact of this treatment on peripheral neuropathy as assessed with nerve conduction velocities (NCVs). Specifically, our investigation focused on delineating the association between baseline characteristics (such as age at treatment and severity of pre-treatment peripheral nervous system involvement), treatment-related factors (including ARSA levels achieved post HSC-GT), and both short-term and long-term alterations observed in nerve conduction studies. Histopathological assessment of dermal nerves from skin biopsies was used as an exploratory endpoint in the absence of sural nerve samples.

Materials and methods

Study Design And Participants

This is a post-hoc analysis of PSLI, enrolled in prospective open-label, single-arm, phase 1/2 interventional trials with fresh (NCT01560182)[20] or cryopreserved (NCT03392987) autologous CD34+ cells transduced with lentiviral vector encoding for human ARSA cDNA, or treated in the context of expanded access frameworks with analogous product. Detailed inclusion criteria of the interventional study have been previously described.[17] Early-juvenile patients were not included in the analysis given the heterogeneity in PNS involvement observed in natural history in EJ and older MLD patients.[8,19,21] Data from treated patients were compared with those from a control group (n=16) extracted from a prospective natural history study conducted in the same center with

observations in the same age range as treated patients .[11] Written informed consent was obtained from parents or guardians of the patients. These trials were approved by the institutional ethical committee of Ospedale San Raffaele and by Agenzia Italiana del Farmaco (AIFA). The study was undertaken according to Good Clinical Practice criteria and the Declaration of Helsinki.

Procedures

Patients were treated and monitored according to the schedule of assessments described in Fumagalli *et al.* 2022 (Appendix). [17] All data were prospectively collected. Before intravenous infusion of arsa-cel, patients received busulfan conditioning.

Determination of ARSA activity

ARSA activity quantification was performed in myeloid subpopulations of CD15+ cells collected from peripheral blood, as previously described.[14] ARSA activity is expressed as nmol/mg/h. Normal reference range is between 41.27 nmol/mg/hr to 153.77 nmol/mg/hr. Of note, ARSA activity profile was previously shown to be similar between CD14+ and CD15+ subpopulations (Fumagalli et al, 2022 Figure S6[17]).

Nerve conduction studies

Motor conduction of right deep peroneal (DPN) and ulnar nerves (UN) and sensory conduction of right sural (SN) and median nerves (MN) were studied at baseline, 3, 6 and 12 months, and every 6 months after the first year post-treatment. The recording site for study of the DPN was the right extensor digitorum brevis muscle, after stimulation of the ankle and the fibular head (the former site was used for the analysis). The recording site for study of the UN was the right abductor digiti minimi muscle (medial hypothenar eminence), after stimulation of the wrist and the elbow (the former site was used for the analysis). The recording site for study of the SN (antidromic) was posterior to the lateral malleolus 4-10 cm below the stimulating site. The recording site for study of the MN (antidromic) was the right index finger, after stimulation at the level of the wrist. In healthy children, a physiological increase in NCV is observed in the first years of life, reflecting the maturation of myelin. Hence, to adjust baseline data we defined PNS severity using a Z-score adjusted for age considering as reference published pediatric normative data.[22,23] In longitudinal models, we considered NCV only if recorded after 12 months of age. For description of baseline

values, when sural nerve was not evocable in patients younger than 12 months due to technical issues related to age, the Z-score was deemed "not applicable".

Skin biopsies

Skin biopsies were performed at baseline, 24, 60 and 96 months after treatment, according to local standards, as previously described.[20] Briefly, a piece of skin was taken using a disposable 3-mm punch from the lateral-malleolar region 10 cm upper from the malleolus. The tissue was fixed with 2% glutaraldehyde in 0.12M phosphate buffer, postfixed with 1% osmium tetroxide, dehydrated with alcohol and embedded in Epon (Fluka). Transverse semithin sections (0.5–1 mm) were stained with toluidine blue and examined by light microscopy. On resin sections, storage material presents as osmiophilic inclusions in the cytoplasm of Schwann cells.[24-26] Skin biopsies were analyzed by optical and/or electronic microscopy to evaluate the 1) presence/absence of neuropathy, 2) its grading (mild/moderate/severe), 3) type (demyelinating vs axonal), and 4) status (active/chronic). For each biopsy, a mean of 2-3 small nerve fascicles were evaluated, typically containing 6-7 fibers on average. The following features were evaluated on biopsies and collected as either categorical (C) or ordinal (O) variables: presence of active demyelination (C), onion bulbs (C), proportion of fibers with normal myelin thickness, axonal degeneration (C), axonal regeneration (C), macrophage activity (C), fiber loss (O), storage material (O), active disease (C) and severity (O).

Outcome measures

Peripheral neuropathy in MLD is mainly demyelinating, thus we considered NCV as the main surrogate parameter of PNS damage, in agreement with the endpoint of the trial (i.e., NCV changes at 2- and 3-years post-treatment). Two motor nerves (UN and DPN) and two sensory nerves (MN and SN) were studied over longitudinal timepoints. The primary outcomes of the analysis were the NCV values recorded at 2 years post GT and longitudinal trajectory afterwards. At the former timepoint the median age of treated PSLI patients in the cohort studied was 37.4 months (interquartile range 34.5-40.9). No clinical parameter was considered in this study. Changes in dermal nerve histopathology in skin biopsies were used as an exploratory outcome.

Statistical analysis

Descriptive statistics, including median and interquartile range (IQR) were generated for all continuous measures as appropriate and categorical data were summarized as frequency and

percentage. In treated patients only, Spearman coefficient was used to evaluate the correlation between ARSA activity measured in CD15+ myeloid cell subpopulation (i.e., CD15+ ARSA) at 3, 6 months and 1 year after treatment and NCV measured at 2 years post-GT. For each nerve, p-values were adjusted with Holm's correction accounting for the tests at multiple time points.

The longitudinal trend of the NCV (in m/s) after 2 years post-GT was analyzed through Linear Mixed-Effects (LME) models, with random-effects set on the intercept in order to account for subject heterogeneity.[27] In the models, only observations after 2 years post-GT were considered, since NCV trajectory showed a linear pattern after this time point. In each analysis, all the outliers of the full model (defined with respect to the normality assumption of the residuals) were removed and then the final model was obtained by performing a backward variable selection of the fixed-effects terms (based on the p-value of the test on the coefficient).

To compare treated vs untreated patients, LME models of NCV were estimated with respect to the chronological age (in months). For NHx patients, only observations within the range of ages of treated patients (at the available timepoints) were used. The time in the model was rescaled to interpret the intercept as the estimated value at age 36 months, which was approximately the age of treated patients at two years post-GT.

LME models were also employed to investigate the influence of baseline variables (i.e., age at GT and the age-matched Z-score of the corresponding nerve pre-GT) and ARSA CD15+ measured at 6 months post-GT on the longitudinal trajectory of NCVs in treated patients. The possible effects of interaction terms between the time and the covariates were also assessed within the model. In these models, the trend was investigated with respect to the time from GT (in months), but this was rescaled so that the intercept of the model could be interpreted as the estimated value at 2 years post-GT. Similarly, in the treated patients, we assessed also the effect of the longitudinal values of ARSA CD15+ on the longitudinal trend of NCVs through LME models. Even in this case, the time from GT (in months) was rescaled so that the intercept of the model could be interpreted as the estimated value at 2 years post-GT.

Whenever present, missing data were not imputed. For all tests, the significance level was set at 0.05 and the test was two-sided. All statistical analyses were performed using R 3.6.2 (http://www.R-project.org/). The R packages used for LME model analysis was nlme 3.1-142.

Results

Cohort description and baseline characteristics

A total of 19 LI patients from 17 unrelated families were screened for the study. One patient was excluded because of the presence of symptoms at the time of treatment, one because of the absence of any longitudinal observation, and two patients because of lack of long-term follow-up data (after 2 years post-GT). The final sample consisted of 15 PSLI patients (4 F, 26.7%), with median age at GT of 13 months (IQR 9.1-14.5) (Supplementary Table 1).

Median age at time of first NCS was 11.7 months (IQR 7.9-13.8). Pre-treatment, 10/15 patients had an age-matched Z-score below -2.5 in at least one nerve, which is neurophysiological evidence of demyelinating peripheral neuropathy. Three additional patients presented with Z-scores between -2.5 and -2 in at least one nerve (Table 1 and Figure 1).

Median follow-up period (i.e. the interval of time between treatment and the last available nerve conduction assessment) was 4.69 years (IQR 3.58-7.96, range 1.95-12.11 years). A median of 6 (IQR 5-11) NCSs were available per patient.

Comparison between patients treated with arsa-cel vs nhx (untreated)

In the first two years after receiving GT we observed a variable decrease in conduction velocities in patients, with 9/14 showing a reduction of more than 5m/s in at least one nerve (Supplementary figure 1). Nevertheless, from the age of 36 months (Figure 2, vertical dashed line) treated patients showed significantly higher NCV values compared to NHx patients, in both motor and sensory nerves. In the LME models (considering only observations of treated patients after 2 years post-GT), the estimated NCV value of DPN was 24.5 vs 10.1 m/s ($p<0.0001$), the estimated UN NCV was 32.1 vs 14.2 m/s ($p<0.0001$), and the estimated sensory MN NCV was 33.2 vs 1.5 m/s ($p<0.0001$) (Supplementary Tables S1A, S1B, S1C). Moreover, despite a further decrease observed after 36 months ($p <0.0001$ for DPN NCV and $p=0.0297$ for UN NCV), treated patients displayed a significantly slower decrease of DPN NCV values with respect to their chronological age, compared to NHx patients ($p=0.0010$) (Figure 2A and Supplementary Tables S1A, S1B). For MN NCV, the model shows a slight increase of the values over time ($p=0.0101$) (Figure 2D and Supplementary Table S1C). No model was estimated for sural nerve, as the value of many observations was zero.

correlation between arsa levels post treatment and nerve conduction velocities at 2 years post-gt

When considering NCVs of all nerves recorded 2 years post-GT, there was a significant correlation between CD15+ ARSA levels measured at 6 and 12 months after treatment and motor conduction velocities (Table 2), with slightly higher correlation using the value at 6 months (Spearman rank correlation coefficient values of 0.77 and 0.60 for DPN and UN, respectively). After adjusting with Holm's correction, significance was maintained only for DPN. In sensory nerves, the correlation with CD15+ ARSA was not significant at any of the considered timepoints.

Influence of baseline variables and CD15+ arsa measured at 6 months post-gt on long-term nerve conduction velocities

We analyzed the influence of baseline variables (i.e., age at GT and pre-GT age-matched Z-score NCV) and CD15+ ARSA at 6 months post-GT on the longitudinal trend of motor and sensory nerve conduction velocities in treated patients starting from 2 years post-GT. No model was estimated for sural nerve, due to the presence of many zero values in the data (individual trajectories are shown in Figure 2).

The estimated LME model of DPN NCV longitudinal values shows that a higher value of CD15+ ARSA at 6 months post-GT corresponds to a higher value of DPN NCV at 2 years post-GT ($p=0.0162$), while the other variables did not have a significant effect because they were not retained in the model after variable selection (Supplementary Table S2A). Moreover, although on average the DPN NCV declines after 2 years post-GT ($p<0.0001$), the value of CD15+ ARSA measured at 6 months post-GT is associated with a slower decline or a slight increase in DPN NCV over time in some patients ($p=0.0030$).

A younger age at treatment corresponds to higher values of UN NCV at 2 years post-GT ($p=0.0208$) (Supplementary Table S2B). Overall, the UN NCV decreases over time after this time point ($p=0.0004$), but the value of CD15+ ARSA at 6 months is associated with a slower decrease or an increase of UN NCV values over time in some patients ($p=0.0006$).

Lastly, a younger age at treatment also corresponds to a higher value of MN NCV at 2 years post-GT ($p=0.0223$) (Supplementary Table S2C). The MN NCV increases significantly over time after

this timepoint, with an increase which is also associated with higher values of CD15+ ARSA at 6 months ($p<0.0001$).

Association between the longitudinal values of CD15+ ARSA and nerve conduction velocities

We also assessed the association between the longitudinal CD15+ ARSA values and the longitudinal NCVs after 2 years post-GT through LME models (Supplementary Tables S3A, S3B, S3C). Regarding the DPN, although the value of NCV slightly decreases with time ($p<0.0001$), the model shows that it assumes higher values the higher is the CD15+ ARSA at the corresponding time point ($p=0.0027$). The value of UN NCV decreases with time ($p=0.0004$), but the values of CD15+ ARSA at the corresponding time point is associated with a slower decline or a slight increase of UN NCV ($p=0.0022$). Lastly, the value of MN NCV increases with time, with a greater increase when the values of CD15+ ARSA at the corresponding time point are higher ($p<0.0001$).

Skin biopsy findings

A total of 24 punch skin biopsies were performed on 7 PSLI patients enrolled in this study. Four of the 7 patients had samples available at baseline, 24, 60, and 96 months post-GT (Table 3). At baseline, histopathological features indicative of a demyelinating neuropathy were found in all patients, either as signs of active demyelination (4/7, 57%), onion bulbs (4/7, 57%) or thinly myelinated fibers (7/7, 100%). Notably, onion bulbs were observed as early as 8 months of age in one patient. Metachromatic storage material was found in 6 out of 7 cases (86%), appearing as osmiophilic inclusions in the cytoplasm of Schwann cells (Figure 3). Fiber loss was present in 6/7 patients, but active signs of axonal degeneration, such as myelin ovoids, increased macrophage activity and/or inflammatory cells were detected in none of the examined sections. Twenty-four months post-treatment most patients showed improvement on histology, driven by the disappearance of active demyelination (n=6/7, 85.76%), storage material (n=4/7, 57.1%) and increase in the number of fibers with thick, well-compacted myelin (5/7, 71.4%). Furthermore, a partial repopulation of myelinated fibers was observed, with patients showing an initial moderate or severe loss of fibers downgrading to a mild loss at 24 months (Table 3). Persistence of storage material at 24 months was observed in three patients, with some of them also showing naked axons (n=1) or onion bulbs (n=2) suggesting ongoing demyelination/remyelination. All three cases (Pt4,

Pt5, Pt6) showed worsening neurophysiological parameters at this timepoint (Supplementary table 2) and 2/3 had lower ARSA levels at 6 months post-treatment. Only one patient still showed isolated onion bulbs in a nerve fascicle with no other signs of active disease, whose NCVs were stable on follow-up.

On extended follow-up at 60 and 96 months after therapy (Figure 3), most patients had stable findings on skin biopsy, characterized by mild axonal loss and normal myelin thickness in most of the residual fibers. Mild demyelination (Pt1) and rare deposits (Pt1 and Pt2) reappeared, but were transient findings and no changes were observed in follow-up of NCS parameters in these two patients. Only one patient (Pt4) showed progressive loss of myelinated fibers, consistent with the parallel worsening in their NCS parameters (Supplementary table 2).

Discussion

In this study, the main objective was to assess the capacity of atidarsagene autotemcel (arsa-cel, a lentiviral-based *ex vivo* autologous HSC gene therapy) to counteract the development of peripheral neuropathy, as assessed with NCV, in a relatively large cohort of LI MLD patients. In contrast with other MLD forms, the severe and rather homogeneous demyelinating neuropathy observed in LI patients in a natural history study [11] makes this sub-population a good group of patients in which to study the effect of treatment.

In the pre-clinical work performed by *Biffi et al.*, an extensive migration of transduced HSC progeny to the PNS and a progressive replacement of endogenous endoneurial macrophages with transgene-expressing cells was observed. [28] Importantly, the study pointed to a better outcome in mice treated with lentiviral-transduced cells compared to wild-type HSC, possibly linked to the overexpression of ARSA achieved with the former approach. A key unmet question regarding both arsa-cel and allo-HSCT is whether myeloid precursors can also effectively repopulate peripheral nervous system in humans,[29] and whether these can provide sufficient ARSA levels to stop the progression of peripheral nerve damage. In fact, in patients treated with allo-HSCT this effect seems limited.[19]

While short-term data from a small subset of PSLI patients had already indicated a potential benefit,[20] we here demonstrate in a larger cohort of LI patients that arsa-cel partially succeeds in preventing motor and sensory nerves from developing the very severe demyelination that is

invariably observed in untreated patients, although this beneficial effect is limited and abnormal NCV values do persist post-GT. Furthermore, in agreement with pre-clinical models, higher levels of ARSA measured in myeloid subpopulations 6 months after treatment are associated with a stronger effect, specifically on NCV in the DPN of patients.

Another contribution of this study is the provision of neurophysiological and neuropathological data regarding the early stages of the disease, when clinical symptoms are not yet overt. In MLD, storage phenomena start during fetal life with an initial compensatory increase of lysosomes, in the absence of myelin damage.[6] During myelination, which is characterized by a very active synthesis of sulfatides, a progressive failure of the catabolic capacity of lysosomes is observed, resulting in a massive accumulation of non-degradable sulfatides affecting oligodendrocytes and Schwann cells. [5,24] Evidence of this phenomenon occurring also in other cell lines such as neurons, microglia and astrocytes suggest that additional pathological mechanisms may be involved. [12,30,31] Despite the fact that the precise toxic threshold of such deposits has not been determined,[6] we here provide evidence that a chronic demyelinating neuropathy can be observed as early as 8 months of age (in the LI population), as demonstrated by the integrated analysis of skin biopsies and NCSs. This is in agreement with a recent study of children identified via newborn screening. [32] Interestingly we observed that, in contrast with later stages of the disease, there is a variable involvement of motor and sensory nerves in LI patients studied before the onset of symptoms, even of the same individual. Of note, we found no apparent association between the severity of peripheral neuropathy at baseline and outcome, but this result should be considered in light of the small size of the study sample.

One challenge in the interpretation of these data is the limited understanding of the progression of peripheral neuropathy in the first years of life; for instance, if the decrease in NCVs is linear or non-linear in untreated patients.[11,19] As such, we cannot establish whether the early and sharp drop of NCVs observed in some patients is attributable to the normal course of the disease occurring before ARSA reconstitution, to a toxic effect of chemotherapy (e.g., busulfan) or to a superimposed inflammatory processes. All these have been proposed as possible mechanisms in patients treated with allo-HSCT.[33] However, the first hypothesis appears to be the most likely given that such decrement was not observed in any of the early-juvenile patients treated with busulfan and arsa-cel (data not shown).

At 36 months of age (corresponding approximately to 2 years post-GT) treated patients showed higher NCVs in all nerves compared to untreated controls. This timepoint was chosen to allow for the comparison with a control group at a time point in which myelin has completed its maturation (i.e. conduction velocities are similar to the ones of an adult). Specifically, there was around 15m/s difference in both DPN and UN and even greater differences observed in sensory nerves. Of note, the observed difference in NCVs between treated and NHx patients appears to be primarily due to an effect of arsa-cel in the first 2 years post-GT. These findings suggest that LI patients experience a combination of early demyelination and abnormal myelin maturation. Therefore, the stabilization or increase in NCVs observed in some patients during early life is likely driven by an improved ability to meet the catabolic demands of myelinogenesis rather than by remyelination. The sustained maintenance of ARSA levels over time appears to benefit the subsequent rate of NCV decline too, although the magnitude of this effect remains limited (Figure 2). Of note, we did not observe significant decline of cMAPs and SAPs (Supplementary Figure 2).

When considering only treated individuals, higher ARSA levels measured in CD15+ cells were correlated with higher conduction velocities of the DPN at 2 years post-GT. Moreover, higher ARSA levels at 6 months, a result which reflects the early reconstitution of enzyme activity by corrected cells, were associated with a slower decrease or with a slight increase in NCVs of DPN, UN and MN. Lastly, a younger age at treatment was also associated with increased NCVs in motor UN and sensory MN nerves. As previously mentioned, the severity of peripheral neuropathy at baseline did not have an influence on outcome, although this was calculated using age-matched Z-score that considers age ranges (i.e., 1-6 months, 6-12 months etc) and the sample size was limited.

In the first clinical study of arsa-cel, skin biopsies were selected due to reduced invasiveness and potential for follow-up studies compared to sural nerve biopsies, although the support of skin biopsy in the detection of pathognomonic features of MLD was considered less robust than the latter approach.[6] Previous studies reported a variable rate of diagnostic success, all of them flawed by the limited number of tested cases.[34] We show here that skin biopsy may be a good biomarker of disease severity, frequently detecting pathological changes occurring in the small dermal nerves, such as demyelination and storage material, which were found in almost all cases tested before treatment. Mirroring the improvement observed in some neurophysiological outcomes, follow-up biopsies demonstrated improvement in myelin thickness and reduction/disappearance of storage material, with a small rescue in the number of myelinated fibers in some cases, thus switching from

an active, chronic demyelinating process to a mild, stable axonal neuropathy. On longer follow-up (60 and 96 months) stable findings were seen in most patients, which again reflected neurophysiological data suggesting persisting beneficial effects of arsa-cel. Mild signs of disease reactivation after initial improvement may be observed but this appeared to be transient and did not lead to overt progression of the neuropathy. Interestingly, the persistence of signs of demyelination and storage material observed in few cases correlated with worsening in NCS parameters, suggesting an active pathologic process in these patients.

The study presents some limitations, mainly related to small sample size and lack of natural history data describing early PNS involvement in MLD. Moreover, we did not provide description of clinical outcomes and their correlation with neurophysiological abnormalities. Unclear correlation between clinical examination and peripheral neuropathy has been described thus far in the disease,[8,13] and the potential for coexisting central involvement likely represents a confounding factor in the interpretation of clinical outcomes.[35] The challenges in interpreting and effectively treating gait difficulties due to such a combination of peripheral neuropathy and central pyramidal tract involvement in a treated LI patient (Patient 5 in this report) have been previously described.[36]

Although we included only LI patients to increase the homogeneity of the cohort, we encountered a higher heterogeneity than expected in terms of PNS involvement, underlining possible differences in disease severity also within the LI subpopulation. This variability, together with sample size, may explain why some results were not replicated in all nerves. Lastly, no NHx control group is available for skin biopsies.

In conclusion, peripheral neuropathy, as revealed by neurophysiology and neuropathology findings, is significantly ameliorated in PSLI patients treated with arsa-cel compared to natural history controls. This "amelioration" primarily occurs during myelin maturation and is likely related to the lack of massive toxic accumulation typically observed in untreated patients during a period when sulfatide synthesis is especially active. In addition to the potential role of early age at treatment, supraphysiological ARSA levels may support the maturation of myelin and slow demyelination of the DPN and other peripheral nerves. Our data indicate that arsa-cel may exert a stronger effect on NCV than allo-HSCT due to its greater ARSA expression.

Data availability

Because of the small number of participants in the study and potential for identification, individual patient data beyond what is included in the manuscript will not be available.

Acknowledgements

We thank the medical and nursing team of the Pediatric Immunohematology Unit, Stem Cell Transplant Program of the IRCCS San Raffaele Scientific Institute for their professional care of patients during hospitalisation; Laura Castagnaro and the quality team; the team of the Department of Anesthesia and Neurointensive Care and Neurophysiology Service for support; Margherita Levi; research nurses; Giuliana Tomaselli and Luisella Meroni for administrative assistance; cultural mediators, and all personnel and volunteers of Fondazione Telethon "Just like home" Program for constant support with families and loving care of the children; the staff of the San Raffaele Telethon Institute of Gene Therapy Clinical Trial Office for its support in trial management; the laboratory staff of the San Raffaele Telethon Institute of Gene Therapy clinical laboratory; and the team of AGC Biologics SpA for manufacturing vector and medicinal product. We thank all employees of Orchard Therapeutics (Europe), in particular Jean Brooks, Kent Christopherson, Sean Moro, Philippa Nutkins for reviewing the manuscript, and the former sponsor GlaxoSmithKline who worked on the arsa-cel Clinical Development Program. We wish to acknowledge the Telethon Foundation for continuous support and strategic guidance. We thank the primary physicians worldwide who referred patients and continued their follow-up with great commitment. This study is dedicated to MLD children and their families who have been involved in clinical studies and supported clinicians and researchers throughout these years. Moreover, we thank Dr Ignazio D. Lopez for his contribution with collecting skin biopsies, Ivan Urban and his colleagues for recording NCSs over more than 20 years. Last but not least we thank Alessandra Biffi and Maria Sessa for their crucial role in designing and conducting the original trial.

Funding

Although we did not receive funding for this specific study, patient data were retrieved from studies sponsored by Orchard Therapeutics (Europe). These studies were previously supported by Telethon Foundation, IRCCS San Raffaele Scientific Institute (until 2016), and GlaxoSmithKline (2016–18), and are currently sponsored by Orchard Therapeutics (Europe).

Competing interests

FFu, AAZ, VC, MGNS, SR, VG, and AA are investigators of gene therapy clinical trials for MLD sponsored by Orchard Therapeutics, the license holder of medicinal product arsa-cel. FFu and VC have acted as ad-hoc consultants for an Orchard Therapeutics advisory board. The MLD gene therapy was licensed to GlaxoSmithKline in 2014, and then to Orchard Therapeutics in 2018. Telethon and Ospedale San Raffaele are entitled to receive milestone payments and royalties for such a therapy. AS is a consultant for Orchard Therapeutics. NDG is an employee of Orchard Therapeutics. All other authors declare that they have no financial interest related to the work described in the manuscript.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Gieselmann V. Metachromatic leukodystrophy: genetics, pathogenesis and therapeutic options. *Acta Paediatr*. Apr 2008;97(457):15-21. doi:10.1111/j.1651-2227.2008.00648.x

2. Gieselmann V, Franken S, Klein D, *et al*. Metachromatic leukodystrophy: consequences of sulphatide accumulation. *Acta paediatrica (Oslo, Norway : 1992) Supplement*. Dec 2003;92(443):74-9; discussion 45.

3. Marcus J, Honigbaum S, Shroff S, Honke K, Rosenbluth J, Dupree JL. Sulfatide is essential for the maintenance of CNS myelin and axon structure. *Glia*. Mar 2006;53(4):372-81. doi:10.1002/glia.20292

4. Yudell A, Gomez MR, Lambert EH, Dockerty MB. The neuropathy of sulfatide lipidosis (metachromatic leukodystrophy). *Neurology*. Feb 1967;17(2):103-11 passim.

5. Martin JJ, Ceuterick C, Mercelis R, Joris C. Pathology of peripheral nerves in metachromatic leucodystrophy. A comparative study of ten cases. *J Neurol Sci*. Jan 1982;53(1):95-112.

6. Gyl Midroni JMB. *Storage Diseases*. 1995 ed. Biopsy Diagnosis of Peripheral Neuropathy 1995.

7. De Silva KL, Pearce J. Neuropathy of metachromatic leucodystrophy. *J Neurol Neurosurg Psychiatry*. Feb 1973;36(1):30-3.

8. Bindu PS, Mahadevan A, Taly AB, Christopher R, Gayathri N, Shankar SK. Peripheral neuropathy in metachromatic leucodystrophy. A study of 40 cases from south India. *J Neurol Neurosurg Psychiatry*. Dec 2005;76(12):1698-701. doi:10.1136/jnnp.2005.063776

9. Fullerton PM. PERIPHERAL NERVE CONDUCTION IN METACHROMATIC LEUKODYSTROPHY (SULPHATIDE LIPIDOSIS). *J Neurol Neurosurg Psychiatry*. Apr 1964;27:100-5.

10. MacFaul R, Cavanagh N, Lake BD, Stephens R, Whitfield AE. Metachromatic leucodystrophy: review of 38 cases. *Arch Dis Child*. Mar 1982;57(3):168-75.

11. Fumagalli F, Zambon AA, Rancoita PMV, *et al*. Metachromatic leukodystrophy: A single-center longitudinal study of 45 patients. *J Inherit Metab Dis*. Apr 15 2021;doi:10.1002/jimd.12388

12. Eckhardt M, Hedayati KK, Pitsch J, *et al*. Sulfatide storage in neurons causes hyperexcitability and axonal degeneration in a mouse model of metachromatic leukodystrophy. *J Neurosci*. Aug 22 2007;27(34):9009-21. doi:10.1523/jneurosci.2329-07.2007

13. Dali CI, Barton NW, Farah MH, *et al*. Sulfatide levels correlate with severity of neuropathy in metachromatic leukodystrophy. *Ann Clin Transl Neurol*. May 2015;2(5):518-33. doi:10.1002/acn3.193

14. Biffi A, Montini E, Lorioli L, *et al.* Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science (New York, NY)*. Aug 23 2013;341(6148):1233158. doi:10.1126/science.1233158

15. Groeschel S, Kuhl JS, Bley AE, *et al.* Long-term Outcome of Allogeneic Hematopoietic Stem Cell Transplantation in Patients With Juvenile Metachromatic Leukodystrophy Compared With Nontransplanted Control Patients. *JAMA Neurol.* Sep 01 2016;73(9):1133-40. doi:10.1001/jamaneurol.2016.2067

16. Boucher AA, Miller W, Shanley R, *et al.* Long-term outcomes after allogeneic hematopoietic stem cell transplantation for metachromatic leukodystrophy: the largest single-institution cohort report. *Orphanet J Rare Dis*. Aug 07 2015;10:94. doi:10.1186/s13023-015-0313-y

17. Fumagalli F, Calbi V, Natali Sora MG, *et al.* Lentiviral haematopoietic stem-cell gene therapy for early-onset metachromatic leukodystrophy: long-term results from a non-randomised, open-label, phase 1/2 trial and expanded access. *Lancet (London, England)*. Jan 22 2022;399(10322):372-383. doi:10.1016/s0140-6736(21)02017-1

18. van den Broek BTA, Page K, Paviglianiti A, *et al.* Early and late outcomes after cord blood transplantation for pediatric patients with inherited leukodystrophies. *Blood advances*. Jan 9 2018;2(1):49-60. doi:10.1182/bloodadvances.2017010645

19. Beerepoot S, Nierkens S, Boelens JJ, Lindemans C, Bugiani M, Wolf NI. Peripheral neuropathy in metachromatic leukodystrophy: current status and future perspective. *Orphanet Journal of Rare Diseases*. 2019/11/04 2019;14(1):240. doi:10.1186/s13023-019-1220-4

20. Sessa M, Lorioli L, Fumagalli F, *et al.* Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. *The Lancet*. 2016;388(10043):476-487. doi:10.1016/S0140-6736(16)30374-9

21. MacFaul R, Cavanagh N, Lake BD, Stephens R, Whitfield AE. Metachromatic leucodystrophy: Review of 38 cases. *Archives of Disease in Childhood*. 1982;57(3):168-175. doi:10.1136/adc.57.3.168

22. Garcia A, Calleja J, Antolin FM, Berciano J. Peripheral motor and sensory nerve conduction studies in normal infants and children. *Clin Neurophysiol*. Mar 2000;111(3):513-20.

23. Parano E, Uncini A, De Vivo DC, Lovelace RE. Electrophysiologic correlates of peripheral nervous system maturation in infancy and childhood. *J Child Neurol*. Oct 1993;8(4):336-8.

24. Meier C, Bischoff A. Sequence of morphological alterations in the nervous system of metachromatic leucodystrophy. Light- and electronmicroscopic observations in the central and peripheral nervous system in a prenatally diagnosed foetus of 22 weeks. *Acta neuropathologica*. Dec 21 1976;36(4):369-79. doi:10.1007/bf00699642

25. Gebhart W, Lassmann H, Niebauer G. Demonstration of specific storage material within cutaneous nerves in metachromatic leukodystrophy. *J Cutan Pathol*. Feb 1978;5(1):5-14. doi:10.1111/j.1600-0560.1978.tb00931.x

26. Bilbao JM, Schmidt, Robert E. *Biopsy diagnosis of peripheral neuropathy, second edition*. second edition ed. © Springer International Publishing Switzerland 2015.; 2015.

27. José C. Pinheiro DMB. *Mixed-Effects Models in S and S-PLUS*. Statistics and Computing. Springer New York; 2000:XVI, 528.

28. Biffi A, De Palma M, Quattrini A, *et al*. Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells. *The Journal of clinical investigation*. Apr 2004;113(8):1118-29. doi:10.1172/jci19205

29. Ydens E, Amann L, Asselbergh B, *et al*. Profiling peripheral nerve macrophages reveals two macrophage subsets with distinct localization, transcriptome and response to injury. *Nature neuroscience*. May 2020;23(5):676-689. doi:10.1038/s41593-020-0618-6

30. Wittke D, Hartmann D, Gieselmann V, Lullmann-Rauch R. Lysosomal sulfatide storage in the brain of arylsulfatase A-deficient mice: cellular alterations and topographic distribution. *Acta Neuropathol*. Oct 2004;108(4):261-71. doi:10.1007/s00401-004-0883-6

31. Dayan AD. Peripheral neuropathy of metachromatic leucodystrophy: observations on segmental demyelination and remyelination and the intracellular distribution of sulphatide. *J Neurol Neurosurg Psychiatry*. Aug 1967;30(4):311-8.

32. Laugwitz L, Mechtler TP, Janzen N, *et al*. Newborn Screening and Presymptomatic Treatment of Metachromatic Leukodystrophy. *N Engl J Med*. Oct 3 2024;391(13):1256-1258. doi:10.1056/NEJMc2407165

33. Beerepoot S, Boelens JJ, Lindemans C, *et al*. Progressive demyelinating polyneuropathy after hematopoietic cell transplantation in metachromatic leukodystrophy: a case series. *Journal of neurology*. Apr 2 2024;doi:10.1007/s00415-024-12322-3

34. Martin JJ, Ceuterick C. Morphological study of skin biopsy specimens: a contribution to the diagnosis of metabolic disorders with involvement of the nervous system. *Journal of neurology, neurosurgery, and psychiatry*. Mar 1978;41(3):232-48. doi:10.1136/jnnp.41.3.232

35. Farah MH, Dali C, Groeschel S, *et al*. Effects of sulfatide on peripheral nerves in metachromatic leukodystrophy. *Annals of clinical and translational neurology*. Feb 2024;11(2):328-341. doi:10.1002/acn3.51954

36. Faccioli S, Sassi S, Pandarese D, *et al*. Preserving Ambulation in a Gene Therapy-Treated Girl Affected by Metachromatic Leukodystrophy: A Case Report. *Journal of personalized medicine*. Apr 6 2023;13(4)doi:10.3390/jpm13040637

Figure legends

Figure 1 Nerve conduction velocity Z-scores in PSLI patients before receiving arsa-cel. Age-matched Z-score of conduction velocities of all nerves at baseline. Each color corresponds to one patient. The dashed line and grey area correspond to a Z-score of -2.5, a threshold selected to define abnormal Z-score values. Abbreviations: DPN, deep peroneal nerve (motor); MN, median nerve (sensory); SN, sural nerve (sensory); UN, ulnar nerve (motor).

Figure 2 Nerve conduction velocity of motor and sensory nerves in treated vs untreated patients. Nerve conduction velocity expressed in m/s of motor (A-B) and sensory (C-D) of lower (A-C) and upper (B-D) limbs plotted with reference to chronological age expressed in months. Treated patients are depicted with colors, NHx patients in grey. Vertical dotted line corresponds to 36 months. Solid line represents the estimated trajectory for treated patients after two years post GT, while dashed line represents the estimated trajectory for NHx patients in the same interval of age. The green areas represent the intervals of normal NCV values with respect to the corresponding age. *A figure focusing on the first 2 years after GT is provided in the supplementary*

material. Abbreviations: LI, late-infantile; NHx, natural history, DPN, deep peroneal nerve (motor); MN, median nerve (sensory); SN, sural nerve (sensory); UN, ulnar nerve (motor).

Figure 3 Longitudinal skin biopsy findings in treated MLD patients. Longitudinal skin biopsy features in three representative patients with MLD. At baseline (A, E, I), pathologic evaluation included the presence of abnormal storage deposits in Schwann cells, signs of demyelination (A, arrow), axonal degeneration (A, I, arrowhead) and reduction in the number of myelinated fibers, with only few showing normal myelin thickness (E and I, arrows). After 24 months of treatment (B, F, J), there was an increase in the number of normal, myelinated fibers (B, F, J, arrows) although degenerating axons (B, arrowhead) and demyelinated fibers (F, J, arrowheads) may still be detected. After 60 months after of treatment (C, G, K), fibers with normal myelin thickness are more representative in the nerve fascicles (C, G, K, arrows), although improvement did not necessarily persist in all patients, witnessed exemplified by the absence of nerve fibers in a fascicle despite treatment (K, asterisk). Still, subclinical pathology may be observed, such as deposition of metachromatic storage material (C, arrowhead), axonal degeneration (G, arrowhead) and demyelination (K, arrowhead). At last follow-up of 96 months (D, I, L), most nerve fascicles were populated by normal or almost normal myelinated fibers (D, I, L, arrows) although fiber density may be variably reduced. Bar = 15 µm.

Figure 4 Electron micrographs of longitudinal skin biopsies in one representative patient with MLD (Pt3). At baseline, ultrastructural analysis shows the presence of signs of demyelination (A) and of storage material within Schwann-cell cytoplasm (B, asterisk) in a myelinated nerve fiber (B, arrow); 24 months-post-treatment, thin myelinated nerve fibers (C, Ax marks the axoplasm) with redundant basal lamina (D, arrowheads) are present. At 60 months-post treatment, EM analysis show many normal myelinated nerve fibers within a nerve bundle (E, arrows) and thin myelinated nerve fibers (E and F, arrows). Panel G (96 months-post-treatment) shows a normal myelinated fibers: normal myelin (My) periodicity can be easily appreciated (insert, high-power). MLD: metachromatic leukodystrophy; Ax: axon; My: myelin. Bar = 2µm (A, B, D, E and F); 1µm (C); 500nm (G); 200nm (insert).

Table 1 Baseline characteristics and CD15+ ARSA activity at 6 months in treated patients

Pt code	Age at gene therapy	Baseline DPN Z-Score	Baseline UN Z-Score	Baseline MN Z-score	Baseline SN Z-score	CD15+ ARSA activity nmol/mg/h at 6 months
Pt1	15.0	−11.10	−9.74	−9.33[a]	−8.99[a]	441.2
Pt2	13.1	−2.31	0.43	1.19	−1.18	184.1
Pt3	7.6	−3.75	−6.83	−1.97	−0.98	331.8
Pt4	17.7	−2.32	1.48	0.61	−0.42	64.7
Pt5	15.8	−5.64	−6.85	−2.77	−8.99[a]	331.8
Pt6	16.8	−6.00	−5.26	−6.84	−5.97	62.5
Pt7	8.2	−3.97	−5.26	−2.56	NA[a]	506.9
Pt8	9.5	−1.68	−0.01	2.29	1.60	1600.6
Pt9	14.1	−8.95	−7.46	−3.37	−4.27	2391.3
Pt10	13.2	−6.89	−4.45	−1.76	−8.99[a]	1068.3
Pt11	8.6	−3.67	−6.22	−1.23	−4.84	1974.4
Pt12	10.5	−1.60	−6.05	1.26	NA[a]	518.7
Pt13	13.0	−5.53	−6.83	1.30	NA[a]	324.8
Pt14	8.5	−1.34	−2.36	1.16	0.78	NA
Pt15	10.0	−1.82	−1.85	NA	0.14	1906.8

Age is expressed in months. DPN, deep peroneal nerve; MN, median nerve; UN, ulnar nerve.

[a]Denotes whether the value of the NCV was zero. If age was <12 months a value of 0 was reported as NA (not available) due to limitations of sural nerve analysis in this age range.

Table 2 Correlation analysis between CD15+ ARSA at different timepoints after GT and NCV at 2-year post treatment

Variable	N	rho[a]	p-value	adj. p-value
Correlation with DPN at 2 years post GT				
CD15+ ARSA at 3 months post GT	13	0.7473	0.0048	0.0066
CD15+ ARSA at 6 months post GT	13	0.7675	0.0022	0.0066
CD15+ ARSA at 12 months post GT	14	0.7538	0.0027	0.0066
Correlation with UN at 2 years post GT				
CD15+ ARSA at 3 months post GT	13	0.4890	0.0929	0.0929
CD15+ ARSA at 6 months post GT	13	0.6052	0.0284	0.0852
CD15+ ARSA at 12 months post GT	14	0.5868	0.0303	0.0852
Correlation with MN at 2 years post GT				
CD15+ ARSA at 3 months post GT	13	0.4649	0.1094	0.3283
CD15+ ARSA at 6 months post GT	13	0.4099	0.1642	0.3283
CD15+ ARSA at 12 months post GT	14	0.4246	0.1302	0.3283
Correlation with SN at 2 years post GT				
CD15+ ARSA at 3 months post GT	13	0.3984	0.1776	0.2433
CD15+ ARSA at 6 months post GT	13	0.5203	0.0684	0.2051
CD15+ ARSA at 12 months post GT	14	0.4334	0.1216	0.2433

DPN, deep peroneal nerve (motor); MN, median nerve (sensory); SN, sural nerve (sensory); UN, ulnar nerve (motor). ARSA activity is expressed as nmol/mg/h

[a]Refers to Spearman's rank correlation coefficient.

Table 3 Skin biopsy findings of treated patients

	Baseline (n=7)	24 months (n=7)	60 months (n=5)	96 months (n=4)
Active demyelination[a]	5 (71%)	1 (14.3%)	1 (20%)	1 (25%)
Onion bulbs	4 (57%)	3 (42.9%)	2 (40%)	1 (25%)
Myelin thickness[b]				
Normal	0	1 (14.3%)	1 (20%)	1 (25%)
Mostly normal	0	4 (57.1%)	3 (60%)	2 (50%)
50%-50%	5 (71%)	2 (28.6%)	0	0
Mostly thin	2 (29%)	0	1 (20%)	1 (25%)
Axonal degeneration	0	0	0	0
Fiber loss				
Normal	1 (14%)	2 (28.6%)	0	1 (25%)
Mild	4 (57%)	5 (71.4%)	4 (80%)	2 (50%)
Moderate	1 (14%)	0	1 (20%)	0
Severe	1 (14%)	0	0	1 (25%)
Macrophage activity	0	0	0	0
Storage material				
None	1 (14%)	4 (57.1%)	2 (40%)	4 (100%)
Some	5 (71%)	3 (42.9%)	3 (40%)	0
Many	1 (14%)	0	0	0
Severity				
Normal	0	1 (14.3%)	0	0
Mild	3 (43%)	5 (71.4%)	3 (60%)	3 (75%)
Moderate	3 (43%)	1 (14.3%)	2 (40%)	1 (25%)
Severe	1 (14%)	0	0	0

Data are presented as N (%).

[a]europathy was defined as active whenever there were signs of active demyelination or axonal degeneration.

[b]Myelin thickness was evaluated depending on the proportion of fibers with well-compacted myelin and defined as following: "normal" if all fibers had normal myelin, "almost normal" if most of fibers had normally thickened myelin, "50%-50%" if roughly half fibers had normal and half thin myelin and "mostly thin" when the majority of fibers had thin myelin sheath.

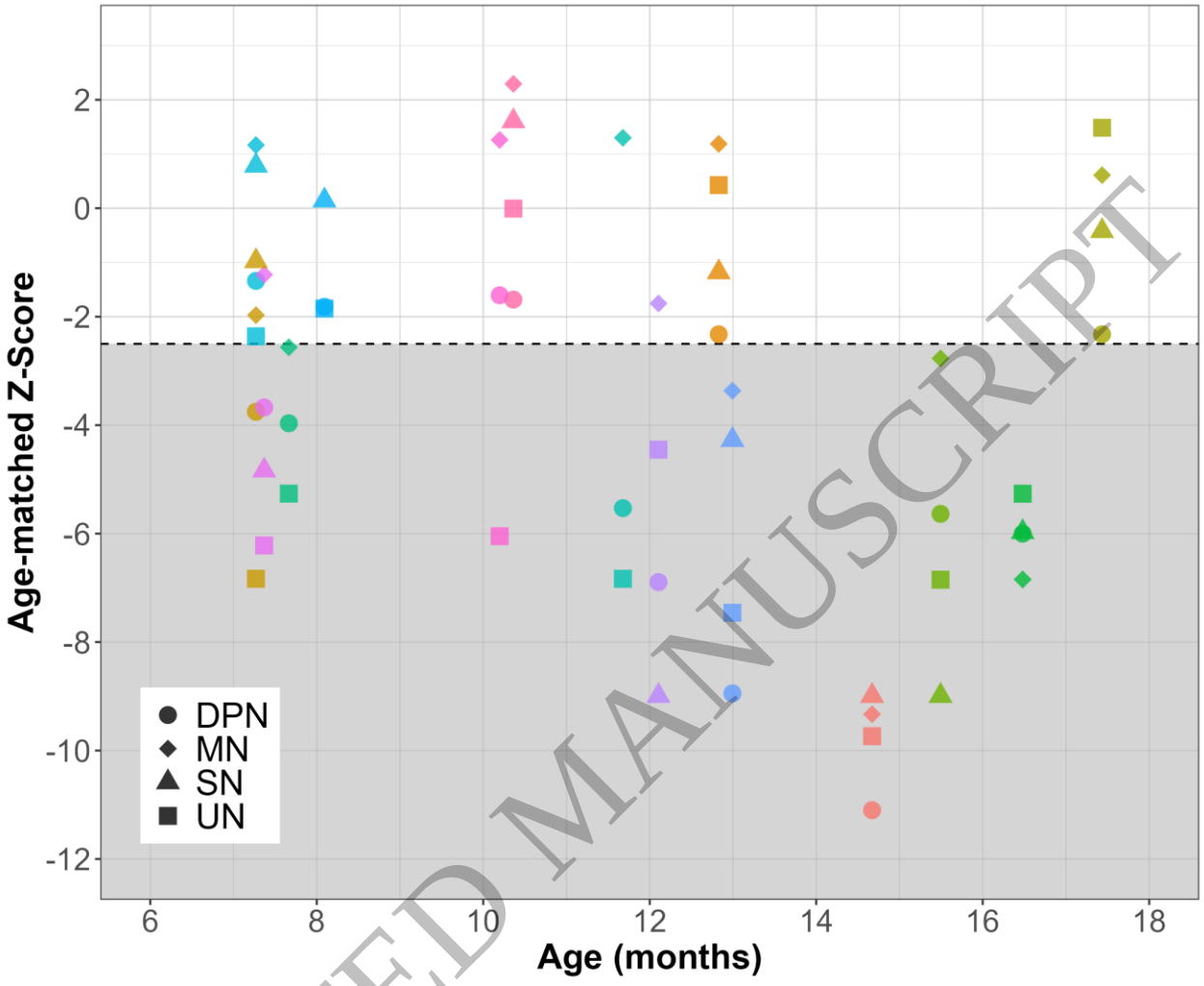

Figure 1
220x180 mm (x DPI)

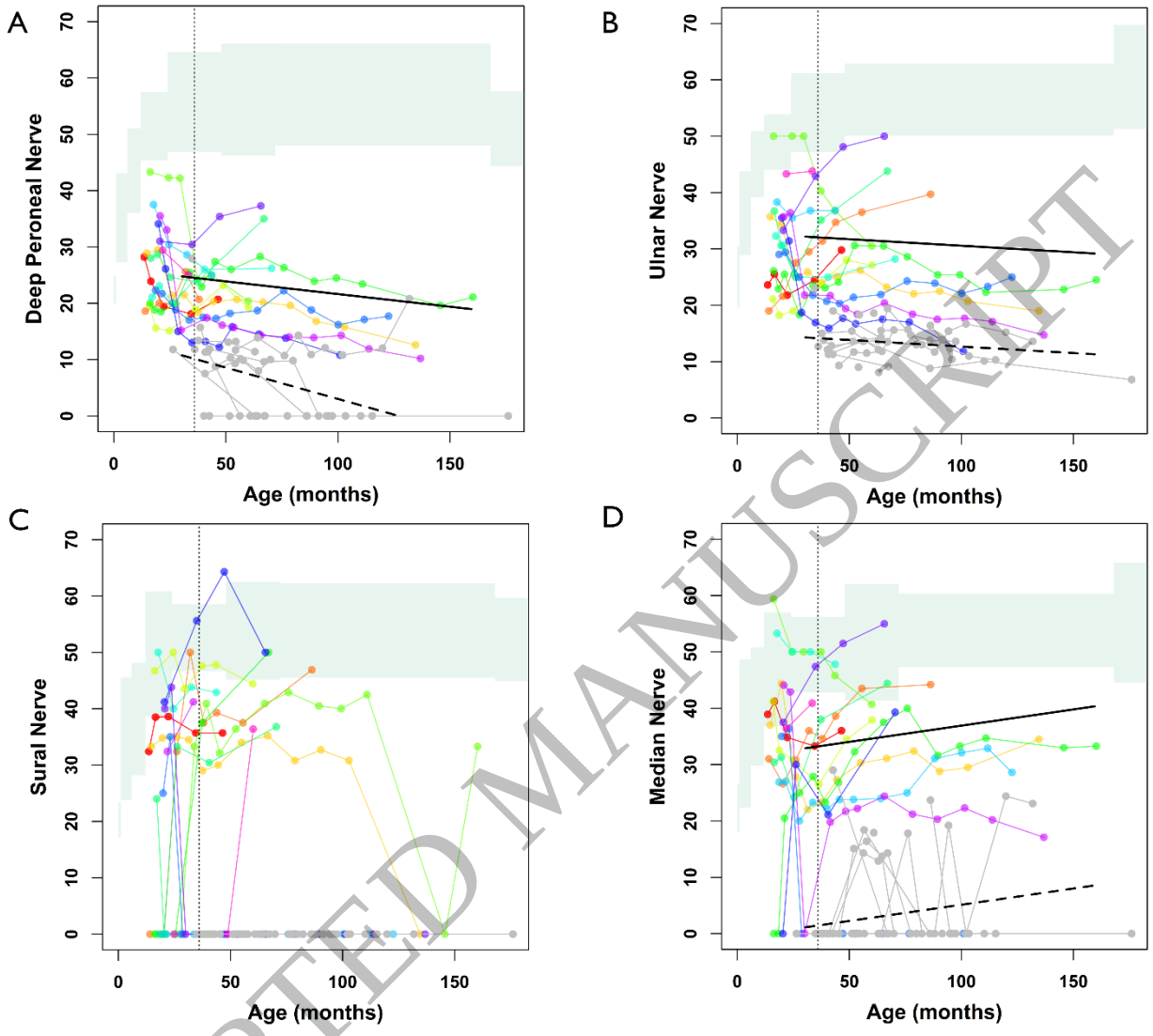

Figure 2
216x190 mm (x DPI)

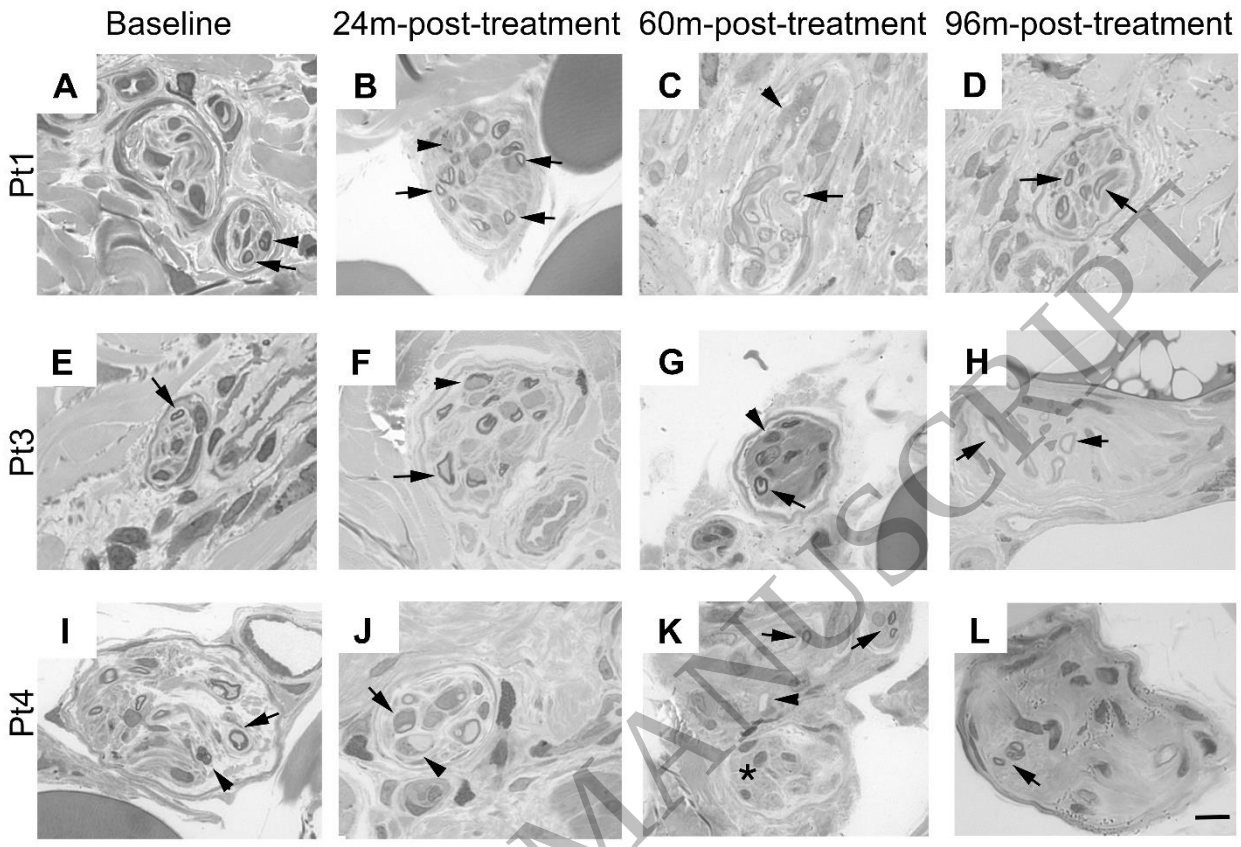

Figure 3
254x173 mm (x DPI)

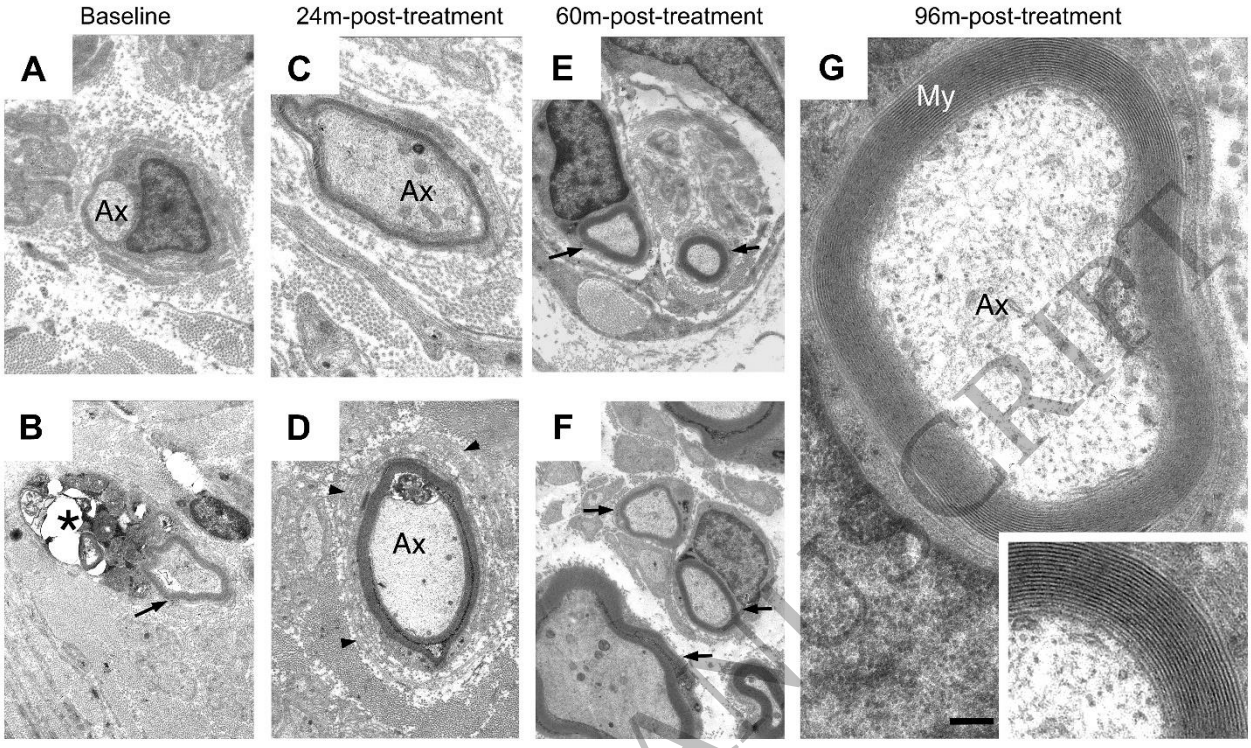

Figure 4
254x154 mm (x DPI)