ORIGINAL ARTICLE

Long-Term Effects of Atidarsagene Autotemcel for Metachromatic Leukodystrophy

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ABSTRACT

BACKGROUND

Metachromatic leukodystrophy (MLD) is an ultrarare, severe lysosomal storage disorder caused by a deficiency of arylsulfatase A (ARSA).

METHODS

We treated patients who had MLD with atidarsagene autotemcel (arsa-cel), a hematopoietic stem-cell–based gene therapy, in two prospective open-label clinical studies and expanded-access programs. We compared their outcomes with those of untreated patients (natural history cohort). The primary end point was survival free from severe motor impairment (the time from birth to the first occurrence of loss of locomotion and of sitting without support or death from any cause).

RESULTS

A total of 39 treated patients and 49 untreated patients were included. The median follow-up was 6.76 years (range, 0.64 to 12.19). Arsa-cel resulted in a significantly lower risk of severe motor impairment or death than no treatment among patients with presymptomatic late-infantile MLD ($P<0.001$), those with presymptomatic early-juvenile MLD ($P=0.04$), and those with early-symptomatic early-juvenile MLD ($P<0.001$). The estimated percentage of patients surviving without severe motor impairment at 6 years of age was 0% (95% confidence interval [CI], not evaluable) among untreated patients with late-infantile MLD and 100% (95% CI, 100 to 100) among treated patients with presymptomatic late-infantile MLD. The estimated percentage of patients surviving without severe motor impairment at 10 years of age was 11.2% (95% CI, 0.9 to 36.4) among untreated patients with early-juvenile MLD and 87.5% (95% CI, 38.7 to 98.1) and 80.0% (95% CI, 40.9 to 94.6) among treated patients with presymptomatic and early-symptomatic early-juvenile MLD, respectively. No evidence of insertional oncogenesis was found. The most common grade 3 or higher adverse event was febrile neutropenia. Anti-ARSA antibodies were detected transiently in 6 of 39 patients (15%). Three deaths occurred, all of which were considered by the investigators to be unrelated to arsa-cel.

CONCLUSIONS

Among patients with presymptomatic late-infantile or early-juvenile MLD and those with early-symptomatic early-juvenile MLD, the risk of severe motor impairment or death was significantly lower among those who received treatment with arsa-cel than in a natural history cohort that did not receive treatment. (Funded by Orchard Therapeutics and others; ClinicalTrials.gov numbers, NCT01560182 and NCT03392987.)

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A list of the investigators in this study is provided in the Supplementary Appendix, available at NEJM.org.

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Metachromatic leukodystrophy (MLD) is an ultrarare autosomal recessive lysosomal storage disorder caused by a deficiency of the enzyme arylsulfatase A (ARSA). ARSA deficiency leads to the accumulation of sulfatides in the central and peripheral nervous system, which results in progressive demyelination, microglial damage, and neurodegeneration, with subsequent loss of motor and cognitive function, and premature death.[1-4] MLD is classified, on the basis of the age at symptom onset, as early-onset (late-infantile [≤30 months] and early juvenile [30 months to <7 years]) and late-onset clinical subtypes.[5]

Atidarsagene autotemcel (arsa-cel, also known as OTL-200) is an autologous hematopoietic stem-cell (HSC)–based gene therapy consisting of CD34+ hematopoietic stem and progenitor cells (HSPCs) transduced ex vivo with a lentiviral vector encoding human *ARSA* complementary DNA, with constitutive ARSA expression driven by a human phosphoglycerate kinase promoter.[6,7] Arsa-cel is the only approved treatment for early-onset MLD.[8,9] Allogeneic HSC transplantation has shown a benefit in patients with late-onset MLD who are presymptomatic or minimally symptomatic at the time of transplantation, but it is not effective in patients with early-onset MLD, even those who are presymptomatic.[9] Before the approval of arsa-cel, no treatment options were available for early-onset MLD beyond supportive care.

Here, we present the results from an integrated analysis of safety and efficacy data from 39 patients with early-onset MLD who were treated with arsa-cel, with a median follow-up of 6.76 years (maximum, 12.19), as compared with data from 49 untreated patients with MLD. Data from these 88 patients constitute the dataset reviewed in the Biologics License Application for arsa-cel.

Methods

Patients and Study Design

The arsa-cel cohort comprised patients with early-onset MLD who received arsa-cel in two prospective open-label clinical studies[10,11] and in expanded-access programs. Data from treated patients (the arsa-cel cohort) were compared with data from untreated patients with early-onset MLD (the natural history cohort). Patients received treatment and completed follow-up at the San Raffaele Hospital in Milan, Italy. The schedule of assessments was similar in each study and expanded-access program, which allowed for an integrated analysis. All the studies were approved by the ethics committee at the San Raffaele Hospital and the Italian Medicines Agency and were conducted in accordance with Good Clinical Practice guidelines and the principles of Declaration of Helsinki. Written informed consent was obtained from the patients or their parents or legal guardians.

Patients were eligible for inclusion if they had a confirmed biochemical and genetic diagnosis of MLD that had been classified as presymptomatic late-infantile MLD, presymptomatic early-juvenile MLD, or early-symptomatic early-juvenile MLD, and if they had no contraindications.[6,7,10,11] The thresholds used to define symptom status in patients with late-infantile and early-juvenile subtypes were refined during the arsa-cel clinical development program. Patient subgroup classifications for the integrated analyses were adjudicated by an independent review committee convened during the program. The integrated analysis was conducted through a collaboration between the sponsor and the principal investigators. For additional details, see the Supplementary Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Procedures

Patients with early-onset MLD were treated with arsa-cel, as described previously.[6,7,12] CD34+ HSPC source material was obtained by means of either bone marrow harvest or mobilized peripheral-blood apheresis after administration of granulocyte colony-stimulating factor with or without plerixafor (or by both methods). CD34+ HSPCs were transduced ex vivo with lentiviral vector encoding *ARSA* and infused as a fresh cell product (in Study 201222 and expanded-access programs) or cryopreserved cell product (in Study 205756). Before undergoing infusion, all the patients received preparative conditioning with busulfan.[6,7,12]

End Points

The primary efficacy end point was survival free from severe motor impairment, defined as the time from birth to the first occurrence of loss of locomotion and of sitting without support (a Gross Motor Function Classification in MLD [GMFC-MLD] level of ≥5) or death from any

cause, up to the age of the oldest treated patient in the analysis set. The GMFC-MLD scale is used globally to measure gross motor function in patients with MLD and ranges from level 0 (normal function) to level 6 (the loss of all gross motor function) (see the Supplementary Methods section).[13,14] Key secondary efficacy end points were severe motor impairment or death at 2 years after treatment and overall survival.

Additional efficacy end points (defined in Table S13) included survival free from motor impairment (a GMFC-MLD level of ≥3), total scores on the 88-item Gross Motor Function Measure (GMFM-88; range 0 to 100%, with higher scores indicating greater motor function), and age-equivalent and standard scores on neuropsychological assessments of cognitive performance and language. Scores for cognitive performance included the performance standard score and developmental quotient for performance (see Table S3 and the study protocols, including the statistical analysis plans, available at NEJM.org), which are used to assess a child's nonverbal, visual-motor, and problem-solving skills. Developmental quotients were calculated for patients whose cognitive impairment necessitated at least one assessment with the use of a tool designed for a younger age than their chronological age. Categories for cognitive performance were based on performance standard scores and included normal cognition (score of ≥85), mild impairment (score of ≥70 to <85), moderate impairment (score of >55 to <70), and severe impairment (score of ≤55). Other clinical efficacy and pharmacodynamic end points included the magnetic resonance imaging (MRI) total score for the brain (brain MRI total score), measured according to a modified Loes assessment (range, 0 to 31.5, with higher scores indicating more extensive disease); vector copy number and ARSA activity in peripheral-blood and bone marrow cell populations; the percentage of lentiviral vector–positive cells in bone marrow; and ARSA activity in cerebrospinal fluid (CSF), measured according to previously described methods.[6,7,12,15] Post hoc analyses included the age at loss of speech and survival free from confirmed severe cognitive impairment (defined as a performance standard score of ≤55 and no subsequent scores of >55). Safety end points included engraftment failure (absolute neutrophil count <500 per microliter at day 60), toxic effects related to preparative conditioning and infusion, immunogenicity (anti-ARSA antibodies), short-term and long-term safety of lentiviral vector use (including replication-competent lentivirus and abnormal clonal expansion), adverse events, and serious adverse events.[6]

STATISTICAL ANALYSIS

Details on the statistical analyses are available in the Supplementary Appendix. The integrated summary of efficacy included treated patients in prespecified subgroups of patients with presymptomatic late-infantile MLD, presymptomatic early-juvenile MLD, or early-symptomatic early-juvenile MLD, whose outcomes were compared with data from patients in the natural history cohort. The sample size was based on the available patients who received treatment in the arsa-cel clinical development program and were determined by the independent review committee to be eligible for analysis. No statistical considerations were applied.

In an analysis of the time-to-event end points, we used unstratified log-rank tests to compare the study groups and Kaplan–Meier plots to show the estimated percentage of patients who were event-free at each year of age. The between-group differences in the percentage of patients with severe motor impairment or death at 2 years and 5 years after treatment were compared with the use of Fisher's exact test. The GMFM-88 and brain MRI total scores at 2 years and 5 years were analyzed with the use of separate analysis of covariance models, adjusted for study group and age at assessment.

All efficacy analyses were conducted within the prespecified patient subgroup classifications of presymptomatic late-infantile, presymptomatic early-juvenile, and early-symptomatic early-juvenile MLD. A testing hierarchy across primary and key secondary efficacy end points was used to control for the type I error rate within patient subgroup classifications, at a two-sided significance level of 5% (see the Supplementary Methods section and the protocol, including the statistical analysis plan).

RESULTS

PATIENT AND PRODUCT CHARACTERISTICS

The arsa-cel cohort comprised 39 patients with early-onset MLD who received arsa-cel in the

Table 1. Patient Characteristics and Duration of Follow-up in the Integrated Summary of Efficacy.

Variable	Arsa-cel*			Untreated†	
	Presymptomatic Late-Infantile MLD (N=18)	Presymptomatic Early-Juvenile MLD (N=8)	Early-Symptomatic Early-Juvenile MLD (N=11)	Late-Infantile MLD (N=26)	Early-Juvenile MLD (N=17)
Sex — no. (%)					
Female	5 (28)	2 (25)	5 (45)	14 (54)	9 (53)
Male	13 (72)	6 (75)	6 (55)	12 (46)	8 (47)
Median age at diagnosis (range) — mo	6.6 (0.4–12.3)	12.6 (0.0–44.1)	64.6 (24.9–131.7)	30.5 (18.6–44.0)	53.2 (30.9–91.3)
Median age at symptomatic disease onset (range) — mo	NA	NA	64 (29–83)	15 (9–26)	47 (18–75)
Median age at arsa-cel treatment or at first contact (range) — mo‡	10.3 (7.6–17.7)	16.1 (11.3–48.9)	69.0 (30.5–139.7)	18.8 (14.5–27.9)	52.6 (19.2–74.1)
Median duration of follow-up (range) — yr	6.7 (2.4–12.2)	3.8 (1.1–9.6)	7.4 (0.6–9.4)	4.4 (0.6–18.8)	5.6 (0.4–20.7)
Median age at last contact or death (range) — yr	7.4 (3.2–13.4)	6.1 (2.1–12.0)	12.6 (5.1–19.1)	6.2 (2.7–20.4)	10.3 (2.8–25.3)

* The results in treated subgroups in the atidarsagene autotemcel (arsa-cel) cohort were adjudicated by the independent review committee. Two patients treated with arsa-cel who were determined by the independent review committee to have symptomatic late-infantile metachromatic leukodystrophy (MLD) (1 patient) or progressively symptomatic early-juvenile MLD (1 patient) were not included the integrated summary of efficacy but were included in the integrated summary of safety (Table S4). NA denotes not applicable.

† Untreated patients included patients in the natural history cohort with the same MLD subtype in Study 204949 (includes 21 untreated siblings of the 39 arsa-cel–treated patients). Note that untreated siblings who were not enrolled in Study 204949 but had data obtained as part of the family history for treated siblings in Study 205756 (6 patients) also contributed to the assessment of severe motor impairment–free survival and overall survival only.

‡ The median age at first contact denotes the age at the time of arsa-cel treatment for treated patients and the age at the earliest assessment (including retrospective assessments) for untreated patients in the natural history cohort.

two prospective open-label clinical studies (20 patients in Study 201222[10] and 10 patients in Study 9205756[11]) and in the expanded-access programs (9 patients). The integrated summary of efficacy included 37 treated patients in prespecified subgroups of patients with presymptomatic late-infantile MLD (18 patients), presymptomatic early-juvenile MLD (8 patients), or early-symptomatic early-juvenile MLD (11 patients), whose outcomes were compared with data from 49 patients in the natural history cohort (Fig. S1A and S1B in the Supplementary Appendix); 2 patients were excluded from the integrated summary of efficacy (described in the Supplementary Appendix). Data from all 39 treated patients were included in the integrated summary of safety. Additional information is provided in Table S1.

The first patient received treatment on May 7, 2010, and the last on April 18, 2015. Data were collected from April 2010 through November 2022. The sample was drawn from patients worldwide with various ethnic and racial backgrounds and is representative of the broader population of patients with early-onset MLD (Table S2). Of the 49 untreated patients in the natural history cohort, 43 were from a natural history study (Study 204949; data collected from January 2000 through November 2021), which included 21 untreated siblings of arsa-cel–treated patients, and 6 were additional untreated siblings of treated patients in Study 205756.[6,10,11,16] Demographic characteristics of the patients and the duration of follow-up are presented in Table 1 and Table S4. The product characteristics of arsa-cel are provided in Table S5.

GROSS MOTOR FUNCTION

Arsa-cel resulted in a significantly lower risk of severe motor impairment (a GMFC-MLD level of ≥5) or death than no treatment among patients with presymptomatic late-infantile MLD (P<0.001), those with presymptomatic early-juvenile MLD (P=0.04), and those with early-symptomatic early-juvenile MLD (P<0.001) (Fig. 1A). The estimated percentage of patients surviving without severe motor impairment at 6 years of age was 0% (95% confidence interval [CI], not evaluable) among untreated patients with late-infantile MLD,

as compared with 100% (95% CI, 100 to 100) among treated patients with presymptomatic late-infantile MLD; severe motor impairment developed in 1 treated patient with presymptomatic late-infantile MLD at 7.2 years of age. Similarly, among patients with early-juvenile MLD, the estimated percentage surviving without severe motor impairment at 10 years of age was 11.2% (95% CI, 0.9 to 36.4) among untreated patients, as compared with 87.5% (95% CI, 38.7 to 98.1) and 80.0% (95% CI, 40.9 to 94.6) among treated patients with presymptomatic early-juvenile MLD and those with early-symptomatic early-juvenile MLD, respectively.

The percentages of treated and age-matched untreated patients who had died or had severe motor impairment at 2 years (a key secondary end point) and 5 years after treatment are provided in Table S6. A significantly greater percentage of arsa-cel–treated patients with presymptomatic late-infantile MLD than age-matched untreated patients with late-infantile MLD were alive and free from severe motor impairment at 2 years; a large difference persisted at 5 years.

The percentage of treated patients with presymptomatic early-juvenile MLD and early-symptomatic early-juvenile MLD who were alive and free from severe motor impairment at 2 years was similar to that of age-matched untreated patients with early-juvenile MLD; the differences were not significant. On the basis of the testing hierarchy, no further claims of statistical significance could be made for comparisons involving the treated patients with presymptomatic early-juvenile MLD and those with early-symptomatic early-juvenile MLD. The differences in the percentages of treated patients with presymptomatic early-juvenile or early-symptomatic early-juvenile MLD and age-matched untreated patients with early-juvenile MLD who had died or had severe motor impairment at 5 years are presented in Table S6.

Kaplan–Meier plots for survival free from motor impairment (the time from birth to the loss of the ability to walk [GMFC-MLD level of ≥3] or death from any cause) in treated patients with presymptomatic late-infantile MLD, those with presymptomatic early-juvenile MLD, and those with early-symptomatic early-juvenile MLD, as compared with untreated patients with matched MLD subtypes, are presented in Figure 1B. The estimated percentage of patients surviving without motor impairment at 6 years of age was 0% (95% CI, not evaluable) among untreated patients with late-infantile MLD, as compared with 93.8% (95% CI, 63.2 to 99.1) among treated patients with presymptomatic late-infantile MLD; motor impairment developed in 1 treated patient with presymptomatic late-infantile MLD at 4.4 years of age. Similarly, the estimated percentage of patients surviving without motor impairment at 10 years of age was 0% (95% CI, not evaluable) among untreated patients with early-juvenile MLD, as compared with 87.5% (95% CI, 38.7 to 98.1) and 57.1% (95% CI, 21.7 to 81.5) among treated patients with presymptomatic early-juvenile MLD and those with early-symptomatic early-juvenile MLD, respectively.

Results of analyses of GMFC-MLD levels and GMFM-88 total scores are consistent with long-term stabilization of motor skills in most treated patients with presymptomatic late-infantile MLD and those with presymptomatic early-juvenile MLD. Details are available in Figures S2 and S3 and Table S7.

OVERALL SURVIVAL

The risk of death was significantly lower among treated patients with presymptomatic late-infantile MLD than among untreated patients with late-infantile MLD (P<0.001) (Fig. 1C). The estimated percentage of patients alive at 6 years of age was 100% (95% CI, 100 to 100) among treated patients with presymptomatic late-infantile MLD, as compared with 59.0% (95% CI, 37.2 to 75.5%) among untreated patients with late-infantile MLD. At the time of this analysis, all 18 treated patients with presymptomatic late-infantile MLD were alive, having reached a median age of 7.4 years (range, 3.2 to 13.4), whereas 19 of 28 untreated patients with late-infantile MLD (68%) had died; the median age of patients at the time of death was 5.9 years (range, 3.5 to 13.4). Overall survival was similar among treated patients with presymptomatic early-juvenile and those with early-symptomatic early-juvenile MLD and untreated patients with early-juvenile MLD (Fig. 1C). One patient with presymptomatic early-juvenile MLD died from an ischemic cerebral infarction at 2.1 years of age, and 2 patients with early-symptomatic early-juvenile MLD died due to disease progression. None of the deaths were considered by the investigators to be related to treatment with arsa-cel.[6]

COGNITIVE PERFORMANCE AND LANGUAGE

Kaplan–Meier plots for survival free from confirmed severe cognitive impairment among treated

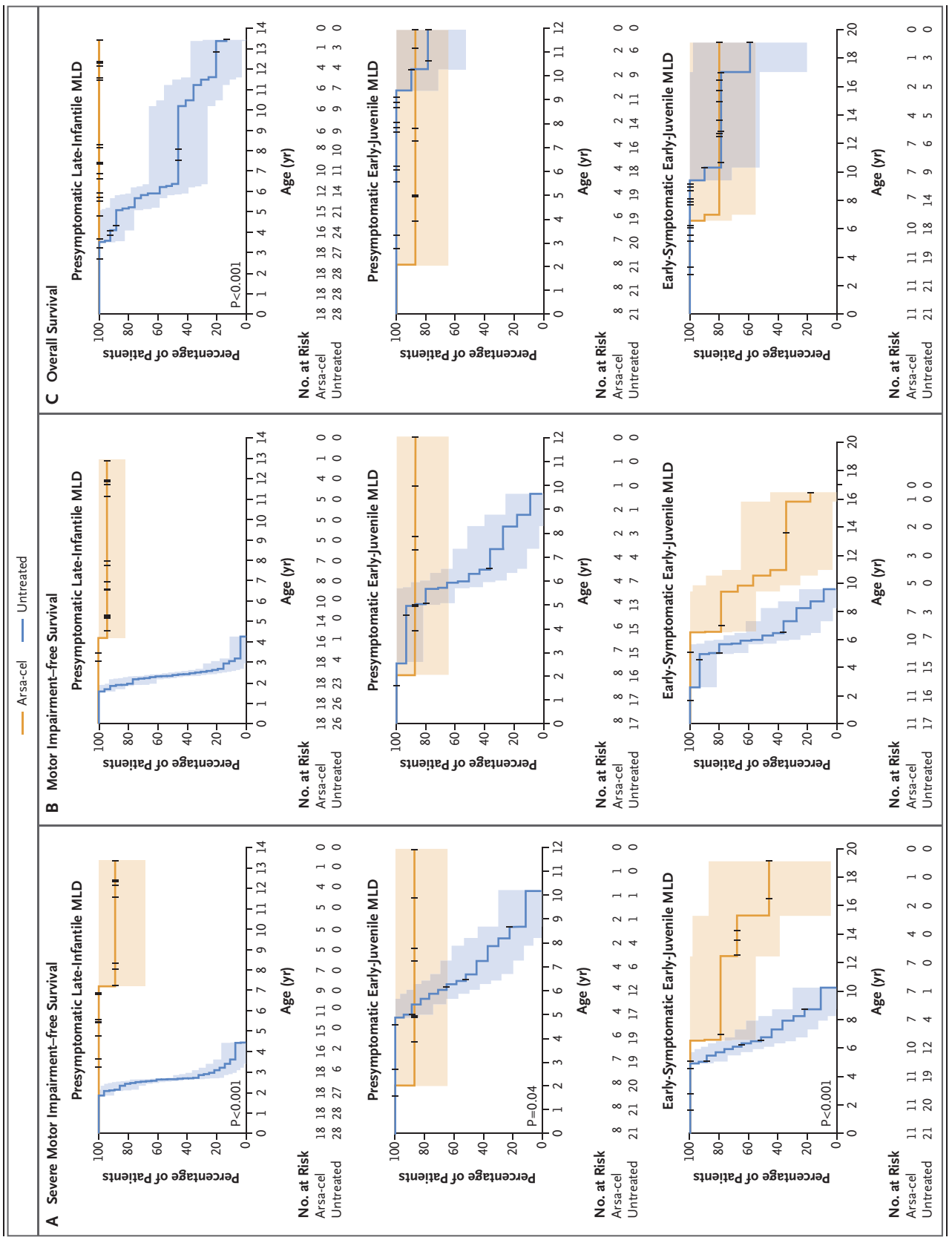


Figure 1 (facing page). Severe Motor Impairment–free Survival, Motor Impairment–free Survival, and Overall Survival.

Shown are Kaplan–Meier estimates of survival free from severe motor impairment among atidarsagene autotemcel (arsa-cel)–treated patients with presymptomatic late-infantile metachromatic leukodystrophy (MLD), those with presymptomatic early-juvenile MLD, and those with early-symptomatic early-juvenile MLD (Panel A); survival free from motor impairment in the three groups (Panel B); and overall survival in the three groups (Panel C), as compared with natural history data from untreated patients with matched MLD subtypes. Survival free from severe motor impairment was defined as the time from birth to the first of loss of locomotion and of sitting without support (a Gross Motor Function Classification for Metachromatic Leukodystrophy [GMFC-MLD] level of ≥5, on a scale that ranges from level 0 [normal function] to level 6 [the loss of all gross motor function]) or death from any cause; otherwise, data were censored at the date of the last GMFC-MLD assessment. Survival free from motor impairment was defined as the time from birth to the loss of the ability to walk (a GMFC-MLD level of ≥3 confirmed at all subsequent assessments) or death from any cause; otherwise, the data were censored at the date of the last GMFC-MLD assessment. Tick marks indicate censored data. P values were calculated from an unstratified log-rank test. The widths of the confidence intervals (shaded areas) have not been adjusted for multiplicity and should not be used in place of hypothesis testing.

patients with presymptomatic late-infantile MLD, those with presymptomatic early-juvenile MLD, and those with early-symptomatic early-juvenile MLD, as compared with those among untreated patients with matched MLD subtypes are presented in Figure 2A. The estimated percentage of patients surviving without severe cognitive impairment at 6 years of age was 8.8% (95% CI, 1.5 to 24.3) among untreated patients with late-infantile MLD, as compared with 100% (95% CI, 100 to 100) among treated patients with presymptomatic late-infantile MLD; 1 patient with presymptomatic late-infantile MLD had confirmed severe cognitive impairment at 6.4 years of age. The estimated percentage of patients surviving without severe cognitive impairment at 10 years of age was 7.5% (95% CI, 0.5 to 28.4) among untreated patients with early-juvenile MLD, whereas 87.5% (95% CI, 38.7 to 98.1) of treated patients with presymptomatic early-juvenile and 64.8% (95% CI, 25.3 to 87.2) with early-symptomatic early-juvenile MLD were estimated to remain event-free at this age.

The majority of surviving patients treated with arsa-cel still had normal cognition (performance standard scores of ≥85) at the last available neuropsychological assessment — 12 of 18 patients with presymptomatic late-infantile MLD, 7 of 7 with presymptomatic early-juvenile MLD, and 8 of 9 with early-symptomatic early-juvenile MLD (Fig. 2B) — and continued to gain cognitive skills, according to the upward trajectory of age-equivalent scores (Fig. S4). Similar results were seen with verbal age-equivalent scores and language standard scores (Figs. S5, S6B, S6D, and S6F). Untreated patients with late-infantile and early-juvenile MLD who completed neurocognitive assessments showed severe cognitive impairment.

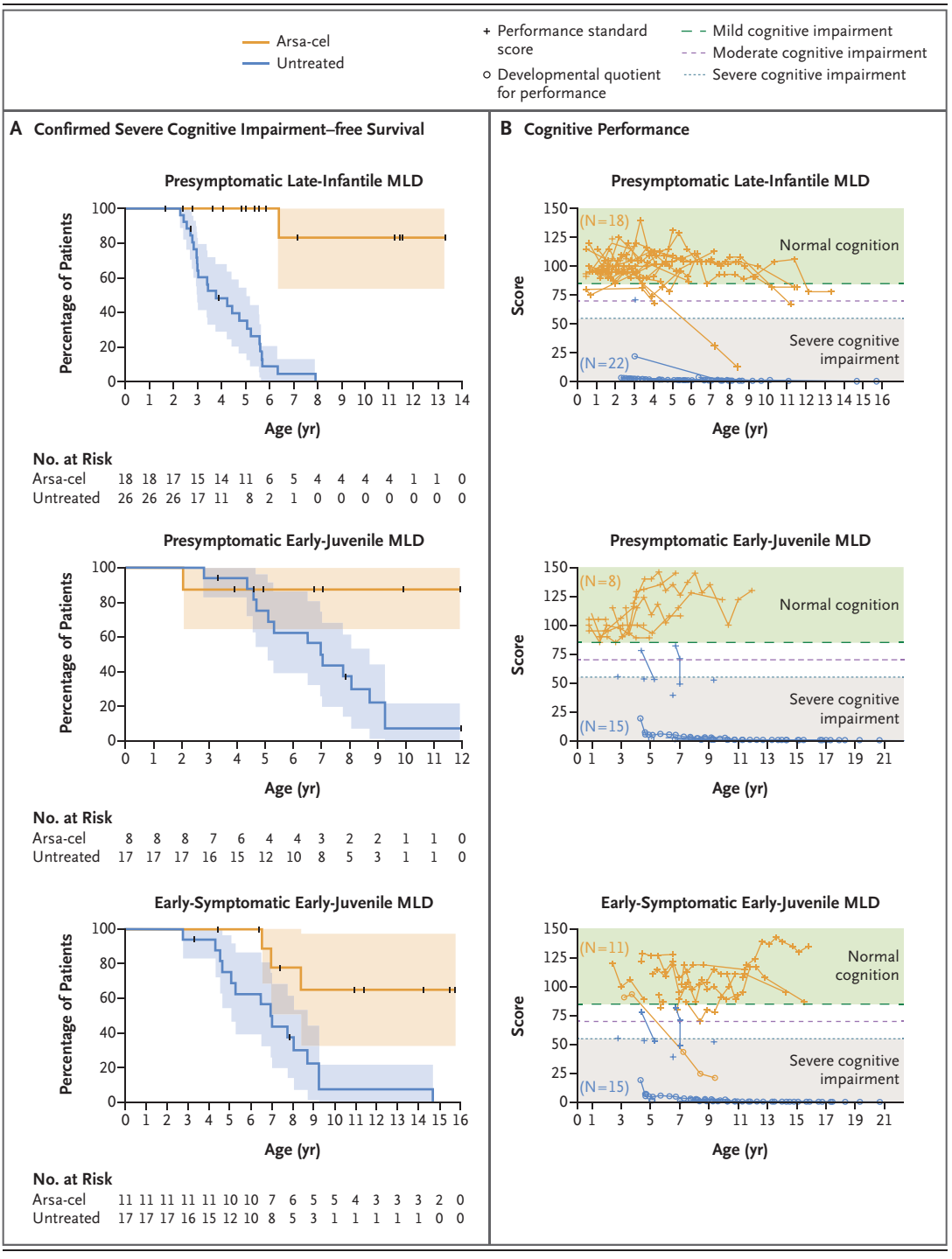
Kaplan–Meier plots for the age at which a loss of speech occurred (treated patients vs. untreated patients with matched MLD subtypes) are presented in Figures S6A, S6C, and S6E. The estimated percentage of patients with no loss of speech at 6 years of age was 4.2% (95% CI, 0.3 to 17.7) among untreated patients with late-infantile MLD, as compared with 100% (95% CI, 100 to 100) among treated patients with presymptomatic late-infantile MLD; 1 patient with presymptomatic late-infantile MLD had a loss of speech at age 6.4 years. The estimated percentage of patients with no loss of speech at 10 years of age was 26.5% (95% CI, 4.6 to 56.3]) among untreated patients with early-juvenile MLD, as compared with 100% (95% CI, 100 to 100) and 66.7% (95% CI, 28.2 to 87.8) among treated patients with presymptomatic early-juvenile and early-symptomatic early-juvenile MLD, respectively.

BRAIN MRI SCORES

Analysis of brain MRI total scores over time showed that scores remained lower among treated patients than among untreated patients with matched MLD subtypes (Fig. S7). The adjusted mean differences in brain MRI total scores between treated subjects and age-matched and subtype-matched untreated patients at 2 years and 5 years are presented in the Supplementary Results section and Table S8.

ENGRAFTMENT AND ARSA ACTIVITY

The geometric mean vector copy number in total peripheral-blood mononuclear cells (PBMCs) and in bone marrow–derived mononuclear cells and the percentage of lentiviral vector–positive cells in bone marrow clonogenic progenitor cells showed



evidence of high and stable engraftment of transduced cells, beginning at 1 month after treatment and remaining stable throughout the course of follow-up (Figs. S8 and S9). The geometric mean vector copy number and cell values in the early-symptomatic early-juvenile subgroup were lower than those in the presymptomatic late-infantile and presymptomatic early-juvenile subgroups. The reason for this difference is unknown but may be related to an effect of patient age on HSC characteristics, bone marrow microenvironment, or response to busulfan (or a combination of the three). Geometric mean ARSA activity levels in total PBMCs and CD14+ (monocyte) and CD15+ (granu-

Figure 2 (facing page). Integrated Efficacy Analyses of Cognitive Performance.

Shown are Kaplan–Meier estimates of survival free from confirmed severe cognitive impairment among arsa-cel–treated patients with presymptomatic late-infantile MLD, those with presymptomatic early-juvenile MLD, and those with early-symptomatic early-juvenile MLD (Panel A), as well as plots of the performance standard score and the developmental quotient for performance according to chronological age among arsa-cel–treated patients with presymptomatic late-infantile MLD, those with presymptomatic early-juvenile MLD, and those with early-symptomatic early-juvenile MLD (Panel B), as compared in each case with natural history data from untreated patients with matched MLD subtypes. Survival free from confirmed severe cognitive impairment was defined as the time from birth to the first occurrence of confirmed severe cognitive impairment (a performance standard score of ≤55 and no subsequent scores of >55) or death from any cause; otherwise, the data were censored (indicated by tick marks) at the time of the last neuropsychological assessment. Survival free from confirmed severe cognitive impairment was a post hoc modification to the prespecified end point of survival free from severe cognitive impairment. Cognitive performance was evaluated according to standardized assessments that were used to calculate a performance standard score or developmental quotient for performance (Table S3). Developmental quotients are shown for patients who had their last assessment performed with the use of a tool designed for a younger age than their chronological age. Reference lines for mild (performance standard score of ≥70 to <85), moderate (score of >55 to <70), and severe (score of ≤55) cognitive impairment are based on standard score categories. Normal cognition was defined as a standard score of ≥85. The widths of the confidence intervals in Panel A (shaded areas) have not been adjusted for multiplicity and should not be used in place of hypothesis testing.

locyte) myeloid cell lineages had increased to within the normal reference range at 1 month after treatment in all patients and subgroups of patients who received arsa-cel, and the levels remained stable (within or above the normal range) throughout the follow-up period (Figs. S10A, S11, and S12A). ARSA activity was also restored in CSF in all treated patients, with geometric mean levels remaining within the normal reference range starting from 3 to 6 months after treatment (Figs. S10B and S12B).

SAFETY

The adverse events and serious adverse events were consistent with the known safety profile of busulfan or symptoms of MLD (Supplementary Results section and Tables S9 and S10). No treatment-related serious adverse events were observed. Three participants had a fatal event, but none of the events were considered by the investigators to be related to arsa-cel. One patient died after an ischemic cerebral infarction on day 414 after treatment, which was considered to be unrelated to arsa-cel owing to its late onset, insufficient causal evidence, and a lack of similar events in the arsa-cel–treated population or in those who received other gene therapy products with the same lentiviral vector backbone.[6] Two patients with early-symptomatic early-juvenile MLD died from disease progression at 15 months and 8 months after treatment, at 7.0 and 6.6 years of age, respectively.[6]

No patients had engraftment failure. The median time to neutrophil and platelet engraftment was 39 days (range, 25 to 50) and 39 days (range, 15 to 109), respectively (Figs. S13 and S14). Delayed platelet engraftment (defined by a platelet count of ≤20,000 per microliter without transfusion support more than 60 days after infusion) was reported in 4 patients.

The adverse event considered by the investigators to be related to arsa-cel was the appearance of anti-ARSA antibodies, which were observed in 6 of 39 treated patients (15%). At the time of data analysis, five of the six events had resolved. Anti-ARSA antibody titers were generally low and transient and did not affect clinical outcomes or the overall safety profile (Supplementary Results section and Table S11).

Integration site analysis of DNA extracted from peripheral-blood and bone marrow samples obtained from treated patients was conducted to monitor the nature and distribution of vector integration sites. These analyses showed high clonal diversity in all patients, with no abnormal clonal proliferation or insertional oncogenesis observed over 251 cumulative patient-years of follow-up (Supplementary Results section and Fig. S15). No evidence of replication-competent lentivirus was found.

DISCUSSION

This study showed a beneficial effect of treatment with arsa-cel in patients with MLD that has been durable — up to 12 years for the earliest treated patient. Moreover, the duration of follow-up is among the longest reported for a lentiviral vector–based gene therapy; 13 patients have been followed for at least 8 years and 7 pa-

tients have been followed for at least 10 years after treatment.

The use of a historical prospective cohort of untreated patients with MLD as the comparison group is appropriate in this study because the rapid disease progression of early-onset MLD is well characterized, and the results from the natural history cohort are consistent with those from published literature.[5,17-20] The natural history data were obtained over a period of 21 years, which overlaps with the 11-year (2010 to 2021) clinical development program of arsa-cel. The inclusion of untreated siblings of treated patients also lends validity to the use of this cohort, since siblings with early-onset MLD typically have a similar natural history of the disease course.[20,21]

The most common adverse events were consistent with the known safety profile of busulfan or known symptoms of MLD. The emergence of anti-ARSA antibodies in some patients did not affect clinical outcomes or the overall safety profile. A highly polyclonal reconstitution of the hematopoietic system was observed in all patients, with no abnormal clonal expansion. Moreover, a recent study involving 23 of the treated patients at 2 years and later time points (up to 7.5 years) after treatment shows the presence of several thousand active HSPCs, a finding consistent with stable long-term hematopoiesis.[22] Stable engraftment of gene-corrected cells was observed across hematopoietic lineages (data not shown), with corresponding durable reconstitution of ARSA activity in the hematopoietic system and the CSF.

The absence of association between clonal abnormalities or oncogenic events and arsa-cel, which uses the ubiquitous human phosphoglycerate kinase promoter, is in line with the safety profile reported in many other clinical trials of HSC-based gene therapies that use lentiviral vectors.[23] The notable exception is the HSC-based gene therapy for cerebral adrenoleukodystrophy, in which hematologic cancers have been reported.[24] The key difference is that the gene therapy for cerebral adrenoleukodystrophy uses a strong promoter derived from a retroviral long terminal repeat, and the transcriptional activity of that virally derived promoter appears to be responsible for the insertional activation of nearby oncogenes.[24]

Motor function, cognitive function, and language skills were better maintained in patients with presymptomatic late-infantile MLD who were treated with arsa-cel than in those with untreated late-infantile MLD. At the last follow-up (median age of patients, 7.4 years; range, 3.2 to 13.4), all but one of the treated patients with presymptomatic late-infantile MLD were able to walk with or without support (Fig. S2 and Table S12); all were older than the median age (2.6 years) and most were older than the oldest age (3.4 years) at which severe motor impairment had developed in untreated patients with late-infantile MLD. Most treated patients with presymptomatic late-infantile MLD had age-appropriate development of cognitive and language abilities and normal or near-normal performance and language standard scores that were maintained over the long term. Untreated patients with late-infantile MLD showed severe cognitive impairment early in the course of the disease. Severe motor and cognitive impairment developed in one treated patient with presymptomatic late-infantile MLD at a later age than was observed in untreated patients with late-infantile MLD, as described previously.[6]

All surviving patients with presymptomatic early-juvenile MLD who were treated with arsa-cel had age-appropriate motor, cognitive, and language skills. Untreated patients with early-juvenile MLD had a rapid loss of motor and cognitive skills early in the course of the disease. At the last follow-up, five of seven surviving treated patients with presymptomatic early-juvenile MLD had surpassed the age at which the onset of symptomatic disease had occurred in their untreated sibling.

Outcomes in patients with early-symptomatic early-juvenile MLD were variable. This study showed significantly longer survival free from severe motor impairment among patients treated with arsa-cel than among untreated patients with early-juvenile MLD. Most (seven of nine) surviving treated patients with early-symptomatic early-juvenile MLD had the ability to sit without support or crawl or roll (a GMFC-MLD level of ≤4) at the last follow-up, and five of seven had surpassed the oldest age (10.2 years) recorded at which severe motor impairment (a GMFC-MLD level of ≥5) developed in untreated patients with early-juvenile MLD. At the last available assess-

ment, eight of nine surviving treated patients with early-symptomatic early-juvenile MLD had normal performance and language standard scores; the ages of these patients ranged from 4.6 to 15.8 years, ages at which almost all untreated patients with early-juvenile MLD had severe cognitive impairment.

In accordance with clinical trial[6,25] and real-world[26] experience, it is essential, when evaluating eligibility for treatment, to determine whether a patient with late-infantile MLD has become symptomatic or a patient with early-symptomatic early-juvenile MLD has entered a phase of rapid disease progression. Such patients are not expected to benefit from arsa-cel.

This long-term follow-up study showed that among patients treated with arsa-cel, mobility, cognition, and communication were preserved in all but one of the patients with presymptomatic late-infantile and presymptomatic early-juvenile MLD, and in most patients with early-symptomatic early-juvenile MLD, which allowed such patients and their caregivers to avoid the tremendous disease burden associated with supportive care only.[17,27] According to U.S. and European clinical consensus guidelines, arsa-cel is recommended for patients with early-onset MLD.[28,29] Because the best outcomes have been observed in patients treated during the presymptomatic period, identifying patients as early as possible is important.[6] In the current real-world scenario, most patients with early-onset MLD are ineligible for treatment with arsa-cel by the time the diagnosis is made.[26] Early diagnosis, especially through newborn screening, is essential to identify children who would receive the greatest benefit from treatment. The feasibility of implementing such an integrated newborn screening and care pathway for MLD has recently been reported.[30]

The results of our study showed that arsa-cel led to a durable, disease-modifying effect in patients with early-onset MLD, especially in children treated before symptoms developed, a finding that contrasts with the rapid decline to severe neurologic disability that has been observed in untreated patients with MLD.

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