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

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Summary

Background Metachromatic leukodystrophy (a deficiency of arylsulfatase A [ARSA]) is a fatal demyelinating lysosomal disease with no approved treatment. We aimed to assess the long-term outcomes in a cohort of patients with early-onset metachromatic leukodystrophy who underwent haemopoietic stem-cell gene therapy (HSC-GT).

Methods This is an ad-hoc analysis of data from an ongoing, non-randomised, open-label, single-arm phase 1/2 trial, in which we enrolled patients with a molecular and biochemical diagnosis of metachromatic leukodystrophy (presymptomatic late-infantile or early-juvenile disease or early-symptomatic early-juvenile disease) at the Paediatric Clinical Research Unit, Ospedale San Raffaele, in Milan. Trial participants received HSC-GT, which consisted of the infusion of autologous HSCs transduced with a lentiviral vector encoding ARSA cDNA, after exposure-targeted busulfan conditioning. The primary endpoints of the trial are safety (toxicity, absence of engraftment failure or delayed haematological reconstitution, and safety of lentiviral vector-tranduced cell infusion) and efficacy (improvement in Gross Motor Function Measure [GMFM] score relative to untreated historical controls, and ARSA activity, 24 months post-treatment) of HSC-GT. For this ad-hoc analysis, we assessed safety and efficacy outcomes in all patients who had received treatment and been followed up for at least 18 months post-treatment on June 1, 2015. This trial is registered with ClinicalTrials.gov, number NCT01560182.

Findings Between April, 2010, and February, 2013, we had enrolled nine children with a diagnosis of early-onset disease (six had late-infantile disease, two had early-juvenile disease, and one had early-onset disease that could not be definitively classified). At the time of analysis all children had survived, with a median follow-up of 36 months (range 18–54). The most commonly reported adverse events were cytopenia (reported in all patients) and mucositis of different grades of severity (in five of nine patients [grade 3 in four of five patients]). No serious adverse events related to the medicinal product were reported. Stable, sustained engraftment of gene-corrected HSCs was observed (a median of 60·4% [range 14·0–95·6] lentiviral vector-positive colony-forming cells across 36 months [range 18–54]). The most commonly reported adverse events were cytopenia (reported in all patients) and mucositis of different grades of severity (in five of nine patients [grade 3 in four of five patients]). No serious adverse events related to the medicinal product were reported. Stable, sustained engraftment of gene-corrected HSCs was observed (a median of 60·4% [range 14·0–95·6] lentiviral vector-positive colony-forming cells across follow-up) and the engraftment level was stable during follow-up; engraftment determinants included the duration of absolute neutropenia and the vector copy number of the medicinal product. A progressive reconstitution of ARSA activity in circulating haemopoietic cells and in the cerebrospinal fluid was documented in all patients in association with a reduction of the storage material in peripheral nerve samples in six of seven patients. Eight patients, seven of whom received treatment when presymptomatic, had prevention of disease onset or halted disease progression as per clinical and instrumental assessment, compared with historical untreated control patients with early-onset disease. GMFM scores for six patients up to the last follow-up showed that gross motor performance was similar to that of normally developing children. The extent of benefit appeared to be influenced by the interval between HSC-GT and the expected time of disease onset. Treatment resulted in protection from CNS demyelination in eight patients and, in at least three patients, amelioration of peripheral nervous system abnormalities, with signs of remyelination at both sites.

Interpretation Our ad-hoc findings provide preliminary evidence of safety and therapeutic benefit of HSC-GT in patients with early-onset metachromatic leukodystrophy who received treatment in the presymptomatic or very early-symptomatic stage. The results of this trial will be reported when all 20 patients have achieved 3 years of follow-up.

Funding Italian Telethon Foundation and GlaxoSmithKline.

Introduction Metachromatic leukodystrophy is a rare autosomal-recessive lysosomal storage disease caused by mutations in the arylsulfatase A (ARSA) gene that result in enzyme deficiency and accumulation of the undegraded substrate cerebroside 3-sulphate (sulphatide) in neural and glial cells in the central nervous system (CNS) and peripheral nervous system (PNS). This accumulation of sulphatide...
Research in context

Evidence before this study
We searched MEDLINE for relevant studies of therapies for metachromatic leukodystrophy, published since the inception of the database until Feb 15, 2016. The literature searches used the following search terms (with acronyms, synonyms, and closely related words): "Metachromatic leukodystrophy" combined with "therapy" or "Hematopoietic stem cell (HSC) transplantation", as well as "mouse model" and "Metachromatic leukodystrophy" and "gene therapy" or "HSC gene therapy". We did not apply any study design or language restrictions. We identified further studies by examining the reference lists of all included articles, and searching relevant websites. No approved treatment is available for patients with metachromatic leukodystrophy. When undertaken before the appearance of symptoms, haemopoietic stem-cell (HSC) transplantation (HCT) from HLA-matched donors can delay onset or attenuate progression of some CNS manifestations of metachromatic leukodystrophy, with no impact on severe peripheral nervous system manifestations. HSC gene therapy, but not HCT, has been shown to be efficacious in preventing and correcting CNS and peripheral nervous system metachromatic leukodystrophy manifestations in the mouse model, and we have reported its safety and early favourable outcome in the short-term follow-up of three patients with metachromatic leukodystrophy with late-infantile onset from our trial.

Added value of this study
Effective treatment for metachromatic leukodystrophy is an unmet medical need. This ad-hoc analysis provides longer-term evidence of safety and, despite the need for longer-term follow-up, therapeutic benefit of HSC gene therapy in patients with early-onset metachromatic leukodystrophy who underwent treatment at a presymptomatic or very early symptomatic stage.

Implications of all the available evidence
The clinical outcome data reported here could pave the way for making HSC gene therapy available to patients with early-onset, presymptomatic, or early-symptomatic metachromatic leukodystrophy in the future. We also provide direct evidence that corrected patients’ HSCs and their progeny can efficiently deliver therapeutics to the nervous system, as proof-of-concept of a novel treatment paradigm for metachromatic leukodystrophy and related disorders. Data from the longer-term follow-up of the entire cohort will be needed to confirm the results of this analysis.

leads to progressive demyelination and neurodegeneration.1
The disease is classified into clinical variants according to the age of the patient at symptom onset. Early-onset metachromatic leukodystrophy consists of late-infantile and early-juvenile variants. Patients affected by the late-infantile variant, who manifest symptoms before 30 months of age, have the most severe presentation, quickly losing motor and cognitive milestones and dying within a few years from onset. Patients presenting symptoms between 30 months and 6 years of age are affected by early-juvenile metachromatic leukodystrophy and usually show a similar, but less rapid evolution than those affected by the late-infantile variant.

Treatment options are scarce, provide benefit only to some patients, and do not halt all disease aspects. When undertaken before the appearance of major symptoms, haemopoietic stem-cell transplantation (HSCT) from HLA-matched donors can delay onset or attenuate progression of some CNS manifestations of the disease;1 however, patients who undergo HSCT still have severe peripheral neuropathy with associated complications. In particular, HSCT appears to mitigate disease progression in patients with late-onset disease, who usually have not had severe peripheral neuropathy since onset,2 whereas its efficacy in patients with early-onset variants is still a matter of debate.3–5 For this reason, metachromatic leukodystrophy remains a devastating disease, resulting in a very poor prognosis, especially in patients who are very young.

We showed in preclinical studies6,7 that haemopoietic stem-cell gene therapy (HSC-GT) using lentiviral vectors, but not HSCT, prevents and corrects disease manifestations in a mouse model. We also reported a preliminary assessment8 of the safety and potential benefit of HSC-GT at 18 months after transplant for the first three patients with late-infantile metachromatic leukodystrophy (MLD01, MLD02, MLD03; the same patient identification codes as used in this report), treated in our open-label clinical trial.

In this report, we provide longer-term, preliminary data for nine patients with early-onset metachromatic leukodystrophy, and assess the safety and therapeutic benefit of HSC-GT in these patients, who received treatment while they were presymptomatic or at a very early symptomatic stage.

Methods

Study design and patients
This is an ad-hoc analysis of data from an ongoing, non-randomised, open-label, single-arm phase 1/2 trial, in which we enrolled patients with a molecular and biochemical diagnosis of metachromatic leukodystrophy from the Paediatric Clinical Research Unit at Ospedale San Raffaele, in Milan, Italy. Patients were eligible if they had presymptomatic late-infantile or early-juvenile disease or early-symptomatic early-juvenile disease (within the first 6 months from symptom onset). Patients were excluded from the present study if they tested positive for HIV, hepatitis C, or hepatitis B; were affected by neoplastic diseases; had cytogenetic alterations typical of myelodysplastic syndrome or acute myelogenous leukaemia; had end-organ functions or any other severe
disease which, as judged by the investigator, would make the patient inappropriate for study entry; were enrolled in other trials; or had undergone allogeneic HSCT in the previous 6 months or had evidence of residual cells of donor origin.

Written informed consent was obtained from the parents or guardians of the patients. This trial was approved by the institutional ethical committee of Ospedale San Raffaele and by Agenzia Italiana del Farmaco. The study was undertaken according to Good Clinical Practice criteria. This study was not overseen by a data monitoring committee.

For the purposes of the analysis, we also used data from a historical cohort of 21 patients with late-infantile metachromatic leukodystrophy and nine patients with early-juvenile metachromatic leukodystrophy who had not received treatment (aged 2–17·5 years), from the LDM1 natural history study approved in 2004 by the institutional ethical committee of Ospedale San Raffaele, upon collection of written informed consent. Additionally, we used Gross Motor Function Measure (GMFM) data from a cohort of 34 healthy children (aged 0–6 years), matched for sex and, to a lesser extent, ethnicity with our metachromatic leukodystrophy patient cohort, within an observational study approved by the institutional ethical committee of Ospedale San Raffaele, upon collection of written informed consent.

Procedures

Patients were treated as described previously. Briefly, bone marrow-derived CD34+ HSCs transduced with a clinical grade lentiviral vector encoding human ARSA cDNA were infused after myeloablative conditioning with intravenous busulfan (Busilvex, Pierre Fabre, Freiberg, Germany) at a targeted cumulative dose of 11·2–16·8 mg/kg, administered every 6 h over 4 days in 14 doses (individual dose area-under-the-curve target 4·8 mg×h/L). Supportive clinical care was delivered in accordance with local standards.

Transduced cell engraftment (assessed as the proportion of lentiviral vector-positive colony-forming cells out of all colonies), vector copy number per diploid genome (VCN), ARSA activity, and lentiviral vector integration sites were analysed in bone marrow, peripheral blood, and cerebrospinal fluid (CSF), every 6 months, as previously described. Each patient also underwent two skin biopsies (at baseline and at 2 years after gene therapy) for morphological analysis of dermal myelinated nerve fibres (appendix p 2).

The disease was monitored in the patients who received treatment in our trial with a standard neurological assessment; GMFM13,15 and Gross Motor Function Classification for metachromatic leukodystrophy (GMFC-MLD), measured every 3 months for the first year then every 6 months; and the Bayley Scale for Infant and Toddler Development, third edition (BSID-III) or Wechsler Preschool and Primary Scale of Intelligence, third edition (WPPSI-III), according to patients’ age, measured every 6 months; and electroneurographic recordings, measured at 3, 6, and 12 months then every 6 months thereafter. We also obtained the corresponding assessment findings from the historical cohort of patients with untreated late-infantile disease and early-juvenile disease, for comparison.

We calculated the nerve conduction velocity (NCV) index, which we used to assess the patients in our trial at baseline and each follow-up visit, using data from healthy children obtained from the scientific literature and internal reference values generated in adult volunteers from a previous study. Because data for healthy children’s NCV showed great variability (because only a small amount of data was available at some of the timepoints in the previous study), we calculated NCV test results obtained during follow-up of the patients in our trial who received treatment using internal adult reference values; analysis using age-matched reference values showed similar trends (data not shown). We undertook brain MRIs, inclusive of spectroscopy and diffusion tensor imaging, with modification of the described metachromatic leukodystrophy scoring system to increase its sensitivity (appendix p 3).

The same trained personnel (two operators per specialty) undertook these assessments along the course of the follow-up.

Outcomes

The objectives of the trial are to assess the safety and efficacy of HSC-GT in patients with metachromatic leukodystrophy. The primary safety endpoints are the absence of engraftment failure or delayed haematological reconstitution (ie, absolute neutrophil count [ANC] <0·5×10⁹/L 60 days after treatment), surveillance of toxicity not related to the haematological regimen, and safety of lentiviral vector-tranduced cell infusion. The primary efficacy endpoints are an improvement of 10% of the total GMFM score, assessed 24 months after treatment compared with corresponding scores in the historical cohort of untreated patients with metachromatic leukodystrophy, and an increase (≥2 SD) in residual ARSA activity compared with pretreatment values, measured in peripheral blood mononuclear cells (PBMCs) or bone marrow progenitors 24 months after treatment. GMFC-MLD was also evaluated as an additional assessment of motor function.

Key secondary endpoints included an improvement (≥2 SD) in the NCV index for electroneurographic recordings and in the total brain MRI score, compared with data obtained from age-matched untreated patients with metachromatic leukodystrophy, assessed 24 months after treatment; engraftment of transduced cells above 4% in PBMCs or bone marrow progenitors 12 months after transplant; and absence of immune responses against the transgene.
**Statistical analysis**

Enrolment was completed when 20 patients were recruited; no formal calculation of sample size was made. For the purposes of this ad-hoc analysis, we analysed safety and efficacy outcomes in all patients who had received treatment and had a follow-up of at least 18 months post-treatment on June 1, 2015. We used Spearman’s correlation to assess the relation between two numerical variables at a fixed time (appendix pp 4–7). We analysed longitudinal data using linear or non-linear mixed-effects models, accounting for the dependency among repeated measures of the same participant. The longitudinal course of each variable was investigated by selecting the appropriate model according to the trajectory shape. Models are described in the appendix, pp 4–7.

A type I error equal to 0.05 was considered for two-tailed hypothesis testing. We calculated 95% CIs. We did all statistical analyses using R 3.0.2. This trial is registered with ClinicalTrials.gov, number NCT01560182.

**Role of the funding source**

Members of the GlaxoSmithKline Rare Disease Unit clinical team contributed to data interpretation and manuscript review. The funders had no role in study design, data collection, or data analysis. AB, MS, and LL had access to the entire dataset and had final responsibility for the decision to submit for publication.

**Results**

Between April, 2010, and February, 2013, we had enrolled nine children with a diagnosis of early-onset metachromatic leukodystrophy, who we assessed in this analysis (table; appendix p 21). At screening, seven patients were classified as presymptomatic on the basis of the age at onset of symptoms in their older sibling(s); in some cases a range instead of a defined age has been reported. Some mild clinical disease manifestations could have been present. NCV index 1: calculated with internal (Ospedale San Raffaele) reference values from adult patients; NCV index 2: calculated using age-matched reference values from the scientific literature. Due to logistical issues, cumulative total. VCN and lentiviral vector-positive CFCs (the proportion of colonies in the CFC assay that harboured the lentiviral vector genome out of all colonies) were measured by in-vitro culture progeny (14 days of in-vitro culture); VCN was measured by liquid culture progeny of the transduced cells. When derived from the cryopreserved apheresis CD34+ cell dose (NCV per diploid genome). CFC=colony-forming cells. Medicinal product

<table>
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<tr>
<th>Variant</th>
<th>MLD01</th>
<th>MLD02</th>
<th>MLD03</th>
<th>MLD04</th>
<th>MLD05</th>
<th>MLD06</th>
<th>MLD07</th>
<th>MLD08</th>
<th>MLD09</th>
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<td>Late infantile</td>
<td>Late infantile</td>
<td>Early juvenile</td>
<td>Late infantile</td>
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<td>827C→T</td>
<td>736C→T</td>
<td>449C→G</td>
<td>1282C→T</td>
<td>465/1G→A</td>
<td>465/1G→A</td>
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<td>1223,1231del</td>
<td>931G→A</td>
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<td>ARSA mutation 2</td>
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<td>737G→A</td>
<td>449C→G</td>
<td>387T→G</td>
<td>980→1G→A</td>
<td>855→1G→A</td>
<td>465→1G→A</td>
<td>1150G→A</td>
<td>931G→A</td>
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<td>(24–27)</td>
<td>(15)</td>
<td>54</td>
<td>(19)</td>
<td>(19)</td>
<td>(20)</td>
<td>35</td>
<td>(24–36)</td>
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<tr>
<td>Age at gene therapy, months</td>
<td>15</td>
<td>13</td>
<td>7</td>
<td>59</td>
<td>18</td>
<td>16</td>
<td>16</td>
<td>39</td>
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**Baseline disease characteristics**

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<th>Symptoms</th>
<th>Yes/No†</th>
<th>No</th>
<th>Yes</th>
<th>No</th>
<th>Yes</th>
<th>No</th>
<th>Yes/No†</th>
<th>Yes</th>
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<tr>
<td>NCV index†</td>
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<td>-1.79</td>
<td>-6.04</td>
<td>-8.26</td>
<td>-1.51</td>
<td>-7.42</td>
<td>-7.09</td>
<td>-4.5</td>
<td>-10.82</td>
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<td>2</td>
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<td>-3.38</td>
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<td>-0.16</td>
<td>-6.11</td>
<td>-6.02</td>
<td>-4.73</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
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<td>GMFM score</td>
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<td>75.6%</td>
<td>27.3%</td>
<td>73.9%</td>
<td>80.1%</td>
<td>75.0%</td>
<td>66.1%</td>
<td>87.1%</td>
<td>77.9%</td>
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<td>Performance</td>
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<td>105</td>
<td>95</td>
<td>100</td>
<td>100</td>
<td>90</td>
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<tr>
<td>Verbal</td>
<td>NA§</td>
<td>83</td>
<td>112</td>
<td>76</td>
<td>109</td>
<td>109</td>
<td>94</td>
<td>103</td>
<td>79</td>
</tr>
</tbody>
</table>

**Busulfan¶**

| Dose, mg/kg | 10.5 | 14.1 | 10.4 | 13.4 | 12.3 | 16.2 | 14.7 | 14.9 | 14.0 |
| Exposure, mg x h/L | 95.9 | 77.7 | 74.5 | 70.5 | 69.5 | 70.7 | 72.4 | 69.8 | 73.2 |

**CD34+ cell dose (×10⁶ cells per kg)**

| CD34+ cell dose (×10⁶ cells per kg) | 11.1 | 7.0 | 7.2 | 9.9 | 4.2 | 6.2 | 18.2 | 7.1 | 16.3 |

**Medicinal product||**

| VCN | 2.5 | 2.5 | 4.4 | 1.9** | 17 | 3.1 | 4.0 | 17.1 | 25 |
| Lentiviral vector-positive CFCs, % | 97% | 90% | 93% | 93%†† | 68% | 91% | 91% | 94% | 95% |

**Duration of neutropenia, days‡‡**

| Severe | 28 | 35 | 25 | 19 | 27 | 24 | 22 | 20 |
| Absolute | 9 | 6 | 4 | 4 | 4 | 8 | 3 | 5 | 5 |

**Sibling survival, months**

| Sibling survival, months | 63 | 69 | 42 | NA§§ | >79 | >74 | 63 | NA§§ | >16 years |

MLD=metachromatic leukodystrophy. NCV=nerve conduction velocity. GMFM=Gross Motor Function Measure. NA=not applicable. VCN=vector copy number per diploid genome. CFC=colony-forming cells.*Age at expected onset was calculated (for presymptomatic patients) on the basis of the age at onset of symptoms in their older sibling(s); in some cases a range instead of a defined age has been reported. ±Some mild clinical disease manifestations could have been present. †NCV index 1: calculated with internal (Ospedale San Raffaele) reference values from adult patients; NCV index 2: calculated using age-matched reference values from the scientific literature. ‡Due to logistical issues. §Cumulative total. ||VCN and lentiviral vector-positive CFCs (the proportion of colonies in the CFC assay that harboured the lentiviral vector genome out of all colonies) were measured by in-vitro culture progeny (14 days of in-vitro culture); VCN was measured by liquid culture progeny of the transduced cells. **3.3 when derived from the cryopreserved apheresis CD34+ haemopoietic stem cells (as opposed to the freshly transduced bone marrow CD34+ cells). ††97% when derived from the cryopreserved apheresis CD34+ haemopoietic stem cells. ‡‡Severe neutropenia was defined as an absolute neutrophil count (ANC) of <500×10⁹ neutrophils per L; absolute neutropenia was defined as an ANC of 0 neutrophils per L. §§No older affected sibling.
of the absence of overt disease manifestations (although two might have had mild clinical disease manifestations); they were diagnosed after symptom onset and diagnosis in an older sibling. Six of them were classified as having late-infantile metachromatic leukodystrophy, whereas patient MLD09 was thought to be affected by an early-onset variant that could not be definitively classified as late-infantile or early juvenile (appendix p 8). Patients MLD04 and MLD08 had early-symptomatic early-juvenile metachromatic leukodystrophy. MLD04 and MLD08 had a widely divergent clinical condition at enrolment (table) and had disease progression in the early pretreatment (within 6 months from disease onset) and post-treatment phases. Patient MLD04 had severe demyelination and motor and cognitive dysfunction at enrolment and baseline, and had rapid disease progression between enrolment and treatment, and during the early post-transplant phase. This early deterioration is described in more detail in the appendix, pp 9–10, 13.

The median age at treatment of the six patients with late-infantile disease was 15·5 months (range 7–18), with a median predicted disease onset, defined as the age at symptom onset in the older affected sibling(s), of 19 months (15–27). Some patients underwent treatment very close to the age of predicted onset (table), which in some cases was probably overestimated as a result of parents not recognising or recalling first symptoms of the disease in their older affected children. Moreover, subtle clinical disease manifestations were conceivably present at the time of treatment in patient MLD01, who had not yet learned to walk independently by age 15 months, and in patient MLD07, who was preferentially walking on his toes. Patient MLD09 underwent treatment 5 months before the lower boundary of the predicted onset, whereas patients MLD04 and MLD08 received HSC-GT within 6 months of disease onset.

A median CD34+ cell dose of 7·2×10⁶ cells per kg (range 4·2–18·2) with a median VCN of 2·5 (1·7–4·4) and transduction efficiency of 92·8% (68–97) was administered (table). Median busulfan exposure was 72·4 mg·h/L (range 68·9–95·9). One patient (MLD04) had a low bone marrow yield and therefore both bone marrow and mobilised peripheral blood-derived CD34+ cells were used to enable infusion of an adequate medicinal product dose (table).

Median duration of post-treatment follow-up was 36 months (range 18–54). At the time of the analysis, all children were alive, with good haematological recovery after HSC-GT (neutrophil engraftment, calculated as the first of three consecutive days at an ANC of greater than 0·5×10⁹/L, which occurred a mean of 35·5 days [SD 4·3] after transplant). The most commonly reported adverse events were cytopenia (reported in all patients) and mucositis of different grades of severity (in five of nine patients, with severity grade 3 in four of five patients, according to Common Terminology Criteria for Adverse Events). Severe neutropenia lasted a median of 24 days (range 19–35), with absolute neutropenia lasting a median of 5 days (3–9; table). No major change from baseline was noted in lymphocyte counts during follow-up (data not shown), whereas the median duration of transfusion-dependent thrombocytopenia and anaemia was 36 days (range 28–45) and 38 days (28–46), respectively.

No serious adverse events related to the medicinal product were reported. No haemopoietic malignancies have been observed so far. Tests for the presence of replication-competent lentivirus, antibodies against ARSA, and HIV p24 antigen were negative in all patients.

The gene-corrected HSCs stably engrafted in the patients (figure 1A, 1B) and a progressive increase in VCN was observed in PBMCs over time (appendix p 14), reflecting the described biphasic marking of the myeloid and lymphoid compartments. Transduced cell engraftment (ie, the proportion of lentiviral vector-positive colony-forming cells) measured in clonogenic progenitors was a median of 60·4% (range 14·0–95·6) in all patients across follow-up, and the engraftment level was stable during follow-up (figure 1B). Multivariate linear mixed-effect model analysis indicated that engraftment determinants include the duration of absolute neutropenia and the VCN of the medicinal product (appendix p 5).

Data for integration site analysis studies of whole bone marrow and peripheral blood of the first seven patients were obtained over a median of 30 months (range 18–48) post-treatment, and revealed a highly polyclonal reconstitution of haemopoiesis in all analysed patients, with patient MLD05 showing a substantially lower number of clones, consistent with the lower VCNs in this patient, without evidence of expanding or dominant clones (appendix pp 11–12, 15). Data for patients MLD08 and MLD09 were not available at the time of analysis.

A progressive reconstitution of ARSA activity at or above normal values in circulating haemopoietic cells was documented (figure 1C, 1D). ARSA activity measured in haemopoietic cells was positively influenced by the transduced cell engraftment (figure 1D). Of note, ARSA activity was also restored in the CSF as early as 6 months after HSC-GT, reaching normal or above normal values at later timepoints in some of the patients (figure 1E). No significant correlation between ARSA activity in the CSF and transduced cell engraftment was observed (figure 1F; appendix).

Although skin biopsy samples at baseline uniformly showed abundant sulphatides within the cytoplasm of Schwann cells, at 2 years after transplant, biopsy samples showed a marked reduction of sulphatides (figure 2; appendix p 2) in the first seven patients (for whom we had data) except MLD07 (who also had worsening demyelination and NCV index). These data provide indirect evidence of functional enzyme delivery
Figure 1: Molecular and biochemical characterisation

VCN per diploid genome in PBMCs (A) and engraftment of the transduced lentiviral vector-positive clonogenic progenitors in the bone marrow (and for some of the timepoints in peripheral blood when bone marrow was not available) (B) of each patient who received HSC-GT, over time. (C) ARSA activity measured by p-nitrocatechol sulphate assay on CD15+ granulocytes from the patients’ peripheral blood, representative of myeloid peripheral blood cells. The activity range measured in a cohort of six healthy volunteers, upon written informed consent collection (approved institutional protocol), is shown in the green box plot (box shows the 5th to the 95th percentile range; central line is the median value; error bars show minimum to maximum). (D) Positive correlation between the transduced cell engraftment (proportion of lentiviral vector-positive colonies) and ARSA enzyme activity measured in PBMCs at the last follow-up (two-tailed p value for Spearman correlation is shown). (E) Measurement of specific ARSA enzyme activity by 4-methylumbellipheryl-sulphate on the protein purified from the CSF of patients sampled at 6, 12, 24, and 36 months after treatment (data at 6 months’ follow-up were recorded for only some of the patients because of a protocol amendment). The activity range measured in a cohort of healthy donors (three pools of three donors [commercial source]) is shown in green (horizontal line shows mean; error bars show the 5th to the 95th percentile range). (F) No correlation between transduced cell engraftment and ARSA activity was measured in the CSF (data are reported at the following timepoints after treatment: triangle, 6 months; circle, 12 months; inverted triangle, 24 months; diamond, 36 months; see appendix for details on the statistical analysis). For (A–C) and (E), a circle indicates late-infantile disease, a diamond indicates early-juvenile disease, and a triangle is used for MLD09 because of the difficulty in classifying this patient. ARSA=arylsulfatase A. CSF=cerebrospinal fluid. HSC-GT=haemopoietic stem-cell gene therapy. MLD=metachromatic leukodystrophy. PBMC=peripheral blood mononuclear cell. VCN=vector copy number.
to PNS resident cells. The baseline skin biopsy samples of the patients, including the presymptomatic patients with late-infantile disease, showed a severe demyelinating neuropathy, with chronic features in some patients (figure 2). Remarkably, 2 years after HSC-GT, most of the biopsy samples (five of the seven examined) showed rare if not absent demyelinated fibres, and fibres surrounded by normal myelin with apparently preserved g-ratio (axon diameter/fibre diameter; figure 2; appendix p 2). Better myelination was evident in patients with a higher transduced cell engraftment—ie, MLD01 and MLD02. Of note, in some of the patients, fibres characterised by redundant processes of the basal membrane and increased g-ratio were observed, suggesting occurrence of remyelination.

Electroneurographic studies documented a mild to severe peripheral neuropathy in most of the patients at baseline, denoted by the NCV index score (table), with patients with late-infantile disease displaying a more severe impairment of motor and sensory conduction than those with early-juvenile disease, as described previously. 13 During follow-up, the NCV index increased in three patients (MLD01, MLD04, and MLD09), over the baseline, denoted by the NCV index score (table).

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MLD06 sib aged 40 months

MLD06 aged 40 months

Coronal FLAIR

Sagittal T2

FA/ADC

FA/ADC

<table>
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remained relatively stable in four (MLD02, MLD03, MLD06, MLD08), or substantially decreased in two (MLD05, MLD07), particularly in the first 6–12 months after treatment (appendix p 17). Nonetheless, in most of the patients with late-infantile metachromatic leukodystrophy, the NCV index remained higher than that measured in their older affected sibling (sibling pairs for whom we had data) and in untreated patients from our historical cohort. Patients MLD04 and MLD08 had a baseline NCV index that was slightly worse than the historical cohort of patients with early-juvenile disease; these values remained relatively stable at follow-up (appendix p 17). The non-linear mixed-effect model analysis showed that the transduced cell engraftment positively affects the plateau reached by the NCV index (appendix pp 6–7).

In the seven presymptomatic patients who received treatment, and in patient MLD08, MRI results at baseline were normal (table). The massive demyelination and severe atrophy observed in untreated patients with late-infantile and early-juvenile metachromatic leukodystrophy and in the patients’ older siblings (characterised by presence of extensive, diffuse, symmetric, hyperintense signal alterations on T2 and fluid-attenuated inversion recovery (FLAIR) images, with a typical tigroid pattern, within supratentorial white matter, at the level of the periventricular areas, centrum semiovale, corpus callosum, and internal capsules, and partial involvement of U fibres and of infratentorial white matter, at the level of corticospinal tracts and deep cerebellar white matter, signs of advanced disease) were not observed in patients who underwent HSC-GT (figure 3A; appendix p 17), whose MR scores remained substantially lower than those of the untreated cohort throughout the follow-up (figure 4; appendix pp 3–4). The only exception was MLD04, who had showed severe MR signal abnormalities since baseline (appendix pp 9–10). Analysis of spectra confirmed these findings (figure 3A). Remarkably, the intensity and extension of white matter signal alteration observed in the MRI of some of the patients who underwent HSC-GT during follow-up partially decreased in the long term, suggesting the occurrence of remyelination also in the CNS (figure 3B). Fractional anisotropy and apparent diffusion coefficient data supported this evidence (figure 3C).

The GMFM scores showed that gross motor performance was similar to that in normally developing children for most of the patients who underwent HSC-GT up to the last follow-up (figure 5A; appendix pp 6–7). Apart from MLD04 who, as discussed already, had a precocious motor and cognitive decline consistent with untreated early-juvenile metachromatic leukodystrophy (appendix pp 9–10), all the other patients who underwent HSC-GT had a progressive increase in GMFM score during the study, consistent with the acquisition of new motor skills. Only patients MLD01 and MLD07, who have the lowest GMFM scores, never reached fully independent walking, requiring external aid. GMFCS-MLD scores in six patients have remained stable at 0 after treatment, corresponding to a normal gross motor function; patients MLD01 and MLD07 had a stable score of 2 and MLD08 had a stable score of 1 up to the latest follow-up (figure 5B). By contrast, at the same chronological ages, untreated children with late-infantile and early-juvenile metachromatic leukodystrophy, and the patients’ siblings, displayed severe motor impairment, and were bedridden and incapable of any voluntary movement (figure 5A, 5B). Change in GMFM score from baseline to last follow-up positively correlated with the time interval between HSC-GT administration and expected or actual disease onset (Spearman r=0.8034; p=0.0138; appendix p 18).

Neurocognitive development as measured by IQ score was within the normal range (plus or minus 1 SD) for all patients except MLD04 during follow-up (figure 5C; appendix p 19), at odds with the natural course of the disease in untreated patients with early-onset metachromatic leukodystrophy and of their siblings (data not shown: IQ values of untreated patients all fell below the minimum value of 40 since first available testing).

Importantly, at the last available follow-up, all children who received HSC-GT had passed the age of their expected symptom onset, and in some cases have also surpassed the age at which their elder siblings died of the disease (table).

**Discussion**

In this trial, in which a lentiviral vector-mediated HSC-GT was used to treat metachromatic leukodystrophy,
all nine patients who underwent treatment survived and were highly engrafted with transduced cells. In eight patients, marked benefit was demonstrated with prevention of disease onset or halted disease progression. Safety of the transplantation of autologous HSCs transduced at high frequency by lentiviral vectors into bone marrow-ablated patients is shown, with a median follow-up of 3 years after treatment (maximum 4·5 years). Clinical data are supported by lentiviral vector integration site analysis, which demonstrates extensive polyclonal repopulation of haemopoiesis by gene-corrected cells without signs of genotoxicity. Factors positively affecting engraftment of transduced HSCs included the exposure to the ablative regimen and efficiency of HSC gene transfer, supporting the need for bone marrow ablation to favour transduced cell engraftment and for the use of efficient vectors such as lentiviral vectors for HSC gene transfer. The choice of lentiviral vectors was also crucial for obtaining robust enzyme production in HSCs and their progeny, and for efficient enzyme delivery to the nervous system. Evidence of HSC-mediated delivery of a functional protein to the CNS and PNS of patients was provided, which likely contributed to protection from massive CNS demyelination, amelioration of PNS morphological and functional features, at least in some patients, and remyelination attempts at both sites. These findings suggest that sulphatide removal from nervous tissues could enable remyelination by local oligodendrocyte and Schwann-cell precursors, at least in young patients and after sufficient time has passed after treatment. Functional consequences of these morphological changes, particularly in the PNS, might need time to develop, particularly in patients with low transduced cell engraftment such as in patient MLD05. Remarkably, the transduced cell engraftment positively affected outcomes in the PNS, reflecting and confirming in patients the advantage of lentiviral vector-driven above-normal enzyme expression in HSCs and their progeny.

**Figure 5:** Effect of the treatment on GMFM score and IQ over time

GMFM (A) and GMFC-MLD (B) scores of patients who underwent HSC-GT (the first datapoint is a baseline value, before treatment was initiated, or [in (B) only] the datapoint measured once the patients had reached age 18 months), respective older affected siblings (open circles and dotted lines), and a historical cohort of untreated patients with late-infantile disease (grey circles in [A], grey boxes in [B] showing minimum, 25%, median, 75%, and maximum values, respectively), and early-juvenile disease (grey diamonds in [A], white boxes in [B] showing minimum, 25%, median, 75%, and maximum values, respectively). The black line in (A) represents the estimated curve obtained from the scores from 34 healthy participants aged between 0 and 6 years. (C) Performance IQ score, assessed by Bayley Scales of Infant Development, and Wechsler Preschool and Primary Scale of Intelligence (depending on age of patient), over time in all patients who underwent HSC-GT; the green shaded areas represent the normal range performance IQ (up to 2 SD); the first datapoint is a baseline value, measured before treatment was initiated. A circle indicates late-infantile disease, a diamond indicates early-juvenile disease, and a triangle is used for MLD09 because of the difficulty in classifying this patient. GMFM=Gross Motor Function Measure. GMFC-MLD= Gross Motor Function Score–metachromatic leukodystrophy. MLD=metachromatic leukodystrophy.
in correcting metachromatic leukodystrophy (and lysosomal storage disease)-associated manifestation over the transplantation of healthy donor HSCs previously described in animal models.29,30–32,37,38 Importantly, these findings resulted in clinical benefit. Indeed, patients given treatment when presymptomatic showed normal-for-age motor and cognitive function during follow-up, and even those who received treatment in the likely presence of early disease manifestations displayed a favourable phenotype with greatly preserved abilities as compared with siblings and age-matched historical controls. Of note, the outcomes of the patients who underwent treatment, and in particular patients MLD01 and MLD07, suggest that the time interval between gene therapy and expected disease onset, among other relevant factors, could affect the extent of therapeutic benefit and GMFM score evolution over time (appendix p 19). Moreover, results in patient MLD04 suggest that patients in the rapidly progressive phase of disease might not benefit from concomitantly administered HSC-GT, other than possibly having the therapy stabilise them at some advanced stage and prevent certain long-term disease complications such as seizures and dysphagia. The findings from patient MLD04 (and another recently treated patient with late-infantile disease; data not shown) resulted in protocol amendments to exclude patients with rapidly progressing clinical symptomatology observed between enrolment and treatment initiation.

Sustained bone marrow engraftment of the corrected cells appeared to positively affect therapeutic efficacy in the PNS, a finding that is not proven in the CNS. These data could be interpreted in light of our research findings showing that long-term microglia reconstitution in the brain after HSCT is driven by local proliferation and differentiation of a proportion of the transplanted HSCs that specifically migrate to the brain, and is maintained independently from the chimerism in the bone marrow. The same bone marrow-independent mechanism might not apply to the turnover of PNS macrophages.

Because of the small number of patients presented in this report and the short follow-up in some patients, confirmatory data from the longer-term follow-up of the entire cohort of 20 patients enrolled and treated within the trial will be needed to confirm the results of this ad-hoc analysis. The final results will also include a larger dataset from patients with early-juvénile disease who underwent treatment both in presymptomatic and symptomatic stages. Understanding of the benefit–risk of HSC-GT in the early-juvénile disease variant is limited by the heterogeneous clinical features of early-juvénile metachromatic leukodystrophy and its less rapidly progressive nature as compared with the late-infantile variant of the disease. As a result, longer follow-up and a greater number of observed patients are needed for proper outcome interpretation.

In conclusion, despite the need for longer-term follow-up to establish the ultimate prognosis of the patients who underwent treatment, the data reported here indicate that HSC-GT results in therapeutic benefit in patients with early-onset metachromatic leukodystrophy treated either before or very early after symptom onset.

Contributors
MS contributed to the study design, data collection and interpretation, and manuscript writing. LL contributed to the generation of the figures, data collection and interpretation, and manuscript writing. MS and LL contributed equally. FF, UdC, SM, AQ, and EM participated in the data collection and interpretation. SA, DR, CB, SC, IDL, FM, MGNS, and AC contributed to data collection and analysis. RF, PS, FB, GA, AAs, and MPC were involved in data collection. PMVR and CDS contributed to data analysis and manuscript writing. MG was involved in study design, and FC, MGR, AAi, and LN contributed to study design and data interpretation. AB contributed to study design, data collection and interpretation, and manuscript writing.

Declaration of interests
The San Raffaele Telethon Institute for Gene Therapy (SR-TIGET) is a joint venture between Telethon and Ospedale San Raffaele (OSR). AB is the principal investigator of the SR-TIGET–Metachromatic Leukodystrophy (MLD) clinical trial of gene therapy for MLD. The MLD gene therapy was licensed to GlaxoSmithKline (GSK) in 2014 and GSK became the financial sponsor of the trial. Telethon and OSR are entitled to receive milestone payments and royalties upon commercialisation of such therapies. All authors declare no other competing interests.

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