Atidarsagene autotemcel for metachromatic leukodystrophy

Abstract

Metachromatic leukodystrophy (MLD) is a rare autosomal recessive disorder of sphingolipid metabolism, due to a deficiency of the enzyme Arylsulfatase A (ARSA). The main clinical signs of the disease are secondary to central and peripheral nervous system demyelination. MLD patients are subdivided into early- and late- onset subtypes based upon the onset of neurological disease. The early onset subtype is associated with a more rapid progression of the disease that leads to death within the first decade of life.

Until recently, no effective treatment was available for MLD. The blood brain barrier (BBB) prevents systemically administered enzyme replacement therapy from reaching target cells in MLD. The evidence for the efficacy of haematopoietic stem cell transplantation is limited to the late onset MLD subtype.

Here, we review the preclinical and clinical studies that facilitated the approval of the *ex vivo* gene therapy for early onset MLD by the European Medicines Agency (EMA) in December 2020.

This approach was studied in an animal model first and then in a clinical trial, eventually proving its efficacy in preventing disease manifestations in pre-symptomatic patients and stabilising its progression in pauci-symptomatic subjects. This new therapeutic consists of patients' CD34+ hematopoietic stem/progenitor cells (HSPCs) transduced with a lentiviral vector encoding functional *ARSA* cDNA. The gene-corrected cells get reinfused into the patients after a cycle of chemotherapy conditioning.

Background

Metachromatic leukodystrophy (MLD) belongs to the broader group of lysosomal storage diseases (LSDs), particularly sphingolipidoses. It is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme arylsulfatase A (ARSA) resulting in accumulation of undegraded galactosyl-3-sulfate ceramide (sulfatide) in microglia, oligodendrocytes and Schwann cells, which triggers apoptosis of glia and neurons resulting in widespread demyelination in the Central Nervous System (CNS) and Peripheral Nervous System (PNS) [1,2].

Clinical manifestations of MLD are characterised by the signs and symptoms of central and peripheral demyelination even though its course and duration are highly variable, depending on the MLD subtypes, which are defined by the age of onset of first symptoms: late infantile (LI), with an onset

before 30 months; juvenile, which itself is subdivided into early (EJ) and late (LJ), when symptoms present between 2.5 and 16 years; and an adult form (AD) in which symptoms present from 16 years of age [3]. Different forms present different clinical manifestations and disease progression. The LI and EJ variants are the most frequent and fastest progressing ones with motor and cognitive impairment leading to death, usually within the second decade of life if left untreated [4–6].

Main aim of an effective treatment should be to restore relevant cell ARSA activity and its breakdown of sulfatides. Enzyme replacement therapy (ERT), haematopoietic stem cell transplantation (HSCT) and gene therapy (GT) have been suggested as potential therapeutic approaches.

The rationale for traditional ERT is to administer the missing enzyme to patients via intravenous infusion. This approach has been effective in the LSDs without CNS involvement. Unfortunately, due to the effective Blood Brain Barrier (BBB), this treatment is not an option for MLD as neurological features are the major manifestations of the disease [3]. The clinical trials testing the efficacy of intrathecal administration of ERT have shown this to have a good safety profile although further evidence is needed to ascertain its efficacy [7,8].

Since bone marrow monocytic cells are able to cross the BBB it was felt that HSC transplantation could be a successful treatment approach for this condition [3]. In fact, once monocytes get into the CNS they differentiate into microglial cells and are able to produce the enzyme. ARSA is then secreted into the extracellular space, endocytosed by oligodendrocytes and neurons and then delivered into the lysosomal compartment via the mannose-6-phosphate receptor system. This process, called "cross correction", allows restoration of the enzyme activity in the relevant cells [9]. Unfortunately, the process of replacing resident microglia happens slowly. By the time the functional enzyme is generated in sufficient amounts to arrest neuropathological events, the signs and symptoms of MLD have progressed. Thus, the consensus of experts who reviewed the patients cohort data concluded that the HSCT approach is not suitable for treatment of rapidly progressing early onset forms and those patients with significant symptoms. Furthermore, HSCT can only be offered to patients with available donors and is not a risk-free procedure with substantial danger even in the post-transplant phase, especially in those patients for whom a closely matched donor cannot be found [3].

The lack of effective treatment for most of the patients with MLD has led to the development of GT as a new treatment approach. The rationale for this treatment is to genetically modify patient's autologous cells so that they express the missing enzyme. *Ex vivo* GT implies the collection of haematopoietic stem cells from the patient which then are genetically modified *in vitro* and are readministered to the same patient. The cells get transduced with a viral vector containing the *ARSA* transgene which gets inserted into the genomic DNA of the host cell. The therapeutic gene then gets

expressed at normal or even supra-normal levels. Subsequently, the gene-corrected cells are infused back into the patient, they find their way back to the bone marrow, engraft, proliferate and fulfil their therapeutic potential [10].

HSC GT for MLD, commercial name Libmeldy, was approved by the European Medicines Agency in December 2020 as treatment for pre-symptomatic LI and EJ and early symptomatic EJ MLD patients. In February 2022 this drug was also approved for reimbursement by the National Institute for Health and Care Excellence (NICE).

Preclinical pharmacology

The medicinal product consists of autologous CD34+ hematopoietic stem/progenitor cells (HSPCs) in which a functional *ARSA* cDNA is introduced by means of 3rd generation VSV-G pseudotyped lentiviral (LV) vectors (https://clinicaltrials.gov/ct2/show/NCT01560182).

Studies in MLD mouse model treated with HSC GT demonstrated an efficient vector transduction of HSCs with a stable transgene expression in the bone marrow and in the visceral organs, showing an extravascular localisation of differentiated HSC derived cells [2].

Reconstitution of enzyme activity in peripheral blood cells, in CNS and PNS originating from migration of transgene-expressing cells and successful crossing of BBB was confirmed in subsequent studies [11]. The scientists showed that donor HSCs in the CNS differentiate into resting microglia, which then becomes a stable source of functional enzyme [11].

After having shown that microglia derived from HSC is stable and the only source of enzyme in the CNS, Biffi et al. demonstrated the occurrence of *in vivo* cross-correction, showing presence of the enzyme in neurons, astrocytes and oligodendrocytes [11]. They also demonstrated that ARSA expression was present in different cell types in the PNS [11].

As a consequence, it was possible to show that the *ex vivo* GT treated mice received a long lasting and almost total protection from motor conduction impairment at nerve conduction studies. The preclinical work in mice demonstrated that overexpression of *ARSA* transgene provided a superior outcome of the lentiviral *ex vivo* gene therapy approach compared to the standard HSCT [2,11]. When compared to wild type HSCs transplanted animals the gene therapy approach showed a statistically significant advantage as shown by a better-preserved peripheral conduction, prevention of motor, learning and coordination impairment [2].

The result was confirmed in subsequent studies when comparing groups of transplanted mice. The animals with higher ARSA expression consistently achieved a better overall outcome, including correction of the already present tissue damage [11].

Histopathological analysis of the CNS and PNS from transplanted mice at 12 months of age demonstrated a marked reduction of sulfatide-containing metachromatic granules in the white matter of the brain and cerebellum of *ARSA*-LV-transplanted mice. These hallmarks of MLD were still present in disease target area in HSC transplanted mice [2]. Similar findings were found in the PNS of mice transplanted with ARSA-transduced cells, with a marked reduction in sulfatide deposits in the sciatic nerve and an almost complete protection from demyelination [2].

The advantage of HSC-GT over HSCT seems to be explained by enzyme overexpression in the former approach [2]. Having confirmed the critical role of HSC-GT in correcting the disease manifestation in subsequent publications, it was also found that supranormal enzyme levels did not impair clonogenic capacity and multilineage differentiation of HSPCs nor did they affect the activities of other sulfatases, both *in vivo* and *in vitro*. Finally, this did not impair an appropriate immunological reconstitution and immune cells differentiation [2,12].

From preclinical studies it was concluded that transplanting genetically engineered HSPCs represented an effective strategy for establishing a source of a bioavailable gene product within the CNS [11].

Clinical studies

A Phase I/II Clinical Trial of Hematopoietic Stem Cell Gene Therapy for the Treatment of Metachromatic Leukodystrophy (NCT01560182)

Biffi et al. [13] performed a phase I/II clinical trial of *ex vivo* gene therapy in MLD patients. The initial publication included the preliminary results of the first three pre-symptomatic LI MLD treated patients. This was followed by a more comprehensive publication by Sessa et al. that included a total of 9 patients affected by early onset MLD [14] and a most recent paper that included all the 29 treated patients, reporting a maximum follow up of 7.5 years [15].

A total of 29 paediatric patients with pre-symptomatic or early-symptomatic early-onset MLD with biochemical and molecular confirmation of diagnoses were treated with gene therapy consisting of an autologous HSPC population transduced *ex vivo* with an LV encoding *ARSA* cDNA (Figure 1). Sixteen were pre-symptomatic LI MLD and 13 were EJ MLD either pre-symptomatic or early

symptomatic. The control population was an untreated natural history cohort of 31 patients with early-onset MLD, matched by age and disease subtype.

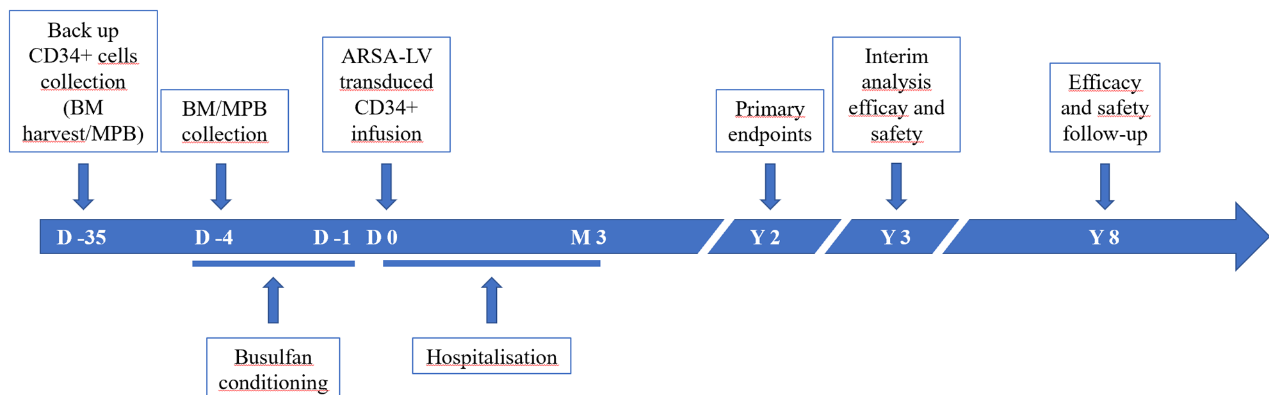

Figure 1. Study protocol schedule of A Phase I/II Clinical Trial of Hematopoietic Stem Cell Gene Therapy for the Treatment of Metachromatic Leukodystrophy

As a safety measure, firstly the patients underwent a collection of a backup HSCs. 20-30 days after the procedure, bone marrow harvest was performed and CD34+ cells were isolated and transduced with transgene-containing lentiviral vector.

All patients but two received only bone marrow-derived CD34+ cells. Two patients were infused with mobilised peripheral blood (MPB)-derived CD34+ cells in addition to the bone marrow derived CD34+ cells and one patient received only MPB-derived CD34+ cells [15,16].

A myeloablative, dose-adjusted busulfan regimen was administered intravenously (IV) from day –4 up to day –1 before HSC-GT to a total of 14 doses, one every 6 hours [14]. The dose was adjusted after the first 9 patients were treated in order to guarantee a more uniform transduced cell engraftment[15].

Twenty four hours after the last busulfan dose, HSCs transduced with a LV encoding human *ARSA* cDNA were infused into patients. Transduced cell engraftment (assessed as the proportion of LV-positive colony-forming cells out of all colonies), vector copy number per diploid genome (VCN) and ARSA activity were analysed in bone marrow, peripheral blood, and cerebrospinal fluid (CSF), every 6 months, LV integration sites was analysed in all the above tissues apart from the CSF. Each patient also underwent at least two skin biopsies (at baseline and at 2 years after HSC-GT) for morphological analysis of dermal myelinated nerve fibres [14,15].

The primary efficacy endpoints were (i) a significant improvement of gross motor function as measured using Gross Motor Function Measurement (GMFM) in treated patients compared to untreated MLD affected children and (ii) increased ARSA activity in peripheral blood cells.

The following assessments were used for monitoring clinical manifestations of the disease:

1) Standard neurological assessment;

2) GMFM and Gross Motor Function Classification for MLD (GMFC-MLD), measured every 3 months for the first year, then every 6 months;

3) Bayley Scale for Infant and Toddler Development, third edition (BSID-III) or Wechsler Preschool and Primary Scale of Intelligence and Wechsler Intelligence Scale for Children (WISC) according to patients' age, measured every 6 months;

4) Electroneurographic recordings, measured at 3, 6, and 12 months, then every 6 months thereafter.

As control population, corresponding measurements were obtained from the historical cohort of patients with untreated LI and EJ forms of the disease. Nerve conduction velocity (NCV) index, used to assess the grade of peripheral neuropathy in enrolled patients at baseline and at follow up visits, was calculated based on data derived from scientific literature for healthy paediatric population and internal standard values obtained in previous studies on a population of adult volunteers [14] .

Brain MRI assessments, including spectroscopy and diffusion tensor imaging, to assess central demyelination and CNS involvement were performed as regular follow up and scored by the same trained personnel throughout the whole study.

Preliminary results on the first three treated patients showed that one year after HSC-GT, ARSA activity in the cerebrospinal fluid from all three patients was comparable to healthy donors. Moreover, no disease progression was detected at the follow up assessments, ranging from 18 to 24 months. The first treated patient continued in their development whereas the other two remained asymptomatic at 18 months follow up. The assessments of peripheral neuropathy showed improvement in the first treated patient and remained stable in the other two. MRI brain scans were normal with a normal progression of myelination. No evidence for activation of a nearby oncogene due to an insertion event was found [13] .

The second and third published papers presented further evidence based on data respectively from 9 and 29 children with a confirmed diagnosis of early-onset metachromatic leukodystrophy who were treated with HSC-GT.

Good haematological recovery after HSC-GT was reported in all patients.

Stable engraftment of the gene-corrected HSCs was reported with stable values of VCN in Peripheral Blood Mononuclear Cells (PBMCs) observed over time[15]. Contributing factors for engraftment were proven to be the absolute neutropoenia duration and the VCN of the medicinal product [14].

At one year from treatment, in LI population, nearly 70% of bone marrow clonogenic progenitors was showing successful LV transduction, in EJ this percentage was above 40%. [15].

ARSA activity was progressively reconstituted at or above normal values in circulating haemopoietic cells as early as at 3 months post treatment, meaning that second primary endpoint was already met at an earlier stage than that foreseen by study protocol at 2 years after treatment. ARSA activity, absent at baseline, was detectable in the CSF at 3 months after treatment, and reached normal levels by 6–12 months. CSF ARSA activity remained within normal range throughout available follow-up (year 3 for EJ patients; year 5 for LI patients) [15]. Functional enzyme was also delivered to the PNS [14]. NCV study showed a stabilisation or a slowdown of disease progression in most of the patients as compared to baseline values or to paired untreated siblings. The positive impact on NCV was much more significant and clearer in treated patients with LI MLD than in EJ patients, who on average had a more impaired pattern at baseline [14,15].

The treated patients showed gross motor skills acquisition within normal limits for age or stabilised their motor performance (maintaining the ability to walk) showing a significant difference when compared to untreated MLD population of the same age and disease subtype. This means that the study met the first primary endpoint. Severe motor impairment-free survival was maintained in most treated patients regardless of disease subtype and symptomatic status at the time of treatment [14,15]. Nevertheless, the treatment benefits were particularly apparent in patients treated before symptoms onset [15].

Most patients displayed normal cognitive development and prevention or delay of central and peripheral demyelination and brain atrophy throughout the follow-up [15].

Overall, brain MRI findings in the treated population did not show signs of massive demyelination and severe atrophy observed in untreated patients with LI and EJ MLD. This pattern was particularly obvious in patients who were treated pre-symptomatically [15].

At least one grade 3 or higher adverse event (AE) was identified in each patient. Most of these AEs being related to conditioning or to background disease[15].

In terms of survival, 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 [14].

Biodistribution, metabolic correction and immune response to treatment

Preclinical studies in MLD mice model demonstrated an efficient transduction process of the HSCs, which were then infused intravenously into irradiated mice. Transgene expression remained stable for up to 12 months in their differentiated progeny. Looking at the vector integration a diffuse hybridization pattern was seen in most primary-transplanted mice. The progeny of transduced donor-derived HSCs were present in all blood lineages 3 months after transplant. They reached CNS by 6 months after transplant, mostly becoming resting microglia, and they were already present in the PNS 3 months after the transplant. This data supported the extensive migration of transduced HSC progeny to the PNS at a faster rate than that observed in the CNS. Vector expression was also demonstrated in the visceral organs. [2].

In preclinical studies, ARSA activity reconstitution in the transduced HSC progeny was demonstrated as breakdown of input sulfatide and the production of its direct metabolite galactosylceramide 7 months after transplant with an overall enzyme activity measured as 4-8 -fold higher than in wild type cells [2].

A total of 29 subjects with LI and EJ MLD were treated in a context of a non-randomized phase I/II clinical trial and expanded access framework[13–15].

From all patients CD34+ cells were collected either as bone marrow harvest or after mobilisation of CD34+ cells in peripheral blood. CD34+ cells were then isolated and transduced with transgene-containing lentiviral vector [15,16]. The cultured progeny of the cells presented a VCN range from 2.5 to 4.4, a transduction efficiency of 90 to 97% and an ARSA activity at ≥10-fold of the level measured in healthy controls [15].

After myeloablative conditioning with intravenous busulfan, the transduced CD34+ cells were then released and infused IV (Sessa et al., 2016).

All patients had high and stable vector copy number values in PBMCs and bone marrow-derived CD34+ cells, and in myeloid-lineage and lymphoid-lineage subpopulations [13–15].

LV and ARSA expression, the first identified by means of in situ hybridization for LV mRNA, and the second by means of immunofluorescence, identified in a high proportion of patients' blood cells. This was consistent with the high VCN detected by means of quantitative PCR in the same cells. ARSA protein was isolated from patients' hematopoietic cells as early as 1 month after treatment at above-normal expression levels in all tested samples. The enzyme isolated from patient cells was

found to hydrolyse the natural substrate sulfatide in vitro, confirming full functionality (0.003mU of ARSA isolated from normal donor and treated patients' hematopoietic cells hydrolysed 3.6 T 1.2 nmol of substrate) [13].

The results from the total 29 treated subjects, summarised in the last paper published by the group in San Raffaele, showed that from 3 months after treatment ARSA activity measured in PBMCs had been restored within or above normal range with a significant increase from baseline at 2 years after GT that remained stable throughout the follow up (increased from baseline by a mean 18.7-fold (95% CI 8.3–42.2; p<0·0001) in patients with LI disease and 5.7-fold (2.6–12.4; p<0·0001) in patients with EJ disease).

Myeloablative or submyeloablative conditioning regimen seemed not to have a significant impact on transduced cells engraftment nor ARSA activity in bloods cells and CSF with no major differences in these parameters in the two subgroups [15].

Engraftment of gene-corrected cells did not differ in those three patients who received a drug product derived from MPB alone or in combination with bone marrow [14,15].

Low titres of anti-ARSA antibodies were reported in four (14%) treated patients in the first year after GT. The antibodies disappeared either spontaneously or after Rituximab, a B-cell depletion therapy. Two patients with anti-ARSA antibodies were also positive for anti-platelet antibodies and in three subjects (10% of treated population) they were associated with veno-occlusive disease or thrombotic microangiopathy [15].

Safety

Preclinical studies showed a reduced risk of mutagenesis and leukemaemogenesis of the LV vector compared to the previous generation (gamma-retroviral). This was most likely due to (i) a self-inactivating structure of the vector and (ii) a less strong promoter used [12].

Although the supranormal enzyme expression was achieved, the preclinical safety studies showed no evidence of organ or cell toxicity caused by increased enzyme activity [11]. Furthermore, it was demonstrated that the supranormal ARSA expression does not impair activation of other sulfatases both *in vitro* and *in vivo* or immune cells response and differentiation [12].

The clinical studies also demonstrated the reassuring safety profile, based on a maximum follow-up of 7.5 years [15]. At least one grade 3 or higher adverse event was reported in each patient. The most

frequently reported grade 3 or higher adverse events were febrile neutropenia, gait disturbance, and stomatitis. Most adverse events were associated with busulfan conditioning or MLD disease progression. Three patients experienced veno-occlusive disease and two thrombotic microangiopathy associated with conditioning [15]. There was no evidence of abnormal clonal proliferation, HIV infections due to replication-competent viral vector or adverse events indicative of oncogenic transformation [14,15].

Engraftment was achieved in all patients with a median neutropoenia duration of 28 days (range 13-39). Only one patient required unmanipulated autologous back-up bone marrow infusion to support haematological recovery [15].

Three deaths occurred during follow-up. Two were due to rapid disease progression in patients with early- symptomatic EJ MLD (at 8 and 15 months after treatment) and were deemed unrelated to the medicinal product. One subject, treated as pre-symptomatic EJ MLD, died following an ischemic event in a context of a systemic infection 13.6 months after gene therapy. At one year follow up patient presented with normal neurological and neurocognitive assessments and neuroimaging. After reviewing clinical summary provided by the clinical team, the study team deemed the event unrelated to the HSCT-GT. In addition, there were no signs of clonal expansion or dominance at integration site analyses [15].

Indications

OTL-200 Gene Therapy is CD34+ cells transduced *ex vivo* with lentiviral vector encoding *ARSA* cDNA approved for treatment of children with a confirmed diagnosis of early onset Metachromatic Leukodystrophy. It has been demonstrated to be effective in preventing symptoms manifestation in children affected by LI form when treated in a pre-symptomatic phase. Early onset EJ patients can be treated when patients do not present cognitive decline (IQ ≥85) and before loss of independent walking, in absence of overt clinical deterioration between screening and infusion.

Summary

The *ex vivo* gene therapy approach, based on transfection of CD34+ hematopoietic stem cells with a LV vector encoding *ARSA* gene, has been proven to be effective in transducing target cells and their differentiated progeny. Restored enzyme activity was reported at normal or above normal levels as early as 6 months after treatment in peripheral blood cells and CSF. Clinical effect was to prevent symptoms manifestation in pre-symptomatic patients and delay the disease progression in early symptomatic EJ patients. Clinical endpoints for this were gross motor development evaluation, IQ,

nerve conduction studies and brain MRI. The effectiveness and clinical advantage of this approach compared to standard HSC transplantation is believed to reside in the enzyme overexpression.

In terms of safety profile, the medicinal product was shown to be safe, with all the adverse events reported related to conditioning regimen or disease progression. Furthermore, no signs of leukemogenesis or viral replication have been reported at maximum 7.5 years follow up.

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