Systemische inflammatie bij ALSP-patiënten en het effect van een allogene hematopoëtische stamceltransplantatie op de inflammatie

English translation: Systemic inflammation in ALSP patients and the effect of an allogenic hematopoietic stem cell transplantation on the inflammation

Version 5

17th of February 2022

PROTOCOL TITLE *Systemische inflammatie bij ALSP-patiënten en het effect van een allogene hematopoëtische stamceltransplantatie op de inflammatie*

English translation: '*Systemic inflammation in ALSP patients and the effect of an allogenic hematopoietic stem cell transplantation on the inflammation*'

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Independent expert(s)	*Dr. Marc Engelen, m.engelen@amsterdamumc.nl*
Laboratory sites <*if applicable*>	*VU Medical Center*

PROTOCOL SIGNATURE SHEET

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee
AE	Adverse Event
ALSP	Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CSF-1R	Colony-stimulating factor 1 receptor
CV	Curriculum Vitae
EU	European Union
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
HCT	Hematopoietic stem cell transplantation
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
(S)AE	(Serious) Adverse Event
Sponsor	The sponsor is the party that commissions the organisation or performance of the research
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a progressive neurodegenerative disease, which primarily affects adults between 30 and 50 years. The median survival is 6.8 years after onset of symptoms. The disease is caused by progressive loss of microglia due to dominant mutations in the gene *CSF1R*, encoding colony stimulating factor-1 receptor (CSF-1R). Treatment with an allogeneic hematopoietic stem cell transplantation (HCT) can result in replacement of patient microglia by healthy donor cells, thereby halting disease progression in ALSP patients. The long-term therapeutic outcomes are still unknown. Previous research into the therapeutic effects of an allogeneic HCT in leukodystrophies and metabolic disorders shows that the effect on systemic and local inflammation plays an important role in HCT treated patients.

Objective: The aims of this study are to examine the degree of systemic inflammation in ALSP patients, and whether there is a change in the degree of systemic inflammation after treatment with an allogeneic HCT.

Study design: Prospective cohort study

Study population: All genetically confirmed ALSP patients who are referred to the Amsterdam UMC, location VUmc or AMC, and are considered to be eligible for HCT.

Intervention: Collection of extra venous blood (±15ml in total, divided over 3 different tubes) during venous blood sampling for standard clinical care.

Main study parameters/endpoints:

The primary outcomes are 1) cytokine profiles in blood samples from ALSP patients before HCT compared to those from healthy people, and 2) change in cytokine profiles over time in HCT treated ALSP patients.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The procedure includes collection of ±15ml extra venous blood at the moment of venous blood sampling for standard clinical care. There is no direct benefit for the patients; there is only benefit for the ALSP patient population by increased knowledge. Risks and burdens of the study will be minimized by collecting blood samples only during venous blood sampling in the context of standard care.

1. INTRODUCTION AND RATIONALE

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a progressive neurodegenerative white matter disease (leukodystrophy), which primarily affects adults between 30 and 50 years. The disease often starts with neuropsychiatric features, followed by progressive motor and gait disturbances, incontinence, speech and swallowing problems, epilepsy, and premature death with a median survival of 6.8 years after onset of symptoms.[1] The disease is caused by dominant mutations in the gene *CSF1R*, encoding colony stimulating factor-1 receptor (CSF-1R). CSF-1R acts as a receptor for cytokines, including CSF-1 and IL-34. These cytokines regulate the production, differentiation and function of various immune cells in the blood and brain, such as macrophages and microglia. In addition, the microglia homeostasis is dependent on CSF-1R. The disease is therefore characterized by microglia loss, resulting in demyelination, axonal loss and inflammation in the central nervous system.[2, 3]

At present, ALSP patients are treated with an allogeneic hematopoietic stem cell transplantation (HCT), if diagnosed rather early in the disease course.[4-6] The theory behind allogenic HCT is that hematopoietic stem cells from bone marrow, peripheral blood or umbilical cord blood of a healthy donor are able to cross the blood-brain barrier, and differentiate into healthy macrophages/microglia to replace the (diseased) patient microglia.[7] There is increasing evidence that HCT may halt disease progression in ALSP patients, but the long-term therapeutic effects are still unknown.[4, 6] The treatment has been proven to be safe and halt disease progression if applied early in the disease in several other leukodystrophies, including metachromatic leukodystrophy and adrenoleukodystrophy, if stable engraftment following HCT has been accomplished.[8, 9] In addition, there is growing recognition that a significant part of the therapeutic effect of HCT in leukodystrophy patients comes from reducing inflammation in the nervous system,[10, 11] and that HCT also reduces systemic inflammation in patients with a metabolic disorders with or without central nervous system involvement.[12, 13] We expect that the anti-inflammatory effects of HCT in ALSP patients might be even greater since the disease affects in particular microglia, the immune cells of the central nervous system, and results in loss of one of the most prevalent cytokine receptors present on immune cells in blood of healthy humans. Nevertheless, scientific data on systemic inflammation and in cytokine profiles in ALSP patients are currently lacking. Therefore, the aims of this study are to examine the degree of systemic inflammation in ALSP patients before and after HCT.

2. OBJECTIVES

The aims of this study are to examine the degree of systemic inflammation in ALSP patients, and whether there is a change in the degree of systemic inflammation after treatment with an allogeneic HCT.

2.1 Primary objective:

In order to study this, we want to answer the following primary research questions:
1. Are cytokine profiles in blood of ALSP patients before HCT different from cytokine profiles in blood of healthy individuals?
2. How do cytokine profiles in blood of ALSP patients change over time after treatment with HCT?

2.2 Secondary objective(s):

Additional (secondary) research questions that will be addressed in this study are:
1. Are changes in cytokine profiles in blood after treatment with HCT related to clinical outcomes?

3. STUDY DESIGN

The study uses a longitudinal cohort study design. It is a multi-center study that will be performed at the Department of Child Neurology in the Amsterdam UMC, location VU medical center (VUmc) (primary site) and in the Emma Children's Hospital, Amsterdam UMC, location Amsterdam Medical Center (AMC), Amsterdam, The Netherlands. Since most Dutch ALSP patients are referred to us to discuss their disease prognosis and treatment possibilities, the ALSP patients will be recruited during a hospital visit to the VUmc or AMC. Sample collection from patients will take place during a period of 5 years (6 times in total) at moment of venous blood sampling for standard clinical care. Reference samples will be acquired via the mini donor bank in the UMC Utrecht.

4. STUDY POPULATION

4.1 Population (base)

Subjects will be drawn from adult ALSP patients (18 years or older), who are referred to us and are capable of giving informed consent. The subjects will be recruited by personal communication during a regular hospital visit. The subject will be informed about the study and asked for his or her consent (see 8.2). There will not be a minimal reflection period to consider their decision. The study population will consist of about 15 adult ALSP patients

(aged between 18 and 80 years old), of whom probably 10 will receive HCT and 5 will not receive HCT for clinical reasons unrelated to this study.
It is possible that some study subjects will lose the formal capacity to consent during the study period due to disease progression. If so, this will most likely happen gradually, allowing us to discuss with the subject whether or not to continue with the study and whether the subject wishes to designate a legal representative. We assume that study continuation after losing formal capacity to consent is still consistent with the subjects' preference and values unless the patient (or legal representative) communicates otherwise.
The likelihood that the planned number of subjects can be recruited from this source population is high, since patients are often intrinsically motivated to contribute to scientific research for this rare but devastating disease and the burden of the study is negligible, as repeated venous blood sampling is part of the standard care for these patients.
In addition, we will also include ALSP patients who have already been treated with HCT previously. Blood samples of the latter patients have already been stored in the HCT biobank of the UMC Utrecht according to their local biobank protocol and can be used for the current study.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Diagnosis of ALSP confirmed by a pathogenic *CSF1R* mutation
- Aged 18 years or older
- Capable of giving informed consent

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- No informed consent given by the patient
- Cognitive capabilities are too low at inclusion of the study to give informed consent

4.4 Sample size calculation

In the last 2 years, 4 HCT treated ALSP patients are known in the VUmc and AMC. Two patients died a few months after HCT, but these patients have agreed to the use of previously collected data and samples (have given informed consent to participate in the PhenoLD study) and autopsy for research purposes. The other two patients are still alive. In addition, 2 recently diagnosed untreated ALSP are known in the VUmc and AMC. These patients will be asked to participate in the study to provide a reliable answer to the research

questions addressed. In addition, we expect to include approximately 10 – 15 new ALSP patients during the research period and anticipate that the majority of them will be treated with HCT. Due to the rarity of ALSP, the only possibility to study our research questions is using a small cohort of patients. This means that we will be able to find only relatively large differences between patients and controls, and can study the change in cytokine profiles over time in treated and untreated patients only in an explanatory way. The effect size for the research question is unknown. Therefore, no conclusive power calculation can be performed. However, we know that for the cluster analysis with Principal Component Analysis (PCA, as described below), the total number of samples (in this case at baseline) is 5 times the number of independent variables. However, the number of independent variables is not equal to the number of markers examined using OLINK. This is because the markers are associated with each other because they are part of the same molecular pathways. A previous publication using composite panels of proteins shows that in an analysis of 184 markers, 24 independent markers / variables were present.[14] Therefore, we estimate that for the 92 markers we want to measure, there are 7 - 10 independent variables. [13] This means that we must have a minimum of 35-50 independent blood samples, including at least 15 from patients and 20-30 from healthy age-matched controls.

5. METHODS

5.1 Study parameters/endpoints

5.1.1 Main study parameter/endpoint

The primary outcomes of objective 1 are cytokine profiles in blood before HCT, expressed in Normalized Protein eXpression (NPX) in Log2 scale, and cytokine profiles in blood over time.

5.1.2 Secondary study parameters/endpoints (if applicable)

Secondary endpoints will be the association of cytokine profiles with clinical outcomes after treatment.

5.1.3 Other study parameters (if applicable)

Other study parameters consist of demographic information such as the patient's age, pathogenic mutation in *CSF1R*, age at disease onset, presenting disease signs and symptoms, disease duration, and family history; and treatment information such as donor, conditioning and graft characteristics, post-transplant immunosuppression and chimerism. This information will be retrieved from patient records.

5.2 Study procedures

There is no interference with any diagnostic procedure or treatment. After agreeing to participate, the patient is asked for an extra 15ml of venous blood collection during venous blood sampling for standard care. The total duration is approximately 5 minutes. There will be no extra risks for the patients. Three tubes (EDTA, heparin and serum separator tube) of ±5 ml venous blood will be collected, and these tubes will be kept at 4 degrees Celsius within 2 hours. Dried blood spots will be obtained from the EDTA tube using regular filter paper and stored at -80 degrees Celsius until analysis. EDTA-plasma, heparin-plasma and serum will be obtained by centrifuging the samples as soon as possible (1800 g, 10 min, room temperature) and filling 0.5 ml aliquots, and will also be stored at -80 degrees Celsius until analysis.

Primary analyses of plasma cytokines will be done at once in collaboration with the Center for Translation Immunology (UMC Utrecht), and is performed by high-throughput, multiplex immunoassay (OLINK inflammation panel). Collected age- and sex-matched plasma samples of healthy controls in the UMC Utrecht will be taken along. Paired measurements in the study participants and retrospective validation in blood of (other) ALSP patients ensure the relevancy and reliance of our results.

5.3 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. Besides, the investigator can decide to withdraw a subject from the study for urgent medical reasons.

5.4 Replacement of individual subjects after withdrawal

There is no procedure to replace individual subjects after withdrawal since all diagnosed ALSP patients who fulfil the inclusion criteria will be asked to participate in this study.

5.5 Follow-up of individual subjects after withdrawal

Subjects will not be followed-up for research purposes after withdrawal from this study.

5.6 Premature termination of the study

The study may be suspended or terminated if there are serious concerns about the protection of the rights and welfare of the patients, and/or when serious or continuing noncompliance has taken place. In the case of premature termination, the Principal Investigator notifies the METC within 15 days of the termination, with the reasons for the

termination and a description of what measures were or will be taken to ensure the safety, rights and welfare of the patients.

6. SAFETY REPORTING

6.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

6.2 AEs, SAEs and SUSARs

6.2.1 Adverse events (AEs)

Adverse events (AE) are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the diagnostic procedures. For this study, the risk of any AEs related to the study procedures is very low. However, the study population consists of patients who are often very ill and have multiple comorbidities. The investigator will record AEs reported spontaneously by the subject or observed by the investigator or his staff, if this AE occurs within a time window of 24 hours after venous blood sampling and is evaluated by the treating paediatric neurologist to be likely caused by the study intervention. This also applies retrospectively to the previously included subjects. This means that also the following AEs, that occur frequently in ALSP patients, will not be reported for this study:

- symptoms that are allocated as side effects (of used medication) by a medical doctor / physician assistant / specialized nurse, including tiredness, drowsiness, nausea, dizziness, constipation and diarrhoea
- pneumonia
- CNS infection
- feeding difficulties
- psychiatric symptoms
- neurological deterioration related to ALSP
- graft versus host disease
- stem cell graft failure
- transplantation induced organ injury/toxicity

- transplantation induced mucositis

6.2.2 Serious adverse events (SAEs)

A serious adverse event (SAE) is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- any other important medical event that did not result in any of the outcomes listed above due to medical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as an SAE.

For this study, the risk of any SAEs related to obtaining extra blood during blood sampling for standard clinical purposes is extremely low. Importantly, the study population consists of patients who undergo a serious procedure (HCT), are often very ill, and have multiple comorbidities. The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol by annual line listing of occurred SAEs that have occurred within a time window of 24 hours after venous blood sampling and were evaluated by the treating paediatric neurologist to be likely caused by the study intervention. This means that also the following SAEs, that occur frequently in ALSP patients, will not be reported for this study:

- death due to disease progression or other non-study related medical causes
- (hospitalisation related to) pneumonia
- (hospitalisation related to) CNS infection
- (hospitalisation related to) feeding difficulties
- (hospitalisation related to) psychiatric symptoms
- (hospitalisation related to) neurological deterioration related to ALSP
- (hospitalisation related to) graft versus host disease
- (hospitalisation related to) stem cell graft failure
- (hospitalisation related to) HCT induced organ injury/toxicity
- (hospitalisation related to) HCT induced mucositis

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that occur within a time window of 24 hours after venous blood sampling, are evaluated by the treating

paediatric neurologist to be likely caused by the study intervention and result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs that occur within a time window of 24 hours after venous blood sampling and are evaluated by the treating paediatric neurologist to be likely caused by the study intervention will be reported within a period of maximum 15 days after the sponsor has first knowledge of the SAEs.

SAEs do not require (expedited) reporting to the accredited METC are SAEs that are not considered to be caused by the study intervention, including those SAE that occur frequently in ALSP patients:

- death due to disease progression or other non-study related medical causes
- (hospitalisation related to) pneumonia
- (hospitalisation related to) CNS infection
- (hospitalisation related to) feeding difficulties
- (hospitalisation related to) psychiatric symptoms
- (hospitalisation related to) neurological deterioration related to ALSP
- (hospitalisation related to) graft versus host disease
- (hospitalisation related to) stem cell graft failure
- (hospitalisation related to) HCT induced organ injury/toxicity
- (hospitalisation related to) HCT induced mucositis

6.3 Follow-up of AEs

All AEs that occur within a time window of 24 hours after venous blood sampling and are evaluated by the treating paediatric neurologist to be likely caused by the study intervention will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

7. STATISTICAL ANALYSIS

Statistical analysis will be performed using R. All primary and secondary study parameters will be presented as quantitative data. Demographic and treatment information will be displayed as both quantitative and qualitative data. Results of continuous data will be presented as means ± standard deviation for normally distributed data or as median + interquartile range for non-normally distributed data (determined by visual inspection of the histograms and Q-Q plots). Results of categorical data will be presented as frequencies and proportions.

Using OLINK, biomarker plasma concentrations in ALSP patients will be calculated from DNA amplicons. The raw data will be normalized and converted into “Normalized Protein Expression Units (NPX)”. These NPX values will be expressed on a Log2 scale, meaning that 1 NPX unit higher equals a doubling of the biomarker concentration. To investigate the degree of systemic inflammation in ALSP patients compared to age-matched healthy controls (primary first objective), a PCA will be performed on the NPX data of (still) untreated patients. With these results, we will determine whether cytokine profiles are distinctive between patients and healthy controls and thus whether the ALSP patients can be separated (or clustered) from the healthy controls based on their cytokine profile. In addition, a Wilcoxon rank-sum test with Benjani-Hochberg correction for multiple testing (controlling the false discovery rate FDR) will be used to compare the NPX values between ALSP patients and healthy controls. Finally, a fold change analysis will be performed on the NPX values while also the fold change will be transformed to a Log2 scale. Markers that exhibit both a minimum log2 fold change of >1.5 and are statistically significant according to the Wilcoxon rank-sum test will be considered as important different biomarkers between ALSP patients and controls. These biomarkers will subsequently be clustered into a heatmap using the Ward's method and the correlation distance.

To determine which markers show a (significant) change over time (trend) (primary objective 2), we will use linear mixed-effect models with fixed effects over time (calculated from the date of HCT for treated patients and from the date of first measurement for untreated patients) for each marker. A random intercept per patient is added to account for variation in biomarker concentrations at baseline. If possible (considering model converging), a random slope (for time) is also included to correct for the correlation between the repeated measurements within one patient. The final coefficients in the model will be estimated using the restricted maximum likelihood method. To answer the secondary objective, similar linear mixed effects models will be used to examine clinical outcomes after treatment. Given the expected low number of patients, we will examine these factors one by one (univariable association) and in an explorative way.

FDR-adjusted p values (q-values) < 0.05 will be considered significant. We expect missing data to be missing at random. We will use multiple imputation techniques to handle missing data.

This statistical plan has been made in consultation with experienced researchers from the Center for Translational Immunology (CTI, UMC Utrecht). They are experienced with (cluster) analyzes of blood biomarkers in very small patient groups. A good example is the publication “Correlation of CXCL10, Tumor Necrosis Factor Receptor Type II, and Galectin 9 With Disease Activity in Juvenile Dermatomyositis” written by F. Bellutti Enders et al. Using Luminex (measurement technique) and cluster analysis, they found statistically significant

differences in plasma concentration of inflammatory biomarkers between well-defined patient groups consisting of only 9 individuals. Another example is a study that is currently being conducted by the CTI and that used the same measurement technique that we want to apply (OLINK). B.T.A. van den Broek et al studied the plasma concentration of 92 biomarkers (https://www.olink.com/products/inflammation/biomarkers/) before HCT in 22 MPS patients, and found that, after adjustment for multiple testing, the concentration of 64 markers differed significantly from age-matched healthy controls. In addition, they were able to demonstrate in smaller patient groups (n = 10) that 12 markers decreased significantly over time after HCT and that the majority of the markers reached the same concentrations as healthy controls after long-term follow-up.

8. ETHICAL CONSIDERATIONS

8.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The study protocol will be submitted to the ethics committee.

8.2 Recruitment and consent

Eligible subjects with an age of 18 years or older will be recruited by us. The subjects will be recruited by personal communication during a regular hospital visit. Thereafter, the subject will be informed about the study and asked for consent by an eligible member of the study team. There will not be a minimal reflection period to consider their decision. When the subject has questions about the study, he or she can contact the treating neurologist, the principal investigator, another member of the study team, or the independent expert by telephone, email or during a next hospital visit. Patients who agree to participate will be asked to sign a written informed consent of which they will receive a copy from an eligible member of the study team. In the informed consent form, a separate thick box is included for the use of material that is left from the analyses described for this research to study other aspects of the disease based on new insights. The patient information letter, informed consent form and recruitment material are attached as a separate document. The first blood collection may occur at the day of the first patient visit (as part of the clinical diagnostic work-up) and thus at the day of recruitment, but only after the patient has read the information letter and signed the informed consent form. The general practitioner of the research patient nor the neurologist from another hospital will be informed if the patient has given informed consent for the study.

8.3 Objection by minors or incapacitated subjects (if applicable)

The study will be conducted according to section 4, subsection 2, of the WMO and the codes of conduct with incapacitated elderly and mentally disabled; minors do not participate in the study. Subjects who have lost the formal capacity to consent cannot be forced to participate in the study nor to undergo a certain study against his or her will. Objections raised by a subject will be analysed at any time during the study. If this analysis shows that the expressed objections are to be interpreted as refusal, the subject's will be respected, and the subject does not have to provide reasons. The investigators are aware of signs of resistance and will evaluate whether these signs are part of the anticipated burden, or that for that subject the experienced burden exceeds the anticipated burden (e.g. distress or fear). The investigator will consult the legal representative and try to reduce the burden. If the analysis concludes that the subject dissents, the subject will be excluded or withdrawn from the study.

8.4 Benefits and risks assessment, group relatedness

The patients included in the study will not have direct personal benefit from the study results. The only benefit for the patient population is increased knowledge. The risks of participation are negligible, as the blood for the study is obtained only during venous blood sampling for clinical care. The risks of AEs during this procedure are very low, and when they do occur, they are non-dangerous and self-limiting.

It is possible that some study subjects will lose the formal capacity to consent during the study period due to disease progression. The study can only be done using this vulnerable patient group, because ALSP is a rare disease and the loss of cognitive capacities is caused by the condition. Nevertheless, we will respect the subjects' preference and values in research participation and protect his or her rights and welfare as much as possible.

8.5 Compensation for injury

The sponsor has obtained dispensation from the statutory obligation to provide insurance covering damage to research subjects, because participating in the study is without additional risks for the participants as only ±15ml extra venous blood is obtained during venous blood sampling for clinical care. The sponsor does have a liability insurance which is in accordance with article 7 of the WMO. This insurance is offered by Onderlinge Waarborgmaatschappij Centramed B.A., PO box 7374, 2701 AJ Zoetermeer. The insurer and the insurance comply with the compulsory insurance policy for medical research with people (Dutch Bulletin of Acts, Orders and Decrees, 2003, 266).

8.6 Incentives (if applicable)

The patients will not receive any incentives for participation in this study.

9. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

9.1 Handling and storage of data and documents

Data will be handled confidentially. A subject identification code list will be used to link the data to the subject: the test results and all samples will be coded based on the identification code. The patient code will only be available to the principal investigator and executive researcher. Data will be stored according to the study number for 15 years. Blood samples will be stored at least until the end of the study. If permission is obtained, blood samples that are left over after analysis will be stored according to study number for 15 years for future research. Raw digital data will be stored in the corresponding patient records in Epic. Raw physical data will be stored in the internal archive at the Department of Child Neurology, Emma Children's Hospital, Amsterdam UMC, VU Medical Center (locked room). Processed data will be saved and stored in a databank file in the VU University Medical Center using Castor EDC and R. These sets of data and files will be stored in a project folder with additional security controls (encryption and user access rights) on a secure "store forever" server \\VUMC\onderzoek$\s4e-gpfs1\kinderneurologie-01\Kinderneurologie-ALSP, together with the versions of scientific publications reporting the analyzed data. Blood samples (and left over samples after analysis) will be stored at the Biobank wittestofziekten (BB CWMD, TcB/METc-nr: 2018.395). The handling of personal data will comply with the Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG. Access is only allowed for research team members and granted by the additional security controls. We will work with patient numbers, containing number, date and researcher's name. We will keep a secure tracking file with the names and the numbers separately. The tracking file is only accessible for available to the principal investigator and the executive researcher. Transfer of sensitive data will only take place through the use of Castor EDC or SURFfilesender with encryption. If permission is obtained, data might be stored in an international database for exchange with other ALSP research groups (outside the EU) and with researchers in the United States (separate permission due to unequal privacy protection).

9.2 Monitoring and Quality Assurance

An independent monitor will monitor the study data according to Good Clinical Practice (GCP). In a selection of the subjects the Informed Consents will be checked. Source Data

verification will also be performed during the onsite monitoring (to check data in the research forms / questionnaires match the source data (patient records, lab results, etc.). The intensity of this verification is related to the risk of the study. The monitor will also check whether all (S)AEs have been adequately reported within the timelines as required by legislation and regulations.

9.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

9.4 Annual progress report

The sponsor/investigator will inform the METC about the start date of the study which corresponds to the first inclusion date.
Thereafter, the sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, SAEs, other problems, and amendments.

9.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the start and the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.
In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.
Within 1 year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

9.6 Public disclosure and publication policy

The results of this study will be disclosed unreservedly as soon as appropriate. Both positive and negative results will be disclosed. Results will be submitted for publication to peer-reviewed scientific journals. If these journals do not consider publication, other ways of

disclosing results will be found, such as trial registers, websites, databases and so on. All parties concerned will justify their actions in this regard.

10. STRUCTURED RISK ANALYSIS

This is a non-therapeutic study. However, in the risk analysis performed for this study we concluded there is a medium risk in this study due to the study group characteristics.

11. REFERENCES

1. Coomans, C., et al., *Early-onset dementia, leukoencephalopathy and brain calcifications: a clinical, imaging and pathological comparison of ALSP and PLOSL/Nasu Hakola disease.* Acta Neurol Belg, 2018. **118**(4): p. 607-615.
2. Oyanagi, K., et al., *Adult onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) and Nasu-Hakola disease: lesion staging and dynamic changes of axons and microglial subsets.* Brain Pathol, 2017. **27**(6): p. 748-769.
3. Chitu, V., et al., *Phenotypic characterization of a Csf1r haploinsufficient mouse model of adult-onset leukodystrophy with axonal spheroids and pigmented glia (ALSP).* Neurobiol Dis, 2015. **74**: p. 219-28.
4. Gelfand, J.M., et al., *Allogeneic HSCT for adult-onset leukoencephalopathy with spheroids and pigmented glia.* Brain, 2020. **143**(2): p. 503-511.
5. Eichler, F.S., et al., *CSF1R mosaicism in a family with hereditary diffuse leukoencephalopathy with spheroids.* Brain, 2016. **139**(Pt 6): p. 1666-72.
6. Mochel, F., et al., *Haematopoietic stem cell transplantation in CSF1R-related adult-onset leukoencephalopathy with axonal spheroids and pigmented glia.* J Neurol Neurosurg Psychiatry, 2019. **90**(12): p. 1375-1376.
7. Capotondo, A., et al., *Brain conditioning is instrumental for successful microglia reconstitution following hematopoietic stem cell transplantation.* Proc Natl Acad Sci U S A, 2012. **109**(37): p. 15018-23.
8. van Rappard, D.F., et al., *Efficacy of hematopoietic cell transplantation in metachromatic leukodystrophy: the Dutch experience.* Blood, 2016. **127**(24): p. 3098-101.
9. Sirrs, S., et al., *Transplantation as disease modifying therapy in adults with inherited metabolic disorders.* J Inherit Metab Dis, 2018. **41**(5): p. 885-896.
10. Wolf, N.I., et al., *Metachromatic leukodystrophy and transplantation: remyelination, no cross-correction.* Ann Clin Transl Neurol, 2020. **7**(2): p. 169-180.
11. Weinstock, N.I., et al., *Macrophages Expressing GALC Improve Peripheral Krabbe Disease by a Mechanism Independent of Cross-Correction.* Neuron, 2020.
12. Thibert, K.A., et al., *Cerebral Spinal Fluid levels of Cytokines are elevated in Patients with Metachromatic Leukodystrophy.* Sci Rep, 2016. **6**(1): p. 24579.
13. Van den Broek, B.T.A. and E. al., *Long-Term Effect of Hematopoietic Cell Transplantation on Systemic Inflammation in Mucopolysaccharidoses Patients*, in *Hematopoietic Cell Transplantation in Inborn Errors of Metabolism Hurdles to 'real' cure*. 2020, Utrecht University: Utrecht.
14. Ghebre, M.A., et al., *Sputum Inflammatory Mediators Are Increased in Aspergillus fumigatus Culture-Positive Asthmatics.* Allergy Asthma Immunol Res, 2017. **9**(2): p. 177-181.