A Statistical Analysis Plan for a phase 1/2 trial to Explore the Safety, Tolerability, Pharmacokinetic Profile, and Potential Efficacy of Guanabenz in Patients With Early-Childhood Onset Vanishing White Matter (VWM)

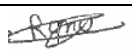
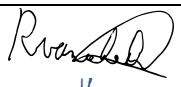
Version of this SAP: 1.9; 07-07-2025

Full title of the study: A Study to Explore the Safety, Tolerability, Pharmacokinetic Profile, and Potential Efficacy of Guanabenz in Patients With Early-Childhood Onset Vanishing White Matter (VWM)
Short title of the study/acronym (optional): The Guanabenz trial
ABR number: 61627.000.18
Research protocol: ID/number: VWM1 Protocol version: 11, 06-02-2025
Trial registration number: NTR 7260 EudraCT number 2017-001438-25 (https://www.clinicaltrialsregister.eu/ctr-search/trial/2017-001438-25/NL) CTIS 2023-503320-89-00

Content

Section 1. Administrative information.

1.1. Names and Signatures

Role of contributor	Name and full affiliation	Signature	Date of signature
Principal investigator	M.S. van der Knaap, professor, Pediatric Neurology, Amsterdam UMC		07-07-2025
Researcher who will perform the statistical analysis	R.J. van Voorst, PhD candidate, Amsterdam UMC E. Gonzato, PhD candidate, Amsterdam UMC	 	07-07-2025 07-07-2025
Senior statisticians consulted	R. van Eekelen, assistant professor, Amsterdam UMC J. Berkhof, professor, Epidemiology and Data Science, Amsterdam UMC	 	07-07-2025 07-07-2025
Contributor to statistical analysis plan			
…			

1.2. Revision history

Non applicable.

1.3. How is the trial registered?

The trial is registered in the EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/ctr-search/trial/2017-001438-25/NL)

1.4. What is the planned period of observation?

The expected date of the inclusion of the first patient is 31-MAY-2021.
The expected data of the completion of follow-up for the last patient is 31-MAY-2025.

Section 2. Introduction.

2.1. What is the background and rationale for the study?

Vanishing white matter (VWM) is a devastating leukodystrophy (genetic brain white matter disorder) caused by recessive mutations in the genes EIF2B1-5, encoding the 5 subunits of eukaryotic translation initiation factor 2B, eIF2B[1-4]. In VWM, variants in the genes EIF2B1-5 decrease eIF2B activity and thereby constitutively activate the downstream integrated stress response (ISR)[5, 6].
Recently, guanabenz has been shown to impact the ISR and currently is the only registered drug with a known effect on the ISR. Guanabenz is an a2-adrenergic antihypertensive drug with proven safety in adults and children above 12 years of age[7]. Guanabenz binds to and inhibits protein phosphatase 1 (PPP1R15A), also known as GADD34[8]. GADD34 constitutes a feedback loop in the ISR. Modulation of GADD34 has shown beneficial effects in nonclinical models of several human diseases, in which the ISR is activated, such as amyotrophic lateral sclerosis[9, 10]. The ameliorating effect of guanabenz that is anticipated on VWM has been substantiated in a mutant VWM mouse model representative for the human disease[11].

The aim of the guanabenz trial is to evaluate the safety, tolerability and efficacy of guanabenz in young children with VWM. For further details on the background and rationale of this study see pages 33 to 39 of the protocol.

2.2. What are the objectives of the study?

- *The primary safety objective is to evaluate the safety and tolerability profile of guanabenz in pediatric patients with VWM.*
- *The primary efficacy objective is to evaluate how disability in terms of ambulation change over time in patients with VWM receiving guanabenz as compared to historical controls.*

- *The secondary objectives are*
    - *to evaluate the PK profile of guanabenz in patients with VWM*
    - *to evaluate overall survival in patients with VWM receiving guanabenz as compared to historical controls*
    - *to evaluate how quantitative MRI parameters relevant to brain white matter integrity change over time in patients with VWM receiving guanabenz.*

- *Exploratory objectives are*
    - *to perform exploratory PK-covariate and PK/PD analyses.*
    - *to perform exploratory biomarker analyses in blood and CSF.*
    - *To perform a Health Technology Assessment (HTA) concerning the use of guanabenz.*

For further details see pages 40 of the protocol.

Section 3. Study Methods.

3.1. What is the study design?

This is an open-label, non-randomized study with a historical control group. All patients enrolled will receive guanabenz at their highest tolerated dose, with a target dose of 2 mg/kg/day, a minimum dose of 1 mg/kg/day, and a maximum dose of 10 mg/kg/day. Guanabenz will be administered as oral capsules once daily before bedtime and in addition to the patient's usual standard of care. The control group consists of historical VWM patients collected from an international longitudinal VWM registry with data collection over 20 years, investigating the natural disease course of VWM.

For more details see pages 41 to 50 of the protocol.

3.2. Will randomization be performed in this study?

No randomization is performed in this study.

3.3. How was the sample size calculated?

The planned sample size for this study (N=20 patients minimum, N=40 patients maximum) is not based on formal hypothesis testing considerations. Considering the low incidence of VWM, N=20-40 is the maximum number that realistically can be achieved over a period of 2-3 years. The planned sample size is deemed sufficient to provide preliminary assessments of safety and tolerability and to allow assessments of PK and major beneficial effects, such as major clinical improvement and any improvement of the brain MRI parameters.
A sample size of 20 patients is deemed necessary to obtain reliable estimates of clinical outcomes and their standard deviation. This information can be used to inform the design of future studies.

The following is an example for a power calculation based on the sample size, and the power might increase based on a 1:1 matching ratio. In the population with onset ≤6 years and eligible for study participation, the proportion of patients who with standard care will be alive and be able to walk without support after 2 years is estimated to be between 50% and 70%. Considering type I error equal to 0.1 and a McNemar test statistic, with 30 patients included within 3 years and 30 historical controls, the study has 80% power to declare guanabenz effective, if the proportion able to walk without support after 2 years increases from 50% to 80.1%, from 60% to 87.8%, or from 70% to 93.5%. With 40 patients included in 2 years and 40 historical controls, the study has 80% power to declare guanabenz effective, if the proportion able to walk without support after 2 years increases from 50% to 75.5%, from 60% to 83.3%, or from 70% to 90.1%. If the data are correlated, the power will increase.

This refers to page 53 of the research protocol.

3.4. What is the hypothesis testing framework for this study?

The guanabenz trial uses a superiority hypothesis testing framework for all primary, secondary and exploratory efficacy outcomes.

3.5. Will interim analyses be performed in this study?

No interim analyses are planned.

3.6. When will the final statistical analysis of the study data be performed?

The statistical analysis of the primary and secondary endpoints assessed during the hospital stay will be conducted after the last patient has been discharged and the database has been closed (scheduled for July 16, 2025). This will occur approximately one year after the final participants were enrolled, as inclusion ended on May 31, 2024. The current estimate is that these statistical analyses will be performed in August and September 2025.

3.7. At which time points are the outcomes measured and which "windows" are allowed?

The time points of the visits are presented in Table 3 on page 71 of the protocol. As stated on page 71, all visits have a visit window of 14 days before and after the nominal time points, except for visits M0, M12, M24, M36, M48, which have a visit window of 28 days before and after the nominal time points. As indicated on page 71, the end of study visit has to be in the last 6 months before the end of study on May 31, 2025.

Section 4. Statistical Principles.

4.1. Which level or levels of statistical significance will be used in the study?
Primary and secondary efficacy outcomes will be considered significantly different between guanabenz treated participants and historical controls if the one-sided p-values are less than 0.05.
4.2. Will the analysis adjust for multiplicity of statistical testing to ensure control of type I error rate?
As there is one primary efficacy endpoint (loss of ambulation) ,the analysis will not adjust for multiplicity of statistical testing. Secondary (and other exploratory) endpoints are considered as exploratory.
4.3. Which confidence intervals will be reported?
Two-sided 95% confidence intervals will be presented for all efficacy parameters.
4.4. How is compliance defined and assessed?
Compliance is defined as the proportion of prescribed doses actually taken by each patient. Pill-count based compliance is assessed by counting the number of left-over pills in the tablet container and dividing this number by the total number of doses in the treatment regime. Additionally, blood levels of guanabenz are monitored every three months to assess medication adherence. Low outliers will be investigated, as they may indicate non-compliance.
4.5. How will compliance be presented?
Pill-count based compliance will be reported as the median and 25th and 75th percentiles of the proportion of prescribed doses actually taken by each GBZ participant of the study. Additionally, compliance will be presented by guanabenz blood levels over time. The relationship between (pill-count and blood-level based) compliance and all outcome measures will be examined.
4.6. What are defined as protocol deviations in this study?
Protocol deviations are defined as: deviations from the eligibility criteria, concurrent trial participation, unscheduled visits, visits outside the allowed time window, and missed mandatory procedures (e.g., lumbar puncture or blood spot collection).
4.7. How will protocol deviations be presented in the reporting of this study?

All protocol deviations will be line-listed for the guanabenz treated participants, including the number and percentage experiencing one or more protocol deviations.

4.8. Which analysis populations will be defined?

The intention to treat population includes all guanabenz treated participants, who gave written informed consent and were given the first dose of medication. The safety population includes all patients, regardless of protocol deviations or use of rescue medication, but excludes patients, who received no study medication.

Section 5. Study populations.

5.1. Which data were collected from participants, who were screened for eligibility for inclusion in the study, and how will these data be presented in study reports?

Screening data will include the number of patients screened, the number meeting the inclusion criteria, and the number approached for informed consent. For all screened patients, data on age at time of screening, age of onset, disease duration at time of screening, sex, and disease severity (as defined by ambulation) were collected. We will report the mean and standard deviation of age, the number and percentage of male patients and percentage of patients with loss of walking ability without or with light support. Age at the time of loss of ambulation with support will be presented as the median and range. These statistics will be presented for all screened patients, those meeting the inclusion criteria, and those approached for informed consent.

5.2. What are the inclusion and exclusion criteria for the study?

Inclusion/exclusion criteria all VWM patients
All patients must be genetically proven to have VWM and a brain MRI compatible with the diagnosis. Patients who are clinically asymptomatic, and those who have comorbidities are excluded.

Inclusion/exclusion criteria GBZ participants
Eligible patients should be within reasonable travel distance from Amsterdam. They must have a disease onset before or at the age of 6 years and a maximum disease duration of 8 years. They should be able to stand and walk 10 steps, either without support of with light support of one hand. Patients that have presence of an unrelated serious condition, are unwilling to travel to Amsterdam, participate in another clinical study with therapeutic intervention, cannot undergo MRI, cannot guarantee adherence to treatment and study visits due to family situation or have allergy/hypersensitivity to guanabenz or any of the other components of the formulations used in this study are excluded.

Inclusion/exclusion criteria historical controls
Historical controls must be matched to GBZ participants based on age at disease onset and a comparable disease severity at the same disease duration. Disease severity is defined by ambulation capabilities. The latter is assessed in multiples ways: eligible historical controls must either retain the ability to walk at least 10 steps without or with support or have a Health Utility Index (HUI) ambulation score of 1, 2, 3 or 4. In this way, historical controls reflect the walking ability of GBZ participants at baseline. To ensure accurate comparisons, historical controls are selected only from time periods where the disease course is comparable to that of the GBZ participants.
For detailed inclusion and exclusion criteria, please refer to pages 51 and 52 of the study protocol.

5.3. Which information will be presented in the flow chart for this study?

Non applicable.

5.4. What is the expected level of, timing of and reasons for withdrawal from the intervention and/or from follow-up and how will this be presented in the study reports?

We expect that 10-20% of patients will stop taking guanabenz before the end of the treatment. We expect that 10-20% of all patients will drop out of the study before assessment of the primary outcome is complete. We will report the number and percentage of GBZ participants who stop taking the allocated medication before the end of the treatment, or drop out before assessment of the primary outcome is complete. We will also report the median and interquartile range of number of weeks actually spent in follow-up and present line listings of the reasons for withdrawal or loss to follow-up".

5.5. Which baseline characteristics of participants will be presented?

** Median age at baseline in years and months*
** Median age of onset in years and months*
** Median disease duration at baseline in years and months*
** Disease severity score/HUI score at baseline*
** Number of participants with loss of walking without support*
** Median age of loss of walking without support*
** Sex*
** Country of residence*

An overview of all gene variants of the GBZ participants and historical controls will be added in a supplementary table.

5.6. How will the baseline characteristics be summarized?

Categorical baseline characteristics will be summarized by presenting the number and percentage in each category. Continuous, normally distributed variables will be summarized by presenting the mean and standard deviation. Continuous, non-normally distributed variables will be summarized by presenting the median and interquartile range. Differences between groups will not be statistically tested, as the historical controls have been matched to the inclusion/exclusion criteria of the guanabenz treated participants.

Section 6. Analysis.

6.1. How are the outcomes of this study defined?

The primary safety outcome is: All adverse events and serious adverse events collected from the start of study treatment until the end of the study and will be summarized for descriptive purposes.

The primary efficacy outcome is the time of loss of ambulation. Loss of ambulation is defined by the loss of walking with support.

The secondary efficacy outcomes are overall survival, HUI attributes and multiscores. Scores on the Vineland, GMFCS-MLD, ELFC-MLD, GMFM, LIPS, GMFCS, MACS CFCS and EDACS will be considered for descriptive purposes.

Other secondary outcomes are Guanabenz PK/PD parameters in plasma and will be summarized for descriptive purposes. Guanabenz exposure will also be correlated with clinical outcome measures.

For more information, please refer to pages 28 to 32 of the study protocol

6.2. Will any calculations or transformations be used to derive any outcome from the original data?

Disease duration will be calculated from age of onset until date of inclusion, screening, baseline, first day of dosing, end of titration, and visits. Ages will be calculated from the date of birth until date of inclusion, screening, baseline, first day of dosing, end of titration, and visits. Time to loss of walking, with or without support, will be calculated from the day of inclusion until the occurrence of the event.
HUI decision tables are used to generate HUI2 and HUI3 single-attribute utility scores, and applied coding algorithms to obtain overall HUI3 Health Index scores. Overall scores on the Vineland and LIPS will be calculated based on their respective domain scores.

6.3. What analysis method will be used and how will the treatment effects be presented?

For the primary efficacy outcome time to loss of ambulation, a comparison of the guanabenz-treated patients will be made with natural history data, adjusting for calendar year of symptoms onset.

For time to loss of ambulation, patients treated with Guanabenz will be matched to historical controls with a 1:2 matching ratio. The matching criteria are:

- *Age of symptoms onset*
- *Degree of handicap regarding ambulation at comparable disease duration*

Other variables might be added if considered crucial.

Primary analysis of the primary efficacy endpoint
In the primary analysis, treated patients will be compared to their matched historical controls. We will analyze time to loss of ambulation using the Kaplan-Meier method and the log-rank test, considering the presence of clustered data. Next, the Cox proportional hazards model will be used to provide an estimate of the effect size, which will contain treatment and will be adjusted for calendar year of symptoms onset. The effects size will be reported using the Hazard Ratio (HR). We will take matching into account when estimating standard errors.

Secondary analysis of the primary efficacy endpoint
In the secondary analysis, we will use historical controls with different matching procedures and different matching ratios. We will use the Kaplan-Meier method and the log-rank test, considering the presence of clustered data . The Cox model will contain treatment and will be adjusted for calendar year of symptoms onset, reporting the effects size using the Hazard Ratio (HR).

Analysis of secondary efficacy endpoints.
The same methods that we used for the primary analysis will be used for overall survival. The HUI scores will be analyzed using linear mixed models adjusting for treatment and calendar year of age of onset. A population pharmacokinetic model will be used to assess the drug exposure (such as AUC or concentration at steady state) and this will then be used to assess the relationship with clinical outcomes using correlation or regression methods.

Descriptive analysis for primary and secondary safety endpoints
A listing of all adverse and serious adverse events will be presented for the guanabenz-treated participants, including the corresponding date and time of collection.

6.4. Will any assumptions for statistical methods be checked?

Proportionality of the hazards in the Cox model will be assessed with visual inspection of Schoenfeld residuals and hypothesis testing.

For linear mixed models, normality of the measurement-level residuals will be assessed by visual inspection of histograms and q-q plots and using formal statistical testing. We will use the Shapiro-Wilk test and regard a p-value less than 0.05 as evidence of non-normality.

6.5. Will sensitivity analyses be performed?

For the primary analysis, we will conduct a sensitivity analysis by using different ratios of matching for guanabenz-treated participants and historical controls or weighted matching. For the secondary analysis, we will conduct a sensitivity analysis by using different trimmed weights Parametric models will also be used as an alternative to the Cox model.

6.6. Will subgroup analyses be performed?

A subgroup analysis will be performed on all primary and secondary efficacy outcomes based on age of onset, with participants categorized into two groups: onset <3 years of age, and onset between 3 and ≤6 years of age.

6.7. How will missing data be reported in the study reports and handled in the statistical analysis?

For incomplete diagnosis dates, a missing day will be imputed as 01. HUI inventories with more than two missing attribute scores (1%) will be excluded. Remaining missing values (up to two domains) will be imputed using pattern similarity hot deck imputation. No imputation of incomplete or missing dates for any other historical data will be done.

6.8. Will additional analyses on the primary or secondary outcomes be performed?

The same methods and reporting measures described in the primary and secondary analysis for the primary endpoint will be used to assess the effect of the treatment on overall survival.

6.9. How will harms be reported?

We will present line listings of safety outcomes. Safety outcomes will be classified by the local primary investigators as: expected complications of treatment using guanabenz; suspected unexpected serious adverse drug reactions; and other serious adverse events. Each class of safety outcome will be presented using tabulations of counts and percentages of events and of patients experiencing one or more of each type of event for each treatment arm. No formal statistical testing will be performed.

6.10. Which statistical software will be used to carry out the statistical analyses?

All outputs will be produced using R version 4.5.0 or a later version.

Section 7. References.

7.1. What is the title, date and version number of the current data management plan?
The current data management plan has the title "GuanabenzinVWM_Data Management Plan_V3.0_25juni2025", version number 3.0, is dated 02-07-2025 and is stored in the trial master file.
7.2. What is the title, date and version number of the current data validation and derivation plan?
The current data validation and derivation plan has the title "Data validation and derivation plan_GBZ_trial", version number 1.0, is dated 02-07-2025 and is stored in the trial master file.
7.3. Where is the study master file stored?
The trial master file is stored at the location G:\divc\kinderneurologie\Data uit VUmc\Trial Master File.
7.4. Where are the syntax files for data extraction, manipulation and preparation and statistical analysis stored?
The syntax files will be stored in the trial master file.
7.5. Which standard operating procedures will be adhered to when using and analysing data from this study?
When using and analysing data from the Guanabenz clinical trial, researchers will adhere to the Amsterdam UMC standard operating procedure RDM001 Research data management.
7.6. Which reporting guidelines will be adhered to when reporting on this study?
When reporting the results of this single-arm open label trial, the researchers will adhere as close as possible to the CONSORT reporting guidelines.

Section 8. Appendix.

Additional Tables, Figures and Documents.

References

1. Leegwater PA, Vermeulen G, Könst AA, et al. Subunits of the translation initiation factor eIF2B are mutant in leukoencephalopathy with vanishing white matter. Nat Genet 2001;29:383-388.

2. van der Knaap MS, Barth PG, Gabreëls FJ, et al. A new leukoencephalopathy with vanishing white matter. Neurology 1997;48:845-855.

3. van der Knaap MS, Kamphorst W, Barth PG, Kraaijeveld CL, Gut E, Valk J. Phenotypic variation in leukoencephalopathy with vanishing white matter. Neurology 1998;51:540-547.

4. van der Knaap MS, Leegwater PA, Könst AA, et al. Mutations in each of the five subunits of translation initiation factor eIF2B can cause leukoencephalopathy with vanishing white matter. Ann Neurol 2002;51:264-270.

5. van der Knaap MS, Pronk JC, Scheper GC. Vanishing white matter disease. Lancet Neurol 2006;5:413-423.

6. Abbink TEM, Wisse LE, Jaku E, et al. Vanishing white matter: deregulated integrated stress response as therapy target. Ann Clin Transl Neurol 2019;6:1407-1422.

7. Holmes B, Brogden RN, Heel RC, Speight TM, Avery GS. Guanabenz. A review of its pharmacodynamic properties and therapeutic efficacy in hypertension. Drugs 1983;26:212-229.

8. Tsaytler P, Harding HP, Ron D, Bertolotti A. Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. Science 2011;332:91-94.

9. Jiang HQ, Ren M, Jiang HZ, et al. Guanabenz delays the onset of disease symptoms, extends lifespan, improves motor performance and attenuates motor neuron loss in the SOD1 G93A mouse model of amyotrophic lateral sclerosis. Neuroscience 2014;277:132-138.

10. Wang L, Popko B, Tixier E, Roos RP. Guanabenz, which enhances the unfolded protein response, ameliorates mutant SOD1-induced amyotrophic lateral sclerosis. Neurobiol Dis 2014;71:317-324.

11. Dooves S, Bugiani M, Wisse LE, Abbink TEM, van der Knaap MS, Heine VM. Bergmann glia translocation: a new disease marker for vanishing white matter identifies therapeutic effects of Guanabenz treatment. Neuropathol Appl Neurobiol 2018;44:391-403.