

Repurposing mirtazapine in Rett syndrome: a multicentric open label Phase II study
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Statistical Analysis Plan

This is an open-label study. No randomization will be required.

All patient data collected on the CRF will be listed by patient and site.

Summaries of continuous variables will include descriptive statistics (number of non-missing observations sample size [n], mean, standard deviation [SD], median, minimum and maximum) and for categorical variables (the number of non-missing observations frequency [n] and percentage), unless otherwise stated in the relevant section. Percentages will be based on the number of patients within the relevant analysis population, or the number of patients with data available where relevant.

A Statistical Analysis Plan (SAP) will be finalized prior to database lock. The SAP will describe in detail study endpoints and statistical analyses, including the analysis of the primary as well as additional endpoints. In case changes to the original primary endpoint or of the original primary analyses occurs during the study, these changes will be the subject of a substantial protocol amendment. All statistical analyses not pre-specified and run after database lock will be considered additional/exploratory analyses.

The default significant level will be (5%); confidence intervals will be 95% and all tests will be two-sided, unless otherwise specified in the description of the analyses.

The study will follow the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline. The statistical analyses will be performed using SPSS version 29.0 (IBM SPSS Statistics for Windows, Version 29.0. Armonk, NY: IBM Corp).

Statistical methods and time-points

For the inferential comparison of primary and secondary endpoints the following tests will be proposed.

Primary endpoint	Inferential test	Time-points
Adverse events (AEs) and serious adverse events (SAEs), both related and non-related.	Descriptive statistics, no inferential statistical test is proposed.	Study follow-up from Baseline (T0) to 24 Weeks (T3).
Motor-Behavior Assessment Scale (MBAS)	The mean change from baseline (T0) in MBAS, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the paired t-test will be replaced by Wilcoxon Signed Rank test.	4 Weeks (T1) 12 Weeks (T2) Primary endpoint analysis: 24 Weeks (T3)
Secondary endpoints	Inferential test	Time-points
Anxiety, Depression, and Mood Scale (ADAMS)	The mean change from baseline (T0) in ADAMS, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the paired t-test will be replaced by Wilcoxon Signed Rank test.	4 Weeks (T1) 12 Weeks (T2) 24 Weeks (T3)
Rett Syndrome Behaviour Questionnaire (RSBQ)	The mean change from baseline (T0) in RSBQ, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the paired t-test will be replaced by Wilcoxon Signed Rank test.	4 Weeks (T1) 12 Weeks (T2) 24 Weeks (T3)
Sleep disturbances scale for children (SDSC)	The mean change from baseline (T0) in SDSC, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the paired t-test will be replaced by Wilcoxon Signed Rank test.	4 Weeks (T1) 12 Weeks (T2) 24 Weeks (T3)

Increased Purposeful Hand Function scale (IPHF)	The mean change from baseline (T0) in IPHF, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the paired t-test will be replaced by Wilcoxon Signed Rank test.	4 Weeks (T1) 12 Weeks (T2) 24 Weeks (T3)
Clinical Global Impression Scale (CGI-C)	The mean change from baseline (T0) in CGI-C, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the paired t-test will be replaced by Wilcoxon Signed Rank test.	4 Weeks (T1) 12 Weeks (T2) 24 Weeks (T3) 1 month after the last dose (T4)
Assessment of the severity (RCSS)	The mean change from baseline (T0) in RCSS, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the paired t-test will be replaced by Wilcoxon Signed Rank test.	4 Weeks (T1) 12 Weeks (T2) 24 Weeks (T3)
Respiratory parameters: number of apnoeas-hypoapnoeas	The mean change from baseline (T0) in the number of apnoeas-hypoapnoeas, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the paired t-test will be replaced by Wilcoxon Signed Rank test.	4 Weeks (T1) 12 Weeks (T2) 24 Weeks (T3)
Parenting Stress Index (PSI-SF)	The mean change from baseline (T0) in PSI-SF, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the paired t-test will be replaced by Wilcoxon Signed Rank test.	12 Weeks (T2) 24 Weeks (T3)
Biomarkers: BDNF, GDNF and PDGF.	The mean change from baseline (T0) in biomarker levels, will be tested using paired t-test. Assumption of Normality will be investigated using Wilk-Shapiro test. If the data is found to be non-Normal, the	12 Weeks (T2) 24 Weeks (T3)

	paired t-test will be replaced by Wilcoxon Signed Rank test.	
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All secondary endpoints will be presented in a descriptive manner using n, mean, median, SD, Q1, Q3, and minimum and maximum. The ITT will be the secondary efficacy analysis set.