1 TITLE PAGE

A multi-centre, randomised, parallel-group, single-blind Phase II trial to evaluate the pharmacokinetics and PKPD relationship of trazodone after single and repeated oral doses in children from 2 to ≤17 years of age, suffering from insomnia, with autism, intellectual disability or attention deficit hyperactivity disorder (ADHD)

Investigational Medicinal Product:	Trazodone
Indication Studied:	Insomnia
Sponsor:	Aziende Chimiche Riunite Angelini Francesco ACRAF S.p.A. (named also Angelini S.p.A.) Viale Amelia 70, 00181 Rome Italy
Protocol Number:	152PO17433
EudraCT Number:	2018-001166-42
Clinical Development Phase:	2
Study Initiation Date:	22 February 2019 (first patient screened)
Study Completion Date:	23 December 2019 (last patient completed)
Coordinating Investigator:	[redacted]
Sponsor Signatory:	Dr. Valeria Tellone Senior Study Manager – Global Clinical Development Specialty Care Dr. Alessandro Comandini Medical Director – Global Clinical Development Specialty Care Dr. Agnese Cattaneo Chief Medical Officer – Global Medical Department ACRAF S.p.A. Viale Amelia 70, 00181 Rome Italy
Version:	Final 1.0
Date of Report:	08 October 2021

This study was performed in compliance with Good Clinical Practice, including the archiving of essential documents.

CONFIDENTIAL

Part or all of the information in this report may be unpublished material. Accordingly, this report should be treated confidentially and restricted to its intended use. Should any portion of this unpublished material be desired for purposes of publication, authorisation must be obtained from Aziende Chimiche Riunite Angelini Francesco ACRAF S.p.A.

2 SYNOPSIS

NAME OF THE SPONSOR: ACRAF S.p.A.	**NAME OF FINISHED PRODUCT:** Trazodone	**NAME OF ACTIVE INGREDIENT:** Trazodone hydrochloride
Title of Study: A multi-centre, randomised, parallel-group, single-blind Phase II trial to evaluate the pharmacokinetics and PKPD relationship of trazodone after single and repeated oral doses in children from 2 to ≤17 years of age, suffering from insomnia, with autism, intellectual disability, or attention deficit hyperactivity disorder (ADHD)		
Coordinating Investigator: [redacted]		
Study Centre(s): 5 centres in Italy and 2 centres in Spain		
Publication(s) (reference): None		
Study Period: 22 February 2019 – 23 December 2019	**Phase of Development**: 2	

Objectives:

Primary Objective:

The primary objective of this study was to assess the pharmacokinetics (PK) of trazodone after single and repeated doses in patients aged from 2 to ≤17 years.

Secondary Objectives:

The secondary objectives of this study were:

- To establish the PK-pharmacodynamic (PKPD) relationship of trazodone, as assessed by actigraphy measures
- To evaluate the concentration-QT interval correlation
- To define the dose rationale in children and adolescents aged from 2 to ≤17 years taking into account the therapeutic exposure range in adults
- To evaluate the safety and tolerability of trazodone in children and adolescents aged from 2 to ≤17 years
- To assess the palatability of trazodone

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Methodology:
This multi-centre, single-blind, parallel-group, randomised Phase II clinical trial was designed to assess the PK and PD of 3 dose levels of trazodone in children with neurodevelopmental disorders (NDDs) (autism, intellectual disability, or attention deficit hyperactivity disorder [ADHD]). After consent from the parent(s) had been obtained and assent from the patients had been documented, the patients were screened for eligibility at the screening visit. Patients qualifying for participation in the study at Visit 1 (Day 1, randomisation; first dose) were randomly assigned to one of 3 treatment arms and received treatment for a maximum of 10 days. Patients were admitted to the clinic for 2 of 3 visits according to a patient-specific PK sampling scheme: Visits 1 + 3 or Visits 2 + 3. Each patient was planned to contribute a total of 5 PK samples collected between 5 to 15 minutes before dosing and up to 12 hours after dosing. Patients were required to remain at the hospital until the subsequent day for all applicable clinical assessments and collection of PK blood samples. The last visit (Visit 4; final assessment) was planned to be performed within 7 days (±2 days) from Visit 3.

At the start of the screening period, an actigraphy device was delivered to the patient and used for at least 1 week to ensure that the patient had become familiarised with device use before the start of the treatment phase. Sleep latency and total sleeping time were recorded by actigraphy starting from 3 consecutive days before Visit 1 up to the end of the treatment. Adaptation to the actigraphy device was monitored by the study staff via a phone call 3 days before Visit 1 to ensure that only patients who complied with the protocol procedures proceeded to the randomisation phase.

The study was conducted in a single-blind fashion. Electrocardiogram (ECG) measurements and readings were performed by persons blinded to associated treatments.

The study protocol was based on the enrolment of at least 30 evaluable patients. An interim analysis was performed after treatment completion of 18 patients to assess whether data collected allowed determination of the relevant PK parameters with the appropriate precision (relative SE < 40%) and establish the need to evaluate a different dose level if systemic exposure in younger patients appeared to be significantly lower than anticipated.

During the treatment phase, patients were provided with a paper diary. The parent(s) were asked to document details about the administration of trazodone (time), adverse events (AEs), concomitant medication use, and sleep-related events (i.e., the time in and out of bed, time for lights out, and when the actigraph was placed and removed) on a daily basis. A visual analogue scale (VAS) for palatability assessment was also included.

Number of Patients (Planned and Analysed):
Planned: A minimum of 36 patients (at least 30 evaluable patients, 10 for each treatment arm); at least 10 evaluable patients aged 2 to ≤5 years, 10 evaluable patients aged 6 to ≤11 years, 10 evaluable patients aged 12 to ≤17 years; at least 3 patients for each age and treatment arm.
Actual: 18 patients received the investigational medicinal product (IMP) (Arm 1: 3 patients; Arm 2: 5 patients; Arm 3: 10 patients)
Completed: 18 patients
Analysed: 18 patients (safety and PK analysis sets); 13 patients (PKPD analysis set)

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Diagnosis and Main Criteria for Inclusion:

Children and adolescents from 2 to ≤17 years of age with insomnia and affected by autism, intellectual disability, or ADHD.

Main inclusion criteria:

- Patient was male or female, 2 to 17 years of age (inclusive)
- Patient diagnosed with autism, intellectual disability, or ADHD according to International Statistical Classification of Diseases (ICD-10) (F84.0, F90.9, F79) or Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) (299.00, 314.01, 319) criteria
- Patient diagnosed with insomnia according to the International Classification of Sleep Disorders, 3rd edition (ICSD-3) criteria
- Sleep Disturbance Scale for Children with a total score of >60
- Patient on a stable therapy for their primary NDDs, apart from medications specified in the exclusion criteria
- Patient taking any sleep-inducing medication had completed the required washout period of 7 days

Main exclusion criteria:

- Patient with ascertained or presumptive hypersensitivity to trazodone and/or its excipients
- Patient with history of anaphylaxis to drugs or allergic reactions in general, which the investigator considered could have affected the outcome of the study
- Patient treated with any form of trazodone within 2 weeks before the inclusion in the study
- Patient not responding to previous trazodone-based therapy based on past medical history records in the last 2 years
- Patient taking any medications (except those foreseen for their primary NDDs) that prolong the QT/corrected QT interval or included in the "Interactions with other medicinal products and other forms of interaction" of trazodone Summary of Product Characteristics within 2 weeks before the start of the study and during the study duration
- Patient was a female affected by Rett syndrome
- Patient with a previous diagnosis of HIV, hepatitis B virus surface antigen, or hepatitis C virus
- Patient with corrected QT interval using Fridericia´s formula (QTcF) value ≥440 msec for male and ≥450 msec for female for all age groups
- Patient with history of risk factors for torsade de pointes (e.g., heart failure, hypokalaemia, family history of long QT syndrome, cardiac arrhythmias, bradycardia, cardiac conduction abnormalities, cardiac hypertrophy, cardiomyopathy, chronic cardiac insufficiency)
- Patient with physical abnormalities or clinically significant abnormal laboratory test results relevant for the study assessments or patient's safety
- Patient with history of significant renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine, or neurological diseases that may have interfered with the aim of the study
- Patient with any condition (surgical or medical) that would have affected the absorption, distribution, metabolism, and/or excretion of the IMP
- Patient with a diagnosis of epilepsy or with 1 or more seizures in the 12 months before screening

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Investigational Medicinal Product, Dose, Mode of Administration, and Batch Number(s):

Initial proposed doses of a new formulation of trazodone hydrochloride (HCl) oral drops (trazodone HCl 1.5% [for patients aged 2 to ≤5 years] and 3% [for patients aged 6 to ≤17 years]), with an escalation step for doses 2 and 3 before administration of the final assigned dose:

Dose 1: 0.25 mg/kg/day (on Days 1 through 10)

Dose 2: 0.4 mg/kg/day (0.25 mg/kg/day on Days 1 through 3 and 0.4 mg/kg/day on Days 4 through 10)

Dose 3: 0.5 mg/kg/day (0.25 mg/kg/day on Days 1 through 3 and 0.5 mg/kg/day on Days 4 through 10)

Duration of Treatment:
Patients received trazodone according to a once daily regimen for 10 days.

Reference Therapy, Dose, Mode of Administration, and Batch Number(s): Not applicable.

Criteria for Evaluation:

Pharmacokinetics: Primary PK parameters for trazodone after single and repeated oral administration of trazodone also included apparent oral clearance, intercompartmental clearance, apparent volume of distribution, and absorption rate constant. Secondary parameters were derived from model-predicted profiles: area under the plasma concentration-time curve extrapolated to infinity (AUC), maximum plasma concentration (C_{max}), minimum plasma concentration (C_{min}), maximum plasma concentration at steady state (C_{ss}), trough plasma concentration, and time at which C_{max} was reached.

Pharmacodynamics: Exploratory analysis of the data included correlation between PK parameters (AUC, C_{ss}, C_{min}, and C_{max}) and clinical endpoints (sleep latency time, sleep efficiency, and total sleeping time), as assessed by actigraphy, and exploratory analysis of concentration-QT interval correlation. If data allowed, PKPD modelling was planned to be applied to derive relevant parameters, such as potency and maximum effects on sleep latency and total sleep time from baseline levels.

Safety: Safety assessments included physical examination, vital signs, 12-lead ECG, clinical laboratory values, and AEs.

Statistical Methods:
All data obtained in this study and documented in the electronic case report forms are listed and summarised using descriptive statistics or frequency tables, as appropriate.

Nonlinear mixed-effects modelling was used to analyse the concentration and clinical response data (i.e., PKPD relationship) and explore a potential correlation between trazodone concentrations and QT interval changes in this population.

Individual AEs are listed in patient data listings. No summary table is provided for pretreatment AEs. Treatment-emergent AEs (TEAEs) are summarised by treatment and overall. Summary statistics for the absolute vital sign values and body weight and the changes from baseline are presented by treatment arm for each visit. Laboratory safety parameters are summarised using descriptive statistics at baseline and each postbaseline time point. Results of the physical examination are listed and summarised. Baseline and change from baseline in ECG parameters (heart rate, PR interval, QRS interval, QT interval, and QTcF interval) are summarised. Results of the palatability assessment are listed and analysed descriptively.

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Summary-Conclusions:

Patient Disposition and Demographics:
Eighteen patients with NDDs and sleep disorders were enrolled and received the IMP. All patients completed the study as planned. The median patient age was 9 years (range: 5 to 17 years); 16 patients (89%) were male, and most were White (17 patients, 94%).

Pharmacokinetics:
The observed trazodone plasma concentration vs. time profiles showed large intra-individual variability. However, proportionality was observed in the dose-concentration relationship when comparing different dosing groups. Despite the lack of details on the exact time of food intake and contents of the meals provided to the patients during clinic visits, the variability in the data does not seem to be associated with food. The samples collected around peak concentrations did not show higher or unexpected variability relative to the remaining data. Evidence of such variation in the data partly determined the choice for the use of noninformative priors for the final PK analysis. The PK could be adequately described by a 2-compartment model, including an allometric function to characterise the effect of body weight on drug disposition. Weight was the only covariate to have a significant effect on clearance, volume of distribution of the central compartment, volume of distribution of the peripheral compartment and intercompartmental clearance.

Whilst allometric principles have been used to describe the effect of body weight on the disposition parameters, the correlation between model parameters and covariate are compounded by other sources of variability. Moreover, the magnitude of the unexplained variability appeared to be independent to the dose administered. Additionally, even though the small sample size does not allow a full assessment of the distributional properties of model parameters, interindividual random effects appeared to be normally distributed around the zero value.

Pharmacodynamics:
Sleep onset latency (SOL) and total sleep duration showed no clear changes over time or differences between treatment arms.

Sleep Onset Latency and Total Sleep Duration (Actigraphy Measurements) (Pharmacokinetic/Pharmacodynamic Analysis Set)

Day	Arm 1 (0.25-0.25 mg/kg) (N = 2) Median (min, max)	Arm 2 (0.25-0.4 mg/kg) (N = 4) Median (min, max)	Arm 3 (0.25-0.5 mg/kg) (N = 7) Median (min, max)	Total (N = 13) Median (min, max)
Sleep onset latency (minutes)				
Day 1	3.0 (2, 5)	4.5 (0, 9)	10.0 (0, 27)	4.5 (0, 27)
Day 9	0.0 (0, 0)	3.0 (2, 33)	24.0 (4, 39)	5.0 (0, 39)
Day 10	10.3 (2, 19)	10.8 (7, 30)	15.0 (0, 198)	12.0 (0, 198)
Total duration of sleep (minutes)				
Day 1	439.8 (404, 476)	511.3 (431, 597)	479.5 (320, 518)	479.5 (320, 597)
Day 9	499.8 (463, 537)	442.8 (421, 612)	489.0 (382, 566)	473.5 (382, 612)
Day 10	273.5 (192, 356)	429.3 (225, 458)	427.5 (151, 499)	416.0 (151, 499)

Abbreviations: max=maximum; min=minimum.

Population Pharmacokinetic and Pharmacokinetic/Pharmacodynamic Modelling, Exposure-Response Analysis for Sleep Parameters, and Concentration-QTcF Analysis:

Correlation between Actigraphy Measures and Trazodone Exposure

Initially, an exploratory analysis was performed with the available actigraphy measurements (i.e., onset latency, sleep efficiency and total sleep time). Based on this initial evaluation, it became evident that at the tested doses, trazodone does not seem to affect SOL. Similarly, no clear correlations were observed between trazodone exposure and sleep efficiency or total sleep time. The lack of clinically relevant changes in these actigraphy measures might be due the enrolment of individuals with relatively long total sleep time and very short sleep latency at baseline. Despite these findings, further inspection of the actigraphy data suggests a shift in the distribution of the total sleep time in subjects receiving the higher dose level.

Concentration-QTcF-Interval Analysis

The concentration-QTcF analysis focused on the correlation between trazodone concentrations in plasma and changes in QTcF relative to baseline (i.e., ΔQTcF). As such, individual data comprised at least one baseline measurement (i.e., pretreatment recording) and one measurement during the treatment period for each subject. Summary statistics of ECG parameters (RR and QTc interval) showed a range of values compatible with normal physiological variation for the age range of the study population. Variation in QTcF interval after the start of the treatment does not differ from QTcF distribution at baseline or study visit (i.e. Visit 1, Visit 2, and Visit 3); similarly, there are no patterns in the change from baseline (ΔQTcF) during the course of treatment.

There was no correlation (R = 0.077, p = 0.37) between trazodone concentrations and QTcF interval. The linear regression indicates that changes relative to baseline in QTcF interval do not reach 10 ms with increasing concentrations up to 500 ng/mL. Even though the current analysis was based on automated readings and time-matched individual predicted concentrations, these results are in agreement with previous findings in a thorough QT study in healthy subjects.

Safety Results:

As shown in the following brief summary of the AEs, there were no serious AEs reported and no AEs that the investigator assessed as possibly or definitely related to the intake of the IMP.

Overview of Adverse Events (Safety Analysis Set)

	Arm 1 (0.25-0.25 mg/kg) (N = 3)	**Arm 2 (0.25-0.4 mg/kg) (N = 5)**	**Arm 3 (0.25-0.5 mg/kg) (N = 10)**	**Total (N = 18)**
	n (%) m	**n (%) m**	**n (%) m**	**n (%) m**
Any AE	1 (33.3) 1	3 (60.0) 9	5 (50.0) 13	9 (50.0) 23
AEs starting before first IMP intake	1 (33.3) 1	2 (40.0) 3	3 (30.0) 5	6 (33.3) 9
Any TEAE	0	1 (20.0) 6	2 (20.0) 5	3 (16.7) 11
Any TEAE related to the IMPa	0	0	0	0
Any SAE	0	0	0	0
Any SAE related to the IMPa	0	0	0	0

Abbreviations: AE=adverse event; m=number of events; IMP=investigational medicinal product; SAE=serious adverse event; TEAE=treatment-emergent adverse event.
Notes: Patients reporting >1 AE were counted only once for the patient count. Percentages are based on total number of patients in each treatment group. Treatment emergence is defined as AEs occurring at/after or worsening after the first administration of the IMP.
[a] IMP-related AEs: AEs that were assessed by the investigator as possibly, probably/likely, or certainly related to administration of the IMP. In case of a missing relationship, the AE was classified as "related".

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All TEAEs were mild, and no AE resulted in death or led to withdrawal of the patient from the study or discontinuation of treatment.

Treatment-Emergent Adverse Events by System Organ Class and Preferred Term (Safety Analysis Set)

Any AEs	0	1 (20.0) 6	2 (20.0) 5	3 (16.7) 11
General disorders and administration site conditions	0	0	1 (10.0) 1	1 (5.6) 1
Pyrexia	0	0	1 (10.0) 1	1 (5.6) 1
Metabolism and nutrition disorders	0	0	1 (10.0) 1	1 (5.6) 1
Decreased appetite	0	0	1 (10.0) 1	1 (5.6) 1
Respiratory, thoracic, and mediastinal disorders	0	0	1 (10.0) 2	1 (5.6) 2
Cough	0	0	1 (10.0) 1	1 (5.6) 1
Oropharyngeal pain	0	0	1 (10.0) 1	1 (5.6) 1
Skin and subcutaneous tissue disorders	0	0	1 (10.0) 1	1 (5.6) 1
Rash erythematous	0	0	1 (10.0) 1	1 (5.6) 1
Vascular disorders	0	1 (20.0) 6	0	1 (5.6) 6
Flushing	0	1 (20.0) 6	0	1 (5.6) 6

Abbreviations: AE=adverse event; m=number of events.
Notes: Adverse events were coded using the Medical Dictionary for Regulatory Activities, Version 21.1. At each level of summation (system organ class and preferred term), patients reporting >1 AE were included only once. The table is sorted by descending patient count in the total column by system organ class and preferred term. Percentages are based on total number of patients in each treatment group. Treatment emergence is defined as an AE occurring at/after or worsening after the first administration of the investigational medicinal product.

No safety issues were detected in ECG data, physical examinations, or other safety assessments.

The overall median palatability rating was (approximately) 50 mm on the 100 mm VAS (range: 0 to 100 mm).

Conclusions:

This study was prematurely stopped after an interim analysis had revealed that an increase of the dose would be needed to obtain the desired effect. From the data obtained, the following conclusions were drawn:

The PK of trazodone in children from 5 to ≤17 years of age could be described using a two-compartment population PK model with first-order absorption and elimination. Even though body weight was implemented as a covariate on disposition parameters based on allometric principles, interindividual variability in the exposure to trazodone could be only partly explained by differences in body weight. Due to the limited samples size, no other baseline demographic characteristics were identified as potential explanatory factors. Overall, the PK of trazodone in this group of patients was dose-proportional. However, only subjects receiving 0.4 and 0.5 mg/kg doses reached exposure levels that are comparable to those observed in adult subjects (studies 44007 and 48449, refer to Section 3.5.5 of the final Clinical Pharmacology Study Report in Appendix 16.1.13) after administration of 30 mg oral dose.

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Actigraphy data showed that despite the diagnosis of insomnia, SOL was zero in many patients, preventing the characterisation of drug effects on this parameter. Similarly, weak or non-significant correlations were observed between trazodone exposure and total sleep time. Assuming that there were no protocol procedures that could interfere with the treatment, the absence of a clinically relevant effect on actigraphy measures may be explained by 2 possible factors, namely, patient population baseline characteristics or insufficient exposure. As a significant proportion of patients included into the study had a total sleep time >7 hours at baseline, one cannot disregard the fact that detection of treatment effect may be more difficult in this subgroup of patients. In contrast to previous findings using polysomnography in adult subjects, the mean peak concentrations in this study were significantly lower than trazodone levels associated with changes in slow wave sleep.

No trends in the ECG measures were observed between titration and maintenance period. Absolute QTcF interval variation appeared to be unrelated with either dose or study visits. No significant correlation was observed between trazodone concentrations and changes relative to baseline in QTcF interval following administration of 0.25, 0.4 and 0.5 mg/kg trazodone oral drops. The absence of any clinical signal across the tested dose range supports the use of 1269 ng/mL, as reference threshold for the evaluation of trazodone effects on QT interval in paediatric patients.

The data collected show that, overall, the investigational medicinal product was safe and well tolerated. However, clear changes over time or differences between treatment arms were not detectable for either sleep parameter.

Date of Report: Final 1.0, 08 October 2021