FORM-STAT-04A-01	Statistical Analysis Plan
Version 1.0	

FAB Trial - Fluoxetine for Autistic Behaviours

Multi-site randomised controlled trial of Fluoxetine versus placebo for the treatment of restricted, repetitive and stereotyped behaviours in children and adolescents with autism

Document Version History

Version Date	Version	Author	Change Description	Reason/Comment
25-Sep-17	Version 1	Francesca Orsini	Initial release.	Not applicable.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
ASD	Autism spectrum disorder
CEBU	Clinical epidemiology and biostatistics unit
CI	Confidence interval
CRF	Case Report Form
CYBOCS-PDD	Children's Yale-Brown Obsessive Compulsion Scale–Pervasive Developmental Disorders
DSM-IV TR	Diagnostic and Statistical Manual
GCP	Good Clinical Practice
ITT	Intent-To-Treat
MAOI	Monoamine oxidase inhibitor
SAE	Serious Adverse Event
SD	Standard Deviation
SSRI	Selective Serotonin Reuptake Inhibitors

1. STUDY OBJECTIVES

1.1. PRIMARY OBJECTIVE

To determine the efficacy of fluoxetine, compared to placebo, for reducing the frequency and severity of restricted, repetitive and stereotypic behaviours in children and adolescents with autism.

1.2. SECONDARY OBJECTIVES

- To determine the frequency and type of adverse events reported for low dose fluoxetine compared to placebo, in children and adolescents with autism.

2. BACKGROUND/INTRODUCTION

2.1. STUDY DESIGN

This study is a randomised double-blind placebo-controlled trial with a parallel group design. Treatment for both the active and control groups will be of 16 weeks duration. Participants will then be weaned off the study medication over 4 weeks and followed up for a further 2 weeks.

2.2. TREATMENT GROUPS

Participants randomised to the active group will receive fluoxetine (fluoxetine hydrochloride dissolved in methocel base). Participants in the control group will receive placebo syrup (methocel base only), which is indistinguishable from the active treatment.

The study will include 146 children and adolescents who will be randomised between the fluoxetine and placebo groups. Block randomisation will be used, and stratified by site, age and IQ. There are three sites:

- Royal Children's Hospital (Victoria)
- Children's Hospital at Westmead (New South Wales)
- State Child Development Centre (Western Australia).

Age will be stratified into:

- 7.5 to <12 years
- 12 to 18 years.

IQ will be stratified into:

- IQ ≥70 (average/borderline range)
- IQ <70 (intellectual disability range).

Parents/guardians, participants, and study investigators will remain blinded to treatment allocation until the database has been locked for analysis and the analysis plan is finalised. The randomisation schedule will be known only to the independent statistician in the clinical epidemiology and biostatistics unit (CEBU) at the Royal Children's Hospital who generated the randomisation list and the clinical trials pharmacist at each site. In an emergency, a participant can be unblinded by a study doctor contacting the clinical trials pharmacist at that site.

2.3. STUDY POPULATION

Children and adolescents aged between 7.5 and less than 18 years with an autism spectrum disorder (ASD), and associated restricted, repetitive and stereotyped behaviours causing functional impairment.

Inclusion criteria

- Males and females aged 7.5 to 18 years.
- A diagnosis of an ASD based on the Autism Diagnostic Interview (ADI-R), and Diagnostic and Statistical Manual (DSM-IV TR) criteria for Autistic Disorder (as above), Asperger's Disorder and Pervasive Developmental Disorder – Not Otherwise Specified.
- Total score of ≥ 6 on the Children's Yale-Brown Obsessive Compulsive Scale – modified for pervasive developmental disorders
- Will be able to comply with the assessments and procedures required for the trial.

Exclusion criteria

- A known DSM-IV diagnosis of Rett's Disorder, Childhood Disintegrative Disorder, Schizophrenia or Major Depression.
- Patients currently prescribed or who have received in the six week period prior to study entry:
    - Fluoxetine and other Selective Serotonin Reuptake Inhibitors (SSRI)
    - Other psychotropic medications, including typical and atypical anti-psychotics, mood stabilisers and anxiolytics.
    - Atomoxetine
    - Monoamine oxidase inhibitor (MAOI) or pimozide
    - Antidepressants
    - Use of St John's Wort
- Co-morbid significant medical conditions (e.g. unstable seizure disorder, cardiac disease, liver failure or renal failure).
- Pregnancy –females of childbearing potential require a urine pregnancy test to exclude pregnancy.

2.4. INTERVENTION

For Study Medication and Dosage refer to Protocol Version 4 (24 May 2015).

2.5. SAMPLE SIZE

The validation of the Children's Yale-Brown Obsessive Compulsion Scale – Modified for Pervasive Developmental Disorders (CYBOCS-PDD) in 172 medication-free children with an ASD found a mean total score of 14.4 with a standard deviation (SD) of 3.86. Based on the study investigators' clinical experience in the management of children with autism, a difference of 2 on the CYBOCS was considered to represent a clinically important improvement in repetitive behaviours. Consequently, we aimed to power our study to find an effect size of 0.5 (corresponding to a difference of 2 on the CYBOCS based on a standard deviation of 3.8). With 80% power and two-sided alpha of 0.05 this required a sample size of 64 per treatment group. Allowing for a 15% drop-out rate, 73 participants were required per treatment group. We therefore aimed to recruit a total of 146 participants to this study.

2.6. STUDY PROCEDURE

The table below provides an overview of the procedures for this study. For complete details on study procedures refer to Protocol Version 4.3 (24 April 2014).

Procedure	Initial Screening	Testing [Pre]	Week 1	Week 2	Week 3	Week 4	Weeks 6,8,10, 12,14,16	Testing [Post]	Week 17-20	Week 22
Inc & Exc Criteria	❑									
Consent		❑								
Randomization		❑								
Trial Questionnaires		❑						❑		
IQ Testing		❑								
ADI-R		❑								
Medication Commenced			❑							
Dose Adjustments				❑	❑	❑				
Side Effects Checklist				❑	❑	❑	❑		❑	❑
Weaning of medication									❑	

2.7. PATIENT COMPLETION AND WITHDRAWAL

Subjects will be considered to have completed the study medication upon completion of 16 weeks of the study drug. Premature termination of the study drug will be defined as completing less than 16 weeks of treatment. Even if the study drug is terminated prior to 16 weeks, every effort will be made by the investigator to continue the follow-up of the patients in the study until study completion (week 22). A patient may withdraw (or be withdrawn) from treatment prematurely for the following reasons:

- Significant adverse events (serious adverse event form must be completed)
- Patient/caregiver decision to discontinue the treatment
- Patient lost to follow-up

Patients may also withdraw their consent to be in the study at which point they will be withdrawn from the study treatment and follow-up. They may also withdraw their consent for previously collected data being used for analysis.

The reason for withdraw from treatment and/or the study will be recorded on the patient completion/withdrawal section of the case report form (CRF). Every effort will be made to follow up patients who withdraw from treatment with drug-related adverse experiences, in order to determine the final outcome.

3. OUTCOME VARIABLES

3.1. PRIMARY OUTCOME

Children's Yale-Brown Obsessive Compulsion Scale – Modified for Pervasive Developmental Disorders (CYBOCS-PDD) - measured at 16 weeks. The CYBOCS-PPD includes a detailed symptom checklist of possible obsessions and compulsions, which are then rated across five severity items (time spent on obsessions, interference, distress, resistance, and degree of control). The total score on the CYBOCS-PDD ranges from 0 to 20, with higher scores indicating higher severity. The CYBOCS was modified for use in children with a pervasive developmental disorder by the RUPP Autism Network. Both the CYBOCS-PDD and YBOCS (adult version) have been widely used in clinical drug trials in autism, and shown to be sensitive to change. The CYBOCS-PDD is the primary outcome measure for this trial, in line with previous trials in this field.

3.2. SECONDARY OUTCOMES

All the secondary outcomes are measured at 16 weeks.

Repetitive Behaviours Scale – Revised (RBS-R). This revised version of the scale captures the breadth of repetitive behaviours in autism. The RBS-R consists of six subscales: stereotypic behaviours (range: 0-18), self injurious behaviours (range: 0-24), compulsive behaviours (range: 0-24), ritualistic behaviours (range: 0-18), sameness behaviours (range: -33), and restricted behaviours (range: 0-12). Higher scores indicate higher severity. Recently the RBS-R was validated in both children and adults with autism.

Spence Children's Anxiety Scale (SCAC). This scale consists of a child and parent version. Each scale contains six subscales (panic/agoraphobia, social anxiety, separation anxiety, generalised anxiety, obsessions/compulsions, and fear of physical injury) and provides a total score which ranges 0-114, with higher score indicating higher severity. The validity and reliability of the Spence (child and parent versions) has been established for both anxiety-disordered and normal control populations.

Aberrant Behaviour Checklist (ABC) – Community Version. The ABC assesses maladaptive behaviours in individuals with a developmental disability or intellectual impairment. The ABC items are grouped into five subscales: irritability/agitation (range: 0-45), lethargy/social withdrawal (range: 0-48), stereotypic behaviour (range: 0-21), hyperactivity/non-compliance (range: 0-48) and inappropriate speech (range: 0-12). Higher scores indicate higher severity. The ABC has been widely used in clinical drug trials in autism. The factor structure of the ABC – community version has been found to be robust in an ASD sample of 275 individuals aged 3 to 21 years.

Clinical Global Impressions Scale (CGI) – Global Improvement and Efficacy Index. The CGI29 is a scale widely used to assess treatment response in psychiatric conditions. It is a three-item scale: severity of illness (rated on a 7-point scale), global improvement (rated on a 7-point scale), and efficacy index (rated on a 16-point scale). The CGI has been widely used in clinical drug trials in autism, and shown to be sensitive to change.

Disruptiveness assessment. This assessment will collect information in regards to Anger/Aggression, Anxiety, Sadness/Depression, Excitability/Overexcited, ADHD each of which will be coded as (0= not a problem, 1= some problem, 2= significant problem). The outcome of interest is the total score of disruptiveness which is a sum of the 5 scores (range: 0-10).

3.3. OTHER PARAMETERS

DEMOGRAPHY AND BASELINE

- Child age (years), weight (kg) and height (m) at baseline
- Gender
- IQ at baseline
- Primary diagnosis (Autism/Asperger's Syndrome/Autism Spectrum Disorder)
- Presence of intellectual disability (y/n)
- Level of intellectual disability (mild/moderate/severe/unknown severity)
- Communication level (Normal language for age/Impaired speech and language/No speech)
- Pre-existing medications (y/n), in particular stimulant medications such as Methylphenidate, Dexamphetamine, Concerta, Atomoxetine, Lysdexamphetamine.
- Medical history: Family history of ASD (y/n), history of seizures (y/n), presence of epilepsy (y/n), frequency of seizures (~1/week, ~1/month, ~1/year, < 1year)

COMPLIANCE

An estimate of compliance to treatment will be calculated for each participant using this formula:

$$\frac{(\text{total amount of doses that should have been taken} - \text{number of missed doses})}{\text{total amount of doses that should have been taken}} * 100$$

SAFETY

- Non-serious adverse event
- Serious adverse event

4. STATISTICAL METHODOLOGY

4.1. GENERAL METHODOLOGY

Data analysis for this study will be performed by Ms Francesca Orsini, an experienced biostatistician who works in CEBU at the Murdoch Children's Research Institute (MCRI).
The details of the randomisation will be unblinded only once the database has been locked and the Statistical Analysis Plan has been finalised and approved by the trial team.

4.1.1. Population for analysis

Analysis will be performed on an intention-to-treat (ITT) basis. Children will be compared according to the group to which they were randomly allocated, regardless of treatment compliance, crossover to other treatments or withdrawal from the study. This approach preserves the prognostic balance in the study arms achieved by randomisation.

4.1.2. Baseline data

Baseline characteristics will be presented separately for children in the active and placebo groups using means and standard deviations (SD) for continuous data (or medians and inter-quartile ranges for non-normal data) and proportions for categorical data.

4.1.3. Distributional assumptions

- *Censored normal distribution outcomes* - Tobit regression models will be used to confirm the sensitivity of linear regression to a non-normal distribution for outcomes with a censored normal distribution (CGI Severity, CGI Improvement, CGI Efficacy, ABI V).

- *Non-normally distributed outcomes* - The outcome will be log-transformed and linear regression model will be run on the logarithmic scale to confirm the sensitivity of linear regression to a non-normal distribution.

4.1.4. Handling of missing data

The primary analysis will be a complete case analysis. Should the proportion of missing data on the primary outcome be more than 5%, the frequency and patterns of missing data will be examined and, if deemed appropriate, a sensitivity analyses will be performed to compare the results of analyses restricted to children with complete data and analyses where those with missing data are considered using multiple imputation. Multiple imputation models will be conducted including all outcome variables in a single imputation model and 50 completed data sets will be imputed by chained equations including all the children initially randomised. The primary outcome and all the secondary ones listed in section 3.2 will be in the imputation model as well as the demography and baseline variable listed in section 3.3, using linear regression for continuous variables, logistic regression for binary variables, ordered logistic regression for ordinal variables, and multinomial logistic regression for nominal variables.

4.1.5. Protocol violations and patient withdrawals

All deviations / violations to the protocol will be summarized separately for children in the active and placebo groups using absolute and relative frequencies. Analogously, all the reason for early withdrawal of the treatment/study will be summarized using absolute and relative frequencies by arm.

4.2. ANALYSIS OF THE PRIMARY OUTCOME

The total score on the CYBOCS-PDD at 16 weeks will be presented separately for children in the active and placebo groups using means and standard deviations (SD). Comparisons between treatments arms will be made using linear regression, with results presented as a mean difference and its 95% confidence interval (CI), adjusted for the stratification factors used during randomization, i.e. site, age at baseline and IQ.

As sensitivity analysis, the same linear regression model will then be re-run adjusting also for the following potentially confounding factors:

- CYBOCS-PDD total score at baseline
- Presence of intellectual disability
- Communication level (verbal versus non-verbal)
- Frequency of seizure (~ 1 /week, ~ 1 /month, ~ 1 /year, < 1 year)
- On stimulant medications at baseline (Methylphenidate, Dexamphetamine, Concerta, Atomoxetine or Lysdexamphetamine)
- Any baseline and demographic variables where an imbalance between groups is found

4.3. ANALYSIS OF THE SECONDARY OUTCOMES

All secondary outcomes (in particular RBS-R: subscales and total score; SCAC: total score; ABC: subscales; CGI: subscales; Disruptiveness Assessment: total score) will be presented separately for children in the active and placebo groups using means and SDs. Comparisons between treatments arms will be made using linear regression, with results presented as a mean difference and its 95%

CI, adjusted for the stratification factors used during randomization, i.e. site, age at baseline and IQ.

As sensitivity analysis, the same linear regression models will then be re-run adjusting also for the following potentially confounding factors:

- correspondent outcome score at baseline
- Presence of intellectual disability
- Communication level (verbal versus non-verbal)
- Frequency of seizure (~ 1 /week, ~ 1 /month, ~ 1 /year, < 1 year)
- On stimulant medications at baseline (Methylphenidate, Dexamphetamine, Concerta, Atomoxetine or Lysdexamphetamine)
- Any baseline and demographic variables where an imbalance between groups is found

4.4. SUBGROUP ANALYSIS

The score of the Spence Anxiety scales described in this document might be affected by the participant communication level, e.g some subscales might be unanswerable by non-verbal children, therefore left blank. For such scales, we will conduct the analysis described in section 4.3 separately for verbal (subgroup 1) and non-verbal children (subgroup 2).

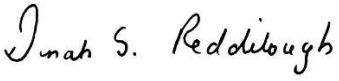
4.5. ANALYSIS OF THE SAFETY DATA

All adverse events (AEs) will be summarized separately for children in the active and placebo groups using absolute and relative frequencies.

4.6. ANALYSIS OF THE COMPLIANCE DATA

Compliance to treatment will be summarized separately for children in the active and placebo groups using absolute and relative frequencies.

SIGNATURES PAGE

Signature of Principal Investigator:  Date 26-09-2017

Print Name Prof Dinah Reddihough

Signature of Statistician performing the analysis:  Date 25-09-2017

Print Name Francesca Orsini

Signature of Trial Statistician:  Date 26-09-2017

Print Name Katherine Lee