The Benefit of Minocycline on Negative Symptoms in Psychosis: Extent and Mechanisms.

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List of Abbreviations

AE	Adverse Event
AIMS	Abnormal Involuntary Movements Scale
ALS	Amyotropic Lateral Sclerosis
ANNSERS	Antipsychotic Non-Neurological Side-Effects Rating Scale
ARMS	at risk mental states
APD	Antipsychotic drug
BMI	Body Mass Index
BOLD	Blood Oxygen Level Dependent
BRC	Biomedical Research Centre
CASE	Computer-Average Causal Effect
CRF	Case Report Form
CSO	Clinical Scientific Officer
DMEC	Data Monitoring and Ethics Committee
DUP	Duration of untreated psychosis
EME	Efficiency & Mechanism Evaluation Programme
EPI	Echo-planar images
fMRI	functional Magnetic Resonance Imaging
GAF	Global Assessment of Function
ITT	Intention to treat
MHRA	Medicines and Healthcare products Regulatory Agency
MHRN	Mental Health Research Network
MHSCT	Manchester Health & Social Care Trust
MINI	Mini-International Neuropsychiatric Interview
MNI	Montreal Neurological Institute
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
NMDA	N-methyl D-aspartate
NPU	Neuroscience and Psychiatry Unit
PANSS	Positive and Negative Syndrome Scale
PET	Positron Emission Tomography
PIL	Patient Information Leaflet
RA	Research Assistant
RCT	Randomised Controlled Trial
REML	Residual Maximum Likelihood
RM	Research Manager
RMO	Responsible Medical Officer
SAE	Serious Adverse Event
SFS	Social Functioning Scale
SLE	Systemic Lupus Erythematosis
SmPC	Summary of Product Characteristics
SMRI	Stanley Medical Research Institute
SURP-NoW	The Northwest Service User Research Advisory Group
SUSAR	Suspected Unexpected Serious Adverse Reaction

TSC	Trial Steering Committee
VF	Verbal Fluency

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1. BACKGROUND

1.1. Health problems to be addressed.

Patients with chronic schizophrenia have an impaired quality of life (QoL) with social isolation, self-neglect, unemployment and reduced activities of daily living, despite current treatments. Symptoms are grouped into positive, negative and disorganised. The negative symptoms reflect the absence or diminution of normal behaviours and functions, and include emotional and social withdrawal, anhedonia, lack of drive and deficiencies in emotional responsiveness. Negative symptoms persist and along with cognitive impairment and the duration of untreated psychosis (DUP) they fairly consistently relate to impaired social functioning (1). Findings such as the correlation between DUP and negative symptoms suggest that active psychosis may reflect a neuropathic process that results in negative symptoms and thus impaired QoL. This has led to interest in directly targeting neuroprotection in early treatment to prevent the development of or alleviate negative symptoms and cognitive decline and thus improve social function and quality of life. Although positive and disorganised symptoms usually respond to drug treatment, no antipsychotic drug (APD) treatment, not even clozapine, is unequivocally effective for negative symptoms.

1.2 Minocycline improves negative symptoms (see appendix 1).

The Stanley Medical Research Institute (SMRI) funded two double-blind randomised placebo-controlled studies of the putative neuroprotective agent minocycline for the negative symptoms of schizophrenia. A two-centre study in Brazil and Pakistan was supervised by the University of Manchester (NCT00916461 www.clinicaltrials.gov). 94 people with schizophrenia on stable medication completed 12 months add-on treatment with placebo or minocycline (2). Minocycline-treated patients showed overall greater improvement on total Positive and Negative Syndrome Scale (PANSS) score (p=.03). However, this was mainly because PANSS negative syndrome score improved more in those taking minocycline (p=.007; Cohen's d =.54) whereas positive symptom subscale scores showed a smaller, trend significant (p=.06) minocycline effect. In a subset of patients, ratings made at 6 months were available and treatment effects were apparent at that time. These data also suggest that improvement in negative symptoms continues to 12 months but that improvement on positive symptoms is maximal at 6 months. Although the improvement in positive symptoms was small it was very marked in 4 patients in the Brazil arm of the study and suggesting there may be significant heterogeneity in the effect of minocycline. A marked acute antipsychotic effect of minocycline has been reported in treatment-resistant patients (3). A second Randomised Controlled Trial (RCT) was carried out in Tel Aviv (4) in 70 relapsed patients. After stabilisation on second generation antipsychotic drugs (APDs), they were randomised to placebo or minocycline in a 1:2 ratio. Significant treatment effects on negative but not positive symptoms were detectable at the first rating at 3 months. No other trials are currently registered on US or UK databases.

1.3 Mechanism of action of minocyline: anti-inflammatory and neuroprotection.

Minocycline has neuroprotective properties in preclinical models of several neurodegenerative diseases. Preliminary clinical trials suggest that stroke and Parkinson's disease are helped by minocycline but that ALS is not (5). Neurodegeneration is not prominent in post-mortem brain studies in schizophrenia which point rather to loss of neuronal branching and loss of astroglial and oligodendroglial cells that support neuronal function. Furthermore, there is a good case for antecedent developmental abnormalities. Nevertheless, subtle loss of grey matter certainly occurs and this continues with transition to psychosis and during the early course of schizophrenia. Lieberman et al (6) reported loss of grey matter in MRI scans in patients treated in their first year of illness and the group of Kahn, over 5 years (7).The brain changes predicted poor functional outcome and DUP predicted the grey matter changes in the Kahn studies. The demonstration that minocycline lessens grey matter loss, and the association with improved negative symptoms would galvanise action on neuroprotection as a major therapeutic target in schizophrenia.

Minocycline has multiple anti-inflammatory and anti-apoptotic actions including decreased production of cytokines and inducible nitric oxide synthase, and inhibition of microglial activation (8). The case for inflammatory mechanisms in schizophrenia has strengthened recently. For example, association with cytokine gene variants looks promising, and a recent meta-analysis of many

studies of circulating cytokine concentrations found medium effect sizes (~0.5) for IL-1RA, SL-2R and IL6 (9). Some post-mortem brain studies and a recent PET imaging study have reported evidence of microglial activation in schizophrenia (10). Although stroke and other neurological disorders are associated with increased circulating cytokines, it remains uncertain whether they originate from brain. We will use MRI structural sequences which are sensitive to brain inflammation and determine whether group differences relate to circulating cytokines.

1.4 Minocyline, glutamate and schizophrenia

Impaired NMDA glutamate receptor function has long been implicated in the pathogenesis of schizophrenia. Drugs such as phencyclidine and ketamine block NMDA function and reproduce predominantly the negative symptoms of schizophrenia in healthy volunteers (11). They cause dysfunctional glutamate release which can exert neurotoxic effects. Atypical APDs lessen their behavioural effects and, remarkably, so does minocycline (12). Furthermore, we have very recently found evidence that a single pre treatment of anaesthetised rats with minocycline caused a highly significant block of ketamine effects on regional brain fMRI responses. Thus minocycline could improve negative symptoms by reversing a disease-related impairment of NMDA function which may directly underlie negative symptoms or cause them via a toxic effect on neuronal branching or glial support cells. Functional glutamate neurotransmission cannot yet be measured in-vivo but we propose tests of the idea that minocycline has rapid on and off effects compatible with a pharmacological action rather than, or in addition to neuroprotective effects. We will also image changes in neural processing underlying cognitive processes relevant to schizophrenia to determine whether they mediate negative symptom change or functional improvement during minocycline treatment or whether minocycline has a direct effect in improving negative symptoms.

2. RISKS AND BENEFITS

2.1 Benefits

Minocycline and potentially also related drugs will be established as a new treatment approach for schizophrenia that can be taken early in the course of the illness to:

- reduce the development and persistence of negative symptoms and
- improve social and occupational functioning
- reduce the need for standard APDs
- reduce the weight-gain and metabolic effects associated with standard APDs

Understanding how minocycline works is likely to reveal entirely new therapeutic principles for antipsychotic drug development and for preventing the onset of schizophrenia in those at familial risk or with prodromal at-risk mental states (ARMS). The effect-sizes on negative subscale PANSS scores was 0.5 in our previous study (2,4). This is defined as moderate and clinically relevant in therapeutics.

2.2 Risks

The common side effects of minocycline are gastro-intestinal upset, dizziness and fatigue. However, these were less common than in the placebo-treated arm in our study. Patches of hyper pigmentation of skin and teeth can occur during long-term therapy, for example after 9 months in 2.4% of 700 acne patients (13). Three patients in our study (6%) reported hyper pigmentation in each treatment arm but those on minocycline dropped out of the study and were the only side-effect related drop-outs. Minocycline-associated SLE is very rare and the number needed to cause one additional case has been estimated at 1/11,364 from a UK database of over 97,000 patients treated for acne (14). No neurological side effects such as extra pyramidal symptoms have been reported.

2.3 Benefit-risk

The risks of minocycline do not appear to be as great as for existing APDs. Extra pyramidal side effects including tardive dyskinesia are common with older drugs and occur but less commonly, with second generation APDs. However the latter are associated with well known metabolic problems, notably weight gain, diabetes (which is unrelated to weight gain), sedation and hyperlipidaemia. These problems are particularly prominent with the most effective antipsychotic

drugs, clozapine and olanzapine. Nevertheless, the benefits of APDs clearly out weight the risks. Crucially this is also true of clozapine's superior benefit in treatment-resistant schizophrenia, despite the relatively common need to discontinue the drug because of falling white cell counts and its specific association with myoclonic jerks and hypersalivation. The effect size for minocycline added to antipsychotic drugs is similar to that reported for clozapine over first generation antipsychotic drugs (15,16).

Evidence suggests that the benefits of clozapine may mainly be improving treatment-resistant positive symptoms. In contrast, the two minocycline studies suggest benefit mainly on negative symptoms, a major unmet need in schizophrenia.

As noted earlier, weight gain was significantly less in our study in those taking minocycline in addition to treatment as usual and this was also noted in the Levkovitz study (4).

3. RATIONALE FOR CURRENT STUDY

3.1 General

The rationale of the study is to use excellent and proven scientific infrastructure to:

i) conduct a multisite, double blind, randomised controlled trial to evaluate the effectiveness of minocycline in addition to standard care, compared to standard care alone, in preventing the development or worsening of negative symptoms of schizophrenia over one year if given early in the course of the illness, and
ii) understand how it works.

The study builds on the demonstrated proof of concept of the efficacy of minocycline on negative symptoms in two placebo-controlled clinical trials in patients on stable APD treatment. Minocycline also lessened weight gain in both RCTs and was well tolerated with a good safety profile. This study will evaluate how rapidly minocycline works on negative symptoms in early (first five treated years) psychosis and whether it has efficacy in reducing positive symptoms - there are no placebo-controlled trials in acute psychosis. Minocycline could therefore reduce the considerable side-effect burden of APDs (eg weight gain, diabetes and hyperlipidaemia) by reducing the dose of APDs necessary to improve psychosis and by lessening drug-induced weight gain. A clinically important health gain is realistic given the effect-size of 0.5 on negative symptoms in the two efficacy trials.

It is not known how minocycline works – it has a number of actions which could be relevant. Each will be assessed by validated biomarkers of potential disease mechanisms. The study will thus add significantly to understanding a very novel mechanism of action, potentially establishing an entirely new scientific and clinical principle in the treatment of psychosis. The study also has broader implications for our understanding of the relationship between brain changes, cognitive function and negative symptoms.

Most of the applicants collaborated on the MRC-funded PsyGrid e-science project. PsyGrid constructed an information systems platform and recruited 960 first-episode psychosis patients in 2 years and collected longitudinal clinical assessments which have been used in the power calculations for this study. Patients were located by Research Assistants (RAs) based in 8 collaborating centres of the Mental Health Research Network (MHRN). The Clinical research Officers (CROs) carried out assessments and transferred anonymised data via their local computer portal of the secure project management software now called OpenCDMS (www.opendcms.org). The proposed study will be run using the same methods, including randomisation and trial management functions already deployed on other multisite trials. PsyGrid addressed the ethical and legal issues involved in the secure and confidential research assessment of people in their first episode of psychosis.

Building on this, the MRC collaborative project NeuroPsyGrid organised the validation of longitudinal structural and functional scanning across 5 UK centres using the same group of 12 volunteers. This has confirmed the feasibility of multicentre scanning and informed the power

calculations for the scanning end-points and these have been published (17). The group has met regularly to coordinate their activities and is cohesive and harmonious.

4. RESEARCH OBJECTIVES:

4.1 Research Questions

If minocycline is started early in schizophrenia does it improve negative symptoms or lessen their development over the next 12 months better than it does in established illnesses, and does this improve quality of life? Does minocycline work by its neuroprotective or anti-inflammatory actions or by its effects on glutamate?

4.2 Primary and subsidiary effectiveness predictions:

Hypothesis 1. Minocycline minimises later negative symptoms when administered during the acute phase of psychosis, compared to standard care alone.
Hypothesis 2. Minocycline reduces weight gain and adverse metabolic changes associated with standard antipsychotic treatments.
Hypothesis 3. Improvements in negative symptoms will translate into improved functioning and quality of life.

4.3 Mechanistic hypotheses:

Hypothesis 1. Minocycline works by lessening a degenerative process, which is most active in the acute phase of psychosis and is responsible for the development of negative symptoms. The hypothesis predicts that the loss of grey matter, known to occur during the early years following onset of psychosis, will be lessened by minocycline treatment and that this will correlate with and explain improved negative symptoms.

Hypothesis 2. Minocycline works by lessening an inflammatory process in the brain which gives rise to negative symptoms, possibly but not necessarily mediated by subtle neurodegeneration (see H1 above). The hypothesis predicts that circulating pro-inflammatory cytokines will be lessened by minocyline treatment.

Hypothesis 3. Minocycline works by ameliorating defective NMDA glutamate receptor function which mediates negative symptoms. The hypothesis predicts that minocycline will improve cortical function as measured by functional MRI (fMRI) activation during a working memory task as resting state connectivity. It also predicts that benefits on negative symptoms wane when the drug is stopped. However, it is possible glutamate actions could also be neuroprotective (see H1 above) whether or not it enhances glutamate function in the short-term.

5. RESEARCH DESIGN

This is a 6-centre, double-blind, randomised placebo-controlled, efficacy and mechanistic study of minocycline added to standard APD treatment, versus standard APD treatment plus placebo for one year in patients in an acute episode of psychosis within 5 years of their first episode of psychosis. It will be carried out in association with relevant Local Research Networks of the English National Institute for Health Research (NIHR) and Scottish Mental Health Research Networks (MHRNs). Project RAs with the assistance of the local MHRN network Clinical Scientific Officers (CSOs) will recruit and assess patients in collaboration with local clinical teams and staff. A Research Manager (RM) will train and oversee the work of the CSO/RAs.

Patients will be recruited while symptomatic within 5 years of onset when an inflammatory or other neurotoxic process may be active and susceptible to the various actions of minocycline. Consenting patients who meet inclusion criteria will be randomised to receive minocycline or matching placebo for one year, added to standard treatment organised by the clinical team. The progress of negative and other symptoms will be monitored at intervals through the year in parallel with a set of cytokine and imaging biomarkers and measures of social functioning. The stability of any changes after treatment is stopped will be assessed 3 months after the end of the trial period.

The trial will be monitored and managed by the PIs and the RM using the ethical, secure, research governance compliant and comprehensive project management procedures established by

PsyGrid for the multi-centre study of first episode psychosis. These procedures are co-ordinated and automated using OpenCDMS software (www.opencdms.org), one of the major deliverables of PsyGrid. The OpenCDMS system organises on- or off-line collection of data onto a secure database, prompts for assessment, quality control, anonymisation of data, and randomisation of treatment allocation.

In this study patients will be allocated to treatment group according to a randomised permuted blocks algorithm, after stratification by centre, as specified by the trial statistician. An experienced clinical trial pharmacist will oversee the blinding and unblinding procedures. Blinded supplies of placebo and active minocycline will be manufactured and distributed to local pharmacies by Catalent who have a long pedigree of involvement in clinical trials. Compliance will be assessed at interview by the CSO/RA or the healthcare team at their monthly contacts and by pill counts by the pharmacies.

The mechanistic biomarkers will probe specific hypotheses about how minocycline works to reduce negative symptoms and whether and how this is translated into improved social functioning.

Effective trial management is crucial. A trial management committee will meet monthly, chaired by the Chief Investigator. The trial will be overseen by an independent Trial Steering Committee (TSC) which meets six monthly, including patient/service-user representation. They will decide at the halfway point of recruitment on the basis of a blinded interim analysis, whether it is ethical or feasible to continue the trial in its current or modified form to achieve its stated objectives.

6. STUDY POPULATION

We will use similar inclusion criteria to those used in PsyGrid and in our previous study:

6.1 Inclusion criteria:

1) Male or female aged 16-35 years
2) Meeting DSMIV criteria for schizophrenia, schizophreniform or schizo-affective psychosis as assessed by the research team.
4) In an episode as defined by the presence of positive symptoms (score >2 on P1,2,3 or 6 PANSS)
5) In contact with early intervention, community or in-patient services,
6) Within 5 years of onset of symptoms
7) IQ greater than 70
8) Male and female patients and their partners must be willing to use effective birth control, as defined in the Patient Information Leaflet, throughout the study and for 7 days after stopping trial medication. Females should have a negative pregnancy test
9) Able to understand and willing to give written informed consent
10) Fluent in English

6.2 Exclusion criteria:

1) Current substance misuse diagnosis that in the opinion of the investigator may interfere with the study.
2) Patients who, in the Investigator's judgment pose a current serious suicidal or violence risk
3) Use of antibiotics of the tetracycline type within 2 months of baseline visit or history of sensitivity or intolerance
4) History of systemic lupus erythematosis (SLE) or a history of SLE in a first-degree relative
5) Use of any investigational drug within 30 days of baseline visit
6) Relevant current or past haematologic, hepatic, renal, neurological or other medical disorder that in the opinion of the PI/RMO may interfere with the study
7) Taking medical treatments that could seriously interact with minocycline as described in the SmPC and judged by the PI/RMO.
8) Clinically significant deviation from the reference range in clinical laboratory test results as judged by the Investigator.
9) Previous randomisation in the present study.
10) Pregnant or breastfeeding.

11) Meeting MR scanning exclusion criteria (Manchester proforma or equivalent)

6.3 Withdrawal criteria:

1) The patient withdraws consent for any or no reason
2) Any adverse event considered to be related to active trial medication which is a threat to health or well being as determined by local PI, responsible medical officer (RMO) or the patient.
3) At the wish of the responsible medical officer
4) Safety reasons as judged by the Investigator, particularly if patient becomes pregnant.
5) Worsening of psychosis considered to be related to active trial medication by local PI, responsible medical officer (RMO) or the patient.
6) The patient is unable to comply with the restrictions on the use of concomitant medications listed on the SmPC.
7) The patient is unable to tolerate the study medication.

6.4 Procedures for discontinuation of a patient

A patient that decides to discontinue investigational product will always be asked about the reason(s) and the presence of any AE. If possible, they will be seen and assessed by an investigator. AEs will be followed up by the RMO.

If a participant wishes to discontinue trial medication after 6 months of treatment, the RA would ask them to complete the 12-month assessments (including MRI scan) at the time of withdrawal, and 3 months later, the 15-month assessments. These data would be included in the main analysis.

7. PLANNED INTERVENTIONS

Minocycline (modified release) or matching placebo will be taken as 100mg capsules t two per day for 2 weeks, and increased to 3 x100mg per day for the remainder of the 12-month study period added to standard APD therapy and routine care. Since it is a modified release preparation, the capsules can be taken once, twice or three times a day to a maximum of three capsules in 24 hours, as preferred. Catalent UK will organise the production of matching placebo and minocycline capsules, quality control, such assays as required by MHRA, labelling and distribution of supplies Trust pharmacies will deal with returned medication.

8. PROPOSED OUTCOME VARIABLES

8.1 Primary clinical outcome variable:

1) Negative symptom severity as defined by negative syndrome subscale score on the PANSS. This is the gold standard for comprehensively rating symptoms of schizophrenia. The negative symptom subscale is composed of 7 items each rated 1-7. (19).

8.2 Primary biomarker outcome variables:

1) Medial prefrontal grey matter volume, (H1)
2) Circulating cytokine IL-6 concentration, (H2).
3) Dorsolateral prefrontal cortex blood oxygen level dependent (BOLD) response, % correct and connectivity during the N-back task, (H3)

8.3 Secondary clinical outcome variables:

1) Body weight and body mass index (BMI),
2) Full scale and positive syndrome subscale score PANSS (18),
3) Functional outcome:
    - Global Assessment of Function (GAF) from DSMIV (19)
    - Social Functioning Scale (SFS) self-rating in 7 domains (20)

4) Cognitive outcome:
    - Blyler WAISIII short form; current IQ (21);
    - IQ decline from premorbid IQ (WTAR) predicts later negative symptoms (22,23).
    - Digit-symbol test; processing speed (24)

- Verbal fluency (VF). Cognitive correlate of negative symptoms (25)
- Auditory Verbal Learning Task; verbal learning (26)

5) APD treatment in chlorpromazine equivalents

8.4 Side-effects and co-morbidity

1) Calgary Depression for Schizophrenia Scale (self- rating)
2) Extrapyramidal symptoms (EPS):
   - The Simpson and Angus scale for pseudo-Parkinsonian symptoms and signs (27)
   - The Barnes Akathisia scale (28)
   - The Abnormal Involuntary Movements Scale for tardive dyskinesia (AIMS, 29)
3) APD subjective side-effects: Antipsychotic Non-Neurological Side-Effects Rating Scale (ANNSERS) developed in the CUtLASS study and used in PsyGrid (30).
4) 7-Point treatment adherence scale (31)

Almost all rating scales are in use in following-up PsyGrid patients and are loaded onto the OpenCDMS system.

8.5 Secondary biomarker outcome variables are changes in:

1) total and other regional grey matter volumes (H1),
2) cytokine screen (H2).
3) resting connectivity and distribution of the Hurst exponent (H3),

8.6 Exploratory analyses

Premorbid and current IQ, DUP and cytokine genotypes will be explored as predictor variables for response to minocycline.

8.7 Cytokine function

At the time of the initial screen a blood sample will be collected into EDTA and sub-aliquots of plasma prepared for measurement of inflammatory markers. Cytokine markers will be quantified using methodology similar to that previously used by this group (32, 33) but adapted for multiplex (Luminex®) assays. CRP will also be measured by sensitive immunoassay, using a modification of an assay previously reported (34). The lowest value will be used as baseline. Further blood samples will be collected at for measurement of IL-6, IL-1RA, MCP-1 (CCL2) and CRP at 6 months and 12 months to evaluate impact on inflammation and then again at 15 months to determine whether the impact is sustained. Changes in cytokine concentration will be related to the effects of minocycline on clinical outcomes and on structural and functional imaging biomarkers.

DNA will be extracted from the Oragene kits and stored in the NPU. Patients will be asked to consent to anonymisation of their DNA for biobanking. DNA will be genotyped for single nucleotide polymorphisms in genes encoding IL-6, the primary cytokine end-point. However, decisions about which genes to genotype will depend on which cytokines are influenced by minocycline treatment.

8.8 Multicentre Imaging

Large numbers of patients are needed to detect subtle biological effects of psychiatric illness and drug action on brain structure and function. Imaging studies conducted across multiple centres offer major opportunities to bring recruitment into a manageable time-frame. However, there are significant operational and statistical challenges, notably the addition of between-centre variance. To address these issues the PsyGrid and NeuroPsyGrid consortia undertook a longitudinal calibration study in which twelve male volunteers were scanned at five centres under the same study protocol as we propose to use.

A voxel-based method of calculating statistical power for multicentre imaging studies was derived from these data and has been used as the basis for power calculations herein (17). In addition, functional and structural MRI data were modelled at each brain voxel to estimate the partitioning of variance between main effects of centre, subject, occasion, within-occasion order as well as interactions of centre-by-occasion, subject-by-occasion and centre-by-subject.

Between-centre variance was limited to around 10% of the total. The main effect of subject was the largest variance partition for structural MRI (70-80%) and error (unexplained) variance the largest for functional MRI (> 80%). Moreover, subject-by-centre interactions were generally 1-2% of the total variance. There are therefore no insurmountable obstacles to MRI as an outcome variable in multicentre trials and including a factor for centre in analysis falls within the guidelines of the Steering Committee of the International Conference on Harmonisation covering the statistical analysis of randomised clinical trials.

8.8.1 Structural imaging

The loss of grey matter will be assessed by computational segmentation of T1-weighted, high-resolution images of the brain. As an adjunct to the measurement of cytokines in peripheral blood, a T2-weighted image (along with a proton density weighted image as part of a dual-echo sequence) will be acquired to observe possible neuroinflammation. Furthermore, in combination with the T1 image we will also use multi-channel texture analysis, pioneered in the assessment of multiple sclerosis lesions (35) to identify areas of abnormal MRI contrast associated with inflammation and observe potential longitudinal changes.

8.8.2 Functional imaging of cognition

Echo-planar images (EPI) depicting blood oxygenation level dependent (BOLD) contrast at a sampling time (TR) of 2 seconds will be acquired during performance of the N-back working memory task (36,37) that engages an executive function network comprising dorso-lateral prefrontal cortex, anterior cingulate and parietal cortex. Impaired functioning in this system has been strongly implicated in the pathogenesis of schizophrenia for several decades (30). Evidence suggests that underlying deficits in intrinsic NMDA glutamate/GABA neurotransmission (38) and impaired connectivity between elements of the network (39) contribute to the impairment. Performance and fMRI measures in the N-back task should therefore be especially sensitive to any NMDA/cognitive enhancing effects of minocycline particularly in schizophrenia. BOLD sensitive data will also be acquired whilst participants are resting (i.e. task absent) to investigate endogenous dynamics and functional connectivity in frontotemporal circuits, widely implicated in schizophrenia (40).

In summary, the MRI scanning sessions will acquire:

- High-resolution T1-weighted structural scan
- High-resolution proton density and T2-weighted dual-echo structural scan
- EPI acquisition (fMRI) during the N-back working memory task
- EPI acquisition (fMRI) during rest

Almost all procedures and tasks are those implemented in the NeuroPsyGrid collaboration.

9. ASSESSMENT AND FOLLOW UP

A schedule of assessments is appended (appendix 2). A case report form (CRF) will be created to hold all source data (eg questionnaires, case note review forms) for each participant.

9.1 Recruitment

This will follow the procedures of PsyGrid. Patients will be recruited by their clinical teams and Responsible Medical Officer (RMO). The RMO or another member of the clinical care team who knows the patient well will make the first approach. The local MHRN CSOs have clinical research contracts with the MHRN Trusts and they assist in ascertaining suitable patients. Records may not be accessed or screened by researchers until consent has been obtained. The RMO will assess diagnostic and other eligibility criteria using a checklist (diagnostic and eligibility checklist) and invite potentially eligible patients take part, at which stage patients will receive the patient information leaflet (PIL). The PIL will also include the information that travel expenses will be paid and their time and effort in attending each scanning session will be compensated with a £30 payment. If patients wish to participate the CSO/RA will arrange a screening visit. The CSO/RA logs the contact into a form on OpenCDMS and receives a patient ID number.

9.2 Screening visit

The CRO will obtain informed consent having verified that the patients understand the PIL and what is involved in the study. Consent will cover case-note review and the procedures in the PIL. Separate consent will be obtained for gifting their DNA in fully anonymised form to the University of Manchester for future genotyping studies. The CSO/RA will apply a diagnostic checklist to the case notes on the basis of a MINI interview (41), reach a consensus diagnosis with the RMO and confirm the presence of psychotic symptoms (>2 PANSS score on items for hallucinations or delusions). The CSO/RA will arrange for blood to be taken for renal and liver function tests. The participant will perform the IQ tests and the CSO/RA will dipstick test urine for drugs and for pregnancy in females.

The CRO will review the inclusion/ exclusion criteria when results are back and arrange the randomisation/scanning visit if indicated. The CROs ensure the CRF checklist for the visit is complete and then log data into OpenCDMS. If there are any uncertainties, the CRO will discuss them with the Principle Investigator.

9.3 Randomisation visit

This covers baseline ratings and 45mins scanning. These activities may be spread over more than one day. The CROs will check consent to continue and complete the items listed. Saliva will be collected using the Oragene kit which will be posted to the NPU. The patient will attend their local scanner unit for a 45 min scan. The session will be run by the radiographer and the CRO who will control computer projection of the N-back task and recording of performance.

The CROs will inform OpenCDMS that the patient should be randomised. OpenCDMS will: i) allocate the patient to a treatment arm; ii) email the local pharmacy about which treatment 'kit' to use to dispense two months supply; and iii) notify Catalent that a local kit has been started. Medication will be collected by the patient, RA or other designated person (for example, friend / relative).

9.4 Two, six and nine month visits

See the table of assessments. The RA will receive emailed prompts for these visits from OpenCDMS. A second dipstick test for drugs of abuse will be carried out at 6 months and action taken according to results.

9.5 Twelve Month final trial visit

This will recap the screening visit safety measures and subsequent effectiveness measures. At all visits the patients cumulative clinical drug treatment will be updated from the case notes. The MINI interview will be repeated to ascertain diagnostic status. The scanning session will be repeated. Trial Medication will cease.

9.6 Fifteen month trial follow-up visit

Safety and efficacy measures will be repeated.

10. ASSESSMENT OF SAFETY

The Principal Investigator is responsible for ensuring that all staff involved in the study are familiar with the content of this section.

10.1 Screening and follow-up safety assessments

The primary purpose of screening is to check for inclusion and exclusion criteria and the absence of relevant disease that could contribute to symptoms or adversely affect the metabolism of minocycline. The clinical team will confirm the absence of symptoms and signs from a checklist and record sitting blood pressure and heart rate. They will send blood for haematology and differential white cell count, and for clinical chemistry to confirm normal renal and liver function. These assessments will be repeated after 12 months in the trial or at the time of withdrawal from

the study. The CROs will test urine for pregnancy in females (about 40% of the sample) with a dipstick test. This will be repeated at each follow up until the end of the treatment phase. In the event of a positive test the patient will leave the study and the GP will be informed.

10.2 Definition of an adverse event (AE)

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered. AE includes both serious and non-serious AEs.

10.3 Definition of a serious adverse event (SAE)

A serious adverse event is an AE occurring during any study phase (screening, treatment, follow-up), that fulfils one or more of the following criteria: 1) results in death; 2) is immediately life-threatening; 3) requires in-patient hospitalisation or prolongation of existing hospitalisation; 4) results in persistent or significant disability or incapacity; 5) is a congenital abnormality or birth defect; 6) is an important medical event that may jeopardise the patient or may require medical intervention to prevent one of the outcomes listed above. If hospitalisation is needed due to the exacerbation of, or for the stabilization of psychosis, it will be reported as a SAE. The psychiatric assessments will reflect the worsening of the patient's condition and the need for hospitalisation. These hospitalisations will be reported in the CRF.

10.4 Time period for collection of adverse events

Adverse Events will be recorded from the randomisation visit to the 3 month non-interventional post-trial follow-up visit. Any AEs that are unresolved at the follow-up visit in the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF. The following variables will be collect for each AE: 1) AE (verbatim); 2) the date and time when the AE started and stopped; 3) maximum intensity (see definitions of intensity below); 4) whether the AE is serious or not; 5) investigator causality rating against the Investigational Product (yes or no); 6) action taken with regard to minocycline; 7) AE caused patient's withdrawal from study *(*yes or no*);* 8) outcome.

In addition, the following variables will be collected for SAEs: 1) date AE met criteria for serious AE; 2) date Investigator became aware of serious AE;3) AE is serious due to….; 4) date of hospitalisation; 5) date of discharge; 6) probable cause of death; 7) date of death; 8) autopsy performed; 9) causality assessment in relation to minocycline; 10) causality in relation to other medication; 11) description of AE.

Intensities will be reported for each AE in the following categories:

- mild (awareness of sign or symptom, but easily tolerated)
- moderate (discomfort sufficient to cause interference with normal activities)
- severe (incapacitating, with inability to perform normal activities)

An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

10.5 Detecting adverse events

The open question "Have you had any health problems since you were last asked?" will be used to evoke spontaneous reports of side effects. Spontaneously reported side-effects will be evaluated for intensity and seriousness. Deterioration in lab or examination safety measures will be recorded

as AE if they are associated with symptoms or signs or if they are sufficiently marked to be clinically significant. Suicidal thoughts, acts or events are AEs that will be rated and recorded. All such events will be notified to the clinical team and carefully monitored by them. Worsening symptoms of the primary study condition should not be recorded as an AE. However, if hospitalization results from worsening psychiatric symptoms, the hospitalization should be reported as an SAE. Diagnoses should be recorded rather than symptoms, signs or lab parameters.

10.6 Causality collection and SUSARS

The Investigator will assess causal relationship between minocycline and each AE, and answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?" For SAEs the patient's allocation to minocycline or placebo will be decoded by contacting the organising pharmacy and the causal relationship will also be assessed for other medications.

If the drug is the suspected cause of the SAE but such events are not listed in the SmPC, the event will be termed a suspected unexpected serious adverse reaction (SUSAR). SUSARs require immediate notification (see 10.8).

10.7 Breaking the code

Authorisation for unblinding is obtained from the local PI, whose contact details are held by local dispensing pharmacies. Pharmacy information will be available on: i) Trial medication bottle; ii) Patient information leaflet; iii) Patient Emergency Contact Card. Once authorised, the pharmacist will enter the patient ID into OpenCDMS which will return minocycline or placebo to the pharmacist. Out of hours code breaks will be delegated to the on-call pharmacist. The Investigators will decide on an individual basis whether and which further BeneMin assessments should be continued, with the patient's continuing consent.

The Chief Investigator will have unblinded access to all research data on OpenCDMS in order to provide reports to the DMEC.

10.8 Reporting and follow-up of adverse events

All SAEs have to be reported, whether or not considered causally related to the trial medication. All SAEs will be recorded in the CRF. If any SAE occurs in the course of the study, then investigators or other site personnel inform the local Principal Investigator and RMO immediately; i.e. within one business day. The Lead Principal Investigator will work with the local PI to ensure that all the necessary information is provided to the NRES committee, Sponsor and EME within one calendar day of initial receipt for fatal and life threatening events and within five calendar days of initial receipt for all other SAEs according to Sponsor SoP RDSOP8A.

10.9 Serious Breaches of GCP

Breaches of the protocol with a serious impact on the safety or scientific quality of the trial will be dealt with according to Sponsor SOP RDSOP9.

11. PROPOSED SAMPLE SIZE

The study will be carried out in Trusts within 4 English MHRN hubs and 1 Scottish MHRN hub. The Northwest (NW) hub has been counted as two recruiting centres because it includes the Lancashire Care NHS Foundation Trust, one of the largest mental health Trusts in the country with a catchment area of 1.5 million covering several hospitals which are located at some distance from Trusts in the central Manchester conurbation. This gives six recruiting sites, 5 with an RA and a sixth with the RA/Research Manager. All NW MRI scans will be carried out in the University of Manchester in Salford which is accessible from central Manchester and Lancashire recruiting sites. There are therefore 5 imaging centres, 4 of which have harmonised procedures through their involvement with the NeuroPsyGrid consortium. In summary, there are 6 recruiting centres and 5 scanning centres nearly all of which have worked together as part of PsyGrid and NeuroPsyGrid.

The study is designed to produce clinical and biomarker data in 170 patients completing one year of placebo or minocycline add-on treatment (85 per group). This will ensure 90% power to detect a standardised effect size of 0.5 in the primary clinical outcome (e.g. a group difference in negative symptom scores of 3 units, assuming the within-group standard deviation is equal to 6 – as estimated from the Manchester-led MRI trial and PsyGrid clinical data) using a 2-tailed t-test at $p<0.05$. A difference of 3 units is the smallest effect we would consider to have any clinical significance. A simple t-test produces a conservative estimate. Power will be greater in practice using a repeated measures design and conditioning on relevant baseline covariates. For statistical reasons we have chosen not to base our sample size calculations on mediator variables or on their hypothesised relationship with the primary outcome. However, based on the NeuroPsyGrid 5-site imaging data, the minimal detectable difference in grey matter is 2% at 80% probability with the sample size calculated above. This is much less than published MRI changes over one year.

We have based our calculations about recruitment on previous experience with the PsyGrid MHRN consortium and our previous minocycline study. In the Chaudhry et al study (2) in Pakistan and Brazil, 25% of those assessed were randomised 29% patients dropped out during the trial to an equal extent in both arms. We have assumed a 25% drop-out rate both from screening and from randomisation onwards. These figures are intended to be pessimistic and drop-out rates may be less than in Pakistan and Brazil because the proposed research is integrated with the clinical care of the patients and through demonstrating the involvement of patients and patient organisations in the design and monitoring of the study. The assumed drop-out rates give figures of 282 at screening and 226 at randomisation to produce completion in 170 (see appended flow chart). Each recruitment centre therefore needs to screen 2.1 a month and randomise 1.7 per month (see appended RA workload calculations). Such figures were achieved by several centres in the PsyGrid study. The RA workload is realistic. It allows a two-month training period and a maximum work rate of one combined MRI scanning and clinical rating session per week and 1-2 clinical follow-up ratings per week.

12. STATISTICAL ANALYSIS

12.1 Clinical efficacy measures

The statistical analysis will be overseen by study statistician, PI G Dunn. A draft statistical analysis plan will be submitted to the TSC for approval as part of their 9 month assessment. There will be only a single round of analyses carried out after the collection of the final outcome measures - there are no planned interim analyses. All initial analyses of treatment effects will be using the Intention-To-Treat (ITT) principle. If there is a non-trivial amount of non-adherence to allocated medication, then treatment efficacy will be estimated through Complier-Average Causal Effect (CACE) estimation – see Dunn et al. (42,43). Both ITT and CACE estimates will allow for missing data (drop outs), either through the use of inverse probability weights (44,45) or the use of maximum likelihood estimation involving joint modelling of treatment effects on the outcome and of the missing data mechanisms (42,43). The presence of missing outcome data is likely to be highly-correlated with treatment non-adherence and non-adherence will be a key component of models generating inverse probability weights and of any explicit missing data model used in the likelihood-based methods. Note that this will be the case even when the main aim of the analysis is the estimation of ITT effects. In both the ITT and CACE approaches, group differences in outcomes and putative mediators will be evaluated using random effects models for longitudinal data (using Residual Maximum Likelihood, REML, as the fitting criterion), allowing for treatment centre and other baseline covariates. Tests of the mechanistic hypotheses (i.e. mediation), and their sensitivity of the results to possible hidden confounding, will use instrumental variable methods from PI Dunn's MRC Methodology Research Programme projects (46). Again, an important component of these analyses and the interpretation of the results will involve making sensible use of data on non-adherence and missing outcomes.

12.2 Imaging data analysis

Multi-channel segmentation of the high-resolution structural MRI data will be used to generate grey and white matter maps for each scan (35) and then co-registered into standard anatomical space of the Montreal Neurological Institute (MNI) template (47). Several analytic approaches will be used to test our mechanistic hypotheses as comprehensively and robustly as possible. We will test for both

differences between treatment arms and associations with outcome in a focused way, using an anatomical parcellation scheme based on the MNI template to measure grey matter volume in anterior cingulate cortex and other a priori regions of interest. All statistical testing will be conducted by robust, well-validated methods of non-parametric inference with appropriate corrections for multiple comparisons (49, 50, 47). Whole brain morphometric methods that do not entail any restriction to prior regions of interest will be used to explore the whole brain for regions of grey or white matter that are sensitive to treatment. We will fit a mixed-effects model (using REML) to the repeated measures of grey or white tissue volume acquired at baseline and following treatment. The model will comprise a main effect of treatment, a main effect of time and a treatment x time interaction. Effects of treatment will be identified by significant treatment x time interactions, which will be further investigated by appropriate post-hoc testing. We will also use the multivariate technique of partial least squares (48) to identify distributed systems of grey matter variation that are optimally correlated with symptomatic response. Additionally we will test for both differences between treatment arms and associations with outcome in a more focused way, using an anatomical parcellation scheme to measure grey matter volume in anterior cingulate cortex, hippocampus, and other regions of interest. All statistical testing will be conducted by robust, well-validated methods of non-parametric inference with appropriate corrections for multiple comparisons (49, 50, 47). Potential biases arising from treatment non-adherence and missing outcomes will be explored and corrected using the methods proposed for the clinical outcomes above. All models will have treatment centre as a baseline covariate.

Structural images depicting T2-contrast will be registered into MNI standard space and an automated parcellation scheme will identify brain regions of interest as described above and texture features extracted (51). A set of texture features optimal for discriminating neuroinflammation surrounding lesions associated with multiple sclerosis has been derived by prior experiment (35). We shall examine this set in the context of schizophrenia and its treatment with minocycline. However, we will also derive a unique set based on the acquired structural MRI data (52). The first principle component of the selected features from each region of interest will be modelled as described above.

All functional MRI datasets will be pre-processed to correct for head movement and analysed (50) using a general linear model at each voxel in MNI standard space. Testing of hypotheses will then proceed conceptually as for structural MRI data analysis identifying voxels that represent significant treatment x time interactions. These whole brain univariate analyses will be complemented by use of partial least squares to identify distributed systems of task-related activation that are related to therapeutic outcome, and by hypothesis-driven region of interest analyses using the same set as defined a priori for analysis of the structural MRI dataset.

The analysis of the resting state fMRI data is analogous to the plan already described for analysis of task-related data. At each voxel in the resting state data we will estimate the Hurst exponent - simply related to the fractal dimension - of the endogenous dynamics, which we have previously shown is sensitive to effects of disease and drug treatment (53, 54). We will then test for a treatment x time interaction in exactly the same ways as outlined for analysis of activation statistics. However, the resting state data will also allow us to measure functional connectivity between anatomically defined components of fronto-temporal systems, and to construct graphical models summarizing the topological properties of fronto-limbic and whole brain networks (55). This network analysis has previously been shown to be sensitive to effects of disorders and drug treatments. We anticipate that the parameters which describe how information flows between nodes of a distributed network may be informative in understanding the mechanism of minocycline in schizophrenia.

13. ETHICAL ARRANGEMENTS

A Patient Information Leaflet approved by a NRES committee will describe the rationale for the study and the possible benefits and risks of taking part. In seeking informed consent the RMO or CSO will establish that the patient had read and understood the PIS and will answer any questions. If this condition is satisfied, the patient will be asked to sign an approved informed consent form. If the patient does not understand the nature of the study, informed consent will not be sought. The

patient will sign that he/she understands that they may withdraw consent at any time without affecting their treatment.

14. RESEARCH GOVERNANCE

14.1 Research Sponsor

The Manchester Mental Health and Social Care Trust (MHSCT) is the research sponsor and its R&D department is responsible for monitoring, audit and pharmacovigilance. It has MHRA approval.

14.2 Monitoring

The OpenCDMS data management software is being increasingly adopted non-commercial clinical trials especially of psychological therapies and by the MHRN. We will use the opportunity of this trial to have it formally validated to MHRA standards before the first patient is entered. Some of its key functions including audit and data provenance are listed in the box. Trial documentation will be retained for 15 years.

OpenCDMS functionality

- Email notification of follow-ups due
- Off-line and on-line data entry
- Multi-centre remote data entry
- Data import from file or URL
- Data anonymisation on import or export
- PKI secured with role-based access control
- Recruitment reports
- Trend analysis reports
- Model driven data set definition
- Data entry forms generated from data set definition
- Data set versioning, publication and resynchronisation
- Data review and approve capability
- Audit trail and data provenance
- NHS N3 compatible
- Call out to 3rd party application data processors
- UKCRN accrual reports
- Fully customisable data set definition including data elements, validation rules and scheduling
- http://www.opencdms.org/try-it

14.3 Data Monitoring and Ethics Committee

A DMEC will be convened which will be attended by the trial statistician Dr Graham Dunn who will have access rights to the entire OpenCDMS unblinded database.

14.4 Trial Steering Committee

A Trial Steering Committee will be chaired by Professor John Geddes, Oxford University. Two other independent experts will be members and a representative of a patient or carer organisation. Observers from the EME will be invited to attend all meetings and will be copied in on all committee papers. We will aim to minimise travel to meetings by using video/Accessgrid conferencing – this proved to be an efficient method of communication for the 8 UK centres of PsyGrid.

A risk management document will be included in submissions to the MHRA and to the TSC. This protocol has outlined several of the risks and protections but they will be listed in the format recommended in the MRC-DH guidelines for the MHRA submission and TSC.

15. PROJECT TIMETABLE AND MILESTONES

The first year milestones concern the formidable administrative hurdles to achieve entry of the first patient into the trial. The second year milestones are quantifiable. The halfway analysis is a critical point. Half the patients required to produce the required sample size should have entered. However, the assumptions about recruitment and retention will have been tested and a new set of predictions about the likelihood of achieving the final sample size can be made. Similarly, the power calculations can be revised in the light of new estimates of variance of the primary efficacy and mechanistic outcome variables. Thus the sample sizes necessary to detect effects of minocyline can be re-calculated together with an assessment of whether they can be achieved.

Year 1	Milestones	Year 2	
Month 1	Physicist starts	Month 19	Half sample randomised (n=143
Month 1	Ethical approval	Month 19	Halfway analysis complete, continuation approved by TSC
Month 1	CTA approval	Month 29	Last patient randomised
Month 4	Research manager starts	**Year 3**	
Month 6	Treatment manufacturing complete	Month 41	Last patient completes
Month 8-9	Research Assistants appointed	Month 44	Last patient followed up
Month10	Treatment supplies distributed	Month45	Database cleaned and locked
Month 10	First patient screened and randomised	Month 47	Final EME report
Month 11	First patient scanned	Month 48	Manuscript submitted

16. EXPERTISE

Seven of the PIs are clinically involved in early intervention services (Joyce, Lewis, Barnes, Dazzan, Husain, Chaudhry, Jones). They have day to day experience of the managing patients with early psychosis. They will be an important component of success in recruitment. Four of these together with Deakin and Dunn are MRC PsyGrid PIs and were closely involved in setting up the methodology and software for gathering clinical and research data in patients with early psychosis. They oversaw the training of CROs in recruiting patients and in diagnostic and symptom ratings. Lawrie, Deakin, Suckling, Williams and Dazzan ran the MRC NeuroPsyGrid feasibility study of longitudinal multi-site MRI imaging. Deakin, Barnes and Lewis are expert in psychopharmacology. Lewis, Jones, Dunn and Barnes were PIs in the HTA funded CUtLASS trials in schizophrenia and have much experience of large-scale treatment studies in psychosis. Joyce and Barnes have published extensive longitudinal studies on negative symptoms, cognition, DUP and quality of life. Husain, Chaudhry and Deakin ran the SMRI trial of minocycline. Hopkins, Reader in Health Science, University of Manchester, will supervise the immunological design and measures of the study. He has published extensively on circulating cytokines as biomarkers of CNS inflammation in recovery from stroke. Dunn is internationally known for his work on clinical trial statistical methodology and has published extensively. He has lead the statistical trial design and analysis in many multicentre clinical trials including in the treatment of schizophrenia. He has formal links with the Christie Hospital Clinical Trials Unit.

17. SERVICE USERS

User involvement helps to lessen stigma and surveys show that a better understanding of their illness and the discovery of better treatments is a high priority. The Northwest Service User Research Advisory Group (SURP-NoW), is very active and is pleased to have been involved at this early stage of planning. We will continue to seek their advice as the project develops. We will also seek the advice collaboration of INVOLVE (www.invo.org.uk) and Rethink (www.rethink.org). We will highlight user involvement in the Patient Information Sheet. We will construct a trial website with links to INVOLVE and Rethink and with their active input into its design. A user representative will be a member of the Trial Steering Committee.

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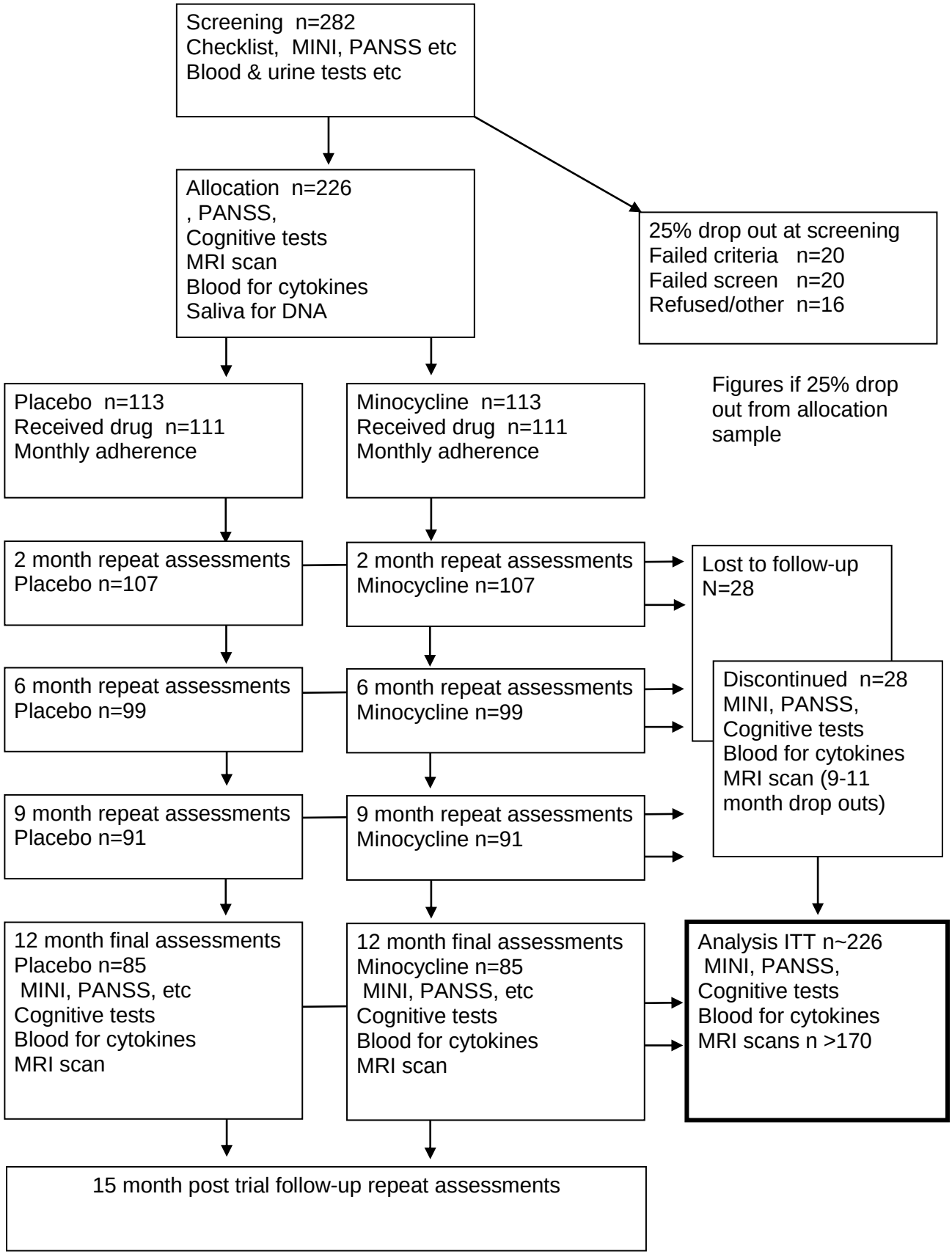
Appendix 1 Flow Chart



Appendix 2
Schedule of assessments

Assessment	Who				When						
x	CSO	RA	RMO team		Screening	Randomisation	Month 2	Month 6	Month 9	Month 12	Post trial f/u
Casenote diagnosis checklist	x	x			x						
Diagnostic and eligibility c'list		x	x		x					x	
DUP			x		x						
MINI interview for psychosis		x			x					x	
Drug treatment history	x	x			x	x	x	x	x	x	x
Body weight & BMI	x	x				x				x	x
BP & HR	x	x			x					x	x
Lab screen	x	x	x		x					x	
Drug screen (urine)	x	x			x			x			
Drug use questionnaire	x	x			x	x	x	x	x	x	x
Pregnancy screen (urine)	x	x			x	x	x	x	x	x	
Inclusion criteria	x	x	x		x						
Exclusion criteria	x	x	x		x						
Withdrawal criteria		x				x	x	x	x	x	
Consent	x	x	x		x						
Consent genetic	x	x	x		x						
Saliva Oragene kit		x				x					
Blood cytokine screen		x	x			x		x		x	x
PANSS		x			x	x	x	x	x	x	x
GAF		x				x	x	x	x	x	x
Social Function Scale	x	x				x		x		x	x
WAIS III (current IQ)	x	x			x					x	x
WTAR (IQ decline)	x	x			x						
Other cognitive tasks	x	x			x					x	x
EPS scales		x				x		x		x	x
Calgary depression scale	x	x				x	x	x	x	x	x
ANNSERS scale (side effects)	x	x				x	x	x	x	x	x
7 point compliance scale		x					x	x	x	x	
MRI screening questionnaire	x	x			x						
MRI scanning		x				x				x	