CLINICAL STUDY REPORT

Minocycline and Depression (MINDEP) study

Understanding the molecular basis for the use of adjunctive anti-inflammatory treatment in treatment resistant depression: a stratified, randomised, placebo-controlled, experimental medicine study using minocycline

Sponsor Protocol Code:	IRAS project ID 189324
EudraCT Number:	2015-003413-26
ClinicalTrials.gov Identifier:	
REC Number:	15/LO/1907
Investigational Drugs (IMPs):	Minocycline
Indication:	medical condition or disease under investigation: major depressive disorder (treatment resistant depression)
Development Phase:	Experimental Medicine trial
Study Begin (FPFV):	15th June 2016
Study End (LPLV):	17th December 2020
Report Version & Issue Date:	V 1.0 18 April 2019
Co-sponsor Name and Address:	1) King's College London, King's Health Partners Clinical Trials Office, F16 Tower Wing Guys Hospital, Great Maze Pond, London SE1 9RT; 2) South London and Maudsley NHS Foundation Trust, : King's Health Partners Clinical Trials Office, F16 Tower Wing Guys Hospital, Great Maze Pond, London SE1 9RT
Co-sponsor contact details:	1)Telephone: 020 7188 5732, Fax: 020 7188 8330, Email: helen.critchley@kcl.ac.uk 2) Telephone: 020 7188 5732, Fax: 020 7188 8330, Email: helen.critchley@kcl.ac.uk
Chief Investigator:	Name: Valeria Mondelli Address: IoPPN, King's College London, The Maurice Wohl Clinical Neuroscience Institute, Room G.30.01, Cutcombe Road, London, SE5 9RT Telephone: 020 7848 0353 Email: valeria.mondelli@kcl.ac.uk

SIGNATURE PAGE

By signing below I approve the contents of this Clinical Study Report, and confirm that to the best of my knowledge it accurately describes the conduct and results of the study. The clinical trial reported herein was conducted in accordance with the principles contained in the Declaration of Helsinki, Good Clinical Practice (GCP) and all applicable laws and regulations.

Chief Investigator:

Printed name	**Signature**	**Date**
Dr Valeria Mondelli		**15/12/2020**

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APPENDICES

1. Ethics

Independent Ethics Committee or Institutional Review Board

The study protocol and amendments were reviewed and approved by a National Research Ethics Service (insert REC name).

Ethical conduct of the study

The trial was conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (1996) as amended, the principles of Good Clinical Practice (GCP) and in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004, as amended, the Research Governance Framework for Health and Social Care, the Data Protection Act 1998 and other regulatory requirements as appropriate. The trial protocol and substantial amendments were reviewed by the United Kingdom (UK) Medicines and Healthcare products Regulatory Agency (MHRA)

Subject information and consent

Recruitment: All patients were recruited from new referrals to primary (e.g., the Improving Access to Psychological Treatments, IAPT) and secondary care services linked to the South London and Maudsley NHS Foundation Trust (SLAM), in London, from primary care services referring to SLAM and other sources such as public advertisement.

Consent: Informed consent were performed by a study investigator or an appropriately trained and suitably qualified member of the research team who received an adequate informed consent training and who observed a minimum of three consents performed by the PI or a suitably qualified study doctor before consenting participants themselves. All researchers were additionally trained in informed consent (as part of their Human Tissue Act Training, this was filed in the TMF. The participant was always be given the opportunity to further discuss the study with a qualified study physician, and this was fully documented in their source data. The PI or a suitably qualified study doctor reviewed all participants' eligibility criteria, their concomitant medication and all relevant medical history before they were randomised and a prescription was issued.

2. Data Monitoring

Data Monitoring Committee was not necessary for this trial.

3. Sponsors, Investigators and Trial Sites

Co-Sponsors	
King's College London	Address: King's Health Partners Clinical Trials Office, F16 Tower Wing Guys Hospital, Great Maze Pond, London SE1 9RT Telephone: 020 7188 5732 Fax: 020 7188 8330 Email: helen.critchley@kcl.ac.uk
South London and Maudsley NHS Foundation Trust	Address: King's Health Partners Clinical Trials Office, F16 Tower Wing Guys Hospital, Great Maze Pond, London SE1 9RT Telephone: 020 7188 5732 Fax: 020 7188 8330 Email: helen.critchley@kcl.ac.uk
Chief Investigator Dr Valeria Mondelli	Address: IoPPN, King's College London, The Maurice Wohl Clinical Neuroscience Institute, Room G.30.01, Cutcombe Road, London, SE5 9RT Telephone: 020 7848 0353 Email: valeria.mondelli@kcl.ac.uk

4. Co-Investigator(s), Statistician, Laboratories, Database Management

Name: Carmine M. Pariante (Co-investigator)	***Address: IoPPN, King's College London, The Maurice Wohl Clinical Neuroscience Institute, Cutcombe Road, London, SE5 9RT*** ***Email: carmine.pariante@kcl.ac.uk***
Name: Allan Young (Co-investigator)	Address: IoPPN, King's College London, De Crespigny Park, London, SE5 8AF Email: allan.young@kcl.ac.uk
Name: Anthony Cleare (Co-investigator)	***Address: IoPPN, King's College London, De Crespigny Park, London, SE5 8AF*** ***Email: anthony.cleare@kcl.ac.uk***

5. Study Synopsis

Title of clinical trial	Understanding the molecular basis for the use of adjunctive anti-inflammatory treatment in treatment resistant depression: a stratified, randomised, placebo-controlled, experimental medicine study using minocycline
Protocol Short Title/Acronym	Minocycline and Depression (MINDEP) study
Study Phase	Experimental Medicine trial
Sponsor name	King's College London & South London and Maudsley NHS Foundation Trust
Chief Investigator	Valeria Mondelli
Eudract number	2015-003413-26
REC number	15/LO/1907
IRAS project ID:	189324
Medical condition or disease under investigation	Major Depressive Disorder (Treatment Resistant Depression)
Purpose of clinical trial	The main aim of the project is to investigate association between changes in inflammatory biomarkers and improvement in depressive symptoms following adjunctive treatment with minocycline in treatment resistant depressed patients selected for increased inflammation.
Primary objective	The primary aim is to investigate association between changes in inflammatory biomarkers and improvement in depressive symptoms following adjunctive treatment with minocycline in treatment resistant depressed patients selected for increased inflammation.
Secondary objective (s)	The secondary aim is to identify molecular inflammatory pathways involved in the response to anti-inflammatory treatment in the same patients.
Trial Design	A stratified, randomised, placebo-controlled trial
Endpoints	Primary Outcome: Changes from baseline to Week 4 for Hamilton Depression Rating Scale total score, including the percentage of patients in the baseline. Secondary Outcomes: Changes from baseline to week 4 for Beck Depression Inventory, State and Trait Anxiety Inventory and Clinical Global Impression

	scale. **Biological outcomes:** Changes from baseline to Week 4 in cytokines and kynurenine pathway metabolites and transcriptomics.
Planned number of subjects	44
Summary of eligibility criteria	The following criteria: 1) have a current DSM-IV diagnosis of nonpsychotic major depressive disorder, confirmed by Mini International Neuropsychiatric Interview (MINI); 2) are non-responders to a current antidepressant taken at therapeutic doses for at least 6 weeks, 3) are tolerant to the current antidepressant, and accepting augmentation with minocycline; 4) have CRP levels ≥ 1 mg/L, indicative of mild-moderate inflammation; 5) additional psychotropic medications will be considered on a case-by-case basis. and 6) have the ability to understand and sign a written consent form prior to participation in any screening procedures and a willingness to comply with all trial requirements.
IMP, dosage and route of administration	Minocycline modified-release 200 mg once daily, administered orally
Active comparator product(s)	Matching placebo
Maximum duration of treatment of a subject	4 weeks
Version and date of protocol amendments	v1.0 13/10/2015 (initial) v1.1 19/11/2015 v1.2 23/11/2015 v2.0 22/03/2016 v3.0 14/10/2016 v4.0 18/07/2017 v4.1 02/08/2018 v5.0 07/11/2019 v5.1 03/03/2020 (final)

6. Glossary of terms

AE adverse event

BRC Biomedical Research Centre

COX cyclo-oxygenase

CRF Clinical Research Facility

CRP C-reactive protein

CTO Clinical Trials Office

CTU Clinical Trials Unit

GMP Good Manufacturing Practise

HAMD Hamilton Depression Rating Scale

IAPT Improving Access to Psychological Treatments

IDO indoleamine 2,3-dioxygenase

IoPPN Institute of Psychiatry, Psychology and Neuroscience

KCL King's College London

KHP King's Health Partners

MHRA Medicines and Healthcare products Regulatory Agency

REC Research Ethics Committee

SAE serious adverse event

SLAM South London And Maudsley

SSRI selective serotonin reuptake inhibitor

SUSAR Suspected Unexpected Serious Adverse Event

7. Publication (reference)

Paper accepted for publication:

First Author: Maria Antonietta Nettis
Journal: Neuropsychopharmacology
Year of submission: 2020
Title: "*Augmentation therapy with Minocycline in treatment-resistant depression patients with low-grade peripheral inflammation: results from a double-blind randomized clinical trial*"

8. Study period (years)

First version of the protocol: 13th October 2015
First Patient Visit: 15th of June 2016
Last Patient Visit: 2nd of September 2019

The trial ended when all patients had their final visits, and all data were entered into the database, and all queries resolved and the database was locked.

Database locked and end of trial declaration: 17th December 2019

Last Patient Visit: 2nd of September 2019

The end of trial date has been extended with the intention to reach the planned number of recruited subjects. Thus, it was not terminated prematurely, and it did not have any interruptions.

9. Phase of development

This is an experimental medicine clinical trial, phased 2 trial.

10. Objectives

The main aim of the study was to investigate association between changes in inflammatory biomarkers and improvement in depressive symptoms following adjunctive treatment with minocycline in treatment resistant depressed patients selected for increased inflammation.
The secondary aim was to identify molecular inflammatory pathways involved in the response to anti-inflammatory treatment in the same patients.

11. Background and Context

Increased inflammation is on the causal pathway to depression: Strong findings have accumulated over the last few years supporting a causal role of increased inflammation in depression, all confirmed by meta-analytical evidence: 1) increased levels of peripheral biomarkers are present in patients with major depression (Hiles et al., 2012); 2) increased levels of inflammation in otherwise healthy and euthymic individuals predict the future onset of depression (Valkanova et al., 2013); 3) treatment with standard antidepressants has anti-inflammatory properties (Hiles et al., 2012); and 4) treatment with anti-inflammatories has antidepressant effects (Na et al., 2014). In addition, other studies have demonstrated that: 1) a history of early life trauma – one of the more powerful risk factors for depression – is associated with increased inflammation (Danese et al., 2007; Danese et al., 2008); 2) depressed patients with higher levels of inflammation are less likely to respond to standard antidepressants (Cattaneo et al., 2013); and 4) even a short (two weeks) treatment with anti-inflammatory compounds has beneficial effects in patients developing depression because of increased inflammation (Su et al., 2010). Taken together, these lines of evidence support a role of increased inflammation in at least some patients with depression: for example, those with a history of early life trauma, those who are less responsive to common antidepressants, or those who have medical conditions associated with inflammation. Therefore, it is not surprising that a number of recent articles have called for further clinical and experimental research into the antidepressant action of anti-inflammatory drugs (Bullmore et al., 2014). Of note for our decision to use a *stratified approach* in our trial, one recent study conducted an exploratory "posthoc" stratification of depressed patients treated with infliximab, a tumor necrosis factor (TNF)-alpha-antagonist; in this study, only patients with higher levels of CRP showed improvement with infliximab, while patients with lower CRP levels

actually got worse with infliximab (Raison et al., 2013). Based on this study, we have decided to recruit patients with higher inflammation (CRP≥1 mg/L), where we are more likely to see a therapeutic effect, and *not* patients with CRP<1 mg/L, where there is a risk of having a detrimental effect.

Minocycline has a broader anti-inflammatory action that is more relevant to the pathogenesis of depression: Using minocycline, a compound with a broader anti-inflammatory action is particularly important in light of evidence from trials of other cyclo-oxygenase (COX) inhibitors in depression, which have shown that these drugs have only modest and not-sustained antidepressant efficacy. Indeed, mechanisms by which inflammation leads to depression appear to include different pathways, for example kynurenine pathway, involving activation of indoleamine 2,3-dioxygenase (IDO) a key enzyme in the metabolism of the serotonin precursor, tryptophan, as well as activation of the p-38 intracellular pathway, which leads to an increase in the expression and function of the serotonin transporter. Of particular relevance is therefore the fact that minocycline has a broader range of anti-inflammatory actions than Aspirin or other COX inhibitors, including the unique ability to inhibit both the IDO and the p-38 components of inflammation-induced depression (Pae et al., 2008; Molina-Hernandez et al., 2008).

12. Methodology

Conceptual Framework

1) Adjunct minocycline will be associated with a normalization of peripheral inflammatory abnormalities at Week 4.

2) Adjunct minocycline will be associated with greater improvement in depressive symptoms, measured at Week 4 (end of treatment) when compared with placebo, and this will be mediated by a normalization of peripheral inflammatory abnormalities at Week 4.

The Reference Group

Patients with high levels of inflammation (C-reactive protein (CRP)≥1 mg/L) and who have not responded to a trial (at least 6 weeks) of a previous antidepressant to the one they are currently taking with major depressive disorder and HAMD-17 score more than 13.

Trial Duration

Overall trial duration: 3 years, 2 months and 2 weeks
Single participant: From baseline (visit 2) to end of treatment (visit 3): 4 weeks

Definition of Trial Time Measurements

Pre-screening phone call: to assess the eligibility to attend the screening visit
Screening visit (Visit 1): to assess the eligibility to take part into the clinical trial
Baseline visit (Visit 2): within four weeks from the screening visit, patients were randomized
Final visit (Visit 3): within two weeks from the last dosage, patients were asked to attend the final visit of the clinical trial.

Table: Schedule of Events

Trial Flowchart

	Screening Visit	**Baseline** ***(within 4 weeks of screening)***	**Week 4** ***(within 14 days of IMP completion)***
Patient information and informed consent	X		
Medical history & demographic data	X		
Mini International Neuropsychiatric Interview 5.0 (MINI)	X		
Hamilton Depression Rating Scale 17 (HAM-D_{17})	X	X	X
Beck Depression Inventory II (BDI-II)		X	X
Snaith–Hamilton Pleasure Scale (SHAPS)		X	X
Childhood Trauma Questionnaire (CTQ)		X	
Spielberger State-Trait Anxiety Rating Scale (STAI)		X	X
Clinical Global Impression (CGI) scale		X	X
Brief Life Events questionnaire		X	X
Perceived Stress Scale (PSS)		X	X
Vital signs (heart rate, blood pressure, height, weight, temp)	X		
Peripheral Blood Sample (for measuring CRP; hepatic and renal function*) ***LFT (TP, ALB, TBIL, ALP, AST, GGT); EUC (Electrolytes, creatinine, urea)**	X		
Review Inclusion/Exclusion criteria	X	X	
Pregnancy Test (urine) ***(for women of child-bearing age only)***	X		X
Randomisation		X	
Study Drug Dispensing ***(& provide patient diary)***		X	
Study Drug Compliance check			X

Peripheral Blood Sample (for measuring inflammatory markers, stress biomarkers, kynurenine pathway metabolites and transcriptomics)		X	X
Concomitant medication review	X	X	X
Adverse events review		X	X

Trial Medication

Investigational Medicinal Product: Minocycline (100 mg x 2) or matching placebo was taken once daily for 4 weeks in adjunct to the current antidepressant. While the common prescribing practice for acne is 100 mg twice/daily, under the guidance of Professor D. Taylor, Head of SLaM Pharmacy, we have decided to use a single oral daily administration of the modified-release formulation, improving compliance without affecting the side-effects profile. Minocycline/placebo were manufactured, packaged and labelled in a Good Manufacturing Practice (GMP)-compliant facility. Only minocycline/placebo were dispensed by SLaM Pharmacy, which also guaranteed distribution of supplies and deal with returned medication, as required by the Medicines and Healthcare products Regulatory Agency (MHRA). The participant was required to collect their antidepressant medication as arranged with their own GP.

Dosing Regimen

After the screening process, the eligible subjects were administered minocycline (200 mg) or matching placebo (oral tablets) for the duration of 4 weeks. The dose was taken once daily.

13. Number of patients (planned and analysed)

13.1 Planned

N = 44 patients proposed in original application (with n=40 patients who complete the trial, assuming an attrition rate of 10%)

13.2 Analysed

N= 124 patients screened
N= 44 randomised patients which started taking the trial drug
N= 5 withdrew from the study
N= 39 completed the trial

Table: The reasons for patient withdrawal from the study

Patient	Study Arm	Comments
1	minocycline	Withdrew from the study but no reason was given
2	minocycline	Lost in follow up
3	minocycline	Withdrew from the study for side effects
4	minocycline	Withdrew from the study for side effects
5	placebo	Withdrew from the study for family reason

14. Diagnosis and main criteria for inclusion

We selected young adult depressed patients both males and females, accordingly to the following criteria:

Inclusion Criteria:

1. Be aged 25-60
2. Have a current DSM-IV diagnosis of nonpsychotic major depressive disorder, confirmed by the Mini International Neuropsychiatric Interview (MINI);
3. Be non-responders to the current antidepressant taken at therapeutic doses for at least 6 weeks, as indicated by a current score of at least 14 on the 17-item Hamilton Depression Rating Scale (HAMD);
4. Be tolerant to the current antidepressant, and accepting augmentation with minocycline;
5. Have CRP levels ≥ 1 mg/L, indicative of mild-moderate inflammation;
6. Have the ability to understand and sign a written informed consent form prior to participation in any screening procedures and a willingness to comply with all trial requirements.

Exclusion Criteria:

1. have active suicidal ideation of significant concern to require intensive monitoring by secondary psychiatry services;
2. have a primary diagnosis of bipolar disorder, obsessive-compulsive disorder, eating disorder, post-traumatic stress disorder, or substance/alcohol misuse disorder;
3. are taking warfarin;
4. have received tetracycline within the previous 2 months, or have a history of sensitivity or intolerance to this class of drugs;
5. have an acute infection; or have an autoimmune or inflammatory disorder, because of both the rare but described association between minocycline and systemic lupus erythematosus, and the potential confounder effects of these conditions on immune biomarkers.
6. have hepatic or renal failure
7. take any other psychotropic medications other than their current antidepressant that has not been approved by a study investigator prior to enrolment
8. Refuse that we contact their General Practitioner (GP) to inform them about their participation in the study.
9. (Females) Have a positive pregnancy test before starting the study/are unwilling to take a pregnancy test and are unwilling to agree to use an acceptable form of contraceptive throughout the study period (e.g. condoms, IUD/IUS, injection, patch, ring). Female participants who use combined oral contraceptives as their main form of birth control were need to use an additional barrier method for the duration of treatment and for 7 days following completion of treatment.
10.Breastfeeding (females)
11.Are currently participating in a clinical trial of an investigational medical product (CTIMP). For individuals who have been recently on CTIMP clinical trials, we decided on case by case basis if they could be included in the trial according to the type of trial they have been involved in.

15. Test product, dose and mode of administration

Baseline therapy

Baseline therapy was assessed as described in the inclusion criteria. Moreover, participants were recruited if they had no planned changes in their current therapy for the duration of the trial.

IMP

Minocycline or Placebo

Table: Dose of IMP administered to each study participant

Table: mean (SD) of total capsules taken

	Study arm	**n**	**mean (SD)**
Total capsules taken	**Minocycline**	18	56,00 (0,00)
	Placebo	21	53,33 (9,59)

Table: Dose for each study participant

ID	**n**	**Study Arm**
1005	56	1*
1011	56	2**
1097	56	1
1020	56	1
1019	56	2
1008	56	1
1022	56	2
1095	56	2
1054	56	1
1112	56	1
1087	56	2
1073	56	1
1001	56	2
1045	56	2
1106	56	2
1053	56	2
1026	56	1
1009	56	1
1119	56	2
1084	56	1
1088	52	2

1048	56	1
1021	56	2
1065	12	2
1016	56	2
1091	56	1
1092	56	1
1116	56	1
1042	56	2
1057	56	1
1069	50	2
1085	56	2
1070	56	1
1064	54	2
1108	56	1
1030	56	1
1028	56	2
1123	56	2
1018	56	2

*Study arm 1: minocycline
**Study arm 2: placebo

16. Duration of treatment

4 weeks

17. Reference therapy, dose and mode of administration

Minocycline (100 mg x 2) or matching placebo were taken orally once daily for 4 weeks (56 capsules) in adjunct to their current antidepressant.

18. Criteria for evaluation: Endpoints

18.1 Efficacy

Primary end-point
Primary clinical outcome: Changes from baseline to Week 4 for Hamilton Depression Rating Scale (HAMD) total score, including the percentage of patients who show response, defined as 50% reduction from baseline

Secondary Efficacy Parameters
Depression Inventory, State and Trait Anxiety Inventory and Clinical Global Impression scale.

Biological outcomes: Changes from baseline to Week 4 in cytokines and kynurenine pathway metabolites and transcriptomics.

18.2 Safety

Assessment of safety

The most common side effects of minocycline are fever and dizziness symptoms. Minocycline is usually prescribed for many months for acne, while our study lasted 4 weeks. This short time frame avoided any risk of hyper-pigmentation of skin and teeth, which can occur during long-term therapy. There is no evidence that minocycline can induce psychiatric adverse effects, and there are no described interactions with antidepressants. Pregnancy could have been a potential problem, and hence the decision to provide pregnancy tests at the beginning and at the end of the trial in female participants. Female participants who were using combined oral contraception as their main method of birth control were advised to use an additional barrier form of contraception for the duration of treatment and for 7 days after completion of the course.

Specification, Timing and Recording of Safety Parameters

We conducted screening assessment for all the subjects before starting the treatment (baseline), including pregnancy test in female participants and excluded patients with history of sensitivity or intolerance to tetracycline. We repeat ed pregnancy test at the end of the trial for female participants. We monitored side effects at the during the 4 weeks of the trial.

19. Statistical Methods

Analysis of Efficacy Variables

The primary analyses included a Pearson's Chi-square test to examine the difference in percentage of treatment response or partial response (defined as 50% or 25% reduction from baseline in the HAM-D-17 score, respectively) between the two study arms, and an independent t-test to test differences in changes in HAM-D-17 scores between the two study arms.

Finally, we further examined differences in changes in HAM-D-17 scores between patients with hsCRP above or below the cut-off 3 mg/L at baseline (3); for this purpose, we divided the sample by patients with hsCRP≥3 mg/L ($hsCRP^+$) and patients with hsCRP<3 mg/L ($hsCRP^-$), and by treatment group, generating 4 final groups: $hsCRP^+$/M (n=6), $hsCRP^+$/P (n=12),

hsCRP$^-$/M (n=12) and hsCRP$^-$/P (n=9) (see Table 2). Then, we performed a one-way ANOVA, to investigate differences among these 4 groups of patients in the HAM-D-17 change.

All of the aforementioned analyses were conducted in both the complete dataset (n=39) and using intention-to-treat approach (n=44). Specifically, we used multiple imputation to handle missing data, generating HAM-D-17 scores at week 4 (end of treatment) for the 5 withdrawn participants.

Finally, we conducted a Receiver Operating Characteristic (ROC) curve analysis, with both parametric and non-parametric methods, to test the ability of baseline hsCRP levels to correctly differentiate treatment response and to identify/confirm the exact threshold point at which hsCRP would correctly identify treatment response.

Analysis of Safety Variables

A chi square test was used to compare frequencies of side effects in the 2 study arms.

20. Summary – Conclusions

20.1 Demographic data

Table: Demographic data for all patients (safety population)

Table: Baseline characteristics of the per protocol population Frequency (%) is displayed unless otherwise specified.

Figure: Gender of the safety population

Figure: Ethnicity of the safety population

The following table summarises the socio-demographic data of the treated subjects:

Socio-demographic variables	**Minocycline n=22**	**Placebo n=22**
Age, mean (SD)	45.4.(9.9)	43.0(10.9)
Gender, F (%)	63.6	59.1
Ethnicity, White (%)	68.1	95.8
BMI, mean (SD)	33.5(10.1)	31.6(6.1)
Current Smoker, yes (%)	18.2(n=4)	31.8(n=7)
Alcohol units per week, mean (SD)	7.3(10.5)	9.7(9.9)
Current Medication:		
1) SSRI (%)	63.6	47.6
2) OTHER AD (%)	22.7	14.3
3) AD+AP (%)	4.5	14.3
4) >2 AD (%)	9.1	23.8
5) AD+ Benzodiazepines (%)	13.6	4.5
Depression duration from onset, years, mean (SD)	20.32 (10.48)	18.05 (12.39)
Baseline HAM-D-17 score, mean (SD)	19.41 (3.66)	16.91 (3.21)
Baseline hs-CRP, mean (SD)	3.96 (4.14)	4.37 (5.11)

Legend: AD (antidepressant); AP (anti-psychotic medication); BMI (body mass index); F (female); HAM-D-17 (Hamilton Depression Rating Scale); hs-CRP (high sensitivity C-reactive protein); n (number); SD (standard deviation).

20.2 Primary outcome

Complete case analysis (n=39)

Both study arms exhibited significant improvement in HAM-D-17 total score (table n 1). We did not find significant differences between the two study arms in the clinical improvement measured in terms of HAM-D-17 change (t=1.57, p=0.13).

We detect 3 subjects in the minocycline group and 2 subjects in the placebo group showing at least 50% improvement in HAM-D-17 scores (table n 2). We then considered the 25% reduction as partial response to treatment. Partial responders in the minocycline group were 44.4 % (8/18), while partial responders in the placebo group were 42.9% (9/21). The Chi square test was not significant (χ^2=0.06, p=0.80) (table n 3).

Table 1. DELTA HAMD-17 changes from baseline to week 4 in the minocycline and in the placebo study arms

Study Arm	n		Time point	mean (SD)	DELTA HAMD-17	Baseline vs Week4 Statistics (bootstrapped)	Minocycline vs Placebo
Minocycline	18	HAM-D-17	Baseline Week 4	19.06 (3.45) 13.44 (5.17)	5.61 (6.36)	t=3.74 p=0.008	t=1.57, p=0.13
Placebo	21	HAM-D-17	Baseline Week 4	17.00 (3.26) 14.10 (5.59)	2.90 (3.88)	t=3.43 p=0.003	

Table 2. Treatment response: 50% of improvement on the HAMD-17 scale

Study Arm		n	% within study arm	% of total
Minocycline	**Responder (≥ 50%)**	3	16.7	7.7
	Non responder (< 50%)	15	83.3	38.5
Placebo	**Responder (≥ 50%)**	2	9.5	5.1
	Non responder (< 50%)	19	90.5	48.7

Table 3. Treatment partial response: 25% of improvement on the HAMD-17 scale

Study Arm		n	% within study arm	% of total
Minocycline	**Responder (≥ 25%)**	8	44.4	20.5
	Non responder (< 25%)	10	55.6	25.6
Placebo	**Responder (≥ 25%)**	9	42.9	23.1
	Non responder (< 25%)	12	57.1	30.8

Hs-CRP baseline levels seemed to play a significant role in the treatment efficacy. Specifically, those patients with CRP ≥ 3 mg/L (CRP+) respond between to minocycline treatment in comparison with the other groups.

Although this was a secondary aim, when we explored differences after further stratification based on CRP levels above or below 3 mg/L, we found some evidence of efficacy for minocycline in the high inflammation group. Specifically, the one-way ANOVA showed a significant difference among the four groups of patients (CRP≥3 mg/L + minocycline (CRP^+/M) n=6, CRP<3 mg/L + minocycline (CRP^-/M) n=12, CRP≥3 mg/L + placebo (CRP^+/P) n=12, CRP<3 mg/L + placebo (CRP^-/P) n=9 ($F_{3,35}$ =8.53, p<0.001). In particular, CRP^+/M patients had the largest HAM-D-17 change from baseline to week 4 (mean±SD=12.00±6.45) compared with CRP^-/M (2.42±3.20, p<0.001, Cohen d=1.9), CRP^+/P (3.50±4.34, p=0.002, Cohen d=1.5) and CRP^-/P (2.11±3.26, p<0.001, Cohen d=1.9) patients (Bonferroni corrected).

Furthermore, the $hsCRP^+$/M group had the highest proportion (83.3%, 5 out of 6) of partial responders (χ^2 =8.27, p=0.04).

These results were confirmed by the Intention-to-treat analysis (n=44).

20.3 Safety results

Table: Listing of Adverse Events for all patients who completed the trial (n=39)

Side effects	*Minocycline n*	*Placebo n*
Acne	0	1
Apathy	0	1
Chest palpitation/pain	2	0
Constipation, flatulence, diarrhoea	4	3
Dizziness	3	1
Dyspepsia/indigestion	1	5
Flu-like symptoms	2	1
General pain/joint pain	1	2
Headache	4	5
Insomnia	0	3
Light bleeding	0	1
Mood fluctuations	1	1
Nausea	4	1
No appetite	0	1
Skin rash	1	0
Sore throat/cold	1	2
Tinnitus	0	2
Tiredness	1	0

Table: Listing of Serious Adverse Events for all patients

Not applicable

Within the per protocol population (n= 39), a total of 21 AEs, including "0" SAE, were identified as treatment-emergent and included in the safety analysis. Summary tables for AEs are presented in the appendix of this synopsis.

Overall, 22 out of 39 patients (56.41%) patients experienced at least one AE. The proportion that experienced at least one SAE was 0% (n=0).

There were 0 Serious Adverse Reactions (SARs), 0 unexpected SARs and 0 SUSARs.

20.4 Conclusion

In conclusion, we found relevant evidence that minocycline was a beneficial as an add-on treatment in TRD patients with MDD with higher levels of inflammation at the baseline (hs-CRP ≥ 3 mg/L).

21. Date of Report

This is version 1.0 of the Clinical Study Report synopsis, dated 15/DEC/2020.

APPENDICES

i) Summary of treatment-emergent AEs in the per protocol population

System Organ Class	Preferred Term	Number of Subjects Experiencing the AE in Active Arm (n=18)	Total Number of Occurrences of the AE	Number of Subjects Experiencing the AE in Placebo Arm (n=21)	Total Number of Occurrences of the AE
Blood and lymphatic system disorders	Leukopenia	0 (0%)	0	0 (0%)	0
Cardiac disorders	Palpitations Cardiac death Sudden death Chest pain Chest discomfort	Chest palpitations/chest pain 1 (5.55%) Chest pain 1 (5.55%)	Chest palpitations/chest pain 1 Chest pain 1	0 (0%)	0
Congenital, familial and genetic	Hydrocele	0 (0%)	0	0 (0%)	0

disorders					
Ear and labyrinth disorders	Ear pain Tinnitus	0 (0%)	0	tinnitus 2 (9.52%)	2
Eye Disorders	Tunnel vision Visual impairment	(0%)	0	(0%)	0
Gastrointestinal disorders	Diarrhoea Dyspepsia Constipation Nausea Paraesthesia oral	Nausea 4 (22.22%) Dyspepsia 1 (5.55%) Constipation/Flatulence/Diarrhoea 4 (22.22%)	Nausea 6 Dyspepsia 1 Constipation/Flatulence/Diarrhoea 4	Nausea 1 (4.76%) Dyspepsia 5 (23.80%) Constipation/Flatulence/Diarrhoea 3 (14.28%) Light bleeding 1 (4.76%) No appetite 1 (4.76%)	Nausea 1 Dyspepsia 5 Constipation/Flatulence/Diarrhoea 6 Light bleeding 1 No appetite 1
General disorders and administration site conditions	Fatigue Impaired healing Oedema peripheral	Tiredness 1 (5.55%)	Tiredness 1	0 (0%)	0
Hepatobiliary disorders		0 (0%)	0	0 (0%)	0
Immune system disorders		0 (0%)	0	0 (0%)	0
Infections and infestations	Viral upper respiratory tract infection	Flu-like symptoms 2 (11.11%)	Flu-like symptoms 2	Flu-like symptoms 1 (4.76%)	Flu-like symptoms 3

	Nasopharyngitis Ear infection Folliculitis Gastroenteritis viral Lower respiratory tract infection Polyomavirus-associated nephropathy Sinusitis Urinary tract infection				
Injury, poisoning and procedural complications	Incision site pain Procedural pain Wound secretion Contusion Post procedural contusion Post procedural haematuria	0 (0%)	0	0 (0%)	0

	Post procedural oedema Procedural nausea Seroma Suture related complication				
Investigations	Polyomavirus test positive Blood creatinine increased Escherichia test positive White blood cell count decreased	0 (0%)	0	0 (0%)	0
Metabolism and nutritional disorders	Glucose tolerance impaired Gout Hypercalcaemia	0 (0%)	0	0 (0%)	0
Musculoskeletal and connective	Myalgia Arthralgia Joint	General pain/joint pain 1 (5.55%)	General pain/joint pain 3	General pain/joint pain 2 (9.52%)	General pain/joint pain 2

tissue disorders	swelling Musculoskeletal discomfort Osteoarthritis Pain in extremity				
Neoplasms benign, malignant and unspecified (incl cysts and polyps)		0 (0%)	0	0 (0%)	0
Nervous system disorders	Dizziness Headache Dysgeusia Paraesthesia Tremor Burning sensation Dizziness postural	Dizziness 3 (16.66%) Headache 4 (22.22%)	Dizziness 7 Headache 5	Dizziness 1 (4.76%) Headache 5 (23.80%)	Dizziness 1 Headache 8
Pregnancy, puerperium and perinatal conditions		0 (0%)	0	0 (0%)	0
Product		0 (0%)	0	0 (0%)	0

issues					
Psychiatric disorders	Anxiety Depression	Mood fluctuation 1 (5.55 %)	Mood fluctuation 1	Mood fluctuation 1 (4.76%) Apathy 1 (4.76%) Insomnia 3 (14.28%)	Mood fluctuation 1 Apathy 3 Insomnia 3
Renal and urinary disorders	Haematuria Pollakiuria Renal cyst haemorrhage Renal cyst ruptured	0 (0%)	0	0 (0%)	0
Reproductive system and breast disorders	Epididymal cyst Erectile dysfunction	0 (0%)	0	0 (0%)	0
Respiratory, thoracic and mediastinal disorders	Cough Dyspnoea exertional Productive cough	Sore throat/cold 1 (5.55 %)	Sore throat/cold 1	Sore throat/cold 2 (9.52%)	Sore throat/cold 2
Skin and subcutaneous tissue disorders	Acne Actinic keratosis Alopecia Dermatitis acneiform Night sweats Pruritus	Skin rash 1 (5.55 %)	Skin rash 2	Acne 1 (4.76%)	Acne 1

	Rash generalised				
Social circumstances		0 (0%)	0	0 (0%)	0
Surgical and medical procedures		0 (0%)	0	0 (0%)	0
Vascular disorders	Hot flush	0 (0%)	0	0 (0%)	0

ii) Summary of treatment-emergent ARs in the per protocol population

See Table above

iii) Summary of treatment-emergent SAEs in the study population

See Table above

iv) Summary of treatment-emergent SARs in the study population

See Table above