Adjunctive Treatment With Pramipexole for Anhedonia Symptoms in Depression – A Pilot Study (PILOT-PRAXOL)

Study protocol

Version: 1.1
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EudraCT: 2019-001907-19
ClinicalTrails.gov ID: NCT04121091

Sponsor: Region Skåne through Adult Psychiatry Clinic, Lund.
Principal Investigator: Daniel Lindqvist.

2 SIGNATURE

I have read this protocol and it contains all the essential elements to carry out the study. By signing the study, I agree to conduct the study in all its parts according to this protocol, the informed consent, and to comply with ICH GCP, the Declaration of Helsinki and the national and international regulations that apply to the clinical study in question.

I will share the protocol and any other important study-related information with my co-workers in order for them to conduct the study properly. I am aware of my responsibility to keep the employees working on the study informed and educated.

I understand that any information provided to me in connection with this study that has not previously been published is considered confidential information.

…….

Signature of the principal investigator

Daniel Lindqvist

Psychiatry Skåne VO Psychiatry Lund

PILOT-PRAXOL /EudraCT version 1.1_2019-06-17

3 ABBREVIATIONS

3T MR	3 Tesla magnetic resonance imaging
AE	Adverse Event/Incident – unwanted medical event
AES	The Apathy Evaluation Scale
BOLD	Blood-oxygen-level dependent imaging
CRF	Case Report Form
CRP	C-reactive Protein
D3 receptor	D3 dopamine receptor
DARS	Dimensional Anhedonia Rating Scale
DSF	European Union General Data Protection Regulation (EU 2016/679) (GDPR)
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5 ed.
eCRF	Electronic Case Report Form
ECT	Electroconvulsive therapy
eGFR	Estimated glomerular filtration rate
EU	European Union
Eu CTR	EU Clinical Trials Register
FASS	Pharmaceutical Specialties in Sweden
fMRI	Functional magnetic resonance imaging
GAD-7	Generalized Anxiety Disorder-7
GCP	Good Clinical Practice
Hb	Hemoglobin
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10 ed.
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IL	Interleukin
Source data	Any information in original and certified copies of original documents of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the study. The source data is contained in source documents (original or certified copies). (ICH-GCP E6)

LPT	The Act on Compulsory Psychiatric Care
LV	Swedish Medical Products Agency
LVFS 2011:19	Guidance to the Medical Products Agency's regulations on clinical trials of medicinal products in humans
MADRS	Montgomery-Åsberg Depression Rating Scale
MAOIs	Monoamine oxidase inhibitors
MAP-SR	Motivation and Pleasure Scale – Self-Report
MID-task	Monetary Incentive Delay task
MINI	Mini International Neuropsychiatric Interview
Monitor	An independent person who reviews that the study is conducted, documented, and reported in accordance with approved study protocols, Standard Operating Procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements.
NAc	Nucleus accumbens
PAL	Patient-responsible doctor at a psychiatric ward or health center
PoC	Proof-of-Concept
Investigator	A licensed physician or licensed dentist performing trial at a trial site
PS	Parkinson's disease
EPM	The Ethical Review Authority
RCT	Randomized controlled trial
SAE	Serious Adverse Event – serious unwanted medical event
SD	Standard Deviation
SHAPS	Snaith–Hamilton Pleasure Scale
SmPC	Summary of Product Characteristics - a document written by the manufacturer at the time of registration of a medicinal product and is a summary of the characteristics and use of the medicinal product.
SSRI	Selective serotonin reuptake inhibitors
SUAS	Suicide Assessment Scale
SUS	Skåne University Hospital
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNF	Tumor Necrosis Factor
YMRS	Young Mania Rating Scale

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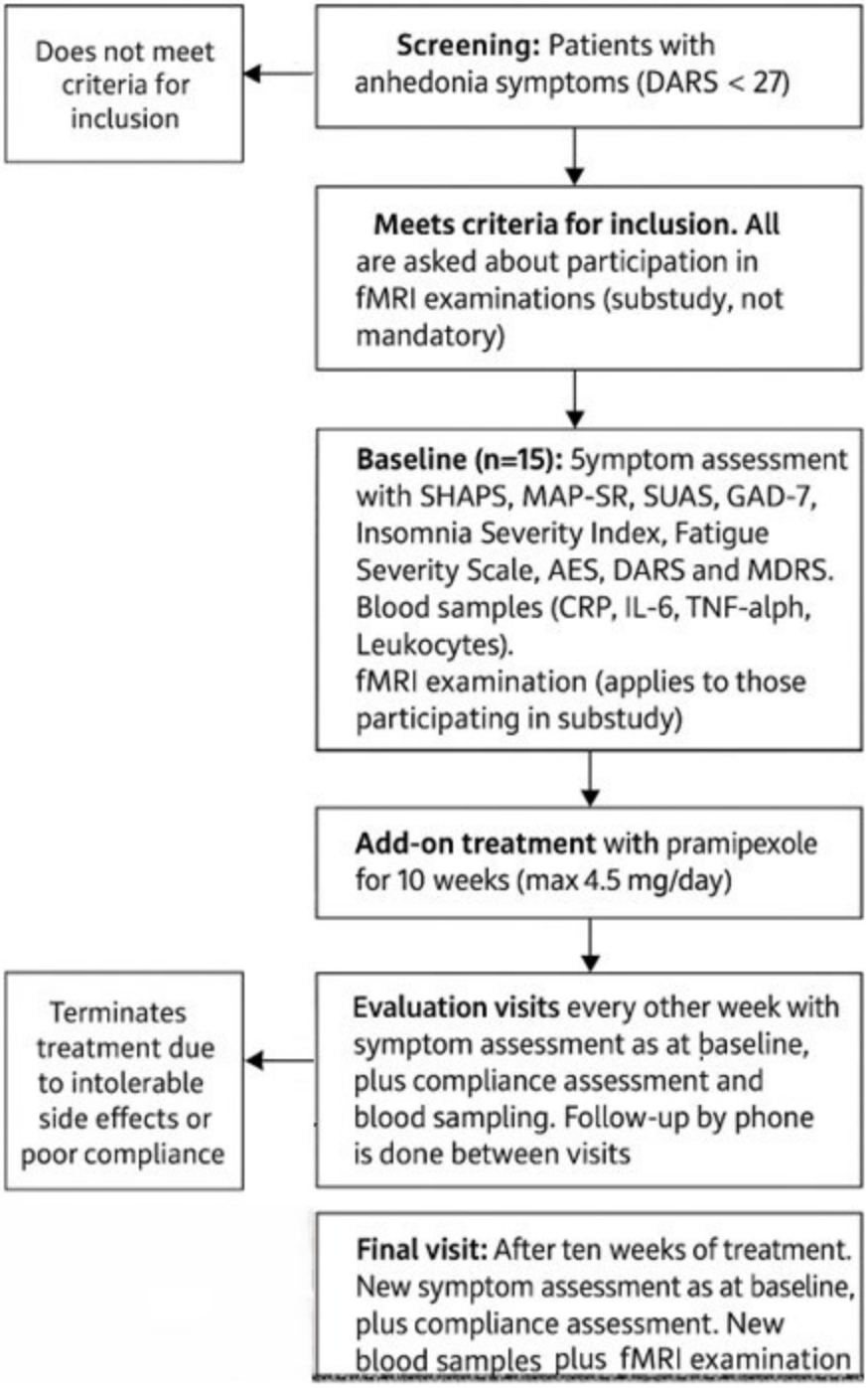
5 SYNOPSIS

Sponsors Protocol ID	1.1 20190619
Title	Adjunctive treatment with Pramipexole for Anhedonia Symptoms in Depression – A Pilot Study
EudraCT nr.	2019-001907-19
Background and expected benefits	The majority of depressed patients do not achieve remission with current treatment methods. Many continue to suffer from severe residual symptoms of anhedonia – the inability to experience pleasure and a reduced sense of drive – after treatment. At present, there is no specific and effective treatment available for anhedonia. Pramipexole, a dopamine agonist used in Parkinson's disease, has been shown to be effective against anhedonic symptoms in that condition. Clinical experience has also demonstrated treatment effects of high-dose pramipexole in treatment-resistant depression; however, randomized clinical trials have only been conducted with low-dose pramipexole and have not specifically examined its effects on anhedonia. This pilot study is expected to serve as a basis for a larger placebo-controlled randomized treatment trial of high-dose pramipexole as an adjunctive therapy in depression with severe anhedonic symptoms.
Objective	**Primary question:** Do anhedonia symptoms, as assessed by the Dimensional Anhedonia Ration Scale (DARS) self-assessment questionnaire, decrease after 10 weeks of additional treatment with pramipexole? **Secondary/Exploratory questions:** 1. Does adjuvant treatment with pramipexole lead to improvement in General Depression symptoms, anxiety symptoms, sleep, apathy and fatigue? 2. Does adjunctive treatment with pramipexole lead to increased activity in the brain's reward system? Is such a change linked to clinical efficacy? 3. Is high-dose treatment with pramipexole a tolerable treatment in terms of side effects?
Variables	**Primary Variable(s):** Total score DARS. **Secondary variable/variables:** Total score Montgomery-Åsberg Depression Rating Scale (MADRS), BOLD signal at MID-task (fMRI), rating scales related to sleep problems, apathy, fatigue and anxiety. AE (Adverse Medical Event).
Investigational drug/treatment	Pramipexole prolonged-release tablet, 0.375 mg to 4.5 mg salt/day for 10 weeks (individually varying dose based on efficacy and side effects titrated during these weeks)

Study population	**Inclusion (not exhaustive list):** Age between 18 years and 75 years Diagnosis of unipolar depressive episode, bipolar disorder in depressive phase or dysthymia. Moderate to severe depression but with pronounced symptoms of anhedonia. To be able to participate, you must have an ongoing stable treatment with at least an antidepressant/mood stabilizer for at least 4 weeks. **Exclusion (not exhaustive list):** Current pregnancy, breastfeeding or planned pregnancy. High suicidal. Substance abuse. Psychotic disorder. Compulsory treatment (LPT). Impulse control disorder. Diagnosis of moderate/severe renal failure or severe cardiovascular disease. Recently started psychotherapy. Ongoing ECT treatment.
Study design	*Proof of concept* study, in which fifteen research subjects are included and receive study treatment (no placebo or control group occurs). All included research subjects will also be asked to participate in the substudy for fMRI examination before and after 10 weeks of treatment with pramipexole.
Timetable	**Study start:** 1 September 2019 **Study end:** 1 December 2020.
Statistical considerations	The objective of the study is to demonstrate feasibility (*proof of concept*) and we hope to generate preliminary data that will form the basis for a larger, definitive, randomized controlled trial. Specifically, we are interested in investigating: i) can we pick up a signal in our material that pramipexole has an anti-anhedonic effect in this study population, (ii) if the treatment doses we proposes are reasonable: treatment effect versus side effects, iii) what the distribution looks like for DARS before and after treatment in order to optimize future dimension calculations.

Method description	Patients will be recruited from outpatient psychiatric clinics or inpatient wards. Those who meet the inclusion criteria and none of the exclusion criteria will, at the screening visit, undergo a baseline assessment including self-report rating scales and blood sampling for inflammatory markers (CRP, IL-6, TNF-alpha, white blood cells). Participants who also consent to the substudy will undergo an fMRI examination of the brain's reward system (MID task). Thereafter, study treatment will begin according to the protocol, with dose escalation once weekly until treatment effect, maximum dose, or intolerable side effects occur. In the event of intolerable side effects, the participant will return to the last tolerable dose. A new attempt at dose escalation may be made after seven days, up to a maximum of two attempts. If serious adverse events occur, the medication will be tapered off. After ten weeks of pramipexole treatment, a final visit will be conducted including new assessment with self-rating scales and blood samples as at baseline visits (including new fMRI examination for those participating in the substudy).
Financing	The study is financed by funds from ALF, the Söderström-Königska Foundation, SUS Donations and Foundation and the Royal Physiographic Society in Lund.

6 STUDY FLOW CHART



7 BACKROUND AND RATIONALE

Affective disorders are psychiatric syndromes whose main symptoms are changes in affectation or mood. Examples of such disorders are major depression (unipolar depression), bipolar disorder and Dysthymia. Today's treatment methods for mood disorders are often inadequate. From the start of drug treatment, it usually takes a few weeks before the treatment effect becomes noticeable, even though any side effects often occur earlier[1]. In addition, a large proportion of patients do not respond at all to drug treatment despite several treatment attempts, and many of those who respond to the treatment have severe and function-limiting residual symptoms in the form of, for example, anhedonia and lack of motivation[2]. It is estimated that up to a third of patients with depression do not reach remission despite repeated treatment attempts with different drugs[3]. One reason for this is that the diagnostic criteria in manuals and systems such as DSM-5 and ICD-10 do not consider the biological causes of the diseases. To better study depression and other affective disorders, psychiatric syndromes can be divided into so-called endophenotypes[4], i.e. characteristic features (in this case, symptoms) with a clear biological correlation that can occur across the diagnostic boundaries and vary within the same diagnosis. In affective disorders, anhedonia has been shown to be such an endophenotype[5].

Anhedonia - the inability to feel pleasure or reduced will/"drive" to seek pleasure - is associated with affective disorders, but also other psychiatric illnesses such as substance addiction and schizophrenia[6]. Anhedonia is found in 20-40% of all cases of major depression and is a risk factor for developing depression with more severe symptoms, longer disease course, treatment resistance and suffering from relapse in depression[7]. Currently, there is no effective and/or specific treatment for the symptoms of anhedonia. The onset of anhedonia is thought to be due to dysfunction in the brain's reward system[8]. An important structure in the brain's reward system is the accumbens nucleus (NAc). fMRI scans have shown an increased neural activity in NAc in healthy individuals with an expectation of receiving a reward; In patients with depression and anhedonia symptoms, the corresponding activity is reduced[9]. In NAc, there are neurons that express the dopaminergic receptor D310. The D3 receptor is considered a potential target for the treatment of anhedonia and in animal studies antidepressant effects of D3 receptor agonists have been demonstrated[11]. Most of today's drug treatments for depression affect dopaminergic neurotransmission, such as monoamine oxidase inhibitors (MAOIs) and bupropion, but none of these act as D3 receptor agonists.

Anhedonia and similar symptoms have also been associated with increased systemic inflammation[12]. For example, depressed patients with increased levels of inflammation markers in the blood have reduced connectivity ("communication") between the reward system and other parts of the brain[13]. Several studies have also shown that inflammation affects dopamine metabolism in the brain, and patients with concomitant low-grade systemic inflammation and depression respond better to bupropion (which increases dopamine in the synapses) compared to other patients with depression without increased inflammation[14]. Thus, drugs that affect dopaminergic nerve transmission are likely to provide better treatment efficacy in depressed patients with signs of systemic inflammation compared to patients with depression as a whole.

Pramipexole is a selective D3 receptor agonist which is primarily used as a treatment for Parkinson's disease (PS) and Willis-Ekbom disease (*Restless Legs syndrome*). Patients with PS often suffer from depression with anhedonia symptoms and it has been suggested that pramipexole has antidepressant effects in these patients by affecting dopaminergic neurotransmission[15]. Pramipexole has also been investigated in a small, randomized trial in unipolar and bipolar depression[16–18]. However, the clinical

effect has been too small to be widely justify treatment with pramipexole instead of usual antidepressants that have fewer side effects. However, in these studies, relatively low doses of pramipexole (an average of 1.0 mg) have been used when treating with pramipexole between 0.375 and 4.5 mg in Parkinson's disease. *The American Journal of Psychiatry* recently published a clinical review of patients with bipolar or unipolar therapy-refractory depression treated with pramipexole as adjunctive therapy. Interestingly, 76% reported an improvement that persisted up to 16 months after the end of treatment with an average dose of 2.5 mg pramipexole[19]. These results are promising because the patients in the study had a high degree of therapy resistance (several had not responded to ECT, for example), but the study was uncontrolled and took place within "clinical routine". Thus, well-controlled and systematic studies are needed before pramipexole can be recommended in the clinic.

In this pilot study, we want to test the efficacy and tolerability of pramipexole in the higher dose range (> 1.5 mg, max 4.5 mg salt) in patients with depression and with significant anhedonia symptoms despite usual treatment. The results of the study will form the basis for designing a larger placebo-controlled randomized trial. We also want to test the conditions for an fMRI scan before and after treatment to be able to demonstrate *"target engagement*". The aim of this is to eventually find biomarkers that can predict the treatment response of pramipexole.

8 RISKS AND BENEFITS

Affective disorders are associated with increased mortality in the form of increased suicide risk, but there is also increased mortality from other diseases such as cardiovascular diseases[20]. The WHO considers depression to be the main cause of disability in the world, and as many as one in four Swedes are at risk of suffering from depression requiring treatment at some point in their lives[21]. Given the often-long duration of the disease, this means severe personal suffering and great costs for society. Affective disorders with anhedonia symptoms have been associated with more severe depressive symptoms, more relapses, and are also particularly strongly linked to suicide[22]. There is also a high risk that the symptoms of anhedonia will persist after the depression has otherwise improved, and there is currently no effective treatment that specifically targets anhedonia. There is therefore a great need in this population (affective disorder with anhedonia) to find effective treatment.

Treatment with pramipexole may cause dose-dependent side effects such as nausea, vomiting, orthostatic hypotension, headache and dizziness. In the elderly after long-term treatment, motor disorders, hallucinations, delusions, confusion, mania (rare) and disturbed impulse control may develop in severe cases, but this is less common[23]. The risk of this is considered to be lower with shorter treatment times and with the use of prolonged-release drugs (as in the current study). Previous psychosis or impulse control disorder are examples of exclusion criteria in our study. In addition to unipolar depression and dysthymia, we will also include patients with bipolar disorder in the depressive phase in our study. A review of the literature has shown that the proportion of patients who make a switch from depression to a manic state during treatment with pramipexole is comparable (or even lower) to those who receive placebo[24]. Unlike other dopamine receptor agonists, pramipexole can induce somnolence (especially at doses above 1.5 mg/day according to FASS) and in rare cases, patients have fallen asleep without warning[23]. Therefore, patients affected by somnolence are instructed to refrain from driving and using machines until this side effect has been resolved. According to FASS, pramipexole is not contraindicated in any specific disease, in the treatment of Parkinson's disease the dose should be reduced in case of concomitant renal impairment (pramipexole is eliminated by the kidneys). Renal failure is another example of exclusion criterion in our study. Caution should also be exercised in patients with psychotic conditions and concomitant administration of antipsychotic medicinal products should be avoided.

Pramipexole is not indicated for the treatment of anhedonia symptoms and will be administered in this study mainly according to the Summary of Product Characteristics (SmPC) for the treatment of Parkinson's disease (maximum dose 4.5 mg of salt/day). This is also what has been done in previous clinical studies on depressed patients[16,18,25]. However, in these studies, pramipexole doses above 4.5 mg have also been treated[16,19]. As this increases the likelihood of neuropsychiatric side effects[23] and is not an approved dose range in Sweden, this does not occur in this study. The study population consists of adults who are able to give informed consent. The drug trial thus does not entail a major risk for research subjects, in which case an external safety committee is not considered justified. Adverse reactions are followed up with regular weekly contacts (bi-weekly telephone contact and bi-weekly physical visit) and are systematically recorded within the framework of the study.

The risk of severe adverse events is very small with dose ranges below 4.5 mg/day, which has been confirmed by several previous clinical studies with pramipexole in depressed patients[19]. Thus, the potential benefit of this pilot study, to demonstrate that this treatment is a feasible and tolerable intervention for depression with pronounced anhedonia symptoms, is greater than the risks for the included patients as it can lead to a successful treatment of the symptom of anhedonia in the future and increase the conditions for individual-based treatment.

9 RESEARCH QUESTIONS

The aim of the project is to treat patients with mood disorders and anhedonia symptoms with pramipexole as an adjunct to usual treatment. This open-label pilot study is intended as *proof of concept* (PoC) and we hope to obtain preliminary data that can form the basis for a larger, more definitive, placebo-controlled study. We evaluate dosing ranges to achieve sufficient treatment effect without obtaining intolerable side effects. According to the literature, this should be an average dose of about 2.5 mg/day but is expected to vary between individuals. For example, it is known that D3 receptors vary with age (fewer at older age) and that older individuals tolerate and need higher dosages to achieve treatment effect. Several previous clinical studies with pramipexole have, like this study, used variable and flexible dosage.

Blood samples are taken before and after treatment for analysis of inflammatory markers in order to investigate whether these can predict treatment response. We also want to investigate how the function of the brain's reward system is linked to anhedonia and treatment response. To this end, an fMRI (MID-task: test of the reward system) is planned to be performed before and after treatment with pramipexole (not mandatory to participate in the treatment study). This data will also form the basis for future follow-up studies aimed at better understanding the neurobiology behind anhedonia and to make prediction analyses of the treatment effect of pramipexole.

9.1 PRIMARY RESEARCH QUESTION AND VARIABLE

Primary question: Change in anhedonia symptoms after ten weeks of treatment with pramipexole at the highest possible dose (without intolerable side effects, max 4.5 mg).

Primary variable: Dimensional Anhedonia Rating Scale *(DARS) score* at *baseline* and week 10 of pramipexole treatment.

9.2 SPECIFIC RESEARCH QUESTIONS AND VARIABLES

Secondary question: Change in depression symptoms after ten weeks of treatment with pramipexole? How many respond to pramipexole (*responders*) and how many reach remission?

Secondary variable: Total-score MADRS. Response = reduction in MADRS by over 50%, remission = MADRS <= 10 after study end.

9.3 EXPLORATORY RESEARCH QUESTIONS AND VARIABLES

Exploratory question: What dosage of pramipexole has an effect on anhedonia symptoms without causing intolerable side effects? Is there a connection between anhedonia symptoms and systemic inflammation in the body? Does pramipexole treatment affect other symptoms associated with depression such as fatigue, anxiety, lethargy, and difficulty sleeping? Correlates estimation of anhedonia symptoms with DARS with estimation of secondary anhedonia scales (SHAPS, MAP-SR). Increases Task in the brain's reward system after treatment with pramipexole and is this linked to clinical efficacy? **Explorative variable:** We will evaluate optimal dosing intervals, i.e. lowest and highest final dose where the patient had clinical efficacy and at the same time no non-tolerable side effects. We will also evaluate the number of *dropouts*, change in blood levels of inflammatory markers, as well as change in anxiety symptoms (GAD-7), fatigue (Fatigue Severity Scale), sleep

problems (Insomnia Severity Index), and apathy (AES). Correlation between Snaith–Hamilton Pleasure Scale (SHAPS) and Motivation and Pleasure Scale (MAP-SR) total score compared to DARS total score. Difference in BOLD signal in ventral striatum (NAc) during *monetary incentive delay task* before and after pramipexole treatment.

10 STUDY DESIGN

This is an open-label, PoC pilot study in which patients are supplemented with pramipexole for 10 weeks. The study is being conducted in Lund, at Psychiatry Skåne, Adult Psychiatry Lund as a single center study. The treatment study is supplemented with a sub-study with fMRI examination in research subjects who wish to participate in this (not mandatory to be included in the main study).

11 STUDY POPULATION

Fifteen research subjects with either unipolar depression, bipolar disorder in depressive phase, or dysthymia are recruited to receive pramipexole treatment. All research subjects are asked to participate in the substudy (fMRI examination). Patients from the outpatient clinics and inpatient wards at the psychiatry in Lund will be asked if they want to participate in the study by treating physicians. Only those who are cared for under the Health and Medical Services Act (i.e. not under compulsory care) may be considered for the study. After the patient has been informed of the study and given consent, the patient will be invited to *screening* visits to the doctor responsible for research at the research clinic or the research physician visits the patient in the ward where the patient is admitted. All examinations/visits in connection with the study will take place at the adult psychiatry in Lund, while the fMRI examination will take place at Skåne University Hospital in Lund.

Research subjects who meet all inclusion criteria and no exclusion criteria as set out below, may be included in this study.

11.1 ELIGIBILITY CRITERIA

11.1.1 Inclusion criteria

1. Age ≥18 years ≤75 years.
2. Diagnosis of; unipolar depressive episode or bipolar disorder in depressive phase or dysthymia.
3. Depression symptoms: total score ≥ 18, as measured by the Montgomery-Åsberg Depression Rating Scale (MADRS).
4. Anhedonia symptoms: total score <27, as measured by the Dimensional Anhedonia Rating Scale (DARS). (Low score = high level of anhedonia symptoms)
5. Have an ongoing treatment of at least one antidepressant medication ≥ 4 weeks without a dose change. For patients with bipolar disorder, there must also be ongoing treatment with mood-stabilizing drugs.
6. The research subject has signed informed consent to participate in the study.

11.1.2 Exclusion criteria

1. Current pregnancy, breastfeeding or planned pregnancy.
2. High risk of suicide according to the research physician's overall clinical assessment.
3. Ongoing substance syndrome (within 12 months).
4. Diagnosis of psychotic disorder.
5. Treatment according to LPT.
6. History of impulse control disorder or a current ADHD diagnosis. The patients are assessed using the Problem Gambling Severity Index (PGSI) where ≤3 points are considered to indicate "moderate risk gambling" which is grounds for exclusion. PGSI will be estimated during follow-up visits and ≥3 points are then reason for discontinuation of the study (with a tapering schedule). In addition to this, at baseline, and at all follow-up visits, we will ask specific questions about impulse control behavior related to sex, shopping and food intake. We will use questions from the Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease[26]
7. Diagnosis of intellectual disability, dementia, or other circumstances that imply difficulty in understanding the meaning of participating in the study and giving informed consent.
8. Diagnosis of renal failure (eGFR <50 mL/min/1.73 m2) or severe cardiovascular disease (specifically

symptomatic heart failure NYHA class 2).

9. Recently started psychotherapy (within 6 weeks) or planning to start such treatment while participating in the study.
10. Ongoing ECT treatment.
11. Other medical conditions or other concomitant drug therapy (see section 13.5) that the investigators believe may affect the evaluable nature of the study or conditions that increase the risk of the trial. For example, Parkinson's disease, liver failure, ongoing cancer that has not gone into remission over a year ago.
12. Known or suspected allergy to any active substance or excipient in the drug product included in the study.
13. Participation in other studies.
14. Other reasons that prevent the research subject's participation, such as the risk that the research subject cannot complete the study (lack of compliance).

11.2 Criteria for discontinuation of study participation

1. Low compliance
2. New acute suicidality
3. Deterioration by more than 25% on MADRS
4. In case of unacceptable side effects such as the development of manic state, impulse control disorder, or symptoms of heart failure
5. Pregnancy
6. The research subject may terminate his/her participation in the study at any time without incurring any consequences for his/her further treatment.

Investigators may at any time discontinue a research subject's participation in the study due to, for example, unacceptable side effects, deteriorating mental health status, or non-compliance with the procedures in the study protocol (see study adherence section 12.8).

If research subjects discontinue prematurely, a plan will be drawn up for the discontinuation of pramipexole (tapering schedule). This is done according to recommendations in FASS but can be adapted individually if needed (e.g. withdrawal side effects that lead to slower joint progression).

12 STUDY DESIGN

Visit	*Screening*	*Baseline (last two weeks after Screening)*	v1, v3, v5, v7, v9 +/- 2 days	V2, V4, V6, V8 +/-2 day	v10 (termination) +/-2 day	Follow-up during Tapering (v14) +/- 2 days
Informed consent	X					
Anamnesis	X					
MINI	X					
SUAS	X					
SHAPS		X		X	X	
MAP-SR		X		X	X	
GAD-7		X		X	X	
YMRS		X		X	X	
PGSI	X	X		X	X	
Insomnia Severity Index		X		X	X	
Fatigue Severity Scale		X		X	X	
Apathy Evaluation Scale		X		X	X	
Physical examination	X					
Blood pressure control	X			X	X	
Incl./exclusion criteria	X	X				
Blood tests β-hCG (if female of childbearing potential), eGFR, liver status, Hb.	X				X	
Inflammation tests (CRP, leukocytes, IL-6, TNF-alpha)		X			X	
fMRI (sub-study)		(X)			(X)	
Distribution of study product		X		X	(X)	
MADRS	X	X		X	X	
DARS	X	X		X	X	
Telephone contact			X			X
Incidents (AEs)			X	X	X	X

12.1 RECRUITMENT/SCREENING

Physicians and nursing staff at general psychiatric clinics and inpatient wards (Region Skåne) are informed about the study and its inclusion criteria. Patients are asked to participate in the study. We will also advertise research subjects. Contact is made with the physician responsible for the patient (PAL) to ensure that he or she is prepared to possibly take responsibility for tapering or continued treatment with pramipexole after the end of the study (tapering can also take place within the framework of the study). The research subject is contacted for booking of *screening* visits that take place at the research clinic. During the visit, anamnesis is recapitulated, somatic diseases, ongoing drug treatment and psychiatric status is performed. Clinical suicide risk assessment is based on psychiatric status and with the help of Suicide *Assessment Scale (*SUAS)[27]. In Mini *International Neuropsychiatric Interview* (MINI)[28] is done for the purpose of confirming (or rediagnosing) psychiatric diagnoses. Assessment of symptoms is made using the MADRS rating scales[29] (depression symptoms) and DARS[30] (anhedonia symptoms). Inclusion and exclusion criteria are systematically assessed and the research subject's initials and year of birth as well as the time of *screening*-visits are documented (*screening*-log). Each research subject is identified by a unique number (enrollment log). The research subject must provide a blood sample in connection with the screening visit (β-hCG [if woman of childbearing potential], eGFR, liver status and Hb). These samples must be checked before a baseline visit can be considered. The research subject will also provide blood samples in connection with the baseline visit for analysis of inflammatory markers (CRP, leukocytes, IL-6 and TNF-alpha). No biological samples are collected for later analysis. A clinical control of status and pulmonary status is performed (including anamnestic questions with a focus on heart disease and auscultation of COR/pulm and blood pressure), and a note is written in the research subject's medical record. If the research subject is to participate in an fMRI examination, information about the procedure is distributed. Before *the baseline* visit has been made, it is ensured that the blood test results (of hCG, eGFR, liver status and Hb) are assessed and cancel the visit if these are abnormal (referral to the appropriate health care provider for further investigation/action is then sent if necessary).

12.2 PROCESS FOR OBTAINING INFORMED CONSENT

During the *screening* visit, before reviewing the medical history and inclusion and exclusion criteria, the research subject is informed orally about what it means to be part of the study. The information is then distributed on paper together with a signature form. The research subject is then allowed to read through the information in peace and quiet. The form is then signed and in connection with that, the research subject is again informed that it is possible to discontinue the study throughout the study and that they will then receive help with tapering off pramipexole if needed. Ethical aspects of processes that deviate from normal practice/statutory statutes are described in section 20.

12.3 PRIOR TO STUDY TREATMENT - *BASELINE*

In connection with the baseline visit, blood samples are taken for TNF-alpha, IL-6, CRP and Leukocytes. New estimation with MADRS and DARS is performed and with *Generalized Anxiety Disorder 7 scale* (GAD-7; anxiety symptoms), *Insomnia Severity Index*[31] (sleep problems), Fatigue Severity Scale[32] (fatigue symptoms), *Apathy Evaluation Scale*[33] (AES; apathy), Snaith-Hamilton Pleasure Scale[34] (SHAPS) The Motivation and Pleasure Scale (MAP-SR)[35] (anhedonia symptoms), and with the Young Mania Rating Scale (YMRS) (manic symptoms)[36]. Study drugs will be dispensed, and the study participant will begin treatment during the day (before bedtime) with the up-titration schedule described in section 13.3. A diary for dosage is handed out and the patient is instructed to bring this filled in at the next visit. On the same day as the start of pramipexole treatment, an fMRI examination is performed for the research subjects as Participating in Sub-studies. On X-ray department Ensures that there are no contraindications

to fMRI (no magnetic implants, normal renal value). Execution of fMRI examination is described in section 26.1. The research subject spends approximately one hour in the scanner and receives compensation for participation after the survey (see section 20.5).

12.4 Allocation of treatment

Study participants are assigned a generic version of pramipexole (Pramipexole Orion, see section 13).

12.5 During treatment

Every two weeks during the study, the research subject is called to the research clinic to discuss up-titration and side effects as well as new assessment with MADRS and DARS. Blood pressure control is performed. A review of the dosing diary and assessment of compliance *is* sufficient for continued participation in the study (number of days of treatment completed, correct dosage, possible change of other antidepressant/mood stabilizing drugs). Every two weeks (between physical visits), telephone contact is made with the research subjects to check the up-titration status and any side effects. In case side effects develop between Visits/telephone contacts the research subject is asked to report this directly. The strategy if side effects occur is to go back to the last tolerable dose and wait seven days before re-attempting to increase (see section 13 for details).

12.6 Final visit

After ten weeks, a final visit is made. New blood samples of inflammation markers are taken in the morning, and a new fMRI scan is performed on the research subjects participating in the substudy. The highest tolerable dose of pramipexole is noted and new estimation with MADRS and DARS is performed. A tapering schedule is drawn up if necessary.

12.7 In case of early discontinuation of study participation

If the research subject wishes to discontinue the study voluntarily or if it is deemed inappropriate to continue (e.g. lack of *compliance,* unacceptable side effects, deterioration of mental health*), the* research subject is offered a new visit or telephone contact to, among other things, establish a de-escalation schedule. Too rapid discontinuation of pramipexole increases the risk of withdrawal side effects in the form of e.g. apathy, anxiety, depression, fatigue, sweating and pain. When tapering becomes relevant, this will be done according to the structure of FASS (vg, see 13.3 below). The research subject is asked if the collected data (from fMRI examination) can continue to be stored and analyzed. Premature discontinuation is recorded, and significant and/or serious incidents are followed up after four weeks until the investigator deems it completed or after two attempts of contact without result.

12.8 Compliance

The requirement for study compliance is that the research subject participates in screening*, baseline and* final visits and taking pramipexole over 75% of the days prescribed regardless of dosage. Every other week, the research subject is questioned about the number of tablets taken (control of dosage diary). Investigators may also terminate the subject's participation if the intake of pramipexole is exceeded.

13 STUDY DRUG

13.1 INVESTIGATIONAL MEDICINAL PRODUCTS

In the study, the investigational medicine contains the active substance pramipexole. In Sweden, it was previously marketed under the name Sifrol but is now available in several generic versions. The dosage of pramipexole can be stated either as a base or as salt. In FASS, it is first and foremost the dosage as a base that is stated and the corresponding concentration in salt is given in parentheses. This protocol specifies dosage in salt form to be consistent with dosage indicated in the literature and abroad. In the diary and patient information for study participants, the dose is stated in base, as is stated on the medicine package and in the FASS.

Pramipexole is approved in the EU and is indicated as a symptomatic treatment of idiopathic Parkinson's disease, alone (without levodopa) or in combination with levodopa, in the maximum dose of 4.5 mg of salt. Pramipexole is also indicated as a symptomatic treatment of moderately to severely idiopathic restless legs syndrome in doses up to 0.75 mg of salt. Both apply to adults and not to children.

Pramipexole has a purely antidepressant effect (in addition to the effect on the underlying disease) in patients with Parkinson's disease, according to clinical studies. It has also been shown to reduce anhedonia symptoms in these patients[37]. However, pramipexole has no approved indication for the treatment of mood disorders. In this study, the research subjects will reach a maximum dose of 4.5 mg of salt/day that is already approved for the treatment of Parkinson's disease.

Common Side Effects According to FASS (Indication Parkinson's Disease):
Very common (≥ 1/10): Somnolence, Dizziness, Dyskinesia and nausea.
Common (≥ 1/100, < 1/10): Insomnia, Hallucinations, Abnormal dreams, Confusion, Behavioral symptoms of disturbed impulse control and compulsive behavior, Headache, Visual impairment incl. diplopia, blurred vision and impaired visual acuity, Hypotension, Constipation, Vomiting, Fatigue, Peripheral oedema, Weight loss incl. decreased appetite.

13.2. HANDLING AND LABELLING OF INVESTIGATIONAL MEDICINAL PRODUCTS

The investigational medicine is ordered from pharmacies that also carry out labelling. The medicine is then distributed in connection with the visits. In the study, prolonged-release tablets manufactured by Orion Pharma are used in strengths (salt) 0.375 mg, 0.75 mg, 1.5 mg, and 3 mg.

13.2.1 Labelling of Investigational Medicinal Products

The specific labelling of the investigational drug will take place in accordance with the Medical Products Agency's regulations.

13.2.2 Traceability of Investigational Medicinal Products

We use the drug accountability log and patients note the intake of drugs in the diary. Leftover medicines are returned to pharmacies.

13.2.3 Storage of Investigational Medicinal Products

Storage takes place in a locked cabinet, room temperature, at a nurse's office at the research unit.

13.3 DOSAGE AND ADMINISTRATION OF MEDICINAL PRODUCTS

The study is based on the hypothesis that it is necessary to reach a high daily dose of pramipexole as possible to achieve the expected effects (reduced anhedonia) reported in other studies[16, 19]. Thus, in the event of intolerable side effects, one will return to a lower dosage and make a new attempt at a dose

increase at a later time (side effects such as nausea, headache, etc. usually disappear after a week).

The study uses prolonged-release tablets that are taken once a day. In a phase I study, the minimum and maximum plasma concentrations and exposure were equivalent to the same daily dose of pramipexole prolonged-release tablets given once daily and pramipexole tablets given three times daily. Once daily administration of pramipexole prolonged-release tablets cause fewer fluctuations in pramipexole plasma concentrations over 24 hours compared to three times daily administration of pramipexole immediate-release tablets.

Up titration takes place according to FASS, that is:

Week 1: 1 tablet in strength 0.375mg for 7 days - daily dose 0.375mg
Week 2: 1 tablet in strength 0.75mg for 7 days - daily dose 0.75mg
Week 3: 1 tablet in strength 1.5 mg for 7 days - daily dose 1.5 mg
Week 4: 1 tablet in strength 1.5 mg and 1 tablet in strength 0.75 mg for 7 days - daily dose 2.25 mg
Week 5: 1 tablet in strength 3 mg for 7 days - daily dose 3 mg

After that, we will make a further increase of 0.75 mg every seven days up to a maximum dosage of 4.5 mg, depending on treatment response and side effects (see below).

The tablets are usually taken at night if the research subject does not have sleep problems. If non-tolerable side effects occur in connection with an increase in dose, the research subject is encouraged to notify this and return to the dosage that did not cause side effects. After that, wait another seven days before a new attempt at dose increase occurs. This is done on a maximum of two occasions before giving up attempts to reach a higher dosage (treatment continues with the lower dosage if possible). An exception to this strategy is if the study participant experiences side effects in the form of impulse control disorder (e.g. pathological gambling addiction or compulsive buying behavior), manic state, or symptoms of heart failure. If such a situation arise, pramipexole will be tapered off and discontinued. Study participants will be assessed with YMRS at baseline, week 2, week 4, week 6, week 8 as well as at week 10 to evaluate any manic symptoms.

The total duration of treatment is ten weeks before an evaluation of efficacy is made and a decision is made on continued treatment or discontinuation (referral is sent to PAL, which is responsible for continued treatment with pramipexole). If the research subject during the study shows a 50% decrease on MADRS or a 50% increase on DARS (=response), further dose increase is stopped, and the research subject then remains on this dose for the rest of the study. Control of *compliance* This is done by asking the research subject to bring the remaining tablets (and dosage diary) to the evaluation visits every two weeks for control counting of the number of tablets.

Pramipexole treatment must not be stopped abruptly due to the risk of dopamine *agonist withdrawal syndrome* (DAWS) with symptoms such as depression, anhedonia, apathy, anxiety, sleep disturbances, and others. The study participant is carefully informed about this. If discontinuation of the drug becomes necessary, this will be done gradually as instructed in FASS: The pramipexole dose is gradually reduced by 0.75 mg of salt per day until the daily dose is reduced to 0.75 mg of salt. The dose should then be reduced by 0.375 mg of salt per day. The patient is instructed to report any withdrawal side effects and then the dose reduction can be individualized to counter them.

13.4. NON-INVESTIGATIONAL MEDICINAL PRODUCTS

The research subject should be on a stable dosage for at least four weeks prior to the start of treatment (not ongoing dose changes) of at least one antidepressant. People with bipolar disorder should also be given a mood stabilizing drug.

To ensure that any clinical effect is dependent on pramipexole, it is recommended that study participants do not alter their ongoing antidepressant or mood stabilizing medication during the study. If the investigator, PAL or the patient himself assesses that the clinical condition nevertheless requires an adjustment of standing medication, this should of course be done. Nevertheless, the study participant can continue with pramipexole as planned, the change is noted in the patient's study log, and this is taken into account in the data analysis.

13.5 CONCOMITANT MEDICATION

Other drugs prescribed by the research subjects are managed as usual through their respective clinics. All concomitant medication is allowed while the research subjects are included in the study, except for those drugs that fall under exclusion criterion no. 11, i.e.:

(i) ongoing treatment with medicinal products that affect plasma levels of pramipexole; For example, cimetidine, amantadine, mexilepine, zidovudine, cisplatin, quinine and procainamide.

(ii) ongoing treatment with medicinal products that have a similar or antagonistic mechanism of action to pramipexole; For example, MAOIs, levodopa, other dopamine agonists, metoclopramide (Primperan), buspirone; This also applies to central stimulants such as methylphenidate and amphetamines (not caffeine).

(iii) ongoing treatment with neuroleptics; For example, olanzapine, haloperidol, risperidone, clozapine, aripiprazole, quetiapine (Seroquel).

After *a wash-out* of > 14 days of all drugs, a patient can be included in the study.

14 METHODS FOR COLLECTING STUDY VARIABLES

The study variables are collected using estimation forms, routine clinical analysis of blood samples and with fMRI examination.

14.1 EFFICACY VARIABLES

Total score DARS (primary variable) and exploratory variables such as total score MADRS, SHAPS, MAP-SR, GAD-7, AES, *Fatigue Severity Scale* and *Insomnia Severity Index* gathered in a self-assessment questionnaire (validation of Swedish translation of DARS is in progress) at *screening*-visit *baseline*-visits and every other week for 10 weeks in connection with visit at the research clinic (a total of six occasions; MADRS and DARS on seven occasions).

Blood inflammation parameters (CRP, IL-6, TNF-alpha, and leukocytes) are collected at *baseline* and final visits. Other exploratory variables are collected during the fMRI examination.

14.2 SAFETY AND TOLERABILITY VARIABLES

14.2.1 Laboratory variables

Routine laboratory tests (blood tests) consist of eGFR, liver status (ASAT, ALAT, GT, ALP), Hb value and β-hCG and are taken between *screening* visits and *baseline* visits and at the final *visits*. Abnormal/unexpected/abnormal metrics after *screening* and termination visits are recorded and reported as incidents in the study. (see paragraph 15.5 below).

14.2.2. Vital metrics

Heart and lung status is performed according to the clinic's standard routine at screening visits. This includes anamnestic questions with direction against heart disease and auscultation of Cor/pulm. Blood pressure control is performed according to the clinic's standard routine at screening visits, v2, v4, v6, v8 and final visit (v10). Abnormal/unexpected/abnormal vital signs are recorded and reported as incidents in the study. (see paragraph 15.5 below).

14.2.3. Tolerability variables

Adverse reactions to pramipexole treatment are collected in connection with visits (biweekly physical visit and biweekly telephone contact). If adverse reactions develop between two visits, the research subject is asked to report this, and the strategy is usually to go back to the last tolerable dose and wait 7 days before making a new attempt at elevation (see section 13.3 for details).

15 SECURITY MONITORING AND REPORTING

15.1 DEFINITION OF INCIDENT

Term	Definition
Incident / *Adverse Event (AE)*	An undesirable medical event or deterioration of existing conditions in a research subject who has received a drug, and which is not necessarily causally related to the given treatment. Examples of incident: symptoms (nausea, somnolence, development of hypertension, development of mania.
Side effect / *Adverse Reaction (AR)*	An adverse medical event that is judged by a medically competent to have a reasonable probability of having a causal relationship with the study drug at any dose
Serious Incident / Serious Adverse Event (SAE)	An undesirable medical event that meets one or more of the following criteria: 1. Death. 2. Life-threatening conditions. The term "life-threatening" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically would have caused death if it were more serious. 3. Requires hospital care or prolongs an already started hospital session. 4. Causes a permanent or significant disability or disability. 5. Constitutes a congenital malformation or congenital defect. 6. Other medically significant event for the research subject that requires an intervention to prevent one of the above-mentioned criteria.
Serious adverse reaction *Serious Adverse Reaction (SAR)*	An adverse medical event that is both serious and is judged by a medically competent person to have a reasonable probability of having a causal relationship with the study medication.
SUSAR *Suspected Unexpected Serious Adverse Reaction (SUSAR)*	A suspected serious side effect that is unexpected, i.e. the nature and severity of which do not correspond to the known information on the medicine as specified in the reference safety information (Investigator*'s Leaflet* [IB]) or Summary of Product Characteristics).

15.2 SAFETY INFORMATION

The Summary of Product Characteristics (SmPC) of pramipexole prolonged-release tablet (Orion Pharma) is used for the assessment of the relationship between the event and the medicine and the assessment of a suspected unexpected serious event (SUSAR).

15.3 ASSESSMENT OF SEVERITY

Mild: short-term/transient symptoms that do not affect the subject's daily activities.

Moderate: tangible/obvious symptoms that to some extent affect the research subject's daily activities.

Severe: symptoms that significantly affect the subject's daily activities.

15.4 Incident relationship assessment

Likely: good grounds and sufficient documentation to assume that a relationship between symptoms and study drug exists.

Possible: a possible causal relationship is conceivable and cannot be ignored.

Unlikely: symptoms that are unlikely to be related to the study drug.

15.5 Registration of incidents

At each contact once a week (every other week physical visit and every other week telephone contact), the research subject will be asked how he/she has been feeling since the previous contact. The research subjects are also informed to report any incident between these visits for discussion of dose changes, among other things. Incidents are recorded in the research subject's medical record and in the study's CRF. Registration of difficulty and relationship is only done in CRF. As a minimum, a description of the event (diagnosis or symptoms), start and stop, relationship, severity, and if it is considered serious, measures and outcome are recorded for each incident.

Incidents are followed from the start of treatment until four weeks after the end of visits. If the research subject wishes to continue with pramipexole after the end of the study, this is done in consultation with the research subject's PAL. In such cases, no dosage decrease takes place, but incidents are followed up for four weeks after the final visit via telephone contact until it is completed or after two attempts with contact without results.

All serious incidents will be followed until they are recovered or no longer necessary to be followed.

15.6 Pregnancy

Pregnant women will not be included in the study. Women of childbearing potential must have a negative serum β-hCG pregnancy test to rule out a pregnancy prior to initiation of treatment. Blood samples are taken between *screening* visits and *baseline* visits and at the final visit. Women who become pregnant during the study are excluded from the study. The effect of pramipexole on the unborn baby is unknown and treatment should be discontinued as early as possible. Clinical data are also lacking for pramipexole and breastfeeding, which is why ongoing breastfeeding is an exclusion criterion.

15.7 Reporting of incidents

15.7.1 Reporting of Incidents and Adverse Events

All incidents and adverse events are recorded by the trial site in the CRF during the study. Collected incidents and adverse events are reported at the end of the study through the final report to the authorities (see below).

15.7.2 Reporting of Serious Adverse Events and Serious Adverse Reactions (SAEs and SARs)

All serious incidents must be reported within 24 hours from knowledge to the investigator responsible and reported in the CRF. Reporting serious incidents and/or serious adverse reactions to the relevant authorities takes place annually through the annual safety report (see below)

15.7.3 Reporting of SUSARs – serious unexpected serious adverse reactions

A suspicious, serious unexpected adverse reaction to the study drug, SUSAR, that is fatal or life-threatening is reported to LV and EPM within 7 days of the event being detected. Supplementation of the report is sent to the authorities within a maximum of 8 days.

A suspicious, serious unexpected adverse reaction to the study drug, SUSAR, that is not fatal or life-threatening is reported to LV and EPM within 15 days of becoming aware of the event. A supplement to the report will be sent to the authorities as soon as possible.

15.8 ANNUAL SAFETY REPORT

The annual safety report is compiled by investigators and sent to LV and EPM, if EPM so wishes. The report summarizes serious incidents that have occurred, as well as a summary assessment of the safety of the research subjects who are still included in the trial, and whether the study's benefit-risk assessment has changed since the study was approved.

16 STATISTICAL CONSIDERATIONS

16.1 POWER CALCULATION

Fifteen individuals (n = 15) are included in the study to ensure that at least ten individuals complete the study (the number of *dropouts* is estimated to be less than one-third). No clinical study has previously used DARS as a primary efficacy variable, but during the development of DARS, the mean standard deviation (SD) was 13.73 when estimating depressed patients[29]. Power calculation with 10 research subjects (r = 0.5, α = .05) results in a difference in mean value of 16.6 at *power* 95% can be detected. This corresponds to the research subject estimating one point less on each question in DARS (a total of 17 questions), which would be a clear clinical improvement.

16.2 POPULATION

Complete Analysis Quantity (FAS) is the primary analysis set. The analyses will also be performed on per protocol amount (PPS).

16.3 STATISTICAL ANALYSIS OF PRIMARY, SECONDARY AND EXPLORATORY VARIABLES

All statistical analysis is performed using the IBM SPPS Statistics v24 software. Paired T-test (2-tailed) is used to analyze the difference in DARS scores between *baseline* and after ten weeks of treatment with pramipexole (primary variable). Normal distribution is expected based on previous studies. Descriptive analysis is performed by response (reduction by at least 50% in MADRS score), remission (MADRS ≤ 10 at study end) of depression symptoms (secondary variable), and number of *dropouts*. Descriptive analysis, mean and *range*, is performed on the dosage of pramipexole at the end of the visit. Of the exploratory variables, similar *paired* T-tests (or equivalent non-parametric method if necessary) are used for the analysis of difference in total scores MADRS, SHAPS, MAP-SR, AES, GAD-7, Fatigue Severity Scale and Insomnia Severity Index.

Analyses may also be made on imputed datasets. If values are missing from the measurements over time, they will be replaced with the means of adjacent values before and after the missing value or with the last observation.

17 DATA MANAGEMENT

17.1 DATA FLOW AND PROCESSING

Data are collected and processed in coded form after the study has ended. Relevant medical record data is transferred to the CRF. Data from CRF, self-assessment forms will then be manually transferred to an Excel file that is password protected for further entry. A manual check that the correct entry has taken place takes place after the study has been completed and is entered as a variable. Data from the Excel file is copied to SPSS database files via automatic functions in the program for statistical analysis by authorized personnel.

17.2 CASE REPORT FORM (CRF, DATA COLLECTION DOCUMENTS)

The data collected in the study will be recorded in a case *record form* (CRF) in paper form that is prepared specifically for recording data for the study in question. A research person form must be available for each research subject and all questions in the research person form must be answered. Questions that cannot be answered will be noted with ND=*Not done*, NA=Not applicable or NK=Not known. Any corrections are made by crossing out the incorrect and dating and signing the change and writing the correct information next to it.

The following rating scales will be added as an appendix to the CRF: MINI, SUAS, SHAPS, MAP-SR, AES, GAD-7, *Insomnia Severity Index*, and *Fatigue Severity Scale*.

17.3 SOURCE DATA DOCUMENTS

1. **Patient record:** Kept in the Melior data journal program according to clinical procedures and the requirements of the
   clinical trial. The minimum level of what must be recorded:
   (i) Name of the examination
   ii) Information that all criteria for inclusion and none for exclusion have been met
   (iii) Date when the patient signed informed consent
   iv) Date when the patient ends or discontinues the study
   (v) Details of diagnosis
   (vi) Data on intake of investigational medicinal products
   (vii) Details of complications
2. **Consent form**
3. **Patient Identification List:** Included patients are assigned research numbers in the order they are included. The patients' names and personal identity numbers are registered on the list in connection with each research number.
4. **Case Report Form *(CRF*) and rating scales**

17.4 INPUT AND ACCESS TO STUDY DATA AND SOURCE DOCUMENTS

Data is entered manually into a data file (Excel and SPSS) in accordance with section 17.1 above. The data file is password protected, and only authorized personnel have access to the file. All information is coded so that no social security numbers, names, initials, addresses, etc. are found in the file. When data was collected, each study participant will be given a specific number, and a code list will be established. This is kept locked up and saved to be able to check the quality of the studies. The code list and source data are kept locked away. Monitors from Clinical Studies Sweden – Forum South will also have access to source data after signing a non-disclosure agreement. All study material is archived for 10 years from the time the study report is written and reported to the authorities.

18 QUALITY CONTROL AND MONITORING

Patients are informed via the patient information that monitoring is taking place and that the monitor and any control authority have access to medical records. A confidentiality agreement must be established between the operations manager, who is formally responsible for the patients' medical records, and the monitor. Monitoring of the study is done by clinical studies Sweden – Forum South according to the GCP principles. A risk-based monitoring plan is drawn up.

Monitor has access to CRF, patient records and originals of laboratory data, etc., to ensure source data relevant to the study, without compromising patient privacy. Inspection by an authority may be carried out.

19 ARCHIVING

Investigators have a study binder with relevant content according to ICH GCP Chapter 8"*Essential documents*". The place of the trial study data, research person identification list, original research subject information and obtained consent for the study will be stored inaccessible to unauthorized persons, but so that research subjects in the study can be identified by those responsible for the study.

A complete trial binder, as well as source documents will be archived at least 10 years after the study report is written and submitted to LV. Source data in patient record systems is stored and archived in accordance with Region Skåne's regulations.

The Archives Act (1990:782) applies to the archiving of research material. The National Archives' regulations and general guidelines on the disposal of documents in the research activities of government agencies, RAFS 1999:1, as well as the region's instructions, will be followed.

20 ETHICAL AND REGULATORY ASPECTS

The study is carried out in accordance with the current version of the Declaration of Helsinki, ICH GCP and applicable international and national regulations. The study can start when written approval from EPM, permission from LV and other approvals required by current regulations are available. The responsible examiner is responsible for ensuring that the content of the individual documents submitted to LV in an application is identical to the content of the corresponding documents when applying for approval at EPM. Approval of notification of personal data processing; approval from KVB for extraction from medical records for research; registration in the DSF for PUG treatment; and approval of the notification to Image and Function Region Skåne must be obtained. Biobank is not needed as blood samples are analyzed and destroyed within 6 months after sampling.

20.1 SIGNIFICANT CHANGES TO THE STUDY

If essential changes in study implementation or the study protocol (including research subject information) is made after approval, an addition "*Amendment*" be written and sent to EPM and/or LV for approval before this change may be implemented (unless performed to prevent a security risk). The respective authority information regarding which changes must be applied for and approved by them will be followed. A significant change can be, for example, a change in an inclusion/exclusion criteria, a change in investigational medicine or if, for example if the principal investigator (the person responsible for the applications) is replaced.

20.2 SERIOUS PROTOCOL DEVIATIONS

Deviations from trial protocols, GCP and other regulations that in a significant way directly affect, or with a high probability would affect, the research subjects in Sweden or the scientific value of the trial must be reported immediately within 7 days to LV. It is the responsibility of the investigator responsible to assess the consequences of deviations that have occurred, and thus also decide whether LV should be informed.

Minor deviations that do not affect the integrity or safety of the research subjects, or significantly affect the scientific value of the trial, are documented.

20.3 REPORT ON CLOSING

Within 90 days after the end of the study (see definition of study completion below), LV and EPM will be notified of termination. The Investigator in charge may at any time discontinue a study if there is a reason to do so. This may be that the study's benefits no longer outweigh its risks. In such cases, LV and EPM must be informed in writing within 7 days.

20.4 PARTICIPANT INFORMATION AND OBTAINING INFORMED CONSENT

All patients are carefully informed in advance about the design of the study and about how the study will be conducted and evaluated. Each research subject must have understood the explanation of the purpose of the study and how the trial is structured and received written and oral information about what the expected benefits and risks are.

Subjects have the opportunity to make their own trade-off between study treatment and therapy given according to clinical practice. They are given the opportunity to ask questions about the study before consent is given. It appears that the subject's participation is voluntary and that participation can be discontinued at any time without deteriorating medical care. The subjects' signatures give consent to

participate in the trial, that the monitor and pharmaceutical authorities (possibly foreign) have access to the study's source data under confidentiality.

The investigator (physician) who informed the patient will certify with their signature that the patient received (and understood) the information and gave their consent to the study.

20.5 Compensation for participation in sub-study

The research subjects who are also included in the substudy (fMRI examination) receive a compensation of SEK 750 for participation plus a maximum of SEK 500 (normally around SEK 250) as "gains" in *the monetary incentive delay task* (MID). SEK 750 is an incentive to participate in the fMRI examination and is normal compensation in fMRI studies[38]. The level is balanced to be sufficient as an incentive for participation in the fMRI examination, but not as an incentive to participate in the main study. SEK 0 to 500 is an accepted remuneration in the form of "winnings" in the MID test itself and is a necessity for the execution as the test examines the activation of the reward system when receiving monetary gains (see appendix 25.1).

21 TIMEPLAN AND DEFINITION OF STUDY COMPLETION

The study is expected to start on 1 September 2019 and is expected to be completed by 1 December 2020 at the latest. *End of the trial* occurs when the last research subject has completed the last study visit and follow-up telephone contact has been made.

22 INSURANCE

Study participants are covered by patient insurance (the Patient Injury Act) as the study is carried out within the framework of the county council's health and medical care. Research subjects are insured by the Swedish Pharmaceutical Insurance Association (LFF).

23 FINANCING

Funding comes from the Söderström-Königska Foundation (500 000 SEK, SLS-851561); SUS donations and foundations (180 000 SEK) and the Royal Physiographic Society in Lund (90 000 SEK). Principal Investigator Daniel Lindqvist also has a grant in the form of ALF Young Researchers, which frees up 50% research time in the clinical position.

24 REGISTRATION, REPORT AND PUBLICATION

24.1 REGISTRATION

The protocol of the study is published in the public registry EU Clinical Trial Register (EU CTR), via EudraCT application to LV before inclusion of the first research subject.

24.2 DISSEMINATION

According to the Declaration of Helsinki, study results will be made publicly available as soon as possible after the end of the study, and no later than one year after completion, regardless of whether the results are positive, negative or neutral via publication and/or public database.

24.3 REPORT AND PUBLICATION

The study is planned to lead to a scientific article/report intended for an international peer-reviewed journal.

Publications will also form the final report to LV and EPM. Authorship is determined in accordance with the guidelines of the International Committee for Medical Journal Editors (ICMJE). All authors must contribute to, review, and accept the final publication.

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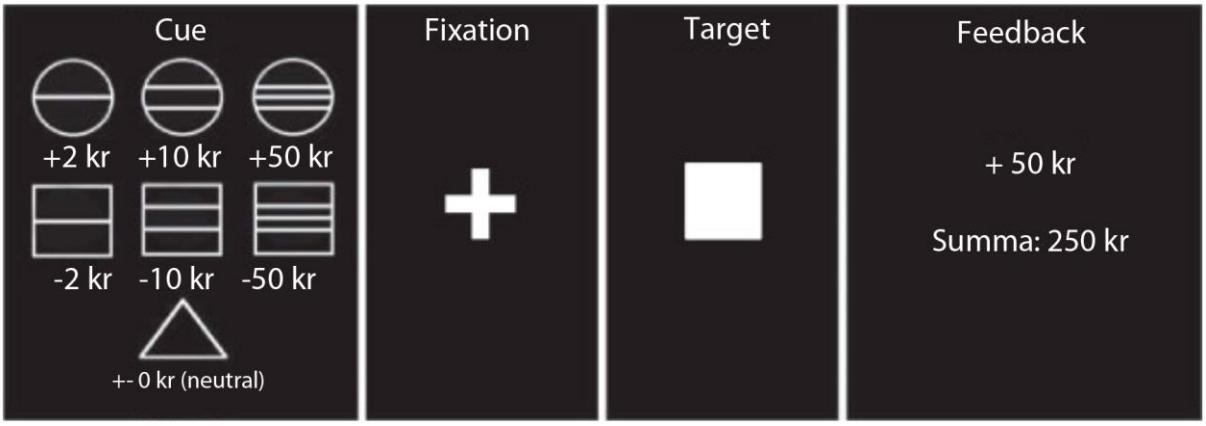
26.1 fMRI

The MRI scans are done with a Siemens 3T scanner or with the Philips 7T. Initially, examinations are performed to obtain structural images of the brain as well as diffusion *tensor imaging (diffusion tensor imaging; DTI).* Thereafter, functional connectivity is examined using the so-called Blood-Oxygen Level Dependent (BOLD) *resting state*. These surveys do not require the research subject to be active in the camera. Finally, a BOLD fMRI paradigm is performed during a test called *the monetary incentive delay task* (MID), which explores different phases of reward processes and requires active participation of the research subject. All examinations are estimated to take a total of less than an hour in the scanner.

During MID, simpler images are displayed on a screen and the subject is provided with a button box. At each examination, the subject is exposed to visual stimuli on 72 occasions. The images consist of a circle, square or triangle (Cue) that indicates how much money can be gained or lost (see image below).



The clue is displayed for 500 ms and is followed by a fixation cross (200 to 2500 ms). After that, the target square (160 to 250 ms) is shown, at which point the research subject is instructed to press the button as quickly as possible to win money or not to lose money. The time is adjusted during the test so that the research subject succeeds around 66% of the time. Feedback is then given on how it went, and the total amount of money raised.

27 AMENDMENTS

No	Date	Change and cause	Author and new version
1	190617	Based on comments from Ethics committee (and because pramipexole can cause impulsive disorders e.g. increased gambling as a side-effect), we changed the protocol and added to the exclusion criteria nr 6 a new scale “Problem Gambling Severity Index”; to ensure the exclusion of subject with risk gambling. The rating takes place at baseline meetings and during every second week during the pramipexole treatment.	Daniel Lindqvist v1.1