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Clinical trial subscription

Title: "Evaluation of the efficacy and safety of Resveratrol in the treatment of depression: a double-blind, randomized, placebo-controlled study in two parallel groups of patients" ("Resveratrol")

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RATIONALE

In July 2015, the predominant involvement of the *SIRT1* gene in the prevalence of depression in the Chinese population was revealed (CONVERGE, Nature, 2015). This gene mediates the activity of the sirtuin-1 protein, which is an antioxidant / cytoprotective enzyme (Kim et al., 2012). For the SIRT1 enzyme, a known activator is natural phytoalexin, a flavonoid-polyphenol resveratrol. Two-thirds of US residents who take dietary supplements (dietary supplements) in their food consume resveratrol-containing dietary supplements (Hubbard, Sinclair, 2014). The clinical effect of resveratrol is being studied in diabetes and obesity, cancer and cardiovascular diseases (www.clinicaltrials.gov). However, no studies have been conducted on the effects of resveratrol on depression (based on an analysis of the PubMed and www.clinicaltrials.gov databases).

During the research, the dependence of the therapeutic effect on the structural features of the *SIRT1* gene and its functional activity will be studied using the example of the sirtuin-1 enzyme. The structural features of the gene were studied in 2016-2017. within the framework of the Russian Science Foundation project No. 16-15-00128 "Structural features of the *SIRT1* gene as a basis for the choice of personalized drug therapy for depression", which includes the Resveratrol study. In patients with MDD and dysthymia, the occurrence of the genotype in the rs2236318, rs7896005 and rs36107781 alleles of the *SIRT1* gene was found to be statistically significantly different than in the population.

At the same time, the safety of taking resveratrol will be monitored despite the fact that there is currently no information in the available literature on its side effects in humans. Resveratrol is metabolized by the liver by sulfation. In addition to resveratrol, active substances in the blood are its metabolites.

The implementation of the study (in the version of the Study Description dated October 19, 2017) was approved by the Ethics Committee of NIIFFM (Protocol No. 13 dated October 27, 2017). The study was registered on December 19, 2017 in the Clinical Trials Registry at www.clinicaltrials.gov (NCT03384329) under the name "RESV-depression".

The difference in HDRS-17 scores between the inclusion point and visit 2 is a key primary outcome measure.

The difference in SIRT1 enzyme activity between the inclusion point and visit 2 is a key primary outcome measure, along with the change in HDRS-17 scores.

The BDI-II score difference between the inclusion point and visit 2 is a secondary outcome measure.

The study goal: To evaluate the efficacy and safety of resveratrol in the treatment of depression.

METHODS

Patients

The study is planned to be carried out from December 2017 to December 2018 on the basis of the NIIFFM (and, possibly, on the basis of the Tomsk NIIPZ - if patients are not recruited quickly enough). The target number of patients in the study is 60.

Inclusion criteria:

- men or women between the ages of 18 and 75;
- body mass index (BMI) ≤30;
- diagnosis of major depressive disorder (single episode or recurrent) with melancholic features or diagnosis of dysthymia (based on DSM-5 classification, Appendix 1);
- current depression (according to DSM-5 criteria);
- the severity of depression is moderate and higher according to the data of clinical examination and data of two scales: the Hamilton scale HDRS-17, completed by the physician-researcher (not less than 16 points), and the BDI-II scale, completed by the patient (not less than 20 points);
- major depressive disorder or dysthymia significantly predominates in the clinical picture over other mental disorders that the patient may have (for example, personality disorder);
- satisfactory and stable state of somatic health; if there are chronic diseases, then compensated, with a selected dose of constantly taken drugs;
- the beginning of sleep – between 21:00–02:00;
- the patient's consent to complete the entire study schedule;
- signed informed consent to participate in the study.

Non-inclusion criteria:

- major depressive disorder with atypical features, anxious distress, with mixed features, with a seasonal pattern, with psychotic features (according to DSM-5 Appendix 1);
- bipolar disorder;
- resistant depression (lack of response to two consecutive treatments with therapeutic doses of antidepressants for at least 6 weeks each);
- suicidal plans and intentions, aggressive behavior, psycho-productive symptoms;
- antidepressant intake in the past: 3 weeks - fluoxetine, 2 weeks - non-selective MAO inhibitors, one week - other antidepressants (including herbal antidepressants);
- taking normotimics and sleeping pills - in the last 2 weeks;
- taking vitamins (especially nicotinamide) and dietary supplements - for the last week;
- treatment with transcranial magnetic stimulation, current stimulation, xenon inhalation, light therapy, psychotherapy - in the last 2 weeks;
- serious (severe) and / or unstabilized diseases: malignant neoplasms, uncontrolled arterial hypertension, stroke, epilepsy, tuberculosis, etc.;
- pregnancy;
- night work, crossing more than 2 time zones and an acute illness within the last week;
- patient characteristics that prevent venipuncture;
- patient refusal to undergo any research;
- clinically significant pathological findings, conditions and circumstances that, in the opinion of the researcher, will negatively affect the patient's well-being, the course or results of the study.

Exclusion criteria:

- failure to conduct key examinations - assessing the severity of depression on the HDRS-17 scale and taking venous blood for the analysis of the activity of the SIRT1 enzyme - before and after the drug intake (inclusion visit and visit No. 2);
- non-adherence to the drug - more than 3 days missed in any half of the study (15 days), admission at the wrong time (in the afternoon or evening instead of morning) most of the days;

• the appearance of moderate to severe adverse events (for example, (hypo) manic state) associated with, or probably associated with, taking the drug;

• the emergence of non-compliance with the inclusion or non-inclusion criteria in the research process, including clinically significant pathological data, conditions and circumstances (for example, the loss of a loved one, etc.), which, in the opinion of the researcher, will negatively affect the patient's well-being, the course or results of the research.

Randomization

The study is a triple-blind, balanced, randomized study on two parallel groups of patients - taking resveratrol (30 people) and taking placebo (30 people).

The medication comes from the manufacturer in two types of vials marked on the back: lot # 50743 (purple caps) or lot # 72005 (white caps). The information about which of them corresponds to the active substance, and which to placebo, is known only to the manufacturer, and will become known to researchers only after the completion of the entire study. Each bottle contains 60 capsules.

The vials received from the manufacturer are kept in custody by the head of the pharmacy.

From these vials, capsules in the amount of 30 pieces are transferred to other vials prepared in advance of the same type, each of which bears a sticker-label with the following information: (1) the name of the study - "Resveratrol", (2) the number of the vial - "1", "2", "3" ... (according to randomization codes).

The list with randomization codes was prepared in advance by one of the study participants (K.V. Danilenko), known only to him and the head of the pharmacy, and is kept out of the reach of others (in a safe). The randomization is block, with a step of 2: each pair will contain 1 bottle of resveratrol and 1 bottle of placebo. The vials are held by the person in charge of randomization ("blinding") and dispensing of the drug (head of the pharmacy).

he prepared vial with the active substance or placebo is issued by the head. pharmacy to the doctor-researcher for each individual patient. The dispensing of the vial is recorded in the Drug Dispensing Report Card (Appendix 3), which is held by the person responsible for the randomization (“blinding”) and dispensing of the drug (head of the pharmacy). At the time the bottle is dispensed, the patient's surname and initials, as well as his code, are applied to the bottle. The Table contains the following information: (1) vial number, (2) date of issue of the vial to the patient, (3) patient's surname and initials, (4) patient's date of birth, (5) patient code, (6) doctor's signature.

Intervention

Resveratrol. Resveratrol from Biotivia (USA), which is a dietary supplement (FDA). Each capsule contains:

- 500 mg 98% trans-resveratrol (trans-resveratrol; polygonum cuspidatum root extract);
- 5 mg 95% piperine (piperine; piper nigrum extract);
- 5 mg 98% polydatin (polydatin; polygonum cuspidatum extract).

The addition of piperine is due to the fact that this alkaloid increases the bioavailability of resveratrol (patent No. 5,536,506 dated July 16, 1996, USA; Johnson JJ et al., "Use of piperine to increase the bioavailability of nutritional compounds", 2011). All constituent ingredients are substances extracted from plants. This drug has already been used in clinical trials (eg in England; link).

Microcrystalline cellulose (MCC) is used as a placebo capsule.

Analytical control of resveratrol and piperine will be performed using preparative chromatography on an outsourced basis. The analysis will be performed for each delivered batch of capsules separately.

The patient is given 30 capsules for a month. It is advisable for the patient to store capsules in the refrigerator at a temperature of +2 to + 8C, but it is also possible at room temperature. Capsules are taken one per day, daily in the morning, preferably on an empty stomach (half an hour before meals), without chewing, swallowing, drinking water. If the patient does not take the drug in the morning, it must be taken as early as possible during the day. Appointment times and missed appointments are noted by the patient in the Diary (Appendix 16). Missing appointments are recorded by the examining physician at visits 1 and 2 by counting the capsules remaining in the vial, and the dates of the missing are recorded on the Patient Registration Card (Appendix 5).

Control over the intake of the drug is carried out by the SMS notification system (see below).

Methods of evaluation and studied variables

The psychometric block consists of a set of questionnaires filled out by a doctor during a clinical interview and by a patient independently under the supervision of a clinical psychologist. Questionnaires are filled in in accordance with the research plan (see below).

The list of questionnaires filled out by the doctor:
1. Mini-International Neuropsychiatric Questionnaire (M.I.N.I.)
2. Montgomery-Asberg Depression Scale (MADRS)
3. Hamilton Depression scale (HDRS-17)
4. Depression Symptom Questionnaire (IDS)
5. Expectation scale
6. Clinical General Impression (CGI) scale
7. Colombian Suicide Severity Scale (C-SSRS)
8. Antidepressant Treatment Response Questionnaire (ATRQ)
9. Maudsley Stage Model (MSM)
10. Scale of side effects (UKU).

The list of questionnaires is filled in by the patient independently under the supervision of a psychologist:
1 Depression Scale (MADRS-SR)
2.Visual Analog Scale (VAS)
3.Positive and Negative Affect Schedule (PANAS)
4 Situational Anxiety (STAI)
5 situational anger (STAXI)
6 Depression Symptom Inventory (IDS-SR)
7 Depression Symptom Questionnaire (QIDS-S)
8 Depression Questionnaire (PHQ-9)
9 Beck Depression Questionnaire (BDI-II)
10 Depression, Anxiety & Stress Scale (DASS)
11 the suicidal ideation scale (ASIQ)
12 Snaith-Hamilton scale of anhedonia (SHAPS)
13 Beck's Scale of Hopelessness (BHS)
14.Pittsburgh Sleep Quality Questionnaire (PSQI)
15.Hospital Anxiety and Depression Scale (HADS)
16.Eating Attitude Test (EAT)

Issued to the patient for self-filling:
1.Hypomania Questionnaire (HCL-32)
2.Barchard Emotional Intelligence Questionnaire (EI-IPIP)
3.Personal Anxiety (STAI)
4.Personal anger (STAXI)
5 Cloninger Temperament and Character Questionnaire (TCI-125)
6 Behavior Activation and Inhibition Systems Questionnaire (BAS / BIS)
7 Goldberg Questionnaire (BFFM-IPIP)
8 Emotion Regulation Scale (ERQ)
9 Thought Suppression Scale (WBSI)
10 The Five-Way Awareness Questionnaire (FFMQ)
11.Toron alexithymia scale (TAS-20)
12 Circadian Rhythm Type Questionnaire (MEQ-SA)
13 Childhood Trauma Assessment Questionnaire (CTQ)
14 Bass-Perry Aggression Questionnaire (BPAQ)
15 Rumination Scale (RRS)

Forms of questionnaires filled in by the doctor and clinical psychologist of the department are pasted into the patient's registration card, forms of questionnaires filled out by the laboratory psychologist are stored in the neurophysiology laboratory.

Psychophysiological research (special psychometry, EEG, EP, eye tracking, stabiloplatform) / performed within 2 days /

1st day:

1. filling out a questionnaire and questionnaires to assess the current state (VAS, PANAS, situational anxiety (STAI), situational anger (STAXI)), photo-documenting Self / 30-40 min /;
2. imposition of an EEG helmet and hardware localization of electrode topography in 3-D space 30 min;
3. resting state electroencephalography / resting state EEG / - registration of bioelectrical activity of the brain / 128 EEG channels, vertical and horizontal electrooculograms, ECG / 2-lead / in association with on-line assessment of the degree of actualization of dysadaptive cognitive strategies / 16 min /;
4. registration of long-latency visual evoked potentials // when perceiving motivationally significant threats and positive reinforcement stimuli / OddBall Pictures paradigm, 40 min /;
5. eye tracking / Self paradigm / - registration of eye movement and gaze fixation when the emotional expression of one's own face and unfamiliar emotional faces is perceived on the computer screen with the expression of discrete emotions of joy, anger and sadness, 20 min;
6. stabilometric test for motor retardation (Dual Cost Task), 30 min;

2nd day:

1. filling out questionnaires (8) to assess the dynamics (BHS, QIDS, PHQ-9, SHAPS, ASIQ, PSQI, HADS, EAT);
2. testing CANTAB;
3. filling in personal and typological questionnaires (15).

Body weight is measured during each visit in the morning on an empty stomach without outerwear on the same scales, the measurement results are entered into the Registration Card. Height is measured once.

Actimetry is performed using a MotionWatch 8 actimeter (CamNTech, England) worn on the wrist of the non-dominant hand throughout the study, with readings taken at the switch-on point and at visits 1, 2 and 3. The actimeter measures the level of motor activity (and lighting) every minute and serves to assess sleep / wakefulness. The actimeter is issued to the patient against a receipt (Appendix 10) along with instructions (Appendix 11).

Blood is taken from a vein and further processed according to the Instruction on the logistics of handling blood samples for analysis (Appendix 12). The amount of blood drawn:

- 5 ml (4 times per study) - for obtaining serum and analysis at NIIFFM;
- 2 ml (4 times per study) - for obtaining serum and analysis in Invitro;
- 2.5 ml (twice) - to determine the expression of the *SIRT1* gene;
- 3 ml (once) - for genetic analysis.

Blood is taken between 11-14 hours, optimally at 12-13 hours (before lunch, on an empty stomach). Before taking blood, the subject must be in a sitting position for at least 15 minutes (and can only get up for a few seconds) to exclude the influence of changes in body position on the concentration of substances in the blood. The following indicators are determined:

(1) the activity of the SIRT1 enzyme in blood serum by the spectrofluorimetric method on a plate spectrofluorometer using commercial kits from Sigma (USA); determined 4 times per research;

(2) concentration of BDNF (brain neurotrophic factor) in blood serum by multiplex analysis on a Luminex-200 device (inclusion point, visits 2 and 3);

(3) pro- and anti-inflammatory cytokines (IL1beta, IL6, TNF-alpha, IL4, IL10) in blood serum by multiplex analysis on a Luminex-200 device (inclusion point, visits 2 and 3);

(4) serum concentration of AST, ALT, CRP (to assess the safety of resveratrol use) (point of inclusion, visits 1, 2 and 3);

(5) the level of expression of the *SIRT1* gene (inclusion visit, visit 2);

(6) three allelic variants of the *SIRT1* gene - rs2236318 T → C, rs7896005 A → G, rs36107781 T → C (inclusion visit);

Samples are stored frozen until analysis after the end of the entire study, with the exception of samples intended for analysis of ALT, AST and CRP, which are transferred on the same or the next day to the appropriate laboratory ("Invitro"). Samples from one patient must be analyzed in one run.

Magnetic resonance imaging (MRI):

The study is carried out on a GE Discovery MR750W 3T MR tomograph, using a 32-channel receiving head coil:

(1) T2-WI (T2-weighted images to exclude gross focal and volumetric pathology, once, 3 minutes),

(2) T2-FLAIR (T2-weighted images with suppression of a signal from free water, to exclude gross focal and volumetric pathology, once, 3 minutes)

(3) 3D T1 SPGR, the number of slices up to a maximum of 256, with an isovoxel of 1 mm3, with the capture of the entire head (6 minutes); performed in 2 repetitions;

(4) diffusion tensor MRI (DTI, with parameters: EPI, scanning plane - axial, 80 diffusion directions, 7 b0, b = 1000; FOV 25.6 cm, matrix 128x128, slice thickness 2.0 mm, 66-72 slices s capture of the whole brain, TR - 12 sec, TE - 110 msec, pepolar - 0; preliminary registration of a short series of diffusion-tensor images with the opposite direction of the phase-encoding gradient for the possibility of correcting EPI distortions; total scan time - 20 min).

(5) functional MRI at rest (resting state mode, with parameters: EPI-BOLD, BrainWave, scanning plane - axial, FOV 24 cm, matrix 80x80, slice thickness 3.0 mm, 42-48 slices with capture of the whole brain, TR - 2.5 sec, TE - 28-30 msec, flip angle - 81, pepolar - 1, 200 volumes, 10 dummy scans; eyes closed; parallel recording of respiration and pulse indicators; scan time - 10 min).

MRI is performed twice - during hospitalization (duration about 60 minutes) and at visit 2 (only point # 5 - fMRI, 10 minutes).

Contraindications for MRI under the protocol:

- inconsistency with the scientific criteria of the project, the results of preliminary express screening in sMRI and fMRI modes;
- impossibility of full audio and / or visual contact with staff;
- the inability to be motionless in the supine position for 1 hour;
- artificial heart rate driver, pacemaker, neurostimulator; conductive or metal implants, braces; metal shards or artifact clips in the tissues of the brain or spinal cord, or near blood vessels; metal-containing tattoos;
- claustrophobia (in case it is not removed by preliminary conditioning in the MRI department).

If the patient has previously undergone Structural MRI, there is no need to repeat the Structural MRI.

Data labeling and analysis

Study abbreviations: "Resveratrol" or "RESV-depression". At visits, the patient is assigned an identification number (ID) in the form "PEC_01", in which "01" is the patient's serial number. The following information is added to the ID:

1. Type of visit: TFR - screening; SEL - selection; ON - enable; or
2. Visit number: 1; 2; 3.

For example: "RES_01_CEL"; "RES_01_3".

These abbreviations are included in the electronic database.

The labeling of biological samples obtained in the course of the study is carried out according to the principle set forth in the "Instruction on the logistics of handling blood samples for analysis" (Appendix 12).

Statistical analysis of the data is performed using the SPSS 21.0 statistical package. The factors to be considered in analyzing their possible effect on the dependent variables (depression severity score or SIRT1 enzyme activity or otherwise) when investigating the effects of resveratrol / placebo are presented in Table 1.

Table 1. Independent variables for statistical analysis of the effect of resveratrol on the severity of MDD.

Factors (categorical variables)	Covariates (continuous variables)
1. Gender 2. Type of depression (MDD or dysthymia) 3. Phase of the menstrual cycle 4. Expectation of treatment 5. Structural variants of the *SIRT1* gene 6. Decrease or increase in hours of sunshine during treatment	1. Age 2. Body mass index 3. Total HDRS-17 score at inclusion visit - when it was different between resveratrol and placebo groups 4. Activity of the SIRT1 enzyme 5. Expression level of the *SIRT1* gene

ПЛАН ПРОВЕДЕНИЯ ИССЛЕДОВАНИЯ

During the study, for each patient, 6 meetings with the research doctor and hospitalization for examination are planned (Scheme 1):

1) screening consultation is carried out for the purpose of the primary selection of patients for research;
2) a selection visit is planned between 7-14 days after the screening consultation;
3) hospitalization for 5-7 days for the examination, it is possible to provide sick leave, an approximate hospitalization plan is shown in diagram 2.
4) the inclusion visit (randomization) chronologically corresponds to the end of hospitalization;
5) visit No. 1 - 14 ± 2 days after starting the drug intake;
6) visit No. 2 - 28 ± 2 days after starting the drug intake;
7) visit # 3 - follow-up visit, 42 ± 2 days after starting the drug.

Table 2. Plan of the study.

Обследование	Screening, day 0	Selection (between days 7-14)	Hospitalization for 5-7 days	inclusion	Visit 1 +14 days	Visit 2 +28 days	Visit 3 +42 days
Clinical part							
Investigator appointment (with an entry in the Outpatient Card in Medialogue)	+	+			+	+	+
Body weight measurement	+	+		+	+	+	+
Complete blood count, ECG, glucose			+				
Therapist's consultation			+				
Neurologist's consultation			+				
Determination of eligibility for (non) inclusion	+	+		+	+	+	
Signing of the Informed Consent to participate in the trial		+					
Completing the case history			+				
Filling in the Registration Card			+	+	+	+	+

Psychometric part							
M.I.N.I.			+				
MADRS			+	+	+	+	+
HDRS-17		+		+	+	+	+
IDS				+	+	+	+
CGI				+	+	+	+
C-SSRS				+	+	+	+
ATRQ			+				
MSM			+		+	+	+
HDRS-17_SR	+						
BDI-II	+		+	+	+	+	+
DASS	+		+	+	+	+	+
IDS-SR			+	+	+	+	+
MADRS-SR			+	+	+	+	+
QIDS-S			+			+	+
VAS			+			+	
PANAS			+			+	
PHQ-9	+		+			+	+
ASIQ			+			+	+
SHAPS			+			+	+
BHS			+			+	+
PSQI			+			+	+
TCI-125			+				
HADS			+			+	+
HCL-32			+				
EAT			+			+	+
CTQ			+				
EI-IPIP			+				
Situational anxiety STAI			+			+	+
Personal anxiety STAI			+				

Situational anger STAXI			+			+	+
Personal anger STAXI			+				
RRS			+				
BAS/BIS			+				
BFFM-IPIP			+				
ERQ			+				
WBSI			+				
FFMQ			+				
TAS-20			+				
MEQ-SA			+				
Expectation scale (filled by the patient)				+	+	+	
Neurophysiological examination			+			+	
CANTAB			+			+	
Actimetry			delivery	+	+	+	+
Laboratory part (taking venous blood for analysis)							
genetic				+			
AST, ALT, CRB-quantitative				+	+	+	+
SIRT1 activity				+	+	+	+
BDNF and cytokines				+		+	+
expression of *SIRT1* gene				+		+	
MRI part							
Screening and structural MRI			+				
Functional MRI (fMRI)			+			+	
Control of drug intake					+	+	
Side effect scale (UKU)					+	+	
The patient brings with him to visits:							
Drug bottle					+	+	
Drug intake and sleep diary					+	+	
Actimeter (on the arm)					+	+	+

1. Screening consultation.

Patients are attracted to the screening consultation by placing approved advertising materials in the media and other advertising media (responsible for providing the advertising campaign - Matveev N.O.).

Additional channels for attracting patients: the existing own patient database, an announcement on the institution's website, a screening survey of the clinic's patients conducted by the clinic's psychologists, referrals of the clinic's doctors for screening consultation, etc.

Call center employees take incoming calls and schedule patients for a screening consultation. The type of admission is noted by the call center staff as "Screening psychotherapist (selection for the study)", duration 30 min. (Responsible for the work of the call center - Matveev N.O.).

At the screening consultation, the psychiatrist conducts a clinical interview (collection of complaints, medical history and life history, mental status assessment), fills out the patient's outpatient card. Establishes a preliminary diagnosis, in the presence of MDD or dysthymia, directs the patient to a screening consultation with a psychologist (responsible - A.A. Markov, E.V. Gadetskaya).

The department nurse measures body weight, height, waist circumference.

A screening consultation with a psychologist lasts 45 minutes. The psychologist independently records the patient's admission in the "Medialogue" information system, creates a visit ticket, the type of admission - "Psychological screening (selection for research)". At the consultation, the psychologist gives the patient to fill in the questionnaires of mental state (HDRS-17-SR (Appendix 27), DASS (Appendix 29), PHQ-9, BDI-II, seasonality questionnaire. After the patient completes the questionnaire results are processed, a conclusion is prepared, forms questionnaires are stored in the patient's outpatient record.

If the patient meets the inclusion criteria, the doctor determines the date of the selection visit and records the patient for the selection visit in the medialogue system. Forms an appointment for a neurologist and therapist examination on the day of the selection visit, after 12:00.

It is important that the appointments of the same patient are carried out by the same doctor in the future. It is advisable that women enter the study in the first half of their menstrual cycle (if any).

2. Selection visit.

The duration of the visit is 30 minutes. The meeting is held no less than 5 and no more than 7 days after the screening consultation. The patient comes to the clinic in the morning on an empty stomach.

During the visit, the investigator conducts a survey on the Hamilton scale (HDRS-17 (Appendix 18). The HDRS-17 depression severity questionnaire is completed by the research physician supervising the patient during the interview with the patient. The questionnaire form is embedded in the patient's medical history, copy - to the Patient Registration Card.

Provided that the required number of points on the HDRS-17 scale (16 or more points) is achieved and other inclusion criteria are met, the doctor conducts a debriefing and signing of the Informed Consent to participate in the study (Appendix 4). When conducting a debriefing, it is imperative to find out the presence / absence of claustrophobia, if necessary, conditioning the patient in the MRI department (conduct, show how the study is carried out).

The moment a patient enters the study is the date of signing the Informed Consent to participate in the study during the selection visit. From this point on, the patient is assigned an identification number ("RES_01" or another). Patients who drop out at any stage of the study are included in and not deleted from

the consolidated data file. The list with patient numbers (patient ID) is kept by the head of the psychotherapy department (Ph.D. Markov A.A.). The list includes the date of selection, full name of the patient, date of birth, number of medical history (Appendix 2). If a patient leaves the study before the randomization code is received and the drug is dispensed, this code is assigned to the next patient.

After obtaining consent to participate in the study, the doctor sends the patient to the registry for registration of the case history.

If the inclusion criteria are not met, the patient should be denied participation in the study and offered alternative treatment on a paid basis in inpatient or outpatient mode.

3. Hospitalization for examination

Hospitalization is carried out after the selection visit on any day of the week except Thursday and Friday. If necessary, a certificate of incapacity for work can be issued for the period of hospitalization.

Hospitalization is issued for up to 10 days, the title page of the medical history is marked "T Resveratrol", a standard consent for hospitalization and personal data processing is drawn up. During hospitalization, the patient undergoes psychometry, neurophysiological examination, and MRI. An approximate hospitalization plan is shown in diagram 2. There may be a break between the third and fourth days (due to weekends if hospitalized on Tuesday or Wednesday), no breaks are allowed between other days.

The nurse enters the patient's height, body weight, and waist circumference in the medical history (observation sheet). The nurse of the department draws up referrals for taking a general blood test, an analysis for glucose concentration, an ECG.

The doctor collects an anamnesis of life and illness, conducts an examination of the somatic and mental state, draws up a record of the initial examination. Fills in the Patient Registration Card (Appendix 5), all fields are required.

When completing the medical history, the doctor gives the patient reminders for conducting neurophysiological research and MRI studies (Appendices 6 and 7). The patient's attention is focused on the fact that it is necessary to exclude the intake of coffee, energy drinks 24 hours before the neurophysiological study, exclude heavy physical exertion, and have a good sleep. It is important that the patient does not have any expectations before undergoing neurophysiologic testing. Before MRI and neurophysiological examination, the patient signs separate Informed Consents for these procedures (Appendices 8 and 9).

The doctor conducts a survey and, based on the patient's answers, fills out clinical questionnaires (M.I.N.I., MADRS, ATRQ, MSM), the questionnaire forms are inserted into the registration card.

On the day the patient is admitted to the clinic, the doctor puts on the patient a MotionWatch 8 actimeter (CamNTech, England). The actimeter must be worn on the wrist of the non-dominant hand during the entire period of admission to hospital and taking the drug, with readings taken at the switch-on point, at visits 1, 2 and 3. The actimeter is issued to the patient against receipt (Appendix 10) along with instructions (Appendix 11).

On the day the patient is admitted to the clinic, the nurse records him for examinations in the "Medialogue" system in accordance with the plan (Scheme 2) prescribed by the doctor and set out in this Study Description. The procedures are included in the schedules of specialists; the study code and patient code (ID) must be indicated in the comments. Responsible - nurse T.A. Skopchenko The working hours of the neurophysiology laboratory are from 9:00 to 17:00, patients can be registered for examination at 9:30 and 13:00. MRI diagnostics should be planned after the psychophysiological examination and blood

sampling. The nurse prepares referral forms.

Psychophysiological examination is carried out on the second and third day of hospitalization for 4 hours every day. The days for performing the neurophysiological study are Tuesday, Wednesday, Thursday, Friday. Moreover, in one patient, both days of the study should take place without interruption. Before the patient is referred for a neurophysiological examination, the doctor conducts an examination for the absence of contraindications. A note about the conduct of neurophysiological research is entered in the history of the disease. The sequence of the neurophysiological study is as follows:

The patient comes to room 610 with a referral (Appendix 14), which indicates the patient's full name, assigned identification number, full name of the attending physician, date.

Patient meeting. The patient is asked how much he slept, whether he had taken coffee in the last 24 hours, taken psychostimulants in the last 48 hours (if he had taken coffee or alcohol, it cannot be examined).

Psychophysiological research (special psychometry, EEG, EP, eye tracking, stabiloplatform) / performed within 2 days /

1st day:

1. filling out a questionnaire and questionnaires to assess the current state (VAS, PANAS, situational anxiety (STAI), situational anger (STAXI)), photo-documenting Self / 30-40 min /;
2. imposition of an EEG helmet and hardware localization of electrode topography in 3-D space 30 min;
3. resting state electroencephalography / resting state EEG / - registration of bioelectrical activity of the brain / 128 EEG channels, vertical and horizontal electrooculograms, ECG / 2-lead / in association with on-line assessment of the degree of actualization of dysadaptive cognitive strategies / 16 min /;
4. registration of long-latency visual evoked potentials // when perceiving motivationally significant threats and positive reinforcement stimuli / OddBall Pictures paradigm, 40 min /;
5. eye tracking / Self paradigm / - registration of eye movement and gaze fixation when the emotional expression of one's own face and unfamiliar emotional faces is perceived on the computer screen with the expression of discrete emotions of joy, anger and sadness, 20 min;
6. stabilometric test for motor retardation (Dual Cost Task), 30 min;

2nd day:

1. filling out questionnaires (8) to assess the dynamics (BHS, QIDS, PHQ-9, SHAPS, ASIQ, PSQI, HADS, EAT);
2. testing CANTAB;
3. filling in personal and typological questionnaires (15).

On the second and third day after lunch, personality questionnaires are filled out under the supervision of a psychologist (Churikova O.S. Kaab 610).

On the fourth day, the attending physician completes the clinical questionnaires (MADRS, IDS, C-SSRS, MSM).

Blood sampling for determination of SIRT activity, *SIRT1* gene expression, ALT, AST, CRP, BDNF concentration, cytokine analysis is performed from a vein according to the Instruction on the logistics of handling blood samples for analysis (Appendix 12). The patient should appear with a referral (Appendix 13) to the procedure room for blood sampling between 11-14 hours, optimally at 12-13 hours (before lunch, on an empty stomach).

Before taking blood, the subject must be in a sitting position for at least 15 minutes (and can only get up for a few seconds) to exclude the influence of changes in body position on the concentration of substances in the blood. Responsible for taking blood - nurse Dechko N.N.

In accordance with the instructions, blood samples are transferred to a clinical laboratory, where they undergo sample preparation and are further stored frozen until analysis. Responsible for the preparation and storage of samples - Zhanaeva S.Ya. and Tenditnik M.The.

Magnetic resonance imaging (express screening, structural and functional MRI) is performed after all examinations and blood sampling on a Tesla GE Discovery MR750W 3 MR tomograph according to protocols corresponding to international protocols (ENIGMA projects, etc.).

The patient comes with a referral to the Department of Radiation Diagnostics (1st floor), in the direction must be indicated the patient's full name, his assigned identification number, full name of the attending physician, date.

4. Inclusion

The doctor gives the patient a diary (Appendix 16), with explanations for keeping the diary.

On the day the patient is discharged from the hospital, after the entire examination plan has been completed and the drug has been dispensed, a plan for further visits is created in the Medialogue Information System. An entry is created for the dates of psychometry, medical examination and blood donation on the same day. The type of intake is indicated: "Resveratrol 1", "Resveratrol 2", "Resveratrol 3" (on the 15th, 30th and 45th days from the moment of starting the drug intake). In addition, the doctor creates an entry in the medialogue information system for psychophysiological examination, an entry for blood sampling, fills in the directions for blood tests and sends them to the treatment room. Further, the doctor transfers information about the discharged patient to the person responsible for the work of the contact center (Matveev N.O.) to organize further reminders to the patient about the need to take the drug.

Control of **drug intake**: to remind patients about taking the drug, they automatically receive an SMS in the morning at 11:00 with a reminder "Please take the drug." If the patient does not report on the receipt of the drug by a response SMS containing any text within an hour, he receives a call from a call center employee to clarify the status of the case. If necessary, the employee of the call center informs the responsible researcher about the violation of the drug intake.

5. Visit 1

The day before visiting the clinic, the call center staff remind the patient about the visit to the clinic by dialing a mobile phone number.

The visit is carried out 14 ± 2 days after the start of the drug intake. The duration of a psychologist's appointment is 60 minutes, and that of a research doctor is 120 minutes.

At the beginning of the visit, the nurse of the department determines the patient's body weight and enters the data into the Registration Card.

During the visit, the psychologist conducts a psychometric study: the patient is given questionnaires (IDS-SR, MADRS-SR, DASS, BDI-II) to fill out, after filling out the questionnaires, the data is processed. The conclusion and questionnaire forms are pasted into the Outpatient Card, copies are inserted into the Patient Registration Card.

After that, the patient is sent for an interview with the research doctor. Based on the results of the conversation, the doctor fills out clinical questionnaires (MADRS, HDRS-17, IDS, CGI, C-SSRS, MSM, UKU), checks the drug intake by reviewing the Drug and Sleep Diary and counting the remaining capsules in the vial, and finds out the emergence of exclusion criteria. The examination data is entered into the Patient Registration Card. During the visit, the doctor reads the actimeter data.

After a doctor's examination, blood is taken to determine the concentration of CRP, ALT, AST, SIRT1 activity.

6. Visit 2

The day before visiting the clinic, the call center staff remind the patient about the visit to the clinic by dialing a mobile phone number.

The visit is carried out 28 ± 2 days after the start of the drug intake. The duration of a psychologist's appointment is 60 minutes, and that of a research doctor is 120 minutes.

The plan of visit 2 is similar to that of visit 1, except that additional blood is taken to determine the expression of the *SIRT1* gene, BDNF concentration, analysis for cytokines, a psychophysiological study is carried out according to the program of the first day (see the description of the psychophysiological study), a set of situational questionnaires and functional MRI.

The visit lasts 2 days; outpatient or hospitalization is possible.

7. Visit 3

During the visit, the psychologist conducts a psychometric study: the patient is given questionnaires (IDS-SR, MADRS-SR, DASS, BDI-II) to fill out, after filling out the questionnaires, the data is processed. The conclusion and questionnaire forms are pasted into the Outpatient Card, copies are inserted into the Patient Registration Card.

After that, the patient is sent for an interview with the research doctor. Based on the results of the conversation, the doctor fills out clinical questionnaires (MADRS, HDRS-17, IDS, CGI, C-SSRS, MSM, UKU). The examination data is entered into the Patient Registration Card.

The doctor reads the actimeter data and directs the patient to donate blood to determine the concentration of CRP, ALT, AST, SIRT1 activity, determination of the concentration of BDNF, cytokines

The patient hands over the self-observation and sleep diary to the doctor.

ORGANIZATIONAL AND FINANCIAL ISSUES

The study is carried out on an outpatient basis. However, when conducting neurophysiological examination in conjunction with MRI and filling out a battery of questionnaires that require a lot of time, hospitalization is provided for up to 10 days. For the period of hospitalization, it is possible to issue a sick leave.

The research documentation is kept in the office of the responsible researcher (A.A. Markov). Registration cards, Ambulatory cards and Medical records are stored in a lockable cabinet, which is accessible only to those directly involved in the execution of the study.

Vials with Resveratrol capsules and placebo are stored at the warehouse of medicines (responsible - the head of the pharmacy).

Vacutainers for taking blood for gene expression analysis are stored in the refrigerator in the treatment room at a temperature of +2 - +8 C.

Meetings for the coordination of the work of departments and researchers are held once a week.

The patient is paid the logistics costs for the scheduled visits (RUB 3000); if the study is not completed, the amount will not be paid. If, at the end of the study, the patient does not show clinical improvement (decrease in the severity of depression), the investigator will suggest that the patient participate in another clinical study or specialized care in accordance with the Standards of Care for Depressive Disorder.

The project is **financed** from the Russian Science Foundation grant No. 16-15-00128 "Structural features of the *SIRT1* gene as a basis for choosing personalized drug therapy for depression" (2016–2018).

The expenses for the patient's stay in the hospital are reimbursed from the grant funds in the amount established in the NIIFFM clinic. The amount of payment to the doctor for the patient at the inclusion visit is equivalent to the cost of the initial consultation with a psychotherapist in the clinic's price list in threefold amount.

Investigators and their functions:

/ section contains tabulated personal data of 35 researchers and is omitted here /

/ Appendices are omitted /