Prospective Randomized Placebo-Controlled Cohort Clinical Study "Personalized Microbial Therapy as an Approach to the Correction of Somatic Pathologies (Metabolic Syndrome and Atherosclerosis)"

Patient Information Sheet and Informed Consent Form Document Status: Version 1.0 in Russian dated April 24, 2023. **Date:** April 24, 2023.

The study is conducted with the support of the Ministry of Science and Higher Education of the Russian Federation under Grant Agreement No. 075-15-2022-302 dated April 20, 2022, aimed at the creation and development of the world-class scientific center "Center for Personalized Medicine".

Patient Information

You are invited to participate in a prospective randomized placebo-controlled cohort clinical study of autoprobiotic therapy. This study aims to develop a new methodology for using autoprobiotics as an adjunct to standard therapy for patients with metabolic syndrome (MS) and concomitant atherosclerosis of the coronary and/or brachiocephalic arteries. The goal is to increase treatment efficacy by restoring the microbiocenosis and the normal functioning of the digestive, cardiovascular, endocrine, immune, and nervous systems.

Intestinal microbiota has a significant impact on human health; it can either prevent or contribute to the development of various diseases, including socially significant conditions such as cardiovascular diseases (CVD), diabetes mellitus (DM), and inflammatory diseases. Several studies have shown that even in individuals without symptoms of cardiovascular, endocrine, or other systemic diseases, but who are overweight, obese, or have lipid metabolism abnormalities based on laboratory tests (lipid profile), there are significant disturbances in the intestinal microbiota. A close link between these disturbances and CVD, insulin resistance (IR), and inflammation has been proven.

Currently, various methods of influencing the microbiome are widely studied worldwide, including the use of various probiotic cultures and fecal microbiota transplantation (FMT). It should be acknowledged that, to date, the results obtained within such approaches remain difficult to reproduce. This is obviously related to the high degree of individual variability of the human microbiome and, consequently, insufficient personalization of therapy. Furthermore, it is necessary to note the significant risks associated with the transplantation of donor microflora.

In this regard, a group of specialists led by the Head of the Department of Molecular Microbiology of the Research Institute of Experimental Medicine (RIEM), Corresponding Member of the Russian Academy of Sciences (RAS), A.N. Suvorov, has developed the concept of autoprobiotic therapy.

The technology of using components of the patient's own microbiota (autoprobiotics), adapted to exist within the patient's microbiocenosis and to which immunological tolerance is present, has undeniable advantages over standard probiotic therapy. The use of autoprobiotics, proposed 20 years ago for the correction of dysbiosis, was largely unused for a long time. However, after substantial modification of their preparation technology by RIEM researchers, "personalized autoprobiotic therapy" has come to be considered an alternative to probiotics, metabiotics, and prebiotics. The main difference in the autoprobiotic production technology developed by RIEM staff lies in the preliminary genetic characterization of indigenous strains, which significantly enhances its safety for the patient and ensures a personalized approach. Previously, the effects of

autoprobiotics were thoroughly studied in biological models and *in vitro* systems. RIEM staff have obtained patents for the method of preparing autoprobiotics and their application in treating several diseases, including irritable bowel syndrome and Parkinson's disease (Invention Patents No. 2460778 dated 30.12.2010, No. 2546253 dated 25.04.2013, No. 2734718 dated 22.10.2020). Currently, an evidence base is being formed to expand the indications for prescribing autoprobiotics; in particular, the application of autoprobiotics in MS is highly relevant and promising.

Aim and Procedure of the Clinical Study

The aim of this study is to develop a new treatment methodology for patients with MS: the use of autoprobiotics as an adjunct to standard therapy to increase its efficacy by restoring the microbiocenosis and the functioning of the digestive, cardiovascular, endocrine, immune, and nervous systems.

If you agree to participate in the study and meet the eligibility criteria, you will be randomly assigned to one of 2 groups:

- **Group 1:** Participants in this group will receive the autoprobiotic.
- **Group 2:** Participants in this group will receive a visually similar product not containing the autoprobiotic (placebo).

RIEM staff will prepare a personalized autoprobiotic for you in the form of a soy milk starter containing beneficial microorganisms (specifically *Enterococcus faecium*, *Lactobacillus* spp., *Bifidobacterium* spp., or bifidobacteria). For this, you will need to collect a stool sample and bring it to the laboratory within a few hours. The preparation of the autoprobiotic takes 7–10 days. Once your autoprobiotic is ready, you will be instructed to take it for 20 days at a dose of 50 ml twice a day, and then repeat the course after 2 months. The studied autoprobiotic is not a medicinal product. It can be considered an element of personalized functional nutrition. For patients randomly assigned to the placebo group, RIEM staff will prepare a soy milk drink with the addition of citric acid for fermentation.

Before study inclusion:

- The doctor will ask you about your medical history, concomitant diseases and conditions, and medications taken previously and currently.
- The doctor will ask you to provide and will analyze the results of previously performed laboratory and instrumental examinations.

After study inclusion:

- Blood will be drawn for subsequent biochemical, hormonal, and genetic analysis, and a stool sample will be collected for subsequent intestinal microbiome analysis (before the start of therapy, as well as at weeks 10, 14, 19, and 24 of the study).
- The doctor will ask you to fill out several questionnaires regarding your well-being and level of physical activity (before the start of therapy and upon completion of the study).
- You will undergo a cardiorespiratory test (a physical stress test on a cycle ergometer) – before the start of therapy and upon completion of the second course of therapy (autoprobiotic intake).
- During the period of taking the autoprobiotic product and for 14 days thereafter, you will need to keep a gastro-diary, weigh yourself, and measure your blood pressure and pulse, recording the readings in the gastro-diary form.

Your doctor will ask you to report any problems or changes in your well-being, if any occur during the program. If necessary, the doctor may make changes to your ongoing treatment.

Risks and Discomforts

The technology of using components of the patient's own microbiota (autoprobiotics), adapted to exist within the patient's microbiocenosis, along with the preliminary genetic characterization of the autoprobiotic strains, significantly enhances the safety of such therapy for the patient and ensures its personalized nature.

- Venous blood sampling is performed using disposable needles under sterile conditions. No invasive manipulations associated with a risk to your health will be performed in this study.
- Vascular ultrasound is a non-invasive, painless examination that poses no risk to your health.
- The cardiorespiratory test (CRT) is a non-invasive study consisting of a physical stress test on a cycle ergometer; it will be performed according to standard methodology. Licensed medical equipment will be used during the CRT. The test will only be performed if your attending physician identifies no contraindications. During stress testing, symptoms such as severe fatigue, shortness of breath, pre-syncope, arrhythmias, ECG changes, chest pain, a drop in blood pressure, or a decrease in oxygen saturation may occasionally occur. If any of the above conditions arise, the stress test will be stopped immediately.
- Filling out the gastro-diary, including measuring pulse, blood pressure, and weighing, will require a time commitment of no more than 30 minutes per day (during the autoprobiotic intake period and for 14 days thereafter).
- Filling out questionnaires regarding your physical activity and quality of life will require a time commitment of no more than 30 minutes during a visit to the investigator physician.

In the event of any unusual symptoms or adverse events, please report them immediately to the investigator physician:

Physician's Full Name: _____

Benefits of Participation

By participating in this clinical study, you will receive recommendations for optimal pharmacological treatment and non-pharmacological correction of cardiovascular risk factors, in accordance with existing clinical guidelines for the treatment of your condition, specifically atherosclerosis of the coronary and brachiocephalic vessels, lipid metabolism disorders, and metabolic syndrome. You will undergo cardiorespiratory testing for an objective assessment of physical load tolerance and metabolic processes in the body during exertion, as well as an intestinal microbiome analysis. These are not routine procedures covered by state health guarantees. These procedures, specialist observation, and the manufacturing of the autoprobiotics will be provided to you free of charge.

Your participation may help us and the medical community clarify the contribution of using autoprobiotics as an adjunct to standard therapy in patients with MS and atherosclerosis to increase the efficacy of treating these pathologies, through the restoration of the microbiocenosis and the functioning of the digestive, cardiovascular, endocrine, immune, and nervous systems.

Is Participation in this Study Mandatory?

You will be included in the study only with your voluntary consent. If you decide not to participate in the study, it will not affect the quality of your medical care.
You may withdraw your participation in the study at any time without giving a reason for your decision. However, you should inform your doctor of this decision. Your decision to withdraw from the study will not affect the quality of your medical care in any way.

Compensation for Harm

This scientific study does not provide for any payments or compensation for medical expenses to participants. The results this study can be published in scientific journals, By signing this form, you do not waive any of your legal rights.

Confidentiality

For the purposes of this scientific study, your personal data and medical history will be kept by the investigator physician. For the purposes of this study, the physician will use medical information containing medical records and examination results that identify you by name or in any other way. Your consent to participate in this study means that you agree that the physician may obtain medical information that they may request from other healthcare facilities for research purposes. You also consent to the physician using this information and providing it to the parties described below.
Except as required by law, your attending physician may grant access to the specified medical information to the Ministry of Science and Higher Education of the Russian Federation (the program sponsor) and its authorized representatives, the Federal Service for Surveillance in Healthcare (Roszdravnadzor), state authorities in other countries, and independent ethics committees. The purpose of using and transferring this information to these parties is to verify the accuracy of the study data. Not all parties that will have access to your Medical Information as part of the study are prohibited by federal laws from further transferring the information; thus, information once obtained by them may no longer be protected by law.
Study results and other information obtained during the study may be provided to the Federal Service for Surveillance in Healthcare and state authorities; however, you will only be identifiable by your initials or a personal patient number assigned in the study. Your personal data will not be mentioned in any reports or publications related to this study.
You have the right to withdraw your consent at any time by notifying your physician. If you withdraw your consent to participate in this study, your physician will no longer use or disclose your medical information, unless required to ensure the scientific integrity of the program. However, withdrawing consent will not affect the prior use and disclosure of your medical information, and your medical information will not be deleted from the program documentation.

Questions

If you have any questions regarding this study, or if you need to report an adverse event, please contact your investigator physician:
Full Name: _____
Phone: _____

Informed Consent Form

For the scientific research protocol: "Personalized Microbial Therapy as an Approach to the Correction of Somatic Pathologies (Metabolic Syndrome and Atherosclerosis)"

Patient Number: _____ Patient Initials: _____

By signing this document, I confirm that I have carefully read and fully understood the information presented above. [ ] YES [ ] NO

I confirm that I have received a copy of the Patient Information Sheet and the Informed Consent Form. [ ] YES [ ] NO

I give my voluntary consent to participate in this clinical study. [ ] YES [ ] NO

I confirm that the results of this clinical trial with my participation as a test patient can be published in scientific journals in particular International Journal of Molecular sciences

? [ ] YES [ ] NO

I have received a copy of the Patient Information Sheet and the Informed Consent Form. [ ] YES [ ] NO

Patient's Full Name (print clearly):

Patient's Signature: _____ Date: _____

Physician's Full Name (print clearly):
