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PROTOCOL AND STATISTICAL PLAN

EVALUATION OF SAFETY AND EFFICIENCY OF METHOD OF EXOSOME INHALATION IN SARS-COV-2 ASSOCIATED PNEUMONIA

(COVID-19EXO)

(COVID-19EXO ver.1 from 06.07.2020)

History of changes

Rev 1.

- Primary document

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Sponsor/Collaborators

Sponsor: State-Financed Health Facility “Samara Regional Medical Center Dinasty”

Responsible Party: Sponsor

Collaborators: Clinics of the Federal State Budgetary Educational Institution SSMU Samara Regional Clinical Hospital V.D. Seredavin

AIM

Evaluation of the safety and efficacy of the method of inhalation administration of exosomes in bilateral pneumonia caused by the new coronavirus infection SARS-CoV-2.

GOALS

1. Develop an algorithm for introducing exosomes in case of pneumonia caused by COVID-19.

2. Formulate patient selection criteria for this technique.

3. To analyze the safety and efficiency of exosomal administration in case of pneumonia caused by COVID-19.

Study Description

Brief Summary: Coronavirus is an acute viral disease with prevailing upper respiratory tract infections caused by the RNA-containing virus of the genus Betacoronavirus of the Coronaviridae family. Most patients with severe COVID-19 develop pneumonia in the first week of the disease. As the infection progresses, the infiltration increases, and the affected areas increases. Excessive and uncontrolled immune system response with rapidly developing fatal cytokine storm plays the main role in the pathogenesis of acute respiratory distress syndrome (ARDS) due to SARS-CoV-2 infection.

According to available data, exosomes can regulate inflammation and regenerative processes due to the change in the concentration of anti-inflammatory cytokines and switch the immune cell to regenerative secretome. Inhalation of exosomes may reduce inflammation and damage to the lung tissue and stimulate the regenerative processes.

This protocol has been developed based on the literature, information about the ongoing tests NCT04276987 (A Pilot Clinical Study on Inhalation of Mesenchymal Stem Cells Exosomes Treating Severe Novel Coronavirus Pneumonia) and NCT04384445 (Organicell Flow for Patients With COVID-19), Patent No 271036826 of 2019. “A method for obtaining and concentrating microRNA-containing exosomal multi-potent mesenchymal-stromal cells for use

in cosmetic and pharmaceutical products to stimulate regenerative processes and slow down aging.

Detailed Description: COVID-19 is an infectious disease caused by the most recently discovered coronavirus. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019. COVID-19 is now a pandemic affecting many countries worldwide. Globally, as of 1:09 pm CEST, 27 July 2020, there have been 16 096 741 confirmed cases of COVID-19, including 646 384 deaths, reported to WHO.

The main and rapidly achievable target of SARS-CoV-2 is lung type II alveolar cells (AT2), which determines the development of diffuse alveolar damage. In the pathogenesis of ARDS due to COVID-19, the main role is played by an over-response of the immune system with rapidly developing severe life-threatening cytokine release syndrome (cytokine storm). Cytokine release syndrome threatens the emergence and progression of ARDS. The key components of the pathogenesis of ARDS also include disruption of cell cytotoxicity mechanisms, excessive activation of cytotoxic lymphocytes and macrophages with a massive release of proinflammatory cytokines (FNO-α, IL-1, IL-2, IL-6, IL-8, IL-10), granulocytic colony-stimulating factor, monocytic chemoattractive protein 1), and inflammatory markers (CRP, serum ferritin), infiltration of internal organs and tissues by activated T-lymphocytes and macrophages, resulting in a hyperinflammatory reaction. Such severe lesions can lead to death or severe lung damage, including long rehabilitation after discharge.

Experimental studies have demonstrated that mesenchymal stem cells (MSCs) may significantly reduce lung inflammation and pathological impairment resulting from different types of lung injury. Many researchers connect the anti-inflammatory effect of MSC with their secretome which includes MSC derived exosomes. It is highly likely that MSC exosomes have the same therapeutic effect on inoculation pneumonia as MSCs themselves. Moreover, exosomes show a strong effect of regenerative stimulation on different wounds so the regenerative effect can be extended on patients with COVID-19 pneumonia.

The purpose of this protocol is to explore the safety and efficiency of aerosol inhalation of the exosomes in the treatment of severe patients hospitalized with novel coronavirus pneumonia (NCP).

Conditions

Conditions: Covid19
SARS-CoV-2 PNEUMONIA
COVID-19

Keywords: Covid-19
SARS-CoV-2
exosomes
MSC

Study Design

Study Type: Interventional

Primary Purpose: Other

Study Phase: Phase 1/Phase 2

Interventional Study Model: Parallel Assignment
The trial has three groups, each with 10 subjects (n=30). All eligible study subjects will be randomized, double-blinded, to either the two treatment groups or placebo

group.

Number of Arms: 3

Masking: Double (Participant, Care Provider)
Two main groups will be provided with exosomes in a specially provided solution, the third group (control) will receive the same solution without exosomes. Due to exosomes are nanoparticles and requires special methods and devices to be detected the hospital staff and patients have no way to check which group receives exosomes.

Allocation: Randomized

Enrollment: 30 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: EXO-1 Participants (n=10) in this group will receive standard therapy and exosomes of the first type.	Procedure/Surgery: EXO 1 inhalation Twice a day during 10 days inhalation of 3 ml special solution contained 0.5-2x10^10 of nanoparticles (exosomes) of the first type.
Experimental: EXO-2 Participants (n=10) in this group will receive standard therapy and exosomes of the second type.	Procedure/Surgery: EXO 2 inhalation Twice a day during 10 days inhalation of 3 ml special solution contained 0.5-2x10^10 of nanoparticles (exosomes) of the second type.
Placebo Comparator: Placebo Participants (n=10) in this group will receive standard therapy and inhalation placebo solution.	Procedure/Surgery: Placebo inhalation Twice a day during 10 days inhalation of 3 ml special solution free of nanoparticles (exosomes).

Eligibility

Minimum Age: 18 Years

Maximum Age: 65 Years

Sex: All

Gender Based: No

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Ability to understand the study objectives and risks and provide signed and dated informed consent;
- Confirmed COVID-19 infection (by PCR or antibody test);
- Pneumonia requiring hospitalization, and oxygen saturation of <94% indoors or a need for auxiliary oxygen. The confirmed volume of lung damage by CT: not less than 30% and not more than 80%;
- ability to proceed with inhalation by self;

Exclusion Criteria:

- Severe respiratory failure at the time of screening due to COVID-19 pneumonia;

• Known to undergo medical resuscitation for 14 days before randomization;
• Any serious medical condition or deviation of the clinical laboratory parameter that, in the opinion of the researcher, prevents safe participation and completion of the study by the participant Confirmed uncontrolled active bacterial, fungal, viral or other infection (other than SARS-CoV-2).
• According to the researcher, the progression to death is inevitable and will occur within the next 24 hours, regardless of the therapy.
• The life expectancy of fewer than 28 days, taking into account a medical condition already existing that cannot be corrected, e.g. participants with the following conditions or suspicions: polyorganic insufficiency, poorly controlled neoplasms, terminal stage heart disease, cardiopulmonary cardiac arrest that required cardiopulmonary resuscitation, or electrical activity not accompanied by a pulse, or asystole within the last 30 days, terminal stage liver disease, terminal stage liver disease, or liver disease;
• Pregnancy or breastfeeding;
• Liver function failure (Class C for Child-Pugh), detected within 24 hours at screening (local laboratory);
• Absolute neutrophil count (ANC) <500 cells/μL at screening (local laboratory);
• Platelet count <50000 cells/μL at screening (based on laboratory data);
• Creatinine level ≥ 1.5 from the upper limit;
• Uncontrolled or untreated arrhythmia with clinical manifestations, myocardial infarction within the last 6 weeks or congestive heart failure (NYHA Degrees 3 or 4);
• Respiratory failure in the last 6 months or home use of oxygen in severe chronic respiratory disease (COPD);
• Quadriplegia;
• Primary immunodeficiency, tuberculosis, progressive multifocal leukoencephalopathy, aspergillosis or other invasive mold/fungal infection in anamnesis, or internal or bone marrow transplantation for 6 months before randomization;
• Known infection with hepatitis B or C viruses requiring therapy;

Research methods and STATISTICAL CONSIDERATIONS

Title	Purpose	Method	Frequency	Statistics
Number of participants with minor and serious side effects	Safety assessment of the procedure.	Collection of information about unwanted reactions to inhalation, for example, 1) temperature increase, 2) allergic reaction in the form of swelling Quincke, rash, seizures, etc.; 3) bronchospasm for inhalation	After each procedure	Numeric data, calculated as a percent from whole patients in the group.
Biochemistry of blood	Evaluation of procedure efficiency	C-reactive protein, LDH	At the beginning of inhalation (day 1) and on next day of last inhalation (day 11)	The difference in each group between baseline and day 11 will be measured by T-test. The data will be presented as a Mean with full range (min-max). The difference will be calculated as Mean

				difference with Standard Deviation (SD)
Time to Clinical Recovery (TTCR)	Evaluation of procedure efficiency	Calculation of days in hospital	Once at the end of trial	The data will be presented as a Mean with Standard Deviation (SD)
SpO2 Concentration Changes	Evaluation of procedure efficiency	The concentration of SpO2 by Pulse oximetry device during procedures and compared to placebo.	Before and after each inhalation (total 4 measures per day). 10 days.	The intraday data (4 measures per day) will be calculated as a Median by day. The Median by day data (10 measures) will be processed by T-test intragroup and between groups. The data in tables will be presented as a Median (Me) with 25-75 quartile range.

The results will be statistically processed using parametric and non-parametric statistics methods using STATA version 9.0, Statistica for Windows version 6.0 and MS Office Excel 2007. Changes from the start of therapy and after will be compared using the T-test, comparing data between the main group and the control group using non-parametric method box-plot (data are presented in Me - median format with an indication of 25% (q1) and 75% (q3) quartile.

STATISTICAL CONSIDERATIONS

This study will enroll patients with PCR confirmed COVID according inclusion criteria.

The trial has three groups, each with 10 subjects (n=30). All eligible study subjects will be randomized, double-blinded, to either the two treatment groups or placebo group. Two main groups will be provided with exosomes in a specially provided solution, the third group (control) will receive the same solution without exosomes. Due to exosomes are nanoparticles and requires special methods and devices to be detected the hospital staff and patients have no way to check which group receives exosomes.

This is interventional, prospective, randomized double-blinded with a control group study.

The primary outcomes will be assessed at discharge of the clinic and 1 month after. Duration of study participation will be 1 months from the time of discharge from the clinic.

It is estimated that up to 2-3 research participants will be enrolled each week and that approximately 4 weeks of accrual will be necessary to enroll 10 participants.

Study Duration

Research participants will be followed for safety for 1 months after the discharge from the clinic.

Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all research participants. Characteristics to be examined include age, sex. The results will be statistically processed using parametric and non-parametric statistics methods using STATA version 9.0, Statistica for Windows version 6.0 and MS Office Excel 2007. Changes from the start of therapy and after will be compared using the T-test, comparing data between the main group and the control group using non-parametric method, box-plot.

POSSIBLE APPLICATION AREA

Specialized hospitals.

DATA TO BE OBTAINED DURING IMPLEMENTATION

- correct selection of criteria for selecting patients for treatment of patients with exosomes;

- developed algorithm for using the method of administration of exosomes;

-information on safety and efficacy of exosomal administration in case of pneumonia caused by SARS-CoV-2;

- appropriateness and effectiveness of this treatment method. To evaluate its impact on the tactics and treatment results;

EXPECTED RESULTS

It is assumed that the inhalation method of injection of exosomes will speed up the rehabilitation of patients, reduce the volume of pulmonary tissue lesions and reduce the time of stay of patients in hospital conditions.