Consiglio Nazionale delle Ricerche		Istituto di Farmacologia Traslazionale Institute of Translational Pharmacology IFT
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Direttore Dott. Vito Michele Fazio		

Study Final Report

1. TITLE PAGE

Study title
Antiviral and Immunomodulatory Interferon-Beta in high-risk COVID-19 patients

Name of investigational product
Rebif® (interferon beta-1a)

Indication studied
COVID-19

Study description
Randomized, Open-Label, Controlled, Phase II Study. The study included one treatment arm and one control arm. Treatment plan consisted in 4 subcutaneous injections of 11 mcg (3MIU) of IFN-β1a, to be given at day 1, 3, 7 and 10 in addition to standard of care to early diagnosed COVID-19 patients..

Name of the sponsor
Institute of Translational Pharmacology (IFT), National Research Council (CNR)

Protocol identification
EudraCT N°: 2020-003872-42

Development phase of study
Phase II

Study initiation date (first patient enrolled, or any other verifiable definition)
First patient enrolled on May 4th, 2021

Date of early study termination:
31st January 2022

Sponsor Scientific Coordinator and Report signatory:
Filippo Belardelli,
IFT, CNR
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Fax: +39 06 45488257
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The study was performed in compliance with Good Clinical Practices (GCP), including the archiving of essential documents

Date of the report
11/08/2022

2. SYNOPSIS

<table>
<tr><td>Name of Sponsor
Institute of Translational Pharmacology (IFT), National Research Council (CNR)</td><td rowspan="3">Individual Study Table Referring to Part of the Dossier
Volume
Page</td><td rowspan="3">(For National Authority Use Only)</td></tr>
<tr><td>Name of Finished Product
Rebif®</td></tr>
<tr><td>Name of Active Ingredient
Interferon beta-1a</td></tr>
<tr><td colspan="3">Title of Study
Antiviral and Immunomodulatory Interferon-Beta in high-risk COVID-19 patients</td></tr>
<tr><td colspan="3">Investigator
Emanuele Nicastri, MD</td></tr>
<tr><td colspan="3">Study Centre
Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani, Rome, Italy</td></tr>
<tr><td colspan="3">Publication (reference)
Not applicable</td></tr>
<tr><td>Studied period
May 4th, 2021 (First patient enrolled)
July 29th, 2021 (Last Protocol amendment approved)</td><td colspan="2">Phase of development
Phase II</td></tr>
<tr><td colspan="3">Objectives
<u>Primary Objective</u>: to evaluate the reduction in disease progression in patients treated with IFN versus control group within 28 days
<u>Secondary Objectives</u>:
1) To assess the reduction in ICU admission in patients treated with IFN versus control group within 28 days of randomization
2) To assess the reduction in number of deaths in IFN compared to control group (day 28)
3) To evaluate the increase in proportion of participants returning to negative SARS-CoV 2 RT-PCR in IFN-treated versus control group at Day 14 and Day 28
4) To assess the increase in SARS-CoV 2-Specific Binding Antibody Titers in IFN compared to control group (day 28)
5) To assess the safety of IFN-treated patients versus control group</td></tr>
<tr><td colspan="3">Methodology
Randomized, Open-Label, Controlled, Phase II Study. The study planned to enroll 60 patients: 40 in the IFN-β1a arm, 20 in the control arm, according to a 2:1 - treated: untreated ratio. Treatment plan foresaw 4 subcutaneous injections of 11 mcg (3MIU) of IFN-β1a, to be given at day 1, 3, 7 and 10 in addition to standard of care. Patients were monitored and disease progression was evaluated by means of the National Early Warning Score (NEWS2).</td></tr>
<tr><td colspan="3">Number of patients (planned and analyzed)
The study planned to enroll 60 patients; however only 2 patients were enrolled: 1 in the IFN-β1a arm, 1 in the control arm</td></tr>
<tr><td colspan="3">Diagnosis and main criteria for inclusion
• ≥ 65 years of age at time of enrolment. NOTE: Age limit was changed to ≥ 50 years of age after the approval of amendment S AMD 01 by regulatory authorities;
• Laboratory-confirmed SARS-CoV 2 infection as determined <72 h prior to randomization in any specimen by PCR. NOTE: Positivity of third generation antigenic test (immunofluorescence with microfluidic reading) if COI >10 was also included (together with the elimination of the 72h limit) after the approval of amendment S AMD 01 by regulatory authorities;
• Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures;
• Understands and agrees to comply with planned study procedures;
• Agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol;
• Being symptomatic for less than 7 days before starting therapy;
• NEWS2 score ≤2</td></tr>
</table>

Test product, dose and mode of administration, batch number

Control arm. No specific antiviral treatment besides standard of care.

Treatment arm. 11 mcg (3MIU) of Rebif® (IFN-β1a) were injected subcutaneously at day 1, 3, 7, and 10 in addition to standard of care. The drug solution (Rebif®), contained in a pre-filled cartridge, was injected by means of the RebiSmart electronic injection device.

Duration of treatment:

Treatment ended at day 10. Patient monitoring was performed over 28 days.

Reference therapy, dose and mode of administration, batch number

Standard of care was administered at the physician discretion, in line with the Ministry of Health official guidelines for early diagnosed COVID-19 patients with mild symptoms.

Criteria for Evaluation

Efficacy endpoints:

Primary endpoint: Proportion of patients experiencing a disease progression, during at least 5 days, according to the National Early Warning Score **(NEWS2).**

Secondary endpoints:

- ICU-free days at 28 days (Day 1 through Day 28)
- All-cause mortality (Day 1 through Day 28)
- Negative SARS-CoV 2 RT-PCR at day 14 post-randomization
- Negative SARS-CoV 2 RT-PCR at day 28 post-randomization
- Change from Baseline in SARS-CoV 2-Specific Binding Antibody Titers at day 14 and 28
- Incidence of adverse events

Safety:

Nature, incidence and severity of adverse events (AEs) and serious adverse events (SAEs). Toxicities were graded using NCI Common Terminology Criteria for adverse Events (CTCAE) version 5. The investigator assessed causality between the study medical product and the occurrence of AE/SAE.

Statistical methods

The primary analysis was planned to be carried out on the primary endpoint on the intention-to-treat (ITT) population defined as all patients randomized receiving at least one dose of treatment.

The percentage of patients undergoing disease progression defined on rate of progression of NEWS2 score lasting more than 5 days would be calculated in two arms (IFN-β1a + standard of care vs standard of care) of the trial. For persons who died, a conservative approach was planned, and death was considered an event. The effect of treatment was planned to be estimated through a logistic regression model including a dummy variable for treatment. The effect of treatment was planned to be estimated through multivariable logistic regression model by accounting for the following covariates: age, gender, co-morbidities and NEWS2 score at baseline.

We planned to carry out all primary and secondary analyses both on ITT population and on per-protocol population, thus including all subjects who were included in the ITT population that received the treatment as defined in the protocol and who completed the study with no major protocol violations. For the secondary endpoint ICU-free days, a competing risk model was planned to be adopted considering death a competing event.

The longitudinal secondary endpoint measured on a continuous scale (the increase in SARS-CoV 2-Specific Binding Antibody Titers in IFN compared to control group) were planned to be analyzed using a Mixed effect Model for Repeat Measure (MMRM) to estimate the difference of mean change from baseline in SARS-CoV 2-Specific Binding Antibody Titers between IFN-β1a + standard of care and standard of care at day 28.

Safety endpoint were planned to be compared by a chi-squared test for discrete variables, by means of analysis of variance (ANOVA) and covariance (ANCOVA) for continuous variables or by the non-parametric Mann-Whitney test when appropriate.

Confidence intervals (95%) were selected for all outcomes and association measures (proportions, means, Odds Ratios and HRs). For all statistical analyses (efficacy and safety), the level of statistical significance was chosen at 0.05 with two-sided p-values.

Summary – Conclusions

Efficacy Results **Safety Results**	Due to the premature discontinuation of the clinical study, and the limited number of patients enrolled per arm, no statistical analysis of the results can be conducted, neither for Efficacy nor for Safety endpoints
Conclusions	Due to the lack of statistically significant results, no conclusion can be drawn

Date of report 11/08/2022

3. TABLE OF CONTENTS

4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
ANCOVA	Analysis of CoVariance
ANOVA	Analysis of Variance
AVPU	Alert, Verbal, Pain, Unresponsive Score
CTCAE	Common Terminology Criteria for Adverse Events
CKD-EPI	Chronic Kidney Disease Epidemiology
CNR	Consiglio Nazionale delle Ricerche
COVID-19	Corona Virus 19 Disease
CRO	Contract Research Organization
EC	Ethics Committee
eCRF	electronic Case Report Form
eGFR	estimated Glomerular Filtration Rate
EMA	European Medicines Agency
GCP	Good Clinical Practice
HR	Hazard Ratio
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IEC	Independent Ethic Committee
IFT	CNR Institute of Translational Pharmacology
IFN	Interferon
IFN-I	Type I Interferons
ITT	Intention To Treat
INMI	Istituto Nazionale Malattie Infettive
IRB	Institutional Review Board
ISS	Istituto Superiore di Sanità
IU	International Units
MMRM	Mixed effect Model for Repeat Measure
MS	Multiple Sclerosis
NCI	National Cancer Institute
NEWS2	National Early Warning Score 2 (2017)
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PP	Per Protocol
RT-PCR	Real Time - Polymerase Chain Reaction
SAE	Serious Adverse Event
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	SARS Corona Virus
SARS-Cov 2	New Corona Virus
SC	Steering Committee
SOCS	Suppressor of cytokine signaling
SpO2	Oxygen Saturation
SUSAR	Suspected Unexpected Serious Adverse Reactions
USCAR	Special Unit for regional continued care
UBP43	Ubiquitin Protease 43

5. ETHICS

5.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

The study and any amendments were reviewed and approved by The Ethics Committee (EC) of the National Institute for Infectious Diseases "Lazzaro Spallanzani", Rome, Italy, which is the National Ethics Committee for evaluation of clinical trials on human drugs in COVID-19 patients.

5.2 Ethical Conduct of the Study

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

5.3 Patient Information and Consent

Written informed consent was obtained prior to patient enrollment, during pre-screening visit.
Representative written information for the patient and a sample patient consent is provided in appendix 16.1.3.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The study Scientific Coordinator was Filippo Belardelli (IFT, CNR), and Principal Investigator was Emanuele Nicastri (INMI). The investigators involved in the studies were MD belonging to the Special Units for Regional Continued Care (USCAR).
The study was designed and the protocol written by Filippo Belardelli (IFT, CNR), Luciano Castiello and Eleonora Aricò (Core Facilities, ISS), with the participation of Laura Bracci and Francesca Urbani (Department of Oncology and Molecular Medicine, ISS).
The statistical design of the study was prepared by Nicola Vanacore, Ilaria Bacigalupo, and Flavia Lombardo (National Centre for Disease Prevention and Health Promotion, ISS).
The Steering committee was chaired by the Scientific Coordinator of the study (Filippo Belardelli) with the cooperation of a coordination team: Giuseppe Sconocchia (IFT, CNR), Emanuele Nicastri (INMI), Pier Luigi Bartoletti (USCAR, INMI), Nicola Vanacore, Eleonora Aricò, Luciano Castiello (ISS).
The CRO FullCro was in charge of clinical study start-up, management and monitoring.
Administrative support was provided by Matilde Paggiolu, and Pamela Papa (IFT, CNR).
Filippo Belardelli, Eleonora Aricò and Luciano Castiello are the authors of this report.

7. INTRODUCTION

The outbreak of Coronavirus disease 2019 (COVID-19) pandemic highlighted the urgent need of developing therapeutic options to control or prevent virus spreading. Priority was given to the repurposing of existing antiviral agents, which allowed to shorten the timelines needed for clinical experimentation and to exploit the clinical experience with other viral infections. Among the many drugs put under evaluation all over the world, Interferon (IFN)-α and β stirred renewed interest against COVID-19 and were evaluated in clinical trials at different dosages and by different delivery systems, either as monotherapy or in combination with other compounds. Notably, IFN-β was considered the most promising, since it proved effective in alleviating COVID-19 symptoms when used in combination with lopinavir and ritonavir and in reducing mortality when combined with hydroxychloroquine and other antivirals (1).
An ensemble of studies, some of them carried out in the proponents' laboratories, have revealed that in addition to the antiviral activity, optimally achieved in the first phase of infection, IFN-I exhibit important immunoregulatory effects, including the increase of neutralizing antibodies and the induction of both innate and adaptive cellular immunity(2–6) .

While the majority of SARS-CoV 2 infected individuals are capable of clearing the virus solely by their own immune response, approximately 20% develops severe COVID-19. Notably, at higher risk of severe COVID-19 are males, people aged >65 years and/or showing some comorbidities (like hypertension and diabetes). An age-related impairment of endogenous IFN-I induction in response to viral infection has been described (7). Data on animal models on SARS-CoV and data emerging from COVID-19 pandemic point out to endogenous IFN-I system as a key player to control early phases of viral replication and prevent disease progression. Moreover, delayed IFN-I signaling activation can contribute to the exacerbation of SARS-CoV hyperinflammation and subsequent viral pathogenesis (Reviewed in (8)).
Rebif® (interferon beta-1a) is a disease-modifying drug used to treat relapsing forms of multiple sclerosis (MS) and is similar to the IFN-beta protein produced by the human body. It was approved in Europe in 1998 and it is used in more than 90 countries worldwide. While current posology of Rebif® in MS (12 MIU 3 times/week) is capable of balancing the neural inflammation typical of MS, the dosing and schedule of Rebif® administration in this study were selected by taking into consideration some features of IFN-I, emerged from many years of clinical use of these cytokines. In fact, several clinical studies reported that an Interferon-induced immune adjuvant activity could be observed already after the administration of intermittent low doses of the cytokine in both cancer and antiviral settings. Instead, the continuous stimulation of IFN-I signaling, exerted by high serum levels of the cytokine, can result in diminished treatment efficacy due to the emergence of refractoriness phenomena caused by receptor internalization/degradation as well as the rapid induction of UBP43 and SOCS negative regulators (9), immunosuppression and can also result in relevant side effects.
In the light of these considerations and evidences, we hypothesized that a short term IFN-β1a administration at the earliest time of SARS-CoV 2 diagnosis could compensate the insufficient or impaired endogenous IFN-I production, thus reducing the disease progression with respect to untreated patients or patients receiving standard of care.
With the aim to tailor the treatment schedule to the early phase of SARS-CoV 2 infection, we selected 3 MIU of IFN-β1a as a dose expected to exploit IFN-mediated antiviral and immunomodulatory properties of the cytokine without causing relevant toxicity or inducing refractoriness phenomena.

8. STUDY OBJECTIVES

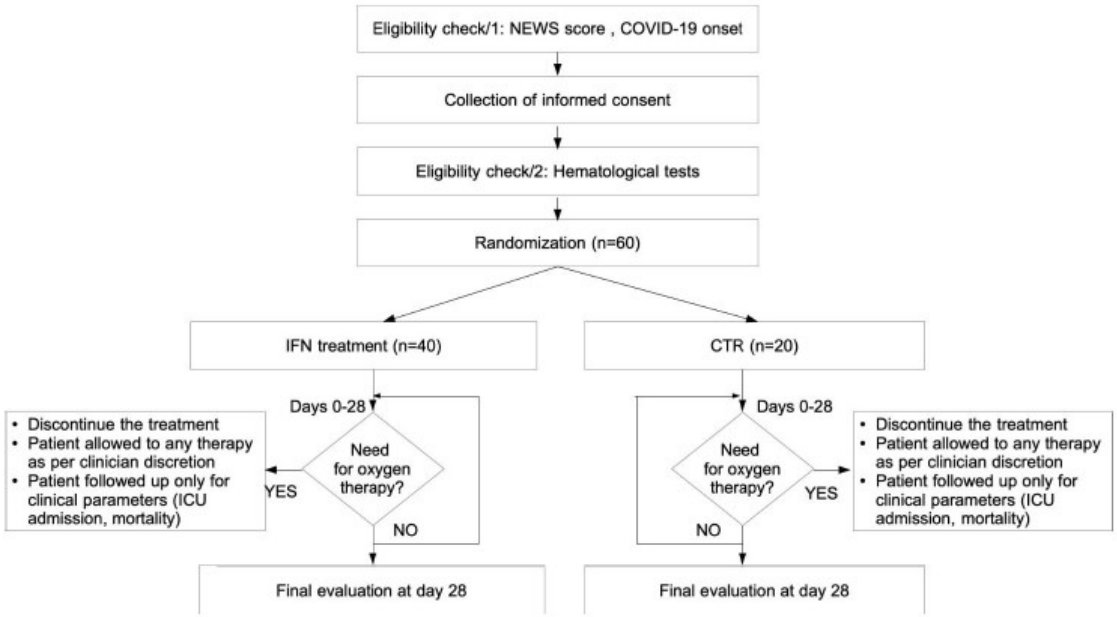
This trial aimed at exploring the use of IFN-β1a in SARS-CoV 2 newly diagnosed patients aged ≥ 65 years (V.2 of protocol) or ≥ 50 years (V.5 of protocol) with increased risk of developing severe COVID-19.
Primary Objective of the study was to evaluate the reduction in disease progression in patients treated with IFN versus control group within 28 days.
Secondary Objectives of the study were: 1) to assess the reduction in ICU admission in patients treated with IFN versus control group; 2) to assess the reduction in number of deaths in IFN compared to control group; 3) to evaluate the increase in proportion of participants returning to negative SARS-CoV 2 RT-PCR in IFN-treated versus control group at Day 14 and Day 28; 4) To assess the increase in SARS-CoV 2-Specific Antibody Titers in IFN-treated compared to control group; 5) to assess the safety of IFN-treated patients.

9. INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan-Description

Randomized, Open-Label, Controlled, Phase II Study. Patients, who satisfied all inclusion criteria and no exclusion criteria, were randomly assigned to one of the two treatment groups in a ratio 2:1. Randomization was planned to be stratified by gender to balance the presence of male and female in both study arms.

Control arm. No specific antiviral treatment besides standard of care.
Treatment arm. 11mcg (3MIU) of IFN-β1administered as subcutaneous injection at day 1, 3, 7, and 10 in addition to standard of care. The drug solution, contained in a pre-filled cartridge, was injected by means of the RebiSmart electronic injection device.
Patients were daily evaluated for 28 days after enrollment by means of the NEWS2 scoring system, assessing body temperature, respiratory rate, oxygen saturation, blood pressure, pulse/heart rate and AVPU response.



9.2 Discussion of Study Design, including the Choice of Control Groups

9.3 Selection of Study Population

9.3.1 Inclusion criteria

- ≥ 65 years of age at time of enrolment
  NOTE: this criterion changed into "≥ 50 years of age at time of enrolment" after the approval of amendment S AMD01 (29/07/21);
- Laboratory-confirmed SARS-CoV 2 infection as determined by PCR, in any specimen < 72 hours prior to randomization
  NOTE: this criterion changed into "Laboratory-confirmed SARS-CoV 2 infection as determined by PCR or third generation antigenic test (immunofluorescence with microfluidic reading) if COI >10, in any specimen prior to randomization" after the approval of amendment S AMD01 (29/07/21);
- Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures;
- Understands and agrees to comply with planned study procedures;
- Agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol;
- Being symptomatic for less than 7 days before starting therapy;
- NEWS2 score ≤2. Details on NEWS2 score are described in 9.5.3

9.3.2 Exclusion criteria

- Hospitalized patients with illness of any duration, and at least one of the following:

- Clinical assessment (evidence of rales/crackles on exam) and SpO2 ≤ 94% on room air at rest or after walking test,

  OR

  -Acute respiratory failure requiring mechanical ventilation and/or supplemental oxygen;

  Patients currently using IFN-beta (e.g., multiple sclerosis patients);
- Patients undergoing chemotherapy or other immunosuppressive treatments
- Patients with chronic kidney diseases;
- Known allergy or hypersensitivity to IFN (including asthma);
- Any autoimmune disease (resulting from patient anamnesis);
- Patients with signs of dementia or neurocognitive disorders;
- Patients with current severe depression and/or suicidal ideations;
- Being concurrently involved in another clinical trial;
- HIV infection (based on the anamnesis);
- Use of any antiretroviral medication;
- Impaired renal function (eGFR calculated by CKD-EPI Creatinine equation < 30 ml/min);
- Any physical or psychological impediment in a patient that could let the investigator to suspect his/her poor compliance;
- Lack or withdrawal of informed consent

NOTE: The following exclusion criteria were introduced after the approval of amendment S AMD01 (29/07/21):

- Pregnant or lactating females;
- Women of childbearing potential defined as all women physiologically capable of becoming pregnant (i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy). Note: A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient

Some of the exclusion criteria were added for safety concerns. The use of IFN-I in hospitalized patients with respiratory failure was discouraged by literature data showing that these cytokines might contribute to the systemic inflammation responsible for SARS-COV2-induced multiorgan failure. Depression and suicidal ideations are considered contraindications for the use of Rebif®, and particular attention must be given when considering the use of IFNβ in patients experiencing kidney dysfunctions and impaired renal function, according to European Medicines Agency (EMA). Patients undergoing immunosuppressive treatments were excluded since the optimal functionality of patient immune system is a requirement to exploit the immunomodulatory properties of IFNβ.

9.3.3 Removal of patients from therapy or assessment

Progressing patients which were in need of oxygen support were maintained in the trial for follow up purposes, but treatment was discontinued and additional treatment was provided at the physician discretion. This criterion was established in light of the possible negative effects of the treatment in later phases of COVID-19.

Another stopping rule includes drug related adverse events grade ≥ 3 according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Patients were also free to withdraw from participation in the study at any time upon request, without any consequence.

9.4 Treatments

9.4.1 Treatments administered

Control arm. No specific antiviral treatment besides standard of care.
Treatment arm. 0.25 ml of a solution containing 11mcg (3MIU) of IFN-β1a were injected subcutaneously at day 1, 3, 7, and 10 in addition to standard of care. The drug solution, contained in a pre-filled cartridge, was injected by means of the RebiSmart electronic injection device.

9.4.2 Identity of investigational product(s)

The investigational product was Rebif® 22 micrograms/0.5 mL solution for injection in cartridge (AIC number EU/1/98/063/008). Its pharmaceutical form is: solution for injection in cartridge. The solution is clear to opalescent, with pH 3.7 to 4.1 and osmolarity 250 to 450 mOsm/L. Each pre-filled cartridge contains 66 micrograms (18 MIU) of interferon beta-1a in 1.5 mL solution, corresponding to 44 micrograms/mL. The strength of the investigational product is measured by cytopathic effect (CPE) bioassay against the in-house interferon beta-1a standard which is calibrated against the current international NIH standard (GB-23-902-531).

9.4.3 Method of assigning patients to treatment groups

Eligible patients were randomised (no later than 36 h after enrolment) by means of a computerized central randomization system. All patients received a unique patient identification number at enrolling visit when signing the informed consent and before any study procedures were performed. A randomization list was prepared by the ISS group by using a validated software and the list was provided to the CRO and was inserted into the eCRF system (blind to the investigators). The randomization was planned to be stratified by sex; for each stratum a sequence of treatments randomly permuted in blocks of variable length (3 or 6) was planned.

9.4.4 Selection of doses in the study

Current posology of Rebif® in MS is 12 MIU 3 times/week. Such dose is meant to balance the neural inflammation typical of MS. The dosing and schedule of Rebif® administration in this study were selected by taking into consideration some features of IFN-I, emerged from many years of clinical use of these cytokines. In fact, several clinical studies reported that an Interferon-induced immune adjuvant activity could be observed already after the administration of intermittent low doses (ranging 1-3 MIU) of the cytokine in both cancer and antiviral settings. Instead, the continuous stimulation of IFN-I signaling, exerted by high serum levels of the cytokine, can result in diminished treatment efficacy due to the emergence of refractoriness phenomena caused by receptor internalization/degradation as well as the rapid induction of UBP43 and SOCS negative regulators, immunosuppression and can also result in relevant side effects.

9.4.5 Selection and timing of dose for each patient

Patients randomly assigned to the treatment arm received the same dose of investigational drug (Rebif® 22 micrograms/0.5 mL solution for injection in cartridge, lot n.BA068568, exp. date 31/03/2022) in addition to the standard of care. Timing was fixed at day 1, 3, 7 and 10. No additional indications regarding timing was established.

9.4.6 Blinding

This was an open-label study. After the randomization, patients were notified whether they were going to receive or not the experimental drug.

9.4.7 Prior and concomitant therapy

Patients did not receive any other antiviral treatment, unless considered needed by the physician. All other treatments including anti-hypertensive drugs, medications for diabetes (insulin and oral drugs), antibiotics, hormone therapy could be provided to patients of both groups according to medical judgments. Patients could not receive nonsteroidal anti-inflammatory drugs apart from paracetamol if needed.

Any previous and concomitant medication was recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

9.4.8 Treatment compliance

Treatment compliance was ensured by the home visits and a strict remote monitoring by the USCAR dedicated units. Rebif® administration was performed by USCAR investigators during home visits. Patients monitoring was auto-performed by the patient either assessed by a caregiver or a family member following training on how to use the provided devices. Measurements were recorded on the clinical diary provided. Patients were contacted daily by USCAR dedicated unit and communicated by phone their health status that was registered on the dedicated Case Report Form.

9.5 Efficacy and Safety Variables

9.5.1 Efficacy and safety measurements assessed and flow chart

Primary efficacy endpoint was the proportion of patients experiencing a disease progression, during at least 5 days, according to the National Early Warning Score **(NEWS2) (see below for details).** Measurement of NEWS2 parameters (body temperature, respiratory rate, oxygen saturation, blood pressure, pulse/heart rate and AVPU response) was either performed by USCAR units during the home visits, or auto-performed by the patient either assessed by a caregiver or a family member and recorded on the clinical diary provided and reported to the USCAR unit during remote monitoring.

For secondary endpoints on treatment efficacy, the following measurements were planned:

1) ICU-free days at 28 days;
2) All-cause mortality within day 0 and day 28;
3) Negative SARS-CoV 2 RT-PCR on an adequate sampling of upper respiratory tract on day 14 and 28 (swab sampling was performed by dedicated USCAR unit, molecular analysis was performed by Synlab Lazio s.r.l. laboratory);
4) Change from Baseline in serum SARS-CoV 2-Specific Binding Antibody on day 14 and 28 (antibody analysis was planned to be performed by the Department of Infectious Diseases, Istituto Superiore di Sanità).

Treatment safety was assessed by addressing the nature, incidence and severity of adverse events (AEs) and serious adverse events (SAEs). Toxicities were recorded and graded under the investigator responsibility using NCI Common Terminology Criteria for adverse Events (CTCAE) version 5. The investigator was obliged to assess the relationship between the study medical product and the occurrence of each AE/SAE and provide the assessment of causality as per instructions on the SAE form in the Investigators File.

Definition of an AE

An AE was defined as any untoward medical occurrence in a patient, temporarily associated with the use of a medicinal product, whether or not it is considered related to the medicinal product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding),

symptom, or disease (new or exacerbated) temporarily associated with the use of a medicinal product. Examples of an AE include:

- Significant or unexpected worsening or exacerbation of the condition/indication under study.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).

Definition of a Serious Adverse Event

A serious adverse event (SAE) was defined as any untoward medical occurrence that, at any dose:

1. Results in death
2. Is life-threatening
3. Requires hospitalization or prolongation of existing hospitalization
4. Results in disability/incapacity
5. Is a congenital anomaly/birth defect
6. Is otherwise considered as medically important.

TIMELINE SCHEME																												
Days	PreTx	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	T27	T28
	Screening	Treatment										Follow-up																	
IFN (ARM2 only)		x		x				x			x																		
Procedures (both ARMS)																													
RT-PCR SARS-CoV 2 positivity assay	x	x													x														x
Demografic Data	x																												
Medical History	x																												
Informed Consent	x																												
Inclusion/Exclusion Criteria	x																												
Signs and symptoms	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Previous/Concomitant Therapy recording	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
NEWS2 score assessment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Safety/Efficacy Evaluation (both ARMS)																													
Adverse Events recording		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Routine laboratory test parameters	x	x		x							x				x							x						x
SARS-CoV 2 Antibodies		x													x													x
Exploratory labtests (ISG/CIM/SIM)		x		x							x				x													

9.5.2 Appropriateness of measurements

NEWS2 scoring system is a well-established measurement to timely identify changes in patients' health status and was commonly used in COVID19 clinical trials.

Same applies to the other measurements selected, such as RT-PCR analysis of SARS-COV2 genetic material in upper respiratory tracts swab, anti-SARS-COV2 serum antibodies and toxicity assessment by CTCAE v.5.

9.5.3 Primary efficacy variable(s)

The NEWS2 score is a standardized approach aimed at promptly detecting signs of clinical deterioration in acutely ill patients and establishing the potential need for higher level of care. It is based on the evaluation of vital signs including respiratory rate, oxygen saturation, temperature, blood pressure, pulse/heart rate, AVPU response. The resulting observations, compared to a normal range, are combined in a single composite "alarm" score. Any other clinical sign clearly indicating a disease worsening will be considered as disease progression.
The NEWS2 score was calculated according to the following Chart. Additional measurements were allowed whenever any sign of disease progression appeared. In case of multiple measurements within a day, the highest score was be considered for patient assessment.

Chart 1: The NEWS scoring system

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

9.5.4 Drug concentration measurements

No drug concentration measurement was planned and performed for this trial. Pharmacodynamic properties of the investigational drug have already been characterized.

9.6 Data Quality Assurance

The clinical protocol and all the monitoring procedures were reviewed and discussed among the scientists involved in the trial design and investigators before activating the trial. Detailed information about the protocol, CRF, ICH.GCP was provided to the involved staff at the initiation meeting.
The Sponsor supplied the access to an online platform for certified eCRF recording to the clinical center.
The investigational product was managed by the clinical center pharmacy delegated by the Principal Investigator. Unused IP was eliminated by the pharmacy of the clinical center. During the trial, CRO monitors visited the clinical center regularly to monitor whether the trial was following the protocol, SOPs, GCP and related regulations and guidelines. Monitors had access to the source documents

including CRFs, copies of laboratory results and medical tests, and verified CRF entries and signed contents against the source document.

9.7 Statistical Methods Planned in the Protocol and Determination of Sample Size

9.7.1 Statistical and analytical plans

The primary analysis was planned to be carried out on the primary endpoint on the intention-to-treat (ITT) population defined as all patients randomized receiving at least one dose of treatment.
The percentage of patients undergoing disease progression defined on rate of progression of NEWS2 score lasting more than 5 days will be calculated in two arms (IFN-β1a + standard of care vs standard of care) of the trial. For persons who died, a conservative approach was planned, and death was considered an event. The effect of treatment was planned to be estimated through a logistic regression model including a dummy variable for treatment. The effect of treatment was planned to be estimated through multivariable logistic regression model by accounting for the following covariates: age, gender, co-morbidities. Moreover, NEWS2 score at baseline and setting of recruitment was planned to be considered.
We planned to carry out all primary and secondary analyses both on ITT population and on per-protocol population. Per-protocol population includes all subjects who were included in the ITT population that received the treatment as defined in the protocol and who completed the study with no major protocol violations. Kaplan-Meier survival analysis and Cox proportional hazards model were intended for time-to-event data, including the following covariates: age, gender, co-morbidities, also considering NEWS2 score at baseline and recruitment. For the secondary endpoint ICU-free days, a competing risk model was planned to be adopted considering death a competing event.
The longitudinal secondary endpoints measured on a continuous scale (the increase in SARS-CoV 2-Specific Binding Antibody Titers in IFN compared to control group) were planned to be analyzed using a Mixed effect Model for Repeat Measure (MMRM) to estimate the difference of mean change from baseline in SARS-CoV 2-Specific Binding Antibody Titers between IFN-β1a + standard of care and standard of care at day 28.
Safety endpoints were planned to be compared by a chi-squared test for discrete variables, by means of analysis of variance (ANOVA) and covariance (ANCOVA) for continuous variables or by the non-parametric Mann-Whitney test when appropriate.
Confidence intervals (95%) were selected for all outcomes and association measures (proportions, means, Odds Ratios and HRs). For all statistical analyses (efficacy and safety), the level of statistical significance was chosen at 0.05 with two-sided p-values.

9.7.2 Determination of sample size

The study was powered to independently assess a potential benefit of IFN-β1a compared with control arm (no specific antiviral treatment besides standard of care) on rate of progression of NEWS2 score lasting more than 5 days.
Sample size was calculated according to the primary endpoint of the study, based on the assumptions of an at least 35% difference in the percentage of patients undergoing disease progression between IFN-β1a and control arm. A sample size of 60 patients total (40 in the IFN-β1a-treated arm and 20 in the control arm, according to a 2:1 randomization ratio) was needed to provide 80% power at significance level of 5% to detect the difference of patients undergoing disease progression between a group 1 proportion of 0.15 (IFN-β1a + standard of care) and a group 2 proportion of 0.50 (standard of care).

9.8 Changes in the Conduct of the Study or Planned Analyses

After the formal opening of the trial for patients' recruitment (April 20 2021), 2 patients were enrolled and treated. In the following weeks, major difficulties in patients' enrolment were encountered, mostly due to three reasons: i) a marked and progressive decrease in the rate of newly infected patients, registered in the Lazio Region as a result of the vaccination campaign; ii) a limited involvement of family doctors, who represent the ideal link for suggesting to patients the enrolment in our clinical trial; iii) the approval of anti-SARS-COV-2 monoclonal antibodies to be used in the early phase of COVID-19 infection in patients with risk factors, including advanced age. The possibility to enroll subjects that underwent partial or full vaccination against SARS-COV2, and/or treated with monoclonal antibodies was excluded as the heterogeneity of the study population would most likely affect the outcome of virus infection, thus impairing the statistical significance of any result collected in the trial in terms of possible Rebif® efficacy.

In June 2021, a substantial amendment to the protocol (*S AMD 01*) was submitted to the regulatory authorities to introduce a change in inclusion criteria and case definition. At that time, literature data had shown that an endogenous impairment of IFN-I system can be triggered by SARS-CoV2 infection at any age, and it can account for patients progression towards severe forms of COVID-19 (8,10–12), Based on these observations, the age limit for patient recruitment was changed from 65 to 50 years of age and older. Moreover, in order to facilitate early patient identification and enrolment, the definition of COVID case was extended to persons with detectable SARS-CoV2 genes, as determined not only by RT-PCR but also by third generation antigenic test (immunofluorescence with microfluidic reading) if COI >10 conducted on respiratory specimen. The amendment was approved on July, the 29th 2021. However, the study continued to experience great difficulties in patient enrolment in the following months.

For all these reasons, the Steering committee agreed to undertake the premature discontinuation of the clinical trial. The enrollment of novel patients was closed on September, the 24th. The trial was formally closed on 31st January 2022.

Due to the extremely limited number of patients enrolled, none of the statistical analysis planned could be performed and no conclusion can be drawn.

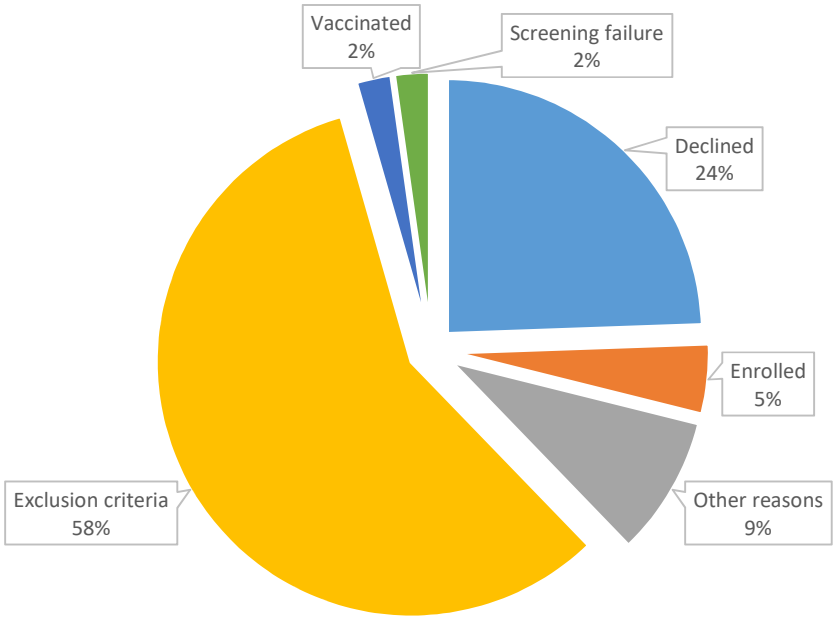
10. STUDY PATIENTS

10.1 Disposition of Patients

45 patients were contacted by phone for preliminary evaluation of inclusion/exclusion criteria. Of these, only 3 received the pre-Tx screening home visit, while the others were excluded for different reasons summarized in Figure 1. Main reason for exclusion was that patients did not meet the inclusion criteria; some patients declined to be enrolled, one had recently received anti-Sars-CoV2 vaccination, that was not listed as criteria for exclusion but was considered inappropriate to avoid biases.

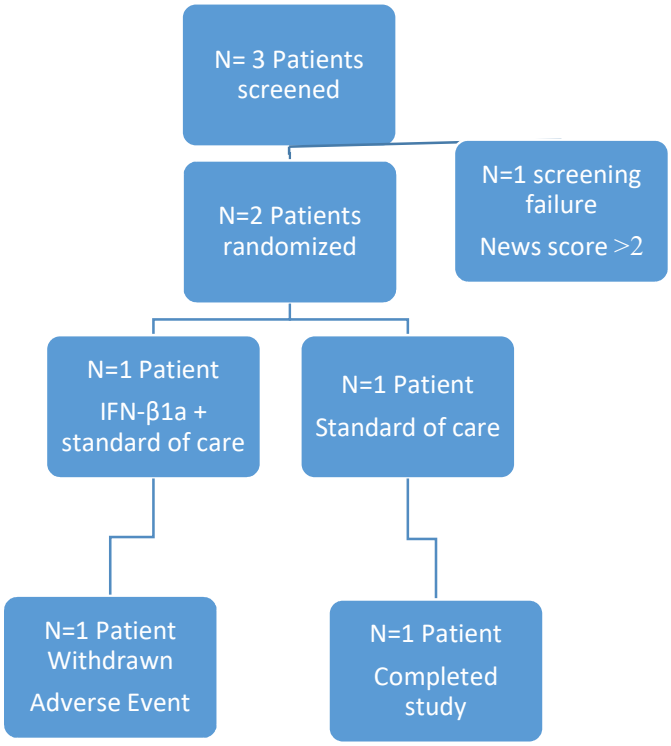
FIGURE 1

Summary of reasons for patient exclusion at screening



Of the three patients that received the Pre-Tx home visit for screening, one patient didn't meet the inclusion criteria for NEWS score>2 and was not randomized (*Screening failure*).

FIGURE 2
Disposition of patients



Only two patients were randomized: one patient was assigned to treatment arm, received home visits for IFNβ administration and remote monitoring according to schedule, until an adverse event occurred and patient required hospitalization. The adverse event ended in the death of patient, which was considered not related to the experimental drug.

Pt#1 IFNβ+ SOC	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Week 1		Pre Tx Visit **Informed Consensus signed**	T1 Visit (Rebif®)	Remote monitoring	T3 Visit (Rebif®)	Remote monitoring	Remote monitoring
Week 2	Remote monitoring	T7 Visit (Rebif®)	Remote monitoring	Remote monitoring	T10 Visit (Rebif®)	Remote monitoring	Remote monitoring ***Study discontinuation for Adverse Event***

The other patient fulfilling the inclusion criteria was randomized in the SOC arm, received all home visits and remote monitoring according to schedule until study conclusion.

Pt#2 SOC	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Week 1	Pre Tx Visit **Informed Consensus signed**	T1 Visit	Remote monitoring	T3 Visit	Remote monitoring	Remote monitoring	Remote monitoring
Week 2	Remote monitoring	Remote monitoring	Remote monitoring	T10 Visit	Remote monitoring	Remote monitoring	Remote monitoring
Week 3	T14 Visit (Swab for SARS-CoV2 RT-PCR)	Remote monitoring	Remote monitoring	Remote monitoring	Remote monitoring	Remote monitoring	Remote monitoring
Week 4	Remote monitoring	Remote monitoring	Remote monitoring	Remote monitoring	Remote monitoring	Remote monitoring	Remote monitoring
Week 5	T28 Visit (Swab for SARS-CoV2 RT-PCR) **Study Conclusion**						

10.2 Protocol Deviations

No deviation from protocol has been reported.

11. EFFICACY EVALUATION

11.1 Data Sets Analyzed

Due to the extremely limited number of patients enrolled, the dataset was not considered suitable for any of the statistical analysis planned.

11.2 Demographic and Other Baseline Characteristics

Patient	Age	Gender	Race	RSA/Home setting	Reported previous diseases	NEWS2 score at enrolment	Days from SARS-CoV2 diagnosis at enrolment
Pt#01	74	F	Caucasian	Home	Musculoskeletal, Endocrine, Cardiovascular, Malignant Neoplasm	1	5
Pt#02	72	F	Caucasian	Home	Musculoskeletal	0	3

11.3 Measurements of Treatment Compliance

No measure of treatment compliance with the treatment regimen under study investigation and drug concentration in body fluids was planned and performed

11.4 Efficacy Results and Tabulations of Individual Patient Data

Due to the extremely limited number of patients enrolled, no efficacy analysis was conducted.

11.4.1 Analysis of efficacy

Not applicable

11.4.2 Statistical/analytical issues

Not applicable

11.4.2.1 Adjustments for Covariates

Not applicable

11.4.2.2 Handling of Dropouts or Missing Data

Not applicable

11.4.2.3 Interim Analyses and Data Monitoring

Not applicable

11.4.2.4 Multicentre Studies

Not applicable

11.4.2.5 Multiple Comparisons/Multiplicity

Not applicable

11.4.2.6 Use of an "Efficacy Subset" of Patients

Not applicable

11.4.2.7 Active-Control Studies Intended to Show Equivalence

Not applicable

11.4.2.8 Examination of Subgroups

Not applicable

11.4.3 Tabulation of individual response data

Not applicable

SARS-CoV2 assay

Pt#01 underwent Adverse Event and study was discontinued before the T14 planned SARS-CoV2 assay.

Pt#02 SARS-CoV2 assay was positive on T14, and negative on T28.

11.4.4 Drug dose, drug concentration, and relationships to response
Not applicable
11.4.5 Drug-drug and drug-disease interactions
Not applicable
11.4.6 By-patient displays
Not applicable
11.4.7 Efficacy conclusions
No conclusion can be drawn

12. SAFETY EVALUATION

12.1 Extent of Exposure

12.2 Adverse Events (AEs)

12.2.1 Brief summary of adverse events

Two days after the last IFNβ administrations, 13 days post enrolment, patient in treatment arm experienced an Adverse Event consisting in severe respiratory crisis. Patient was hospitalized to receive the appropriate treatments, including oxygen support, and was later admitted to ICU. Four days later, the patient died. The event was considered a consequence of disease progression and, as such, unrelated to treatment.

12.2.2 Display of adverse events

AE description	Initial/Follow up	Patient ID	Start date	End date	Seriousness	Serious Criteria	Severity	Casualty	Outcome
Severe respiratory crisis	Initial	Pt#1	16 May 2021		Y	Hospitalization	Severe	Unrelated	Not recovered/not resolved
Severe respiratory crisis	Follow-up	Pt#1	16 May 2021	20 May 2021	Y	Death		Unrelated	resolved

12.2.3 Analysis of adverse events

Refer to 12.2.1

12.2.4 Listing of adverse events by patient

Refer to 12.2.1 and 12.2.2

12.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

Refer to 12.2.1 and 12.2.2

12.3.1 Listing of deaths, other serious adverse events, and other significant adverse events

Refer to 12.2.1 and 12.2.2

12.3.1.1 Deaths

Refer to 12.2.1 and 12.2.2

12.3.1.2 Other Serious Adverse Events

Not Applicable

12.3.1.3 Other Significant Adverse Events

Not Applicable

12.3.2 Narratives of deaths, other serious adverse events,and certain other significant adverse events

Refer to 12.2.1 and 12.2.2

12.3.3 Analysis and discussion of deaths, other serious adverse events, and other significant adverse events

Refer to 12.2.1 and 12.2.2

12.4 Clinical Laboratory Evaluation

12.4.1 Listing of individual laboratory measurements by patient (16.2.8) and each abnormal laboratory value (14.3.4)

Refer to Appendix 16.2.8

12.4.2 Evaluation of each laboratory parameter
Refer to Appendix 16.2.8
12.4.2.1 Laboratory Values Over Time
Refer to Appendix 16.2.8
12.4.2.2 Individual Patient Changes
Not applicable
12.4.2.3 Individual Clinically Significant Abnormalities
Refer to Appendix 16.2.8
12.5 Vital Signs, Physical Findings, and Other Observations Related to Safety
Not Applicable
12.6 Safety Conclusions
Due to the extremely limited number of patients enrolled, no conclusion can be drawn on investigational treatment safety.

13. DISCUSSION AND OVERALL CONCLUSIONS

The innovative characteristic of the study (i.e: treatment of patients in a very early phase of infection during a pandemic) implied the need to overcome several difficulties due to uncertainties on patient management in an evolving scenario of continuous changes in the criteria for hospitalization versus home care of patients > 65 years of age, depending on the variations in infection rate in the months September-December 2020 in the "Regione Lazio". Therefore, the activation of the study took longer than expected, mostly due to the complexity of the home-care setting for the clinical experimentation. In spite of several efforts to increase the possibility to enrol patients aged over 50 years, likely to benefit from IFNβ treatment, the implementation of the vaccination campaign in Italy and the standard use of monoclonal antibodies closed any chance to keep the trial open in view of the impossibilities to recruit patients with inclusion criteria compatible with the original rationale of the protocol design. We regret that the study had to be closed, in spite of many and intensive efforts to overcome several difficulties and delays.

A brief overview of the protocol of the study was published in 2021 in the Journal "TRIALS"(13) (Appendix 16.1.7). In principle, we believe that the protocol is still potentially valid from the scientific point of view, even in the context of the very recent progress in the development of virus-specific antiviral drugs, as we have now discussed in detail in a review article published in "Cytokines & Growth Factors Reviews" in February 2022(8).

14. TABLES, FIGURES AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

None

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16. LIST OF APPENDICES

16.1 Study Information
- 16.1.1 Protocol and protocol amendments
- 16.1.2 Sample case report form (unique pages only)
- 16.1.3 List of IECs or IRBs (plus the name of the committee Chair) - representative written information for patient and sample consent forms
- 16.1.4 List and description of investigators and other important participants in the study
- 16.1.5 Signature of Sponsor Scientific Coordinator
- 16.1.6 Randomization scheme and codes (patient identification and treatment assigned)
- 16.1.7 Publication based on the study

16.2. Patient Data Listings
- 16.2.1 Discontinued patients
- 16.2.2. Listing of individual laboratory measurements by patient

Appendix 16.1.1 Protocol and protocol amendments

- ANTIICIPATE study | version: 02 date:02/10/2020
- ANTIICIPATE study | version: 03 date:18/03/2021
- ANTIICIPATE study | version: 04 date:31/05/2021

CLINICAL STUDY PROTOCOL

Study Title: Antiviral and Immunomodulatory Interferon-Beta in high-risk COVID-19 patients

Short title: (ANTiviral and **I**mmunomodulatory **I**nterferon-Beta in high-risk **C**ov**I**d-19 **PAT**i**E**nts**)**
ANTIICIPATE

EudraCT N°: 2020-003872-42

Sponsor: Institute of Translational Pharmacology (IFT), National Research Council (CNR)

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Investigational Product Interferon β1a (Rebif™)

Clinical Study Phase: II

Version: 2.0

Issue Date: 02/10/2020

Protocol Signature form

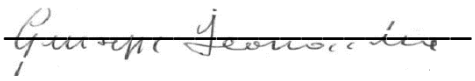
Protocol Title:

Antiviral and Immunomodulatory Interferon-Beta in high-risk COVID-19 patients (ANTIICIPATE)

Version: 2.0

Version Date: 02/10/2020

I have read the protocol described below and agree to conduct this study in accordance with procedures described therein. I also agree to conduct the study in compliance with all applicable regulations.



Principal Investigator's printed name

Giuseppe Sconocchia, MD

Date: 02/10/2020

Summary

List of abbreviations

AE	Adverse event
AIFA	Italian medicines agency
ALT	Alanine AminoTransferase
ANCOVA	Analysis of CoVariance
ANOVA	Analysis of Variance
AVPU	Alert, Verbal, Pain, Unresponsive Score
AST	Aspartate AminoTransferase
CT	Coordination Team
CTCAE	Common Terminology Criteria for Adverse Events
CIM	Cellular Immune Monitoring
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CNR	Consiglio Nazionale delle Ricerche
COVID-19	Corona Virus 19 Disease
CRO	Contract Research Organization
CRP	C-Reactive Protein
EC	Ethical Committee
eCRF	electronic Case Report Form
eGFR	estimated Glomerular Filtration Rate
ELISA	Enzyme Linked ImmunoSorbent Assay
FFP	Filtering Face Mask
FKN	Fractalkine
GCP	Good Clinical Practice
Hb	Haemoglobin
ICAM-1	Intercellular Adhesion Molecule 1
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IEC	Independent Ethic Committee
IFT	CNR Institute of Translational Pharmacology
IFN	Interferon

IL-6	Interleukin-6
ITT	Intention To Treat
LDH	Lactate DeHydrogenase
LSRCHs	long-stay residential care homes
INMI	Istituto Nazionale Malattie Infettive
ISG	Interferon Stimulated Genes
ISS	Istituto Superiore di Sanità
IU	International Units
MAR	Missing At Random
MERS	Middle East respiratory syndrome
MFC	Multiparametric Flow Cytometry
MS	Multiple Sclerosis
NCI	National Cancer Institute
NEWS2	National Early Warning Score 2 (2017)
NK	Natural Killer
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PP	Per Protocol
RCP	Riassunto delle Caratteristiche del Prodotto
RT-PCR	Real Time - Polymerase Chain Reaction
SAE	Serious Adverse Event
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	SARS Corona Virus
SARS-Cov 2	New Corona Virus
SC	Steering Committee
SIM	Systemic Inflammatory Markers
SOCS	Suppressor of cytokine signaling
SpO_2	Oxygen Saturation
SUSAR	Suspected Unexpected Serious Adverse Reactions
TNF	Tumor Necrosis Factor

USCAR	Special Unit for regional continued care
VCAM-1	Vascular Cell Adhesion Molecule 1
VPA-1	Vascular Adhesion Protein 1
UBP43	Ubiquitin Protease 43
WBC	White Blood Cells
WHO	World Health Organization

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Administrative clinical research support on inter-institutional agreements, material transfer agreements, institutional tenders.

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This is an investigator-initiated study. The Steering Committee will take responsibility for study design and data analysis and will operate actions necessary to guarantee that the trial is conducted in accordance with procedures described in this document and good clinical practice. The study is partially funded by Merck. Merck has no role in study design, data collection, management, analysis, data interpretation, manuscript writing, or in the decision to submit manuscripts for publication.

The Steering committee will include at least one representative from all units participating to the study and will be chaired by the Scientific Coordinator of the study (Filippo Belardelli) with the cooperation of a coordination team (Giuseppe Sconocchia, Emanuele Nicastri, Pier Luigi Bartoletti, Nicola Vanacore, Eleonora Aricò, Luciano Castiello). The Steering committee will oversee all the aspects of the project's life: decision about safety, decision for stopping rule, diagnostics issues, capacity development, financial, schedule, partnership, dissemination and exploitation. The Steering committee will hold at least one meeting a week on teleconference. In addition, extraordinary sessions will be held in case of critical issues.

1. Synopsis

1.1 BACKGROUND

The rapid and devastating outbreak of Coronavirus disease 2019 (COVID-19) pandemic highlighted the urgent need of developing therapeutic options to control or prevent virus spreading. In this regard, priority should be given to the repurposing of existing antiviral agents, thus shortening the timelines needed for clinical experimentation while exploiting the clinical experience with other viral infections (1). Among the many drugs under evaluation all over the world, Interferon (IFN)-α and β stirred renewed interest against COVID-19 and are presently being evaluated in clinical trials at different dosages and by different delivery systems, either as monotherapy or in combination with other compounds. Notably, IFN-β proved effective in alleviating COVID-19 symptoms when used in combination with lopinavir and ritonavir (2) and in reducing mortality when combined with hydroxychloroquine and other antivirals (3).

IFN-α and β, thereafter referred to as type I IFN (IFN-I), are cytokines with a long record of clinical use in patients with infectious disease (4), multiple sclerosis, and cancer (5). They are pleiotropic factors endowed with multiple activities, including both a broad spectrum antiviral activity and a remarkable immunoregulatory function (6). IFN-I are expressed at very low levels under basal physiological conditions, while they are generally abundantly produced in response to virus infections, when they play a crucial role in limiting viral replication and spread (7). In fact, many viruses, including Coronaviruses, evolved evasion strategies to counteract IFN-I system activation (8,9).

An ensemble of studies, some of them carried out in the proponents' laboratories, have revealed that in addition to the antiviral activity, optimally achieved in the first phase of infection, IFN-I exhibit important immunoregulatory effects, including the increase of neutralizing antibodies and the induction of both innate and adaptive cellular immunity (10–15).

While the majority of SARS-CoV 2 infected individuals are capable of clearing the virus solely with their own immune response, approximately 20% develops severe COVID-19. Notably, at higher risk of severe COVID-19 are males, people aged >65 years and/or showing some comorbidities (like hypertension and diabetes). An age-related impairment of endogenous IFN-I induction in response to viral infection has been described (16). Data on animal models on SARS-CoV (17,18) and data emerging from COVID-19 pandemic (19–22) point out to endogenous IFN-I system as a key player

to control early phases of viral replication and prevent disease progression. Moreover, delayed IFN-I signaling activation can contribute to the exacerbation of SARS-CoV hyperinflammation and subsequent viral pathogenesis (20,23,24).

In the light of these considerations and evidences, we hypothesize that elderly patients will greatly benefit from a short term IFN-β1a administration at the earliest time of SARS-CoV 2 diagnosis, thus compensating the insufficient or impaired endogenous IFN-I production.

In these patients, the antiviral and immunomodulatory effects of this cytokine could be efficiently exploited against COVID-19 through a short-term, discontinuous treatment with IFN-β1a in the early phases of infection, thus minimizing the relevant side effects (refractoriness and toxicity) associated to IFN continuous treatment schedules.

1.2 Objectives

This trial aims at exploring the efficacy of IFN-β1a in reducing the risk of SARS-CoV 2 recently infected elderly patients to progress towards severe COVID-19. In particular, this study will evaluate the consequences of a low and discontinuous use of IFN-β1a in the early phase of infection, and to exploit its immune activating properties in addition to its antiviral effects. Such regimen is expected to prevent any toxicity and refractoriness phenomena often occurring during IFN-I chronic administration.

Primary Objective of the study is to evaluate the role of IFN-β1a in reducing the disease progression in treated patients versus control group.

Secondary Objectives of the study are: 1) to assess the reduction in ICU admission in patients treated with IFN versus control group; 2) to assess the reduction in number of deaths in IFN compared to control group; 3) to evaluate the increase in proportion of participants returning to negative SARS-CoV 2 RT-PCR in IFN-treated *versus* control group at Day 14 and Day 28; 4) To assess the increase in SARS-CoV 2-Specific Antibody Titers in IFN-treated compared to control group; 5) to assess the safety of IFN-treated patients.

1.3 Methodology

Randomized, Open-Label, Controlled, Phase II Study. The study plans to enroll 60 patients: 40 in the IFN-β1a arm, 20 in the control arm, according to a 2:1 - treated: untreated ratio. Treatment plan foresees 4 subcutaneous injections of 3MIU of IFN-β1a, to be given at day 1, 3, 7 and 10 in addition to standard of care. Patients will be monitored and disease progression will be evaluated by means of the National Early Warning Score (NEWS2).

1.4 Expected results

Data emerging from the ongoing pandemic show that the management of advanced stage COVID-19 is mostly critical for elderly patients. This study is expected to provide information about the efficacy of a timely administration of IFN-β to elderly patients in achieving a more efficient control of SARS-CoV 2 infection, thus preventing the progression towards severe forms of the disease. The results of this study will provide a treatment option for high-risk elderly patients experiencing mild symptoms, for which no approved therapy is available so far (besides support therapy and a strict clinical monitoring).

The proposed treatment, upon demonstration of efficacy and safety, could be administered not only to hospitalized patients, but also during isolation at home or in long-stay residential care homes (LSRCHs), with the support of the territorial medical units. Therefore, this treatment protocol will represent an important tool to protect the elderly population in every pandemic scenario that will occur in the near future.

2. Background

The rapid and devastating outbreak of Coronavirus disease 2019 (COVID-19) pandemic and the lack of approved treatments for any human coronavirus (CoV) infection highlighted the urgent need of developing therapeutic options to control or prevent virus spreading. Several options can be envisaged ranging from prophylactic vaccine to targeted antiviral drugs. However, new interventions are likely to require months to years to be developed, and priority is being given to the repurposing of existing antiviral agents (1). Since COVID-19 outbreak, more than 3000 clinical trials have been authorized to identify the drugs or drug combinations capable of attenuating the

virulence of the disease (25). Some of these trials include the use of type I Interferons (IFN-I), mainly α and β, alone or in combination with other compounds. Interestingly, a randomized clinical trial testing the combination of Lopinavir, Ritonavir plus IFN-β in COVID-19 patients showed that only the triple combination was effective in alleviating symptoms and shortening the duration of viral shedding and hospitalization (2). A significant reduction of mortality was observed when IFN-β was administered together with hydroxychloroquine and other antivirals (3). Notably, data suggest that the timing of IFN therapy during SARS-CoV 2 infection can determine treatment efficacy and clinical outcome (26).

IFN-I were first discovered and characterized more than 60 years ago as antiviral substances produced by influenza virus-infected cells, capable of markedly inhibiting viral replication in target cells (27). These cytokines were the firsts to be cloned and extensively used in patients with some viral diseases (28) and cancer (IFN-α) (5). IFN-I are pleiotropic factors endowed with multiple activities, including both a broad-spectrum antiviral activity (27,28) and a remarkable immunoregulatory function (6). The antiviral activity of IFN-I has been extensively exploited for the treatment of viral chronic infections (28) Nevertheless, as highlighted by the long clinical records of IFN-I use, caution is required in terms of route, timing and dose of administration to balance clinical efficacy and side effects.

As many other viruses, Coronaviruses have developed multiple mechanisms to prevent IFN-I induction and subsequent signaling (29), particularly during the early phase of infection, ultimately leading to a dysregulated immune response and increased immunopathogenesis (20,30,31). Diminished levels of IFN-I have been detected in patients during the course of SARS and MERS (32–34). Similar results were also achieved with aged macaques infected with SARS-CoV, that exhibited considerably lower levels of IFN-β and a more severe pathology than young animals (17). Interestingly, when the deficiency in IFN-I production in CoV-infected macaques was remedied by IFN-α2 treatment in combination with ribavirin, lower levels of systemic (serum) and local (lung) proinflammatory markers were observed, in addition to fewer viral genome copies and less severe histopathological changes in the lungs (18). More relevantly, the results of a recent work clearly showed an impaired IFN-I signaling, associated with persistent blood virus load and an exacerbated inflammatory response in patients with severe COVID-19 (35). Impaired IFN-I response was also observed in young men experiencing severe COVID-19, in which a loss-of function genetic mutation in Toll Like Receptor 7 caused impaired IFN-I response (21). Overall, these observations outline the

critical role of IFN-I in both protective and pathogenic events during CoV infections, thus strengthening the need of fine tuning the IFN-I signaling with respect to the kinetics of CoV replication for an optimal protective response.

3. Rationale

In the light of the current information on SARS-CoV 2 pathogenesis, we speculate that the majority of SARS-CoV 2- infected patients are capable of clearing the virus by means of their effective endogenous IFN-I system and do not require hospitalization. We assume that in a minority of people a defective IFN-I system may favor SARS-CoV 2 spread, eventually causing the development of severe forms of COVID-19 and dismal prognosis. People aged >65 years, for which an impairment of IFN-I induction in response to viral infection has been documented (16,36,37), are at higher risk of severe COVID-19 (38).

In these patients, a delayed IFN-I response and the loss of viral control might contribute in early phases of infection to disease outcome. Data suggest that the IFN-β subtype appears to be the most suited for COVID-19 treatment (39). Thus, we hypothesize that elderly patients will greatly benefit from a short term IFN-I administration at the earliest time of SARS-CoV 2 infection, thus compensating the insufficient or impaired endogenous IFN-I production and preventing COVID-19 progression to severe forms of disease. In light of its immunomodulatory properties, IFN-β administered at the early phases of infection can represent a valuable tool to enhance humoral and cellular immunity in addition to its direct antiviral treatment restricting early viral spread, thus halting virus replication and preventing the progression towards severe forms of disease.

4. Impact for the National Health System

Italy was the first European country to experience COVID-19 pandemic, when the information about viral pathogenesis and therapeutic options were scarcely available. Moreover, Italian demographic structure, with a high percentage of population above 65 years of age, greatly affected the outcome and the death toll of the first epidemic wave. In fact, data show that not only sex and comorbidities, but also age increases the risk of developing severe COVID-19 (38,40) needing hospitalization and intensive care support. Since the first case, recorded in Italy on February 21th, COVID-19 represented a big challenge for the Italian National Health System, which underwent an increasing pressure until

restriction measures were undertaken to avoid its collapse. However, the interruption of non-essential economic and social activities has a serious impact on global economy and people quality of life in the long term. For elderly people, isolation can result not only in increased risks of cardiovascular, autoimmune and neurocognitive disorders, but also induce or exacerbate mental health problems, such as depression and anxiety. The introduction of a new phase, in which the restriction measures were gradually released and economic activities restarted, required some strategies to be undertaken to keep an acceptable risk for all population. A reinforced surveillance system was developed and is currently in use to ensure a prompt diagnosis of new cases. Nevertheless, it is urgent to develop and test new treatment options that can be administered during the early infection to reduce viral shedding, and consequent contagion, and to hamper disease progression toward severe forms, thus diminishing the impact on the National Health System.

In this trial, particular attention is given to aged patients with a recent diagnosis of COVID-19 in the presence of mild symptoms. In these patients, a strict medical control during home isolation, or a precautionary hospitalization are both appropriate choices, to monitor the possible rapid evolution of the infection. However, no therapeutic regimen specifically designed for these patients is available. Therefore, the risk of developing severe forms of the disease requiring intensive care or ending in fatalities is still high.

This trial will test the efficacy of IFN-β administered to aged patients during the early phase of the infection, in limiting viral replication and preventing the evolution of COVID-19 towards severe and critical diseases. Individual infectivity is directly associated with disease severity and time of viral shedding. Moreover, preventing severe COVID-19 will directly reduce lethality and will immediately mitigate the hospitals overworking, thus overall reducing the potential impact of COVID-19 on the National Health System.

5. Objectives of the study

This trial aims at exploring the use of IFN-β1a in SARS-CoV 2 newly diagnosed elderly patients with increased risk of developing severe COVID-19. In particular, this study will evaluate low-dose and discontinuous use of IFN-β1a in the early phase of infection, in order to exploit not only its antiviral,

but also its immune activating and anti-inflammatory properties. Such regimen should avoid any toxicity and refractoriness phenomena often occurring during IFN-I chronic administration.

5.1 Primary Objective

Primary Objective of the study is to evaluate the reduction in disease progression in patients treated with IFN versus control group within 28 days.

5.1.1 Primary endpoint and outcome

Primary endpoint of the study is the proportion of patients experiencing a disease progression, during at least 5 days, according to the National Early Warning Score **(NEWS2).** The **NEWS2** score is a standardized approach aimed at promptly detecting signs of clinical deterioration in acutely ill patients and establishing the potential need for higher level of care. It is based on the evaluation of vital signs including respiratory rate, oxygen saturation, temperature, blood pressure, pulse/heart rate, AVPU response. The resulting observations, compared to a normal range, are combined in a single composite "alarm" score. Any other clinical sign clearly indicating a disease worsening will be considered as disease progression.

5.2 Secondary Objectives and Endpoints

The following table 1 contains the secondary objectives and endpoint of the study

Objective	Endpoint
1) To assess the reduction in ICU admission in patients treated with IFN versus control group within 28 days of randomization	ICU-free days at 28 days (Day 1 through Day 28)
2) To assess the reduction in number of deaths in IFN compared to control group (day 28)	All-cause mortality (Day 1 through Day 28)
3) To evaluate the increase in proportion of participants returning to negative SARS-CoV 2 RT-PCR in IFN-treated versus control group at Day 14 and Day 28	Negative SARS-CoV 2 RT-PCR at day 14 post-randomization Negative SARS-CoV 2 RT-PCR at day 28 post-randomization

4) To assess the increase in SARS-CoV 2-Specific Binding Antibody Titers in IFN compared to control group (day 28)	Change from Baseline in SARS-CoV 2-Specific Binding Antibody Titers at day 14 and 28
5) To assess the safety of IFN-treated patients versus control group	Incidence of adverse events

For secondary endpoints, more detailed descriptions follow:

1) ICU-free days at 28 days will be calculated as the number of days a patient is not in an ICU. Time Frame will be: Admission (day 0) to 28 days after admission (day 28). In case of death, it will be counted as 0 day;

2) All-cause mortality will be: total number of death events occurring within day 0 and day 28;

3) Negative SARS-CoV 2 RT-PCR is defined as an undetectable presence of SARS-CoV 2 genes, as determined by PCR on an adequate sampling of upper respiratory tract.

4) Change from Baseline in SARS-CoV 2-Specific Binding Antibody Titers is defined as the difference in anti-SARS-CoV 2-specific antibody levels measured at day 28 versus day 0;

5) Details on safety event are described in paragraph 9.4

5.3 Exploratory Endpoints

Exploratory studies will be also performed on blood samples collected before and after treatment to assess:

- IFN-I signaling activation
- Cellular immune monitoring
- Systemic inflammatory markers

5.3.1 IFN-I Signaling

Pioneer studies in animal models showed that the complete absence of IFN-I signalling, by deletion of IFN-I receptor, enhanced mice susceptibility and mortality from several viral infections (7). IFN-I signalling downregulation may occur during viral infections as a consequence of viral-specific evasion mechanisms that Coronaviruses mainly establish during the early phase of infection (29,31). Diminished levels of IFN-I or Interferon Stimulated Genes (ISG) expression have been detected in the peripheral blood mononuclear cells of SARS and MERS patients (32,33). More relevantly, the

results of a very recent work clearly showed an impaired IFN-I signaling, associated with persistent blood virus load and an exacerbated inflammatory response in patients with severe COVID-19 (35). A diminished level of endogenous IFN-I activation and signalling may also occur as a consequence of aging, as reported in several *in vitro* and *in vivo* settings (17,41). In light of these considerations, the level of expression of selected ISG will be analysed in patients PBMC as surrogate markers of IFN-I signalling activation. Samples will be collected before, during and after the completion of IFN-β1a treatment in order to assess 1) possible correlations between IFN-I activation status and patient clinical outcome *per se*; 2) treatment-induced modifications of IFN-I signalling activation possibly associated with clinical improvement.

5.3.2 Cellular Immune-Monitoring

A decrease in peripheral lymphocyte count (with lower frequencies and absolute counts of CD3, CD4, CD8 T cells as well as of NK subsets) and an inflammatory cytokine storm may be the main reasons for rapid disease progression and poor treatment response in severe COVID-19. The neutrophil-to-CD8+ T cell ratio and the neutrophil-to-lymphocyte ratio were identified as prognostic factors affecting the prognosis for severe COVID-19 (42). Besides quantitative alteration, T cell maturation status was found to be modified since the percentage of naïve helper T cells increases and memory helper T cells decreases in severe cases. Patients with COVID-19 have also low levels of regulatory T cells, showing damaged features in severe cases (43). In general, COVID-19 patients show marked T cell activation, senescence, exhaustion and skewing towards Th17, if compared to healthy subjects (44).

The innovative technology MFC will help in elucidating the immunomodulatory *in vivo* effect of IFN β1a treatment. Leukocyte subpopulation frequency, activation status and functionality will be explored in pre- and post-treatment patients' blood samples. MFC results will be correlated with clinical outcome in order to identify potential peripheral immune markers of response to treatment.

5.3.3 Systemic inflammation

It was reported that in some COVID-19 patients, the immune response elicited against SARS-CoV 2 results in an increase in systemic inflammatory cytokines, which may eventually progress to a "cytokine storm," followed by multi-organ system dysfunction (45). In fact, some of the severe manifestations of COVID-19 are linked to the excess of circulating pro-inflammatory cytokines: acute respiratory distress syndrome, thromboembolic diseases such as acute ischemic strokes caused by

large vessel occlusion and myocardial infarction, encephalitis, acute kidney injury, and vasculitis (46). The chronic activation of pro-inflammatory pathways documented in the elderly, especially men, and named “inflamm-aging”, represents a risk factor *per se* for the development of COVID-19 complications (47).

We believe the restoration of a functional IFN-I response, through the administration of IFN-β1a during the early phase of SARS-CoV 2 infection, may affect systemic hyper inflammation both directly, by means of the immunomodulatory properties of the cytokines, and indirectly as an effect of reduced SARS-CoV 2 replication.

The level of inflammatory markers known to have a prognostic role in COVID-19 progression, such as IL-6, CRP, TNF-α (48) together with some endothelial cell adhesion molecules whose expression levels correlate with COVID-19 severity (FKN, VCAM-1, ICAM-1, VAP-1 (49)), will be analysed in the blood collected from IFN and control arm before and 10 days after enrolment. Data will be integrated with the results of routine lab analysis on coagulations factors (Fibrinogen, D-dimers) also involved in COVID-19 pathogenesis. The comparative analysis between groups will address treatment-induced modulations and possible correlation with clinical outcome.

5.4 Statistical hypothesis

The trial power has been calculated by the ISS group. The study was powered to independently assess a potential benefit of IFN-β1a compared with control arm (no specific antiviral treatment besides standard of care) on rate of progression of NEWS2 score lasting more than 5 days.

Sample size was calculated according to the primary endpoint of the study. In particular, the sample size calculation is based on the assumptions of an at least 35% difference in the percentage of patients undergoing disease progression between IFN-β1a and control arm. A sample size of 60 patients total (40 in the IFN-β1a-treated arm and 20 in the control arm, according to a 2:1 randomization ratio) will be needed to provide 80% power at significance level of 5% to detect the difference of patients undergoing disease progression between a group 1 proportion of 0.15 (IFN-β1a + standard of care) and a group 2 proportion of 0.50 (standard of care).

Sample Size: ANTIICIPATE trial

Two-sided significance level (1-α)	95
Power (1-β, % chance of detecting)	80
Ratio of sample size, Unexposed/Exposed	0.5
Percent of Unexposed with Outcome	50
Percent of Exposed with Outcome	15
Risk Ratio	0.3
Risk difference	-35

	Kelsey	**Fleiss**	**Fleiss (CC)**
Sample Size - Exposed	39	40	48
Sample Size-Unexposed	20	20	24
Total sample size	59	**60**	72

6. Study design

Randomized, Open-Label, Controlled, Phase II Study. Patients, who satisfy all inclusion criteria and no exclusion criteria, will be randomly assigned to one of the two treatment groups in a ratio 2:1. Randomization will be stratified by gender. Stratified randomization will balance the presence of male and female in both study arms. The planned study duration is 12 months including study set up, enrollment, follow up and data analysis as indicated in Appendix 3.

7. Study Population

Male and female adults aged 65 years or older with newly diagnosed mild COVID-19 are eligible for the study.

7.1 Case definition

For the purpose of the study, the following definition is applied: a case of COVID-19 is a person with detectable SARS-CoV 2 genes, as determined by PCR on an adequate sampling of upper respiratory tract.

7.2. Criteria for eligibility

7.2.1 Inclusion criteria

- ≥ 65 years of age at time of enrolment;
- Laboratory-confirmed SARS-CoV 2 infection as determined by PCR, in any specimen < 72 hours prior to randomization;
- Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures;
- Understands and agrees to comply with planned study procedures;
- Agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol;
- Being symptomatic for less than 7 days before starting therapy;
- NEWS2 score ≤2

7.2.2 Exclusion criteria

- Hospitalized patients with illness of any duration, and at least one of the following:

  -Clinical assessment (evidence of rales/crackles on exam) AND SpO2 ≤ 94% on room air at rest or after walking test,

  OR

  -Acute respiratory failure requiring mechanical ventilation and/or supplemental oxygen;
- Patients currently using IFN-beta (e.g., multiple sclerosis patients);
- Patients undergoing chemotherapy or other immunosuppressive treatments
- Patients with chronic kidney diseases;
- Known allergy or hypersensitivity to IFN (including asthma);
- Any autoimmune disease (resulting from patient anamnesis);
- Patients with signs of dementia or neurocognitive disorders;

- Patients with current severe depression and/or suicidal ideations;
- Being concurrently involved in another clinical trial;
- HIV infection (based on the anamnesis);
- Use of any antiretroviral medication;
- Impaired renal function (eGFR calculated by CKD-EPI Creatinine equation < 30 ml/min);
- Presence of other severe diseases impairing life expectancy (e.g. patients are not expected to survive 28 days given their pre-existing medical condition);
- Any physical or psychological impediment in a patient that could let the investigator to suspect his/her poor compliance;
- Lack or withdrawal of informed consent

7.3 Recruitment strategy

The management of elderly patients with COVID-19 needs to take into consideration the presence of comorbidities that increases their fatality risk, but it is also affected by the epidemiological situation of SARS-CoV 2 infection (see Feasibility section). Our study plans to enroll either hospitalized and non-hospitalized newly diagnosed COVID-19 patients, as well as patients hosted in long-stay residential care homes.

The Special Unit for regional continued care (USCAR), having the role of early detecting clusters of infection within Regione Lazio, will be responsible for screening and enrolling eligible patients that after SARS-CoV 2 positivity notification are not hospitalized, but remain in isolation at home or in a long-stay residential care homes. When dealing with patients older than 65, USCAR will be responsible of informing the patient of the current study, of having the Informed Consent signed and of collecting the blood sample to assess eligibility criteria. After the enrolment, the patient will be followed by a dedicated USCAR team that will: i) give to the patient/family-caregiver the kit of devices for home monitoring (i.e., 1 pulse oxymeter, 1 digital sphygmomanometer, 1 thermometer), ii) perform training for the use of devices, iii) perform treatments and collect samples for monitoring patients according to the timeline described in Appendix 2. The USCAR team will receive daily updates from non-hospitalized patients to determine their NEWS2 score values.

Patients that, at discretion of the general practitioner, are directed to Spallanzani Hospital for hospitalization, will be there assessed for inclusion/exclusion criteria and, in case of eligibility, enrolled in the study. Patients will be monitored according to standard hospital protocol in addition to the timeline described in Appendix 2.

8. Intervention

8.1 Experimental Drug and justification for dose

Rebif® (interferon beta-1a) is a disease-modifying drug used to treat relapsing forms of multiple sclerosis (MS) and is similar to the IFN-beta protein produced by the human body. It was approved in Europe in 1998 and it is used in more than 90 countries worldwide. While current posology of Rebif in MS (12 MIU 3 times/week) is capable of balancing the neural inflammation typical of MS, the dosing and schedule of Rebif® administration in this study were selected by taking into consideration some features of IFN-I, emerged from many years of clinical use of these cytokines. In fact, several clinical studies reported that an Interferon-induced immune adjuvant activity could be observed already after the administration of intermittent low doses of the cytokine in both cancer and antiviral settings (15,50–52). Instead, the continuous stimulation of IFN-I signaling, exerted by high serum levels of the cytokine, can result in diminished treatment efficacy due to the emergence of refractoriness phenomena caused by receptor internalization/degradation as well as the rapid induction of UBP43 and SOCS negative regulators (4), immunosuppression and can also result in relevant side effects.

With the aim to tailor the treatment schedule to the early phase of SARS-CoV 2 infection in elderly patients, we selected 3 MIU of IFN-β1a as a dose expected to exploit IFN-mediated antiviral and immunomodulatory properties of the cytokine without causing relevant toxicity or inducing refractoriness phenomena (53).

8.2 Treatment arms

Control arm. No specific antiviral treatment besides standard of care.

Treatment arm. 11ug (3MIU) of IFN-β1a will be injected subcutaneously at day 1, 3, 7, and 10 in addition to standard of care. The drug solution, contained in a pre-filled cartridge, will be injected by means of the RebiSmart electronic injection device, as described in Appendix 6.

8.3 Standard patients monitoring

Patients will be daily evaluated for body temperature, respiratory rate, oxygen saturation, blood pressure, pulse/heart rate and AVPU response. The NEWS2 score will be then calculated following the table 2. Additional measurements are allowed whenever any sign of disease progression appears. In case of multiple measurements within a day, the highest score will be considered for patient assessment.

Table 2. NEWS2 Score

Chart 1: The NEWS scoring system

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

For non-hospitalized patients, measurements will be auto-performed by the patient either assessed by a caregiver or a family member. Training on how to use the provided devices will be performed by USCAR unit at T1, written instructions will be also provided, and additional help will be given

upon request by phone or videocall. Measurements will be recorded on the clinical diary that will be provided (Appendix 5). Patients will be contacted daily by USCAR dedicated unit and will communicate by phone their health status that will be registered on a dedicated clinical records form (Appendix 5). USCAR unit approaching COVID-19 patients will use personal protective equipment including a FFP3 (or FFP2) mask, gloves, gown and googles. FFP3 will be used always in case of any procedure on respiratory tract (including nasopharyngeal swab).

Hospitalized patients can be discharged from the hospital considering the ongoing national and regional recommendations to discharge COVID-19 patient at home. The USCAR unit will then responsible of continuing follow up of the patient according to the timeline described in Appendix 2.

8.4 Other therapies allowed

Patients will not receive any other antiviral treatment, unless considered needed by the physician. All other treatments including anti-hypertensive drugs, medications for diabetes (insulin and oral drugs), antibiotics, hormone therapy can be provided to patients of both groups according to medical judgments. Patients should not receive nonsteroidal anti-inflammatory drugs apart from paracetamol if needed.

Any previous and concomitant medication will be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

8.5 Safety monitoring and individual stopping rules

Any sign or symptom associated to drug adverse events will be daily reported.

Progressing patients which are in need of oxygen support will be maintained in the trial for follow up purposes, but treatment will be discontinued (Appendix 1). Progressing patients will receive standard of care or additional treatment at the physician discretion.

Another stopping rule includes drug related adverse events grade ≥ 3 according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

For progressing patients, we will record the admission to the intensive care unit, days in ICU, and the disease outcome (either survivor or non-survivors) by using the Regional surveillance systems. Patients are free to withdraw from participation in the study at any time upon request, without any consequence. Patients should be listed as having withdrawn consent only when they no longer wish to participate in the study and no longer authorize the Investigators to make efforts to continue to obtain their outcome data. Every effort should be made to encourage patients to remain in the study for the duration of their planned outcome assessments. Patients should be educated on the continued scientific importance of their data, even if they discontinue the study. In the case of a patients becoming lost to follow-up, attempts to contact the patient should be made and documented in the patient's medical records.

9. Methods

9.1 Randomization

Sixty patients will be randomized 2:1 to receive IFN-β1a or control arm. Eligible patients will be randomised (no later than 36 h after enrolment) by means of a computerized central randomization system. All patients will receive a unique patient identification number at enrolling visit when signing the informed consent and before any study procedures are performed. This number must remain constant throughout the entire study.

ISS will prepare a randomization list by using a validated software and the list will be managed by the CRO. The randomization of patients will be closed when 60 patients have been randomized. The randomization will be stratified by sex; for each stratum a sequence of treatments randomly permuted in blocks of variable length (3 or 6) will be generated.

9.2 Blinding

This is an open-label study. After the randomization, patient will be notified whether will receive or not the experimental drug.

9.3 Electronic case report form

Patients' data will be recorded in an *ad hoc* online database. The Electronic case report form will be provided by a Clinical Research Organization and implemented according to the study design. An example of the information to be recorded in the e-CRF is provided as Appendix 4.

9.4. Safety Criteria Evaluation

9.4.1 Safety profile

Subjects participating in this trial who received at least one dose of the trial medication are considered to be included in the safety population (full analysis population). Safety population not include subjects who drop out prior to receiving any treatment. Data on safety profile, nature, incidence and severity of adverse events (AEs) and serious adverse events (SAEs) will be collected as detailed in both this section of the protocol and in the AE/SAE section of the CRF. Any reason for drug interruption, reduction and discontinuation will be collected. Toxicities will be graded using NCI Common Terminology Criteria for adverse Events (CTCAE) version 5. The investigator is responsible for detecting, documenting and reporting AEs and SAEs, according to the criteria defined in this protocol.

The safety profile of experimental drug (i.e., IFN-β1a, Rebif®) has been well established. Below are the very common and common adverse reactions as reported in the Summary of Product Characteristic 2010:

Very common (≥1/10)	**Common (≥1/100 to <1/10)**
Neutropenia, lymphopenia, leukopenia, thrombocytopenia, anaemia	Diarrhoea, vomiting, nausea
Asymptomatic transaminase increase	Severe elevations in transaminases
Headache	Pruritus, rash, erythematous rash, maculo-papular rash, alopecia
Injection site inflammation, injection site reaction, influenza-like symptoms	Myalgia, arthralgia
	Depression, insomnia
	Injection site pain, fatigue, rigors, fever

9.4.2 Adverse events (AE) and serious adverse events (SAE)

9.4.2.1 Definition of an AE

An AE is defined as any untoward medical occurrence in a patient, temporarily associated with the use of a medicinal product, whether or not it is considered related to the medicinal product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporarily associated with the use of a medicinal product. Examples of an AE include:

· Significant or unexpected worsening or exacerbation of the condition/indication under study.

· Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.

· New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.

· Signs, symptoms, or the clinical sequelae of a suspected interaction.

· Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).

9.4.2.2 Definition of a Serious Adverse Event

A serious adverse event (SAE) is defined as any untoward medical occurrence that, at any dose:

1. Results in death
2. Is life-threatening
3. Requires hospitalization or prolongation of existing hospitalization
4. Results in disability/incapacity
5. Is a congenital anomaly/birth defect
6. Is otherwise considered as medically important.

9.4.2.3 Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE or SAE on the eCRF. Any AEs or SAEs occurring during the study must be documented in the subject's medical records and on the appropriate page of the eCRF. Each AE or SAE is to be recorded individually. All AEs which occur

during the course of the study should be recorded in the eCRF. Information on the AE must be recorded on a specific AE form (appendix 5).

9.4.2.4 Evaluating AEs and SAEs

9.4.2.4.1 Assessment of intensity

The investigator will make an assessment of intensity of each AE and SAE reported. In this protocol, the intensity of AEs and SAEs will be graded on a scale of 1 to 5 according the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE) Version 5 and are available at https://evs.nci.nih.gov/ftp1/CTCAE/About.html .

For SAEs, the maximum intensity (or grade) will be reported in the eCRF. For non-serious AEs, each change in intensity (or grade) will be reported in the eCRF.

9.4.2.4.2 Assessment of causality

The investigator is obliged to assess the relationship between the study medical product and the occurrence of each AE/SAE and provide the assessment of causality as per instructions on the SAE form in the Investigators File.

9.4.2.4.3 Follow-Up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide further information on the subject's condition by any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. AEs that are ongoing with a toxicity of Grade 3 or 4, or have a relationship to study drug that is suspected (Reasonable Possibility) will be queried for resolution at study conclusion and at approximately 30 days after the last dose of study. New or updated information will be recorded on the originally completed SAE form in the Investigator's File, with all changes signed and dated by the Investigator.

9.4.3 Regulatory reporting requirements for adverse events

The Investigator must report immediately (within 24 hours from the knowledge) to the study Sponsor any SAE, occurred during the study whether related to the investigational product or not.

The study Sponsor has the legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. The study Sponsor will comply with the Italian regulatory requirements related to the reporting of SAEs to regulatory authorities and the Independent Ethics Committee (IEC). In particular, all the Suspected Unexpected Serious Adverse Reactions (SUSARs) that occur while on treatment and within 30 days since the last investigational drug administration, and that have a

suspected relationship to study's drug (Reasonable Possibility) will be notified with an urgency procedure to the local regulatory Agency (AIFA) and IEC with the following timelines:

- SUSARs that are considered life-threatening: notification within 7 days.
- SUSARs that are not considered life-threatening: notification within 15 days.

The notification with urgency procedure is not required for SAEs that are expected with the drugs used in the protocol, and for non-serious AEs, both expected and unexpected. For these events (expected SAEs and AEs), the CT will notify the local regulatory agency and IECs by an annual safety report.

9.5 Secondary and Exploratory endpoints

Dedicated blood samples will be collected at different time points (see APPENDIX 2: Timeline scheme) and processed at the biobank of the INMI.

9.5.1 SARS-CoV-2 Antibodies

The development of a specific humoral response will be monitored by measuring specific anti SARS-CoV-2 antibodies in the sera of patients collected at day 14 and 28 post randomization. Commercially available tests will be used to detect IgM specific for S antigen, IgG specific for the N and S antigens, and IgA specific for S antigen. Sera resulting reactive with the S antigen will be tested for the capacity of viral neutralization using standardized methods.

9.5.2 Molecular IFN-I signaling

Blood samples will be collected at T1 prior first treatment, during treatment (T3 prior second treatment) and post treatment (T14) and processed at the biobank of the INMI. Isolated PBMC will be aliquoted, submerged with RNA stabilization reagent and cryopreserved. For analysis, total RNA will be isolated and the transcriptional analysis of over 500 general immunology genes will be performed by means of Nanostring technology. Data analysis will determine the transcriptional modifications occurring during the course of IFN-β1a treatment as well as to identify molecular patterns potentially correlated with clinical outcome. Particular focus will be given to the ISG score reported to be differentially expressed among mild to severe COVID-19 (22). This exploratory analysis will be conducted by Dr Aricò and Dr Castiello, having a relevant background on IFN signaling analysis (51,54)

9.5.3 Cellular Immune monitoring

Pre-(T1 prior treatment) and post-treatment (T14) blood samples will be monitored by MFC-based assays through different antibody panels in order to analyze frequency of major leukocyte subpopulations associated with naïve/memory, co-activation and co-inhibition markers; polyfunctional properties of T cell specific response against virus antigens will be evaluated after short term in vitro culture.

Stained samples will be acquired on a Beckman Coulter CytoFlex Cytometer and analyzed by CytExpert and/or Kaluza software as well as by advanced machine learning algorithms such as FlowSOM and CITRUS (Cytobank online platform). Dr. Francesca Urbani and Dr. Iole Macchia, co-investigators at ISS unit, have long lasting experience in MFC assays and immune-monitoring (52,55,56).

9.5.4 Systemic Inflammatory markers

Pre- (T1 prior treatment) and during-treatment (day 10 prior last treatment) blood samples will be collected from Treatment and control group to monitor the levels of soluble factors involved in inflammation (e.g., cytokines and chemokines) and endothelial cell adhesion molecules. At the selected time points, plasma will be isolated from peripheral blood and cryopreserved until analysis that will be simultaneously conducted by means of specific ELISA assays. Data will be integrated with the results of routine lab tests on coagulation factors and factors involved in COVID-19 pathogenesis (CRP, IL-6, TNF-α, Fibrinogen and D-Dimer).

10. Statistical Plan

The primary analysis will be carried out on the primary endpoint on the intention-to-treat (ITT) population defined as all patients randomized receiving at least one dose of treatment.

The percentage of patients undergoing disease progression defined on rate of progression of NEWS2 score lasting more than 5 days will be calculated in two arms (IFN-β1a + standard of care vs standard of care) of the trial. For persons who died, a conservative approach will be adopted and death will be considered an event. The effect of treatment will be estimated through a logistic regression model including a dummy variable for treatment. The effect of treatment will be estimated through multivariable logistic regression model by accounting for the following

covariates: age, gender, co-morbidities. Moreover, NEWS2 score at baseline and setting of recruitment will be also considered.

All primary and secondary analyses will be carried out both on ITT population and on per-protocol population. Per-protocol population includes all subjects who were included in the ITT population that received the treatment as defined in the protocol and who completed the study with no major protocol violations.

Kaplan-Meier survival analysis and Cox proportional hazards model will be used for time-to-event data. The following covariates will be included in the Cox model: age, gender, co-morbidities. Moreover, NEWS2 score at baseline and setting of recruitment will be also considered. For the secondary endpoint ICU-free days, a competing risk model will be adopted considering death a competing event, following the method proposed by Fine and Gray (57). Moreover, the median difference will be reported.

The longitudinal secondary endpoint measured on a continuous scale (the increase in SARS-CoV 2-Specific Binding Antibody Titers in IFN compared to control group) will be analysed using a Mixed effect Model for Repeat Measure (MMRM) to estimate the difference of mean change from baseline in SARS-CoV 2-Specific Binding Antibody Titers between IFN-β1a + standard of care and standard of care at day 28. In case of data sporadically missing during the course of trial we will assume they were Missing At Random (MAR). A sensitivity analysis will be carried out by conducting the statistical test after imputing missing, including the worst-case imputation. All missing data will be imputed within treatment groups defined by randomized treatment.

Safety endpoint will be compared by a chi-squared test for discrete variables, by means of analysis of variance (ANOVA) and covariance (ANCOVA) for continuous variables or by the non-parametric Mann-Whitney test when appropriate.

Confidence intervals (95%) will be reported for all outcomes and association measures (proportions, means, Odds Ratios and HRs).

For all statistical analyses (efficacy and safety), the level of statistical significance will be kept at 0.05 with two-sided p-values. Statistical analyses and related reports will be in full compliance with ICH E9 guidance (https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-9-statistical-principles-clinical-trials-step-5_en.pdf).

11. Timing

See APPENDIX 2: Timeline scheme and APPENDIX 3: GANTT

12. Feasibility

Our study plans to enroll either hospitalized and non-hospitalized newly diagnosed COVID-19 patients, as well as patients hosted in long-stay residential care homes. The possible scenario of the Italian pandemic occurring during the conduction of the study will likely affect the proportion of patients that will be enrolled in the different settings. In fact, for patients presenting with mild illness, the decision to undertake hospitalization vs home care should be carefully evaluated to take into account patient risk of rapid deterioration, but also the burden on the health care system.

On august 11th, the ISS, together with the Ministry of Health and the Coordination of Italian regions and autonomous provinces, issued a document called "Elementi di preparazione e risposta a COVID-19 nella stagione autunno-invernale". The document, aimed at providing general elements and suggesting preparedness frameworks to strengthen the response and optimally cope with any increase in the number of new infections by SARS-CoV 2 in the autumn-winter 2020-2021 season, foresees four possible scenarios, characterized by increasing SARS-CoV 2 transmission rate and related risk of SSN collapse.

To ensure that patients' enrolment is duly completed in any of the possible scenarios, the study will count on a network of collaborating institutions directly involved in the identification and management of COVID-19 patients in Rome. A campaign will be held to inform Family doctors (Medici di Medicina Generale) and long-stay residential care homes (LSRCHs), whose collaboration will ensure the precocious identification of eligible patients throughout the urban area of Rome. The Special Unit for regional continued care (USCAR) are currently involved in the prompt identification of COVID-19 clusters within Regione Lazio. In this study, a group of physicians belonging to USCAR will be specifically trained and will be responsible for the screening, enrolling and clinical monitoring of patients kept under home isolation or hosted in long-stay residential care homes (LSRCHs).

All experts involved in the project are highly motivated, have complementary expertise and a strong background in the fields of IFN biology and infectious diseases. The CNR group, including the PI and Scientific Coordinator of the study, has a long-lasting expertise on immunology, IFN biology and clinical studies with IFN-I. The Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani

(clinical center) is one of the five clinical hubs for COVID-19 management in town and has a longstanding experience in multicentre clinical studies. The ISS group includes scientists with a background on IFN-I biology in both basic research and clinical trials, with particular focus on patients immunomonitoring (Francesca Urbani, Iole Macchia) and molecular studies on IFN-I signalling (Luciano Castiello, Eleonora Aricò). Moreover, the Clinical Epidemiology group of the ISS has a strong background on statistical analysis and was involved in the recent survey on COVID-19 infection in long-stay residential care homes (58) . To ensure the full feasibility and the high quality performance of the study the Sponsor will soon finalize a contract with a CRO, highly specialised in clinical studies involving IFN-β, which will support the PI (Giuseppe Sconocchia) and the Scientific Coordinator (Filippo Belardelli) with regard to specific services and for the implementation and monitoring of the entire study.

13. Good clinical practices and ethics

13.1. Good clinical practice

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (1964) and subsequent amendments and updates (Fortaleza, Brazil, October 2013), in the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and in the appropriate regulatory requirements. The drug used in this trial is already registered and its toxicity profile is very well known, since it is largely used for the treatment of Multiple Sclerosis.

13.2 Ethical aspects

The entire study protocol, including informative material for the patients and modules for the informed consent, will be evaluated by The Ethics Committee (EC) of the National Institute for Infectious Diseases “Lazzaro Spallanzani”, Rome, Italy, which is the National Ethics Committee for evaluation of clinical trials on human drugs in COVID-19 patients.

The study will not start before obtaining a favorable opinion from the EC, the Competent Authority Authorization and any other authorization required by local regulation. Every intention to modify any element of the original protocol after the first approval will be promptly notified to the EC and will be applied only after its written authorization. Investigators will be responsible for submitting

any amendments to the protocol to the EC. Any modifications to the protocol, which may impact on the conduct of the study, may affect patient safety, including changes of study objectives, study design, patient population, sample size, study procedures or significant administrative aspects will require a formal amendment to the protocol. Such amendments will be agreed and approved by the Ethics Committee of the National Institute for Infectious Diseases "Lazzaro Spallanzani", Rome, Italy, and the health authorities prior to implementation, in accordance with local regulation. Administrative changes of the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be documented in a memorandum.

13.2.1 Written informed consent

The Investigators will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects will also be notified that they are free to discontinue from the study at any time. The subject's signed and dated informed consent will be obtained prior to conduct any procedure specific for the study. The original signed Written Informed Consent Form will be stored, and a copy will be given to the patient.

13.2.2 Subject data protection

In order to protect the subjects' identity, the Investigator will assign a subject identification number to each enrolled subject to be used instead of subject name when reporting all study related data and adverse events.

The Written Informed Consent Form will explain that the study data will be stored at Spallanzani Hospital maintaining confidentiality in accordance with national data legislation. However, the personal information must be available to authorized personnel of Study Sponsor (clinical monitor and auditor), Ethics Committee and Regulatory Authorities. In addition, consent to allow direct access to original medical records to ensure data verification will be obtained from the subject before participation in the study.

Enrolment log must be kept strictly confidential to enable patient identification at the site.

13.2.3 Audits and inspections

The Principal Investigator and the SC will provide all the necessary information and material to the participating centers in order to standardize all the protocol-related procedures and to avoid

unexpected variability. Printed and electronic informative material (complete original protocol, informed consent modules, informative modules for patients and relatives, recruitment checklist, graphic timeline of interventions and visits, order list for physicians and nurses) will be distributed to Spallanzani Hospital and USCAR. Source data/documents must be available to inspections by the designee or Health Authorities.

13.2.4 Monitoring

The monitoring activities will be performed by a Clinical Research Organization. Clinical Monitor will perform the monitoring activities according to "Note of Guidance on Good Clinical Practice" (ICH E6 (R2), EMA/CHMP/ICH/135/1995).

The clinical monitor will maintain contacts between Investigators and Study Sponsor; furthermore, during the study the clinical monitor will verify that informed consent was obtained from all subjects, that the data were adequately documented in medical records and that the Investigators were compliant with the protocol. The clinical monitor will inform the study Sponsor and the Investigators about all detected protocol deviations, all facilities and technical Staff detected problems. The Investigators will provide direct access to source data/documents for data verification.

13.2.5 Declaration of interest

The study participants declare no financial and/or other conflicts of interest related to the study.

13.2.6 Dissemination policy

The Circ. Min. Health N° 6 of 09/02/2002 obliges each researcher who gets any results of interest to public health, to publish the results within 12 months from the end of the study. All the patients will freely agree or disagree to participate in the study in the belief that the results will be useful to improve knowledge about their pathologies, for health benefit from themselves or other patients. To respect their will and in the maximum interest of honest clinical research, the investigators agree on the need to ensure the wide publication and diffusion of their results in a consistent and responsible way under their responsibility. The Study Coordinator is the official data owner. The Study Coordinator has the right to present methods and results of the study at public symposia and conferences. The principal publications from the trial will be in the name of Investigators with full credit assigned to all collaborating investigators and institutions.

13.3 Insurance

The study will be conducted according to the law about the study insurance agreement (DM 14 luglio 2009); *ad hoc* insurance will be established.

14. Budget

Materiale/Utilità	Costo unitario	Numero per paziente	N. pazienti	Quantità	Totale
Costi coordinamento medico	75000				75000
Rebif/Rebismart	0	1	60	60	0
Servizi svolti da CRO					60000
Saturimetro	25	1	30	30	750
Sfingomanometro	20	1	30	30	600
DPI completo FFP3	10	10	30	300	3000
Assistenza domiciliare	400	1	30	30	12000
Teleassistenza	10	28	30	840	8400
Analisi del sangue	40	7	60	420	16800
Test Sars-CoV2	85	3	60	180	15300
Test sierologico	60	3	60	180	10800
Markers infiammatori	100	2	60	120	12000
Ddimero	6	2	60	120	720
Proteina C Reattiva	6	2	60	120	720
Systemic immune profiling	356	3	60	180	64080
RT-PCR per IFN signaling	280	3	60	180	40000
Costi processamento campioni biologici	30	7	60	420	12600
Assicurazione				1	9500
Costi etichettatura e gestione farmaco				1	5000
Costi generali per struttura coordinatrice					47730
Medical Writing and Statistical Data Analysis (all endpoints)					50000
Totale					**445000**

The study is co-funded by Merck Healthcare KGaA with a support equal to 40% of total costs.

15. Institutions agreement

The Study Sponsor will submit in the Clinical Trials all the documentation required by law to AIFA, as the Competent Authority and to Ethics Committee within a week after approval. Also, the Study Sponsor will comply in all respects with the standards of Good Clinical Practice, as defined in the

"Note of Guidance on Good Clinical Practice (CPMP/ICH 135/95)" and related Guidelines, as well as with all applicable regulatory requirements including national drug law and data protection law.
A collaboration agreement between all the Institutions involved in the study (CNR, INMI and ISS) will be signed before the enrollment of the first patient.

16. Participating Centers

- IFT, CNR, Rome;
- ISS, Rome
- INMI, Rome

17. Publications and data properties

Clinical trial data are considered the property of the investigators involved. Publications generated from the study will be sent to peer-reviewed international journals. The name and order of the authors will be decided by the working group.

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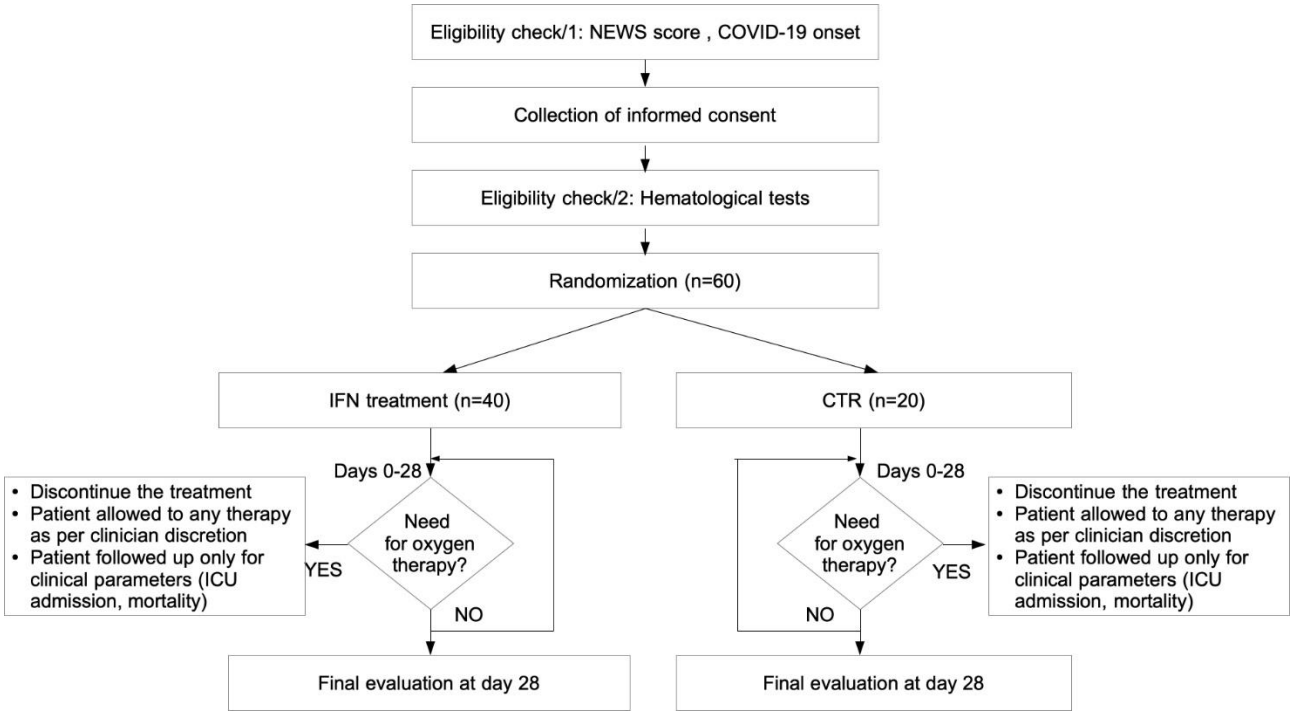
List of Appendices

APPENDIX 1: Flow Chart of the Study

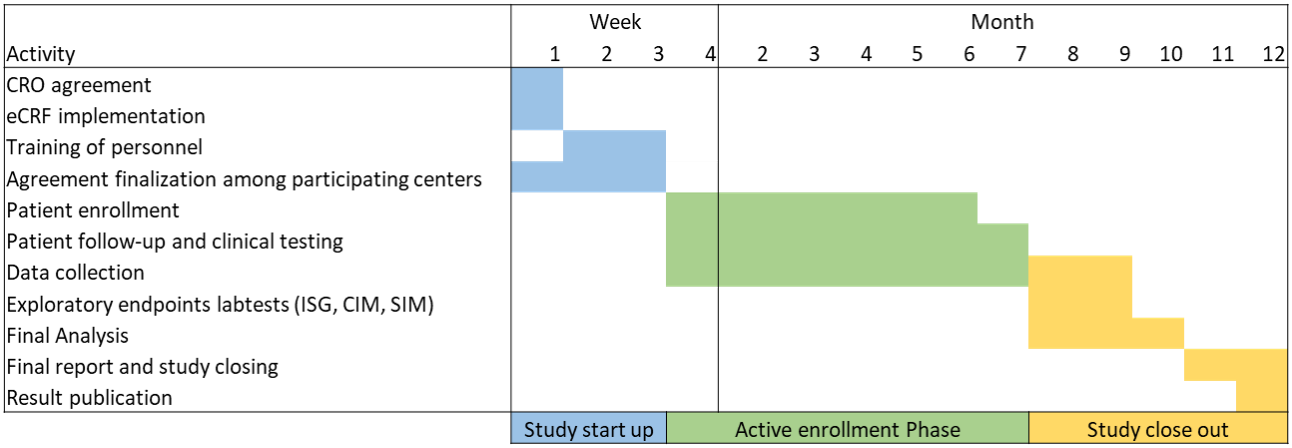


APPENDIX 2: Timeline scheme

TIMELINE SCHEME																															
Days	PreTx		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10		T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	T27	T28
	Screening		**Treatment**											**Follow-up**																	
IFN (ARM2 only)			x		x				x			x																			
Procedures (both ARMS)																															
RT-PCR SARS-CoV 2 positivity assay	x		x														x														x
Demografic Data	x																														
Medical History	x																														
Informed Consent	x																														
Inclusion/Exclusion Criteria	x																														
Signs and symptoms	x		x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Previous/Concomitant Therapy recording	x		x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
NEWS2 score assessment	x		x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Safety/Efficacy Evaluation (both ARMS)																															
Adverse Events recording			x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Routine laboratory test parameters	x		x		x							x					x							x						x
SARS-CoV 2 Antibodies			x														x														x
Exploratory labtests (ISG/CIM/SIM)			x		x							x					x														

As clinically indicated, laboratory and instrumental tests can be performed on other time points that will be recorded.

APPENDIX 3: GANTT chart



APPENDIX 4: eCRF design

The CRO will implement and validate eCRF on a Gamp 5 21 CFR part 11 compliant platform and share it among participants, endowed with different appropriate access privileges: CRO will have complete data control, while data input permission only will be assigned to local data managers.

eCRF will be designed on the basis of the following structure:

CRF sections by time of visit

Screening **PreTx**	***Treatment*** **T1**
Id Number	Date
SARS-CoV-2 positivity assay	Id Number
Demografic Data	IFN (ARM2 only)
Medical History	SARS-CoV-2 positivity assay
Informed Consent	Signs and symptoms
Inclusion/Exclusion Criteria	Previous/Concomitant Therapy
Signs and symptoms	NEWS2 score assessment
Previous/Concomitant Therapy	Adverse Events
NEWS2 score assessment	Routine laboratory test parameters
Routine laboratory test parameters	SARS-CoV-2 Antibodies
Randomization	Exploratory labtests (ISG/CIM/SIM)
	Study Discontinuation or Withdrawal

Treatment **T2/T4/T5/T6/T8/T9**	***Treatment*** **T3/T10**	***Treatment*** **T7**
Date Id Number Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Study Discontinuation or Withdrawal	Date Id Number IFN (ARM2 only) Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters Exploratory labtests (ISG/CIM/SIM) Study Discontinuation or Withdrawal	Date Id Number IFN (ARM2 only) Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Study Discontinuation or Withdrawal

Follow up **T11/T12/T13/T15/T16/T17/T18/T19/T20/T22/T23/T24/T25/T26/T27**	***Follow up*** **T14**
Date Id Number Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Study Discontinuation or Withdrawal	Date Id Number SARS-CoV-2 positivity assay Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters SARS-CoV-2 Antibodies Exploratory labtests (ISG/CIM/SIM) Study Discontinuation or Withdrawal

Follow up **T21**	***Follow up*** **T28**
Date Id Number Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters Study Discontinuation or Withdrawal	Date Id Number SARS-CoV-2 positivity assay Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters SARS-CoV-2 Antibodies Study Discontinuation or Withdrawal

CRF - section data		
SARS-CoV-2 positivity assay	date	RT-PCR: Gene name, CT number, Laboratory (INMI or other, to be specified); rapid antigen test: positive/negative (executed by: operator ID)
Demografic Data	date	Sex at birth, Date of birth, Race/Ethnicity; i) hospitalized, ii) RSA, iii) home patient
Medical History	date	see TABLE 1
Informed Consent	signature date	Y/N
Inclusion/Exclusion Criteria	date	see TABLE 2
Previous/Concomitant Therapy	date	Any drug/medicament name, reason for use, dose, frequency, duration of consumption
NEWS2 score assessment	date	SCORE, Systolic and diastolic arterial pressure, heart rate (HR), respiratory rate (RR), systemic body temperature, ACVPU, SpO_2
Randomization	date	ARM1/ARM2, random number
Signs and symptoms		see TABLE 3
IFN (ARM2 only)		Y/N, expiration date, batch number
Adverse Events		TABLE 4 AE and any other AE will be recorded using NCI Common Terminology Criteria for adverse Events (CTCAE) version 5. SUSAR will be recorded in specific CRF.
Routine laboratory test parameters		see TABLE 5
SARS-CoV-2 Antibodies		anti-S IgG, anti-N IgG, anti-S IgM, anti S IgA, neutralizing anti-S Ab titer
Exploratory labtests (ISG/CIM/SIM)		ISG: IFI44L, IFI27, RSAD2, SIGLEC1, IFIT1, IS15. CIM: see TABLE 6. SIM: see TABLE 7.
Study Discontinuation or Withdrawal		Y/N, reason: withdrawal of subjects for non-compliance/adherence, for AE, consent withdrawal, other (to be specified)

TABLE 1 - Medical History		
		Tobacco, Alcohol, other recreational drug use (dose, frequency, duration of comsumption) Flu vaccine (in the last year): Y/N, date
By System	Respiratory	Chronic pulmonary disease, Asthma, Tuberculosis (active/previous), other (to be specified)
	Cardiovascular/Circulatory	Chronic cardiac disease (not hypertension), Hypertension, other (to be specified)
	Musculoskeletal	to be specified
	Endocrine	to be specified
	Hematopoietic	to be specified
	Nervous	Chronic neurological disorder, other (to be specified)
	Dermatological Integumentary System/Exocrine System	to be specified
	Genitourinary	Chronic kidney disease, other (to be specified)
	Lymphatic System/Immune System	to be specified
	Digestive	Chronic liver disease, other (to be specified)
	Metabolic disease	Diabetes, other (to be specified)
	Ear, Nose, Throath	to be specified
	Psychiatric disease	to be specified
	Allergy	to be specified
	Malignant neoplasm	to be specified
	Infectious disease	HIV, HCV, other (to be specified)
	Other (to be specified)	

Note: For any disease, onset date and duration as well as indication about disease current status will be recorded.

TABLE 2 - Inclusion/Exclusion Criteria	
Inclusion criteria (all required):	
≥ 65 years of age at time of enrolment	Y/N
Subject has laboratory-confirmed SARS-CoV-2 infection as determined by PCR, in any specimen < 72 hours prior to randomization	Y/N
Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures	Y/N
Subject understands and agrees to comply with planned study procedures	Y/N
Subject agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol	Y/N
Being symptomatic for less than 7 days before starting therapy	Y/N
NEWS2 score ≤2	Y/N
Exclusion criteria:	
Hospitalized patients with illness of any duration, and at least one of the following: Clinical assessment (evidence of rales/crackles on exam) AND SpO2 ≤ 94% on room air at rest or after walking test, OR Acute respiratory failure requiring mechanical ventilation and/or supplemental oxygen.	Y/N
Patients currently using interferon-beta (e.g., multiple sclerosis patients)	Y/N
Patients with chronic kidney diseases	Y/N
Known allergy or hypersensitivity to interferon (including asthma)	Y/N
Any autoimmune disease (based on the anamnesis)	Y/N
Patients with signs of dementia or neurocognitive disorders	Y/N
Patients with current severe depression and/or suicidal ideations	Y/N
Being concurrently involved in another trial for COVID-19	Y/N
HIV infection (based on the anamnesis)	Y/N
Use of any antiretroviral medication	Y/N
Impaired renal function (eGFR calculated by CKD-EPI Creatinine equation < 30 ml/min)	Y/N
Presence of other severe diseases impairing life expectancy (e.g. patients are not expected to survive 28 days given their pre-existing medical condition)	Y/N
Any physical or psychological impediment in a patient that could let the investigator to suspect his/her poor compliance	Y/N
Lack or withdrawal of informed consent	Y/N

TABLE 3 - Signs and symptoms
History of fever
Lower chest indrawing
Cough (with sputum production/with emoptysis)
Headache
Altered consciousness/confusion
Seizures
Sore throat
Abdominal pain
Runny nose
Vomiting/nausea
Wheezing
Diarrhoea
Chest pain
Conjunctivitis
Muscle aches
Skin rash
Joint pain (arthralgia)
Skin ulcers
Fatigue/malaise
Lymphadenopathy
Loss of taste
Inability to walk
Loss of smell
Bleeding (ischaemic stroke, entracerebral haemorrhage)
Shortness of breath

Note: If present, onset date and duration as well as indication about sign or symptom current status will be recorded.

TABLE 4 - REBIF common AE	
Very common (≥1/10)	Common (≥1/100 to <1/10)
Neutropenia, lymphopenia, leukopenia, thrombocytopenia, anaemia Asymptomatic transaminase increase Headache Injection site inflammation, injection site reaction, influenza-like symptoms	Diarrhoea, vomiting, nausea Severe elevations in transaminases Pruritus, rash, erythematous rash, maculo-papular rash, alopecia Myalgia, arthralgia Depression, insomnia Injection site pain, fatigue, rigors, fever

TABLE 5 - Routine laboratory test parameters	
Haemoglobin	Ferritin
Haematocrit	Creatine kinase
Full Blood count	ALT/SGPT
Creatinine	Troponin
Sodium	AST/SGOT
Potassium	ESR
Procalcitonin	Total bilirubin
Lactate	Urea (BUN)
Glucose	Albumin
Lipase	BNP (brain natriuretic peptide)
LDH	presepsin
PT (seconds)	INR
fibrinogen	APTT/APTR
D-dimer	CRP
IL-6	

TABLE 6 - Cellular Immune Monitoring		
leu_linfociti	CD4_CM	Vd2_TD
lym_CD3	CD4_EM	Vd2_EM
CD3_CD4SP	CD4_N	Vd2_N
CD3_CD8SP	CD4_TD	Vd2_CM
CD8_CM	CD4_CD28pos27neg	lym_CD19
CD8_EM	CD4_CD28pos27pos	lym_NK
CD8_N	CD4_CD28neg27neg	lym_NKT
CD8_TD	CD4_CD28neg27pos	leu_CD14pos
CD8_CD28pos27neg	CD4_CD57pos27neg	leu_Treg
CD8_CD28pos27pos	CD4_CD57posPD1neg	lym_Treg
CD8_CD28neg27neg	CD4_CD57posPD1pos	CD3_Treg
CD8_CD28neg27pos	CD4_CD57negPD1pos	CD4_Treg
CD8_CD57pos CD27neg	gpSpike_CD8	Treg_CD45RAnegCD39neg
CD8_CD57posPD1neg	Vd2	Treg_CD45RAnegCD39pos
CD8_CD57posPD1pos		Treg_CD45RAposCD39neg
CD8_CD57negPD1pos		Treg_CD45RAposCD39pos

TABLE 7 - Systemic Inflammation Markers	
IL-2	IP-10
IL-7	MCP1
IL-10	MIP1a
G-CSF	VCAM-1
ICAM-1	VAP-1
Fractalkine	TNF-α

APPENDIX 5: Patient Diary and clinical record template

DIARIO CLINICO

NOME: _____ COGNOME: _____

DATA DI NASCITA: _____

CODICE IDENTIFICATIVO: _____

CONSEGNATO DA: _____

DATA: _____ FIRMA:

_____

PER COMUNICAZIONI URGENTI

CHIAMARE:_____

STUDIO CLINICO: "**Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 anziani**"

Numero EUDRACT: 2020-003872-42

Promotore: Istituto di Farmacologia Traslazionale – Consiglio Nazionale delle Ricerche

Sperimentatore Principale: Dott Giuseppe Sconocchia

Telefono: xxxxxxxxxxxxx

Sezione a cura del paziente o del *care giver*

REGISTRAZIONE PUNTEGGIO NEWS2 (da giorno 1 a giorno 28)

DATA:_____ ORARIO:_____

MISURE EFFETTUATE DA:_____

TEMPERATURA CORPOREA: _____
SATURAZIONE 1:_____
SATURAZIONE 2:_____
FREQUENZA CARDIACA:_____
PRESSIONE:_____
FREQUENZA RESPIRATORIA:_____
NECESSITA OSSIGENO?_____
È VIGILE?_____

Per valutare lo stato di salute complessivo per ogni misurazione mettere una X nella casella corretta, riportare nell'ultima colonna il punteggio corrispondente. Poi fare la somma dei numeri scritti sulla colonna di destra.

SE IL PUNTEGGIO È 3 O MAGGIORE CONTATTARE IL MEDICO.

Parametri fisiologici	Punteggio							
	3	2	1	0	1	2	3	
Frequenza respiratoria	≤ 8		9-11	12-20		21-24	≥ 25	
Saturazione 1 (%)	≤ 91	92-93	94-95	≥ 96				
Saturazione 2 (%)	≤ 83	84-85	86-87	88-92 ≥ 93 senza ossigeno	93-94 con ossigeno	95-96 con ossigeno	≥ 97 con ossigeno	
Necessità di ossigeno?		SI		NO				
Pressione	≤ 90	91-100	101-110	111-219			≥ 220	
Frequenza cardiaca	≤ 40		41-50	51-90	91-110	111-130	≥ 131	
È vigile?				SI			NO	
Temperatura corporea	≤ 35,0		35,1-36,0	36,1-38,0	38,1-39,0	≥ 39,1		
							Totale	

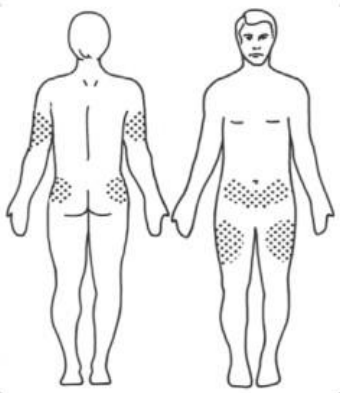
Sezione a cura del medico

REGISTRAZIONE SOMMINISTRAZIONI REBIF 11 mcg
(giorni: 1 – 3 – 7 – 10)

(sbarrare per i pazienti del gruppo di controllo)

Numero Somministrazione (I II III IV): ___

Sito iniezione



Data: _____ Ora: _____

Modificati settaggi Rebismart? SI NO

Se SI, specificare:_____

_____

_____

Nome e Cognome del medico: _____

Firma: _____

Registro terapie concomitanti, segni e sintomi (da giorno 1 a giorno 28)

Ha assunto farmaci nelle ultime 24 ore? SI NO

Se sì, indicare quali:

	Nome del farmaco	Posologia
1		
2		
3		
4		
5		
6		
...		

Segno o sintomo	**SI**	**NO**	**NOTE**
Tosse (catarrosa/con presenza di sangue)			
Rientramento toracico			
Cefalea			
Stato confusionale			
Attacchi epilettici			
Mal di gola			
Dolore addominale			
Rinorrea (abbondante muco nasale)			
Vomito/nausea			
Respiro sibilante			
Diarrea			
Dolore toracico			
Congiuntivite			
Dolori muscolari			
Eruzione cutanea			
Dolori articolari			
Ulcere della pelle			
Stato di affaticamento/malessere			
Linfo-adenopatia (ingrossamento dei linfonodi)			
Perdita del gusto			
Incapacità o difficoltà a camminare			
Perdita dell'odorato			
Sanguinamento			
Affanno			
Altro			

Eventi Avversi (da giorno 1 a giorno 28)

Il paziente ha mostrato eventi avversi? SI NO

Se sì, compilare la tabella:

Descrizione	Data Inizio	Data fine	Frequenza£	Grado$	Farmaco relazione %	Terapia adottata&	Esito ç	Gravità§

£ 1=Una volta; 2=Occasionale; 3=Frequente; 4=Permanente

$ Classificazione ECOG

% 0=Assente; 1=Improbabile; 2= Possibile; 3= Probabile; 4= Certa; 9= Non classificata

& 0=Nessuna; 1=Interruzione temporanea; 2= Riduzione dose prodotto; 3= Interruzione permanente; 4= Trattamento medico; 5=Ospedalizzazione

ç 1=Risoluzione; 2=Risoluzione con sequele; 3= Non risoluzione; 4=Morte; 5= Ignoto

§ 0= Non grave; 1=grave. **Registrare nella scheda EA gravi e contattare il responsabile DSMB**

Tampone nasofaringeo (giorni 1 – 14 – 28)

Il tampone è stato effettuato? SI NO

È stato prelevato correttamente? SI NO

NOTE:

Prelievi di sangue

Prelievo per analisi routinarie (emocromo, biochimica) – 4 ml + 4 ml EDTA (giorni: 1 – 3 – 10 – 14 – 21 – 28)

Il prelievo è stato effettuato? SI NO

È stato prelevato il volume previsto? SI NO

NOTE:

Prelievo per analisi anticorpi anti-SARS CoV 2 – 4 ml (giorni: 1 – 14 – 28)

Il prelievo è stato effettuato? SI NO

È stato prelevato il volume previsto? SI NO

NOTE:

Prelievo per analisi esplorative – 20 ml (giorni: 1 – 3 – 10 – 14)

Il prelievo è stato effettuato? SI NO

È stato prelevato il volume previsto? SI NO

NOTE:

APPENDIX 6: Standard operating procedure for drug management

MODALITÀ DI APPROVVIGIONAMENTO E SOMMINISTRAZIONE DEL FARMACO REBIF TRAMITE INIETTORE REBISMART

Documento Redatto da: Luciano Castiello Firma e data _____

Documento Approvato da:

Ruolo	Nominativo	Firma e Data
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Co-Sperimentatore INMI "Lazzaro Spallazani"	Dr. Emanuele Nicastri	
Co-Sperimentatore USCAR	Dr. Pier Luigi Bartoletti	
Coordinatore Scientifico	Dr. Filippo Belardelli	
Project Manager - FullCRO	Dr.ssa Moira Cordisco	
ISS	Dr.ssa Eleonora Aricò	
Farmacia INMI "Lazzaro Spallanani	Dr.ssa Silvia Murachelli	

Lista di distribuzione:

Ruolo	Nominativo	N° copie	Firma e Data
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ISS	Dr.ssa Eleonora Aricò	1	
Farmacia INMI "Lazzaro Spallanani	Dr.ssa Silvia Murachelli	1	

MODALITÀ DI APPROVVIGIONAMENTO E SOMMINISTRAZIONE DEL FARMACO REBIF TRAMITE INIETTORE REBISMART

1. Scopo

La presente procedura descrive le operazioni che gli sperimentatori devono eseguire per l'approvvigionamento del farmaco e per il corretto utilizzo dell'autoiniettore Rebismart nella sperimentazione clinica ANTIICIPATE sia nel setting ospedaliero che in quello non-ospedaliero.

2. Abbreviazioni e definizioni

Farmaco: Rebif (Interferon beta-1a)

Farmacia: Farmacia dell'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani"

CRO: FullCRO

Promotore: Istituto di Farmacologia Traslazionale

3. Approvvigionamento del farmaco e dell'iniettore

Per i soli pazienti inclusi nel braccio di trattamento, il giorno previsto per l'inizio del trattamento l'sperimentatore, o un suo delegato, deve presentarsi presso la Farmacia e richiedere una dose di farmaco e un dispositivo per l'autoiniezione del farmaco utilizzando il modulo Allegato 1. Il modulo originale deve essere conservato dalla Farmacia mentre una copia deve essere inviata digitalmente (Fax o email) alla CRO incaricata di seguire lo studio.

Per i soli pazienti non ospedalizzati, l'sperimentatore dovrà accertarsi di essere inoltre in possesso di:

- Sei aghi Serofine 29G, 30G o 31G;
- Un contenitore per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti);
- Un diario clinico del paziente (fornito dal Promotore);
- Uno sfingomanometro digitale e un saturimetro per la misurazione dei parametri vitali (fornito dal Promotore);
- Un cacciavite a stella e quattro pile al litio 1,5 V AAA (se necessari forniti dal Promotore);
- Un manuale d'uso del Rebismart (da usare in caso di necessità);
- Salviettine o tamponcini imbevuti di alcol o batuffoli di ovatta e alcol per frizione;
- Cerotti (classici e a rocchetto)

Nota bene: la cartuccia di farmaco può essere trasportata a temperatura ambiente per una durata temporale inferiore alle due ore. Qualora, fosse necessario un trasporto per un tempo superiore alle due ore riporre la cartuccia all'interno di un contenitore termoisolante dotato di piastra eutettica (siberino) fornito dal Promotore.

4. Attivazione e settaggio dell'iniettore Rebismart e caricamento cartuccia Rebif

4.1 Inserimento pile

In caso il dispositivo abbia le batterie scariche, prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Svitare la vite del coperchio dell'alloggiamento pile con un cacciavite e far scivolare verticalmente il coperchio. Inserire 4 pile al litio nuove. Verificare che siano orientate come mostrato sul dispositivo e stringere la vite del coperchio dell'alloggiamento delle pile per chiuderlo. Una sequenza illustrata della attività da eseguire è riportata nell'allegato 2.

4.2 Settaggio Rebismart

4.2.1 Settaggio cartuccia e dose

Prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Accendere RebiSmart e premere il pulsante 'Menu'. Scorrere su 'Impostaz. iniezione' e premere il pulsante 'Apri' per selezionare. Sarà visualizzata una schermata di avvertenza e selezionare 'Si'.
Selezionare 'Cartuccia' premendo su 'Cambia' e scorrere al dosaggio della cartuccia 22mcg. Premere 'OK' per selezionare e confermare la selezione.

Dal menù 'Impostaz. Iniezione' scorrere e selezionare 'Riduzione dose' e premere il pulsante 'Cambia' per selezionare. Inserire il codice PIN del dispositivo. Il dispositivo visualizzerà il menu 'Riduzione dose'. Selezionare '50% della dose'. Premere 'Ok' e confermare la selezione. Premere due volte il pulsante 'Esci' per tornare alla schermata informativa.

4.2.2 Settaggio ago

Prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Accendere RebiSmart. E premere il pulsante 'Menu'. Scorrere su 'Impostaz. pers.' e premere il pulsante 'Apri' per selezionare. Si apre la schermata di avvertenza e selezionare 'Si'. Selezionare 'Velocità ago' premendo su 'Cambia'. Selezionare velocità 'Media'. Premere 'OK' per selezionare.

Scorrere su 'Velocità iniez.' e premere il pulsante 'Cambia'. Selezionare velocità 'Media'. Premere 'OK' per selezionare. Scorrere in basso su 'Profondità iniez.' e premere il pulsante 'Cambia'. Selezionare '4 mm' e premere 'OK' per selezionare.

Scorrere in basso su 'Durata iniezione' e premere il pulsante 'Cambia'. Selezionare '3 secondi' premere 'OK' per selezionare. Premere due volte il pulsante 'Esci/Esci' per tornare alla schermata informativa.

Premere il pulsante 'Menu', scorrere su 'Impostaz. iniezione' e premere il pulsante 'Apri' per selezionare. Scorrere su 'Tipo di ago' e premere il pulsante 'Cambia' per selezionare. Selezionare il tipo di ago utilizzato. Premere 'OK' per selezionare. Premere due volte il pulsante 'Esci' per tornare alla schermata informativa.

4.3 Caricamento cartuccia

Prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Estrarre la cartuccia dal confezionamento secondario. Accendere RebiSmart tenendo premuto il pulsante 'Acceso' fino a che compare la schermata di "Benvenuto (Ciao)". Premere su 'Inizio' e aprire lo sportello dell'alloggiamento della cartuccia facendo scorrere verso l'alto il pulsante posto sul lato sinistro del dispositivo. Inserire la cartuccia di Rebif nell'alloggiamento cartuccia con la parte metallica rivolta verso il basso. Chiudere lo sportello dell'alloggiamento cartuccia fino ad udire un "clic". Una sequenza illustrata della attività da eseguire è riportata nell'allegato 3. Staccare dal confezionamento secondario la parte staccabile dell'etichetta e applicarla sul Rebismart..

5. Somministrazione farmaco

5.1 Preparazione somministrazione

Prima di iniziare, estrarre RebiSmart. dal frigorifero e dalla scatola di conservazione almeno 30 minuti prima dell'utilizzo previsto. Disporre, su una superficie stabile, come per esempio un tavolo, quanto segue:

- RebiSmart. contenente una cartuccia di Rebif in posizione verticale;
- Ago Serofine™ (29G, 30G o 31G, in base alla prescrizione);
- Salviettine o tamponcini imbevuti di alcol o batuffoli di ovatta e alcol per frizione;
- Piccolo cerotto;
- Contenitore per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti);

Sanitizzare i guanti accuratamente. Indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Accendere RebiSmart tenendo premuto il pulsante 'Acceso' fino a che compare la schermata di "Benvenuto (Ciao)". Premere il pulsante 'Inizio'.

Qualora RebiSmart visualizzi il messaggio "Meno di 48 ore dall'ultima iniezione. Procedere con l'iniezione", selezionare 'Si'. Prelevare un ago e rimuovere il sigillo di sterilità.

Inserire il cappuccio che contiene l'ago direttamente nell'alloggiamento dell'ago fino a che si blocca con un 'clic'. Togliere il cappuccio dell'ago spingendolo di lato fino a che non venga rimosso e conservare il cappuccio. Una sequenza illustrata della attività da eseguire è riportata nell'allegato 4.

5.2 Somministrazione farmaco

Posizionare RebiSmart sulla cute in posizione verticale nel sito di iniezione più idoneo (in base alle caratteristiche del paziente scegliere tra la parte esterna superiore delle braccia, la zona periombelicale dell'addome, la parte anteriore delle cosce). Assicurarsi che il sensore cutaneo sia completamente a contatto con la cute. Quando RebiSmart è posizionato correttamente sulla cute, la luce del pulsante di iniezione diventa verde e RebiSmart emette un bip.

Premere il pulsante per iniziare l'iniezione. La spia del pulsante di iniezione verde durante l'iniezione lampeggia. Tenere RebiSmart a contatto con la pelle per tutta la durata dell'iniezione.

Al termine dell'iniezione, la spia del pulsante verde si spegne e RebiSmart emette due bip.

Sollevare delicatamente RebiSmart dalla cute. Premere su 'OK' per confermare che l'iniezione è stata praticata correttamente.
Registrare sul diario clinico del paziente le informazioni sulla somministrazione.

5.3 Eliminazione ago e spegnimento Rebismart

Inserire il cappuccio vuoto direttamente nell'alloggiamento dell'ago fino a che si blocca con un 'clic'. RebiSmar. emette un bip. Premere e mantenere premuto il pulsante di rilascio dell'ago fino a che RebiSmart emette due bip. Togliere il cappuccio contenente l'ago spingendolo di lato fino a che si stacca per essere facilmente rimosso.
Controllare l'interno del cappuccio dell'ago per vedere l'ago rimosso. Gettare gli aghi usati nel contenitore per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti).

Premere e tenere premuto il pulsante 'Spento' fino a che RebiSmart si spegne e la schermata informativa si chiude.

6. Conservazione del farmaco e dell'iniettore

Al termine dell'iniezione riposizionare RebiSmart in posizione verticale nella sua custodia all'interno del frigorifero.

7. Recupero dell'iniettore e riconsegna

Al termine della quarta iniezione, eliminare la cartuccia in uso. Prima di spegnere Rebismart, premere il pulsante 'Menu'. Scorrere in basso su 'Rimuovere cartuc.' e premere il pulsante 'Apri'. Premere su 'Si' per confermare la selezione. Attendere che Rebismart visualizza il messaggio 'Aprire sportello alloggiamento cartuccia' ed emette due bip. Far scorrere verso l'alto il pulsante dello sportello alloggiamento cartuccia e rimuovere la cartuccia. Gettare la cartuccia nel contenitore

per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti). Selezionare 'No' sul Rebismart e spegnerlo. Rimuovere il confezionamento secondario dalla custodia del Rebismart.

Riconsegnare il Rebismart alla Farmacia, registrando la consegna sul modulo Allegato 1 utilizzato per il ritiro.

8. Allegati

Allegato 1: Modulo richiesta ritiro/riconsegna Rebif e Rebismart

Allegato 2: Schema dei passaggi da effettuare per la sostituzione pile

Allegato 3: Schema dei passaggi da effettuare per l'inserimento di una cartuccia di Rebif

Allegato 4: Schema dei passaggi da effettuare per somministrare il farmaco

MODULO RICHIESTA RITIRO/RICONSEGNA REBIF E REBISMART

Inviare a:
Moira Cordisco - FullCRO
Fax:06xxxxxxx email:

e p.c.:
Giuseppe Sconocchia - IFT

DATI RICHIEDENTE
NOME E COGNOME:_____ UNITÀ: INMI USCAR

RICHIEDE n° 1 cartuccia/e Rebif 66 mcg e n° 1 dispositivo Rebismart per effettuare il trattamento al paziente

Codice _____ **Data di Nascita:** _____

DATA:_____ FIRMA: _____

Spazio da compilare a cura della Farmacia
Numero lotto Rebif:_____ Data di scadenza:_____
Numero seriale cartuccia:_____
Identificativo Rebismart:_____

Nome e Cognome:_____ Data:_____ Ora:_____
Firma:_____

Spazio da compilare alla riconsegna del dispositivo Rebismart a cura della Farmacia

Identificativo dispositivo Rebismart riconsegnato: _____
Nome e Cognome:_____ Data:_____ Ora:_____
Firma:_____

SCHEMA DEI PASSAGGI DA EFFETTUARE PER LA SOSTITUZIONE PILE

Svitare la vite del coperchio dell'alloggiamento pile con un cacciavite.

Afferrare il coperchio sui due lati e farlo scivolare via.

Inserire 4 pile al litio nuove. Verificare che siano orientate come mostrato sul dispositivo.

Far scorrere il coperchio dell'alloggiamento delle pile nella posizione di chiusura, verificando che entri nelle fessure.

Stringere la vite del coperchio dell'alloggiamento delle pile per chiuderlo.

SCHEMA DEI PASSAGGI DA EFFETTUARE PER L'INSERIMENTO DI UNA CARTUCCIA DI REBIF

Accendere RebiSmart® tenendo premuto il pulsante **'Acceso'** fino a che compare la schermata di **"Benvenuto (Ciao)"**, in genere dopo 3-5 secondi.

Premere su **'Inizio'** e aprire lo sportello dell'alloggiamento della cartuccia facendo scorrere il pulsante verso l'alto.

Inserire una nuova cartuccia di Rebif® nell'alloggiamento cartuccia, verificando che la parte metallica sia rivolta verso il basso.

Chiudere lo sportello dell'alloggiamento cartuccia fino ad udire un "clic".

SCHEMA DEI PASSAGGI DA EFFETTUARE PER SOMMINISTRARE IL FARMACO

Accendere il dispositivo premendo e tenendo premuto il pulsante **'Acceso'** fino a che compare la schermata di **"Benvenuto (Ciao)"**, in genere dopo 3-5 secondi.

Premere il pulsante **'Inizio'**.

Verificare che la misura in gauge (G) indicata sulla scatola degli aghi Serofine™ corrisponda a quella indicata nella schermata di RebiSmart®.

Inserire il cappuccio che contiene l'ago direttamente nell'alloggiamento dell'ago fino a che si blocca con un 'clic'.

Togliere il cappuccio dell'ago spingendolo di lato fino a che venga rimosso.

SCHEMA DEI PASSAGGI DA EFFETTUARE PER SOMMINISTRARE IL FARMACO

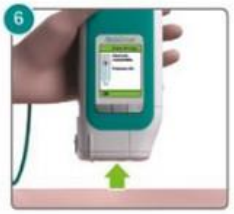
Posizionare RebiSmart® sulla cute in posizione verticale nel sito di iniezione preparato come indicato dal medico o dall'infermiere.

Quando RebiSmart® è posizionato correttamente sulla cute, la luce del pulsante di iniezione diventa verde e RebiSmart® emette un bip. 1

Premere il pulsante per iniziare l'iniezione.

La spia del pulsante di iniezione verde durante l'iniezione lampeggia. Tenere RebiSmart® a contatto con la pelle per tutta la durata dell'iniezione. Non è necessario mantenere premuto il pulsante di iniezione.

Al termine dell'iniezione, la spia del pulsante verde si spegne e RebiSmart® emette due bip. 2



Sollevare delicatamente RebiSmart® dalla cute.

Premere su 'OK' per confermare che l'iniezione è stata praticata correttamente.

SCHEMA DEI PASSAGGI DA EFFETTUARE PER SOMMINISTRARE IL FARMACO

CLINICAL STUDY PROTOCOL

Study Title: Antiviral and Immunomodulatory Interferon-Beta in high-risk COVID-19 patients

Short title: (ANTiviral and **I**mmunomodulatory **I**nterferon-Beta in high-risk **C**ov**I**d-19 **PAT**i**E**nts**)**

ANTIICIPATE

EudraCT N°: 2020-003872-42

Sponsor: Institute of Translational Pharmacology (IFT), National Research Council (CNR)

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Investigational Product Interferon β1a (Rebif™)

Clinical Study Phase: II

Version: 3.0

Issue Date: 18/03/2021

Protocol Signature form

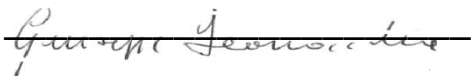
Protocol Title:

Antiviral and Immunomodulatory Interferon-Beta in high-risk COVID-19 patients (ANTIICIPATE)

Version: 3.0

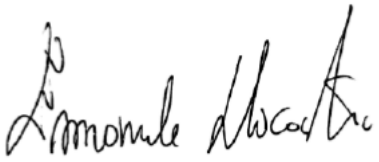
Version Date: 18/03/2021

I have read the protocol described below and agree to conduct this study in accordance with procedures described therein. I also agree to conduct the study in compliance with all applicable regulations.



Sponsor coordinator's printed name

Giuseppe Sconocchia, MD



Principal Investigator's printed name

Emanuele Nicastri, MD

Date: 18/03/2021

Summary

List of abbreviations

AE	Adverse event
AIFA	Italian medicines agency
ALT	Alanine AminoTransferase
ANCOVA	Analysis of CoVariance
ANOVA	Analysis of Variance
AVPU	Alert, Verbal, Pain, Unresponsive Score
AST	Aspartate AminoTransferase
CT	Coordination Team
CTCAE	Common Terminology Criteria for Adverse Events
CIM	Cellular Immune Monitoring
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CNR	Consiglio Nazionale delle Ricerche
COVID-19	Corona Virus 19 Disease
CRO	Contract Research Organization
CRP	C-Reactive Protein
EC	Ethical Committee
eCRF	electronic Case Report Form
eGFR	estimated Glomerular Filtration Rate
ELISA	Enzyme Linked ImmunoSorbent Assay
FFP	Filtering Face Mask
FKN	Fractalkine
GCP	Good Clinical Practice
Hb	Haemoglobin
ICAM-1	Intercellular Adhesion Molecule 1
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IEC	Independent Ethic Committee
IFT	CNR Institute of Translational Pharmacology
IFN	Interferon

IL-6	Interleukin-6
ITT	Intention To Treat
LDH	Lactate DeHydrogenase
LSRCHs	long-stay residential care homes
INMI	Istituto Nazionale Malattie Infettive
ISG	Interferon Stimulated Genes
ISS	Istituto Superiore di Sanità
IU	International Units
MAR	Missing At Random
MERS	Middle East respiratory syndrome
MFC	Multiparametric Flow Cytometry
MS	Multiple Sclerosis
NCI	National Cancer Institute
NEWS2	National Early Warning Score 2 (2017)
NK	Natural Killer
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PP	Per Protocol
RCP	Riassunto delle Caratteristiche del Prodotto
RT-PCR	Real Time - Polymerase Chain Reaction
SAE	Serious Adverse Event
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	SARS Corona Virus
SARS-Cov 2	New Corona Virus
SC	Steering Committee
SIM	Systemic Inflammatory Markers
SOCS	Suppressor of cytokine signaling
SpO_2	Oxygen Saturation
SUSAR	Suspected Unexpected Serious Adverse Reactions
TNF	Tumor Necrosis Factor

USCAR	Special Unit for regional continued care
VCAM-1	Vascular Cell Adhesion Molecule 1
VPA-1	Vascular Adhesion Protein 1
UBP43	Ubiquitin Protease 43
WBC	White Blood Cells
WHO	World Health Organization

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Administrative clinical research support on inter-institutional agreements, material transfer agreements, institutional tenders.

This is an investigator-initiated study. The Steering Committee will take responsibility for study design and data analysis and will operate actions necessary to guarantee that the trial is conducted in accordance with procedures described in this document and good clinical practice. The study is partially funded by Merck. Merck has no role in study design, data collection, management, analysis, data interpretation, manuscript writing, or in the decision to submit manuscripts for publication.

The Steering committee will include at least one representative from all units participating to the study and will be chaired by the Scientific Coordinator of the study (Filippo Belardelli) with the cooperation of a coordination team (Giuseppe Sconocchia, Emanuele Nicastri, Ombretta Papa, Nicola Vanacore, Eleonora Aricò, Luciano Castiello). The Steering committee will oversee all the aspects of the project's life: decision about safety, decision for stopping rule, diagnostics issues, capacity development, financial, schedule, partnership, dissemination and exploitation. The Steering committee will hold at least one meeting a week on teleconference. In addition, extraordinary sessions will be held in case of critical issues.

1. Synopsis

1.1 BACKGROUND

The rapid and devastating outbreak of Coronavirus disease 2019 (COVID-19) pandemic highlighted the urgent need of developing therapeutic options to control or prevent virus spreading. In this regard, priority should be given to the repurposing of existing antiviral agents, thus shortening the timelines needed for clinical experimentation while exploiting the clinical experience with other viral infections (1). Among the many drugs under evaluation all over the world, Interferon (IFN)-α and β stirred renewed interest against COVID-19 and are presently being evaluated in clinical trials at different dosages and by different delivery systems, either as monotherapy or in combination with other compounds. Notably, IFN-β proved effective in alleviating COVID-19 symptoms when used in combination with lopinavir and ritonavir (2) and in reducing mortality when combined with hydroxychloroquine and other antivirals (3).

IFN-α and β, thereafter referred to as type I IFN (IFN-I), are cytokines with a long record of clinical use in patients with infectious disease (4), multiple sclerosis, and cancer (5). They are pleiotropic factors endowed with multiple activities, including both a broad spectrum antiviral activity and a remarkable immunoregulatory function (6). IFN-I are expressed at very low levels under basal physiological conditions, while they are generally abundantly produced in response to virus infections, when they play a crucial role in limiting viral replication and spread (7). In fact, many viruses, including Coronaviruses, evolved evasion strategies to counteract IFN-I system activation (8,9).

An ensemble of studies, some of them carried out in the proponents' laboratories, have revealed that in addition to the antiviral activity, optimally achieved in the first phase of infection, IFN-I exhibit important immunoregulatory effects, including the increase of neutralizing antibodies and the induction of both innate and adaptive cellular immunity (10–15).

While the majority of SARS-CoV 2 infected individuals are capable of clearing the virus solely with their own immune response, approximately 20% develops severe COVID-19. Notably, at higher risk of severe COVID-19 are males, people aged >65 years and/or showing some comorbidities (like hypertension and diabetes). An age-related impairment of endogenous IFN-I induction in response to viral infection has been described (16). Data on animal models on SARS-CoV (17,18) and data emerging from COVID-19 pandemic (19–22) point out to endogenous IFN-I system as a key player

to control early phases of viral replication and prevent disease progression. Moreover, delayed IFN-I signaling activation can contribute to the exacerbation of SARS-CoV hyperinflammation and subsequent viral pathogenesis (20,23,24).

In the light of these considerations and evidences, we hypothesize that elderly patients will greatly benefit from a short term IFN-β1a administration at the earliest time of SARS-CoV 2 diagnosis, thus compensating the insufficient or impaired endogenous IFN-I production.

In these patients, the antiviral and immunomodulatory effects of this cytokine could be efficiently exploited against COVID-19 through a short-term, discontinuous treatment with IFN-β1a in the early phases of infection, thus minimizing the relevant side effects (refractoriness and toxicity) associated to IFN continuous treatment schedules.

1.2 Objectives

This trial aims at exploring the efficacy of IFN-β1a in reducing the risk of SARS-CoV 2 recently infected elderly patients to progress towards severe COVID-19. In particular, this study will evaluate the consequences of a low and discontinuous use of IFN-β1a in the early phase of infection, and to exploit its immune activating properties in addition to its antiviral effects. Such regimen is expected to prevent any toxicity and refractoriness phenomena often occurring during IFN-I chronic administration.

Primary Objective of the study is to evaluate the role of IFN-β1a in reducing the disease progression in treated patients versus control group.

Secondary Objectives of the study are: 1) to assess the reduction in ICU admission in patients treated with IFN versus control group; 2) to assess the reduction in number of deaths in IFN compared to control group; 3) to evaluate the increase in proportion of participants returning to negative SARS-CoV 2 RT-PCR in IFN-treated *versus* control group at Day 14 and Day 28; 4) To assess the increase in SARS-CoV 2-Specific Antibody Titers in IFN-treated compared to control group; 5) to assess the safety of IFN-treated patients.

1.3 Methodology

Randomized, Open-Label, Controlled, Phase II Study. The study plans to enroll 60 patients: 40 in the IFN-β1a arm, 20 in the control arm, according to a 2:1 - treated: untreated ratio. Treatment plan foresees 4 subcutaneous injections of 3MIU of IFN-β1a, to be given at day 1, 3, 7 and 10 in addition to standard of care. Patients will be monitored and disease progression will be evaluated by means of the National Early Warning Score (NEWS2).

1.4 Expected results

Data emerging from the ongoing pandemic show that the management of advanced stage COVID-19 is mostly critical for elderly patients. This study is expected to provide information about the efficacy of a timely administration of IFN-β to elderly patients in achieving a more efficient control of SARS-CoV 2 infection, thus preventing the progression towards severe forms of the disease. The results of this study will provide a treatment option for high-risk elderly patients experiencing mild symptoms, for which no approved therapy is available so far (besides support therapy and a strict clinical monitoring).

The proposed treatment, upon demonstration of efficacy and safety, could be administered not only to hospitalized patients, but also during isolation at home or in long-stay residential care homes (LSRCHs), with the support of the territorial medical units. Therefore, this treatment protocol will represent an important tool to protect the elderly population in every pandemic scenario that will occur in the near future.

2. Background

The rapid and devastating outbreak of Coronavirus disease 2019 (COVID-19) pandemic and the lack of approved treatments for any human coronavirus (CoV) infection highlighted the urgent need of developing therapeutic options to control or prevent virus spreading. Several options can be envisaged ranging from prophylactic vaccine to targeted antiviral drugs. However, new interventions are likely to require months to years to be developed, and priority is being given to the repurposing of existing antiviral agents (1). Since COVID-19 outbreak, more than 3000 clinical trials have been authorized to identify the drugs or drug combinations capable of attenuating the

virulence of the disease (25). Some of these trials include the use of type I Interferons (IFN-I), mainly α and β, alone or in combination with other compounds. Interestingly, a randomized clinical trial testing the combination of Lopinavir, Ritonavir plus IFN-β in COVID-19 patients showed that only the triple combination was effective in alleviating symptoms and shortening the duration of viral shedding and hospitalization (2). A significant reduction of mortality was observed when IFN-β was administered together with hydroxychloroquine and other antivirals (3). Notably, data suggest that the timing of IFN therapy during SARS-CoV 2 infection can determine treatment efficacy and clinical outcome (26).

IFN-I were first discovered and characterized more than 60 years ago as antiviral substances produced by influenza virus-infected cells, capable of markedly inhibiting viral replication in target cells (27). These cytokines were the firsts to be cloned and extensively used in patients with some viral diseases (28) and cancer (IFN-α) (5). IFN-I are pleiotropic factors endowed with multiple activities, including both a broad-spectrum antiviral activity (27,28) and a remarkable immunoregulatory function (6). The antiviral activity of IFN-I has been extensively exploited for the treatment of viral chronic infections (28) Nevertheless, as highlighted by the long clinical records of IFN-I use, caution is required in terms of route, timing and dose of administration to balance clinical efficacy and side effects.

As many other viruses, Coronaviruses have developed multiple mechanisms to prevent IFN-I induction and subsequent signaling (29), particularly during the early phase of infection, ultimately leading to a dysregulated immune response and increased immunopathogenesis (20,30,31). Diminished levels of IFN-I have been detected in patients during the course of SARS and MERS (32–34). Similar results were also achieved with aged macaques infected with SARS-CoV, that exhibited considerably lower levels of IFN-β and a more severe pathology than young animals (17). Interestingly, when the deficiency in IFN-I production in CoV-infected macaques was remedied by IFN-α2 treatment in combination with ribavirin, lower levels of systemic (serum) and local (lung) proinflammatory markers were observed, in addition to fewer viral genome copies and less severe histopathological changes in the lungs (18). More relevantly, the results of a recent work clearly showed an impaired IFN-I signaling, associated with persistent blood virus load and an exacerbated inflammatory response in patients with severe COVID-19 (35). Impaired IFN-I response was also observed in young men experiencing severe COVID-19, in which a loss-of function genetic mutation in Toll Like Receptor 7 caused impaired IFN-I response (21). Overall,

these observations outline the critical role of IFN-I in both protective and pathogenic events during CoV infections, thus strengthening the need of fine tuning the IFN-I signaling with respect to the kinetics of CoV replication for an optimal protective response.

3. Rationale

In the light of the current information on SARS-CoV 2 pathogenesis, we speculate that the majority of SARS-CoV 2- infected patients are capable of clearing the virus by means of their effective endogenous IFN-I system and do not require hospitalization. We assume that in a minority of people a defective IFN-I system may favor SARS-CoV 2 spread, eventually causing the development of severe forms of COVID-19 and dismal prognosis. People aged >65 years, for which an impairment of IFN-I induction in response to viral infection has been documented (16,36,37), are at higher risk of severe COVID-19 (38).

In these patients, a delayed IFN-I response and the loss of viral control might contribute in early phases of infection to disease outcome. Data suggest that the IFN-β subtype appears to be the most suited for COVID-19 treatment (39). Thus, we hypothesize that elderly patients will greatly benefit from a short term IFN-I administration at the earliest time of SARS-CoV 2 infection, thus compensating the insufficient or impaired endogenous IFN-I production and preventing COVID-19 progression to severe forms of disease. In light of its immunomodulatory properties, IFN-β administered at the early phases of infection can represent a valuable tool to enhance humoral and cellular immunity in addition to its direct antiviral treatment restricting early viral spread, thus halting virus replication and preventing the progression towards severe forms of disease.

4. Impact for the National Health System

Italy was the first European country to experience COVID-19 pandemic, when the information about viral pathogenesis and therapeutic options were scarcely available. Moreover, Italian demographic structure, with a high percentage of population above 65 years of age, greatly affected the outcome and the death toll of the first epidemic wave. In fact, data show that not only sex and comorbidities, but also age increases the risk of developing severe COVID-19 (38,40) needing hospitalization and intensive care support. Since the first case, recorded in Italy on

February 21th, COVID-19 represented a big challenge for the Italian National Health System, which underwent an increasing pressure until restriction measures were undertaken to avoid its collapse. However, the interruption of non-essential economic and social activities has a serious impact on global economy and people quality of life in the long term. For elderly people, isolation can result not only in increased risks of cardiovascular, autoimmune and neurocognitive disorders, but also induce or exacerbate mental health problems, such as depression and anxiety. The introduction of a new phase, in which the restriction measures were gradually released and economic activities restarted, required some strategies to be undertaken to keep an acceptable risk for all population. A reinforced surveillance system was developed and is currently in use to ensure a prompt diagnosis of new cases. Nevertheless, it is urgent to develop and test new treatment options that can be administered during the early infection to reduce viral shedding, and consequent contagion, and to hamper disease progression toward severe forms, thus diminishing the impact on the National Health System.

In this trial, particular attention is given to aged patients with a recent diagnosis of COVID-19 in the presence of mild symptoms. In these patients, a strict medical control during home isolation, or a precautionary hospitalization are both appropriate choices, to monitor the possible rapid evolution of the infection. However, no therapeutic regimen specifically designed for these patients is available. Therefore, the risk of developing severe forms of the disease requiring intensive care or ending in fatalities is still high.

This trial will test the efficacy of IFN-β administered to aged patients during the early phase of the infection, in limiting viral replication and preventing the evolution of COVID-19 towards severe and critical diseases. Individual infectivity is directly associated with disease severity and time of viral shedding. Moreover, preventing severe COVID-19 will directly reduce lethality and will immediately mitigate the hospitals overworking, thus overall reducing the potential impact of COVID-19 on the National Health System.

5. Objectives of the study

This trial aims at exploring the use of IFN-β1a in SARS-CoV 2 newly diagnosed elderly patients with increased risk of developing severe COVID-19. In particular, this study will evaluate low-dose and discontinuous use of IFN-β1a in the early phase of infection, in order to exploit not only its

antiviral, but also its immune activating and anti-inflammatory properties. Such regimen should avoid any toxicity and refractoriness phenomena often occurring during IFN-I chronic administration.

5.1 Primary Objective

Primary Objective of the study is to evaluate the reduction in disease progression in patients treated with IFN versus control group within 28 days.

5.1.1 Primary endpoint and outcome

Primary endpoint of the study is the proportion of patients experiencing a disease progression, during at least 5 days, according to the National Early Warning Score **(NEWS2).** The **NEWS2** score is a standardized approach aimed at promptly detecting signs of clinical deterioration in acutely ill patients and establishing the potential need for higher level of care. It is based on the evaluation of vital signs including respiratory rate, oxygen saturation, temperature, blood pressure, pulse/heart rate, AVPU response. The resulting observations, compared to a normal range, are combined in a single composite "alarm" score. Any other clinical sign clearly indicating a disease worsening will be considered as disease progression.

5.2 Secondary Objectives and Endpoints

The following table 1 contains the secondary objectives and endpoint of the study

Objective	Endpoint
1) To assess the reduction in ICU admission in patients treated with IFN versus control group within 28 days of randomization	ICU-free days at 28 days (Day 1 through Day 28)
2) To assess the reduction in number of deaths in IFN compared to control group (day 28)	All-cause mortality (Day 1 through Day 28)
3) To evaluate the increase in proportion of participants returning to negative SARS-CoV 2 RT-PCR in IFN-treated versus control group at Day 14 and Day 28	Negative SARS-CoV 2 RT-PCR at day 14 post-randomization Negative SARS-CoV 2 RT-PCR at day 28 post-randomization
4) To assess the increase in SARS-CoV 2-	Change from Baseline in SARS-CoV 2-Specific

Specific Binding Antibody Titers in IFN compared to control group (day 28)	Binding Antibody Titers at day 14 and 28
5) To assess the safety of IFN-treated patients versus control group	Incidence of adverse events

For secondary endpoints, more detailed descriptions follow:

1) ICU-free days at 28 days will be calculated as the number of days a patient is not in an ICU. Time Frame will be: Admission (day 0) to 28 days after admission (day 28). In case of death, it will be counted as 0 day;

2) All-cause mortality will be: total number of death events occurring within day 0 and day 28;

3) Negative SARS-CoV 2 RT-PCR is defined as an undetectable presence of SARS-CoV 2 genes, as determined by PCR on an adequate sampling of upper respiratory tract.

4) Change from Baseline in SARS-CoV 2-Specific Binding Antibody Titers is defined as the difference in anti-SARS-CoV 2-specific antibody levels measured at day 28 versus day 0;

5) Details on safety event are described in paragraph 9.4

5.3 Exploratory Endpoints

Exploratory studies will be also performed on blood samples collected before and after treatment to assess:

- IFN-I signaling activation
- Cellular immune monitoring
- Systemic inflammatory markers

5.3.1 IFN-I Signaling

Pioneer studies in animal models showed that the complete absence of IFN-I signalling, by deletion of IFN-I receptor, enhanced mice susceptibility and mortality from several viral infections (7). IFN-I signalling downregulation may occur during viral infections as a consequence of viral-specific evasion mechanisms that Coronaviruses mainly establish during the early phase of infection (29,31). Diminished levels of IFN-I or Interferon Stimulated Genes (ISG) expression have been detected in the peripheral blood mononuclear cells of SARS and MERS patients (32,33). More relevantly, the results of a very recent work clearly showed an impaired IFN-I signaling, associated

with persistent blood virus load and an exacerbated inflammatory response in patients with severe COVID-19 (35). A diminished level of endogenous IFN-I activation and signalling may also occur as a consequence of aging, as reported in several *in vitro* and *in vivo* settings (17,41). In light of these considerations, the level of expression of selected ISG will be analysed in patients PBMC as surrogate markers of IFN-I signalling activation. Samples will be collected before, during and after the completion of IFN-β1a treatment in order to assess 1) possible correlations between IFN-I activation status and patient clinical outcome *per se*; 2) treatment-induced modifications of IFN-I signalling activation possibly associated with clinical improvement.

5.3.2 Cellular Immune-Monitoring

A decrease in peripheral lymphocyte count (with lower frequencies and absolute counts of CD3, CD4, CD8 T cells as well as of NK subsets) and an inflammatory cytokine storm may be the main reasons for rapid disease progression and poor treatment response in severe COVID-19. The neutrophil-to-CD8+ T cell ratio and the neutrophil-to-lymphocyte ratio were identified as prognostic factors affecting the prognosis for severe COVID-19 (42). Besides quantitative alteration, T cell maturation status was found to be modified since the percentage of naïve helper T cells increases and memory helper T cells decreases in severe cases. Patients with COVID-19 have also low levels of regulatory T cells, showing damaged features in severe cases (43). In general, COVID-19 patients show marked T cell activation, senescence, exhaustion and skewing towards Th17, if compared to healthy subjects (44).

The innovative technology MFC will help in elucidating the immunomodulatory *in vivo* effect of IFN β1a treatment. Leukocyte subpopulation frequency, activation status and functionality will be explored in pre- and post-treatment patients' blood samples. MFC results will be correlated with clinical outcome in order to identify potential peripheral immune markers of response to treatment.

5.3.3 Systemic inflammation

It was reported that in some COVID-19 patients, the immune response elicited against SARS-CoV 2 results in an increase in systemic inflammatory cytokines, which may eventually progress to a "cytokine storm," followed by multi-organ system dysfunction (45). In fact, some of the severe manifestations of COVID-19 are linked to the excess of circulating pro-inflammatory cytokines: acute respiratory distress syndrome, thromboembolic diseases such as acute ischemic strokes

caused by large vessel occlusion and myocardial infarction, encephalitis, acute kidney injury, and vasculitis (46). The chronic activation of pro-inflammatory pathways documented in the elderly, especially men, and named “inflamm-aging”, represents a risk factor *per se* for the development of COVID-19 complications (47).

We believe the restoration of a functional IFN-I response, through the administration of IFN-β1a during the early phase of SARS-CoV 2 infection, may affect systemic hyper inflammation both directly, by means of the immunomodulatory properties of the cytokines, and indirectly as an effect of reduced SARS-CoV 2 replication.

The level of inflammatory markers known to have a prognostic role in COVID-19 progression, such as IL-6, CRP, TNF-α (48) together with some endothelial cell adhesion molecules whose expression levels correlate with COVID-19 severity (FKN, VCAM-1, ICAM-1, VAP-1 (49)), will be analysed in the blood collected from IFN and control arm before and 10 days after enrolment. Data will be integrated with the results of routine lab analysis on coagulations factors (Fibrinogen, D-dimers) also involved in COVID-19 pathogenesis. The comparative analysis between groups will address treatment-induced modulations and possible correlation with clinical outcome.

5.4 Statistical hypothesis

The trial power has been calculated by the ISS group. The study was powered to independently assess a potential benefit of IFN-β1a compared with control arm (no specific antiviral treatment besides standard of care) on rate of progression of NEWS2 score lasting more than 5 days.

Sample size was calculated according to the primary endpoint of the study. In particular, the sample size calculation is based on the assumptions of an at least 35% difference in the percentage of patients undergoing disease progression between IFN-β1a and control arm. A sample size of 60 patients total (40 in the IFN-β1a-treated arm and 20 in the control arm, according to a 2:1 randomization ratio) will be needed to provide 80% power at significance level of 5% to detect the difference of patients undergoing disease progression between a group 1 proportion of 0.15 (IFN-β1a + standard of care) and a group 2 proportion of 0.50 (standard of care).

Sample Size: ANTIICIPATE trial

Two-sided significance level (1-α)	95
Power (1-β, % chance of detecting)	80
Ratio of sample size, Unexposed/Exposed	0.5
Percent of Unexposed with Outcome	50
Percent of Exposed with Outcome	15
Risk Ratio	0.3
Risk difference	-35

	Kelsey	**Fleiss**	**Fleiss (CC)**
Sample Size - Exposed	39	40	48
Sample Size-Unexposed	20	20	24
Total sample size	59	**60**	72

6. Study design

Randomized, Open-Label, Controlled, Phase II Study. Patients, who satisfy all inclusion criteria and no exclusion criteria, will be randomly assigned to one of the two treatment groups in a ratio 2:1. Randomization will be stratified by gender. Stratified randomization will balance the presence of male and female in both study arms. The planned study duration is 12 months including study set up, enrollment, follow up and data analysis as indicated in Appendix 3.

7. Study Population

Male and female adults aged 65 years or older with newly diagnosed mild COVID-19 are eligible for the study.

7.1 Case definition

For the purpose of the study, the following definition is applied: a case of COVID-19 is a person with detectable SARS-CoV 2 genes, as determined by PCR on an adequate sampling of upper respiratory tract.

7.2. Criteria for eligibility

7.2.1 Inclusion criteria

- ≥ 65 years of age at time of enrolment;
- Laboratory-confirmed SARS-CoV 2 infection as determined by PCR, in any specimen < 72 hours prior to randomization;
- Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures;
- Understands and agrees to comply with planned study procedures;
- Agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol;
- Being symptomatic for less than 7 days before starting therapy;
- NEWS2 score ≤2

7.2.2 Exclusion criteria

- Hospitalized patients with illness of any duration, and at least one of the following:

  -Clinical assessment (evidence of rales/crackles on exam) AND SpO2 ≤ 94% on room air at rest or after walking test,

  OR

  -Acute respiratory failure requiring mechanical ventilation and/or supplemental oxygen;
- Patients currently using IFN-beta (e.g., multiple sclerosis patients);
- Patients undergoing chemotherapy or other immunosuppressive treatments
- Patients with chronic kidney diseases;
- Known allergy or hypersensitivity to IFN (including asthma);
- Any autoimmune disease (resulting from patient anamnesis);
- Patients with signs of dementia or neurocognitive disorders;

- Patients with current severe depression and/or suicidal ideations;
- Being concurrently involved in another clinical trial;
- HIV infection (based on the anamnesis);
- Use of any antiretroviral medication;
- Impaired renal function (eGFR calculated by CKD-EPI Creatinine equation < 30 ml/min);
- Presence of other severe diseases impairing life expectancy (e.g. patients are not expected to survive 28 days given their pre-existing medical condition);
- Any physical or psychological impediment in a patient that could let the investigator to suspect his/her poor compliance;
- Lack or withdrawal of informed consent

7.3 Recruitment strategy

The management of elderly patients with COVID-19 needs to take into consideration the presence of comorbidities that increases their fatality risk, but it is also affected by the epidemiological situation of SARS-CoV 2 infection (see Feasibility section). Our study plans to enroll either hospitalized and non-hospitalized newly diagnosed COVID-19 patients, as well as patients hosted in long-stay residential care homes.

The Special Unit for regional continued care (USCAR), having the role of early detecting clusters of infection within Regione Lazio, will be responsible for screening and enrolling eligible patients that after SARS-CoV 2 positivity notification are not hospitalized, but remain in isolation at home or in a long-stay residential care homes. When dealing with patients older than 65, USCAR will be responsible of informing the patient of the current study, of having the Informed Consent signed and of collecting the blood sample to assess eligibility criteria. After the enrolment, the patient will be followed by a dedicated USCAR team that will: i) give to the patient/family-caregiver the kit of devices for home monitoring (i.e., 1 pulse oxymeter, 1 digital sphygmomanometer, 1 thermometer), ii) perform training for the use of devices, iii) perform treatments and collect samples for monitoring patients according to the timeline described in Appendix 2. The USCAR team will receive daily updates from non-hospitalized patients to determine their NEWS2 score values.

Patients that, at discretion of the general practitioner, are directed to Spallanzani Hospital for hospitalization, will be there assessed for inclusion/exclusion criteria and, in case of eligibility, enrolled in the study. Patients will be monitored according to standard hospital protocol in addition to the timeline described in Appendix 2.

8. Intervention

8.1 Experimental Drug and justification for dose

Rebif® (interferon beta-1a) is a disease-modifying drug used to treat relapsing forms of multiple sclerosis (MS) and is similar to the IFN-beta protein produced by the human body. It was approved in Europe in 1998 and it is used in more than 90 countries worldwide. While current posology of Rebif in MS (12 MIU 3 times/week) is capable of balancing the neural inflammation typical of MS, the dosing and schedule of Rebif® administration in this study were selected by taking into consideration some features of IFN-I, emerged from many years of clinical use of these cytokines. In fact, several clinical studies reported that an Interferon-induced immune adjuvant activity could be observed already after the administration of intermittent low doses of the cytokine in both cancer and antiviral settings (15,50–52). Instead, the continuous stimulation of IFN-I signaling, exerted by high serum levels of the cytokine, can result in diminished treatment efficacy due to the emergence of refractoriness phenomena caused by receptor internalization/degradation as well as the rapid induction of UBP43 and SOCS negative regulators (4), immunosuppression and can also result in relevant side effects.

With the aim to tailor the treatment schedule to the early phase of SARS-CoV 2 infection in elderly patients, we selected 3 MIU of IFN-β1a as a dose expected to exploit IFN-mediated antiviral and immunomodulatory properties of the cytokine without causing relevant toxicity or inducing refractoriness phenomena (53).

8.2 Treatment arms

Control arm. No specific antiviral treatment besides standard of care.

Treatment arm. 11ug (3MIU) of IFN-β1a will be injected subcutaneously at day 1, 3, 7, and 10 in addition to standard of care. The drug solution, contained in a pre-filled cartridge, will be injected by means of the RebiSmart electronic injection device, as described in Appendix 6.

8.3 Standard patients monitoring

Patients will be daily evaluated for body temperature, respiratory rate, oxygen saturation, blood pressure, pulse/heart rate and AVPU response. The NEWS2 score will be then calculated following the table 2. Additional measurements are allowed whenever any sign of disease progression appears. In case of multiple measurements within a day, the highest score will be considered for patient assessment.

Table 2. NEWS2 Score

Chart 1: The NEWS scoring system

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

For non-hospitalized patients, measurements will be auto-performed by the patient either assessed by a caregiver or a family member. Training on how to use the provided devices will be performed by USCAR unit at T1, written instructions will be also provided, and additional help will

be given upon request by phone or videocall. Measurements will be recorded on the clinical diary that will be provided (Appendix 5). Patients will be contacted daily by USCAR dedicated unit and will communicate by phone their health status that will be registered on a dedicated clinical records form (Appendix 5). USCAR unit approaching COVID-19 patients will use personal protective equipment including a FFP3 (or FFP2) mask, gloves, gown and googles. FFP3 will be used always in case of any procedure on respiratory tract (including nasopharyngeal swab).

Hospitalized patients can be discharged from the hospital considering the ongoing national and regional recommendations to discharge COVID-19 patient at home. The USCAR unit will then responsible of continuing follow up of the patient according to the timeline described in Appendix 2.

8.4 Other therapies allowed

Patients will not receive any other antiviral treatment, unless considered needed by the physician. All other treatments including anti-hypertensive drugs, medications for diabetes (insulin and oral drugs), antibiotics, hormone therapy can be provided to patients of both groups according to medical judgments. Patients should not receive nonsteroidal anti-inflammatory drugs apart from paracetamol if needed.

Any previous and concomitant medication will be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

8.5 Safety monitoring and individual stopping rules

Any sign or symptom associated to drug adverse events will be daily reported.

Progressing patients which are in need of oxygen support will be maintained in the trial for follow up purposes, but treatment will be discontinued (Appendix 1). Progressing patients will receive standard of care or additional treatment at the physician discretion.

Another stopping rule includes drug related adverse events grade ≥ 3 according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

For progressing patients, we will record the admission to the intensive care unit, days in ICU, and the disease outcome (either survivor or non-survivors) by using the Regional surveillance systems. Patients are free to withdraw from participation in the study at any time upon request, without any consequence. Patients should be listed as having withdrawn consent only when they no longer wish to participate in the study and no longer authorize the Investigators to make efforts to continue to obtain their outcome data. Every effort should be made to encourage patients to remain in the study for the duration of their planned outcome assessments. Patients should be educated on the continued scientific importance of their data, even if they discontinue the study. In the case of a patients becoming lost to follow-up, attempts to contact the patient should be made and documented in the patient's medical records.

9. Methods

9.1 Randomization

Sixty patients will be randomized 2:1 to receive IFN-β1a or control arm. Eligible patients will be randomised (no later than 36 h after enrolment) by means of a computerized central randomization system. All patients will receive a unique patient identification number at enrolling visit when signing the informed consent and before any study procedures are performed. This number must remain constant throughout the entire study.

ISS will prepare a randomization list by using a validated software and the list will be managed by the CRO. The randomization of patients will be closed when 60 patients have been randomized. The randomization will be stratified by sex; for each stratum a sequence of treatments randomly permuted in blocks of variable length (3 or 6) will be generated.

9.2 Blinding

This is an open-label study. After the randomization, patient will be notified whether will receive or not the experimental drug.

9.3 Electronic case report form

Patients' data will be recorded in an *ad hoc* online database. The Electronic case report form will be provided by a Clinical Research Organization and implemented according to the study design. An example of the information to be recorded in the e-CRF is provided as Appendix 4.

9.4. Safety Criteria Evaluation

9.4.1 Safety profile

Subjects participating in this trial who received at least one dose of the trial medication are considered to be included in the safety population (full analysis population). Safety population not include subjects who drop out prior to receiving any treatment. Data on safety profile, nature, incidence and severity of adverse events (AEs) and serious adverse events (SAEs) will be collected as detailed in both this section of the protocol and in the AE/SAE section of the CRF. Any reason for drug interruption, reduction and discontinuation will be collected. Toxicities will be graded using NCI Common Terminology Criteria for adverse Events (CTCAE) version 5. The investigator is responsible for detecting, documenting and reporting AEs and SAEs, according to the criteria defined in this protocol.

The safety profile of experimental drug (i.e., IFN-β1a, Rebif®) has been well established. Below are the very common and common adverse reactions as reported in the Summary of Product Characteristic 2010:

Very common (≥1/10)	**Common (≥1/100 to <1/10)**
Neutropenia, lymphopenia, leukopenia, thrombocytopenia, anaemia	Diarrhoea, vomiting, nausea
Asymptomatic transaminase increase	Severe elevations in transaminases
Headache	Pruritus, rash, erythematous rash, maculo-papular rash, alopecia
Injection site inflammation, injection site reaction, influenza-like symptoms	Myalgia, arthralgia
	Depression, insomnia
	Injection site pain, fatigue, rigors, fever

9.4.2 Adverse events (AE) and serious adverse events (SAE)

9.4.2.1 Definition of an AE

An AE is defined as any untoward medical occurrence in a patient, temporarily associated with the use of a medicinal product, whether or not it is considered related to the medicinal product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporarily associated with the use of a medicinal product. Examples of an AE include:

· Significant or unexpected worsening or exacerbation of the condition/indication under study.

· Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.

· New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.

· Signs, symptoms, or the clinical sequelae of a suspected interaction.

· Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).

9.4.2.2 Definition of a Serious Adverse Event

A serious adverse event (SAE) is defined as any untoward medical occurrence that, at any dose:

1. Results in death
2. Is life-threatening
3. Requires hospitalization or prolongation of existing hospitalization
4. Results in disability/incapacity
5. Is a congenital anomaly/birth defect
6. Is otherwise considered as medically important.

9.4.2.3 Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE or SAE on the eCRF. Any AEs or SAEs occurring during the study must be documented in the subject's medical records and on the appropriate page of the eCRF. Each AE or SAE is to be recorded individually. All AEs which

occur during the course of the study should be recorded in the eCRF. Information on the AE must be recorded on a specific AE form (appendix 5).

9.4.2.4 Evaluating AEs and SAEs

9.4.2.4.1 Assessment of intensity

The investigator will make an assessment of intensity of each AE and SAE reported. In this protocol, the intensity of AEs and SAEs will be graded on a scale of 1 to 5 according the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE) Version 5 and are available at https://evs.nci.nih.gov/ftp1/CTCAE/About.html .

For SAEs, the maximum intensity (or grade) will be reported in the eCRF. For non-serious AEs, each change in intensity (or grade) will be reported in the eCRF.

9.4.2.4.2 Assessment of causality

The investigator is obliged to assess the relationship between the study medical product and the occurrence of each AE/SAE and provide the assessment of causality as per instructions on the SAE form in the Investigators File.

9.4.2.4.3 Follow-Up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide further information on the subject's condition by any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. AEs that are ongoing with a toxicity of Grade 3 or 4, or have a relationship to study drug that is suspected (Reasonable Possibility) will be queried for resolution at study conclusion and at approximately 30 days after the last dose of study. New or updated information will be recorded on the originally completed SAE form in the Investigator's File, with all changes signed and dated by the Investigator.

9.4.3 Regulatory reporting requirements for adverse events

The Investigator must report immediately (within 24 hours from the knowledge) to the study Sponsor any SAE, occurred during the study whether related to the investigational product or not.

The study Sponsor has the legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. The study Sponsor will comply with the Italian regulatory requirements related to the reporting of SAEs to regulatory authorities and the Independent Ethics Committee (IEC). In particular, all the Suspected Unexpected Serious Adverse Reactions (SUSARs) that occur while on treatment and within 30 days since the last investigational drug administration, and that have a

suspected relationship to study's drug (Reasonable Possibility) will be notified with an urgency procedure to the local regulatory Agency (AIFA) and IEC with the following timelines:

- SUSARs that are considered life-threatening: notification within 7 days.
- SUSARs that are not considered life-threatening: notification within 15 days.

The notification with urgency procedure is not required for SAEs that are expected with the drugs used in the protocol, and for non-serious AEs, both expected and unexpected. For these events (expected SAEs and AEs), the CT will notify the local regulatory agency and IECs by an annual safety report.

9.5 Secondary and Exploratory endpoints

Dedicated blood samples will be collected at different time points (see APPENDIX 2: Timeline scheme) and processed at the biobank of the INMI.

9.5.1 SARS-CoV-2 Antibodies

The development of a specific humoral response will be monitored by measuring specific anti SARS-CoV-2 antibodies in the sera of patients collected at day 14 and 28 post randomization. Commercially available tests will be used to detect IgM specific for S antigen, IgG specific for the N and S antigens, and IgA specific for S antigen. Sera resulting reactive with the S antigen will be tested for the capacity of viral neutralization using standardized methods.

9.5.2 Molecular IFN-I signaling

Blood samples will be collected at T1 prior first treatment, during treatment (T3 prior second treatment) and post treatment (T14) and processed at the biobank of the INMI. Isolated PBMC will be aliquoted, submerged with RNA stabilization reagent and cryopreserved. For analysis, total RNA will be isolated and the transcriptional analysis of over 500 general immunology genes will be performed by means of Nanostring technology. Data analysis will determine the transcriptional modifications occurring during the course of IFN-β1a treatment as well as to identify molecular patterns potentially correlated with clinical outcome. Particular focus will be given to the ISG score reported to be differentially expressed among mild to severe COVID-19 (22). This exploratory analysis will be conducted by Dr Aricò and Dr Castiello, having a relevant background on IFN signaling analysis (51,54)

9.5.3 Cellular Immune monitoring

Pre-(T1 prior treatment) and post-treatment (T3 and T14) blood samples will be monitored by MFC-based assays through different antibody panels in order to analyze frequency of major leukocyte subpopulations associated with naïve/memory, co-activation and co-inhibition markers; polyfunctional properties of T cell specific response against virus antigens will be evaluated after short term in vitro culture.

Stained samples will be acquired on a Beckman Coulter CytoFlex Cytometer and analyzed by CytExpert and/or Kaluza software as well as by advanced machine learning algorithms such as FlowSOM and CITRUS (Cytobank online platform). Dr. Francesca Urbani and Dr. Iole Macchia, co-investigators at ISS unit, have long lasting experience in MFC assays and immune-monitoring (52,55,56).

9.5.4 Systemic Inflammatory markers

Pre- (T1 prior treatment) and after-treatment (T14) blood samples will be collected from Treatment and control group to monitor the levels of soluble factors involved in inflammation (e.g., cytokines and chemokines) and endothelial cell adhesion molecules. At the selected time points, plasma will be isolated from peripheral blood and cryopreserved until analysis that will be simultaneously conducted by means of specific ELISA assays. Data will be integrated with the results of routine lab tests on coagulation factors and factors involved in COVID-19 pathogenesis (CRP, IL-6, TNF-α, Fibrinogen and D-Dimer).

10. Statistical Plan

The primary analysis will be carried out on the primary endpoint on the intention-to-treat (ITT) population defined as all patients randomized receiving at least one dose of treatment.

The percentage of patients undergoing disease progression defined on rate of progression of NEWS2 score lasting more than 5 days will be calculated in two arms (IFN-β1a + standard of care vs standard of care) of the trial. For persons who died, a conservative approach will be adopted and death will be considered an event. The effect of treatment will be estimated through a logistic regression model including a dummy variable for treatment. The effect of treatment will be estimated through multivariable logistic regression model by accounting for the following

covariates: age, gender, co-morbidities. Moreover, NEWS2 score at baseline and setting of recruitment will be also considered.

All primary and secondary analyses will be carried out both on ITT population and on per-protocol population. Per-protocol population includes all subjects who were included in the ITT population that received the treatment as defined in the protocol and who completed the study with no major protocol violations.

Kaplan-Meier survival analysis and Cox proportional hazards model will be used for time-to-event data. The following covariates will be included in the Cox model: age, gender, co-morbidities. Moreover, NEWS2 score at baseline and setting of recruitment will be also considered. For the secondary endpoint ICU-free days, a competing risk model will be adopted considering death a competing event, following the method proposed by Fine and Gray (57). Moreover, the median difference will be reported.

The longitudinal secondary endpoint measured on a continuous scale (the increase in SARS-CoV 2-Specific Binding Antibody Titers in IFN compared to control group) will be analysed using a Mixed effect Model for Repeat Measure (MMRM) to estimate the difference of mean change from baseline in SARS-CoV 2-Specific Binding Antibody Titers between IFN-β1a + standard of care and standard of care at day 28. In case of data sporadically missing during the course of trial we will assume they were Missing At Random (MAR). A sensitivity analysis will be carried out by conducting the statistical test after imputing missing, including the worst-case imputation. All missing data will be imputed within treatment groups defined by randomized treatment.

Safety endpoint will be compared by a chi-squared test for discrete variables, by means of analysis of variance (ANOVA) and covariance (ANCOVA) for continuous variables or by the non-parametric Mann-Whitney test when appropriate.

Confidence intervals (95%) will be reported for all outcomes and association measures (proportions, means, Odds Ratios and HRs).

For all statistical analyses (efficacy and safety), the level of statistical significance will be kept at 0.05 with two-sided p-values. Statistical analyses and related reports will be in full compliance with ICH E9 guidance (https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-9-statistical-principles-clinical-trials-step-5_en.pdf).

11. Timing

See APPENDIX 2: Timeline scheme and APPENDIX 3: GANTT

12. Feasibility

Our study plans to enroll either hospitalized and non-hospitalized newly diagnosed COVID-19 patients, as well as patients hosted in long-stay residential care homes. The possible scenario of the Italian pandemic occurring during the conduction of the study will likely affect the proportion of patients that will be enrolled in the different settings. In fact, for patients presenting with mild illness, the decision to undertake hospitalization vs home care should be carefully evaluated to take into account patient risk of rapid deterioration, but also the burden on the health care system.

On august 11th, the ISS, together with the Ministry of Health and the Coordination of Italian regions and autonomous provinces, issued a document called “Elementi di preparazione e risposta a COVID-19 nella stagione autunno-invernale”. The document, aimed at providing general elements and suggesting preparedness frameworks to strengthen the response and optimally cope with any increase in the number of new infections by SARS-CoV 2 in the autumn-winter 2020-2021 season, foresees four possible scenarios, characterized by increasing SARS-CoV 2 transmission rate and related risk of SSN collapse.

To ensure that patients’ enrolment is duly completed in any of the possible scenarios, the study will count on a network of collaborating institutions directly involved in the identification and management of COVID-19 patients in Rome. A campaign will be held to inform Family doctors (Medici di Medicina Generale) and long-stay residential care homes (LSRCHs), whose collaboration will ensure the precocious identification of eligible patients throughout the urban area of Rome. The Special Unit for regional continued care (USCAR) are currently involved in the prompt identification of COVID-19 clusters within Regione Lazio. In this study, a group of physicians belonging to USCAR will be specifically trained and will be responsible for the screening, enrolling and clinical monitoring of patients kept under home isolation or hosted in long-stay residential care homes (LSRCHs).

All experts involved in the project are highly motivated, have complementary expertise and a strong background in the fields of IFN biology and infectious diseases. The CNR group, including

the Sponsor Coordinator (Giuseppe Sconocchia) and Scientific Coordinator (Filippo Belardelli) of the study, has a long-lasting expertise on immunology, IFN biology and clinical studies with IFN-I. The Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani (clinical center) is one of the five clinical hubs for COVID-19 management in town and has a longstanding experience in multicentre clinical studies and the PI (Emanuele Nicastri) is a well-recognized clinician deeply involved in the clinical management of COVID-19 patients. The ISS group includes scientists with a background on IFN-I biology in both basic research and clinical trials (Eleonora Aricò, Laura Bracci, Luciano Castiello, Iole Macchia, Francesca Urbani) and with long record expertise on antibody response measurement (Annarita Ciccaglione, Roberto Nisini). Moreover, the Clinical Epidemiology group of the ISS (Ilaria Bacigalupo, Flavia Lombardo, Nicola Vanacore, Antonio Ancidoni) has a strong background on statistical analysis and was involved in the recent survey on COVID-19 infection in long-stay residential care homes (58) . To ensure the full feasibility and the high quality performance of the study the Sponsor finalized a contract with a CRO, highly specialised in clinical studies involving IFN-β, which will support the Sponsor Coordinator and the Scientific Coordinator with regard to specific services and for the implementation and monitoring of the entire study.

13. Good clinical practices and ethics

13.1. Good clinical practice

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (1964) and subsequent amendments and updates (Fortaleza, Brazil, October 2013), in the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and in the appropriate regulatory requirements. The drug used in this trial is already registered and its toxicity profile is very well known, since it is largely used for the treatment of Multiple Sclerosis.

13.2 Ethical aspects

The entire study protocol, including informative material for the patients and modules for the informed consent, will be evaluated by The Ethics Committee (EC) of the National Institute for

Infectious Diseases “Lazzaro Spallanzani”, Rome, Italy, which is the National Ethics Committee for evaluation of clinical trials on human drugs in COVID-19 patients.

The study will not start before obtaining a favorable opinion from the EC, the Competent Authority Authorization and any other authorization required by local regulation. Every intention to modify any element of the original protocol after the first approval will be promptly notified to the EC and will be applied only after its written authorization. Investigators will be responsible for submitting any amendments to the protocol to the EC. Any modifications to the protocol, which may impact on the conduct of the study, may affect patient safety, including changes of study objectives, study design, patient population, sample size, study procedures or significant administrative aspects will require a formal amendment to the protocol. Such amendments will be agreed and approved by the Ethics Committee of the National Institute for Infectious Diseases “Lazzaro Spallanzani”, Rome, Italy, and the health authorities prior to implementation, in accordance with local regulation. Administrative changes of the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be documented in a memorandum.

13.2.1 Written informed consent

The Investigators will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects will also be notified that they are free to discontinue from the study at any time. The subject’s signed and dated informed consent will be obtained prior to conduct any procedure specific for the study. The original signed Written Informed Consent Form will be stored, and a copy will be given to the patient.

13.2.2 Subject data protection

In order to protect the subjects’ identity, the Investigator will assign a subject identification number to each enrolled subject to be used instead of subject name when reporting all study related data and adverse events.

The Written Informed Consent Form will explain that the study data will be stored at Spallanzani Hospital maintaining confidentiality in accordance with national data legislation. However, the personal information must be available to authorized personnel of Study Sponsor (clinical monitor and auditor), Ethics Committee and Regulatory Authorities. In addition, consent to allow direct

access to original medical records to ensure data verification will be obtained from the subject before participation in the study.
Enrolment log must be kept strictly confidential to enable patient identification at the site.

13.2.3 Audits and inspections

The Principal Investigator and the SC will provide all the necessary information and material to the participating centers in order to standardize all the protocol-related procedures and to avoid unexpected variability. Printed and electronic informative material (complete original protocol, informed consent modules, informative modules for patients and relatives, recruitment checklist, graphic timeline of interventions and visits, order list for physicians and nurses) will be distributed to Spallanzani Hospital and USCAR. Source data/documents must be available to inspections by the designee or Health Authorities.

13.2.4 Monitoring

The monitoring activities will be performed by a Clinical Research Organization. Clinical Monitor will perform the monitoring activities according to "Note of Guidance on Good Clinical Practice" (ICH E6 (R2), EMA/CHMP/ICH/135/1995).
The clinical monitor will maintain contacts between Investigators and Study Sponsor; furthermore, during the study the clinical monitor will verify that informed consent was obtained from all subjects, that the data were adequately documented in medical records and that the Investigators were compliant with the protocol. The clinical monitor will inform the study Sponsor and the Investigators about all detected protocol deviations, all facilities and technical Staff detected problems. The Investigators will provide direct access to source data/documents for data verification.

13.2.5 Declaration of interest

The study participants declare no financial and/or other conflicts of interest related to the study.

13.2.6 Dissemination policy

The Circ. Min. Health N° 6 of 09/02/2002 obliges each researcher who gets any results of interest to public health, to publish the results within 12 months from the end of the study. All the patients will freely agree or disagree to participate in the study in the belief that the results will be useful

to improve knowledge about their pathologies, for health benefit from themselves or other patients. To respect their will and in the maximum interest of honest clinical research, the investigators agree on the need to ensure the wide publication and diffusion of their results in a consistent and responsible way under their responsibility. The Study Coordinator is the official data owner. The Study Coordinator has the right to present methods and results of the study at public symposia and conferences. The principal publications from the trial will be in the name of Investigators with full credit assigned to all collaborating investigators and institutions.

13.3 Insurance

The study will be conducted according to the law about the study insurance agreement (DM 14 luglio 2009); *ad hoc* insurance n. A1202150060-LB has been established with Lloyd's Insurance Company S.A

14. Budget

Materiale/Utilità	Costo unitario	Numero per paziente	N. pazienti	Quantità	Totale
Costi coordinamento medico	75000				75000
Rebif/Rebismart	0	1	60	60	0
Servizi svolti da CRO					60000
Saturimetro	25	1	30	30	750
Sfingomanometro	20	1	30	30	600
DPI completo FFP3	10	10	30	300	3000
Assistenza domiciliare	400	1	30	30	12000
Teleassistenza	10	28	30	840	8400
Analisi del sangue	40	7	60	420	16800
Test Sars-CoV2	85	3	60	180	15300
Test sierologico	60	3	60	180	10800
Markers infiammatori	100	2	60	120	12000
Ddimero	6	2	60	120	720
Proteina C Reattiva	6	2	60	120	720
Systemic immune profiling	356	3	60	180	64080
RT-PCR per IFN signaling	280	2	60	120	33600
Costi processamento campioni biologici	30	7	60	420	12600
Assicurazione				1	9500
Costi etichettatura e gestione farmaco				1	5000
Costi generali per struttura coordinatrice					47730
Medical Writing and Statistical Data Analysis (all endpoints)					50000
Totale					**438600**

The study is co-funded by Merck Healthcare KGaA with a support equal to 40% of total costs.

15. Institutions agreement

The Study Sponsor will submit in the Clinical Trials all the documentation required by law to AIFA, as the Competent Authority and to Ethics Committee within a week after approval. Also, the Study Sponsor will comply in all respects with the standards of Good Clinical Practice, as defined in the

"Note of Guidance on Good Clinical Practice (CPMP/ICH 135/95)" and related Guidelines, as well as with all applicable regulatory requirements including national drug law and data protection law. A collaboration agreement between all the Institutions involved in the study (CNR, INMI and ISS) will be signed before the enrollment of the first patient.

16. Participating Centers

- IFT, CNR, Rome;
- ISS, Rome
- INMI, Rome
- Synlab srl
- FullCro srl

17. Publications and data properties

Clinical trial data are considered the property of the investigators involved. Publications generated from the study will be sent to peer-reviewed international journals. The name and order of the authors will be decided by the working group.

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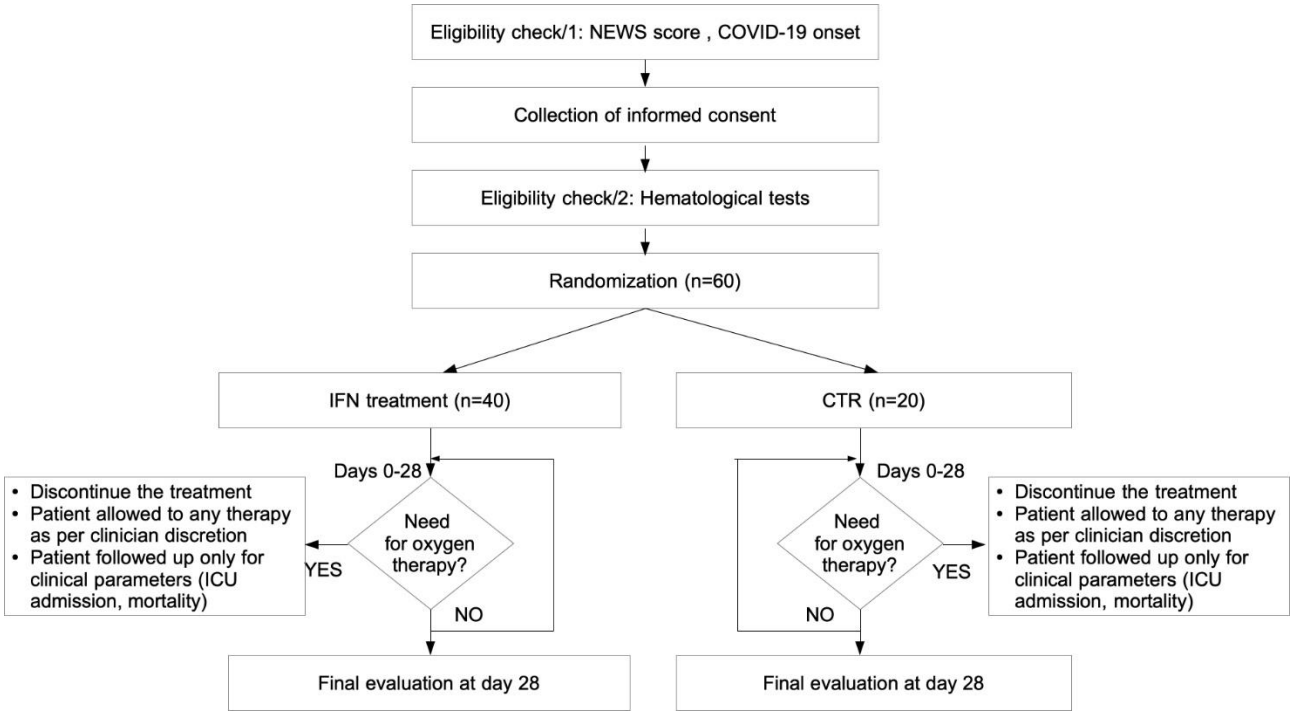
List of Appendices

APPENDIX 1: Flow Chart of the Study

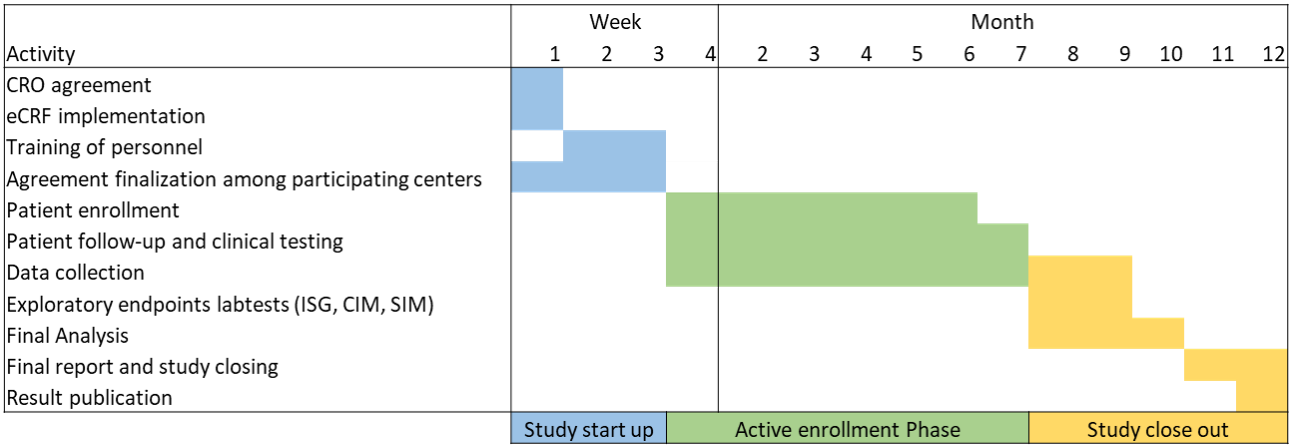


APPENDIX 2: Timeline scheme

TIMELINE SCHEME																															
Days	PreTx	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	T27	T28
	Screening	**Treatment**										**Follow-up**																	
IFN (ARM2 only)		x		x				x			x																		
Procedures (both ARMS)																															
RT-PCR SARS-CoV 2 positivity assay															x														x
Demografic Data	x																												
Medical History	x																												
Informed Consent	x																												
Inclusion/Exclusion Criteria	x																												
Signs and symptoms	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Previous/Concomitant Therapy recording	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
NEWS2 score assessment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Safety/Efficacy Evaluation (both ARMS)																															
Adverse Events recording		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Routine laboratory test parameters	x	x		x							x				x														x
SARS-CoV 2 Antibodies		x													x														x
Exploratory labtests (ISG/CIM/SIM)		x		x											x														

As clinically indicated, laboratory and instrumental tests can be performed on other time points that will be recorded.

APPENDIX 3: GANTT chart



APPENDIX 4: eCRF design

The CRO will implement and validate eCRF on a Gamp 5 21 CFR part 11 compliant platform and share it among participants, endowed with different appropriate access privileges: CRO will have complete data control, while data input permission only will be assigned to local data managers.

eCRF will be designed on the basis of the following structure:

CRF sections by time of visit

Screening **PreTx**	***Treatment*** **T1**
Id Number Demografic Data Medical History Informed Consent Inclusion/Exclusion Criteria Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Routine laboratory test parameters: Blood count, potassium, sodium, glucose, lipase, LDH, creatinine, ALT GPT, AST GOT, Bilirubin, Urea, Albumin Randomization	Date Id Number IFN (ARM2 only) SARS-CoV-2 positivity assay Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters: Procalcitonin, Reactive C protein, Protrombin time (INR), functional fibrinogen, D-dimer SARS-CoV-2 Antibodies Exploratory labtests (ISG/CIM/SIM) Study Discontinuation or Withdrawal

Treatment **T2/T4/T5/T6/T8/T9**	***Treatment*** **T3**	***Treatment*** **T7**	***Treatment*** **T10**
Date Id Number Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Study Discontinuation or Withdrawal ,	Date Id Number IFN (ARM2 only) Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters: blood count Exploratory labtests (ISG/CIM/SIM) Study Discontinuation or Withdrawal	Date Id Number IFN (ARM2 only) Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Study Discontinuation or Withdrawal	Date Id Number IFN (ARM2 only) Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters: Blood count, potassium, sodium, glucose, lipase, LDH, creatinine, ALT GPT, AST GOT, Bilirubin, Urea, Albumin, Reactive C protein, Protrombin time (INR), functional fibrinogen, D-dimer, Procalcitonin Study Discontinuation or Withdrawal

Follow up **T11/T12/T13/T15/T16/T17/T18/T19/T20/T21/T22/T23/T24/T25/T26/T27**	***Follow up*** **T14**	***Follow up*** **T28**
Date Id Number Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Study Discontinuation or Withdrawal	Date Id Number SARS-CoV-2 positivity assay Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters: Blood count, potassium, sodium, glucose, lipase, LDH, creatinine, ALT GPT, AST GOT, Bilirubin, Urea, Albumin, SARS-CoV-2 Antibodies Exploratory labtests (CIM/SIM) Study Discontinuation or Withdrawal	Date Id Number SARS-CoV-2 positivity assay Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters: Blood count, potassium, sodium, glucose, lipase, LDH, creatinine, ALT GPT, AST GOT, Bilirubin, Urea, Albumin, Reactive C protein, Protrombin time (INR), functional fibrinogen, D-dimer, Procalcitonin SARS-CoV-2 Antibodies Study Discontinuation or Withdrawal

CRF - section data		
SARS-CoV-2 positivity assay	date	RT-PCR: Gene name, CT number, Laboratory (INMI or other, to be specified); rapid antigen test: positive/negative (executed by: operator ID)
Demografic Data	date	Sex at birth, Date of birth, Race/Ethnicity; i) hospitalized, ii) RSA, iii) home patient
Medical History	date	see TABLE 1
Informed Consent	signature date	Y/N
Inclusion/Exclusion Criteria	date	see TABLE 2
Previous/Concomitant Therapy	date	Any drug/medicament name, reason for use, dose, frequency, duration of consumption
NEWS2 score assessment	date	SCORE, Systolic and diastolic arterial pressure, heart rate (HR), respiratory rate (RR), systemic body temperature, ACVPU, SpO_2
Randomization	date	ARM1/ARM2, random number
Signs and symptoms		see TABLE 3
IFN (ARM2 only)		Y/N, expiration date, batch number
Adverse Events		TABLE 4 AE and any other AE will be recorded using NCI Common Terminology Criteria for adverse Events (CTCAE) version 5. SUSAR will be recorded in specific CRF.
Routine laboratory test parameters		see TABLE 5
SARS-CoV-2 Antibodies		anti-S IgG, anti-N IgG, anti-S IgM, anti S IgA, neutralizing anti-S Ab titer
Exploratory labtests (ISG/CIM/SIM)		ISG: IFI44L, IFI27, RSAD2, SIGLEC1, IFIT1, IS15. CIM: see TABLE 6. SIM: see TABLE 7.
Study Discontinuation or Withdrawal		Y/N, reason: withdrawal of subjects for non-compliance/adherence, for AE, consent withdrawal, other (to be specified)

TABLE 1 - Medical History		
		Tobacco, Alcohol, other recreational drug use (dose, frequency, duration of comsumption) Flu vaccine (in the last year): Y/N, date
By System	Respiratory	Chronic pulmonary disease, Asthma, Tuberculosis (active/previous), other (to be specified)
	Cardiovascular/Circulatory	Chronic cardiac disease (not hypertension), Hypertension, other (to be specified)
	Musculoskeletal	to be specified
	Endocrine	to be specified
	Hematopoietic	to be specified
	Nervous	Chronic neurological disorder, other (to be specified)
	Dermatological Integumentary System/Exocrine System	to be specified
	Genitourinary	Chronic kidney disease, other (to be specified)
	Lymphatic System/Immune System	to be specified
	Digestive	Chronic liver disease, other (to be specified)
	Metabolic disease	Diabetes, other (to be specified)
	Ear, Nose, Throath	to be specified
	Psychiatric disease	to be specified
	Allergy	to be specified
	Malignant neoplasm	to be specified
	Infectious disease	HIV, HCV, other (to be specified)
	Other (to be specified)	

Note: For any disease, onset date and duration as well as indication about disease current status will be recorded.

TABLE 2 - Inclusion/Exclusion Criteria	
Inclusion criteria (all required):	
≥ 65 years of age at time of enrolment	Y/N
Subject has laboratory-confirmed SARS-CoV-2 infection as determined by PCR, in any specimen < 72 hours prior to randomization	Y/N
Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures	Y/N
Subject understands and agrees to comply with planned study procedures	Y/N
Subject agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol	Y/N
Being symptomatic for less than 7 days before starting therapy	Y/N
NEWS2 score ≤2	Y/N
Exclusion criteria:	
Hospitalized patients with illness of any duration, and at least one of the following: Clinical assessment (evidence of rales/crackles on exam) AND SpO2 ≤ 94% on room air at rest or after walking test, OR Acute respiratory failure requiring mechanical ventilation and/or supplemental oxygen.	Y/N
Patients currently using interferon-beta (e.g., multiple sclerosis patients)	Y/N
Patients with chronic kidney diseases	Y/N
Known allergy or hypersensitivity to interferon (including asthma)	Y/N
Any autoimmune disease (based on the anamnesis)	Y/N
Patients with signs of dementia or neurocognitive disorders	Y/N
Patients with current severe depression and/or suicidal ideations	Y/N
Being concurrently involved in another trial for COVID-19	Y/N
HIV infection (based on the anamnesis)	Y/N
Use of any antiretroviral medication	Y/N
Impaired renal function (eGFR calculated by CKD-EPI Creatinine equation < 30 ml/min)	Y/N
Presence of other severe diseases impairing life expectancy (e.g. patients are not expected to survive 28 days given their pre-existing medical condition)	Y/N
Any physical or psychological impediment in a patient that could let the investigator to suspect his/her poor compliance	Y/N
Lack or withdrawal of informed consent	Y/N

TABLE 3 - Signs and symptoms
History of fever
Lower chest indrawing
Cough (with sputum production/with emoptysis)
Headache
Altered consciousness/confusion
Seizures
Sore throat
Abdominal pain
Runny nose
Vomiting/nausea
Wheezing
Diarrhoea
Chest pain
Conjunctivitis
Muscle aches
Skin rash
Joint pain (arthralgia)
Skin ulcers
Fatigue/malaise
Lymphadenopathy
Loss of taste
Inability to walk
Loss of smell
Bleeding (ischaemic stroke, entracerebral haemorrhage)
Shortness of breath

Note: If present, onset date and duration as well as indication about sign or symptom current status will be recorded.

TABLE 4 - REBIF common AE	
Very common (≥1/10)	Common (≥1/100 to <1/10)
Neutropenia, lymphopenia, leukopenia, thrombocytopenia, anaemia Asymptomatic transaminase increase Headache Injection site inflammation, injection site reaction, influenza-like symptoms	 Diarrhoea, vomiting, nausea Severe elevations in transaminases Pruritus, rash, erythematous rash, maculo-papular rash, alopecia Myalgia, arthralgia Depression, insomnia Injection site pain, fatigue, rigors, fever

TABLE 5 - Routine laboratory test parameters	
Haemoglobin	
Haematocrit	
Full Blood count	ALT/SGPT
Creatinine	
Sodium	AST/SGOT
Potassium	ESR
Procalcitonin	Total bilirubin
	Urea (BUN)
Glucose	Albumin
Lipase	
LDH	
PT (seconds)	INR
fibrinogen	
D-dimer	CRP

TABLE 6 - Cellular Immune Monitoring		
leu_linfociti	CD4_CM	Vd2_TD
lym_CD3	CD4_EM	Vd2_EM
CD3_CD4SP	CD4_N	Vd2_N
CD3_CD8SP	CD4_TD	Vd2_CM
CD8_CM	CD4_CD28pos27neg	lym_CD19
CD8_EM	CD4_CD28pos27pos	lym_NK
CD8_N	CD4_CD28neg27neg	lym_NKT
CD8_TD	CD4_CD28neg27pos	leu_CD14pos
CD8_CD28pos27neg	CD4_CD57pos27neg	leu_Treg
CD8_CD28pos27pos	CD4_CD57posPD1neg	lym_Treg
CD8_CD28neg27neg	CD4_CD57posPD1pos	CD3_Treg
CD8_CD28neg27pos	CD4_CD57negPD1pos	CD4_Treg
CD8_CD57pos CD27neg	gpSpike_CD8	Treg_CD45RAnegCD39neg
CD8_CD57posPD1neg	Vd2	Treg_CD45RAnegCD39pos
CD8_CD57posPD1pos		Treg_CD45RAposCD39neg
CD8_CD57negPD1pos		Treg_CD45RAposCD39pos

TABLE 7 - Systemic Inflammation Markers	
IL-2	IP-10
IL-7	MCP1
IL-10	MIP1a
G-CSF	VCAM-1
ICAM-1	VAP-1
Fractalkine	TNF-α

APPENDIX 5: Patient Diary

DIARIO CLINICO

NOME: _____ COGNOME: _____ DATA DI NASCITA:_____

CODICE IDENTIFICATIVO: _____

PER COMUNICAZIONI URGENTI CHIAMARE:_____

STUDIO CLINICO: **"Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 anziani"**

Numero EUDRACT: 2020-003872-42

Promotore: Istituto di Farmacologia Traslazionale – Consiglio Nazionale delle Ricerche

Sperimentatore Principale: Dott Emanuele Nicastri

Telefono: xxxxxxxxxxxx

GIORNO														
ORARIO														
TEMPERATURA CORPOREA														
SATURAZIONE														
FREQUENZA CARDIACA														
PRESSIONE														
FREQUENZA RESPIRATORIA														
NECESSITA OSSIGENO?														
È VIGILE?														

APPENDIX 6: Standard operating procedure for drug management

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MODALITÀ DI APPROVVIGGIONAMENTO E SOMMINISTRAZIONE DEL FARMACO REBIF TRAMITE INIETTORE REBISMART

Documento Redatto da: Luciano Castiello Firma e data _____

Documento Approvato da:

Ruolo	Nomimativo	Firma e Data
Sperimentatore Principale	Dr. Emanuele Nicastri	
Coordinatore Scientifico	Dr. Filippo Belardelli	
Project Manager - FullCRO	Dr.ssa Moira Cordisco	
ISS	Dr.ssa Eleonora Aricò	
Farmacia INMI "Lazzaro Spallanani	Dr.ssa Silvia Murachelli	

Lista di distribuzione:

Ruolo	Nomimativo	N° copie	Firma e Data
Sperimentatore Principale	Dr. Emanuele Nicastri	1	
Coordinatore Scientifico	Dr. Filippo Belardelli	1	
Project Manager - FullCRO	Dr.ssa Moira Cordisco	1	
ISS	Dr.ssa Eleonora Aricò	1	
Farmacia INMI "Lazzaro Spallanani	Dr.ssa Silvia Murachelli	1	

MODALITÀ DI APPROVVIGGIONAMENTO E SOMMINISTRAZIONE DEL FARMACO REBIF TRAMITE INIETTORE REBISMART

1. Scopo

La presente procedura descrive le operazioni che gli sperimentatori devono eseguire per recuperare il farmaco e per utilizzare correttamente l'autoiniettore Rebismart nella sperimentazione clinica ANTIICIPATE sia nel setting ospedaliero che in quello non-ospedaliero.

2. Abbreviazioni e definizioni

Farmaco: Rebif (Interferon beta-1a)

Farmacia: Farmacia dell'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani"

CRO: FullCRO

Promotore: Istituto di Farmacologia Traslazionale

3. Approvviggionamento del farmaco e dell'iniettore

Per i soli pazienti rientranti nel braccio di trattamento, il giorno previsto per l'inizio del trattamento l'investigatore o un suo delegato deve presentarsi presso la Farmacia e richiedere una dose di farmaco e un dispositivo per l'autoiniezione del farmaco utilizzando il modulo Allegato 1. Il modulo originale deve essere conservato dalla Farmacia fino al ritorno del dispositivo per l'autoiniezione, dopodichè il modulo viene conservato nella cartella clinica del paziente.

Per i soli pazienti non ospedalizzati, l'investigatore dovrà accertarsi di essere inoltre in possesso di:

- Sei aghi Serofine 29G, 30G o 31G;
- Un contenitore per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti);
- Un diario clinico del paziente (fornito dal Promotore);
- Uno sfingomanometro digitale, un termometro e un saturimetro per la misurazione dei parametri vitali (fornito dal Promotore);
- Un manuale d'uso del Rebismart (da usare in caso di necessità);
- Salviettine o tamponcini imbevuti di alcol o batuffoli di ovatta e alcol per frizione;
- Cerotti (classici e a rocchetto)

4. Attivazione e settaggio dell'iniettore Rebismart e caricamento cartuccia Rebif

4.1 Inserimento pile

In caso il dispositivo abbia le batterie scariche, prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Svitare la vite del coperchio dell'alloggiamento pile con un cacciavite e far scivolare verticalmente il coperchio. Inserire 4 pile al litio nuove. Verificare che siano orientate come mostrato sul dispositivo e stringere la vite del coperchio dell'alloggiamento delle pile per chiuderlo. Una sequenza illustrata della attività da eseguire è riportata nell'allegato 2.

4.2 Settaggio Rebismart

4.2.1 Settaggio cartuccia e dose

Prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Accendere RebiSmart e premere il pulsante 'Menu'. Scorrere su 'Impostaz. iniezione' e premere il pulsante 'Apri' per selezionare. Sarà visualizzata una schermata di avvertenza e selezionare 'Si'.
Selezionare 'Cartuccia' premendo su 'Cambia' e scorrere al dosaggio della cartuccia 22mcg. Premere 'OK' per selezionare e confermare la selezione.

Dal menù 'Impostaz. Iniezione' scorrere e selezionare 'Riduzione dose' e premere il pulsante 'Cambia' per selezionare. Inserire il codice PIN del dispositivo. Il dispositivo visualizzerà il menu 'Riduzione dose'. Selezionare '50% della dose'. Premere 'Ok' e confermare la selezione. Premere due volte il pulsante 'Esci' per tornare alla schermata informativa.

4.2.2 Settaggio ago

Prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Accendere RebiSmart. E premere il pulsante 'Menu'. Scorrere su 'Impostaz. pers.' e premere il pulsante 'Apri' per selezionare. Si apre la schermata di avvertenza e selezionare 'Si'. Selezionare 'Velocità ago' premendo su 'Cambia'. Selezionare velocità 'Media'. Premere 'OK' per selezionare.

Scorrere su 'Velocità iniez.' e premere il pulsante 'Cambia'. Selezionare velocità 'Media'. Premere 'OK' per selezionare. Scorrere in basso su 'Profondità iniez.' e premere il pulsante 'Cambia'. Selezionare '4 mm' e premere 'OK' per selezionare.

Scorrere in basso su 'Durata iniezione' e premere il pulsante 'Cambia'. Selezionare '3 secondi' premere 'OK' per selezionare. Premere due volte il pulsante 'Esci/Esci' per tornare alla schermata informativa.

Premere il pulsante 'Menu', scorrere su 'Impostaz. iniezione' e premere il pulsante 'Apri' per selezionare. Scorrere su 'Tipo di ago' e premere il pulsante 'Cambia' per selezionare. Selezionare il tipo di ago utilizzato. Premere 'OK' per selezionare. Premere due volte il pulsante 'Esci' per tornare alla schermata informativa.

4.3 Caricamento cartuccia

Prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Estrarre la cartuccia dal confezionamento secondario. Accendere RebiSmart tenendo premuto il pulsante 'Acceso' fino a che compare la schermata di "Benvenuto (Ciao)". Premere su 'Inizio' e aprire lo sportello dell'alloggiamento della cartuccia facendo scorrere verso l'alto il pulsante posto sul lato sinistro del dispositivo. Inserire la cartuccia di Rebif nell'alloggiamento cartuccia con la parte metallica rivolta verso il basso. Chiudere lo sportello dell'alloggiamento cartuccia fino ad udire un "clic". Una sequenza illustrata della attività da eseguire è riportata nell'allegato 3. Staccare dal confezionamento secondario la parte staccabile dell'etichetta e applicarla sul Rebismart..

5. Somministrazione farmaco

5.1 Preparazione somministrazione

Prima di iniziare, estrarre RebiSmart. dal frigorifero e dalla scatola di conservazione almeno 30 minuti prima dell'utilizzo previsto. Disporre, su una superficie stabile, come per esempio un tavolo, quanto segue:

- RebiSmart. contenente una cartuccia di Rebif in posizione verticale;
- Ago Serofine™ (29G, 30G o 31G, in base alla prescrizione);
- Salviettine o tamponcini imbevuti di alcol o batuffoli di ovatta e alcol per frizione;
- Piccolo cerotto;
- Contenitore per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti);

Sanitizzare i guanti accuratamente. Indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Accendere RebiSmart tenendo premuto il pulsante 'Acceso' fino a che compare la schermata di "Benvenuto (Ciao)". Premere il pulsante 'Inizio'.

Qualora RebiSmart visualizzi il messaggio "Meno di 48 ore dall'ultima iniezione. Procedere con l'iniezione", selezionare 'Si'. Prelevare un ago e rimuovere il sigillo di sterilità.

Inserire il cappuccio che contiene l'ago direttamente nell'alloggiamento dell'ago fino a che si blocca con un 'clic'. Togliere il cappuccio dell'ago spingendolo di lato fino a che non venga rimosso e conservare il cappuccio. Una sequenza illustrata della attività da eseguire è riportata nell'allegato 4.

5.2 Somministrazione farmaco

Posizionare RebiSmart sulla cute in posizione verticale nel sito di iniezione più idoneo (in base alle caratteristiche del paziente scegliere tra la parte esterna superiore delle braccia, la zona periombelicale dell'addome, la parte anteriore delle cosce). Assicurarsi che il sensore cutaneo sia completamente a contatto con la cute. Quando RebiSmart è posizionato correttamente sulla cute, la luce del pulsante di iniezione diventa verde e RebiSmart emette un bip.

Premere il pulsante per iniziare l'iniezione. La spia del pulsante di iniezione verde durante l'iniezione lampeggia. Tenere RebiSmart a contatto con la pelle per tutta la durata dell'iniezione.

Al termine dell'iniezione, la spia del pulsante verde si spegne e RebiSmart emette due bip.

Sollevare delicatamente RebiSmart dalla cute. Premere su 'OK' per confermare che l'iniezione è stata praticata correttamente.
Registrare sul diario clinico del paziente le informazioni sulla somministrazione.

5.3 Eliminazione ago e spegnimento Rebismart

Inserire il cappuccio vuoto direttamente nell'alloggiamento dell'ago fino a che si blocca con un 'clic'. RebiSmar. emette un bip. Premere e mantenere premuto il pulsante di rilascio dell'ago fino a che RebiSmart emette due bip. Togliere il cappuccio contenente l'ago spingendolo di lato fino a che si stacca per essere facilmente rimosso.
Controllare l'interno del cappuccio dell'ago per vedere l'ago rimosso. Gettare gli aghi usati nel contenitore per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti).

Premere e tenere premuto il pulsante 'Spento' fino a che RebiSmart si spegne e la schermata informativa si chiude.

6. Conservazione del farmaco e dell'iniettore

Al termine dell'iniezione riposizionare RebiSmart in posizione verticale nella sua custodia all'interno del frigorifero.

7. Recupero dell'iniettore e riconsegna

Al termine della quarta iniezione, eliminare la cartuccia in uso. Prima di spegnere Rebismart, premere il pulsante 'Menu'. Scorrere in basso su 'Rimuovere cartuc.' e premere il pulsante 'Apri'. Premere su 'Si' per confermare la selezione. Attendere che Rebismart visualizza il messaggio 'Aprire sportello alloggiamento cartuccia' ed emette due bip. Far scorrere verso l'alto il pulsante dello sportello alloggiamento cartuccia e rimuovere la cartuccia. Gettare la cartuccia nel contenitore

per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti). Selezionare 'No' sul Rebismart e spegnerlo. Rimuovere il confezionamento secondario dalla custodia del Rebismart.

Riconsegnare il Rebismart alla Farmacia, registrando la consegna sul modulo Allegato 1 utilizzato per il ritiro.

8. Allegati

Allegato 1: Modulo richiesta ritiro/riconsegna Rebif e Rebismart

Allegato 2: Schema dei passaggi da effettuare per la sostituzione pile

Allegato 3: Schema dei passaggi da effettuare per l'inserimento di una cartuccia di Rebif

Allegato 4: Schema dei passaggi da effettuare per somministrare il farmaco

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MODULO RICHIESTA RITIRO/RICONSEGNA REBIF E REBISMART

DATI RICHIEDENTE

NOME E COGNOME:_____ UNITÀ: INMI USCAR

RICHIEDE n° 1 cartuccia/e Rebif 66 mcg e n° 1 dispositivo Rebismart per effettuare il trattamento al paziente

Codice _____ **Data di Nascita:** _____

DATA:_____ FIRMA: _____

Spazio da compilare a cura della Farmacia

Numero lotto Rebif:_____ Data di scadenza:_____

Numero seriale cartuccia:_____

Identificativo Rebismart:_____

Nome e Cognome:_____ Data:_____ Ora:_____

Firma:_____

Spazio da compilare alla riconsegna del dispositivo Rebismart a cura della Farmacia

Identificativo dispositivo Rebismart riconsegnato: _____

Nome e Cognome:_____ Data:_____ Ora:_____

Firma:_____

SCHEMA DEI PASSAGGI DA EFFETTUARE PER LA SOSTITUZIONE PILE

Svitare la vite del coperchio dell'alloggiamento pile con un cacciavite.

Afferrare il coperchio sui due lati e farlo scivolare via.

Inserire 4 pile al litio nuove. Verificare che siano orientate come mostrato sul dispositivo.

Far scorrere il coperchio dell'alloggiamento delle pile nella posizione di chiusura, verificando che entri nelle fessure.

Stringere la vite del coperchio dell'alloggiamento delle pile per chiuderlo.

SCHEMA DEI PASSAGGI DA EFFETTUARE PER L'INSERIMENTO DI UNA CARTUCCIA DI REBIF

Accendere RebiSmart® tenendo premuto il pulsante **'Acceso'** fino a che compare la schermata di **"Benvenuto (Ciao)"**, in genere dopo 3-5 secondi.

Premere su **'Inizio'** e aprire lo sportello dell'alloggiamento della cartuccia facendo scorrere il pulsante verso l'alto.

Inserire una nuova cartuccia di Rebif® nell'alloggiamento cartuccia, verificando che la parte metallica sia rivolta verso il basso.

Chiudere lo sportello dell'alloggiamento cartuccia fino ad udire un "clic".

SCHEMA DEI PASSAGGI DA EFFETTUARE PER SOMMINISTRARE IL FARMACO

Accendere il dispositivo premendo e tenendo premuto il pulsante **'Acceso'** fino a che compare la schermata di **"Benvenuto (Ciao)"**, in genere dopo 3-5 secondi.

Premere il pulsante **'Inizio'**.

Verificare che la misura in gauge (G) indicata sulla scatola degli aghi Serofine™ corrisponda a quella indicata nella schermata di RebiSmart®.

Inserire il cappuccio che contiene l'ago direttamente nell'alloggiamento dell'ago fino a che si blocca con un 'clic'.

Togliere il cappuccio dell'ago spingendolo di lato fino a che venga rimosso.

SCHEMA DEI PASSAGGI DA EFFETTUARE PER SOMMINISTRARE IL FARMACO

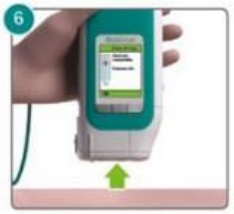
Posizionare RebiSmart® sulla cute in posizione verticale nel sito di iniezione preparato come indicato dal medico o dall'infermiere.

Quando RebiSmart® è posizionato correttamente sulla cute, la luce del pulsante di iniezione diventa verde e RebiSmart® emette un bip. 1

Premere il pulsante per iniziare l'iniezione.

La spia del pulsante di iniezione verde durante l'iniezione lampeggia. Tenere RebiSmart® a contatto con la pelle per tutta la durata dell'iniezione. Non è necessario mantenere premuto il pulsante di iniezione.

Al termine dell'iniezione, la spia del pulsante verde si spegne e RebiSmart® emette due bip. 2



Sollevare delicatamente RebiSmart® dalla cute.

Premere su 'OK' per confermare che l'iniezione è stata praticata correttamente.

SCHEMA DEI PASSAGGI DA EFFETTUARE PER SOMMINISTRARE IL FARMACO

CLINICAL STUDY PROTOCOL

Study Title: Antiviral and Immunomodulatory Interferon-Beta in high-risk COVID-19 patients

Short title: (ANTiviral and **I**mmunomodulatory **I**nterferon-Beta in high-risk **C**ov**I**d-19 **PAT**i**E**nts**)**
ANTIICIPATE

EudraCT N°: 2020-003872-42

Sponsor: Institute of Translational Pharmacology (IFT), National Research Council (CNR)

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Investigational Product Interferon β1a (Rebif™)

Clinical Study Phase: II

Version: 4.0

Issue Date: 31/05/2021

Protocol Signature form

Protocol Title:

Antiviral and Immunomodulatory Interferon-Beta in high-risk COVID-19 patients (ANTIICIPATE)

Version: 4.0

Version Date: 31/05/2021

I have read the protocol described below and agree to conduct this study in accordance with procedures described therein. I also agree to conduct the study in compliance with all applicable regulations.

_____

Sponsor coordinator's printed name

Giuseppe Sconocchia, MD

_____

Principal Investigator's printed name

Emanuele Nicastri, MD

Date: 31/05/2021

Summary

List of abbreviations

AE	Adverse event
AIFA	Italian medicines agency
ALT	Alanine AminoTransferase
ANCOVA	Analysis of CoVariance
ANOVA	Analysis of Variance
AVPU	Alert, Verbal, Pain, Unresponsive Score
AST	Aspartate AminoTransferase
CT	Coordination Team
CTCAE	Common Terminology Criteria for Adverse Events
CIM	Cellular Immune Monitoring
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CNR	Consiglio Nazionale delle Ricerche
COVID-19	Corona Virus 19 Disease
CRO	Contract Research Organization
CRP	C-Reactive Protein
EC	Ethical Committee
eCRF	electronic Case Report Form
eGFR	estimated Glomerular Filtration Rate
ELISA	Enzyme Linked ImmunoSorbent Assay
FFP	Filtering Face Mask
FKN	Fractalkine
GCP	Good Clinical Practice
Hb	Haemoglobin
ICAM-1	Intercellular Adhesion Molecule 1
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IEC	Independent Ethic Committee
IFT	CNR Institute of Translational Pharmacology
IFN	Interferon

IL-6	Interleukin-6
ITT	Intention To Treat
LDH	Lactate DeHydrogenase
LSRCHs	long-stay residential care homes
INMI	Istituto Nazionale Malattie Infettive
ISG	Interferon Stimulated Genes
ISS	Istituto Superiore di Sanità
IU	International Units
MAR	Missing At Random
MERS	Middle East respiratory syndrome
MFC	Multiparametric Flow Cytometry
MS	Multiple Sclerosis
NCI	National Cancer Institute
NEWS2	National Early Warning Score 2 (2017)
NK	Natural Killer
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PP	Per Protocol
RCP	Riassunto delle Caratteristiche del Prodotto
RT-PCR	Real Time - Polymerase Chain Reaction
SAE	Serious Adverse Event
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	SARS Corona Virus
SARS-Cov 2	New Corona Virus
SC	Steering Committee
SIM	Systemic Inflammatory Markers
SOCS	Suppressor of cytokine signaling
SpO_2	Oxygen Saturation
SUSAR	Suspected Unexpected Serious Adverse Reactions
TNF	Tumor Necrosis Factor

USCAR	Special Unit for regional continued care
VCAM-1	Vascular Cell Adhesion Molecule 1
VPA-1	Vascular Adhesion Protein 1
UBP43	Ubiquitin Protease 43
WBC	White Blood Cells
WHO	World Health Organization

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Administrative clinical research support on inter-institutional agreements, material transfer agreements, institutional tenders.

This is an investigator-initiated study. The Steering Committee will take responsibility for study design and data analysis and will operate actions necessary to guarantee that the trial is conducted in accordance with procedures described in this document and good clinical practice. The study is partially funded by Merck. Merck has no role in study design, data collection, management, analysis, data interpretation, manuscript writing, or in the decision to submit manuscripts for publication.

The Steering committee will include at least one representative from all units participating to the study and will be chaired by the Scientific Coordinator of the study (Filippo Belardelli) with the cooperation of a coordination team (Giuseppe Sconocchia, Emanuele Nicastri, Ombretta Papa, Nicola Vanacore, Eleonora Aricò, Luciano Castiello). The Steering committee will oversee all the aspects of the project's life: decision about safety, decision for stopping rule, diagnostics issues, capacity development, financial, schedule, partnership, dissemination and exploitation. The Steering committee will hold at least one meeting a week on teleconference. In addition, extraordinary sessions will be held in case of critical issues.

1. Synopsis

1.1 BACKGROUND

The rapid and devastating outbreak of Coronavirus disease 2019 (COVID-19) pandemic highlighted the urgent need of developing therapeutic options to control or prevent virus spreading. In this regard, priority should be given to the repurposing of existing antiviral agents, thus shortening the timelines needed for clinical experimentation while exploiting the clinical experience with other viral infections (1). Among the many drugs under evaluation all over the world, Interferon (IFN)-α and β stirred renewed interest against COVID-19 and are presently being evaluated in clinical trials at different dosages and by different delivery systems, either as monotherapy or in combination with other compounds. Notably, IFN-β proved effective in alleviating COVID-19 symptoms when used in combination with lopinavir and ritonavir (2) and in reducing mortality when combined with hydroxychloroquine and other antivirals (3).

IFN-α and β, thereafter referred to as type I IFN (IFN-I), are cytokines with a long record of clinical use in patients with infectious disease (4), multiple sclerosis, and cancer (5). They are pleiotropic factors endowed with multiple activities, including both a broad spectrum antiviral activity and a remarkable immunoregulatory function (6). IFN-I are expressed at very low levels under basal physiological conditions, while they are generally abundantly produced in response to virus infections, when they play a crucial role in limiting viral replication and spread (7). In fact, many viruses, including Coronaviruses, evolved evasion strategies to counteract IFN-I system activation (8,9).

An ensemble of studies, some of them carried out in the proponents' laboratories, have revealed that in addition to the antiviral activity, optimally achieved in the first phase of infection, IFN-I exhibit important immunoregulatory effects, including the increase of neutralizing antibodies and the induction of both innate and adaptive cellular immunity (10–15).

While the majority of SARS-CoV 2 infected individuals are capable of clearing the virus solely with their own immune response, approximately 20% develops severe COVID-19. Notably, at higher risk of severe COVID-19 are males, people aged >65 years and/or showing some comorbidities (like hypertension and diabetes). An age-related impairment of endogenous IFN-I induction in response to viral infection has been described (16). Data on animal models on SARS-CoV (17,18) and data emerging from COVID-19 pandemic (19–22) point out to endogenous IFN-I system as a key player

to control early phases of viral replication and prevent disease progression. Moreover, delayed IFN-I signaling activation can contribute to the exacerbation of SARS-CoV hyperinflammation and subsequent viral pathogenesis (20,23,24).

In the light of these considerations and evidences, we hypothesize that patients aged 50 years or older will greatly benefit from a short term IFN-β1a administration at the earliest time of SARS-CoV 2 diagnosis, thus compensating the insufficient or impaired endogenous IFN-I production.

In these patients, the antiviral and immunomodulatory effects of this cytokine could be efficiently exploited against COVID-19 through a short-term, discontinuous treatment with IFN-β1a in the early phases of infection, thus minimizing the relevant side effects (refractoriness and toxicity) associated to IFN continuous treatment schedules.

1.2 Objectives

This trial aims at exploring the efficacy of IFN-β1a in reducing the risk of SARS-CoV 2 recently infected elderly patients to progress towards severe COVID-19. In particular, this study will evaluate the consequences of a low and discontinuous use of IFN-β1a in the early phase of infection, and to exploit its immune activating properties in addition to its antiviral effects. Such regimen is expected to prevent any toxicity and refractoriness phenomena often occurring during IFN-I chronic administration.

Primary Objective of the study is to evaluate the role of IFN-β1a in reducing the disease progression in treated patients versus control group.

Secondary Objectives of the study are: 1) to assess the reduction in ICU admission in patients treated with IFN versus control group; 2) to assess the reduction in number of deaths in IFN compared to control group; 3) to evaluate the increase in proportion of participants returning to negative SARS-CoV 2 RT-PCR in IFN-treated *versus* control group at Day 14 and Day 28; 4) To assess the increase in SARS-CoV 2-Specific Antibody Titers in IFN-treated compared to control group; 5) to assess the safety of IFN-treated patients.

1.3 Methodology

Randomized, Open-Label, Controlled, Phase II Study. The study plans to enroll 60 patients: 40 in the IFN-β1a arm, 20 in the control arm, according to a 2:1 - treated: untreated ratio. Treatment plan foresees 4 subcutaneous injections of 3MIU of IFN-β1a, to be given at day 1, 3, 7 and 10 in addition to standard of care. Patients will be monitored and disease progression will be evaluated by means of the National Early Warning Score (NEWS2).

1.4 Expected results

Data emerging from the ongoing pandemic show that the management of advanced stage COVID-19 is mostly critical for elderly patients. This study is expected to provide information about the efficacy of a timely administration of IFN-β to elderly patients in achieving a more efficient control of SARS-CoV 2 infection, thus preventing the progression towards severe forms of the disease. The results of this study will provide a treatment option for high-risk elderly patients experiencing mild symptoms, for which no approved therapy is available so far (besides support therapy and a strict clinical monitoring).

The proposed treatment, upon demonstration of efficacy and safety, could be administered not only to hospitalized patients, but also during isolation at home or in long-stay residential care homes (LSRCHs), with the support of the territorial medical units. Therefore, this treatment protocol will represent an important tool to protect the elderly population in every pandemic scenario that will occur in the near future.

2. Background

The rapid and devastating outbreak of Coronavirus disease 2019 (COVID-19) pandemic and the lack of approved treatments for any human coronavirus (CoV) infection highlighted the urgent need of developing therapeutic options to control or prevent virus spreading. Several options can be envisaged ranging from prophylactic vaccine to targeted antiviral drugs. However, new interventions are likely to require months to years to be developed, and priority is being given to the repurposing of existing antiviral agents (1). Since COVID-19 outbreak, more than 3000 clinical trials have been authorized to identify the drugs or drug combinations capable of attenuating the

virulence of the disease (25). Some of these trials include the use of type I Interferons (IFN-I), mainly α and β, alone or in combination with other compounds. Interestingly, a randomized clinical trial testing the combination of Lopinavir, Ritonavir plus IFN-β in COVID-19 patients showed that only the triple combination was effective in alleviating symptoms and shortening the duration of viral shedding and hospitalization (2). A significant reduction of mortality was observed when IFN-β was administered together with hydroxychloroquine and other antivirals (3). More recently, treatment with IFNβ1a (in combination with Lopinavir/Ritonavir as standard treatment) was reported to significantly reduce the time to clinical improvement in hospitalized COVID-19 patients aged 55-82 years (26). Notably, data suggest that the timing of IFN therapy during SARS-CoV 2 infection can determine treatment efficacy and clinical outcome (27).

IFN-I were first discovered and characterized more than 60 years ago as antiviral substances produced by influenza virus-infected cells, capable of markedly inhibiting viral replication in target cells (28). These cytokines were the firsts to be cloned and extensively used in patients with some viral diseases (29) and cancer (IFN-α) (5). IFN-I are pleiotropic factors endowed with multiple activities, including both a broad-spectrum antiviral activity (28,29) and a remarkable immunoregulatory function (6). The antiviral activity of IFN-I has been extensively exploited for the treatment of viral chronic infections (29) Nevertheless, as highlighted by the long clinical records of IFN-I use, caution is required in terms of route, timing and dose of administration to balance clinical efficacy and side effects.

As many other viruses, Coronaviruses have developed multiple mechanisms to prevent IFN-I induction and subsequent signaling (30), particularly during the early phase of infection, ultimately leading to a dysregulated immune response and increased immunopathogenesis (20,31,32). Diminished levels of IFN-I have been detected in patients during the course of SARS and MERS (33–35). Similar results were also achieved with aged macaques infected with SARS-CoV, that exhibited considerably lower levels of IFN-β and a more severe pathology than young animals (17). Interestingly, when the deficiency in IFN-I production in CoV-infected macaques was remedied by IFN-α2 treatment in combination with ribavirin, lower levels of systemic (serum) and local (lung) proinflammatory markers were observed, in addition to fewer viral genome copies and less severe histopathological changes in the lungs (18). More relevantly, the results of a recent work clearly showed an impaired IFN-I signaling, associated with persistent blood virus load and an exacerbated inflammatory response in patients with severe COVID-19 (36). Impaired IFN-I response was also

observed in young men experiencing severe COVID-19, in which a loss-of function genetic mutation in Toll Like Receptor 7 caused impaired IFN-I response (21). Among the studies exploring the reasons behind the heterogeneity of COVID-19 different outcomes, two papers from the group of Jean-Laurent Casanova and many collaborators worldwide pointed out that endogenous individual factors linked to an impaired IFN-I response account for patients susceptibility to severe COVID-19 (37,38).

Overall, these observations outline the critical role of IFN-I in both protective and pathogenic events during CoV infections, thus strengthening the need of fine tuning the IFN-I signaling with respect to the kinetics of CoV replication for an optimal protective response.

3. Rationale

In the light of the current information on SARS-CoV 2 pathogenesis, we speculate that the majority of SARS-CoV 2- infected patients are capable of clearing the virus by means of their effective endogenous IFN-I system and do not require hospitalization. We assume that in a minority of people a defective IFN-I system may favor SARS-CoV 2 spread, eventually causing the development of severe forms of COVID-19 and dismal prognosis. People aged >65 years, for which an impairment of IFN-I induction in response to viral infection has been documented (16,39,40), are at higher risk of severe COVID-19 (41). Nevertheless, an endogenous impairment of IFN-I system can be triggered by SARS-CoV 2 infection at any age, and it can account for patients progression towards severe forms of COVID-19 (37,38,42).

In these patients, a delayed IFN-I response and the loss of viral control might contribute in early phases of infection to disease outcome. Data suggest that the IFN-β subtype appears to be the most suited for COVID-19 treatment (43). Thus, we hypothesize that patients aged 50 years and older will greatly benefit from a short term IFN-I administration at the earliest time of SARS-CoV 2 infection, thus compensating the insufficient or impaired endogenous IFN-I production and preventing COVID-19 progression to severe forms of disease. In light of its immunomodulatory properties, IFN-β administered at the early phases of infection can represent a valuable tool to enhance humoral and cellular immunity in addition to its direct antiviral treatment restricting early viral spread, thus halting virus replication and preventing the progression towards severe forms of disease.

4. Impact for the National Health System

Italy was the first European country to experience COVID-19 pandemic, when the information about viral pathogenesis and therapeutic options were scarcely available. Moreover, Italian demographic structure, with a high percentage of population above 65 years of age, greatly affected the outcome and the death toll of the first epidemic wave. In fact, data show that not only sex and comorbidities, but also age increases the risk of developing severe COVID-19 (41,44) needing hospitalization and intensive care support. Since the first case, recorded in Italy on February 21th, COVID-19 represented a big challenge for the Italian National Health System, which underwent an increasing pressure until restriction measures were undertaken to avoid its collapse. However, the interruption of non-essential economic and social activities has a serious impact on global economy and people quality of life in the long term. For elderly people, isolation can result not only in increased risks of cardiovascular, autoimmune and neurocognitive disorders, but also induce or exacerbate mental health problems, such as depression and anxiety. The release of the restriction measures and the restart of the economic activitiesrequired some strategies to be undertaken to keep an acceptable risk for all population. A reinforced surveillance system was developed and is currently in use to ensure a prompt diagnosis of new cases. Moreover, although the vaccination campaign is rapidly progressing, in Lazio region, there is still 50% of the target population awaiting to be vaccinated (source: www.salutelazio.it). Of note, the vaccination program prioritized elderly and people with comorbidities. For these reasons, , it is urgent to develop and test new treatment options that can be administered during the early infection to reduce viral shedding, and consequent contagion, and to hamper disease progression toward severe forms, thus diminishing the impact on the National Health System.

In this trial, particular attention is given to patients aged ≥50 years with a recent diagnosis of COVID-19 in the presence of mild symptoms. In these patients, a strict medical control during home isolation, or a precautionary hospitalization are both appropriate choices, to monitor the possible rapid evolution of the infection. However, no therapeutic regimen specifically designed for these patients is available. Therefore, the risk of developing severe forms of the disease requiring intensive care or ending in fatalities is still high.

This trial will test the efficacy of IFN-β administered to patients aged ≥ 50 years during the early phase of the infection, in limiting viral replication and preventing the evolution of COVID-19 towards

severe and critical diseases. Individual infectivity is directly associated with disease severity and time of viral shedding. Moreover, preventing severe COVID-19 will directly reduce lethality and will immediately mitigate the hospitals overworking, thus overall reducing the potential impact of COVID-19 on the National Health System.

5. Objectives of the study

This trial aims at exploring the use of IFN-β1a in SARS-CoV 2 newly diagnosed patients aged ≥ 50 years with increased risk of developing severe COVID-19. In particular, this study will evaluate low-dose and discontinuous use of IFN-β1a in the early phase of infection, in order to exploit not only its antiviral, but also its immune activating and anti-inflammatory properties. Such regimen should avoid any toxicity and refractoriness phenomena often occurring during IFN-I chronic administration.

5.1 Primary Objective

Primary Objective of the study is to evaluate the reduction in disease progression in patients treated with IFN versus control group within 28 days.

5.1.1 Primary endpoint and outcome

Primary endpoint of the study is the proportion of patients experiencing a disease progression, during at least 5 days, according to the National Early Warning Score **(NEWS2).** The **NEWS2** score is a standardized approach aimed at promptly detecting signs of clinical deterioration in acutely ill patients and establishing the potential need for higher level of care. It is based on the evaluation of vital signs including respiratory rate, oxygen saturation, temperature, blood pressure, pulse/heart rate, AVPU response. The resulting observations, compared to a normal range, are combined in a single composite “alarm” score. Any other clinical sign clearly indicating a disease worsening will be considered as disease progression.

5.2 Secondary Objectives and Endpoints

The following table 1 contains the secondary objectives and endpoint of the study

Objective	Endpoint

1) To assess the reduction in ICU admission in patients treated with IFN versus control group within 28 days of randomization	ICU-free days at 28 days (Day 1 through Day 28)
2) To assess the reduction in number of deaths in IFN compared to control group (day 28)	All-cause mortality (Day 1 through Day 28)
3) To evaluate the increase in proportion of participants returning to negative SARS-CoV 2 RT-PCR in IFN-treated versus control group at Day 14 and Day 28	Negative SARS-CoV 2 RT-PCR at day 14 post-randomization Negative SARS-CoV 2 RT-PCR at day 28 post-randomization
4) To assess the increase in SARS-CoV 2-Specific Binding Antibody Titers in IFN compared to control group (day 28)	Change from Baseline in SARS-CoV 2-Specific Binding Antibody Titers at day 14 and 28
5) To assess the safety of IFN-treated patients versus control group	Incidence of adverse events

For secondary endpoints, more detailed descriptions follow:

1) ICU-free days at 28 days will be calculated as the number of days a patient is not in an ICU. Time Frame will be: Admission (day 0) to 28 days after admission (day 28). In case of death, it will be counted as 0 day;

2) All-cause mortality will be: total number of death events occurring within day 0 and day 28;

3) Negative SARS-CoV 2 RT-PCR is defined as an undetectable presence of SARS-CoV 2 genes, as determined by PCR on an adequate sampling of upper respiratory tract.

4) Change from Baseline in SARS-CoV 2-Specific Binding Antibody Titers is defined as the difference in anti-SARS-CoV 2-specific antibody levels measured at day 28 versus day 0;

5) Details on safety event are described in paragraph 9.4

5.3 Exploratory Endpoints

Exploratory studies will be also performed on blood samples collected before and after treatment to assess:

- IFN-I signaling activation

- Cellular immune monitoring
- Systemic inflammatory markers

5.3.1 IFN-I Signaling

Pioneer studies in animal models showed that the complete absence of IFN-I signalling, by deletion of IFN-I receptor, enhanced mice susceptibility and mortality from several viral infections (7). IFN-I signalling downregulation may occur during viral infections as a consequence of viral-specific evasion mechanisms that Coronaviruses mainly establish during the early phase of infection (30,32). Diminished levels of IFN-I or Interferon Stimulated Genes (ISG) expression have been detected in the peripheral blood mononuclear cells of SARS and MERS patients (33,34). More relevantly, the results of a very recent work clearly showed an impaired IFN-I signaling, associated with persistent blood virus load and an exacerbated inflammatory response in patients with severe COVID-19 (36). A diminished level of endogenous IFN-I activation and signalling may also occur as a consequence of aging, as reported in several *in vitro* and *in vivo* settings (17,45). In light of these considerations, the level of expression of selected ISG will be analysed in patients PBMC as surrogate markers of IFN-I signalling activation. Samples will be collected before, during and after the completion of IFN-β1a treatment in order to assess 1) possible correlations between IFN-I activation status and patient clinical outcome *per se*; 2) treatment-induced modifications of IFN-I signalling activation possibly associated with clinical improvement.

5.3.2 Cellular Immune-Monitoring

A decrease in peripheral lymphocyte count (with lower frequencies and absolute counts of CD3, CD4, CD8 T cells as well as of NK subsets) and an inflammatory cytokine storm may be the main reasons for rapid disease progression and poor treatment response in severe COVID-19. The neutrophil-to-CD8+ T cell ratio and the neutrophil-to-lymphocyte ratio were identified as prognostic factors affecting the prognosis for severe COVID-19 (46). Besides quantitative alteration, T cell maturation status was found to be modified since the percentage of naïve helper T cells increases and memory helper T cells decreases in severe cases. Patients with COVID-19 have also low levels of regulatory T cells, showing damaged features in severe cases (47). In general, COVID-19 patients show marked T cell activation, senescence, exhaustion and skewing towards Th17, if compared to healthy subjects (48).

The innovative technology MFC will help in elucidating the immunomodulatory *in vivo* effect of IFN β1a treatment. Leukocyte subpopulation frequency, activation status and functionality will be explored in pre- and post-treatment patients' blood samples. MFC results will be correlated with clinical outcome in order to identify potential peripheral immune markers of response to treatment.

5.3.3 Systemic inflammation

It was reported that in some COVID-19 patients, the immune response elicited against SARS-CoV 2 results in an increase in systemic inflammatory cytokines, which may eventually progress to a "cytokine storm," followed by multi-organ system dysfunction (49). In fact, some of the severe manifestations of COVID-19 are linked to the excess of circulating pro-inflammatory cytokines: acute respiratory distress syndrome, thromboembolic diseases such as acute ischemic strokes caused by large vessel occlusion and myocardial infarction, encephalitis, acute kidney injury, and vasculitis (50). The chronic activation of pro-inflammatory pathways documented in the elderly, especially men, and named "inflamm-aging", represents a risk factor *per se* for the development of COVID-19 complications (51).

We believe the restoration of a functional IFN-I response, through the administration of IFN-β1a during the early phase of SARS-CoV 2 infection, may affect systemic hyper inflammation both directly, by means of the immunomodulatory properties of the cytokines, and indirectly as an effect of reduced SARS-CoV 2 replication.

The level of inflammatory markers known to have a prognostic role in COVID-19 progression, such as IL-6, CRP, TNF-α (52) together with some endothelial cell adhesion molecules whose expression levels correlate with COVID-19 severity (FKN, VCAM-1, ICAM-1, VAP-1 (53)), will be analysed in the blood collected from IFN and control arm before and 10 days after enrolment. Data will be integrated with the results of routine lab analysis on coagulations factors (Fibrinogen, D-dimers) also involved in COVID-19 pathogenesis. The comparative analysis between groups will address treatment-induced modulations and possible correlation with clinical outcome.

5.4 Statistical hypothesis

The trial power has been calculated by the ISS group. The study was powered to independently assess a potential benefit of IFN-β1a compared with control arm (no specific antiviral treatment besides standard of care) on rate of progression of NEWS2 score lasting more than 5 days.

Sample size was calculated according to the primary endpoint of the study. In particular, the sample size calculation is based on the assumptions of an at least 35% difference in the percentage of patients undergoing disease progression between IFN-β1a and control arm. A sample size of 60 patients total (40 in the IFN-β1a-treated arm and 20 in the control arm, according to a 2:1 randomization ratio) will be needed to provide 80% power at significance level of 5% to detect the difference of patients undergoing disease progression between a group 1 proportion of 0.15 (IFN-β1a + standard of care) and a group 2 proportion of 0.50 (standard of care).

Sample Size: ANTIICIPATE trial

Two-sided significance level (1-α)	95
Power (1-β, % chance of detecting)	80
Ratio of sample size, Unexposed/Exposed	0.5
Percent of Unexposed with Outcome	50
Percent of Exposed with Outcome	15
Risk Ratio	0.3
Risk difference	-35

	Kelsey	**Fleiss**	**Fleiss (CC)**
Sample Size - Exposed	39	40	48
Sample Size-Unexposed	20	20	24
Total sample size	59	**60**	72

6. Study design

Randomized, Open-Label, Controlled, Phase II Study. Patients, who satisfy all inclusion criteria and no exclusion criteria, will be randomly assigned to one of the two treatment groups in a ratio 2:1. Randomization will be stratified by gender. Stratified randomization will balance the presence of male and female in both study arms. The planned study duration is 12 months including study set up, enrollment, follow up and data analysis as indicated in Appendix 3.

7. Study Population

Male and female adults aged 50 years or older with newly diagnosed mild COVID-19 are eligible for the study.

7.1 Case definition

For the purpose of the study, the following definition is applied: a case of COVID-19 is a person with detectable SARS-CoV 2 genes, as determined by PCR or third generation antigenic test (immunofluorescence with microfluidic reading) if COI >10 on an adequate sampling of upper respiratory tract.

7.2. Criteria for eligibility

7.2.1 Inclusion criteria

- ≥ 50 years of age at time of enrolment;
- Laboratory-confirmed SARS-CoV 2 infection as determined by PCR or third generation antigenic test (immunofluorescence with microfluidic reading) if COI >10, in any specimen ≤ 5 daysprior to randomization;
- Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures;
- Understands and agrees to comply with planned study procedures;
- Agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol;
- Being symptomatic for less than 7 days before starting therapy;
- NEWS2 score ≤2

7.2.2 Exclusion criteria

- Hospitalized patients with illness of any duration, and at least one of the following:

  -Clinical assessment (evidence of rales/crackles on exam) AND SpO2 ≤ 94% on room air at rest or after walking test,

  OR

  -Acute respiratory failure requiring mechanical ventilation and/or supplemental oxygen;

- Patients currently using IFN-beta (e.g., multiple sclerosis patients);
- Patients undergoing chemotherapy or other immunosuppressive treatments
- Patients with chronic kidney diseases;
- Known allergy or hypersensitivity to IFN (including asthma);
- Any autoimmune disease (resulting from patient anamnesis);
- Patients with signs of dementia or neurocognitive disorders;
- Patients with current severe depression and/or suicidal ideations;
- Being concurrently involved in another clinical trial;
- HIV infection (based on the anamnesis);
- Use of any antiretroviral medication;
- Impaired renal function (eGFR calculated by CKD-EPI Creatinine equation < 30 ml/min);
- Pregnant or lactating females;
- Women of childbearing potential based on the presence of ≥1 menstrual cycle 12 months before randomization
- Presence of other severe diseases impairing life expectancy (e.g. patients are not expected to survive 28 days given their pre-existing medical condition);
- Any physical or psychological impediment in a patient that could let the investigator to suspect his/her poor compliance;
- Lack or withdrawal of informed consent

7.3 Recruitment strategy

The management of elderly patients with COVID-19 needs to take into consideration the presence of comorbidities that increases their fatality risk, but it is also affected by the epidemiological situation of SARS-CoV 2 infection (see Feasibility section). Our study plans to enroll either hospitalized and non-hospitalized newly diagnosed COVID-19 patients, as well as patients hosted in long-stay residential care homes.

The Special Unit for regional continued care (USCAR), having the role of early detecting clusters of infection within Regione Lazio, will be responsible for screening and enrolling eligible patients that after SARS-CoV 2 positivity notification are not hospitalized, but remain in isolation at home or in a long-stay residential care homes. When dealing with patients older than

50, USCAR will be responsible of informing the patient of the current study, of having the Informed Consent signed and of collecting the blood sample to assess eligibility criteria. After the enrolment, the patient will be followed by a dedicated USCAR team that will: i) give to the patient/family-caregiver the kit of devices for home monitoring (i.e., 1 pulse oxymeter, 1 digital sphygmomanometer, 1 thermometer), ii) perform training for the use of devices, iii) perform treatments and collect samples for monitoring patients according to the timeline described in Appendix 2. The USCAR team will receive daily updates from non-hospitalized patients to determine their NEWS2 score values.

Patients that, at discretion of the general practitioner, are directed to Spallanzani Hospital for hospitalization, will be there assessed for inclusion/exclusion criteria and, in case of eligibility, enrolled in the study. Patients will be monitored according to standard hospital protocol in addition to the timeline described in Appendix 2.

8. Intervention

8.1 Experimental Drug and justification for dose

Rebif® (interferon beta-1a) is a disease-modifying drug used to treat relapsing forms of multiple sclerosis (MS) and is similar to the IFN-beta protein produced by the human body. It was approved in Europe in 1998 and it is used in more than 90 countries worldwide. While current posology of Rebif in MS (12 MIU 3 times/week) is capable of balancing the neural inflammation typical of MS, the dosing and schedule of Rebif® administration in this study were selected by taking into consideration some features of IFN-I, emerged from many years of clinical use of these cytokines. In fact, several clinical studies reported that an Interferon-induced immune adjuvant activity could be observed already after the administration of intermittent low doses of the cytokine in both cancer and antiviral settings (15,54–56). Instead, the continuous stimulation of IFN-I signaling, exerted by high serum levels of the cytokine, can result in diminished treatment efficacy due to the emergence of refractoriness phenomena caused by receptor internalization/degradation as well as the rapid induction of UBP43 and SOCS negative regulators (4), immunosuppression and can also result in relevant side effects.

With the aim to tailor the treatment schedule to the early phase of SARS-CoV 2 infection in elderly patients, we selected 3 MIU of IFN-β1a as a dose expected to exploit IFN-mediated antiviral and immunomodulatory properties of the cytokine without causing relevant toxicity or inducing refractoriness phenomena (57).

8.2 Treatment arms

Control arm. No specific antiviral treatment besides standard of care.

Treatment arm. 11ug (3MIU) of IFN-β1a will be injected subcutaneously at day 1, 3, 7, and 10 in addition to standard of care. The drug solution, contained in a pre-filled cartridge, will be injected by means of the RebiSmart electronic injection device, as described in Appendix 6.

8.3 Standard patients monitoring

Patients will be daily evaluated for body temperature, respiratory rate, oxygen saturation, blood pressure, pulse/heart rate and AVPU response. The NEWS2 score will be then calculated following the table 2. Additional measurements are allowed whenever any sign of disease progression appears. In case of multiple measurements within a day, the highest score will be considered for patient assessment.

Table 2. NEWS2 Score

Chart 1: The NEWS scoring system

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

For non-hospitalized patients, measurements will be auto-performed by the patient either assessed by a caregiver or a family member. Training on how to use the provided devices will be performed by USCAR unit at T1, written instructions will be also provided, and additional help will be given upon request by phone or videocall. Measurements will be recorded on the clinical diary that will be provided (Appendix 5). Patients will be contacted daily by USCAR dedicated unit and will communicate by phone their health status that will be registered on a dedicated clinical records form (Appendix 5). USCAR unit approaching COVID-19 patients will use personal protective equipment including a FFP3 (or FFP2) mask, gloves, gown and googles. FFP3 will be used always in case of any procedure on respiratory tract (including nasopharyngeal swab).

Hospitalized patients can be discharged from the hospital considering the ongoing national and regional recommendations to discharge COVID-19 patient at home. The USCAR unit will then responsible of continuing follow up of the patient according to the timeline described in Appendix 2.

8.4 Other therapies allowed

Patients will not receive any other antiviral treatment, unless considered needed by the physician. All other treatments including anti-hypertensive drugs, medications for diabetes (insulin and oral drugs), antibiotics, hormone therapy can be provided to patients of both groups according to medical judgments. Patients should not receive nonsteroidal anti-inflammatory drugs apart from paracetamol if needed.

Any previous and concomitant medication will be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

8.5 Safety monitoring and individual stopping rules

Any sign or symptom associated to drug adverse events will be daily reported.

Progressing patients which are in need of oxygen support will be maintained in the trial for follow up purposes, but treatment will be discontinued (Appendix 1). Progressing patients will receive standard of care or additional treatment at the physician discretion.

Another stopping rule includes drug related adverse events grade ≥ 3 according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

For progressing patients, we will record the admission to the intensive care unit, days in ICU, and the disease outcome (either survivor or non-survivors) by using the Regional surveillance systems.

Patients are free to withdraw from participation in the study at any time upon request, without any consequence. Patients should be listed as having withdrawn consent only when they no longer wish to participate in the study and no longer authorize the Investigators to make efforts to continue to obtain their outcome data. Every effort should be made to encourage patients to remain in the study for the duration of their planned outcome assessments. Patients should be educated on the continued scientific importance of their data, even if they discontinue the study. In the case of a patients becoming lost to follow-up, attempts to contact the patient should be made and documented in the patient's medical records.

9. Methods

9.1 Randomization

Sixty patients will be randomized 2:1 to receive IFN-β1a or control arm. Eligible patients will be randomised (no later than 36 h after enrolment) by means of a computerized central randomization system. All patients will receive a unique patient identification number at enrolling visit when signing the informed consent and before any study procedures are performed. This number must remain constant throughout the entire study.

ISS will prepare a randomization list by using a validated software and the list will be managed by the CRO. The randomization of patients will be closed when 60 patients have been randomized. The randomization will be stratified by sex; for each stratum a sequence of treatments randomly permuted in blocks of variable length (3 or 6) will be generated.

9.2 Blinding

This is an open-label study. After the randomization, patient will be notified whether will receive or not the experimental drug.

9.3 Electronic case report form

Patients' data will be recorded in an *ad hoc* online database. The Electronic case report form will be provided by a Clinical Research Organization and implemented according to the study design. An example of the information to be recorded in the e-CRF is provided as Appendix 4.

9.4. Safety Criteria Evaluation

9.4.1 Safety profile

Subjects participating in this trial who received at least one dose of the trial medication are considered to be included in the safety population (full analysis population). Safety population not include subjects who drop out prior to receiving any treatment. Data on safety profile, nature, incidence and severity of adverse events (AEs) and serious adverse events (SAEs) will be collected as detailed in both this section of the protocol and in the AE/SAE section of the CRF. Any reason for

drug interruption, reduction and discontinuation will be collected. Toxicities will be graded using NCI Common Terminology Criteria for adverse Events (CTCAE) version 5. The investigator is responsible for detecting, documenting and reporting AEs and SAEs, according to the criteria defined in this protocol.

The safety profile of experimental drug (i.e., IFN-β1a, Rebif®) has been well established. Below are the very common and common adverse reactions as reported in the Summary of Product Characteristic 2010:

Very common (≥1/10)	**Common (≥1/100 to <1/10)**
Neutropenia, lymphopenia, leukopenia, thrombocytopenia, anaemia	Diarrhoea, vomiting, nausea
Asymptomatic transaminase increase	Severe elevations in transaminases
Headache	Pruritus, rash, erythematous rash, maculo-papular rash, alopecia
Injection site inflammation, injection site reaction, influenza-like symptoms	Myalgia, arthralgia
	Depression, insomnia
	Injection site pain, fatigue, rigors, fever

9.4.2 Adverse events (AE) and serious adverse events (SAE)

9.4.2.1 Definition of an AE

An AE is defined as any untoward medical occurrence in a patient, temporarily associated with the use of a medicinal product, whether or not it is considered related to the medicinal product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporarily associated with the use of a medicinal product. Examples of an AE include:

· Significant or unexpected worsening or exacerbation of the condition/indication under study.

· Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.

· New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.

· Signs, symptoms, or the clinical sequelae of a suspected interaction.

· Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).

9.4.2.2 Definition of a Serious Adverse Event

A serious adverse event (SAE) is defined as any untoward medical occurrence that, at any dose:

1. Results in death
2. Is life-threatening
3. Requires hospitalization or prolongation of existing hospitalization
4. Results in disability/incapacity
5. Is a congenital anomaly/birth defect
6. Is otherwise considered as medically important.

9.4.2.3 Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE or SAE on the eCRF. Any AEs or SAEs occurring during the study must be documented in the subject's medical records and on the appropriate page of the eCRF. Each AE or SAE is to be recorded individually. All AEs which occur during the course of the study should be recorded in the eCRF. Information on the AE must be recorded on a specific AE form (appendix 5).

9.4.2.4 Evaluating AEs and SAEs

9.4.2.4.1 Assessment of intensity

The investigator will make an assessment of intensity of each AE and SAE reported. In this protocol, the intensity of AEs and SAEs will be graded on a scale of 1 to 5 according the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE) Version 5 and are available at https://evs.nci.nih.gov/ftp1/CTCAE/About.html .

For SAEs, the maximum intensity (or grade) will be reported in the eCRF. For non-serious AEs, each change in intensity (or grade) will be reported in the eCRF.

9.4.2.4.2 Assessment of causality

The investigator is obliged to assess the relationship between the study medical product and the occurrence of each AE/SAE and provide the assessment of causality as per instructions on the SAE form in the Investigators File.

9.4.2.4.3 Follow-Up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide further information on the subject's condition by any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. AEs that are ongoing with a toxicity of Grade 3 or 4, or have a relationship to study drug that is suspected (Reasonable Possibility) will be queried for resolution at study conclusion and at approximately 30 days after the last dose of study. New or updated information will be recorded on the originally completed SAE form in the Investigator's File, with all changes signed and dated by the Investigator.

9.4.3 Regulatory reporting requirements for adverse events

The Investigator must report immediately (within 24 hours from the knowledge) to the study Sponsor any SAE, occurred during the study whether related to the investigational product or not.

The study Sponsor has the legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. The study Sponsor will comply with the Italian regulatory requirements related to the reporting of SAEs to regulatory authorities and the Independent Ethics Committee (IEC). In particular, all the Suspected Unexpected Serious Adverse Reactions (SUSARs) that occur while on treatment and within 30 days since the last investigational drug administration, and that have a suspected relationship to study's drug (Reasonable Possibility) will be notified with an urgency procedure to the local regulatory Agency (AIFA) and IEC with the following timelines:

- SUSARs that are considered life-threatening: notification within 7 days.
- SUSARs that are not considered life-threatening: notification within 15 days.

The notification with urgency procedure is not required for SAEs that are expected with the drugs used in the protocol, and for non-serious AEs, both expected and unexpected. For these events (expected SAEs and AEs), the CT will notify the local regulatory agency and IECs by an annual safety report.

9.5 Secondary and Exploratory endpoints

Dedicated blood samples will be collected at different time points (see APPENDIX 2: Timeline scheme) and processed at the biobank of the INMI.

9.5.1 SARS-CoV-2 Antibodies

The development of a specific humoral response will be monitored by measuring specific anti SARS-CoV-2 antibodies in the sera of patients collected at day 14 and 28 post randomization. Commercially available tests will be used to detect IgM specific for S antigen, IgG specific for the N and S antigens, and IgA specific for S antigen. Sera resulting reactive with the S antigen will be tested for the capacity of viral neutralization using standardized methods.

9.5.2 Molecular IFN-I signaling

Blood samples will be collected at T1 prior first treatment, during treatment (T3 prior second treatment) and post treatment (T14) and processed at the biobank of the INMI. Isolated PBMC will be aliquoted, submerged with RNA stabilization reagent and cryopreserved. For analysis, total RNA will be isolated and the transcriptional analysis of over 500 general immunology genes will be performed by means of Nanostring technology. Data analysis will determine the transcriptional modifications occurring during the course of IFN-β1a treatment as well as to identify molecular patterns potentially correlated with clinical outcome. Particular focus will be given to the ISG score reported to be differentially expressed among mild to severe COVID-19 (22). This exploratory analysis will be conducted by Dr Aricò and Dr Castiello, having a relevant background on IFN signaling analysis (55,58)

9.5.3 Cellular Immune monitoring

Pre-(T1 prior treatment) and post-treatment (T3 and T14) blood samples will be monitored by MFC-based assays through different antibody panels in order to analyze frequency of major leukocyte subpopulations associated with naïve/memory, co-activation and co-inhibition markers; polyfunctional properties of T cell specific response against virus antigens will be evaluated after short term in vitro culture.

Stained samples will be acquired on a Beckman Coulter CytoFlex Cytometer and analyzed by CytExpert and/or Kaluza software as well as by advanced machine learning algorithms such as FlowSOM and CITRUS (Cytobank online platform). Dr. Francesca Urbani and Dr. Iole Macchia, co-investigators at ISS unit, have long lasting experience in MFC assays and immune-monitoring (56,59,60).

9.5.4 Systemic Inflammatory markers

Pre- (T1 prior treatment) and after-treatment (T14) blood samples will be collected from Treatment and control group to monitor the levels of soluble factors involved in inflammation (e.g., cytokines and chemokines) and endothelial cell adhesion molecules. At the selected time points, plasma will be isolated from peripheral blood and cryopreserved until analysis that will be simultaneously conducted by means of specific ELISA assays. Data will be integrated with the results of routine lab tests on coagulation factors and factors involved in COVID-19 pathogenesis (CRP, IL-6, TNF-α, Fibrinogen and D-Dimer).

10. Statistical Plan

The primary analysis will be carried out on the primary endpoint on the intention-to-treat (ITT) population defined as all patients randomized receiving at least one dose of treatment.

The percentage of patients undergoing disease progression defined on rate of progression of NEWS2 score lasting more than 5 days will be calculated in two arms (IFN-β1a + standard of care vs standard of care) of the trial. For persons who died, a conservative approach will be adopted and death will be considered an event. The effect of treatment will be estimated through a logistic regression model including a dummy variable for treatment. The effect of treatment will be estimated through multivariable logistic regression model by accounting for the following covariates: age, gender, co-morbidities. Moreover, NEWS2 score at baseline and setting of recruitment will be also considered.

All primary and secondary analyses will be carried out both on ITT population and on per-protocol population. Per-protocol population includes all subjects who were included in the ITT population that received the treatment as defined in the protocol and who completed the study with no major protocol violations.

Kaplan-Meier survival analysis and Cox proportional hazards model will be used for time-to-event data. The following covariates will be included in the Cox model: age, gender, co-morbidities. Moreover, NEWS2 score at baseline and setting of recruitment will be also considered. For the secondary endpoint ICU-free days, a competing risk model will be adopted considering death a competing event, following the method proposed by Fine and Gray (57). Moreover, the median difference will be reported.

The longitudinal secondary endpoint measured on a continuous scale (the increase in SARS-CoV 2-Specific Binding Antibody Titers in IFN compared to control group) will be analysed using a Mixed effect Model for Repeat Measure (MMRM) to estimate the difference of mean change from baseline in SARS-CoV 2-Specific Binding Antibody Titers between IFN-β1a + standard of care and standard of care at day 28. In case of data sporadically missing during the course of trial we will assume they were Missing At Random (MAR). A sensitivity analysis will be carried out by conducting the statistical test after imputing missing, including the worst-case imputation. All missing data will be imputed within treatment groups defined by randomized treatment.

Safety endpoint will be compared by a chi-squared test for discrete variables, by means of analysis of variance (ANOVA) and covariance (ANCOVA) for continuous variables or by the non-parametric Mann-Whitney test when appropriate.

Confidence intervals (95%) will be reported for all outcomes and association measures (proportions, means, Odds Ratios and HRs).

For all statistical analyses (efficacy and safety), the level of statistical significance will be kept at 0.05 with two-sided p-values. Statistical analyses and related reports will be in full compliance with ICH E9 guidance (https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-9-statistical-principles-clinical-trials-step-5_en.pdf).

11. Timing

See APPENDIX 2: Timeline scheme and APPENDIX 3: GANTT

12. Feasibility

Our study plans to enroll either hospitalized and non-hospitalized newly diagnosed COVID-19 patients, as well as patients hosted in long-stay residential care homes. The possible scenario of the Italian pandemic occurring during the conduction of the study will likely affect the proportion of patients that will be enrolled in the different settings. In fact, for patients presenting with mild illness, the decision to undertake hospitalization vs home care should be carefully evaluated to take into account patient risk of rapid deterioration, but also the burden on the health care system.

On august 11th, the ISS, together with the Ministry of Health and the Coordination of Italian regions and autonomous provinces, issued a document called “Elementi di preparazione e risposta a COVID-

19 nella stagione autunno-invernale". The document, aimed at providing general elements and suggesting preparedness frameworks to strengthen the response and optimally cope with any increase in the number of new infections by SARS-CoV 2 in the autumn-winter 2020-2021 season, foresees four possible scenarios, characterized by increasing SARS-CoV 2 transmission rate and related risk of SSN collapse.

To ensure that patients' enrolment is duly completed in any of the possible scenarios, the study will count on a network of collaborating institutions directly involved in the identification and management of COVID-19 patients in Rome. A campaign will be held to inform Family doctors (Medici di Medicina Generale) and long-stay residential care homes (LSRCHs), whose collaboration will ensure the precocious identification of eligible patients throughout the urban area of Rome. The Special Unit for regional continued care (USCAR) are currently involved in the prompt identification of COVID-19 clusters within Regione Lazio. In this study, a group of physicians belonging to USCAR will be specifically trained and will be responsible for the screening, enrolling and clinical monitoring of patients kept under home isolation or hosted in long-stay residential care homes (LSRCHs).

All experts involved in the project are highly motivated, have complementary expertise and a strong background in the fields of IFN biology and infectious diseases. The CNR group, including the Sponsor Coordinator (Giuseppe Sconocchia) and Scientific Coordinator (Filippo Belardelli) of the study, has a long-lasting expertise on immunology, IFN biology and clinical studies with IFN-I. The Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani (clinical center) is one of the five clinical hubs for COVID-19 management in town and has a longstanding experience in multicentre clinical studies and the PI (Emanuele Nicastri) is a well-recognized clinician deeply involved in the clinical management of COVID-19 patients. The ISS group includes scientists with a background on IFN-I biology in both basic research and clinical trials (Eleonora Aricò, Laura Bracci, Luciano Castiello, Iole Macchia, Francesca Urbani) and with long record expertise on antibody response measurement (Annarita Ciccaglione, Roberto Nisini). Moreover, the Clinical Epidemiology group of the ISS (Ilaria Bacigalupo, Flavia Lombardo, Nicola Vanacore, Antonio Ancidoni) has a strong background on statistical analysis and was involved in the recent survey on COVID-19 infection in long-stay residential care homes (61) . To ensure the full feasibility and the high quality performance of the study the Sponsor finalized a contract with a CRO, highly specialised in clinical studies involving IFN-β, which will support the Sponsor Coordinator and the Scientific Coordinator with regard to specific services and for the implementation and monitoring of the entire study.

13. Good clinical practices and ethics

13.1. Good clinical practice

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (1964) and subsequent amendments and updates (Fortaleza, Brazil, October 2013), in the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and in the appropriate regulatory requirements. The drug used in this trial is already registered and its toxicity profile is very well known, since it is largely used for the treatment of Multiple Sclerosis.

13.2 Ethical aspects

The entire study protocol, including informative material for the patients and modules for the informed consent, will be evaluated by The Ethics Committee (EC) of the National Institute for Infectious Diseases "Lazzaro Spallanzani", Rome, Italy, which is the National Ethics Committee for evaluation of clinical trials on human drugs in COVID-19 patients.

The study will not start before obtaining a favorable opinion from the EC, the Competent Authority Authorization and any other authorization required by local regulation. Every intention to modify any element of the original protocol after the first approval will be promptly notified to the EC and will be applied only after its written authorization. Investigators will be responsible for submitting any amendments to the protocol to the EC. Any modifications to the protocol, which may impact on the conduct of the study, may affect patient safety, including changes of study objectives, study design, patient population, sample size, study procedures or significant administrative aspects will require a formal amendment to the protocol. Such amendments will be agreed and approved by the Ethics Committee of the National Institute for Infectious Diseases "Lazzaro Spallanzani", Rome, Italy, and the health authorities prior to implementation, in accordance with local regulation. Administrative changes of the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be documented in a memorandum.

13.2.1 Written informed consent

The Investigators will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects will also be notified

that they are free to discontinue from the study at any time. The subject's signed and dated informed consent will be obtained prior to conduct any procedure specific for the study. The original signed Written Informed Consent Form will be stored, and a copy will be given to the patient.

13.2.2 Subject data protection

In order to protect the subjects' identity, the Investigator will assign a subject identification number to each enrolled subject to be used instead of subject name when reporting all study related data and adverse events.

The Written Informed Consent Form will explain that the study data will be stored at Spallanzani Hospital maintaining confidentiality in accordance with national data legislation. However, the personal information must be available to authorized personnel of Study Sponsor (clinical monitor and auditor), Ethics Committee and Regulatory Authorities. In addition, consent to allow direct access to original medical records to ensure data verification will be obtained from the subject before participation in the study.

Enrolment log must be kept strictly confidential to enable patient identification at the site.

13.2.3 Audits and inspections

The Principal Investigator and the SC will provide all the necessary information and material to the participating centers in order to standardize all the protocol-related procedures and to avoid unexpected variability. Printed and electronic informative material (complete original protocol, informed consent modules, informative modules for patients and relatives, recruitment checklist, graphic timeline of interventions and visits, order list for physicians and nurses) will be distributed to Spallanzani Hospital and USCAR. Source data/documents must be available to inspections by the designee or Health Authorities.

13.2.4 Monitoring

The monitoring activities will be performed by a Clinical Research Organization. Clinical Monitor will perform the monitoring activities according to "Note of Guidance on Good Clinical Practice" (ICH E6 (R2), EMA/CHMP/ICH/135/1995).

The clinical monitor will maintain contacts between Investigators and Study Sponsor; furthermore, during the study the clinical monitor will verify that informed consent was obtained from all subjects, that the data were adequately documented in medical records and that the Investigators

were compliant with the protocol. The clinical monitor will inform the study Sponsor and the Investigators about all detected protocol deviations, all facilities and technical Staff detected problems. The Investigators will provide direct access to source data/documents for data verification.

13.2.5 Declaration of interest

The study participants declare no financial and/or other conflicts of interest related to the study.

13.2.6 Dissemination policy

The Circ. Min. Health N° 6 of 09/02/2002 obliges each researcher who gets any results of interest to public health, to publish the results within 12 months from the end of the study. All the patients will freely agree or disagree to participate in the study in the belief that the results will be useful to improve knowledge about their pathologies, for health benefit from themselves or other patients. To respect their will and in the maximum interest of honest clinical research, the investigators agree on the need to ensure the wide publication and diffusion of their results in a consistent and responsible way under their responsibility. The Study Coordinator is the official data owner. The Study Coordinator has the right to present methods and results of the study at public symposia and conferences. The principal publications from the trial will be in the name of Investigators with full credit assigned to all collaborating investigators and institutions.

13.3 Insurance

The study will be conducted according to the law about the study insurance agreement (DM 14 luglio 2009); *ad hoc* insurance n. A1202150060-LB has been established with Lloyd's Insurance Company S.A

14. Budget

Materiale/Utilità	Costo unitario	Numero per paziente	N. pazienti	Quantità	Totale
Costi coordinamento medico	75000				75000
Rebif/Rebismart	0	1	60	60	0
Servizi svolti da CRO					60000
Saturimetro	25	1	30	30	750
Sfingomanometro	20	1	30	30	600
DPI completo FFP3	10	10	30	300	3000
Assistenza domiciliare	400	1	30	30	12000
Teleassistenza	10	28	30	840	8400
Analisi del sangue	40	7	60	420	16800
Test Sars-CoV2	85	3	60	180	15300
Test sierologico	60	3	60	180	10800
Markers infiammatori	100	2	60	120	12000
Ddimero	6	2	60	120	720
Proteina C Reattiva	6	2	60	120	720
Systemic immune profiling	356	3	60	180	64080
RT-PCR per IFN signaling	280	2	60	120	33600
Costi processamento campioni biologici	30	7	60	420	12600
Assicurazione				1	9500
Costi etichettatura e gestione farmaco				1	5000
Costi generali per struttura coordinatrice					47730
Medical Writing and Statistical Data Analysis (all endpoints)					50000
Totale					**438600**

The study is co-funded by Merck Healthcare KGaA with a support equal to 40% of total costs.

15. Institutions agreement

The Study Sponsor will submit in the Clinical Trials all the documentation required by law to AIFA, as the Competent Authority and to Ethics Committee within a week after approval. Also, the Study Sponsor will comply in all respects with the standards of Good Clinical Practice, as defined in the

"Note of Guidance on Good Clinical Practice (CPMP/ICH 135/95)" and related Guidelines, as well as with all applicable regulatory requirements including national drug law and data protection law.
A collaboration agreement between all the Institutions involved in the study (CNR, INMI and ISS) will be signed before the enrollment of the first patient.

16. Participating Centers

- IFT, CNR, Rome;
- ISS, Rome
- INMI, Rome
- Synlab Lazio srl
- FullCro srl

17. Publications and data properties

Clinical trial data are considered the property of the investigators involved. Publications generated from the study will be sent to peer-reviewed international journals. The name and order of the authors will be decided by the working group.

18. References

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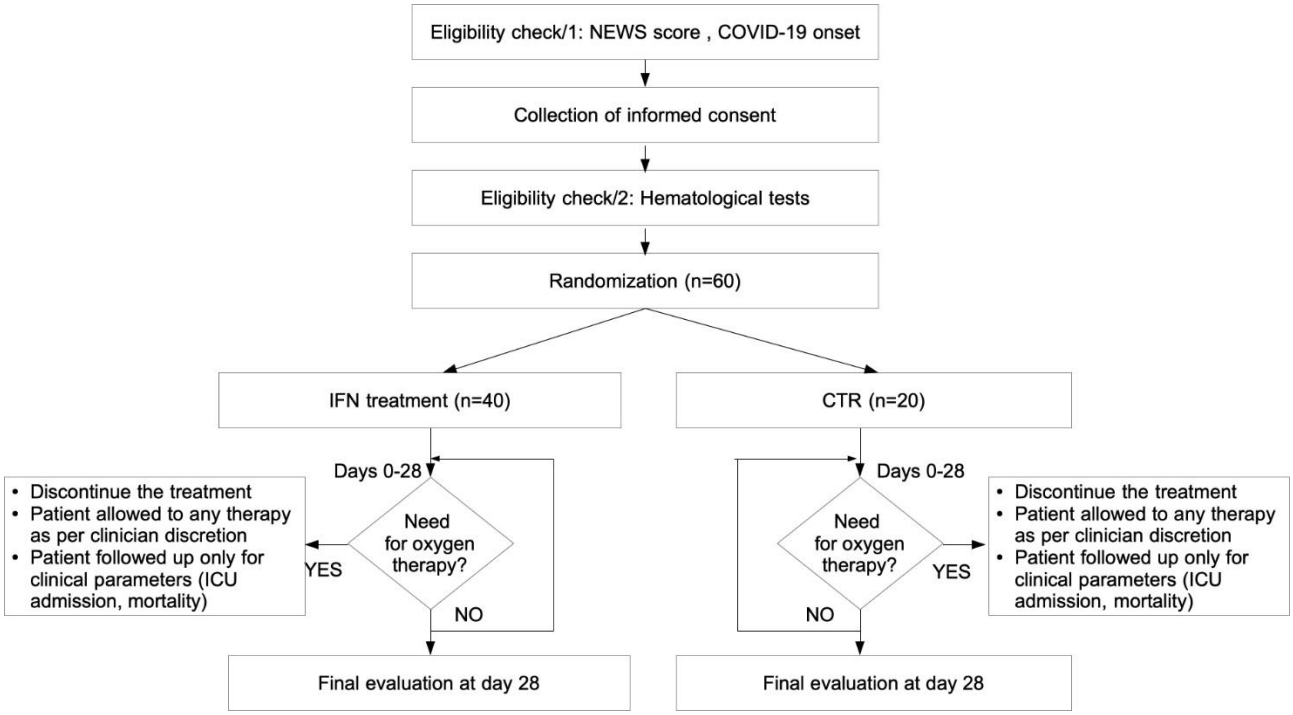
List of Appendices

APPENDIX 1: Flow Chart of the Study

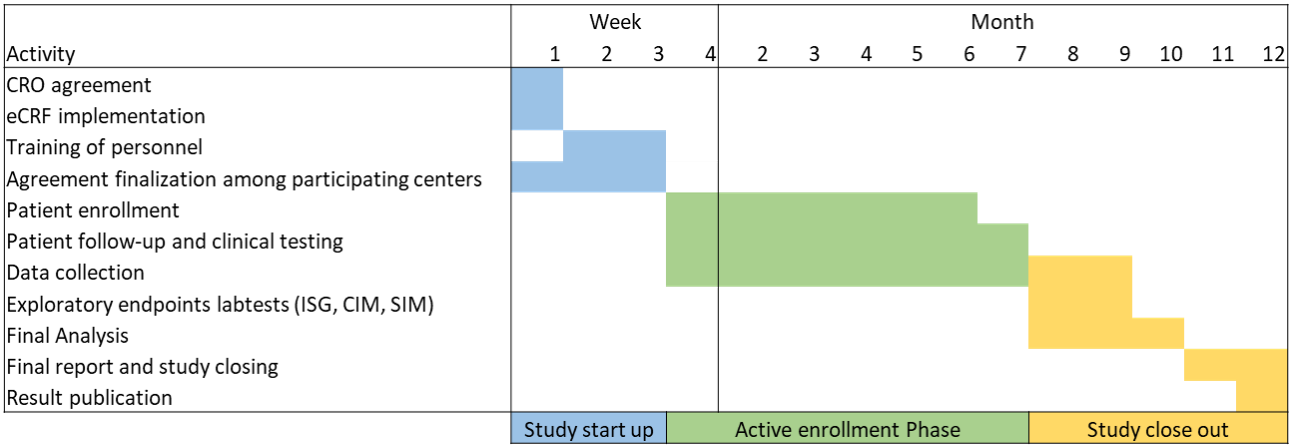


APPENDIX 2: Timeline scheme

TIMELINE SCHEME																																
Days	PreTx	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24	T25	T26	T27	T28
	Screening	**Treatment**										**Follow-up**																	
IFN (ARM2 only)		x		x				x			x																		
Procedures (both ARMS)																																
RT-PCR SARS-CoV 2 positivity assay															x														x
Demografic Data	x																												
Medical History	x																												
Informed Consent	x																												
Inclusion/Exclusion Criteria	x																												
Signs and symptoms	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Previous/Concomitant Therapy recording	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
NEWS2 score assessment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Safety/Efficacy Evaluation (both ARMS)																																
Adverse Events recording		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Routine laboratory test parameters	x	x		x							x				x														x
SARS-CoV 2 Antibodies		x													x														x
Exploratory labtests (ISG/CIM/SIM)		x		x											x														

As clinically indicated, laboratory and instrumental tests can be performed on other time points that will be recorded.

APPENDIX 3: GANTT chart



APPENDIX 4: eCRF design

The CRO will implement and validate eCRF on a Gamp 5 21 CFR part 11 compliant platform and share it among participants, endowed with different appropriate access privileges: CRO will have complete data control, while data input permission only will be assigned to local data managers.

eCRF will be designed on the basis of the following structure:

CRF sections by time of visit

Screening **PreTx**	***Treatment*** **T1**
Id Number	Date
SARS-CoV-2 positivity assay	Id Number
Demografic Data	IFN (ARM2 only)
Medical History	
Informed Consent	Signs and symptoms
Inclusion/Exclusion Criteria	Previous/Concomitant Therapy
Signs and symptoms	NEWS2 score assessment
Previous/Concomitant Therapy	Adverse Events
NEWS2 score assessment	Routine laboratory test parameters: Procalcitonin, Reactive C protein, Protrombin time (INR), functional fibrinogen, D-dimer
Routine laboratory test parameters: Blood count, potassium, sodium, glucose, lipase, LDH, creatinine, ALT GPT, AST GOT, Bilirubin, Urea, Albumin	SARS-CoV-2 Antibodies
Randomization	Exploratory labtests (ISG/CIM/SIM)
	Study Discontinuation or Withdrawal

Treatment **T2/T4/T5/T6/T8/T9**	***Treatment*** **T3**	***Treatment*** **T7**	***Treatment*** **T10**
Date Id Number Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Study Discontinuation or Withdrawal ,	Date Id Number IFN (ARM2 only) Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters: blood count Exploratory labtests (ISG/CIM/SIM) Study Discontinuation or Withdrawal	Date Id Number IFN (ARM2 only) Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Study Discontinuation or Withdrawal	Date Id Number IFN (ARM2 only) Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters: Blood count, potassium, sodium, glucose, lipase, LDH, creatinine, ALT GPT, AST GOT, Bilirubin, Urea, Albumin, Reactive C protein, Protrombin time (INR), functional fibrinogen, D-dimer, Procalcitonin Study Discontinuation or Withdrawal

Follow up **T11/T12/T13/T15/T16/T17/T18/T19/T20/T21/T22/T23/T24/T25/T26/T27**	***Follow up*** **T14**	***Follow up*** **T28**
Date Id Number Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Study Discontinuation or Withdrawal	Date Id Number SARS-CoV-2 positivity assay Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters: Blood count, potassium, sodium, glucose, lipase, LDH, creatinine, ALT GPT, AST GOT, Bilirubin, Urea, Albumin, SARS-CoV-2 Antibodies Exploratory labtests (/CIM/SIM) Study Discontinuation or Withdrawal	Date Id Number SARS-CoV-2 positivity assay Signs and symptoms Previous/Concomitant Therapy NEWS2 score assessment Adverse Events Routine laboratory test parameters: Blood count, potassium, sodium, glucose, lipase, LDH, creatinine, ALT GPT, AST GOT, Bilirubin, Urea, Albumin, Reactive C protein, Protrombin time (INR), functional fibrinogen, D-dimer, Procalcitonin SARS-CoV-2 Antibodies Study Discontinuation or Withdrawal

CRF - section data		
SARS-CoV-2 positivity assay	date	RT-PCR: Gene name, CT number, Laboratory (INMI or other, to be specified); rapid antigen test: positive/negative (executed by: operator ID)
Demografic Data	date	Sex at birth, Date of birth, Race/Ethnicity; i) hospitalized, ii) RSA, iii) home patient
Medical History	date	see TABLE 1
Informed Consent	signature date	Y/N
Inclusion/Exclusion Criteria	date	see TABLE 2
Previous/Concomitant Therapy	date	Any drug/medicament name, reason for use, dose, frequency, duration of consumption
NEWS2 score assessment	date	SCORE, Systolic and diastolic arterial pressure, heart rate (HR), respiratory rate (RR), systemic body temperature, ACVPU, SpO_2
Randomization	date	ARM1/ARM2, random number
Signs and symptoms		see TABLE 3
IFN (ARM2 only)		Y/N, expiration date, batch number
Adverse Events		TABLE 4 AE and any other AE will be recorded using NCI Common Terminology Criteria for adverse Events (CTCAE) version 5. SUSAR will be recorded in specific CRF.
Routine laboratory test parameters		see TABLE 5
SARS-CoV-2 Antibodies		anti-S IgG, anti-N IgG, anti-S IgM, anti S IgA, neutralizing anti-S Ab titer
Exploratory labtests (ISG/CIM/SIM)		ISG: IFI44L, IFI27, RSAD2, SIGLEC1, IFIT1, IS15. CIM: see TABLE 6. SIM: see TABLE 7.
Study Discontinuation or Withdrawal		Y/N, reason: withdrawal of subjects for non-compliance/adherence, for AE, consent withdrawal, other (to be specified)

TABLE 1 - Medical History		
		Tobacco, Alcohol, other recreational drug use (dose, frequency, duration of comsumption) Flu vaccine (in the last year): Y/N, date
By System	Respiratory	Chronic pulmonary disease, Asthma, Tuberculosis (active/previous), other (to be specified)
	Cardiovascular/Circulatory	Chronic cardiac disease (not hypertension), Hypertension, other (to be specified)
	Musculoskeletal	to be specified
	Endocrine	to be specified
	Hematopoietic	to be specified
	Nervous	Chronic neurological disorder, other (to be specified)
	Dermatological Integumentary System/Exocrine System	to be specified
	Genitourinary	Chronic kidney disease, other (to be specified)
	Lymphatic System/Immune System	to be specified
	Digestive	Chronic liver disease, other (to be specified)
	Metabolic disease	Diabetes, other (to be specified)
	Ear, Nose, Throath	to be specified
	Psychiatric disease	to be specified
	Allergy	to be specified
	Malignant neoplasm	to be specified
	Infectious disease	HIV, HCV, other (to be specified)
	Other (to be specified)	

Note: For any disease, onset date and duration as well as indication about disease current status will be recorded.

TABLE 2 - Inclusion/Exclusion Criteria	
Inclusion criteria (all required):	
≥ 50 years of age at time of enrolment	Y/N
Subject has laboratory-confirmed SARS-CoV-2 infection as determined by PCR or third generation antigenic test, in any specimen ≤ 5 days prior to randomization	Y/N
Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures	Y/N
Subject understands and agrees to comply with planned study procedures	Y/N
Subject agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol	Y/N
Being symptomatic for less than 7 days before starting therapy	Y/N
NEWS2 score ≤2	Y/N
Exclusion criteria:	
Hospitalized patients with illness of any duration, and at least one of the following: Clinical assessment (evidence of rales/crackles on exam) AND SpO2 ≤ 94% on room air at rest or after walking test, OR Acute respiratory failure requiring mechanical ventilation and/or supplemental oxygen.	Y/N
Patients currently using interferon-beta (e.g., multiple sclerosis patients)	Y/N
Patients with chronic kidney diseases	Y/N
Known allergy or hypersensitivity to interferon (including asthma)	Y/N
Any autoimmune disease (based on the anamnesis)	Y/N
Patients with signs of dementia or neurocognitive disorders	Y/N
Patients with current severe depression and/or suicidal ideations	Y/N
Being concurrently involved in another trial for COVID-19	Y/N
HIV infection (based on the anamnesis)	Y/N
Use of any antiretroviral medication	Y/N
Impaired renal function (eGFR calculated by CKD-EPI Creatinine equation < 30 ml/min)	Y/N
Presence of other severe diseases impairing life expectancy (e.g. patients are not expected to survive 28 days given their pre-existing medical condition)	Y/N
Any physical or psychological impediment in a patient that could let the investigator to suspect his/her poor compliance	Y/N
Lack or withdrawal of informed consent	Y/N

TABLE 3 - Signs and symptoms
History of fever
Lower chest indrawing
Cough (with sputum production/with emoptysis)
Headache
Altered consciousness/confusion
Seizures
Sore throat
Abdominal pain
Runny nose
Vomiting/nausea
Wheezing
Diarrhoea
Chest pain
Conjunctivitis
Muscle aches
Skin rash
Joint pain (arthralgia)
Skin ulcers
Fatigue/malaise
Lymphadenopathy
Loss of taste
Inability to walk
Loss of smell
Bleeding (ischaemic stroke, entracerebral haemorrhage)
Shortness of breath

Note: If present, onset date and duration as well as indication about sign or symptom current status will be recorded.

TABLE 4 - REBIF common AE	
Very common (≥1/10)	Common (≥1/100 to <1/10)
Neutropenia, lymphopenia, leukopenia, thrombocytopenia, anaemia Asymptomatic transaminase increase Headache Injection site inflammation, injection site reaction, influenza-like symptoms	Diarrhoea, vomiting, nausea Severe elevations in transaminases Pruritus, rash, erythematous rash, maculo-papular rash, alopecia Myalgia, arthralgia Depression, insomnia Injection site pain, fatigue, rigors, fever

TABLE 5 - Routine laboratory test parameters	
Haemoglobin	CRP
Haematocrit	INR
Full Blood count	ALT/SGPT
Creatinine	Albumin
Sodium	AST/SGOT
Potassium	ESR
fibrinogen	Total bilirubin
D-dimer	Urea (BUN)
Glucose	LDH
Lipase	PT (seconds)

TABLE 6 - Cellular Immune Monitoring		
leu_linfociti	CD4_CM	Vd2_TD
lym_CD3	CD4_EM	Vd2_EM
CD3_CD4SP	CD4_N	Vd2_N
CD3_CD8SP	CD4_TD	Vd2_CM
CD8_CM	CD4_CD28pos27neg	lym_CD19
CD8_EM	CD4_CD28pos27pos	lym_NK
CD8_N	CD4_CD28neg27neg	lym_NKT
CD8_TD	CD4_CD28neg27pos	leu_CD14pos
CD8_CD28pos27neg	CD4_CD57pos27neg	leu_Treg
CD8_CD28pos27pos	CD4_CD57posPD1neg	lym_Treg
CD8_CD28neg27neg	CD4_CD57posPD1pos	CD3_Treg
CD8_CD28neg27pos	CD4_CD57negPD1pos	CD4_Treg
CD8_CD57pos CD27neg	gpSpike_CD8	Treg_CD45RAnegCD39neg
CD8_CD57posPD1neg	Vd2	Treg_CD45RAnegCD39pos
CD8_CD57posPD1pos		Treg_CD45RAposCD39neg
CD8_CD57negPD1pos		Treg_CD45RAposCD39pos

TABLE 7 - Systemic Inflammation Markers	
IL-2	IP-10
IL-7	MCP1
IL-10	MIP1a
G-CSF	VCAM-1
ICAM-1	VAP-1
Fractalkine	TNF-α

APPENDIX 5: Patient Diary

DIARIO CLINICO

NOME: _____ COGNOME: _____ DATA DI NASCITA:_____

CODICE IDENTIFICATIVO: _____

PER COMUNICAZIONI URGENTI CHIAMARE:_____

STUDIO CLINICO: "**Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 a Over 50**

Numero EUDRACT: 2020-003872-42

Promotore: Istituto di Farmacologia Traslazionale – Consiglio Nazionale delle Ricerche

Sperimentatore Principale: Dott Emanuele Nicastri

Telefono: xxxxxxxxxxxx

GIORNO														
ORARIO														
TEMPERATURA CORPOREA														
SATURAZIONE														
FREQUENZA CARDIACA														
PRESSIONE														
FREQUENZA RESPIRATORIA														
NECESSITA OSSIGENO?														
È VIGILE?														

APPENDIX 6: Standard operating procedure for drug management

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MODALITÀ DI APPROVVIGGIONAMENTO E SOMMINISTRAZIONE DEL FARMACO REBIF TRAMITE INIETTORE REBISMART

Documento Redatto da: Luciano Castiello Firma e data _____

Documento Approvato da:

Ruolo	Nomimativo	Firma e Data
Sperimentatore Principale	Dr. Emanuele Nicastri	
Coordinatore Scientifico	Dr. Filippo Belardelli	
Project Manager - FullCRO	Dr.ssa Moira Cordisco	
ISS	Dr.ssa Eleonora Aricò	
Farmacia INMI "Lazzaro Spallanani	Dr.ssa Silvia Murachelli	

Lista di distribuzione:

Ruolo	Nomimativo	N° copie	Firma e Data
Sperimentatore Principale	Dr. Emanuele Nicastri	1	
Coordinatore Scientifico	Dr. Filippo Belardelli	1	
Project Manager - FullCRO	Dr.ssa Moira Cordisco	1	
ISS	Dr.ssa Eleonora Aricò	1	
Farmacia INMI "Lazzaro Spallanani	Dr.ssa Silvia Murachelli	1	

MODALITÀ DI APPROVVIGGIONAMENTO E SOMMINISTRAZIONE DEL FARMACO REBIF TRAMITE INIETTORE REBISMART

1. Scopo

La presente procedura descrive le operazioni che gli sperimentatori devono eseguire per recuperare il farmaco e per utilizzare correttamente l'autoiniettore Rebismart nella sperimentazione clinica ANTIICIPATE sia nel setting ospedaliero che in quello non-ospedaliero.

2. Abbreviazioni e definizioni

Farmaco: Rebif (Interferon beta-1a)

Farmacia: Farmacia dell'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani"

CRO: FullCRO

Promotore: Istituto di Farmacologia Traslazionale

3. Approvviggionamento del farmaco e dell'iniettore

Per i soli pazienti rientranti nel braccio di trattamento, il giorno previsto per l'inizio del trattamento l'investigatore o un suo delegato deve presentarsi presso la Farmacia e richiedere una dose di farmaco e un dispositivo per l'autoiniezione del farmaco utilizzando il modulo Allegato 1. Il modulo originale deve essere conservato dalla Farmacia fino al ritorno del dispositivo per l'autoiniezione, dopodichè il modulo viene conservato nella cartella clinica del paziente.

Per i soli pazienti non ospedalizzati, l'investigatore dovrà accertarsi di essere inoltre in possesso di:

- Sei aghi Serofine 29G, 30G o 31G;
- Un contenitore per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti);
- Un diario clinico del paziente (fornito dal Promotore);
- Uno sfingomanometro digitale, un termometro e un saturimetro per la misurazione dei parametri vitali (fornito dal Promotore);
- Un manuale d'uso del Rebismart (da usare in caso di necessità);
- Salviettine o tamponcini imbevuti di alcol o batuffoli di ovatta e alcol per frizione;
- Cerotti (classici e a rocchetto)

4. Attivazione e settaggio dell'iniettore Rebismart e caricamento cartuccia Rebif

4.1 Inserimento pile

In caso il dispositivo abbia le batterie scariche, prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Svitare la vite del coperchio dell'alloggiamento pile con un cacciavite e far scivolare verticalmente il coperchio. Inserire 4 pile al litio nuove. Verificare che siano orientate come mostrato sul dispositivo e stringere la vite del coperchio dell'alloggiamento delle pile per chiuderlo. Una sequenza illustrata della attività da eseguire è riportata nell'allegato 2.

4.2 Settaggio Rebismart

4.2.1 Settaggio cartuccia e dose

Prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Accendere RebiSmart e premere il pulsante 'Menu'. Scorrere su 'Impostaz. iniezione' e premere il pulsante 'Apri' per selezionare. Sarà visualizzata una schermata di avvertenza e selezionare 'Si'.
Selezionare 'Cartuccia' premendo su 'Cambia' e scorrere al dosaggio della cartuccia 22mcg. Premere 'OK' per selezionare e confermare la selezione.

Dal menù 'Impostaz. Iniezione' scorrere e selezionare 'Riduzione dose' e premere il pulsante 'Cambia' per selezionare. Inserire il codice PIN del dispositivo. Il dispositivo visualizzerà il menu 'Riduzione dose'. Selezionare '50% della dose'. Premere 'Ok' e confermare la selezione. Premere due volte il pulsante 'Esci' per tornare alla schermata informativa.

4.2.2 Settaggio ago

Prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Accendere RebiSmart. E premere il pulsante 'Menu'. Scorrere su 'Impostaz. pers.' e premere il pulsante 'Apri' per selezionare. Si apre la schermata di avvertenza e selezionare 'Si'. Selezionare 'Velocità ago' premendo su 'Cambia'. Selezionare velocità 'Media'. Premere 'OK' per selezionare.

Scorrere su 'Velocità iniez.' e premere il pulsante 'Cambia'. Selezionare velocità 'Media'. Premere 'OK' per selezionare. Scorrere in basso su 'Profondità iniez.' e premere il pulsante 'Cambia'. Selezionare '4 mm' e premere 'OK' per selezionare.

Scorrere in basso su 'Durata iniezione' e premere il pulsante 'Cambia'. Selezionare '3 secondi' premere 'OK' per selezionare. Premere due volte il pulsante 'Esci/Esci' per tornare alla schermata informativa.

Premere il pulsante 'Menu', scorrere su 'Impostaz. iniezione' e premere il pulsante 'Apri' per selezionare. Scorrere su 'Tipo di ago' e premere il pulsante 'Cambia' per selezionare. Selezionare il tipo di ago utilizzato. Premere 'OK' per selezionare. Premere due volte il pulsante 'Esci' per tornare alla schermata informativa.

4.3 Caricamento cartuccia

Prelevare il dispositivo Rebismart dalla scatola contenitore e indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Estrarre la cartuccia dal confezionamento secondario. Accendere RebiSmart tenendo premuto il pulsante 'Acceso' fino a che compare la schermata di "Benvenuto (Ciao)". Premere su 'Inizio' e aprire lo sportello dell'alloggiamento della cartuccia facendo scorrere verso l'alto il pulsante posto sul lato sinistro del dispositivo. Inserire la cartuccia di Rebif nell'alloggiamento cartuccia con la parte metallica rivolta verso il basso. Chiudere lo sportello dell'alloggiamento cartuccia fino ad udire un "clic". Una sequenza illustrata della attività da eseguire è riportata nell'allegato 3. Staccare dal confezionamento secondario la parte staccabile dell'etichetta e applicarla sul Rebismart..

5. Somministrazione farmaco

5.1 Preparazione somministrazione

Prima di iniziare, estrarre RebiSmart. dal frigorifero e dalla scatola di conservazione almeno 30 minuti prima dell'utilizzo previsto. Disporre, su una superficie stabile, come per esempio un tavolo, quanto segue:

- RebiSmart. contenente una cartuccia di Rebif in posizione verticale;
- Ago Serofine™ (29G, 30G o 31G, in base alla prescrizione);
- Salviettine o tamponcini imbevuti di alcol o batuffoli di ovatta e alcol per frizione;
- Piccolo cerotto;
- Contenitore per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti);

Sanitizzare i guanti accuratamente. Indossare il cinturino per impedire che il dispositivo possa cadere accidentalmente. Accendere RebiSmart tenendo premuto il pulsante 'Acceso' fino a che compare la schermata di "Benvenuto (Ciao)". Premere il pulsante 'Inizio'.

Qualora RebiSmart visualizzi il messaggio "Meno di 48 ore dall'ultima iniezione. Procedere con l'iniezione", selezionare 'Si'. Prelevare un ago e rimuovere il sigillo di sterilità.

Inserire il cappuccio che contiene l'ago direttamente nell'alloggiamento dell'ago fino a che si blocca con un 'clic'. Togliere il cappuccio dell'ago spingendolo di lato fino a che non venga rimosso e conservare il cappuccio. Una sequenza illustrata della attività da eseguire è riportata nell'allegato 4.

5.2 Somministrazione farmaco

Posizionare RebiSmart sulla cute in posizione verticale nel sito di iniezione più idoneo (in base alle caratteristiche del paziente scegliere tra la parte esterna superiore delle braccia, la zona periombelicale dell'addome, la parte anteriore delle cosce). Assicurarsi che il sensore cutaneo sia completamente a contatto con la cute. Quando RebiSmart è posizionato correttamente sulla cute, la luce del pulsante di iniezione diventa verde e RebiSmart emette un bip.

Premere il pulsante per iniziare l'iniezione. La spia del pulsante di iniezione verde durante l'iniezione lampeggia. Tenere RebiSmart a contatto con la pelle per tutta la durata dell'iniezione.

Al termine dell'iniezione, la spia del pulsante verde si spegne e RebiSmart emette due bip.

Sollevare delicatamente RebiSmart dalla cute. Premere su 'OK' per confermare che l'iniezione è stata praticata correttamente.
Registrare sul diario clinico del paziente le informazioni sulla somministrazione.

5.3 Eliminazione ago e spegnimento Rebismart

Inserire il cappuccio vuoto direttamente nell'alloggiamento dell'ago fino a che si blocca con un 'clic'. RebiSmar. emette un bip. Premere e mantenere premuto il pulsante di rilascio dell'ago fino a che RebiSmart emette due bip. Togliere il cappuccio contenente l'ago spingendolo di lato fino a che si stacca per essere facilmente rimosso.
Controllare l'interno del cappuccio dell'ago per vedere l'ago rimosso. Gettare gli aghi usati nel contenitore per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti).

Premere e tenere premuto il pulsante 'Spento' fino a che RebiSmart si spegne e la schermata informativa si chiude.

6. Conservazione del farmaco e dell'iniettore

Al termine dell'iniezione riposizionare RebiSmart in posizione verticale nella sua custodia all'interno del frigorifero.

7. Recupero dell'iniettore e riconsegna

Al termine della quarta iniezione, eliminare la cartuccia in uso. Prima di spegnere Rebismart, premere il pulsante 'Menu'. Scorrere in basso su 'Rimuovere cartuc.' e premere il pulsante 'Apri'. Premere su 'Si' per confermare la selezione. Attendere che Rebismart visualizza il messaggio 'Aprire sportello alloggiamento cartuccia' ed emette due bip. Far scorrere verso l'alto il pulsante dello sportello alloggiamento cartuccia e rimuovere la cartuccia. Gettare la cartuccia nel contenitore

per rifiuti sanitari pericolosi a rischio infettivo (taglienti e pungenti). Selezionare 'No' sul Rebismart e spegnerlo. Rimuovere il confezionamento secondario dalla custodia del Rebismart.

Riconsegnare il Rebismart alla Farmacia, registrando la consegna sul modulo Allegato 1 utilizzato per il ritiro.

8. Allegati

Allegato 1: Modulo richiesta ritiro/riconsegna Rebif e Rebismart

Allegato 2: Schema dei passaggi da effettuare per la sostituzione pile

Allegato 3: Schema dei passaggi da effettuare per l'inserimento di una cartuccia di Rebif

Allegato 4: Schema dei passaggi da effettuare per somministrare il farmaco

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MODULO RICHIESTA RITIRO/RICONSEGNA REBIF E REBISMART

DATI RICHIEDENTE

NOME E COGNOME:_____ UNITÀ: INMI USCAR

RICHIEDE n° 1 cartuccia/e Rebif 66 mcg e n° 1 dispositivo Rebismart per effettuare il trattamento al paziente

Codice _____ **Data di Nascita:** _____

DATA:_____ FIRMA: _____

Spazio da compilare a cura della Farmacia

Numero lotto Rebif:_____ Data di scadenza:_____

Numero seriale cartuccia:_____

Identificativo Rebismart:_____

Nome e Cognome:_____ Data:_____ Ora:_____

Firma:_____

Spazio da compilare alla riconsegna del dispositivo Rebismart a cura della Farmacia

Identificativo dispositivo Rebismart riconsegnato: _____

Nome e Cognome:_____ Data:_____ Ora:_____

Firma:_____

SCHEMA DEI PASSAGGI DA EFFETTUARE PER LA SOSTITUZIONE PILE

Svitare la vite del coperchio dell'alloggiamento pile con un cacciavite.

Afferrare il coperchio sui due lati e farlo scivolare via.

Inserire 4 pile al litio nuove. Verificare che siano orientate come mostrato sul dispositivo.

Far scorrere il coperchio dell'alloggiamento delle pile nella posizione di chiusura, verificando che entri nelle fessure.

Stringere la vite del coperchio dell'alloggiamento delle pile per chiuderlo.

SCHEMA DEI PASSAGGI DA EFFETTUARE PER L'INSERIMENTO DI UNA CARTUCCIA DI REBIF

Accendere RebiSmart® tenendo premuto il pulsante **'Acceso'** fino a che compare la schermata di **"Benvenuto (Ciao)"**, in genere dopo 3-5 secondi.

Premere su **'Inizio'** e aprire lo sportello dell'alloggiamento della cartuccia facendo scorrere il pulsante verso l'alto.

Inserire una nuova cartuccia di Rebif® nell'alloggiamento cartuccia, verificando che la parte metallica sia rivolta verso il basso.

Chiudere lo sportello dell'alloggiamento cartuccia fino ad udire un "clic".

SCHEMA DEI PASSAGGI DA EFFETTUARE PER SOMMINISTRARE IL FARMACO

Accendere il dispositivo premendo e tenendo premuto il pulsante **'Acceso'** fino a che compare la schermata di **"Benvenuto (Ciao)"**, in genere dopo 3-5 secondi.

Premere il pulsante **'Inizio'**.

Verificare che la misura in gauge (G) indicata sulla scatola degli aghi Serofine™ corrisponda a quella indicata nella schermata di RebiSmart®.

Inserire il cappuccio che contiene l'ago direttamente nell'alloggiamento dell'ago fino a che si blocca con un 'clic'.

Togliere il cappuccio dell'ago spingendolo di lato fino a che venga rimosso.

SCHEMA DEI PASSAGGI DA EFFETTUARE PER SOMMINISTRARE IL FARMACO

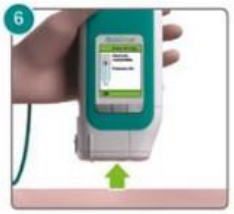
Posizionare RebiSmart® sulla cute in posizione verticale nel sito di iniezione preparato come indicato dal medico o dall'infermiere.

Quando RebiSmart® è posizionato correttamente sulla cute, la luce del pulsante di iniezione diventa verde e RebiSmart® emette un bip. 1

Premere il pulsante per iniziare l'iniezione.

La spia del pulsante di iniezione verde durante l'iniezione lampeggia. Tenere RebiSmart® a contatto con la pelle per tutta la durata dell'iniezione. Non è necessario mantenere premuto il pulsante di iniezione.

Al termine dell'iniezione, la spia del pulsante verde si spegne e RebiSmart® emette due bip. 2



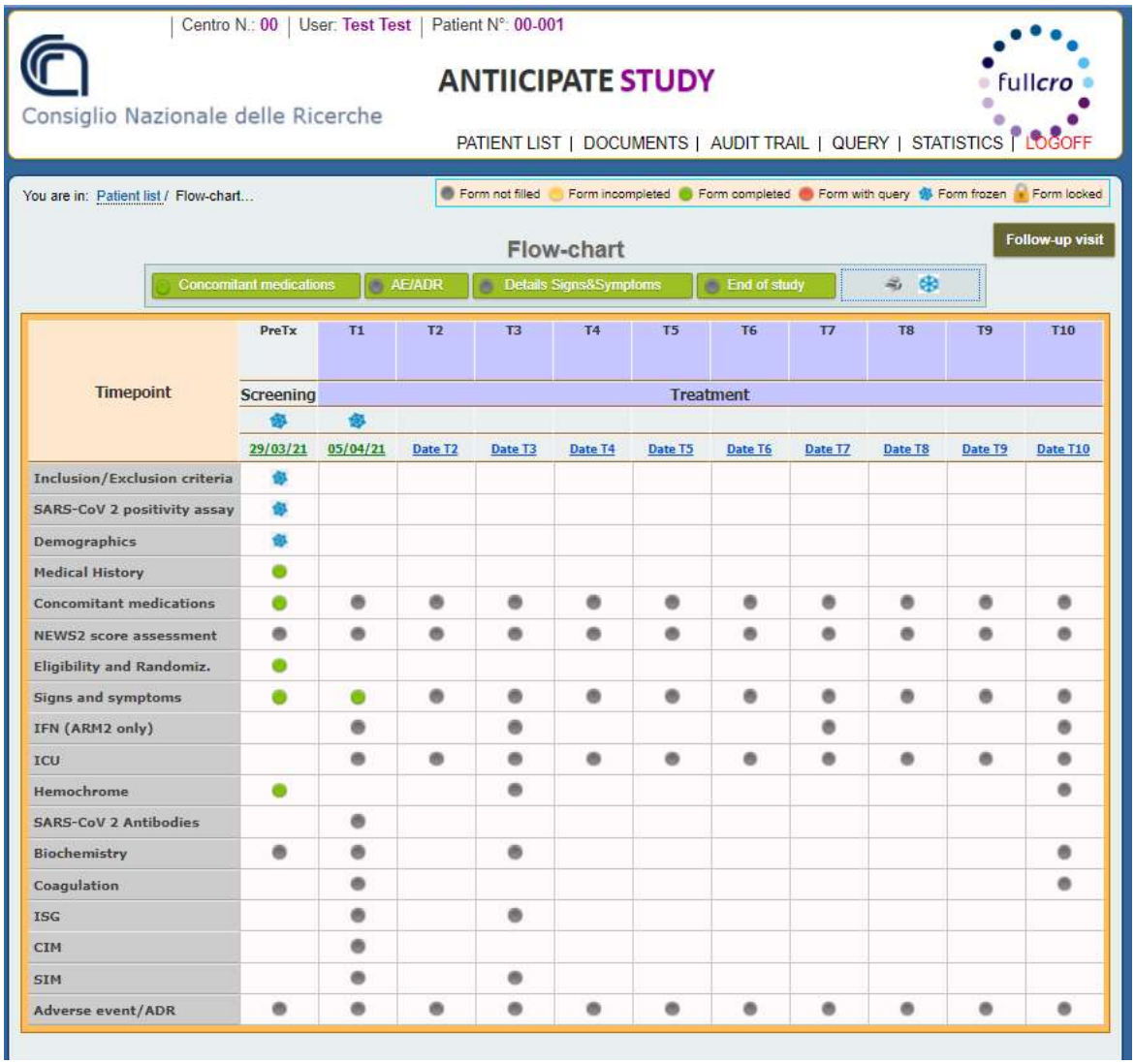
Sollevare delicatamente RebiSmart® dalla cute.

Premere su 'OK' per confermare che l'iniezione è stata praticata correttamente.

SCHEMA DEI PASSAGGI DA EFFETTUARE PER SOMMINISTRARE IL FARMACO

Final Report Appendix 16.1.2

Sample case report form



| Centro N.: 00 | User: Test Test | Patient N°: 00-001

Consiglio Nazionale delle Ricerche

ANTIICIPATE STUDY

fullcro

PATIENT LIST | DOCUMENTS | AUDIT TRAIL | QUERY | STATISTICS | LOGOFF

You are in: Patient list / Flow-chart...

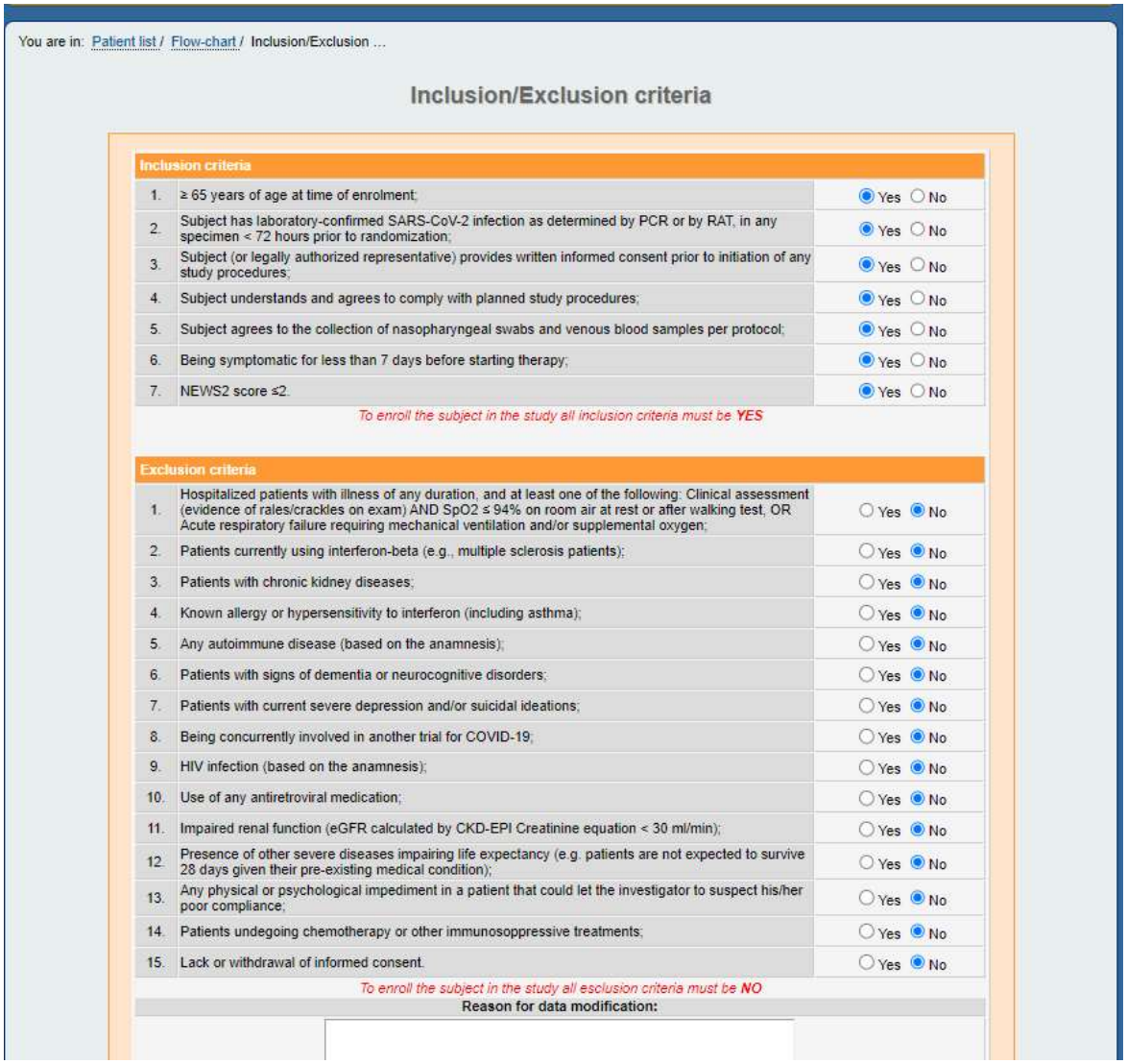
Form not filled | Form incompleted | Form completed | Form with query | Form frozen | Form locked

Flow-chart

Follow-up visit

Concomitant medications | AE/ADR | Details Signs&Symptoms | End of study

Timepoint	PreTx	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
	Screening	Treatment									
	29/03/21	05/04/21	Date T2	Date T3	Date T4	Date T5	Date T6	Date T7	Date T8	Date T9	Date T10
Inclusion/Exclusion criteria	❄										
SARS-CoV 2 positivity assay	❄										
Demographics	❄										
Medical History	●										
Concomitant medications	●	●	●	●	●	●	●	●	●	●	●
NEWS2 score assessment	●	●	●	●	●	●	●	●	●	●	●
Eligibility and Randomiz.	●										
Signs and symptoms	●	●	●	●	●	●	●	●	●	●	●
IFN (ARM2 only)		●		●				●			●
ICU		●	●	●	●	●	●	●	●	●	●
Hemochrome	●			●							●
SARS-CoV 2 Antibodies		●									
Biochemistry	●	●		●							●
Coagulation		●									●
ISG		●		●							
CIM		●									
SIM		●		●							
Adverse event/ADR	●	●	●	●	●	●	●	●	●	●	●



You are in: Patient list / Flow-chart / Inclusion/Exclusion ...

Inclusion/Exclusion criteria

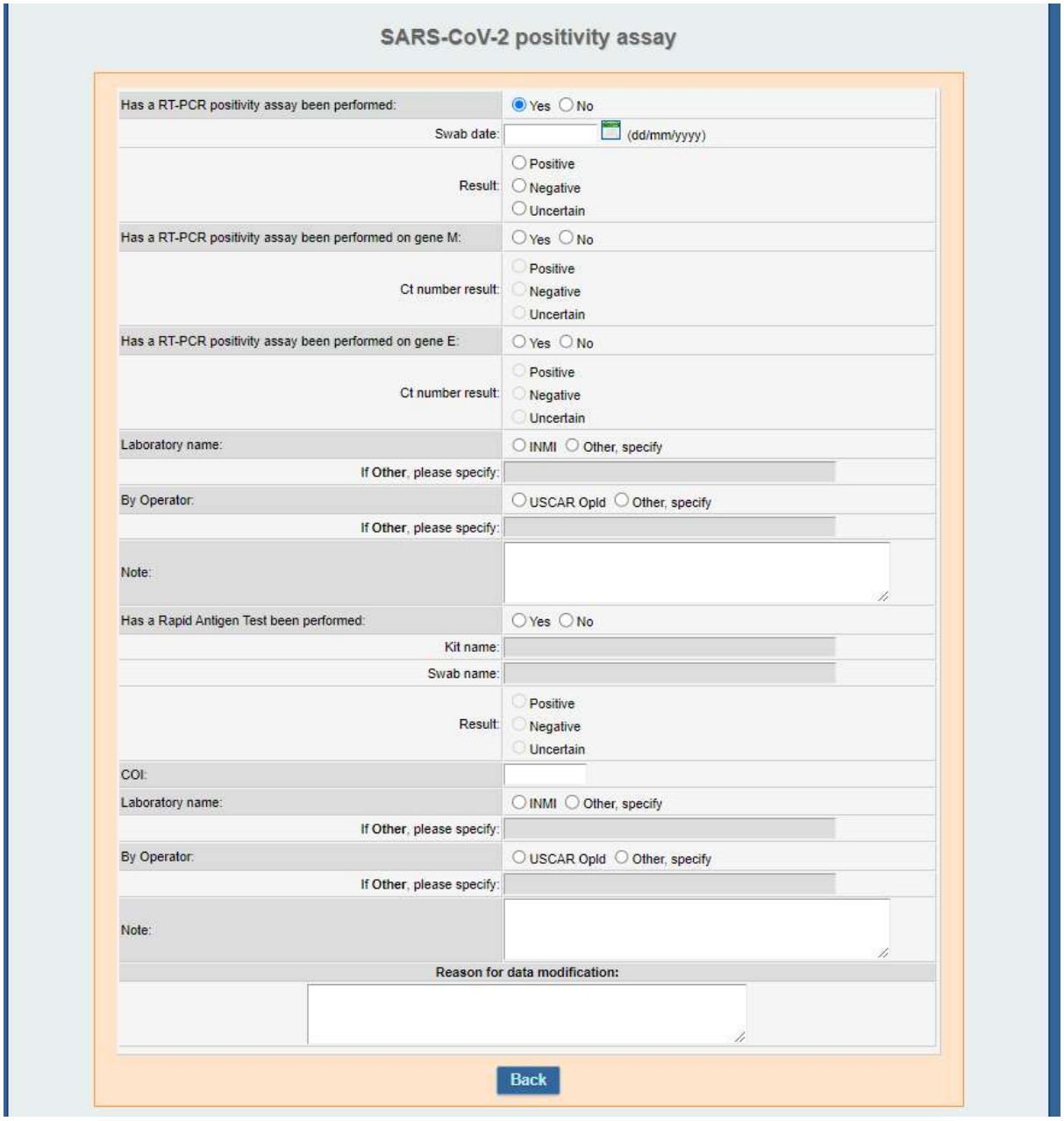
Inclusion criteria		
1.	≥ 65 years of age at time of enrolment;	Yes / No
2.	Subject has laboratory-confirmed SARS-CoV-2 infection as determined by PCR or by RAT, in any specimen < 72 hours prior to randomization;	Yes / No
3.	Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures;	Yes / No
4.	Subject understands and agrees to comply with planned study procedures;	Yes / No
5.	Subject agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol;	Yes / No
6.	Being symptomatic for less than 7 days before starting therapy;	Yes / No
7.	NEWS2 score ≤2.	Yes / No

*To enroll the subject in the study all inclusion criteria must be **YES***

Exclusion criteria		
1.	Hospitalized patients with illness of any duration, and at least one of the following: Clinical assessment (evidence of rales/crackles on exam) AND SpO2 ≤ 94% on room air at rest or after walking test; OR Acute respiratory failure requiring mechanical ventilation and/or supplemental oxygen;	Yes / No
2.	Patients currently using interferon-beta (e.g., multiple sclerosis patients);	Yes / No
3.	Patients with chronic kidney diseases;	Yes / No
4.	Known allergy or hypersensitivity to interferon (including asthma);	Yes / No
5.	Any autoimmune disease (based on the anamnesis);	Yes / No
6.	Patients with signs of dementia or neurocognitive disorders;	Yes / No
7.	Patients with current severe depression and/or suicidal ideations;	Yes / No
8.	Being concurrently involved in another trial for COVID-19;	Yes / No
9.	HIV infection (based on the anamnesis);	Yes / No
10.	Use of any antiretroviral medication;	Yes / No
11.	Impaired renal function (eGFR calculated by CKD-EPI Creatinine equation < 30 ml/min);	Yes / No
12.	Presence of other severe diseases impairing life expectancy (e.g. patients are not expected to survive 28 days given their pre-existing medical condition);	Yes / No
13.	Any physical or psychological impediment in a patient that could let the investigator to suspect his/her poor compliance;	Yes / No
14.	Patients undegoing chemotherapy or other immunosoppressive treatments;	Yes / No
15.	Lack or withdrawal of informed consent.	Yes / No

*To enroll the subject in the study all esclusion criteria must be **NO***

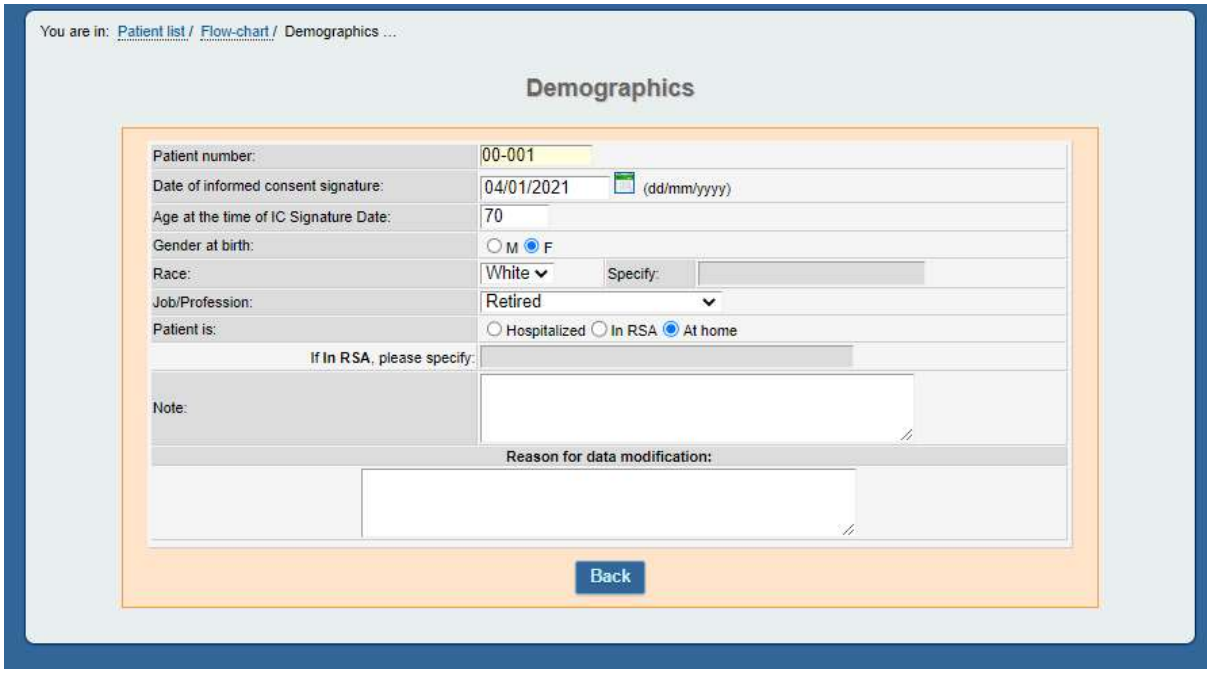
Reason for data modification:



SARS-CoV-2 positivity assay

Has a RT-PCR positivity assay been performed:	Yes No
Swab date:	(dd/mm/yyyy)
Result:	Positive Negative Uncertain
Has a RT-PCR positivity assay been performed on gene M:	Yes No
Ct number result:	Positive Negative Uncertain
Has a RT-PCR positivity assay been performed on gene E:	Yes No
Ct number result:	Positive Negative Uncertain
Laboratory name:	INMI Other, specify
If Other, please specify:	
By Operator:	USCAR OpId Other, specify
If Other, please specify:	
Note:	
Has a Rapid Antigen Test been performed:	Yes No
Kit name:	
Swab name:	
Result:	Positive Negative Uncertain
COI:	
Laboratory name:	INMI Other, specify
If Other, please specify:	
By Operator:	USCAR OpId Other, specify
If Other, please specify:	
Note:	
Reason for data modification:	

Back

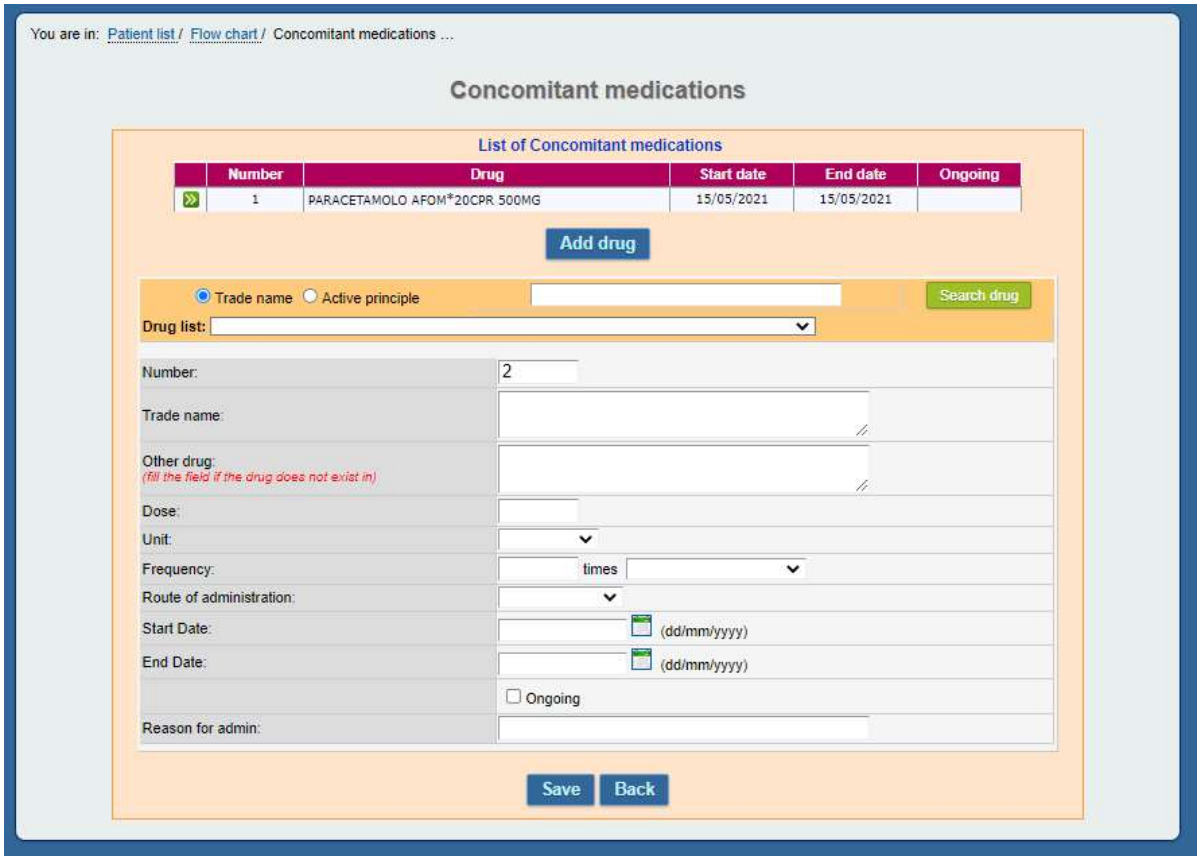

You are in: Patient list / Flow-chart / Demographics ...
Demographics
Patient number:
00-001
Date of informed consent signature:
04/01/2021
(dd/mm/yyyy)
Age at the time of IC Signature Date:
70
Gender at birth:
M
F
Race:
White
Specify:
Job/Profession:
Retired
Patient is:
Hospitalized
In RSA
At home
If In RSA, please specify:
Note:
Reason for data modification:
Back

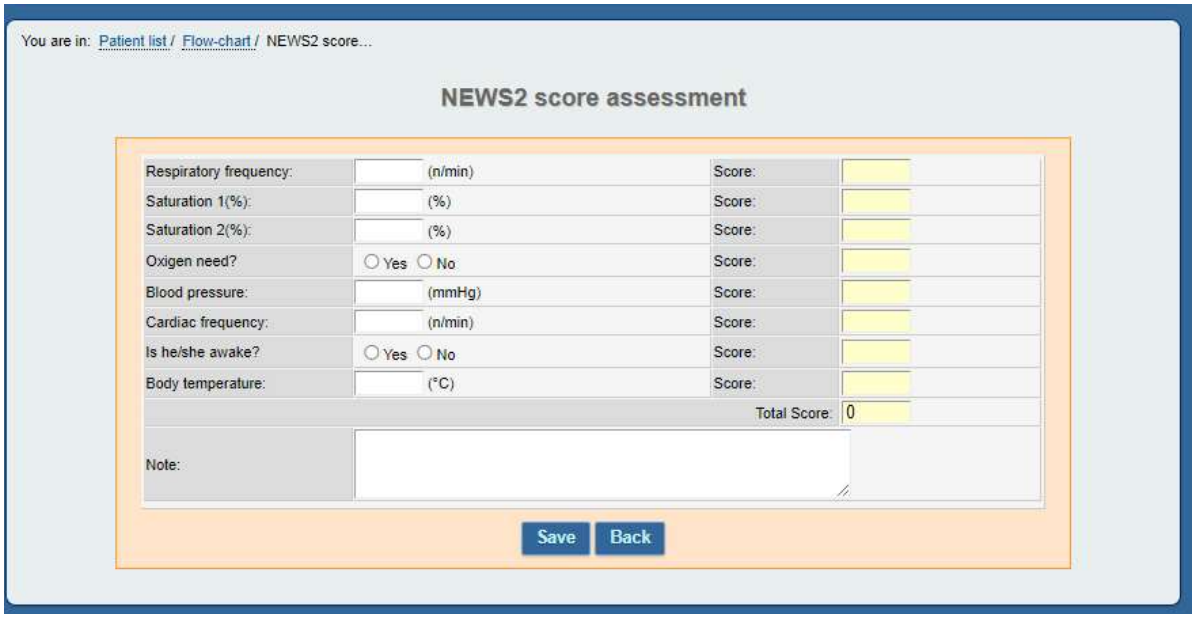
Medical History

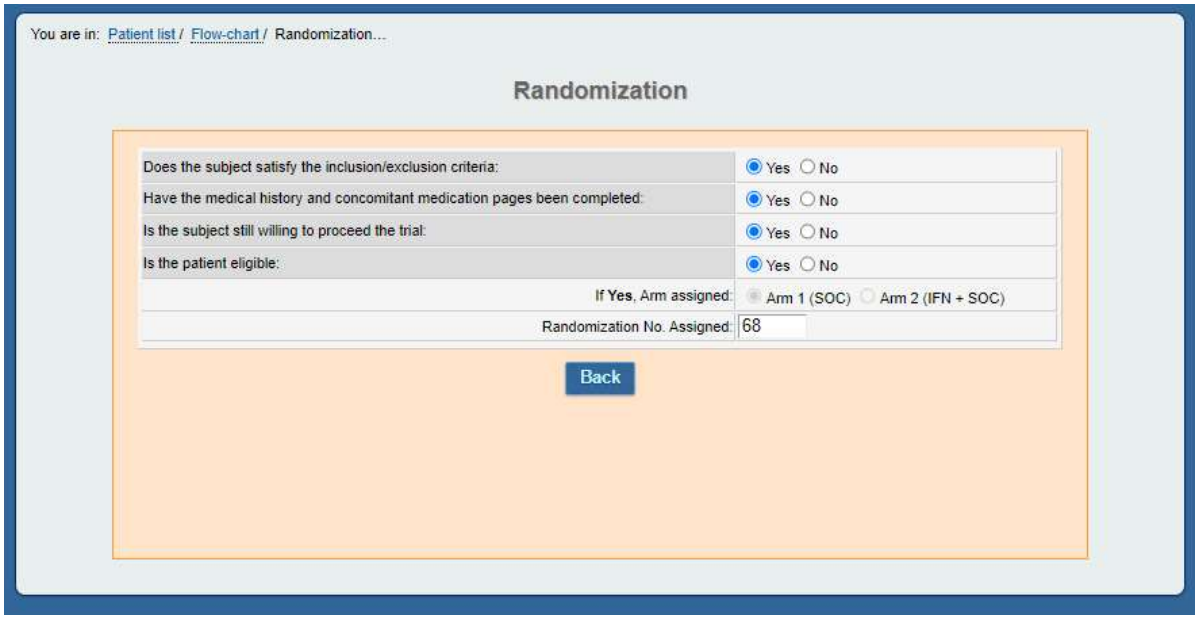
Pathologies	Onset date	DURATION (years)	Specify
☐ Respiratory disease			
☐ Chronic pulmonary disease			
☐ Asthma			
☐ Tuberculosis (active/previous)			
☐ Other			
☐ Cardiovascular/Circulatory disease			
☐ Chronic cardiac disease			
☐ Hypertension			
☐ Other			
☐ Musculoskeletal disease			
☐ Endocrine disease			
☐ Hematopoietic disease			
☐ Nervous disease			
☐ Chronic neurological disorder			
☐ Other			
☐ Dermatological disease			
☐ Integumentary System/Exocrine System disease			
☐ Genitourinary disease			
☐ Chronic kidney disease			
☐ Other			
☐ Lymphatic System/Immune System disease			
☐ Digestive disease			
☐ Chronic liver disease			
☐ Other			
☐ Obesity			
☐ Metabolic disease			
☐ Diabetes			
☐ Other			
☐ Ear, Nose, Throath disease			
☐ Psychiatric disease			
☐ Allergy			
☐ Malignant neoplasm			
☐ Infectious disease			
☐ HIV			
☐ HCV			
☐ Other			

Reason for change:

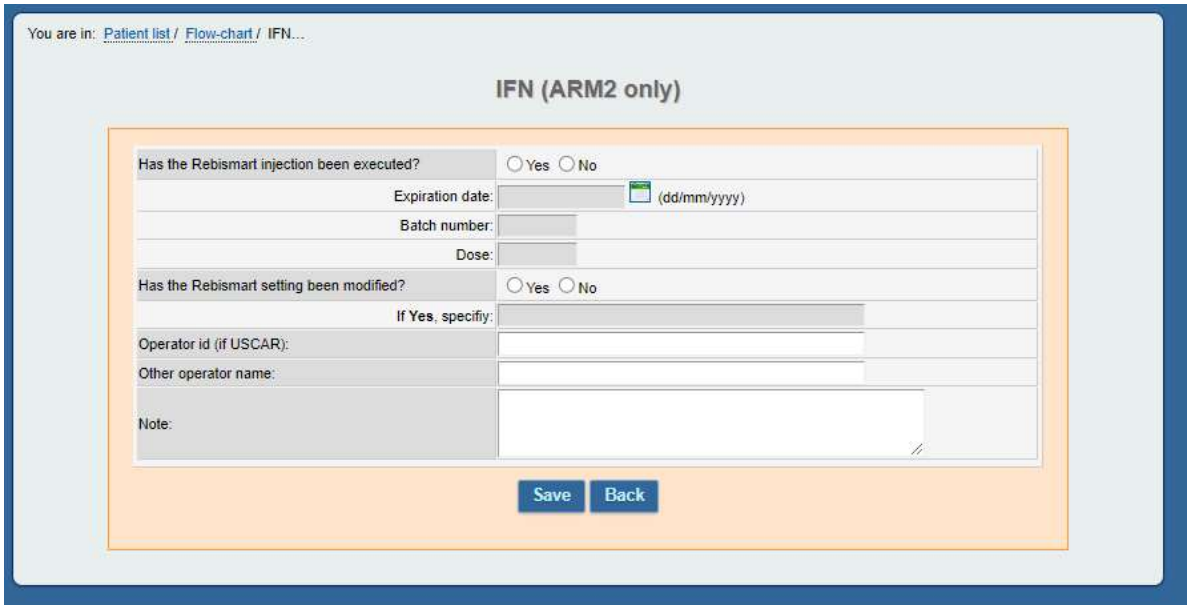
Save Back

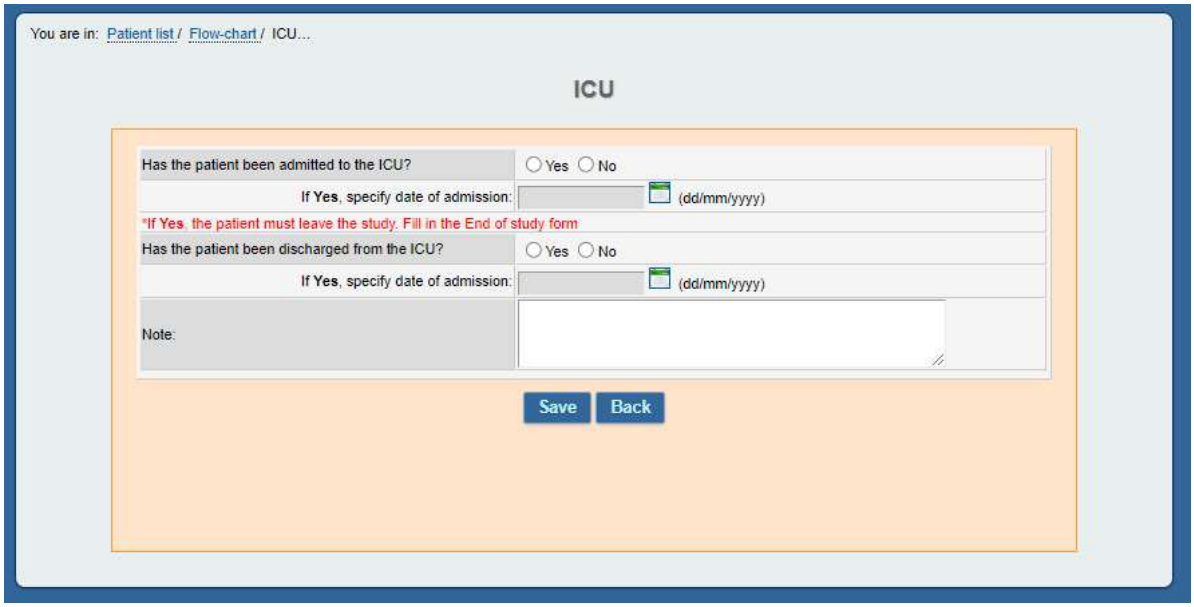

You are in: Patient list / Flow chart / Concomitant medications ...
Concomitant medications
List of Concomitant medications
Number
Drug
Start date
End date
Ongoing
1
PARACETAMOLO AFOM*20CPR 500MG
15/05/2021
15/05/2021
Add drug
Trade name
Active principle
Search drug
Drug list:
Number:
2
Trade name:
Other drug:
(fill the field if the drug does not exist in)
Dose:
Unit:
Frequency:
times
Route of administration:
Start Date:
(dd/mm/yyyy)
End Date:
(dd/mm/yyyy)
Ongoing
Reason for admin:
Save
Back

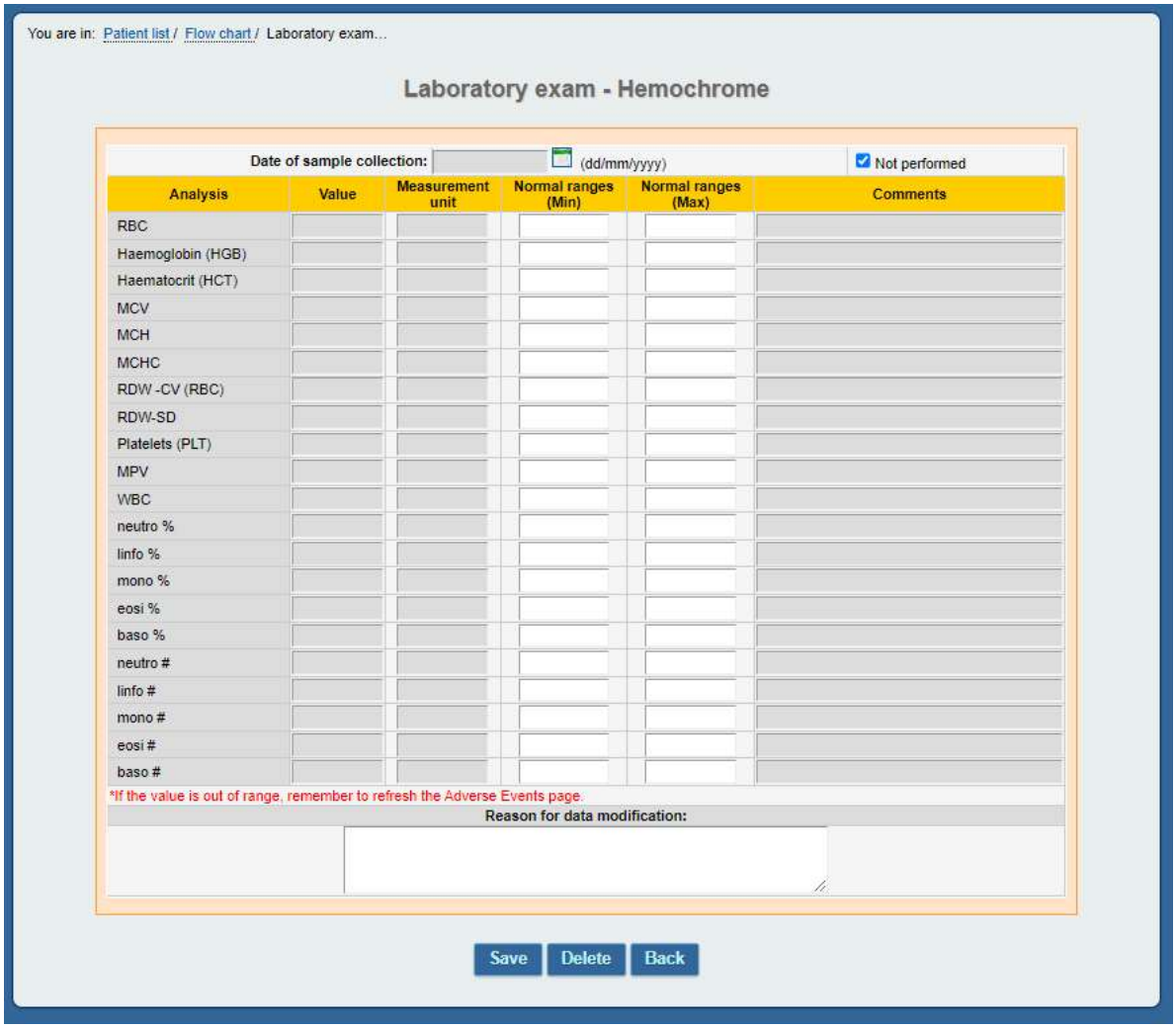

You are in: Patient list / Flow-chart / NEWS2 score...
NEWS2 score assessment
Respiratory frequency:
(n/min)
Score:
Saturation 1(%):
(%)
Score:
Saturation 2(%):
(%)
Score:
Oxigen need?
Yes
No
Score:
Blood pressure:
(mmHg)
Score:
Cardiac frequency:
(n/min)
Score:
Is he/she awake?
Yes
No
Score:
Body temperature:
(°C)
Score:
Total Score:
0
Note:
Save
Back

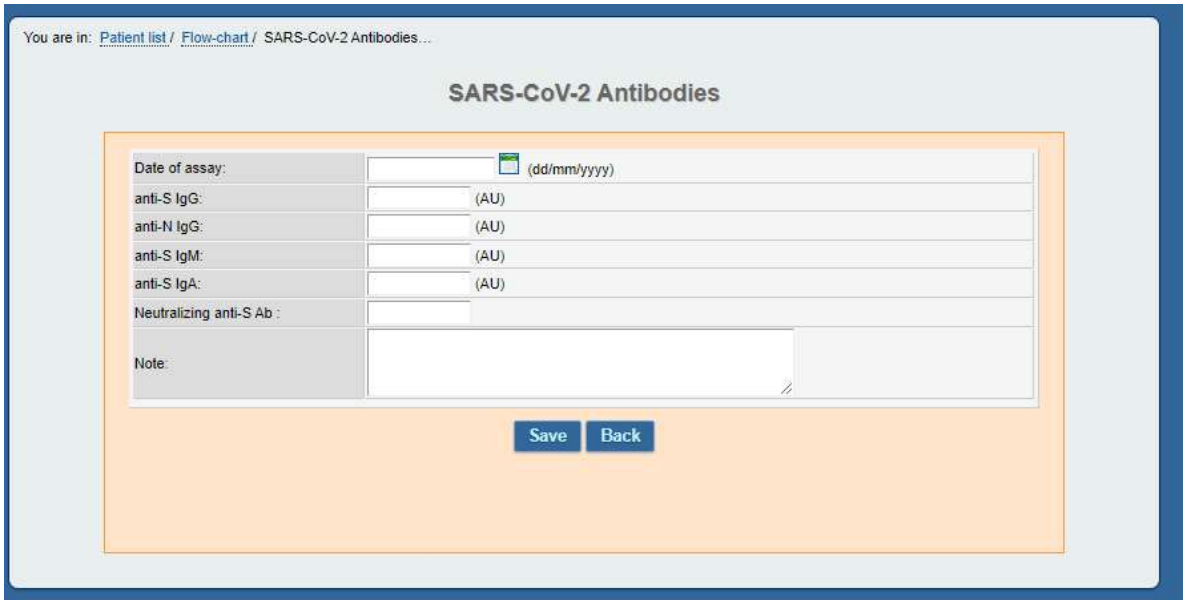

You are in: Patient list / Flow-chart / Randomization...
Randomization
Does the subject satisfy the inclusion/exclusion criteria:
Yes No
Have the medical history and concomitant medication pages been completed:
Yes No
Is the subject still willing to proceed the trial:
Yes No
Is the patient eligible:
Yes No
If Yes, Arm assigned:
Arm 1 (SOC) Arm 2 (IFN + SOC)
Randomization No. Assigned:
68
Back

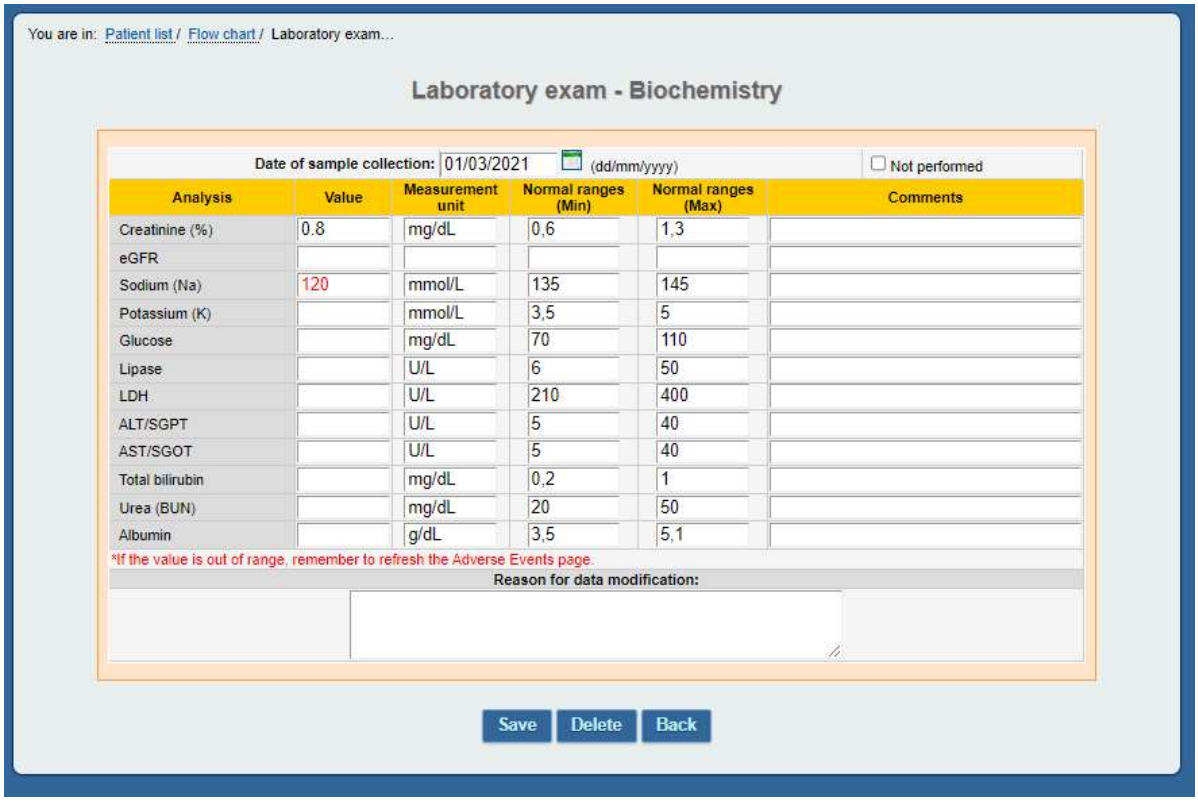

You are in: Patient list / Flow-chart / Sign and symptoms ...
Sign and symptoms
Add Sign and symptoms
Sign and symptoms:
If Other, specify:
Onset date:
(dd/mm/yyyy)
End date:
(dd/mm/yyyy)
Ongoing
Current Status:
Save
Back

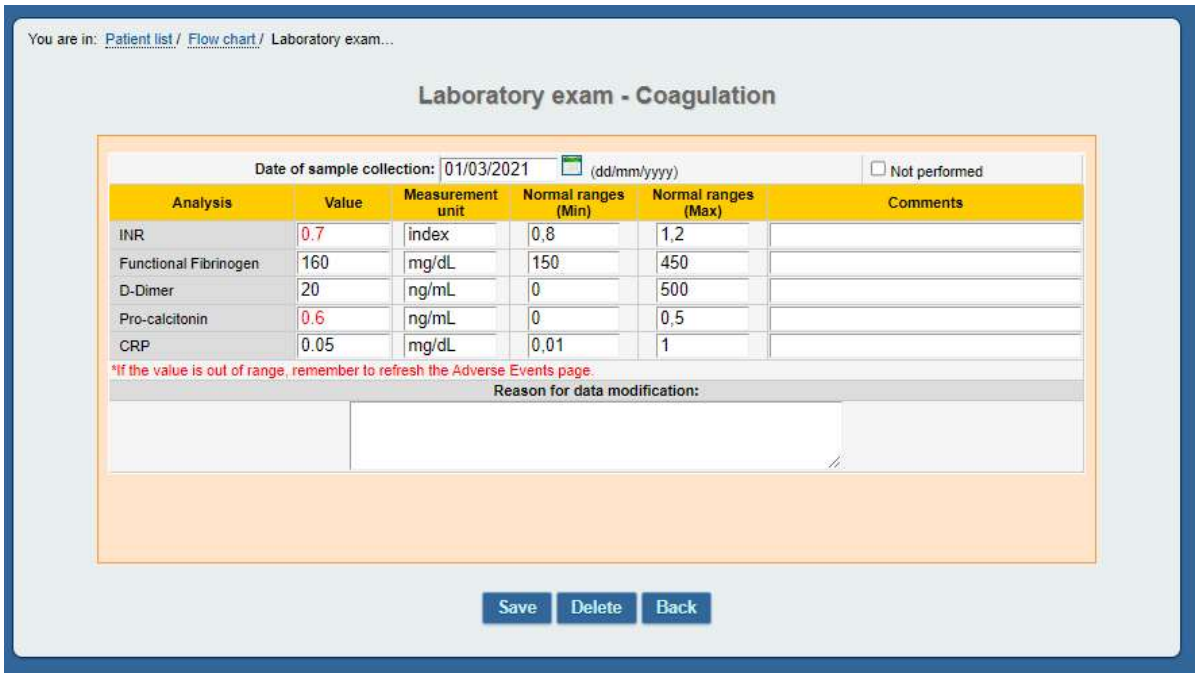

You are in: Patient list / Flow-chart / IFN...
IFN (ARM2 only)
Has the Rebismart injection been executed?
Yes No
Expiration date:
(dd/mm/yyyy)
Batch number:
Dose:
Has the Rebismart setting been modified?
Yes No
If Yes, specifiy:
Operator id (if USCAR):
Other operator name:
Note:
Save
Back


You are in: Patient list / Flow-chart / ICU...
ICU
Has the patient been admitted to the ICU?
Yes No
If Yes, specify date of admission:
(dd/mm/yyyy)
*If Yes, the patient must leave the study. Fill in the End of study form
Has the patient been discharged from the ICU?
Yes No
If Yes, specify date of admission:
(dd/mm/yyyy)
Note:
Save
Back


You are in: Patient list / Flow chart / Laboratory exam...
Laboratory exam - Hemochrome
Date of sample collection: (dd/mm/yyyy)
Not performed
Analysis
Value
Measurement unit
Normal ranges (Min)
Normal ranges (Max)
Comments
RBC
Haemoglobin (HGB)
Haematocrit (HCT)
MCV
MCH
MCHC
RDW -CV (RBC)
RDW-SD
Platelets (PLT)
MPV
WBC
neutro %
linfo %
mono %
eosi %
baso %
neutro #
linfo #
mono #
eosi #
baso #
*If the value is out of range, remember to refresh the Adverse Events page.
Reason for data modification:
Save
Delete
Back


You are in: Patient list / Flow-chart / SARS-CoV-2 Antibodies...
SARS-CoV-2 Antibodies
Date of assay: (dd/mm/yyyy)
anti-S IgG: (AU)
anti-N IgG: (AU)
anti-S IgM: (AU)
anti-S IgA: (AU)
Neutralizing anti-S Ab :
Note:
Save
Back


You are in: Patient list / Flow chart / Laboratory exam...
Laboratory exam - Biochemistry
Date of sample collection: 01/03/2021 (dd/mm/yyyy)
Not performed
Analysis | Value | Measurement unit | Normal ranges (Min) | Normal ranges (Max) | Comments
Creatinine (%) | 0.8 | mg/dL | 0,6 | 1,3
eGFR
Sodium (Na) | 120 | mmol/L | 135 | 145
Potassium (K) | mmol/L | 3,5 | 5
Glucose | mg/dL | 70 | 110
Lipase | U/L | 6 | 50
LDH | U/L | 210 | 400
ALT/SGPT | U/L | 5 | 40
AST/SGOT | U/L | 5 | 40
Total bilirubin | mg/dL | 0,2 | 1
Urea (BUN) | mg/dL | 20 | 50
Albumin | g/dL | 3,5 | 5,1
*If the value is out of range, remember to refresh the Adverse Events page.
Reason for data modification:
Save
Delete
Back

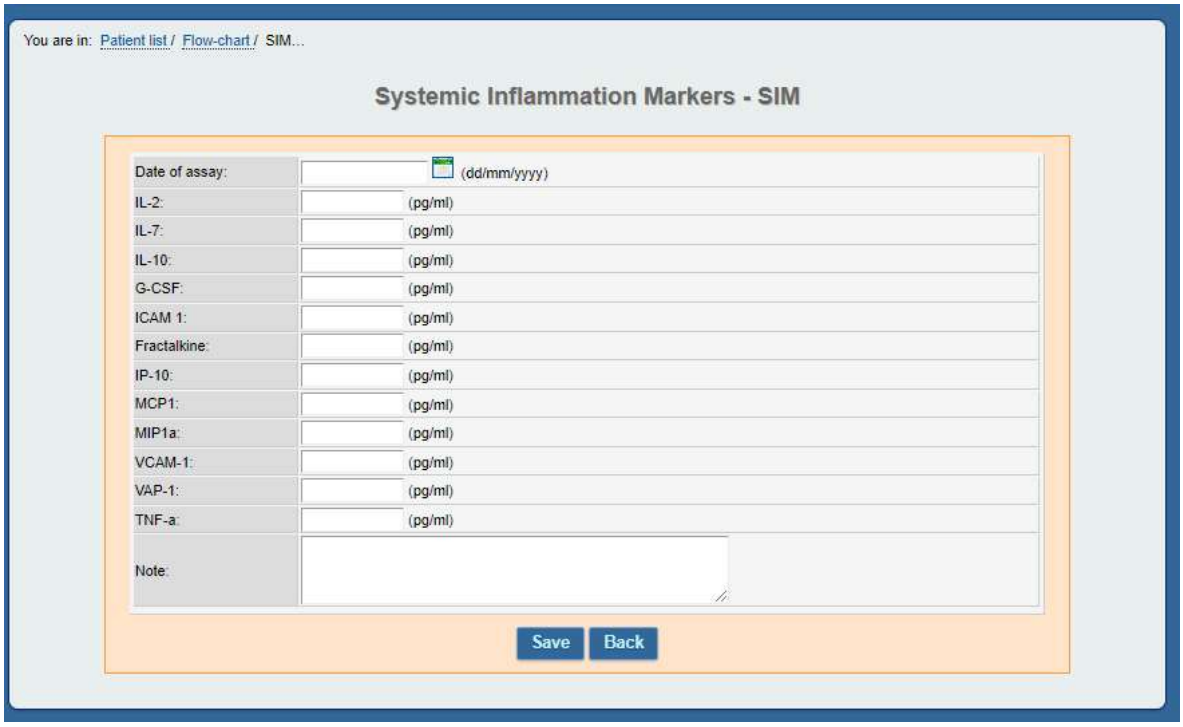

You are in: Patient list / Flow chart / Laboratory exam...
Laboratory exam - Coagulation
Date of sample collection: 01/03/2021 (dd/mm/yyyy)
Not performed
Analysis | Value | Measurement unit | Normal ranges (Min) | Normal ranges (Max) | Comments
INR | 0.7 | index | 0,8 | 1,2
Functional Fibrinogen | 160 | mg/dL | 150 | 450
D-Dimer | 20 | ng/mL | 0 | 500
Pro-calcitonin | 0.6 | ng/mL | 0 | 0,5
CRP | 0.05 | mg/dL | 0,01 | 1
*If the value is out of range, remember to refresh the Adverse Events page.
Reason for data modification:
Save
Delete
Back

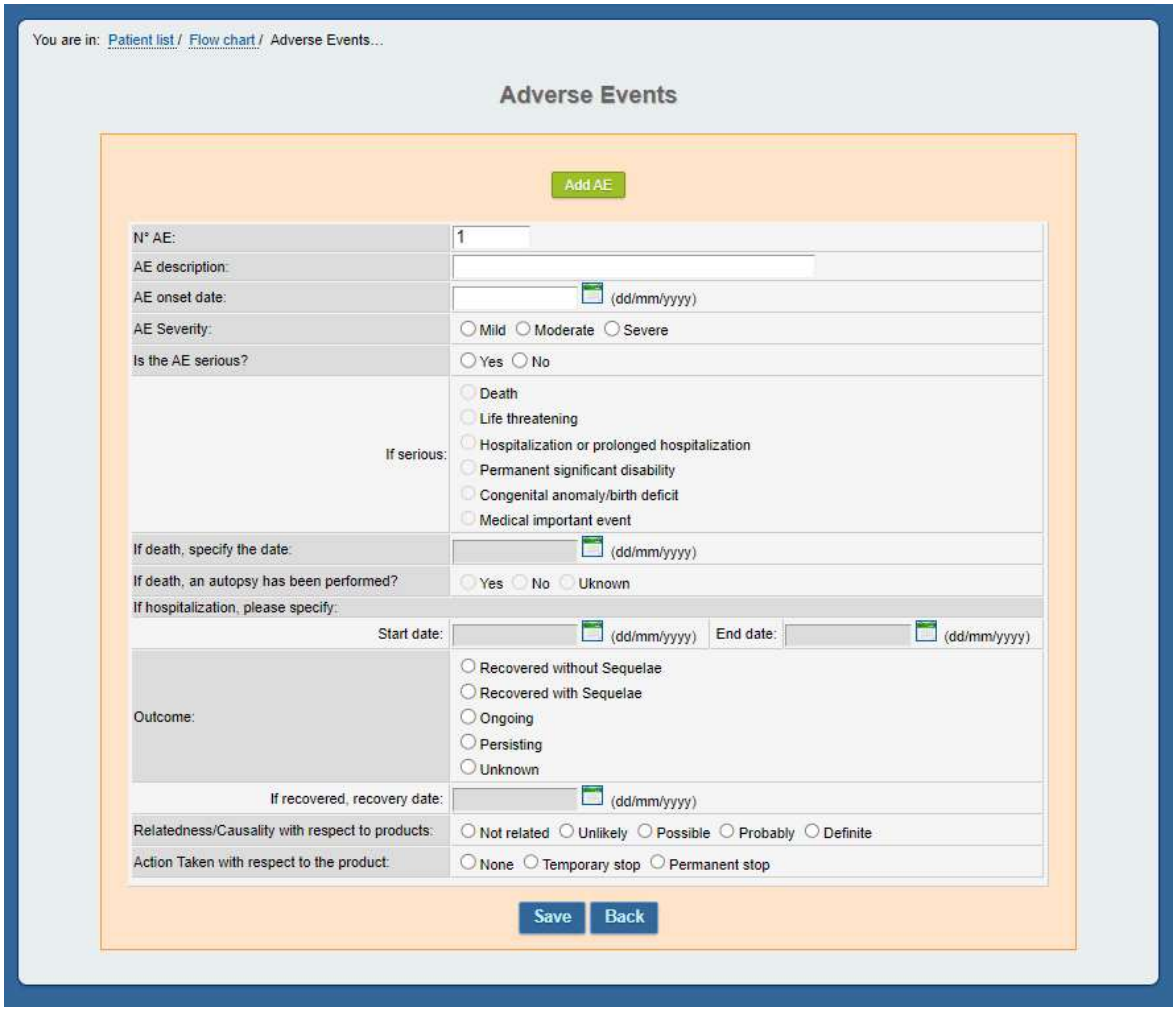

You are in: Patient list / Flow-chart / ISG...
Interferon Signature - ISG
Date of assay:
(dd/mm/yyyy)
IFI44L:
(RCN)
IFI27:
(RCN)
RSAD2:
(RCN)
SIGLEC1:
(RCN)
IFIT1:
(RCN)
ISG15:
(RCN)
Note:
Save
Back

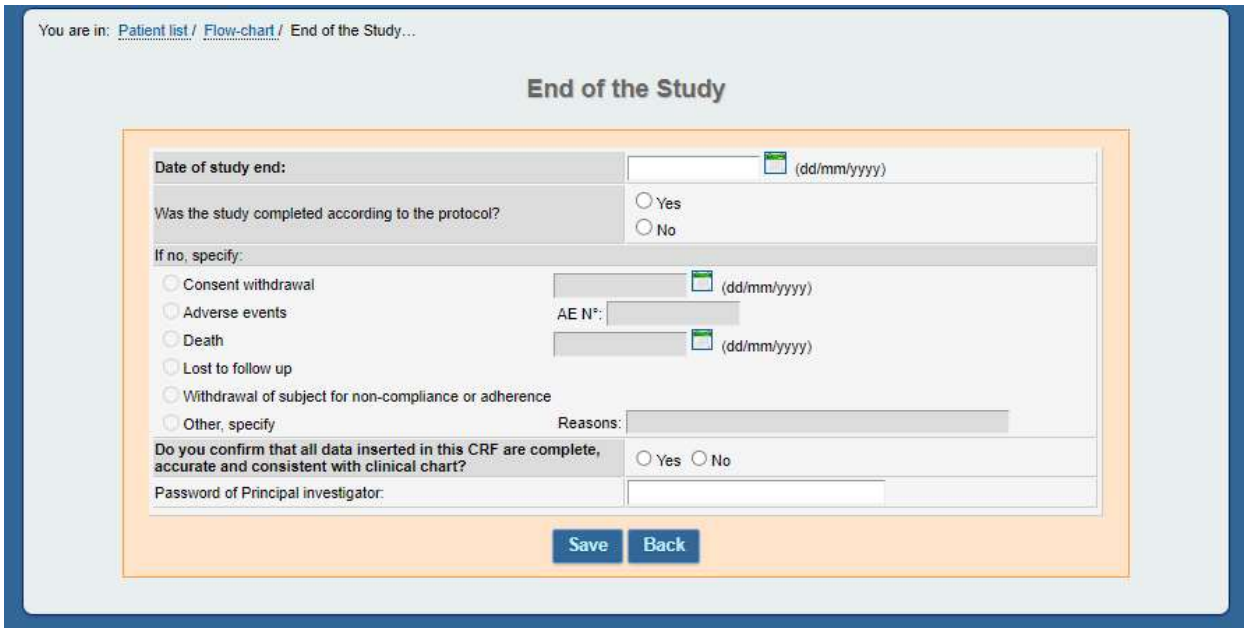
Cellular Immune Monitoring - CIM

Date of assay:	(dd/mm/yyyy)
leu_linfociti:	(%)
lym_CD3:	(%)
CD3_CD4SP:	(%)
CD3_CD8SP:	(%)
CD8_CM:	(%)
CD8_EM:	(%)
CD8_EM:	(%)
CD8_TD:	(%)
CD8_CD28pos27neg:	(%)
CD8_CD28pos27pos:	(%)
CD8_CD28neg27neg:	(%)
CD8_CD28neg27pos:	(%)
CD8_CD57posCD57neg:	(%)
CD8_CD57posPD1neg:	(%)
CD8_CD57posPD1pos:	(%)
CD8_CD57negPD1pos:	(%)
CD4_CM:	(%)
CD4_EM:	(%)
CD4_N:	(%)
CD4_TD:	(%)
CD4_CD28pos27neg:	(%)
CD4_CD28pos27pos:	(%)
CD4_CD28neg27neg:	(%)
CD4_CD28neg27pos:	(%)
CD4_CD57pos27neg:	(%)
CD4_CD57posPD1neg:	(%)
CD4_CD57posPD1pos:	(%)
CD4_CD57negPD1pos:	(%)
gpSpike_CD8:	(%)
Vd2:	(%)
Vd2_TD:	(%)
Vd2_EM:	(%)
Vd2_N:	(%)
Vd2_CM:	(%)
lym_CD19:	(%)
lym_NK:	(%)
lym_NKT:	(%)
leu_CD14pos:	(%)
leu_Treg:	(%)
lym_Treg:	(%)
CD3_Treg:	(%)
CD4_Treg:	(%)
Treg_CD45RAnegCD39neg:	(%)
Treg_CD45RAnegCD39pos:	(%)
Treg_CD45RAposCD39neg:	(%)
Treg_CD45RAposCD39pos:	(%)
Note:	

Save Back


You are in: Patient list / Flow-chart / SIM...
Systemic Inflammation Markers - SIM
Date of assay:
(dd/mm/yyyy)
IL-2:
(pg/ml)
IL-7:
(pg/ml)
IL-10:
(pg/ml)
G-CSF:
(pg/ml)
ICAM 1:
(pg/ml)
Fractalkine:
(pg/ml)
IP-10:
(pg/ml)
MCP1:
(pg/ml)
MIP1a:
(pg/ml)
VCAM-1:
(pg/ml)
VAP-1:
(pg/ml)
TNF-a:
(pg/ml)
Note:
Save
Back


You are in: Patient list / Flow chart / Adverse Events...
Adverse Events
Add AE
N° AE: 1
AE description:
AE onset date: (dd/mm/yyyy)
AE Severity: Mild Moderate Severe
Is the AE serious? Yes No
If serious: Death Life threatening Hospitalization or prolonged hospitalization Permanent significant disability Congenital anomaly/birth deficit Medical important event
If death, specify the date: (dd/mm/yyyy)
If death, an autopsy has been performed? Yes No Uknown
If hospitalization, please specify:
Start date: (dd/mm/yyyy) End date: (dd/mm/yyyy)
Outcome: Recovered without Sequelae Recovered with Sequelae Ongoing Persisting Unknown
If recovered, recovery date: (dd/mm/yyyy)
Relatedness/Causality with respect to products: Not related Unlikely Possible Probably Definite
Action Taken with respect to the product: None Temporary stop Permanent stop
Save Back


You are in: Patient list / Flow-chart / End of the Study...
End of the Study
Date of study end: (dd/mm/yyyy)
Was the study completed according to the protocol? Yes No
If no, specify:
Consent withdrawal (dd/mm/yyyy)
Adverse events AE N°:
Death (dd/mm/yyyy)
Lost to follow up
Withdrawal of subject for non-compliance or adherence
Other, specify Reasons:
Do you confirm that all data inserted in this CRF are complete, accurate and consistent with clinical chart? Yes No
Password of Principal investigator:
Save Back

Final Report Appendix 16.1.3

- List of IECs (plus the name of the committee Chair)
- Representative written information for patient and sample consent forms

List of IECs:

Name of EC

COMITATO ETICO DELL'ISTITUTO NAZIONALE PER LE MALATTIE INFETTIVE " LAZZARO SPALLANZANI" IRCCS, VIA PORTUENSE, Via Portuense, 292 - 00149 Roma

Name of the Committee chair: Dott.ssa Cinzia Caporale, Annarita Vestri

Documents name and version	Amendment	Date of transmission by Sponsor	Date of approval by IEC	Name of the Committee chair
• Study Protocol V.2.0 • Foglio Informativo e Consenso Informato per il paziente V. 1.0	n.a.	05/10/2020	6/11/2020	Cnzia Caporale
• Study Protocol V. 3.0 • Foglio Informativo e Consenso Informato per il paziente V. 2.0	NS AMD 01	18/03/2021	23/3/2021	Cinzia Caporale
• Study Protocol V. 4.0 • Foglio Informativo e Consenso Informato per il paziente V. 3.0	S AMD 01	31/5/2021	29/7/2021	Annarita Vestri

Representative written information for patient and sample consent forms

- Foglio Informativo e Consenso Informato per il paziente V. 1.0
- Foglio Informativo e Consenso Informato per il paziente V. 2.0
- Foglio Informativo e Consenso Informato per il paziente V. 3.0

Consiglio Nazionale delle Ricerche		**Istituto di Farmacologia Traslazionale** Institute of Translational Pharmacology **IFT**
Via Fosso del Cavaliere, 100 - 00133 Roma, Italy Tel: +39 06- 45488487 fax: +39 06-45488257		
Direttore Dott. Vito Michele Fazio		

FOGLIO INFORMATIVO PER IL PAZIENTE

TITOLO DELLO STUDIO:

"Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 anziani"

Numero EUDRACT: 2020-003872-42

Promotore dello Studio:

Istituto di Farmacologia Traslazionale – Consiglio Nazionale delle Ricerche

Sperimentatore Principale:

Dott. Giuseppe Sconocchia

Versione n. 01 del 15/09/2020

Gent.mo/gent.ma paziente,

con il presente documento la invitiamo a partecipare allo studio no profit dal titolo "Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 anziani" promosso dall'Istituto di Farmacologia Traslazionale — Consiglio Nazionale delle Ricerche.

Prima che Lei prenda una decisione in merito, è importante che comprenda il motivo dello studio e cosa Le sarà chiesto di fare, qualora decidesse di prendervi parte. Lo sperimentatore ed i suoi collaboratori, oltre alle spiegazioni che Le forniranno durante questo colloquio, sono a Sua completa disposizione per qualsiasi chiarimento.

Questo documento ha lo scopo di fornirle un'informazione corretta e completa affinché Lei possa esprimere una scelta libera e consapevole. La partecipazione allo studio è a carattere volontario e Lei avrà a disposizione un tempo adeguato per riflettere e porre domande di chiarimento prima di dare la sua adesione. Avrà inoltre il diritto di ritirare il proprio consenso in qualsiasi momento senza dover fornire alcuna giustificazione e senza perdere alcun diritto e beneficio. In caso di ritiro del consenso, nessuna nuova informazione sarà raccolta e aggiunta ai dati esistenti.

Lei ha il diritto di venire a conoscenza di ogni nuova informazione che possa modificare la sua decisione di partecipare allo studio.

NOTA INFORMATIVA

Qual è lo scopo dello studio?

Lo studio ha lo scopo di sperimentare un trattamento che, nei pazienti con più di 65 anni positivi all'infezione da nuovo Coronavirus 19 (SARS-CoV2), possa ridurre il rischio di progressione verso forme più gravi di malattia, e la conseguente necessità di ricovero in terapia intensiva. Questo studio sperimentale sarà condotto in un centro clinico (Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani"), in alcune Residenze Sanitarie Assistenziali e in regime domiciliare, e vi parteciperanno 60 pazienti nel Lazio.

Qual è il farmaco che viene testato?

Il farmaco che verrà testato è l'Interferone beta 1a, disponibile in commercio con il nome Rebif e ad oggi utilizzato nel trattamento della Sclerosi Multipla.

In diversi studi recentemente pubblicati su riviste scientifiche internazionali, il trattamento con Interferone (da solo oppure in combinazione con altri farmaci) ha dimostrato di ridurre la durata dell'infezione da nuovo Coronavirus 19 e la progressione verso forme più gravi di malattia. Dai dati emersi nel corso della pandemia da COVID-19, risulta che i pazienti anziani abbiano maggiore possibilità di trarre vantaggio dal trattamento con questo farmaco.

Per quale motivo sono stato scelto?

Le è stata diagnosticata l'infezione da nuovo Coronavirus 19 ed il medico ritiene che possieda i requisiti necessari per entrare nello studio. Le è stato chiesto di considerare la possibilità di aderirvi per valutare se il nuovo trattamento può contribuire a migliorare il Suo stato di salute ed impedire la progressione dell'infezione verso stati più gravi di malattia.

Sono obbligato a partecipare?

No. La decisione di partecipare allo studio dipende solo da Lei. È completamente volontaria. Se preferisce non partecipare non deve fornire spiegazioni. Riceverà comunque tutte le indagini, le visite e le terapie attualmente disponibili per la Sua malattia.

Cosa accadrà se decido di partecipare allo studio?

Se desidera prendere in considerazione la possibilità di parteciparvi, Le sarà consegnata questa scheda informativa, da leggere e conservare. Avrà la possibilità di chiedere tutte le spiegazioni che desidera a riguardo. Le sarà chiesto di firmare il modulo di consenso, in allegato e l'informativa per il trattamento dei dati personali. Solo dopo che Lei avrà firmato la dichiarazione di consenso, inizierà la valutazione medica per accertare la Sua idoneità a partecipare allo studio, che si baserà sulla risposta ad alcune domande riguardanti la sua condizione medica personale e la relativa terapia, sulla sua storia (anamnesi) medica personale e familiare e anche sui risultati delle analisi che verranno effettuate su alcuni campioni di sangue che le verranno prelevati dal personale dello studio.

Come si svolgerà lo studio? A quale trattamento sarò sottoposto?

Lo studio sarà condotto "in aperto", cioè sia Lei, sia il medico, sarete a conoscenza del trattamento/del gruppo di appartenenza. Lo studio prevede di arruolare 60 pazienti, di cui 40 riceveranno il trattamento sperimentale con Interferone e 20 pazienti riceveranno solo lo "standard di cura", cioè le terapie che i medici coinvolti riterranno opportuno somministrare per alleviare eventuali sintomi. L'assegnazione dei pazienti al gruppo sperimentale o al gruppo di controllo verrà affidata ad un protocollo "di randomizzazione", e sarà quindi frutto del caso. Lei avrà il 66% di possibilità di essere trattato con Interferone ed il 33% di ricevere le cure standard. Sia che venga assegnato al gruppo sperimentale o a quello di controllo, nel caso il medico deciderà che lei può rimanere nel suo domicilio, le forniremo un kit di strumenti con cui potrà monitorare il Suo stato di salute per rilevare tempestivamente l'insorgenza di sintomi di allerta. Il personale medico spiegherà a Lei (e ai Suoi familiari) il corretto uso degli strumenti.

Quale sarà il mio impegno? Cosa dovrò fare?

Per entrare nello studio, il medico dovrà verificare che Lei soddisfi tutti i criteri previsti per la partecipazione. Le chiederà pertanto di sottoporsi a visita medica, durante la quale Le saranno rilevati e registrati i seguenti parametri: temperatura corporea, pressione del sangue, frequenza respiratoria, saturazione di ossigeno. Le verranno inoltre prelevati 10 ml di sangue per valutare alcuni parametri (emocromo, funzionalità epatica e renale) rilevanti per l'ingresso al presente studio.

Lei dovrà fornire al medico sperimentatore tutte le informazioni che riguardano la Sua storia clinica, i medicinali che ha assunto e quelli sta assumendo attualmente. Dovrà riferire anche in merito a eventuali studi clinici, cui sta partecipando.

Qualora tutti i criteri previsti dallo studio siano soddisfatti, il medico la informerà del Suo ingresso nello studio.

All'ingresso dello studio, Le verrà assegnato un codice e Lei verrà assegnato/a ad un gruppo (sperimentale o di controllo). Se capiterà nel gruppo di pazienti trattati con farmaco sperimentale, Le verrà somministrato Interferone beta a basso dosaggio attraverso un inoculo sottocute per 4 volte nel corso di dieci giorni (giorno 1, 3, 7 e 10).

Indipendentemente dal gruppo di trattamento in cui Lei capiterà, verranno effettuati una serie di controlli del Suo stato di salute, sia durante la malattia, sia nella fase successiva alla Sua guarigione, nell'esclusivo interesse di monitorare il Suo andamento clinico. Personale specializzato le effettuerà alcuni prelievi di sangue (circa 10-30 ml) e i tamponi nasofaringei necessari per misurare la presenza del nuovo Coronavirus 19, in aggiunta a quelli eseguiti nella usuale pratica clinica, secondo la tempistica seguente:

Giorno 1: 30 ml di sangue + tampone nasofaringeo

Giorno 3: 30 ml di sangue

Giorno 10: 15 ml di sangue

Giorno 14: 30 ml di sangue + tampone nasofaringeo

Giorno 21: 10 ml di sangue

Giorno 28: 15 ml di sangue + tampone nasofaringeo

I campioni raccolti verranno trasferiti all'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani" per essere analizzati e/o conservati per successive analisi. Alcune delle analisi

sui campioni di sangue verranno effettuate presso l'Istituto Superiore di Sanità, da personale coinvolto nello studio clinico.

Lei dovrà seguire attentamente le istruzioni che Le saranno fornite. Dovrà sottoporsi agli inoculi di farmaco sottocute (solo se capiterà nel gruppo di trattamento) e ai prelievi (entrambi i gruppi) da parte del personale specializzato coinvolto nello studio; con l'assistenza di personale infermieristico oppure, se lei resterà nel proprio domicilio, con l'aiuto di un suo familiare, dovrà farsi monitorare due volte al giorno la temperatura corporea, la pressione arteriosa, la frequenza respiratoria, la frequenza cardiaca e la saturazione attraverso la strumentazione che le verrà consegnata, seguendo le modalità che le verranno illustrate, annotandole sull'apposito diario che le verrà fornito; dovrà informare il medico di eventuali problemi/disturbi che potranno insorgere e di ogni variazione riguardante la Sua salute. Dovrà consultare sempre il medico responsabile dello studio prima di sottoporsi ad altre terapie, o assumere farmaci da banco, onde evitare incompatibilità/effetti/possibili interazioni tra Interferone ed altri prodotti.

Quali benefici potrò attendermi dalla partecipazione allo studio?

Con l'utilizzo dell'Interferone ci si aspetta una guarigione più veloce dall'infezione da nuovo Coronavirus 19, una minore necessità di ricovero in Rianimazione e di intubazione, una riduzione della mortalità. Tale beneficio, in ogni caso, non può esserle garantito e Lei potrebbe non avere alcun vantaggio personale. Quantunque Lei non ottenga benefici diretti da questa ricerca, le informazioni raccolte grazie alla Sua partecipazione a questo studio saranno comunque di grande importanza per la lotta contro il virus responsabile dell'attuale pandemia da COVID-19.

Rischi ed effetti collaterali che possono derivare dalla partecipazione allo studio

L'interferone beta può causare effetti collaterali simil-influenzali, come mal di testa, febbre, brividi, dolori muscolari ed articolari, affaticamento e nausea. Questi sintomi sono generalmente di lieve entità soprattutto alle dosi che verranno utilizzate in questo studio; sono più frequenti all'inizio del trattamento e diminuiscono con il suo proseguimento.

È anche possibile che si verifichi un certo arrossamento al sito di iniezione.

I rischi fisici e i disturbi correlati al prelievo di sangue sono identici a quelli riguardanti ogni genere di prelievo di campione ematico da vena, ovvero possibilità di piccoli lividi e irritazioni locali, con rari casi di infezione.

Sono disponibili altre terapie?

No. Al momento presente non sono disponibili altri farmaci approvati per la sua patologia.

Infatti, trattandosi di un nuovo virus, tutto il mondo scientifico sta lavorando per cercare di trovare la cura adatta per rallentare la malattia COVID19, soprattutto nei pazienti anziani. Se deciderà di non partecipare allo studio proposto, il Suo stato di salute verrà monitorato e le verrà somministrata la terapia standard di supporto per ridurre eventuali sintomi.

Cosa accadrà se nuove conoscenze o nuove informazioni si rendessero disponibili?

Qualora si rendessero disponibili nuove informazioni sul farmaco, o sullo studio, che potrebbero influenzare la Sua sicurezza, o la Sua volontà a continuare il trattamento, Le saranno comunicate tempestivamente dal medico sperimentatore.

Potrò cambiare idea dopo aver accettato di partecipare?

Si. Lei potrà decidere di ritirare il consenso e interrompere il trattamento, in qualsiasi momento, anche a studio avviato, senza dover fornire giustificazioni a meno che la decisione non derivi dalla comparsa di disturbi o effetti indesiderati o non previsti, nel qual caso dovrà fornire al medico sperimentatore tutte le informazioni del caso. La Sua decisione non avrà ripercussioni sull'assistenza e sulle cure che dovrà ricevere in futuro. I medici continueranno a seguirla con la migliore assistenza sanitaria possibile. Qualora decidesse di ritirare il consenso Le chiediamo di informare il medico sperimentatore e di acconsentire alla valutazione finale.

Il mio medico di fiducia sarà informato?

Previa sua autorizzazione, il Suo medico di fiducia sarà informato con apposita lettera della Sua partecipazione e potrà anche contattare il responsabile dello studio per qualsiasi informazione.

Quanto dura lo studio?

La Sua partecipazione allo studio avrà una durata di circa un mese.

Il trattamento sperimentale potrebbe essere interrotto o sospeso?

Si. Il medico sperimentatore potrebbe interrompere lo studio in qualsiasi momento, anche contro la Sua volontà, qualora lo ritenesse necessario per la Sua salute, o per la corretta

conduzione della ricerca (es. se Lei non assume regolarmente il farmaco, non si attiene alle istruzioni ricevute, o non rispetta il programma delle visite). Lo studio potrebbe essere sospeso/interrotto anche dallo Sponsor, o dalle Autorità Regolatorie per cause attualmente non prevedibili.

Cosa accadrà se le mie condizioni fisiche dovessero peggiorare?

Qualora il monitoraggio delle Sue condizioni di salute dovesse evidenziare un significativo peggioramento, il medico sperimentatore disporrà l'interruzione della sua partecipazione allo studio. Il Suo medico di Medicina Generale verrà tempestivamente informato, e le verranno assicurati i migliori trattamenti disponibili per la sua patologia.

Cosa accadrà se subentrassero problemi: infortunio o danni correlati allo studio?

Qualora dovessero verificarsi effetti collaterali, indesiderati, o danni alla Sua salute, riconducibili allo studio, Lei dovrà informare tempestivamente il medico sperimentatore che Le fornirà le relative informazioni. Come previsto dalla normativa vigente (D.M. 14.07.2009), lo studio è coperto da un'apposita assicurazione. Inoltre, nel caso si manifestassero complicanze correlate alla partecipazione allo studio saranno garantiti tutti i migliori trattamenti medici necessari e l'assicurazione si farà carico di tutti i costi relativi all'assistenza e al trattamento non coperti dall'assistenza sanitaria ordinaria. Sono esclusi i trattamenti per danni o patologie che risultino indipendenti da questo studio.

Dovrò sostenere spese aggiuntive?

No. La Sua partecipazione allo studio di ricerca non comporterà per Lei alcun aggravio di spesa. Il farmaco, tutte le visite e gli esami supplementari richiesti, saranno a carico dell'Istituzione che sponsorizza la ricerca.

Riceverò un compenso per la mia partecipazione allo studio?

No. La vigente normativa regola la partecipazione agli studi clinici esclusivamente su base spontanea, senza possibilità di ricevere alcun compenso economico.

Chi organizza e finanzia lo studio di ricerca?

Lo studio è organizzato e sponsorizzato dall'Istituto di Farmacologia Traslazionale (Consiglio Nazionale delle Ricerche, CNR), che verserà all'Ospedale /all'Unità Operativa/al Medico sperimentatore un compenso per l'impegno a seguire la Sua partecipazione alla ricerca.

Chi ha esaminato lo studio?

Il protocollo dello studio è stato stilato in conformità alle Norme di Buona Pratica Clinica dell'Unione Europea e alla revisione corrente della Dichiarazione di Helsinki ed è stato approvato dal Comitato Etico Unico per gli studi COVID dell'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", dalle competenti Autorità Sanitarie o dalle Istituzioni da queste delegate.

La mia partecipazione resterà riservata? Come saranno usati i miei dati personali?

La Sua partecipazione allo studio sarà obbligatoriamente registrata nella cartella clinica, tuttavia, il medico sperimentatore e i suoi collaboratori gestiranno i Suoi dati personali e tutte le informazioni relative al Suo stato di salute in modo strettamente riservato e soltanto nella misura in cui essi saranno necessari in relazione all'obiettivo dello studio. I Suoi dati e tutte le informazioni saranno trattate in accordo a quanto è stabilito nella "nota informativa per la tutela dei dati personali" (vedi allegato N° … …). il Titolare del trattamento dei dati personali è l'Istituto di Farmacologia Traslazionale del Consiglio Nazionale delle Ricerche, nella persona del suo XXX. Il Delegato interno alla gestione delle attività di trattamento dei dati personali e degli adempimenti previsti dal Regolamento UE n. 2016/679, ai sensi del D.lgs. 196/2003 come modificato dal D.lgs. 101/2018, è il dr.XXX, in qualità di XXX

Lei dovrà prestare il consenso al trattamento dei dati personali all'atto della firma del presente modulo, altrimenti non sarà possibile garantire la sua partecipazione allo studio

Come saranno utilizzati i miei campioni?

I campioni di sangue e i tamponi che le verranno prelevati saranno essenziali per valutare il Suo stato di salute e per comprendere meglio gli effetti del farmaco nel contrastare il nuovo Coronavirus 19. Per tutelare la sua privacy personale, i campioni che La riguardano non verranno identificati con i Suoi dati anagrafici ma con un codice e saranno utilizzati unicamente per supportare le evidenze cliniche raccolte da questo studio. I campioni saranno conservati presso l'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani" e l'Istituto Superiore di Sanità per un tempo massimo di due anni dalla chiusura dello studio (prevista per settembre 2021).

Come saranno utilizzati/diffusi i risultati?

Alla fine della ricerca i dati raccolti durante lo studio potranno essere presentati a congressi o in pubblicazioni scientifiche, ma la Sua identità resterà anonima.

Normativa di riferimento

Tutte le informazioni raccolte durante lo studio sono confidenziali e verranno trattate in ottemperanza al D. Lgs. 196 del 30 giugno 2003 ”Codice in materia di protezione dei dati personali”, alla Deliberazione n. 52 del 24.07.2008 ”Linee Guida per il trattamento dei dati nell’ambito delle sperimentazioni cliniche di medicinali”, del Garante per la Privacy e ai Regolamenti Europei 2016/679 e 536/2014.

Posso richiedere altre informazioni oppure essere informato circa i risultati dello studio?

Per qualsiasi informazione o per ricevere ulteriori chiarimenti o informazioni può rivolgersi a:

Filippo Belardelli, email: filippo.belardelli@ift.cnr.it

Giuseppe Sconocchia, email: giuseppe.sconocchia@ift.cnr.it, Tel: +39 06- 45488487

La ringraziamo per la collaborazione

****
****

ESPRESSIONE DEL CONSENSO – SPERIMENTAZIONE CLINICA

Luogo e data

Io sottoscritto/a (NOME E COGNOME) nato il

dichiaro di accettare la proposta di sottopormi alla

sperimentazione clinica

"Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti

COVID-19 anziani"

Sono stato/a adeguatamente informato/a circa gli scopi dello studio e le metodiche dello stesso, in particolare sono consapevole della necessità di osservare le indicazioni e le regole che mi sono state illustrate e che ho perfettamente compreso.

Sono a conoscenza dei benefici che mi possono derivare dalla partecipazione allo studio, ma anche degli eventuali rischi e di tutti i disagi connessi.

Mi è stato spiegato che dal nuovo trattamento potrebbero attendersi dei miglioramenti rispetto agli approcci terapeutici ad oggi in uso, ma che questi non possono essermi garantiti.

Sono consapevole che in qualsiasi momento potrò sospendere la sperimentazione ed esigere di essere curato/a con le terapie ordinarie per la patologia di cui soffro, senza obbligo da parte mia di motivare la decisione, a meno che la stessa non derivi dalla comparsa di disturbi o effetti indesiderati o non previsti, nel qual caso mi impegno sin da ora a comunicarne tempestivamente al medico sperimentatore natura ed entità.

Dichiaro che il mio consenso è espressione di una libera decisione, non influenzata da promesse di denaro o di altri benefici, né da obblighi di gratitudine o di amicizia e/o parentela nei confronti del medico sperimentatore.

•*acconsento*

•non acconsento

che le notizie riguardanti la sperimentazione, limitatamente a quelle che potrebbero rivelarsi utili ai fini della mia salute, vengano trasmesse al mio medico curante, dott.

Autorizzo il trasferimento dei campioni biologici, prelevati nel corso della sperimentazione, all' Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani" e all'Istituto Superiore di Sanità, per condurre le analisi previste dallo studio.

Autorizzo sin d'ora l'utilizzo e la divulgazione, in forma anonima e per sole finalità scientifiche e amministrative e nell'osservanza delle vigenti norme sulla tutela della riservatezza, dei risultati della sperimentazione, compresi i dati clinici che mi riguardano.

Acconsento infine di rendere disponibili i miei dati personali riservati per le procedure di controllo della qualità e per le ispezioni da parte delle autorità/istituzioni competenti e da parte del Comitato Etico.

Luogo, Data, …/…/… Firma del paziente……

Luogo, Data, …/…/… Firma del Rappresentante Legale…… (se appropriato)

Luogo, Data, …/…/… Firma del Testimone ……

(se appropriato)

Luogo, Data, …/…/… Firma dello Sperimentatore ……

DICHIARAZIONE DI CHI INFORMA

Io sottoscritta/o ……. dichiaro di aver informato il/la paziente e discusso dello scopo e della natura dello studio clinico in oggetto, di aver risposto ad ogni sua domanda riguardo la natura, l'impegno, le procedure, i rischi e i benefici della partecipazione al presente studio di ricerca.

Dal colloquio sono emersi elementi sufficienti, per affermare che il paziente ha compreso natura, scopo e quant'altro gli/le viene chiesto conseguentemente alla Sua partecipazione

Luogo, Data, …/…/…Firma del professionista …….

**** La sezione relativa ai testimoni imparziali va compilata solo se il/la paziente non è in grado di fornire per iscritto il consenso informato.***

Se due Testimoni imparziali indipendenti prestano il consenso per conto del/della paziente, il/la paziente dovrà firmare il consenso informato non appena sarà in grado di farlo.

REDATTO IN DUE COPIE, L'ORIGINALE DA CONSERVARSI A CURA DEL MEDICO DELLO STUDIO, E LA COPIA DA CONSEGNARE AL/ALLA PAZIENTE, ALL'EVENTUALE RAPPRESENTANTE LEGALE / AMMINISTRATORE DI SOSTEGNO O AGLI EVENTUALI TESTIMONI IMPARZIALI.

Consiglio Nazionale delle Ricerche		**Istituto di Farmacologia Traslazionale** Institute of Translational Pharmacology **IFT**
Via Fosso del Cavaliere, 100 - 00133 Roma, Italy Tel: +39 06- 45488487 fax: +39 06-45488257		
Direttore Dott. Vito Michele Fazio		

FOGLIO INFORMATIVO PER IL PAZIENTE

TITOLO DELLO STUDIO:

"Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 anziani"

Numero EUDRACT: 2020-003872-42

Promotore dello Studio:

Istituto di Farmacologia Traslazionale – Consiglio Nazionale delle Ricerche

Sperimentatore Principale:

Dott. Emanuele Nicastri, U.O.C. Malattie Infettive ad alta Intensità di cura ed altamente contagiose, Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani (Centro di Sperimentazione)

Versione n. 02 del 18/03/2021

Gent.mo/gent.ma paziente,

con il presente documento la invitiamo a partecipare allo studio no profit dal titolo "Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 anziani" promosso dall'Istituto di Farmacologia Traslazionale — Consiglio Nazionale delle Ricerche.

Prima che Lei prenda una decisione in merito, è importante che comprenda il motivo dello studio e cosa Le sarà chiesto di fare, qualora decidesse di prendervi parte. Lo sperimentatore ed i suoi collaboratori, oltre alle spiegazioni che Le forniranno durante questo colloquio, sono a Sua completa disposizione per qualsiasi chiarimento.

Questo documento ha lo scopo di fornirle un'informazione corretta e completa affinché Lei possa esprimere una scelta libera e consapevole. La partecipazione allo studio è a carattere volontario e Lei avrà a disposizione un tempo adeguato per riflettere e porre domande di chiarimento prima di dare la sua adesione. Avrà inoltre il diritto di ritirare il proprio consenso in qualsiasi momento senza dover fornire alcuna giustificazione e senza perdere alcun diritto e beneficio. In caso di ritiro del consenso, nessuna nuova informazione sarà raccolta e aggiunta ai dati esistenti.

Lei ha il diritto di venire a conoscenza di ogni nuova informazione che possa modificare la sua decisione di partecipare allo studio.

NOTA INFORMATIVA

Qual è lo scopo dello studio?

Lo studio ha lo scopo di sperimentare un trattamento che, nei pazienti con più di 65 anni positivi all'infezione da nuovo Coronavirus 19 (SARS-CoV2), possa ridurre il rischio di progressione verso forme più gravi di malattia, e la conseguente necessità di ricovero in terapia intensiva. Questo studio sperimentale sarà condotto in un centro clinico (Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani"), in alcune Residenze Sanitarie Assistenziali e in regime domiciliare, e vi parteciperanno 60 pazienti nel Lazio.

Qual è il farmaco che viene testato?

Il farmaco che verrà testato è l'Interferone beta 1a, disponibile in commercio con il nome Rebif e ad oggi utilizzato nel trattamento della Sclerosi Multipla.

In diversi studi recentemente pubblicati su riviste scientifiche internazionali, il trattamento con Interferone (da solo oppure in combinazione con altri farmaci) ha dimostrato di ridurre la durata dell'infezione da nuovo Coronavirus 19 e la progressione verso forme più gravi di malattia. Dai dati emersi nel corso della pandemia da COVID-19, risulta che i pazienti anziani abbiano maggiore possibilità di trarre vantaggio dal trattamento con questo farmaco.

Per quale motivo sono stato scelto?

Le è stata diagnosticata l'infezione da nuovo Coronavirus 19 ed il medico ritiene che possieda i requisiti necessari per entrare nello studio. Le è stato chiesto di considerare la possibilità di aderirvi per valutare se il nuovo trattamento può contribuire a migliorare il Suo stato di salute ed impedire la progressione dell'infezione verso stati più gravi di malattia.

Sono obbligato a partecipare?

No. La decisione di partecipare allo studio dipende solo da Lei. È completamente volontaria. Se preferisce non partecipare non deve fornire spiegazioni. Riceverà comunque tutte le indagini, le visite e le terapie attualmente disponibili per la Sua malattia.

Cosa accadrà se decido di partecipare allo studio?

Se desidera prendere in considerazione la possibilità di parteciparvi, Le sarà consegnata questa scheda informativa, da leggere e conservare. Avrà la possibilità di chiedere tutte le spiegazioni che desidera a riguardo. Le sarà chiesto di firmare il modulo di consenso, in allegato e l'informativa per il trattamento dei dati personali. Solo dopo che Lei avrà firmato la dichiarazione di consenso, inizierà la valutazione medica per accertare la Sua idoneità a partecipare allo studio, che si baserà sulla risposta ad alcune domande riguardanti la sua condizione medica personale e la relativa terapia, sulla sua storia (anamnesi) medica personale e familiare e anche sui risultati delle analisi che verranno effettuate su alcuni campioni di sangue che le verranno prelevati dal personale dello studio.

Come si svolgerà lo studio? A quale trattamento sarò sottoposto?

Lo studio sarà condotto "in aperto", cioè sia Lei, sia il medico, sarete a conoscenza del trattamento/del gruppo di appartenenza. Lo studio prevede di arruolare 60 pazienti, di cui 40 riceveranno il trattamento sperimentale con Interferone e 20 pazienti riceveranno solo lo "standard di cura", cioè le terapie che i medici coinvolti riterranno opportuno somministrare per alleviare eventuali sintomi. L'assegnazione dei pazienti al gruppo sperimentale o al gruppo di controllo verrà affidata ad un protocollo "di randomizzazione", e sarà quindi frutto del caso. Lei avrà il 66% di possibilità di essere trattato con Interferone ed il 33% di ricevere le cure standard. Sia che venga assegnato al gruppo sperimentale o a quello di controllo, nel caso il medico deciderà che lei può rimanere nel suo domicilio, le forniremo un kit di strumenti con cui potrà monitorare il Suo stato di salute per rilevare tempestivamente l'insorgenza di sintomi di allerta. Il personale medico spiegherà a Lei (e ai Suoi familiari) il corretto uso degli strumenti.

Quale sarà il mio impegno? Cosa dovrò fare?

Per entrare nello studio, il medico dovrà verificare che Lei soddisfi tutti i criteri previsti per la partecipazione. Le chiederà pertanto di sottoporsi a visita medica, durante la quale Le saranno rilevati e registrati i seguenti parametri: temperatura corporea, pressione del sangue, frequenza respiratoria, saturazione di ossigeno. Le verranno inoltre prelevati 10 ml di sangue per valutare alcuni parametri (emocromo, funzionalità epatica e renale) rilevanti per l'ingresso al presente studio.

Lei dovrà fornire al medico sperimentatore tutte le informazioni che riguardano la Sua storia clinica, i medicinali che ha assunto e quelli sta assumendo attualmente. Dovrà riferire anche in merito a eventuali studi clinici, cui sta partecipando.

Qualora tutti i criteri previsti dallo studio siano soddisfatti, il medico la informerà del Suo ingresso nello studio.

All'ingresso dello studio, Le verrà assegnato un codice e Lei verrà assegnato/a ad un gruppo (sperimentale o di controllo). Se capiterà nel gruppo di pazienti trattati con farmaco sperimentale, Le verrà somministrato Interferone beta a basso dosaggio attraverso un inoculo sottocute per 4 volte nel corso di dieci giorni (giorno 1, 3, 7 e 10).

Indipendentemente dal gruppo di trattamento in cui Lei capiterà, verranno effettuati una serie di controlli del Suo stato di salute, sia durante la malattia, sia nella fase successiva alla Sua guarigione, nell'esclusivo interesse di monitorare il Suo andamento clinico. Personale specializzato le effettuerà alcuni prelievi di sangue (circa 10-30 ml) e i tamponi nasofaringei necessari per misurare la presenza del nuovo Coronavirus 19, in aggiunta a quelli eseguiti nella usuale pratica clinica, secondo la tempistica seguente:

Giorno 1: 30 ml di sangue

Giorno 3: 30 ml di sangue

Giorno 10: 15 ml di sangue

Giorno 14: 30 ml di sangue + tampone nasofaringeo Giorno

28: 15 ml di sangue + tampone nasofaringeo

I campioni raccolti verranno trasferiti all'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani" per essere analizzati e/o conservati per successive analisi. Alcune delle analisi sui campioni di sangue verranno effettuate presso l'Istituto Superiore di Sanità, da personale coinvolto nello studio clinico. I tamponi nasofaringei verranno portati dai medici USCAR presso il laboratorio SYNLAB ITALIA s.r.l., uno dei laboratori autorizzati dalla Regione Lazio all'esecuzione di tamponi molecolari per la ricerca di Sars-CoV2. Il laboratorio SYNLAB

si occuperà dell'analisi dei tamponi, il cui esito verrà comunicato al medico sperimentatore e quindi a Lei.

Lei dovrà seguire attentamente le istruzioni che Le saranno fornite. Dovrà sottoporsi agli inoculi di farmaco sottocute (solo se capiterà nel gruppo di trattamento) e ai prelievi (entrambi i gruppi) da parte del personale specializzato coinvolto nello studio; con l'assistenza di personale infermieristico oppure, se lei resterà nel proprio domicilio, con l'aiuto di un suo familiare, dovrà farsi monitorare due volte al giorno la temperatura corporea, la pressione arteriosa, la frequenza respiratoria, la frequenza cardiaca e la saturazione attraverso la strumentazione che le verrà consegnata, seguendo le modalità che le verranno illustrate, annotandole sull'apposito diario che le verrà fornito; dovrà informare il medico di eventuali problemi/disturbi che potranno insorgere e di ogni variazione riguardante la Sua salute. Dovrà consultare sempre il medico responsabile dello studio prima di sottoporsi ad altre terapie, o assumere farmaci da banco, onde evitare incompatibilità/effetti/possibili interazioni tra Interferone ed altri prodotti.

Quali benefici potrò attendermi dalla partecipazione allo studio?

Con l'utilizzo dell'Interferone ci si aspetta una guarigione più veloce dall'infezione da nuovo Coronavirus 19, una minore necessità di ricovero in Rianimazione e di intubazione, una riduzione della mortalità. Tale beneficio, in ogni caso, non può esserle garantito e Lei potrebbe non avere alcun vantaggio personale. Quantunque Lei non ottenga benefici diretti da questa ricerca, le informazioni raccolte grazie alla Sua partecipazione a questo studio saranno comunque di grande importanza per la lotta contro il virus responsabile dell'attuale pandemia da COVID-19.

Rischi ed effetti collaterali che possono derivare dalla partecipazione allo studio

L'interferone beta può causare effetti collaterali simil-influenzali, come mal di testa, febbre, brividi, dolori muscolari ed articolari, affaticamento e nausea. Questi sintomi sono generalmente di lieve entità soprattutto alle dosi che verranno utilizzate in questo studio; sono più frequenti all'inizio del trattamento e diminuiscono con il suo proseguimento.

È anche possibile che si verifichi un certo arrossamento al sito di iniezione.

I rischi fisici e i disturbi correlati al prelievo di sangue sono identici a quelli riguardanti ogni genere di prelievo di campione ematico da vena, ovvero possibilità di piccoli lividi e irritazioni locali, con rari casi di infezione.

Sono disponibili altre terapie?

No. Al momento presente non sono disponibili altri farmaci approvati per la sua patologia. Infatti, trattandosi di un nuovo virus, tutto il mondo scientifico sta lavorando per cercare di trovare la cura adatta per rallentare la malattia COVID19, soprattutto nei pazienti anziani. Se deciderà di non partecipare allo studio proposto, il Suo stato di salute verrà monitorato e le verrà somministrata la terapia standard di supporto per ridurre eventuali sintomi.

Cosa accadrà se nuove conoscenze o nuove informazioni si rendessero disponibili?

Qualora si rendessero disponibili nuove informazioni sul farmaco, o sullo studio, che potrebbero influenzare la Sua sicurezza, o la Sua volontà a continuare il trattamento, Le saranno comunicate tempestivamente dal medico sperimentatore.

Potrò cambiare idea dopo aver accettato di partecipare?

Si. Lei potrà decidere di ritirare il consenso e interrompere il trattamento, in qualsiasi momento, anche a studio avviato, senza dover fornire giustificazioni a meno che la decisione non derivi dalla comparsa di disturbi o effetti indesiderati o non previsti, nel qual caso dovrà fornire al medico sperimentatore tutte le informazioni del caso. La Sua decisione non avrà ripercussioni sull'assistenza e sulle cure che dovrà ricevere in futuro. I medici continueranno a seguirla con la migliore assistenza sanitaria possibile. Qualora decidesse di ritirare il consenso Le chiediamo di informare il medico sperimentatore e di acconsentire alla valutazione finale.

Il mio medico di fiducia sarà informato?

Previa sua autorizzazione, il Suo medico di fiducia sarà informato con apposita lettera della Sua partecipazione e potrà anche contattare il responsabile dello studio per qualsiasi informazione.

Quanto dura lo studio?

La Sua partecipazione allo studio avrà una durata di circa un mese.

Il trattamento sperimentale potrebbe essere interrotto o sospeso?

Si. Il medico sperimentatore potrebbe interrompere lo studio in qualsiasi momento, anche contro la Sua volontà, qualora lo ritenesse necessario per la Sua salute, o per la corretta

conduzione della ricerca (es. se Lei non assume regolarmente il farmaco, non si attiene alle istruzioni ricevute, o non rispetta il programma delle visite). Lo studio potrebbe essere sospeso/interrotto anche dallo Sponsor, o dalle Autorità Regolatorie per cause attualmente non prevedibili.

Cosa accadrà se le mie condizioni fisiche dovessero peggiorare?

Qualora il monitoraggio delle Sue condizioni di salute dovesse evidenziare un significativo peggioramento, il medico sperimentatore disporrà l'interruzione della sua partecipazione allo studio. Il Suo medico di Medicina Generale verrà tempestivamente informato, e le verranno assicurati i migliori trattamenti disponibili per la sua patologia.

Cosa accadrà se subentrassero problemi: infortunio o danni correlati allo studio?

Qualora dovessero verificarsi effetti collaterali, indesiderati, o danni alla Sua salute, riconducibili allo studio, Lei dovrà informare tempestivamente il medico sperimentatore che Le fornirà le relative informazioni. Come previsto dalla normativa vigente (D.M. 14.07.2009), lo studio è coperto da un'apposita assicurazione. Inoltre, nel caso si manifestassero complicanze correlate alla partecipazione allo studio saranno garantiti tutti i migliori trattamenti medici necessari e l'assicurazione si farà carico di tutti i costi relativi all'assistenza e al trattamento non coperti dall'assistenza sanitaria ordinaria. Sono esclusi i trattamenti per danni o patologie che risultino indipendenti da questo studio.

Dovrò sostenere spese aggiuntive?

No. La Sua partecipazione allo studio di ricerca non comporterà per Lei alcun aggravio di spesa. Il farmaco, tutte le visite e gli esami supplementari richiesti, saranno a carico dell'Istituzione che sponsorizza la ricerca.

Riceverò un compenso per la mia partecipazione allo studio?

No. La vigente normativa regola la partecipazione agli studi clinici esclusivamente su base spontanea, senza possibilità di ricevere alcun compenso economico.

Chi organizza e finanzia lo studio di ricerca?

Lo studio è organizzato e sponsorizzato dall'Istituto di Farmacologia Traslazionale (Consiglio Nazionale delle Ricerche, CNR), che verserà al Centro di Sperimentazione un compenso per l'impegno a seguire la Sua partecipazione alla ricerca.

Chi ha esaminato lo studio?

Il protocollo dello studio è stato stilato in conformità alle Norme di Buona Pratica Clinica dell'Unione Europea e alla revisione corrente della Dichiarazione di Helsinki ed è stato approvato dal Comitato Etico Unico per gli studi COVID dell'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani", dalle competenti Autorità Sanitarie o dalle Istituzioni da queste delegate.

La mia partecipazione resterà riservata? Come saranno usati i miei dati personali?

La Sua partecipazione allo studio sarà obbligatoriamente registrata nella cartella clinica, tuttavia, il medico sperimentatore e i suoi collaboratori gestiranno i Suoi dati personali e tutte le informazioni relative al Suo stato di salute in modo strettamente riservato e soltanto nella misura in cui essi saranno necessari in relazione all'obiettivo dello studio. I Suoi dati e tutte le informazioni saranno trattate in accordo a quanto è stabilito nel documento "Informazioni privacy e manifestazione del consenso al trattamento dei dati personali", che le verrà sottomesso contestualmente a questo documento. Lei dovrà prestare il consenso al trattamento dei dati personali all'atto della firma del presente modulo, altrimenti non sarà possibile garantire la sua partecipazione allo studio

Come saranno utilizzati i miei campioni?

I campioni di sangue e i tamponi che le verranno prelevati saranno essenziali per valutare il Suo stato di salute e per comprendere meglio gli effetti del farmaco nel contrastare il nuovo Coronavirus 19. Per tutelare la sua privacy personale, i campioni che La riguardano non verranno identificati con i Suoi dati anagrafici ma con un codice e saranno utilizzati unicamente per supportare le evidenze cliniche raccolte da questo studio. I campioni saranno conservati presso l'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani" e l'Istituto Superiore di Sanità per un tempo massimo di due anni dalla chiusura dello studio

Come saranno utilizzati/diffusi i risultati?

Alla fine della ricerca i dati raccolti durante lo studio potranno essere presentati a congressi o in pubblicazioni scientifiche, ma la Sua identità resterà anonima.

Normativa di riferimento

Tutte le informazioni raccolte durante lo studio sono confidenziali e verranno trattate in ottemperanza al D. Lgs. 196 del 30 giugno 2003 "Codice in materia di protezione dei dati personali", alla Deliberazione n. 52 del 24.07.2008 "Linee Guida per il trattamento dei dati nell'ambito delle sperimentazioni cliniche di medicinali", del Garante per la Privacy e ai Regolamenti Europei 2016/679 e 536/2014.

Posso richiedere altre informazioni oppure essere informato circa i risultati dello studio?

Per qualsiasi informazione o per ricevere ulteriori chiarimenti può rivolgersi a:

Emanuele Nicastri, email: emanuele.nicastri@inmi.it tel. 0655170393

Nazario Bevilacqua, email: nazario.bevilacqua@inmi.it tel.06 55170232

La ringraziamo per la collaborazione

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ESPRESSIONE DEL CONSENSO – SPERIMENTAZIONE CLINICA

*Io sottoscritto/a (NOME E COGNOME)*_____

nato il _____ *dichiaro di accettare la proposta di sottopormi*

alla sperimentazione clinica

"Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 anziani"

Sono stato/a adeguatamente informato/a circa gli scopi dello studio e le metodiche dello stesso, in particolare sono consapevole della necessità di osservare le indicazioni e le regole che mi sono state illustrate e che ho perfettamente compreso.

Sono a conoscenza dei benefici che mi possono derivare dalla partecipazione allo studio, ma anche degli eventuali rischi e di tutti i disagi connessi.

Mi è stato spiegato che dal nuovo trattamento potrebbero attendersi dei miglioramenti rispetto agli approcci terapeutici ad oggi in uso, ma che questi non possono essermi garantiti.

Sono consapevole che in qualsiasi momento potrò sospendere la sperimentazione ed esigere di essere curato/a con le terapie ordinarie per la patologia di cui soffro, senza obbligo da parte mia di motivare la decisione, a meno che la stessa non derivi dalla comparsa di disturbi o effetti indesiderati o non previsti, nel qual caso mi impegno sin da ora a comunicarne tempestivamente al medico sperimentatore natura ed entità.

Dichiaro che il mio consenso è espressione di una libera decisione, non influenzata da promesse di denaro o di altri benefici, né da obblighi di gratitudine o di amicizia e/o parentela nei confronti del medico sperimentatore.

☐ *acconsento* ☐

non acconsento

che le notizie riguardanti la sperimentazione, limitatamente a quelle che potrebbero rivelarsi utili ai fini della mia salute, vengano trasmesse al mio medico curante

Autorizzo il trasferimento dei campioni biologici, prelevati nel corso della sperimentazione, all' Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani" e all'Istituto Superiore di Sanità, per condurre le analisi previste dallo studio.

Autorizzo sin d'ora l'utilizzo e la divulgazione, in forma anonima e per sole finalità scientifiche e amministrative e nell'osservanza delle vigenti norme sulla tutela della riservatezza, dei risultati della sperimentazione, compresi i dati clinici che mi riguardano.

Acconsento infine di rendere disponibili i miei dati personali riservati per le procedure di controllo della qualità e per le ispezioni da parte delle autorità/istituzioni competenti e da parte del Comitato Etico.

Luogo, Data, _____

Firma del paziente_____

Luogo, Data, _____

Firma del Rappresentante Legale_____ (se appropriato)

Luogo, Data,_____

Firma del Testimone _____ (se appropriato)

DICHIARAZIONE DI CHI INFORMA

Io sottoscritta/o _____ dichiaro di aver informato il/la paziente e discusso dello scopo e della natura dello studio clinico in oggetto, di aver risposto ad ogni sua domanda riguardo la natura, l'impegno, le procedure, i rischi e i benefici della partecipazione al presente studio di ricerca.

Dal colloquio sono emersi elementi sufficienti, per affermare che il paziente ha compreso natura, scopo e quant'altro gli/le viene chiesto conseguentemente alla Sua partecipazione

Luogo, Data, _____

Firma dello Sperimentatore _____.

**** La sezione relativa ai testimoni imparziali va compilata solo se il/la paziente non è in grado di fornire per iscritto il consenso informato.***

Se due Testimoni imparziali indipendenti prestano il consenso per conto del/della paziente, il/la paziente dovrà firmare il consenso informato non appena sarà in grado di farlo.

REDATTO IN DUE COPIE, L'ORIGINALE DA CONSERVARSI A CURA DEL MEDICO DELLO STUDIO, E LA COPIA DA CONSEGNARE AL/ALLA PAZIENTE, ALL'EVENTUALE RAPPRESENTANTE LEGALE / AMMINISTRATORE DI SOSTEGNO O AGLI EVENTUALI TESTIMONI IMPARZIALI.

Consiglio Nazionale delle Ricerche		**Istituto di Farmacologia Traslazionale** Institute of Translational Pharmacology **IFT**
Via Fosso del Cavaliere, 100 - 00133 Roma, Italy Tel: +39 06- 45488487 fax: +39 06-45488257		
Direttore Dott. Vito Michele Fazio		

FOGLIO INFORMATIVO PER IL PAZIENTE

TITOLO DELLO STUDIO:

"Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 ad alto rischio"

Numero EUDRACT: 2020-003872-42

Promotore dello Studio:

Istituto di Farmacologia Traslazionale – Consiglio Nazionale delle Ricerche

Sperimentatore Principale:

Dott. Emanuele Nicastri, U.O.C. Malattie Infettive ad alta Intensità di cura ed altamente contagiose, Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani (Centro di Sperimentazione)

Versione n. 03del 31/05/2021

Gent.mo/gent.ma paziente,

con il presente documento la invitiamo a partecipare allo studio no profit dal titolo "Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti COVID-19 ad alto rischio" promosso dall'Istituto di Farmacologia Traslazionale — Consiglio Nazionale delle Ricerche.

Prima che Lei prenda una decisione in merito, è importante che comprenda il motivo dello studio e cosa Le sarà chiesto di fare, qualora decidesse di prendervi parte. Lo sperimentatore ed i suoi collaboratori, oltre alle spiegazioni che Le forniranno durante questo colloquio, sono a Sua completa disposizione per qualsiasi chiarimento.

Questo documento ha lo scopo di fornirle un'informazione corretta e completa affinché Lei possa esprimere una scelta libera e consapevole. La partecipazione allo studio è a carattere volontario e Lei avrà a disposizione un tempo adeguato per riflettere e porre domande di chiarimento prima di dare la sua adesione. Avrà inoltre il diritto di ritirare il proprio consenso in qualsiasi momento senza dover fornire alcuna giustificazione e senza perdere alcun diritto e beneficio. In caso di ritiro del consenso, nessuna nuova informazione sarà raccolta e aggiunta ai dati esistenti.

Lei ha il diritto di venire a conoscenza di ogni nuova informazione che possa modificare la sua decisione di partecipare allo studio.

NOTA INFORMATIVA

Qual è lo scopo dello studio?

Lo studio ha lo scopo di sperimentare un trattamento che, nei pazienti con più di 65 anni positivi all'infezione da nuovo Coronavirus 19 (SARS-CoV2), possa ridurre il rischio di progressione verso forme più gravi di malattia, e la conseguente necessità di ricovero in terapia intensiva. Questo studio sperimentale sarà condotto in un centro clinico (Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani"), in alcune Residenze Sanitarie Assistenziali e in regime domiciliare, e vi parteciperanno 60 pazienti nel Lazio.

Qual è il farmaco che viene testato?

Il farmaco che verrà testato è l'Interferone beta 1a, disponibile in commercio con il nome Rebif e ad oggi utilizzato nel trattamento della Sclerosi Multipla.

In diversi studi recentemente pubblicati su riviste scientifiche internazionali, il trattamento con Interferone (da solo oppure in combinazione con altri farmaci) ha dimostrato di ridurre la durata dell'infezione da nuovo Coronavirus 19 e la progressione verso forme più gravi di malattia. Dai dati emersi nel corso della pandemia da COVID-19, risulta che i pazienti anziani abbiano maggiore possibilità di trarre vantaggio dal trattamento con questo farmaco, poichè la capacità di produrre autonomamente l'interferone necessario al nostro organismo per combattere le infezioni virali diminuisce con l'età. Dati recenti sembrano suggerire che anche alcuni adulti mostrano una ridotta capacità di produrre interferone, e questa caratteristica li rende più a rischio di sviluppare forme gravi di COVID19.

Per quale motivo sono stato scelto?

Le è stata diagnosticata l'infezione da nuovo Coronavirus 19 ed il medico ritiene che possieda i requisiti necessari per entrare nello studio. Le è stato chiesto di considerare la possibilità di

aderirvi per valutare se il nuovo trattamento può contribuire a migliorare il Suo stato di salute ed impedire la progressione dell'infezione verso stati più gravi di malattia.

Sono obbligato a partecipare?

No. La decisione di partecipare allo studio dipende solo da Lei. È completamente volontaria. Se preferisce non partecipare non deve fornire spiegazioni. Riceverà comunque tutte le indagini, le visite e le terapie attualmente disponibili per la Sua malattia.

Cosa accadrà se decido di partecipare allo studio?

Se desidera prendere in considerazione la possibilità di parteciparvi, Le sarà consegnata questa scheda informativa, da leggere e conservare. Avrà la possibilità di chiedere tutte le spiegazioni che desidera a riguardo. Le sarà chiesto di firmare il modulo di consenso, in allegato e l'informativa per il trattamento dei dati personali. Solo dopo che Lei avrà firmato la dichiarazione di consenso, inizierà la valutazione medica per accertare la Sua idoneità a partecipare allo studio, che si baserà sulla risposta ad alcune domande riguardanti la sua condizione medica personale e la relativa terapia, sulla sua storia (anamnesi) medica personale e familiare e anche sui risultati delle analisi che verranno effettuate su alcuni campioni di sangue che le verranno prelevati dal personale dello studio.

Come si svolgerà lo studio? A quale trattamento sarò sottoposto?

Lo studio sarà condotto "in aperto", cioè sia Lei, sia il medico, sarete a conoscenza del trattamento/del gruppo di appartenenza. Lo studio prevede di arruolare 60 pazienti, di cui 40 riceveranno il trattamento sperimentale con Interferone e 20 pazienti riceveranno solo lo "standard di cura", cioè le terapie che i medici coinvolti riterranno opportuno somministrare per alleviare eventuali sintomi. L'assegnazione dei pazienti al gruppo sperimentale o al gruppo di controllo verrà affidata ad un protocollo "di randomizzazione", e sarà quindi frutto del caso. Lei avrà il 66% di possibilità di essere trattato con Interferone ed il 33% di ricevere le cure standard. Sia che venga assegnato al gruppo sperimentale o a quello di controllo, nel caso il medico deciderà che lei può rimanere nel suo domicilio, le forniremo un kit di strumenti con cui potrà monitorare il Suo stato di salute per rilevare tempestivamente l'insorgenza di sintomi di allerta. Il personale medico spiegherà a Lei (e ai Suoi familiari) il corretto uso degli strumenti.

Quale sarà il mio impegno? Cosa dovrò fare?

Per entrare nello studio, il medico dovrà verificare che Lei soddisfi tutti i criteri previsti per la partecipazione. Le chiederà pertanto di sottoporsi a visita medica, durante la quale Le saranno

rilevati e registrati i seguenti parametri: temperatura corporea, pressione del sangue, frequenza respiratoria, saturazione di ossigeno. Le verranno inoltre prelevati 10 ml di sangue per valutare alcuni parametri (emocromo, funzionalità epatica e renale) rilevanti per l'ingresso al presente studio.

Lei dovrà fornire al medico sperimentatore tutte le informazioni che riguardano la Sua storia clinica, i medicinali che ha assunto e quelli sta assumendo attualmente. Dovrà riferire anche in merito a eventuali studi clinici, cui sta partecipando.

Qualora tutti i criteri previsti dallo studio siano soddisfatti, il medico la informerà del Suo ingresso nello studio.

All'ingresso dello studio, Le verrà assegnato un codice e Lei verrà assegnato/a ad un gruppo (sperimentale o di controllo). Se capiterà nel gruppo di pazienti trattati con farmaco sperimentale, Le verrà somministrato Interferone beta a basso dosaggio attraverso un inoculo sottocute per 4 volte nel corso di dieci giorni (giorno 1, 3, 7 e 10).

Indipendentemente dal gruppo di trattamento in cui Lei capiterà, verranno effettuati una serie di controlli del Suo stato di salute, sia durante la malattia, sia nella fase successiva alla Sua guarigione, nell'esclusivo interesse di monitorare il Suo andamento clinico. Personale specializzato le effettuerà alcuni prelievi di sangue (circa 10-30 ml) e i tamponi nasofaringei necessari per misurare la presenza del nuovo Coronavirus 19, in aggiunta a quelli eseguiti nella usuale pratica clinica, secondo la tempistica seguente:

Giorno 1: 30 ml di sangue

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Giorno 28: 15 ml di sangue + tampone nasofaringeo

I campioni raccolti verranno trasferiti all'Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani" per essere analizzati e/o conservati per successive analisi. Alcune delle analisi sui campioni di sangue verranno effettuate presso l'Istituto Superiore di Sanità, da personale coinvolto nello studio clinico. I tamponi nasofaringei verranno portati dai medici USCAR presso il laboratorio SYNLAB ITALIA s.r.l., uno dei laboratori autorizzati dalla Regione Lazio all'esecuzione di tamponi molecolari per la ricerca di Sars-CoV2. Il laboratorio SYNLAB si occuperà dell'analisi dei tamponi, il cui esito verrà comunicato al medico sperimentatore e quindi a Lei.

Lei dovrà seguire attentamente le istruzioni che Le saranno fornite. Dovrà sottoporsi agli inoculi di farmaco sottocute (solo se capiterà nel gruppo di trattamento) e ai prelievi (entrambi i gruppi) da parte del personale specializzato coinvolto nello studio; con l'assistenza di personale infermieristico oppure, se lei resterà nel proprio domicilio, con l'aiuto di un suo

familiare, dovrà farsi monitorare due volte al giorno la temperatura corporea, la pressione arteriosa, la frequenza respiratoria, la frequenza cardiaca e la saturazione attraverso la strumentazione che le verrà consegnata, seguendo le modalità che le verranno illustrate, annotandole sull'apposito diario che le verrà fornito; dovrà informare il medico di eventuali problemi/disturbi che potranno insorgere e di ogni variazione riguardante la Sua salute. Dovrà consultare sempre il medico responsabile dello studio prima di sottoporsi ad altre terapie, o assumere farmaci da banco, onde evitare incompatibilità/effetti/possibili interazioni tra Interferone ed altri prodotti.

Quali benefici potrò attendermi dalla partecipazione allo studio?

Con l'utilizzo dell'Interferone ci si aspetta una guarigione più veloce dall'infezione da nuovo Coronavirus 19, una minore necessità di ricovero in Rianimazione e di intubazione, una riduzione della mortalità. Tale beneficio, in ogni caso, non può esserle garantito e Lei potrebbe non avere alcun vantaggio personale. Quantunque Lei non ottenga benefici diretti da questa ricerca, le informazioni raccolte grazie alla Sua partecipazione a questo studio saranno comunque di grande importanza per la lotta contro il virus responsabile dell'attuale pandemia da COVID-19.

Rischi ed effetti collaterali che possono derivare dalla partecipazione allo studio

L'interferone beta può causare effetti collaterali simil-influenzali, come mal di testa, febbre, brividi, dolori muscolari ed articolari, affaticamento e nausea. Questi sintomi sono generalmente di lieve entità soprattutto alle dosi che verranno utilizzate in questo studio; sono più frequenti all'inizio del trattamento e diminuiscono con il suo proseguimento.

È anche possibile che si verifichi un certo arrossamento al sito di iniezione.

I rischi fisici e i disturbi correlati al prelievo di sangue sono identici a quelli riguardanti ogni genere di prelievo di campione ematico da vena, ovvero possibilità di piccoli lividi e irritazioni locali, con rari casi di infezione.

Sono disponibili altre terapie?

No. Al momento presente non sono disponibili altri farmaci approvati per la sua patologia. Infatti, trattandosi di un nuovo virus, tutto il mondo scientifico sta lavorando per cercare di trovare la cura adatta per rallentare la malattia COVID19, soprattutto nei pazienti anziani. Se deciderà di non partecipare allo studio proposto, il Suo stato di salute verrà monitorato e le verrà somministrata la terapia standard di supporto per ridurre eventuali sintomi.

Cosa accadrà se nuove conoscenze o nuove informazioni si rendessero disponibili?

Qualora si rendessero disponibili nuove informazioni sul farmaco, o sullo studio, che potrebbero influenzare la Sua sicurezza, o la Sua volontà a continuare il trattamento, Le saranno comunicate tempestivamente dal medico sperimentatore.

Potrò cambiare idea dopo aver accettato di partecipare?

Si. Lei potrà decidere di ritirare il consenso e interrompere il trattamento, in qualsiasi momento, anche a studio avviato, senza dover fornire giustificazioni a meno che la decisione non derivi dalla comparsa di disturbi o effetti indesiderati o non previsti, nel qual caso dovrà fornire al medico sperimentatore tutte le informazioni del caso. La Sua decisione non avrà ripercussioni sull'assistenza e sulle cure che dovrà ricevere in futuro. I medici continueranno a seguirla con la migliore assistenza sanitaria possibile. Qualora decidesse di ritirare il consenso Le chiediamo di informare il medico sperimentatore e di acconsentire alla valutazione finale.

Il mio medico di fiducia sarà informato?

Previa sua autorizzazione, il Suo medico di fiducia sarà informato con apposita lettera della Sua partecipazione e potrà anche contattare il responsabile dello studio per qualsiasi informazione.

Quanto dura lo studio?

La Sua partecipazione allo studio avrà una durata di circa un mese.

Il trattamento sperimentale potrebbe essere interrotto o sospeso?

Si. Il medico sperimentatore potrebbe interrompere lo studio in qualsiasi momento, anche contro la Sua volontà, qualora lo ritenesse necessario per la Sua salute, o per la corretta conduzione della ricerca (es. se Lei non assume regolarmente il farmaco, non si attiene alle istruzioni ricevute, o non rispetta il programma delle visite). Lo studio potrebbe essere sospeso/interrotto anche dallo Sponsor, o dalle Autorità Regolatorie per cause attualmente non prevedibili.

Cosa accadrà se le mie condizioni fisiche dovessero peggiorare?

Qualora il monitoraggio delle Sue condizioni di salute dovesse evidenziare un significativo peggioramento, il medico sperimentatore disporrà l'interruzione della sua partecipazione allo studio. Il Suo medico di Medicina Generale verrà tempestivamente informato, e le verranno assicurati i migliori trattamenti disponibili per la sua patologia.

Cosa accadrà se subentrassero problemi: infortunio o danni correlati allo studio?

Qualora dovessero verificarsi effetti collaterali, indesiderati, o danni alla Sua salute, riconducibili allo studio, Lei dovrà informare tempestivamente il medico sperimentatore che Le fornirà le relative informazioni. Come previsto dalla normativa vigente (D.M. 14.07.2009), lo studio è coperto da un'apposita assicurazione. Inoltre, nel caso si manifestassero complicanze correlate alla partecipazione allo studio saranno garantiti tutti i migliori trattamenti medici necessari e l'assicurazione si farà carico di tutti i costi relativi all'assistenza e al trattamento non coperti dall'assistenza sanitaria ordinaria. Sono esclusi i trattamenti per danni o patologie che risultino indipendenti da questo studio.

Dovrò sostenere spese aggiuntive?

No. La Sua partecipazione allo studio di ricerca non comporterà per Lei alcun aggravio di spesa. Il farmaco, tutte le visite e gli esami supplementari richiesti, saranno a carico dell'Istituzione che sponsorizza la ricerca.

Riceverò un compenso per la mia partecipazione allo studio?

No. La vigente normativa regola la partecipazione agli studi clinici esclusivamente su base spontanea, senza possibilità di ricevere alcun compenso economico.

Chi organizza e finanzia lo studio di ricerca?

Lo studio è organizzato e sponsorizzato dall'Istituto di Farmacologia Traslazionale (Consiglio Nazionale delle Ricerche, CNR), che verserà al Centro di Sperimentazione un compenso per l'impegno a seguire la Sua partecipazione alla ricerca.

Chi ha esaminato lo studio?

Il protocollo dello studio è stato stilato in conformità alle Norme di Buona Pratica Clinica dell'Unione Europea e alla revisione corrente della Dichiarazione di Helsinki ed è stato approvato dal Comitato Etico Unico per gli studi COVID dell'Istituto Nazionale per le Malattie

Infettive “Lazzaro Spallanzani”, dalle competenti Autorità Sanitarie o dalle Istituzioni da queste delegate.

La mia partecipazione resterà riservata? Come saranno usati i miei dati personali?

La Sua partecipazione allo studio sarà obbligatoriamente registrata nella cartella clinica, tuttavia, il medico sperimentatore e i suoi collaboratori gestiranno i Suoi dati personali e tutte le informazioni relative al Suo stato di salute in modo strettamente riservato e soltanto nella misura in cui essi saranno necessari in relazione all’obiettivo dello studio. I Suoi dati e tutte le informazioni saranno trattate in accordo a quanto è stabilito nel documento “Informazioni privacy e manifestazione del consenso al trattamento dei dati personali”, che le verrà sottomesso contestualmente a questo documento. Lei dovrà prestare il consenso al trattamento dei dati personali all'atto della firma del presente modulo, altrimenti non sarà possibile garantire la sua partecipazione allo studio

Come saranno utilizzati i miei campioni?

I campioni di sangue e i tamponi che le verranno prelevati saranno essenziali per valutare il Suo stato di salute e per comprendere meglio gli effetti del farmaco nel contrastare il nuovo Coronavirus 19. Per tutelare la sua privacy personale, i campioni che La riguardano non verranno identificati con i Suoi dati anagrafici ma con un codice e saranno utilizzati unicamente per supportare le evidenze cliniche raccolte da questo studio. I campioni saranno conservati presso l’Istituto Nazionale per le Malattie Infettive “Lazzaro Spallanzani” e l’Istituto Superiore di Sanità per un tempo massimo di due anni dalla chiusura dello studio

Come saranno utilizzati/diffusi i risultati?

Alla fine della ricerca i dati raccolti durante lo studio potranno essere presentati a congressi o in pubblicazioni scientifiche, ma la Sua identità resterà anonima.

Normativa di riferimento

Tutte le informazioni raccolte durante lo studio sono confidenziali e verranno trattate in ottemperanza al D. Lgs. 196 del 30 giugno 2003 ”Codice in materia di protezione dei dati personali”, alla Deliberazione n. 52 del 24.07.2008 ”Linee Guida per il trattamento dei dati nell’ambito delle sperimentazioni cliniche di medicinali”, del Garante per la Privacy e ai Regolamenti Europei 2016/679 e 536/2014.

Posso richiedere altre informazioni oppure essere informato circa i risultati dello studio?

Per qualsiasi informazione o per ricevere ulteriori chiarimenti può rivolgersi a:

Emanuele Nicastri, email: emanuele.nicastri@inmi.it tel. 0655170393

Nazario Bevilacqua, email: nazario.bevilacqua@inmi.it tel.06 55170232

La ringraziamo per la collaborazione

****

ESPRESSIONE DEL CONSENSO – SPERIMENTAZIONE CLINICA

*Io sottoscritto/a (NOME E COGNOME)*_____

nato il _____ *dichiaro di accettare la proposta di sottopormi*

alla sperimentazione clinica

"Valutazione dell'attività antivirale e immunomodulatoria di Interferone-Beta in pazienti

COVID-19 ad alto rischio"

Sono stato/a adeguatamente informato/a circa gli scopi dello studio e le metodiche dello stesso, in particolare sono consapevole della necessità di osservare le indicazioni e le regole che mi sono state illustrate e che ho perfettamente compreso.

Sono a conoscenza dei benefici che mi possono derivare dalla partecipazione allo studio, ma anche degli eventuali rischi e di tutti i disagi connessi.

Mi è stato spiegato che dal nuovo trattamento potrebbero attendersi dei miglioramenti rispetto agli approcci terapeutici ad oggi in uso, ma che questi non possono essermi garantiti.

Sono consapevole che in qualsiasi momento potrò sospendere la sperimentazione ed esigere di essere curato/a con le terapie ordinarie per la patologia di cui soffro, senza obbligo da parte mia di motivare la decisione, a meno che la stessa non derivi dalla comparsa di disturbi o effetti indesiderati o non previsti, nel qual caso mi impegno sin da ora a comunicarne tempestivamente al medico sperimentatore natura ed entità.

Dichiaro che il mio consenso è espressione di una libera decisione, non influenzata da promesse di denaro o di altri benefici, né da obblighi di gratitudine o di amicizia e/o parentela nei confronti del medico sperimentatore.

☐ *acconsento* ☐

non acconsento

che le notizie riguardanti la sperimentazione, limitatamente a quelle che potrebbero rivelarsi utili ai fini della mia salute, vengano trasmesse al mio medico curante

Autorizzo il trasferimento dei campioni biologici, prelevati nel corso della sperimentazione, all' Istituto Nazionale per le Malattie Infettive "Lazzaro Spallanzani" e all'Istituto Superiore di Sanità, per condurre le analisi previste dallo studio.

Autorizzo sin d'ora l'utilizzo e la divulgazione, in forma anonima e per sole finalità scientifiche e amministrative e nell'osservanza delle vigenti norme sulla tutela della riservatezza, dei risultati della sperimentazione, compresi i dati clinici che mi riguardano.

Acconsento infine di rendere disponibili i miei dati personali riservati per le procedure di controllo della qualità e per le ispezioni da parte delle autorità/istituzioni competenti e da parte del Comitato Etico.

Luogo, Data, _____

Firma del paziente_____

Luogo, Data, _____

Firma del Rappresentante Legale_____ (se appropriato)

Luogo, Data,_____

Firma del Testimone _____ (se appropriato)

DICHIARAZIONE DI CHI INFORMA

Io sottoscritta/o _____ dichiaro di aver informato il/la paziente e discusso dello scopo e della natura dello studio clinico in oggetto, di aver risposto ad ogni sua domanda riguardo la natura, l'impegno, le procedure, i rischi e i benefici della partecipazione al presente studio di ricerca.

Dal colloquio sono emersi elementi sufficienti, per affermare che il paziente ha compreso natura, scopo e quant'altro gli/le viene chiesto conseguentemente alla Sua partecipazione

Luogo, Data, _____

Firma dello Sperimentatore _____.

**** La sezione relativa ai testimoni imparziali va compilata solo se il/la paziente non è in grado di fornire per iscritto il consenso informato.***

Se due Testimoni imparziali indipendenti prestano il consenso per conto del/della paziente, il/la paziente dovrà firmare il consenso informato non appena sarà in grado di farlo.

REDATTO IN DUE COPIE, L'ORIGINALE DA CONSERVARSI A CURA DEL MEDICO DELLO STUDIO, E LA COPIA DA CONSEGNARE AL/ALLA PAZIENTE, ALL'EVENTUALE RAPPRESENTANTE LEGALE / AMMINISTRATORE DI SOSTEGNO O AGLI EVENTUALI TESTIMONI IMPARZIALI.

Final Report Appendix 16.1.4

List and description of investigators and other important participants in the study

Principal investigator

Emanuele Nicastri, MD

National Institute for Infectious Diseases "Lazzaro Spallanzani"
Via Portuense 292, 00149 Rome, Italy

Co-Principal investigator

Pier Luigi Bartoletti, MD

Coordinator of the Special Units for Regional Continued Care (USCAR),

Sponsor Coordinator

Giuseppe Sconocchia, MD

Institute of Translational Pharmacology (IFT)
National Research Council (CNR), Roma, Italy
Via Fosso del Cavaliere 100 - 00133 Rome - Italy
Responsible for the coordination of study protocol

Sponsor Scientific Coordinator

Filippo Belardelli, PhD

Institute of Translational Pharmacology (IFT)
National Research Council (CNR), Rome, Italy
Responsible for the management of the MERCK Grant and of the scientific coordination of the entire project

Co-investigators:

Nazario Bevilacqua, MD
National Institute for Infectious Diseases "Lazzaro Spallanzani"
Via Portuense 292, 00149 Rome, Italy
Responsible for patients enrollment and management

Nicola Vanacore, Ilaria Bacigalupo, Flavia Lombardo and Antonio Ancidoni
National Centre for Disease Prevention and Health Promotion
Istituto Superiore di Sanità
Viale Regina Elena, 299
Roma, Italy

Responsible for Statistical design, data management and analysis

Eleonora Aricò and **Luciano Castiello**

FaBioCell, Core Facilities
Istituto Superiore di Sanità
Responsible for study design, protocol writing and for the exploratory analysis on IFN signaling

Laura Bracci

Department of Oncology and Molecular Medicine
Istituto Superiore di Sanità
Participation to protocol writing and responsible for inflammatory cytokine analysis

Francesca Urbani

Department of Oncology and Molecular Medicine
Istituto Superiore di Sanità
Responsible for CRF design, participation to protocol writing and responsible, together with Iole Macchia, of the exploratory analysis on cellular immunomonitoring

Roberto Nisini and **Anna Rita Ciccaglione**

Department of Infectious Diseases
Istituto Superiore di Sanità
Responsible for SARS-CoV 2-Specific Binding Antibody analysis

Ombretta Papa,

Special Units for Regional Continued Care (USCAR)
Participating in the enrollment and management of non-hospitalized patients. Responsible for the establishment of the network of family doctors for the early detection of non-hospitalized patients

Concetta Castilletti and **Maria R. Capobianchi**

Laboratory of Virology
National Institute for Infectious Diseases “Lazzaro Spallanzani”
Responsible for diagnostic analyses of COVID-19 patients enrolled at INMI

Antonino Di Caro, Stefania Carrara and Donatella Vincenti

Microbiology Laboratory and Infectious Diseases Biobank
National Institute for Infectious Diseases “Lazzaro Spallanzani”
Responsible for processing and storage of biological samples at INMI BioBank

Silvia Murachelli

Pharmacy Unit
National Institute for Infectious Diseases “Lazzaro Spallanzani”
Responsible for experimental drug storage at INMI pharmacy

Other laboratories involved:

Synlab Lazio srl
Via San Polo Dei Cavalieri 20 00159 Roma
Responsible for SARS-CoV-2 RT-PCR analysis on nasopharyngeal swabs

Clinical research organization:

FullCro srl
Via Ignazio Guidi 3, 00147 Roma

Administrative support:

Matilde Paggiolu, **Giuseppina Ozzella** and **Pamela Papa**
Institute of Translational Pharmacology (IFT)
National Research Council (CNR)
Administrative clinical research support on inter-institutional agreements, material transfer agreements, institutional tenders.

Final Report Appendix 16.1.5

Signature of Sponsor Scientific Coordinator

Protocol Title:

Antiviral and Immunomodulatory Interferon-Beta in high-risk COVID-19 patients (ANTIICIPATE)

Version: 1.0

Date of Report: 11/08/2022

Sponsor Scientific Coordinator

Filippo Belardelli,

IFT, CNR

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Fax: +39 06 45488257

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Final Report Appendix 16.1.6

A. Randomisation scheme and codes

Randomization Scheme and codes

NrRandom	Treatment	Assigned (0: No; 1: Yes)
1	SOC	0
2	IFN	0
3	IFN	0
4	IFN	0
5	SOC	0
6	IFN	0
7	SOC	0
8	IFN	0
9	IFN	0
10	IFN	0
11	IFN	0
12	SOC	0
13	IFN	0
14	SOC	0
15	IFN	0
16	SOC	0
17	IFN	0
18	IFN	0
19	SOC	0
20	IFN	0
21	IFN	0
22	SOC	0
23	IFN	0
24	IFN	0
25	SOC	0
26	IFN	0
27	IFN	0
28	IFN	0
29	SOC	0
30	IFN	0
31	IFN	0
32	SOC	0
33	IFN	0
34	SOC	0
35	IFN	0
36	IFN	0
37	IFN	0
38	SOC	0
39	IFN	0
40	IFN	0

Randomization Scheme and codes

NrRandom	Treatment	Assigned (0: No; 1: Yes)
41	IFN	0
42	SOC	0
43	IFN	0
44	SOC	0
45	IFN	0
46	IFN	0
47	IFN	0
48	SOC	0
49	IFN	0
50	IFN	0
51	SOC	0
52	IFN	0
53	IFN	0
54	SOC	0
55	IFN	0
56	SOC	0
57	IFN	0
58	IFN	0
59	IFN	0
60	SOC	0
61	IFN	1
62	SOC	1
63	IFN	0
64	IFN	0
65	SOC	0
66	IFN	0
67	IFN	0
68	SOC	0
69	IFN	0
70	IFN	0
71	SOC	0
72	IFN	0
73	IFN	0
74	IFN	0
75	SOC	0
76	IFN	0
77	SOC	0
78	IFN	0
79	SOC	0
80	IFN	0

Randomization Scheme and codes

NrRandom	Treatment	Assigned (0: No; 1: Yes)
81	IFN	0
82	IFN	0
83	SOC	0
84	IFN	0
85	IFN	0
86	SOC	0
87	IFN	0
88	IFN	0
89	IFN	0
90	SOC	0
91	IFN	0
92	SOC	0
93	IFN	0
94	SOC	0
95	IFN	0
96	IFN	0
97	SOC	0
98	IFN	0
99	IFN	0
100	SOC	0
101	IFN	0
102	IFN	0
103	IFN	0
104	IFN	0
105	SOC	0
106	SOC	0
107	IFN	0
108	IFN	0
109	SOC	0
110	IFN	0
111	IFN	0
112	IFN	0
113	IFN	0
114	SOC	0
115	IFN	0
116	IFN	0
117	SOC	0
118	SOC	0
119	IFN	0
120	IFN	0

Aricò et al. Trials (2021) 22:584
https://doi.org/10.1186/s13063-021-05367-6

Trials

LETTER Open Access

Antiviral and immunomodulatory interferon-beta in high-risk COVID-19 patients: a structured summary of a study protocol for a randomised controlled trial

Eleonora Aricò[1*†], Luciano Castiello[1†], Laura Bracci[2], Francesca Urbani[2,3], Flavia Lombardo[4], Ilaria Bacigalupo[4], Antonio Ancidoni[4], Nicola Vanacore[4], Alessandro Falcione[5], Chiara Reggiani[5], Giovanni Marco Dutti[5], Maria Grazia Maglie[5], Ombretta Papa[5], Pier Luigi Bartoletti[5], Giuseppina Ozzella[6], Nazario Bevilacqua[7], Emanuele Nicastri[7†], Filippo Belardelli[6*†] and Giuseppe Sconocchia[6†]

Abstract

Objectives: The primary objective of the study is to demonstrate the efficacy of low-dose IFN-β in reducing the risk of SARS-CoV-2 recently infected elderly patients to progress towards severe COVID-19 *versus* control group within 28 days. Secondary objectives are:

1) To assess the reduction in Intensive Care Unit (ICU) admission in patients treated with IFN-β *versus* control group within 28 days of randomization
2) To assess the reduction in number of deaths in IFN- β compared to control group (day 28)
3) To evaluate the increase in proportion of participants returning to negative SARS-CoV-2 RT-PCR in IFN-β -treated *versus* control group at Day 14 and Day 28
4) To assess the increase in SARS-CoV-2-specific binding antibody titers in IFN-β compared to control group (day 28)
5) To assess the safety of IFN-β -treated patients *versus* control group

Trial design: Randomized, Open-Label, Controlled, Superiority Phase II Study. Patients, who satisfy all inclusion criteria and no exclusion criteria, will be randomly assigned to one of the two treatment groups in a ratio 2:1 (IFN-treated *versus* control patients). Randomization will be stratified by gender. Stratified randomization will balance the presence of male and female in both study arms.

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Full list of author information is available at the end of the article

Participants: Male and female adults aged 65 years or older with newly diagnosed SARS-CoV-2 infection and mild COVID-19 symptoms are eligible for the study. The trial is being conducted in Rome.
Participants will be either hospitalized or home isolated. A group of physicians belonging to the Special Unit for Regional Continued Care (USCAR), specifically trained for the study and under the supervision of the National Institute for Infectious Diseases "Lazzaro Spallanzani", will be responsible for the screening, enrolment, treatment and clinical monitoring of patients, thus acting as a bridge between clinical centers and territorial health management.
Inclusion criteria are as follows:

- ≥ 65 years of age at time of enrolment;
- Laboratory-confirmed SARS-CoV-2 infection as determined by PCR, in any specimen < 72 hours prior to randomization;
- Subject (or legally authorized representative) provides written informed consent prior to initiation of any study procedures;
- Understands and agrees to comply with planned study procedures;
- Agrees to the collection of nasopharyngeal swabs and venous blood samples per protocol;
- Being symptomatic for less than 7 days before starting therapy;
- NEWS2 score ≤2.

Exclusion criteria are as follows:

- Hospitalized patients with illness of any duration, and at least one of the following:
  - Clinical assessment (evidence of rales/crackles on exam) and SpO2 ≤ 94% on room air at rest or after walking test,
    OR
  - Acute respiratory failure requiring mechanical ventilation and/or supplemental oxygen;
- Patients currently using IFN-β (e.g., multiple sclerosis patients);
- Patients undergoing chemotherapy or other immunosuppressive treatments;
- Patients with chronic kidney diseases;
- Known allergy or hypersensitivity to IFN (including asthma);
- Any autoimmune disease (resulting from patient anamnesis);
- Patients with signs of dementia or neurocognitive disorders;
- Patients with current severe depression and/or suicidal ideations;
- Being concurrently involved in another clinical trial;
- HIV infection (based on the anamnesis);
- Use of any antiretroviral medication;
- Impaired renal function (eGFR calculated by CKD-EPI Creatinine equation < 30 ml/min);
- Presence of other severe diseases impairing life expectancy (e.g. patients are not expected to survive 28 days given their pre-existing medical condition);
- Any physical or psychological impediment in a patient that could let the investigator to suspect his/her poor compliance;
- Lack or withdrawal of informed consent

Intervention and comparator: Control arm: No specific antiviral treatment besides standard of care.
Treatment arm: 11μg (3MIU) of IFN-β1a will be injected subcutaneously at day 1, 3, 7, and 10 in addition to standard of care. The drug solution, contained in a pre-filled cartridge, will be injected by means of the RebiSmart® electronic injection device.
Interferon β1a (Rebif®, Merck KGaA, Darmstadt, Germany) is a disease-modifying drug used to treat relapsing forms of multiple sclerosis (MS). The dose selected for this study is expected to exploit the antiviral and immunomodulatory properties of the cytokine without causing relevant toxicity or inducing refractoriness

phenomena sometimes observed after high-dose and/or chronic IFNβ treatments.

Main outcomes: Primary endpoint of the study is the proportion of patients experiencing a disease progression, during at least 5 days, according to the National Early Warning Score (NEWS2). The NEWS2 score is a standardized approach aimed at promptly detecting signs of clinical deterioration in acutely ill patients and establishing the potential need for higher level of care. It is based on the evaluation of vital signs, including respiratory rate, oxygen saturation, temperature, blood pressure, pulse/heart rate, AVPU response. The resulting observations, compared to a normal range, are combined in a single composite "alarm" score. Any other clinical sign clearly indicating a disease worsening will be considered as disease progression.

Randomization: Sixty patients will be randomized 2:1 to receive IFN-β1a plus the standard of care or the standard of care only. Eligible patients will be randomized (no later than 36 h after enrolment) by means of a computerized central randomization system. All patients will receive a unique patient identification number at enrolling visit when signing the informed consent and before any study procedure is performed. This number remains constant throughout the entire study. The randomization of patients will be closed when 60 patients have been enrolled. The randomization will be stratified by sex; for each stratum a sequence of treatments randomly permuted in blocks of variable length (3 or 6) will be generated.

Blinding (masking): This is an open-label study. After the randomization, patients will be notified whether they will be in the experimental arm or in the control arm.

Numbers to be randomised (sample size): The study plans to enrol 60 patients: 40 in the IFN-β1a arm, 20 in the control arm, according to a 2:1 - treated: untreated ratio.

Trial Status: Protocol Version: 3.0
Version Date: 18/03/2021
The study is open for recruitment since 16/04/2021.Recruitment is expected to l be completed before 15/08/2021.

Trial registration: EudraCT N°: 2020-003872-42, registration date: 19/10/2020.

Full protocol: The full protocol is attached as an additional file, accessible from the Trials website (Additional file 1). In the interest in expediting dissemination of this material, the familiar formatting has been eliminated; this Letter serves as a summary of the key elements of the full protocol."

Keywords: COVID-19, Randomised controlled trial, protocol, Interferon-beta, antiviral, immunomodulation, non-hospitalized patients

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-021-05367-6.

Additional file 1. Full study protocol.

Acknowledgements

We are grateful to Matilde Paggiolu and Pamela Papa for administrative support.

Authors' contributions

E.A., L.C., L.B., F.U., F.B. conceived and designed the trial; E.A., L.C., L.B., F.U., G.O., G.S. wrote the trial protocol and prepared the manuscript for submission; F.L., I.B., A.A., N.V., led the statistical analysis plan; O.P., P.L.B., N.B., E.N. provided clinical advice and revised the trial protocol. F.B. received funding by Merck KGaA. All authors approved the final version of the protocol and the manuscript.

Funding

The study is partially funded by Merck KGaA, who also provided the investigational drug. Merck has no role in study design, data collection, management, analysis, data interpretation, manuscript writing, or in the decision to submit manuscripts for publication.

Availability of data and materials

Not applicable

Declarations

Ethics approval and consent to participate

The study was approved on 06/11/2020 by the Ethics Committee of the National Institute for Infectious Diseases "Lazzaro Spallanzani" in Rome, designed as National Committee for evaluation of clinical trials on human drugs in COVID-19 patients. Version 3, including non-substantial amendment to the original protocol, was approved on 23/03/2021 by the same committee.
Prior to inclusion, potential patients will first receive written and verbal information about the nature, purpose, procedures required by the protocol, possible risks and benefits of the study. Patients will have the time to carefully review the consent form and ask questions prior to completing. Informed consent to participate in the study will be obtained from all participants before enrolment. The original signed informed consent form will be retained in the patient's records and a copy of the informed consent form will be provided to the patient.
We herein certify that this trial has received ethical approval from the appropriate ethical committee as described above.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Received: 3 June 2021 Accepted: 9 June 2021
Published online: 03 September 2021

Publisher's Note

Final Report Appendix 16.2.1

Discontinued patients

Pt #	Study conclusion date	Study discontinuation	Reason for study discontinuation	Number of AE
1	16-May-21	NO	AE	1
3	17-May-21	NO	screening failure	0

Final Report Appendix 16.2.2

Listing of individual laboratory measurements by patient

Pt #1

Date	Time Point	Parameter	Result	Unit	Comment	Value range	
04-May-21	PreTx	RBC	4.49	x10e6/uL		4.4	5.8
04-May-21		Haemoglobin (HGB)	14.3	g/dL		13.5	17.2
04-May-21		Haematocrit (HCT)	45.6	%		39	52
04-May-21		MCV	101.6	fL	ncs	80	94
04-May-21		MCH	31.8	pg		27	32
04-May-21		MCHC	31.4	g/dL		30	36
04-May-21		RDW -CV (RBC)	14	%		11	16.5
04-May-21		RDW-SD	52.5	fL	ncs	38	48
04-May-21		Platelets (PLT)	253	x10e3/uL		150	450
04-May-21		MPV	10.8	fL		6.3	11
04-May-21		WBC	7.22	x10e3/uL		4	10
04-May-21		neutro %	74.5	%		40	75
04-May-21		linfo %	16.5	%	cs due to covid infection	20	45
04-May-21		mono %	7.6	%		2	10
04-May-21		eosi %	0.7	%		0	6
04-May-21		baso %	0.70	%		0	1.5
04-May-21		neutro #	5.38	x10e3/uL		1.8	7.5
04-May-21		linfo #	1.19	x10e3/uL		1	4.5
04-May-21		mono #	0.55	x10e3/uL		0.1	0.8
04-May-21		eosi #	0.05	x10e3/uL		0	0.4
04-May-21		baso #	0.05	x10e3/uL		0	0.2
04-May-21		Creatinine (%)	1.33	mg/dL	ncs	0.6	1.3
04-May-21		eGFR	38				
04-May-21		Sodium (Na)	n.a.	mmol/L		135	145
04-May-21		Potassium (K)	n.a.	mmol/L		3.5	5
04-May-21		Glucose	n.a.	mg/dL		70	110
04-May-21		Lipase	n.a.	U/L		6	50
04-May-21		LDH	n.a.	U/L		210	400
04-May-21		ALT/SGPT	n.a.	U/L		5	40
04-May-21		AST/SGOT	n.a.	U/L		5	40
04-May-21		Total bilirubin	n.a.	mg/dL		0.2	1
04-May-21		Urea (BUN)	n.a.	mg/dL		20	50
04-May-21		Albumin	n.a.	g/dL		3.5	5.1
05-May-21		INR	1.01	index		0.8	1.2
05-May-21	T1	Functional Fibrinogen	413	mg/dL		150	450
05-May-21		D-Dimer	n.a.	ng/mL		0	500
05-May-21		Pro-calcitonin	n.a.	ng/mL		0	0.5
05-May-21		CRP	0.16	mg/dL		0.01	1

07-May-21		RBC	4.69	x10e6/uL		4.4	5.8
07-May-21		Haemoglobin (HGB)	14.6	g/dL		13.5	17.2
07-May-21		Haematocrit (HCT)	46.3	%		39	52
07-May-21		MCV	98.7	fL	NCS	80	94
07-May-21		MCH	31.1	pg		27	32
07-May-21		MCHC	31.5	g/dL		30	36
07-May-21		RDW -CV (RBC)	14.2	%		11	16.5
07-May-21		RDW-SD	51.8	fL	NCS	38	48
07-May-21		Platelets (PLT)	198	x10e3/uL		150	450
07-May-21		MPV	11.1	fL	NCS	6.3	11
07-May-21	T3	WBC	7.08	x10e3/uL		4	10
07-May-21		neutro %	64	%		40	75
07-May-21		linfo %	22.2	%		20	45
07-May-21		mono %	12.4	%	NCS	2	10
07-May-21		eosi %	1.1	%		0	6
07-May-21		baso %	0.30	%		0	1.5
07-May-21		neutro #	4.53	x10e3/uL		1.8	7.5
07-May-21		linfo #	1.57	x10e3/uL		1	4.5
07-May-21		mono #	0.88	x10e3/uL	NCS	0.1	0.8
07-May-21		eosi #	0.08	x10e3/uL		0	0.4
07-May-21		baso #	0.02	x10e3/uL		0	0.2
07-May-21		Creatinine (%)	0.87	mg/dL		0.6	1.3
07-May-21		eGFR	65.6				
07-May-21		Sodium (Na)	139	mmol/L		135	145
07-May-21		Potassium (K)	4.70	mmol/L		3.5	5
07-May-21		Glucose	96	mg/dL		70	110
07-May-21		Lipase	36	U/L		6	50
07-May-21		LDH	204	U/L		210	400
07-May-21		ALT/SGPT	15	U/L		5	40
07-May-21		AST/SGOT	25	U/L		5	40
07-May-21		Total bilirubin	0.77	mg/dL		0.2	1
07-May-21		Urea (BUN)	n.a.	mg/dL		20	50
07-May-21		Albumin	4	g/dL		3.5	5.1
14-May-21		Creatinine (%)	1.08	mg/dL		0.6	1.3
14-May-21		Sodium (Na)	137	mmol/L		135	145
14-May-21		Potassium (K)	4.30	mmol/L		3.5	5
14-May-21		Glucose	136	mg/dL		70	110
14-May-21		Lipase	60	U/L		6	50
14-May-21		LDH	224	U/L		210	400
14-May-21		ALT/SGPT	37	U/L		5	40
14-May-21	T10	AST/SGOT	46	U/L		5	40
14-May-21		Total bilirubin	0.45	mg/dL		0.2	1
14-May-21		Urea (BUN)	n.a.	mg/dL		20	50
14-May-21		Albumin	3	g/dL		3.5	5.1
14-May-21		Creatinine (%)	1.18	index		0.8	1.2
14-May-21		Functional Fibrinogen	529	mg/dL		150	450
14-May-21		D-Dimer	1830	ng/mL		0	500
14-May-21		Pro-calcitonin	n.a.	ng/mL		0	0.5
14-May-21		CRP	n.a.	mg/dL		0.01	1
14-May-21		RBC	4.52	x10e6/uL		4.4	5.8
14-May-21		Haemoglobin (HGB)	14	g/dL		13.5	17.2
14-May-21		Haematocrit (HCT)	43.5	%		39	52
14-May-21		MCV	96.2	fL	NCS	80	94
14-May-21		MCH	31	pg		27	32
14-May-21		MCHC	32.2	g/dL		30	36
14-May-21		RDW -CV (RBC)	14	%		11	16.5
14-May-21		RDW-SD	50.1	fL	NCS	38	48
14-May-21		Platelets (PLT)	182	x10e3/uL		150	450
14-May-21		MPV	10.7	fL		6.3	11

14-May-21		WBC	4.52	x10e3/uL		4	10
14-May-21		neutro %	73.7	%		40	75
14-May-21		linfo %	18.1	%	CS DUE TO COVID INFECTION	20	45
14-May-21		mono %	8	%		2	10
14-May-21		eosi %	0	%		0	6
14-May-21		baso %	0.2	%		0	1.5
14-May-21		neutro #	3.33	x10e3/uL		1.8	7.5
14-May-21		linfo #	0.82	x10e3/uL	CS DUE TO COVID INFECTION	1	4.5
14-May-21		mono #	0.36	x10e3/uL		0.1	0.8
14-May-21		eosi #	0	x10e3/uL		0	0.4
14-May-21		baso #	0.01	x10e3/uL		0	0.2
14-May-21		Creatinine (%)	1.08	mg/dL		0.6	1.3
14-May-21		eGFR	50.5				
14-May-21		Sodium (Na)	137	mmol/L		135	145
14-May-21		Potassium (K)	4.3	mmol/L		3.5	5
14-May-21		Glucose	136	mg/dL	NCS	70	110
14-May-21		Lipase	60	U/L	NCS DUE TO MANY CONCOMITANT TREATMENT	6	50
14-May-21		LDH	224	U/L		210	400
14-May-21		ALT/SGPT	37	U/L		5	40
14-May-21		AST/SGOT	46	U/L	NCS DUE TO MANY CONCOMITANT TREATMENT	5	40
14-May-21		Total bilirubin	0.45	mg/dL		0.2	1
14-May-21		Urea (BUN)	n.a.	mg/dL		20	50
14-May-21		Albumin	3	g/dL	NCS	3.5	5.1
14-May-21		INR	1.18	index		0.8	1.2
14-May-21		Functional Fibrinogen	529	mg/dL	CS DUE TO COVID INFECTION	150	450
14-May-21		D-Dimer	1830	ng/mL	UNRELIABLE DATUM	0	500
14-May-21		Pro-calcitonin		ng/mL		0	0.5
14-May-21		CRP	4.21	mg/dL	CS DUE TO COVID INFECTION	0.01	1