# SUPPLEMENT 1

1. **ConPlas-19 Study Team and organization**
2. **Supplementary Methods**
3. **Supplementary Results**
4. **Clinical Trial Protocol**

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## 1. ConPlas-19 Study Team and organization

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# 2. Supplementary Methods

## Additional details on laboratory assays

### -RT-PCR assays

Specimens were taken from each donor and patient at admission and were sent for virologic investigation to the Respiratory Viruses and Influenza Laboratory (CNM-ISCIII). Samples were stored at 4°C and processed within 24 hours after collection. Upon reception, 3 aliquots were prepared and stored at −80°C. Both the reception and the sample processing areas are separated from those defined as working areas. RNA from 200-µL aliquots of oro/nasopharyngeal swab specimens and 400--µL aliquots of blood sample were extracted by using the QIAamp Mini Elute Virus spin kit in an automated extractor (QIAcube; Qiagen, Valencia, CA).

Every specimen from donors and patients were analysed by RT–PCR assay with targets in the E and N genes as previously described (1). This protocol was modified in order to include a simultaneous amplification of human DNA as internal control and was modified and adapted at the reagents and CFX96 termociclator at the Respiratory Virus and Influenza Laboratory at the Microbiology National Centre (CNM, ISCIII). Values of Cts showed a semiquantitative measure of the viral load in the non-homogeneous respiratory samples.

1. Corman, V. M. et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT–PCR. Euro Surveill. 25, 1–8 (2020).

**- Immunoassay for SARS-CoV-2 antibody testing**

Euroimmun

The Euroimmun Anti-SARS-CoV-2 ELISA IgG assays (Euroimmun, Luebeck, Germany) were performed on serum samples according to the manufacturer's instructions. The microplate wells are coated with recombinant structural protein 1 (S1) of SARS-CoV-2 and the assay detects anti-SARS-CoV-2 IgG against the viral spike protein. The results are evaluated semi-quantitatively by calculation of the ratio between the extinction of the sample and calibrator. A ratio <0.8 is considered negative, ≥0.8 and <1.1 borderline, and ≥1.1 positive.

ORTHO

The VITROS Immunodiagnostic Anti-SARS-CoV-2 ELISA IgG assays (Ortho-Clinical Diagnostics, Rochester, New York, USA) is a chemiluminescent immunoassay (CLIA) utilizing a recombinant structural protein 1 (S1) of SARS-CoV-2 to measure total antibody present in serum and plasma. The light signal is read by the system and the results are interpreted qualitatively. An index S/C (signal-to-cutoff) <1.0 is considered negative, ≥1.0 positive.

**-Pseudovirus Assay**.

Materials.

The Vero E6 (African green monkey kidney) cell line were kindly provided by Dr. A. Alcami (CBM Severo Ochoa, Madrid). HEK-293T (National Institute for Biological Standards and Control (NIBSC]) and Vero E6 cells were cultured in DMEM supplemented with 10% FCS, 2 mM L-glutamine and 100 units/ml penicillin and streptomycin (Lonza).

For the generation of the expression plasmid for SARS-Cov-2 S glycoprotein, the codon-optimized cDNA (QHU36824.1) lacking the last 19 amino acids (1) was synthesized (GeneArt Gene Synthesis, ThermoFisher Scientific), PCR-amplified and cloned into the pcDNA3.1 expression vector (Invitrogen). Complete DNA sequence of S glycoprotein in pcDNA3.1-SARS-Cov2-SΔ19-D614 was confirmed by sequencing. The pcDNA-VSV-G plasmid contains the cDNA encoding the vesicular stomatitis virus G protein and was obtained from Dr. Arenzana-Seisdedos (Institute Pasteur, Paris, France).

Pseudovirus neutralization assay.

NL4.3 pseudotypes were generated with the previously described plasmid pNL4-3ΔenvRen (2). Briefly, Renilla luciferase reporter pseudovirus were prepared by co-transfecting HEK-293T cells with pNL4-3ΔenvRen backbone and viral envelope protein expression plasmid pcDNA3.1-SARS-Cov2-SΔ19-D614 or pcDNA-VSV-G using the calcium phosphate method. The medium was changed 18 hours after transfection, and 48 hours post-transfection cell culture supernatants were harvested, clarified by centrifugation at 500 x g for 5 min, and frozen at -80 °C. The amount of p24 antigen in the supernatants was quantified by electrochemiluminescence Immunoassay (Roche Diagnostic).

To measure neutralization activity of sera from COVID-19 patients or healthy donors, two-fold serial dilutions of heat-inactivated sera (range 1:16 -1:8192) were preincubated with titrated pseudoviruses (10 ng p24 Gag/well) for 1 hour at 37ºC. Thereafter, 100 µl of the mixture was added to Vero E6 cells plated at 5 x $10^3$ cells/well in 100 µl medium in 96-well plates the previous day. The culture medium was refreshed after 16 hours. At 48 h post-infection, cells were lysed, and viral infectivity was assessed by measuring luciferase activity (Renilla Luciferase Assay, Promega, Madison, WI) using a 96-well plate luminometer "Orion II" (Berthold, Oak Ridge, TN). The titers of neutralizing antibodies were calculated as 50% inhibitory dose (ID50), expressed as the highest dilution of plasma (reciprocal dilution), which resulted in a 50% reduction of luciferase activity compared to control without serum. Sigmoid curves were generated and ID50 neutralization titers (NT50) were calculated by non-linear regression using GraphPad Prism version 7.02 (GraphPad Software, Inc.). VSV-G pseudoviruses were used as a specificity control virus in neutralization testing.

1. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020;11: 1620.

2. Garcia-Perez J, Sanchez-Palomino S, Perez-Olmeda M, Fernandez B, Alcami J. A new strategy based on recombinant viruses as a tool for assessing drug susceptibility of human immunodeficiency virus type 1. J Med Virol. 2007 Feb;79(2):127-37.

## Additional details on protocol changes

The final protocol is included in the supplementary material to this publication. Changes made to the protocol are summarised below:

| Protocol versions | Amendment description |
|---|---|
| v.1 22/03/2020 | Version first approved by Research Ethics Committee. No approval by the Medicines Competent Authority was deemed necessary (blood product) |
| v.2 06/04/2020 | Amendment to change inclusion criteria:<br><br>-The limit of maximum of three days from admission to randomization was withdrawn. It was considered the relevant limits were those based on the days elapsed since the beginning of the symptoms until randomization.<br><br>-The 72-hour window for the SARS-CoV-2 positive RT-PCR was withdrawn and changed for a valid positive RT-PCR obtained in the current course of the disease. This was prompted by the fact that some patients experienced worsening and admission a few days after their diagnosis together with the huge overload of local laboratories. A new basal RT-PCR was to be collected and sent to the central laboratory of the trial, but there were no need to repeat the local RT-PCR for inclusion.<br><br>-The limit of 10 days of symptoms was extended to 12 days of symptoms. This was due to the evidence of a number of candidate patients being admitted around 10 days of symptoms together with data published of series of cases informing of no risk in patients with longer disease evolution.<br><br>The possibility to include patients with longer duration of symptoms if negative for SARS-CoV-2 antibodies was removed by logistical reasons. The need to confirm negative for SARS-CoV-2 antibodies for patients with more than 10 days of symptoms was removed based on the absence of safety concerns in the series of patients treated with CP (with much longer disease evolution). |

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| | -Explicit mention to the need to include ABO group determination at baseline was added. |
| v.2.1 14/04/2020 | -Description of categories 4 and 5 of the ordinal scale was reworded to state that patients with oxygen mask with reservoir bag were considered category 4.<br><br>-Determination of IL-6 was added to the laboratory parameters |
| v.2.2 22/04/2020 | -The requirement that plasma donors must submit a negative PCR for SARS-CoV2 in nasopharyngeal or oropharyngeal smears was removed. The change followed the publication of the revised EU Guidance on collection, testing, processing, storage, distribution and monitored use of COVID-19 convalescent plasma, which does not include this requirement from its Version 1.0 April 4 2020.<br><br>As it was not required anymore, it is a bothersome test for the donor and the plasma inactivation gives further reassurance about viral elimination, the test was removed from the protocol.<br><br>-The description of Adverse Events of Special Interest (TRALI, ADE and TACO) was included in the protocol |
| Amendment on date 26/05/2020. No change in protocol version | The amendment aims to allow an extended follow up of patients by adding a substudy on COVID-19 recrudescences. The objective of this substudy is to describe the frequency and the clinical, analytical, radiological and microbiological characteristics of recrudescences in patients with Covid-19. The amendment includes a new version of the Informed Consent with the mention to the substudy and a brief protocol for the substudy. The original protocol was not amended. |
| v.3.0 31/08/2020 | -Selection of patients with a maximum evolution time of 7 days of symptoms (instead of max 12) in order to decrease the rate of patients already seroconverted at inclusion.<br><br>-Extension of the follow-up of patients from 28 days to 60 days in order to better capture thrombotic events and other COVID-19 complications and readmissions.<br><br>-Significant simplification of virus and serology determinations in patients: Elimination of serial PCR in naso / oropharyngeal smears, annoying for patients and with a low contribution in terms of characterizing virus clearance. Only serial PCR will be kept in blood in those patients (10% or less) who show viral RNA in blood, which allow a more reliable quantitative analysis for viral clerance.<br><br>- Collection of race and ethnicity (Latin American yes / no), potential risk factors linked to racial and ethnic factors.<br><br>-Some secondary variables are added that do not involve additional procedures beyond the telephone call at 60 days or new determinations in extractions for analytics already planned, adding CPK and troponin, which were not included. Collection of additional variables of respiratory distress in intubated patients (pO2 / FIO2 or SpO2 / FIO2, use of vasopressors, dialysis). |
| v.3.1 30/09/2020 | - The inclusion criteria of local laboratory confirmation for SARS-CoV-2 was modified to accept positivity with new tests (i.e rapid antigenic tests) alternative to RT-PCR, provided that an adequate specificity of that particular test has been accepted by the sponsor. Nevertheless, a RT-PCR in oro/nasopharyngeal swab at the centralized laboratory remains as a mandatory baseline procedure for all patients. |

## Additional details on statistical analysis

The version 1.0 of the Statistical Analysis Plan was approved on 2 June, 2020. A second version 2.0 was prepared to include the secondary variables added to protocol v.3.0, 31/08/2020 and approved on 2 Feb 21. The second version of the SAP did not amend any previously planned analysis. The most relevant changes were to add several secondary variables (rehospitalization rate, follow-up to 60 days, 11 points ordinal scale), the addition of a new subgroup analysis to assess the possible effect of the different waves of pandemics and some wording improvements.

DSMB assessments and Steering Committee decisions:

An assessment, restricted to safety objectives, was performed by the DSMB after first 20 patients and a recommendation to follow with the study was issued. On 10 June, the DSMB performed the first planned safety and futility analysis (at 20% recruitment). The analysis was based on data cut-off of 3 June with 77 recruited patients. The recommendation of the DSMB was to follow without changes. However, on 10 July, the Steering Committee decided to stop recruitment due to the drastic fall in recruitment related to pandemic control in Spain (only 6 new patients in 5 weeks). An amendment was performed in case of trial restart, with the intention to select patients with earlier disease (7 days or less) and to extend patients follow-up to capture potential effect on late and serious COVID-19 complications. The trial was effectively resumed on September 7, 2020 with the surge of the second wave.

The second DSMB meeting took place on November 13 and the last one on December 3, 2020. On their last meeting the DSMB recommended to increase the sample size in at least a 20% in order to maintain a reasonable power and the new sample size was set by the Steering Committee at 350 patients.

## 3. Supplementary Results

Table S1. Patients recruited by site

| | | Plasma (N=179) | Standard of Care (N=171) | Total (N=350) |
|---|---|---|---|---|
| Hospital Universitario Puerta de Hierro Majadahonda | n (%) | 48 (26.82) | 47 (27.49) | 95 (27.14) |
| Hospital Universitario 12 de Octubre | n (%) | 13 (7.26) | 14 (8.19) | 27 (7.71) |
| Complejo Asistencial Universitario de León | n (%) | 12 (6.70) | 12 (7.02) | 24 (6.86) |
| Hospital Clínico San Carlos | n (%) | 9 (5.03) | 9 (5.26) | 18 (5.14) |
| Hospital General de Ciudad Real | n (%) | 9 (5.03) | 8 (4.68) | 17 (4.86) |
| Hospital Universitario HM Sanchinarro | n (%) | 8 (4.47) | 9 (5.26) | 17 (4.86) |
| Hospital Universitario de La Princesa | n (%) | 6 (3.35) | 7 (4.09) | 13 (3.71) |
| Complejo Hospitalario de Navarra | n (%) | 6 (3.35) | 5 (2.92) | 11 (3.14) |
| Hospital Universitario Marqués de Valdecilla | n (%) | 5 (2.79) | 6 (3.51) | 11 (3.14) |
| Hospital Universitario Donostia | n (%) | 5 (2.79) | 5 (2.92) | 10 (2.86) |
| Hospital General Universitario de Valencia | n (%) | 6 (3.35) | 4 (2.34) | 10 (2.86) |
| Hospital General Universitario Gregorio Marañón | n (%) | 4 (2.23) | 6 (3.51) | 10 (2.86) |
| Hospital Universitario Miguel Servet | n (%) | 5 (2.79) | 5 (2.92) | 10 (2.86) |
| Hospital Universitario Son Espases | n (%) | 6 (3.35) | 4 (2.34) | 10 (2.86) |
| Hospital Clínico Universitario Lozano Blesa | n (%) | 4 (2.23) | 4 (2.34) | 8 (2.29) |
| Hospital Universitario de Salamanca | n (%) | 4 (2.23) | 4 (2.34) | 8 (2.29) |
| Hospital Universitario de Gran Canaria Doctor Negrín | n (%) | 4 (2.23) | 4 (2.34) | 8 (2.29) |
| Clínica Universidad de Navarra | n (%) | 4 (2.23) | 3 (1.75) | 7 (2.00) |
| Hospital General Universitario de Albacete | n (%) | 4 (2.23) | 3 (1.75) | 7 (2.00) |
| Hospital San Pedro | n (%) | 3 (1.68) | 4 (2.34) | 7 (2.00) |
| Hospital Universitario Ramón y Cajal | n (%) | 4 (2.23) | 2 (1.17) | 6 (1.71) |
| Hospital del Mar | n (%) | 2 (1.12) | 2 (1.17) | 4 (1.14) |
| Hospital Clínico Universitario de Valladolid | n (%) | 2 (1.12) | 1 (0.58) | 3 (0.86) |
| Complejo Hospitalario de Toledo | n (%) | 2 (1.12) | 1 (0.58) | 3 (0.86) |
| Hospital Universitario Arnau de Vilanova | n (%) | 2 (1.12) | 0 (0.00) | 2 (0.57) |
| Hospital Universitario de Girona Dr. Josep Trueta | n (%) | 1 (0.56) | 1 (0.58) | 2 (0.57) |
| Hospital Sant Joan de Déu de Manresa | n (%) | 1 (0.56) | 1 (0.58) | 2 (0.57) |

Table S2. Distribution of ordinal scale status over time and per treatment groups.

| | Group PLASMA N=179 | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day1[a] (Bas) | Day2 | Day3 | Day4 | Day5 | Day6 | Day7 | Day8 | Day9 | Day10 | Day11 | Day12 | Day13 | Day14 | Day15 | Day 15[a] cum[b] | Day29 | Day 29[a] cum[b] |
| | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) |
| Not evaluated | 0 (0) | 1 (0.6) | 2 (1.1) | 10 (5.6) | 12 (6.7) | 28 (15.6) | 40 (22.3) | 43 (24) | 72 (40.2) | 86 (48) | 74 (41.3) | 101 (56.4) | 109 (60.9) | 115 (64.2) | 5 (2.8) | 0 (0) | 9 (5) | 0 (0) |
| 1. Not hospitalized, no limitations on activities | 0 (0) | 0 (0) | 5 (2.8) | 3 (1.7) | 14 (7.8) | 8 (4.5) | 4 (2.2) | 16 (8.9) | 9 (5) | 5 (2.8) | 18 (10.1) | 3 (1.7) | 3 (1.7) | 2 (1.1) | 81 (45.3) | 81 (45.3) | 106 (59.2) | 107 (59.8) |
| 2. Not hospitalized, limitation on activities | 0 (0) | 1 (0.6) | 2 (1.1) | 4 (2.2) | 5 (2.8) | 6 (3.4) | 13 (7.3) | 15 (8.4) | 9 (5) | 8 (4.5) | 10 (5.6) | 7 (3.9) | 2 (1.1) | 5 (2.8) | 41 (22.9) | 41 (22.9) | 44 (24.6) | 45 (25.1) |
| 3. Hospitalized, not requiring supplemental oxygen | 39 (21.8) | 40 (22.3) | 44 (24.6) | 38 (21.2) | 35 (19.6) | 27 (15.1) | 23 (12.8) | 18 (10.1) | 17 (9.5) | 14 (7.8) | 11 (6.1) | 11 (6.1) | 15 (8.4) | 12 (6.7) | 8 (4.5) | 8 (4.5) | 1 (0.6) | 1 (0.6) |
| 4. Hospitalized, requiring supplemental oxygen | 140 (78.2) | 129 (72.1) | 109 (60.9) | 104 (58.1) | 86 (48) | 80 (44.7) | 73 (40.8) | 59 (33) | 47 (26.3) | 42 (23.5) | 44 (24.6) | 39 (21.8) | 35 (19.6) | 32 (17.9) | 28 (15.6) | 28 (15.6) | 11 (6.1) | 11 (6.1) |
| 5. Hospitalized, on non-invasive ventilation or high flow oxygen devices | 0 (0) | 6 (3.4) | 14 (7.8) | 13 (7.3) | 18 (10.1) | 17 (9.5) | 11 (6.1) | 12 (6.7) | 10 (5.6) | 9 (5) | 7 (3.9) | 4 (2.2) | 2 (1.1) | 2 (1.1) | 3 (1.7) | 4 (2.2) | 1 (0.6) | 2 (1.1) |
| 6. Hospitalized, on invasive mechanical ventilation or ECMO | 0 (0) | 1 (0.6) | 3 (1.7) | 7 (3.9) | 9 (5) | 13 (7.3) | 15 (8.4) | 15 (8.4) | 15 (8.4) | 15 (8.4) | 14 (7.8) | 13 (7.3) | 13 (7.3) | 11 (6.1) | 11 (6.1) | 11 (6.1) | 6 (3.4) | 6 (3.4) |
| 7. Death | 0 (0) | 1 (0.6) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (0.6) | 0 (0) | 0 (0) | 1 (0.6) | 1 (0.6) | 0 (0) | 0 (0) | 2 (1.1) | 6 (3.4) | 1 (0.6) | 7 (3.9) |

[a] Day 1 is randomization day. Outcome at trial day 15 means 14 days after randomization. Outcome at trial day 29 means 28 days after randomization and plasma administration.

[b] Assessments were performed daily until discharge and at trial day 15 and 29 in all patients. Data are cumulative for death and current status for clinical categories. If discharged before, scheduled visits at the outpatient clinics or by phone call were performed. Window of ±2 days for 14 days visit and ±3 days for 28 days visit was accepted. Eight missing data at day 29 were imputed according to rules established at the Statistical Analysis Plan ("Composite" strategy for all missing potentially related to the treatment or bad evolution and "Hypothetical" multiple imputation for those not related to treatment)

| | Group Standard of Care N=171 | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day1[a] (Bas) | Day2 | Day3 | Day4 | Day5 | Day6 | Day7 | Day8 | Day9 | Day10 | Day11 | Day12 | Day13 | Day14 | Day15 | Day 15[a] cum[b] | Day29 | Day 29[a] cum[b] |
| | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) |
| Not evaluated | 0 (0) | 2 (1.2) | 5 (2.9) | 15 (8.8) | 16 (9.4) | 30 (17.5) | 49 (28.7) | 56 (32.7) | 74 (43.3) | 87 (50.9) | 85 (49.7) | 107 (62.6) | 120 (70.2) | 125 (73.1) | 13 (7.6) | 0 (0) | 18 (10.5) | 0 (0) |
| 1. Not hospitalized, no limitations on activities | 0 (0) | 1 (0.6) | 6 (3.5) | 3 (1.8) | 8 (4.7) | 10 (5.8) | 5 (2.9) | 13 (7.6) | 7 (4.1) | 9 (5.3) | 19 (11.1) | 6 (3.5) | 3 (1.8) | 4 (2.3) | 88 (51.5) | 88 (51.5) | 102 (59.6) | 102 (59.6) |
| 2. Not hospitalized, limitation on activities | 0 (0) | 1 (0.6) | 1 (0.6) | 1 (0.6) | 6 (3.5) | 12 (7) | 10 (5.8) | 10 (5.8) | 12 (7) | 11 (6.4) | 9 (5.3) | 9 (5.3) | 5 (2.9) | 6 (3.5) | 37 (21.6) | 37 (21.6) | 31 (18.1) | 32 (18.7) |
| 3. Hospitalized, not requiring supplemental oxygen | 34 (19.9) | 34 (19.9) | 31 (18.1) | 33 (19.3) | 31 (18.1) | 26 (15.2) | 26 (15.2) | 24 (14) | 19 (11.1) | 12 (7) | 9 (5.3) | 6 (3.5) | 6 (3.5) | 5 (2.9) | 4 (2.3) | 4 (2.3) | 2 (1.2) | 2 (1.2) |
| 4. Hospitalized, requiring supplemental oxygen | 137 (80.1) | 124 (72.5) | 112 (65.5) | 102 (59.6) | 92 (53.8) | 76 (44.4) | 61 (35.7) | 51 (29.8) | 40 (23.4) | 34 (19.9) | 31 (18.1) | 26 (15.2) | 22 (12.9) | 17 (9.9) | 14 (8.2) | 14 (8.2) | 6 (3.5) | 6 (3.5) |
| 5. Hospitalized, on non-invasive ventilation or high flow oxygen devices | 0 (0) | 6 (3.5) | 9 (5.3) | 9 (5.3) | 8 (4.7) | 7 (4.1) | 7 (4.1) | 8 (4.7) | 9 (5.3) | 5 (2.9) | 4 (2.3) | 3 (1.8) | 2 (1.2) | 1 (0.6) | 3 (1.8) | 6 (3.5) | 4 (2.3) | 8 (4.7) |

| | Group Standard of Care N=171 | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day1[a] (Bas) | Day2 | Day3 | Day4 | Day5 | Day6 | Day7 | Day8 | Day9 | Day10 | Day11 | Day12 | Day13 | Day14 | Day15 | Day 15[a] cum[b] | Day29 | Day 29[a] cum[b] |
| | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) |
| 6. Hospitalized, on invasive mechanical ventilation or ECMO | 0 (0) | 1 (0.6) | 5 (2.9) | 8 (4.7) | 9 (5.3) | 10 (5.8) | 10 (5.8) | 9 (5.3) | 9 (5.3) | 13 (7.6) | 14 (8.2) | 13 (7.6) | 13 (7.6) | 13 (7.6) | 12 (7) | 12 (7) | 7 (4.1) | 7 (4.1) |
| 7. Death | 0 (0) | 2 (1.2) | 2 (1.2) | 0 (0) | 1 (0.6) | 0 (0) | 3 (1.8) | 0 (0) | 1 (0.6) | 0 (0) | 0 (0) | 1 (0.6) | 0 (0) | 0 (0) | 0 (0) | 10 (5.8) | 1 (0.6) | 14 (8.2) |

[a] Day 1 is randomization day. Outcome at trial day 15 means 14 days after randomization. Outcome at trial day 29 means 28 days after randomization and plasma administration.

[b] Assessments were performed daily until discharge and at trial day 15 and 29 in all patients. Data are cumulative for death and current status for clinical categories. If discharged before, scheduled visits at the outpatient clinics or by phone call were performed. Window of ±2 days for 14 days visit and ±3 days for 28 days visit was accepted. Eight missing data at day 29 were imputed according to rules established at the Statistical Analysis Plan ("Composite" strategy for all missing potentially related to the treatment or bad evolution and "Hypothetical"multiple imputation for those not related to treatment)

Table S3. Serious or Grade 3-4 adverse events by treatment group.

| MedDRA System Organ Class | Preferred Term | Convalescent Plasma (n=179) n (%) | Standard of Care (n=172) n (%) |
|---|---|---|---|
| **Patients with at least one serious or grade 3-4 adverse event[a]** | | 15 (8.38) | 16 (9.30) |
| **Description of any grade 3-4 events** | | | |
| Cardiac disorders | Heart failure | 1 (0.56) | 0 (0.00) |
| | Myocardial infarction | 1 (0.56) | 0 (0.00) |
| Gastrointestinal disorders | Gastrointestinal hemorrhage | 0 (0.00) | 1 (0.58) |
| General disorders and administration site conditions | Pyrexia | 1 (0.56) | 0 (0.00) |
| Infections, infestations | Bacterial Pneumonia | 2 (01.12) | 0 (0.00) |
| Nervous system disorders | Cerebral infarction | 0 (0.00) | 1 (0.58) |
| | Hypoxic-ischemic encephalopathy | 1 (0.56) | 0 (0.00) |
| Renal Disorders | Renal disfunction | 0 (0.00) | 1 (0.58) |
| Respiratory, thoracic and mediastinal disorders | Acute respiratory distress syndrome[b] | 1 (0.56) | 1 (0.58) |
| | Respiratory failure[b] | 2 (1.12) | 5 (2.91) |
| | Pulmonary embolism | 1 (0.56) | 4 (2.33) |
| | Dyspnea[b,c] | 0 (0.00) | 1 (0.58) |
| | Stridor | 0 (0.00) | 1 (0.58) |
| Skin and subcutaneous tissue disorders | Skin rash | 1 (0.56) | 0 (0.00) |
| Vascular disorders | Thromboembolism | 1 (0.56) | 0 (0.00) |
| **Description of reported thrombotic events (n, %)** | | | |
| Arterial Thrombotic events | | | |

| | | | |
|---|---|---|---|
| | *Myocardial infarction* | 1 (0.56) | 0 (0.00) |
| | *Cerebral infarction* | 0 (0.0) | 1 (0.58) |
| Venous Thrombotic events | | | |
| | *Pulmonary embolism* | 1 (1.12) | 4 (2.33) |
| | *Thromboembolism* | 1 (1.12) | 0 (0.00) |
| **Description of CP infusion reactions (n, %)** | | | |
| | | Fever | 2 (1.12) |
| | | Nausea/vomiting | 1 (0.56) |
| | | Dyspnea | 5 (2.79) |
| | | Allergic reaction (immediate) | 2 (1.12) |
| | | TRALI | 0 (0.00) |
| | | Fever | 2 (1.12) |
| | | Nausea/vomiting | 1 (0.56) |
| | | Dyspnea | 5 (2.79) |
| | | Allergic reaction (immediate) | 2 (1.12) |
| | | TRALI | 0 (0.00) |

N/A

[a] Four patients (3 in CP, 1 in SOC) experienced two events each.

[b] Reports of worsening dyspnea, respiratory failure or ADRS in the table are those spontaneously reported by clinical investigators as adverse events, following the instruction received to promptly report any sudden worsening that could be a suspected TRALI (transfusion-related acute lung injury) or ADE (antibody-dependent enhancement of disease). In all reported cases, worsening was finally attributed by the investigator to the COVID disease and TRALI was ruled out after a full assessment.

[c] Reported as "dyspnea worsening". This term is not included in MedDRA dictionary, and for data management was coded as "dyspnea".

Abbreviations. TRALI: Transfusion-related acute lung injury. NA: not applicable.

Table S4. Standard of Care in COVID-19 pneumonia based on National recomendations [1,2], according to severity.

| **Treatment in place during the study period 9th March 2020 to 9th July 2020 (first wave).** | | | | |
|---|---|---|---|---|
| | Mild<br>Clinical or radiological diagnosis of pneumonia with adequate respiratory status (SatO2 ≥95%; Brescia[2] scale 0) | Moderate<br>Clinical or radiological diagnosis of pneumonia with mild respiratory compromise (SatO2 <95% or Brescia scale 0-1) | Severe<br>SatO2 <90%; PaO2 <60 mmHg or Brescia's scale [4] | Respiratory worsening with PaO2/FiO2 ≤ 200 or SatO2/FiO2 ≤235 > 118 or Brescia scale ≥3 or radiological progression of ARDS despite steroid treatment |
| Hospital admission | No | Yes | Yes | Yes |
| Recommended | HYDROXYCHLOROQUINE 400 mg/12 hours the first day and then 200 mg/12 hours for 5 days plus<br><br>AZITHROMYCIN 500 mg/qd for 3 days. | HYDROXYCHLOROQUINE 400 mg/12 hours the first day and then 200 mg/12 hours for 5 days plus<br><br>LOPINAVIR/RITONAVIR (200mg/50 mg)2 tablets/12 hours for 5 days. | HYDROXYCHLOROQUINE 400 mg/12 hours the first day and then 200 mg/12 hours for 5 days plus<br><br>LOPINAVIR/RITONAVIR (200mg/50 mg)2 tablets/12 hours for 5 days. | METHYLPREDNISOLONE 1mg/kg/day i.v. for 5 days.<br>or DEXAMETHASONE 20 mg/24 hours for 5 days followed by 10 mg/24 hours for another 5 days |
| Elective therapy [3] | | AZITHROMYCIN 500 mg/24 hours for 3-5 days | METHYLPREDNISOLONE 1mg/kg/day i.v. for 5 days.<br>or DEXAMETHASONE 20 mg/24 hours for 5 days followed by 10 mg/24 hours for another 5 days<br><br>INTERFERON- β1a (Rebif®) 22 mcg s.c. single dose (symptoms < 7 days)<br><br>AZITHROMYCIN 500 mg/24 hours for 3-5 days | METHYLPREDNISOLONE (bolus) 500 mg/24 hours i.v. for 3-5 days.<br><br>TOCILIZUMAB 400 – 600 m mg i.v. single dose.<br>A second dose depending on the response<br><br>ANAKINRA 100 mg/12 hours sc for up to 15 days. |
| Thromboprophylaxis | No | Yes | Yes | Yes |
| **Treatment in place during the study period 7th September 2020 to 5th December2020 (second wave).** | | | | |
| | Mild<br>Clinical or radiological diagnosis of pneumonia with adequate respiratory status (SatO2 ≥95%; Brescia[2] scale 0) | Moderate<br>Clinical or radiological diagnosis of pneumonia with mild respiratory compromise (SatO2 <95% or Brescia scale 0-1) | Severe<br>SatO2 <90%; PaO2 <60 mmHg or Brescia's scale [4] | Respiratory worsening with PaO2/FiO2 ≤ 200 or SatO2/FiO2 ≤235 > 118 or Brescia scale ≥3 or radiological progression of ARDS despite steroid treatment |
| Hospital admission | No | Yes | Yes | Yes |
| Recommended | Symptomatic treatment | METHYLPREDNISOLONE 1mg/kg/day i.v. for 5 days.<br>or DEXAMETHASONE 6-20 mg/24 hours for 5 days followed by 10 mg/24 hours for another 5 days | METHYLPREDNISOLONE 1mg/kg/day i.v. for 5 days.<br>or DEXAMETHASONE 6-20 mg/24 hours for 5 days followed by 10 mg/24 hours for another 5 days | TOCILIZUMAB 400 – 600 m mg i.v. single dose.<br>A second dose depending on the response<br><br>ANAKINRA 200 mg/12 hours sc for up to 15 days |
| Optional or elective therapy [3] | | INTERFERON- β1a (Rebif®) 22 mcg s.c. single dose (symptoms < 7 days)<br><br>AZITHROMYCIN 500 mg/qd for 3 days. | INTERFERON- β1a (Rebif®) 22 mcg s.c. single dose<br><br>REMDESIVIR 200 mg/24 hours i.v. (day 1) → 100 mg/24 hours (days 2 → 5) | METHYLPREDNISOLONE (bolus) 500 mg/24 hours i.v. for 3-5 days. |

| | | | | |
|---|---|---|---|---|
| | | | AZITHROMYCIN 500 mg/24 hours for 3-5 days | |
| Thromboprophylaxis | No | Yes | Yes | Yes |
| **Treatment in place during the study period 6th December 2020 to 5th February 2021 (third wave).** | | | | |
| | Mild<br>Clinical or radiological diagnosis of pneumonia with adequate respiratory status (SatO2 ≥95%; Brescia[2] scale 0) | Moderate<br>Clinical or radiological diagnosis of pneumonia with mild respiratory compromise (SatO2 <95% or Brescia scale 0-1) | Severe<br>SatO2 <90%; PaO2 <60 mmHg or Brescia's scale [4] | Respiratory worsening with PaO2/FiO2 ≤ 200 or SatO2/FiO2 ≤235 > 118 or Brescia scale ≥3 or radiological progression of ARDS despite steroid treatment |
| Hospital admission | No | Yes | Yes | Yes |
| Recommended | Symptomatic treatment | METHYLPREDNISOLONE 1mg/kg/day i.v. for 5 days.<br>or DEXAMETHASONE 6-20 mg/24 hours for 5 days followed by 10 mg/24 hours for another 5 days | METHYLPREDNISOLONE 1mg/kg/day i.v. for 5 days.<br>or DEXAMETHASONE 6-20 mg/24 hours for 5 days followed by 10 mg/24 hours for another 5 days | TOCILIZUMAB 400 – 600 m mg i.v. single dose.<br>A second dose depending on the response<br><br>ANAKINRA 200 mg/12 hours sc for up to 15 days |
| Optional or elective therapy [3] | | REMDESIVIR 200 mg/24 hours i.v. (day 1) → 100 mg/24 hours (days 2 → 5). | REMDESIVIR 200 mg/24 hours i.v. (day 1) → 100 mg/24 hours (days 2 → 5). | |

1Ministry of Health of Spain. Recomendations available at https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov/documentos.htm

[2] Spanish Society for Infectious Disease (SEIMC). Recomendations available at https://covid19.seimc.org/index.php/recomendaciones-de-tratamiento/

[3] Not used in all participating hospitals. [4] Duca A, Piva S, Focà E, Latronico N, Rizzi M. Calculated Decisions: Brescia-COVID Respiratory Severity Scale (BCRSS)/Algorithm. Emerg Med Pract. 2020 Apr 16;22(5 Suppl):CD1-CD.

Table S5. Conplas-19 Study patients. Change over time of the percentage of patients with relevant treatments at baseline.

| | **First wave[1]** | **Second wave[1]** | **Third wave[1]** | **Total** |
|---|---|---|---|---|
| **Patients with concomitant treatment at baseline —N (%)** | | | | |
| Corticosteroids | 41 (50.6) | 136 (72.7) | 72 (87.8) | 249 (71.1) |
| Anticoagulants | NA[2] | 119 (63.6) | 61 (74.4) | 180 (66.9) |
| Remdesivir | 2 (2.5) | 60 (32.1) | 27 (32.9) | 89 (25.4) |
| Hydroxychloroquine | 71 (87.7) | 0 (0.0) | 0 (0.0) | 71 (20.3) |
| Lopinavir/ritonavir | 34 (42.0) | 1 (0.5) | 1 (1.2) | 36 (10.3) |
| Tocilizumab | 15 (18.5) | 6 (3.2) | 5 (6.1) | 26 (7.4) |
| **Total patients per wave** | **81** | **187** | **82** | **350** |

[1]1st wave: 4 april 2020- 9 July 2020; 2nd wave:7 sept 2020- 5 dec 2020; 3rd wave:10 dec 2020-5 feb 2021

[2]Data not available for first 81 patients /1st wave). Anticoagulation was included as a mandatory variable to be registered in the eCRF with the September 2020 amendment.

Table S6. COVID-related treatments received by patients after randomization

| Patients with treatments started after randomization | Plasma + SOC (N=179) | SOC (N=171) |
|---|---|---|
| Overall | 86 (48.0) | 80 (46.8) |
| Treatments of special interest[a] for review | | |
| Anakinra | 2 (1.1) | 3 (1.8) |
| Anticoagulation | 13 (7.3) | 9 (5.3) |
| Baricitinib | 1 (0.6) | 0 (0.0) |
| Colchicine | 2 (1.1) | 0 (0.0) |
| Corticosteroids | 24 (13.4) | 20 (11.7) |
| Immunoglobulins | 0 (0.0) | 1 (0.6) |
| Interferon Beta | 0 (0.0) | 1 (0.6) |
| Convalescent Plasma[b] | 1 (0.6) | 5 (2.9) |
| Remdesivir | 19 (10.6) | 13 (7.6) |
| Sarilumab | 1 (0.6) | 0 (0.0) |
| Tocilizumab | 28 (15.6) | 23 (13.5) |

[a] All covid-related medications were of mandatory recording, both at baseline and throughout the trial. The proportion of patients treated after randomization with therapies that could be considered rescue therapies (corticosteroids, corticosteroids bolus added to initial corticosteroids prescription, anti IL-6, baricitinib, anakinra, plasma units) are used similarly, in 57/179 (31,8%) in CP group and 57/171 (29,8) in control group.
b One patient in CP group received an additional CP Unit and 5 patients in the control arm received CP as rescue treatment according to their physicians medical decision.

Table S7. Mean change in clinical status ordinal scale by group. Proportional odds model for change against baseline

**Estimates**

| Label | Estimate | Standard Error | DF | t Value | Pr > \|t\| | Exponentiated Estimate |
|---|---|---|---|---|---|---|
| CP Vs SOC | 0.05932 | 0.285 | 4306 | 0.21 | 0.8351 | 1.0611 |

**Cochran-Mantel-Haenszel Statistics (Modified Ridit Scores)**

| Statistic | Alternative Hypothesis | DF | Value | Prob |
|---|---|---|---|---|
| 1 | Nonzero Correlation | 1 | 1.3426 | 0.2466 |
| 2 | Row Mean Scores Differ | 1 | 1.2838 | 0.2572 |

Table S8. Proportion of patients in categories 5-7 (non-invasive ventilation or high flow oxygen devices, ICU; invasive mechanical ventilation or ECMO, death). Subgroup analysis by factors with potential impact either on COVID severity or on plasma effect. Data at 14 and 28 days.

| **14 days** | **Convalescent Plasma** | | | **Standard of Care** | | | **0dds ratio** | **(95% CI) INF** | **(95% CI) SUP** | **P value for Interaction** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **n[a]** | **N[a]** | **%** | **n[a]** | **N[a]** | **%** | | | | |
| Overall | 21 | 179 | 11.73 | 28 | 171 | 16.37 | 0.716 | 0.424 | 1.212 | |
| Age | | | | | | | | | | |
| < 75 years | 15 | 130 | 11.54 | 17 | 127 | 13.39 | 0.862 | 0.450 | 1.651 | 0.195 |
| ≥ 75 years | 6 | 49 | 12.24 | 11 | 44 | 25.00 | 0.490 | 0.198 | 1.214 | |
| Sex | | | | | | | | | | |
| Male | 16 | 118 | 13.56 | 21 | 111 | 18.92 | 0.717 | 0.385 | 1.301 | 0.809 |
| Female | 5 | 61 | 8.20 | 7 | 60 | 11.67 | 0.703 | 0.236 | 2.092 | |
| Period of inclusion[c] | | | | | | | | | | |
| 1st wave | 0 | 38 | 0.00 | 6 | 43 | 13.95 | | | | 0.281 |
| 2nd wave | 13 | 101 | 12.87 | 15 | 86 | 17.44 | 0.738 | 0.372 | 1.464 | |
| 3rd wave | 8 | 40 | 20.00 | 7 | 42 | 16.67 | 1.200 | 0.480 | 3.003 | |
| Days of symptoms | | | | | | | | | | |
| ≤ 4 days | 8 | 51 | 15.69 | 7 | 44 | 15.91 | 0.986 | 0.389 | 2.501 | 0.760 |
| 5-6 days | 10 | 84 | 11.90 | 15 | 82 | 18.29 | 0.651 | 0.319 | 1.364 | |
| ≥ 7days | 3 | 44 | 6.82 | 6 | 45 | 13.33 | 0.511 | 0.136 | 1.918 | |
| Score on ordinal scale | | | | | | | | | | |
| 3. Hospitalized, not requiring supplemental oxygen | 2 | 40 | 5.00 | 2 | 34 | 5.88 | 0.850 | 0.126 | 5.717 | 0.626 |
| 4. Hospitalized, requiring supplemental oxygen by mask or nasal prongs | 19 | 139 | 13.67 | 26 | 137 | 18.98 | 0.720 | 0.419 | 1.239 | |
| Corticosteroids[d] | | | | | | | | | | |
| Yes | 18 | 127 | 14.17 | 20 | 122 | 16.39 | 0.865 | 0.481 | 1.554 | 0.310 |
| No | 3 | 52 | 5.77 | 8 | 49 | 16.33 | 0.353 | 0.099 | 1.256 | |
| Patients Ab at baseline[e] | | | | | | | | | | |
| Positive | 1 | 48 | 2.08 | 5 | 61 | 8.20 | 0.254 | 0.031 | 2.104 | 0.186 |
| Negative | 19 | 130 | 14.62 | 22 | 107 | 20.56 | 0.711 | 0.407 | 1.242 | |
| Plasma IgG Values ORTHO[f] | | | | | | | | | | |
| < 9.5 S/C | 10 | 111 | 9.01 | 28 | 171 | 16.37 | 0.550 | 0.278 | 1.088 | 0.348 |
| ≥ 9.5 S/C | 9 | 64 | 14.06 | 28 | 171 | 16.37 | 0.859 | 0.429 | 1.719 | |
| Plasma IgG Values Euroimmun[f] | | | | | | | | | | |
| < 3.5 | 9 | 92 | 9.78 | 28 | 171 | 16.37 | 0.597 | 0.295 | 1.212 | 0.671 |
| ≥ 3.5 | 10 | 83 | 12.95 | 28 | 171 | 16.37 | 0.736 | 0.375 | 1.442 | |
| Plasma Neutralizing Ab Titer[f] | | | | | | | | | | |
| Titer ≤ 80 | 8 | 53 | 15.09 | 28 | 171 | 16.37 | 0.922 | 0.447 | 1.899 | 0.205 |
| > 80 Titer ≤ 336 | 3 | 63 | 4.76 | 28 | 171 | 16.37 | 0.291 | 0.092 | 0.923 | |
| Titer > 336 | 8 | 59 | 13.56 | 28 | 171 | 16.37 | 0.828 | 0.400 | 1.715 | |

a. "n" means patients in categories 5-7 (non-invasive ventilation or high flow oxygen devices, ICU; invasive mechanical ventilation or ECMO, death) at that time point. "N" means the total number of patients in the group, following intention to treat principle.

b. Periods were established according to official information on waves and actual trial recruitment. 1st wave: 4 april 2020 - 9 July 2020; 2nd wave:7 sept 2020- 5 dec 2020; 3rd wave:10 dec 2020-5 feb 2021

c. Includes patients receiving corticosteroids on the day of randomization or before

d. Four patients had missing serology values, 146 patients included

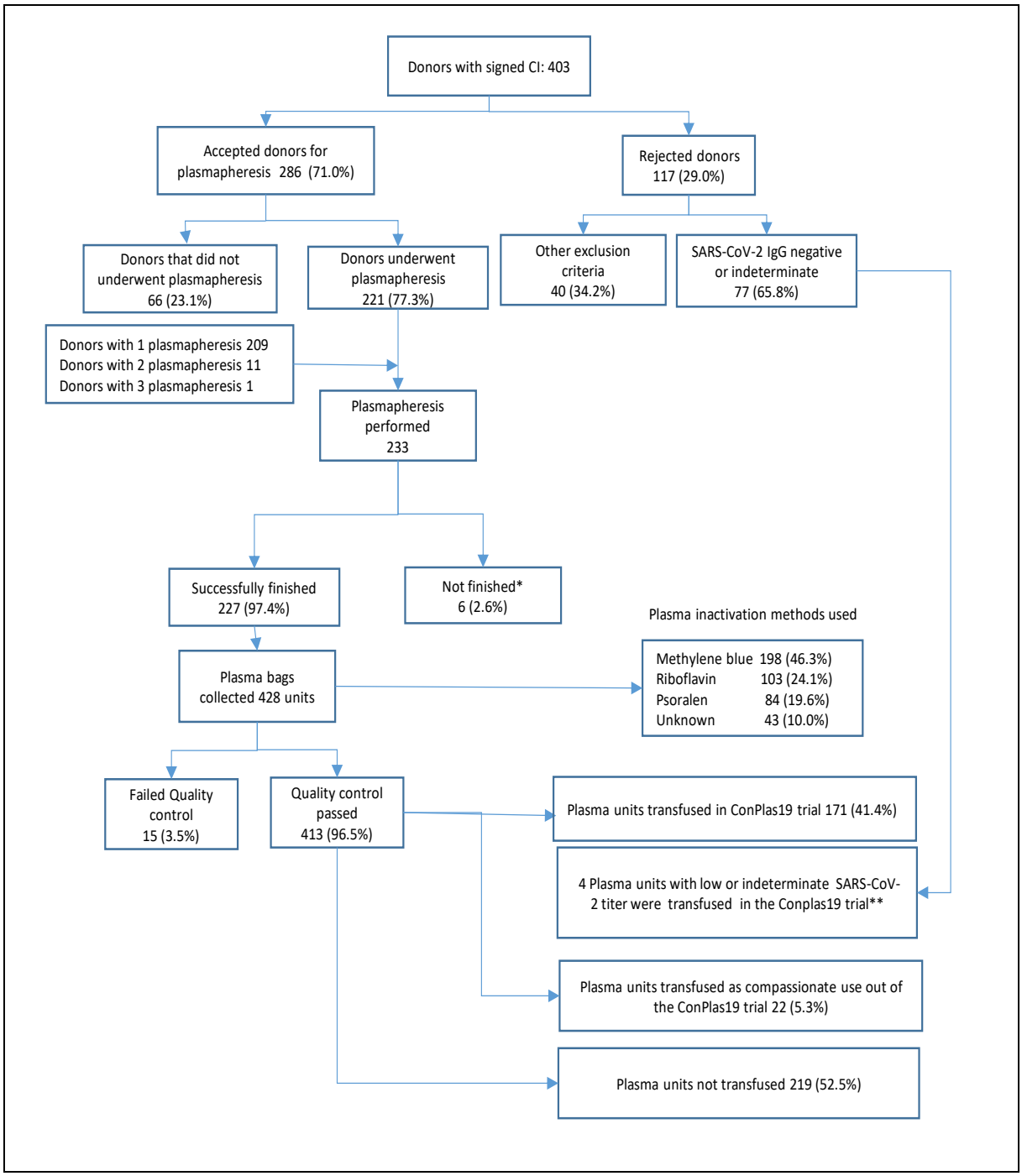
e. Four CP patients did not receive plasma (consent withdrawal, not plasma available,..) and only results from 175 used plasma units are available

| 28 days | Convalescent Plasma | | | Standard of Care | | | 0dds ratio | (95% CI) INF | (95% CI) SUP | P value for Interaction |
|---|---|---|---|---|---|---|---|---|---|---|
| | n[a] | N[a] | % | n[a] | N[a] | % | | | | |
| Overall | 15 | 179 | 8.38 | 29 | 171 | 16.96 | 0.494 | 0.275 | 0.889 | |
| Age | | | | | | | | | | |
| < 75 years | 10 | 130 | 7.69 | 16 | 127 | 12.60 | 0.611 | 0.288 | 1.294 | 0.069 |
| ≥ 75 years | 5 | 49 | 10.20 | 13 | 44 | 29.55 | 0.345 | 0.134 | 0.891 | |
| Sex | | | | | | | | | | |
| Male | 12 | 118 | 10.17 | 22 | 111 | 19.82 | 0.513 | 0.267 | 0.987 | 0.695 |
| Female | 3 | 61 | 4.92 | 7 | 60 | 11.67 | 0.422 | 0.114 | 1.554 | |
| Period of inclusion[c] | | | | | | | | | | |
| 1st wave | 0 | 38 | 0.00 | 7 | 43 | 16.28 | | | | 0.471 |
| 2nd wave | 10 | 101 | 9.90 | 15 | 86 | 17.44 | 0.508 | 0.269 | 1.198 | |
| 3rd wave | 5 | 40 | 12.50 | 7 | 42 | 16.67 | 0.750 | 0.259 | 2.171 | |
| Days of symptoms | | | | | | | | | | |
| ≤ 4 days | 6 | 51 | 11.76 | 9 | 44 | 20.45 | 0.575 | 0.222 | 1.489 | 0.341 |
| 5-6 days | 7 | 84 | 8.33 | 14 | 82 | 17.07 | 0.488 | 0.208 | 1.147 | |
| ≥ 7days | 2 | 44 | 4.55 | 6 | 45 | 13.33 | 0.341 | 0.134 | 1.599 | |
| Score on ordinal scale | | | | | | | | | | |
| 3. Hospitalized, not requiring supplemental oxygen | 0 | 40 | 0.00 | 2 | 34 | 5.88 | | | | 0.724 |
| 4. Hospitalized, requiring supplemental oxygen by mask or nasal prongs | 15 | 139 | 10.79 | 27 | 137 | 19.71 | 0.548 | 0.305 | 0.983 | |
| Corticosteroids[d] | | | | | | | | | | |
| Yes | 12 | 127 | 9.45 | 21 | 122 | 17.21 | 0.549 | 0.282 | 1.067 | 0.720 |
| No | 3 | 52 | 5.77 | 8 | 49 | 16.33 | 0.353 | 0.099 | 1.256 | |
| Patients Ab at baseline[e] | | | | | | | | | | |
| Positive | 0 | 48 | 0.00 | 5 | 61 | 8.20 | | | | 0.506 |
| Negative | 14 | 130 | 10.77 | 22 | 107 | 20.56 | 0.524 | 0.282 | 0.973 | |
| Plasma IgG Values ORTHO[f] | | | | | | | | | | |
| < 9.5 S/C | 5 | 111 | 4.50 | 29 | 171 | 16.96 | 0.266 | 0.106 | 0.666 | 0.126 |
| ≥ 9.5 S/C | 9 | 64 | 14.06 | 29 | 171 | 16.96 | 0.829 | 0.416 | 1.654 | |
| Plasma IgG Values Euroimmun[f] | | | | | | | | | | |
| < 3.5 | 4 | 92 | 4.35 | 29 | 171 | 16.96 | 0.256 | 0.093 | 0.707 | 0.923 |
| ≥ 3.5 | 10 | 83 | 12.95 | 29 | 171 | 16.96 | 0.710 | 0.364 | 1.387 | |
| Plasma Neutralizing Ab Titer[f] | | | | | | | | | | |
| Titer ≤ 80 | 2 | 53 | 3.77 | 29 | 171 | 16.96 | 0.223 | 0.055 | 0.902 | 0.452 |
| > 80 Titer ≤ 336 | 4 | 63 | 6.35 | 29 | 171 | 16.96 | 0.374 | 0.137 | 1.022 | |
| Titer > 336 | 8 | 59 | 13.56 | 29 | 171 | 16.96 | 0.800 | 0.387 | 1.650 | |

a. "n" means patients in categories 5-7 (non-invasive ventilation or high flow oxygen devices, ICU; invasive mechanical ventilation or ECMO, death) at that time point. "N" means the total number of patients in the group, following intention to treat principle.
b. Periods were established according to official information on waves and actual trial recruitment. 1st wave: 4 april 2020- 9 July 2020; 2nd wave:7 sept 2020- 5 dec 2020; 3rd wave:10 dec 2020-5 feb 2021
c. Includes patients receiving corticosteroids on the day of randomization or before
d. Four patients had missing serology values, 146 patients included

e. Four CP patients did not receive plasma (consent withdrawal, not plasma available,..) and only results from 175 used plasma units are available

Figure S1. Hyperinmune Convalescent Plasma (CP) donors, apheresis and CP units.




*Plasmapheresis not finished due to adverse event or technical failure.

** First donors were assessed with several ELISA IgG assays that were being used and validated, before EUROIMMUN was set as the standard assay for the trial. Two donors (4 units) were accepted positive with other AntiSARS-CoV-2 assays but resulted negative when reanalyzed with EUROIMMUN. Two of those units were administered to trial patients.

Figure S2. Serology results in Convalescent Plasma (CP) donors. Anti SARS-CoV-2 anti spike protein by Euroimmun assay

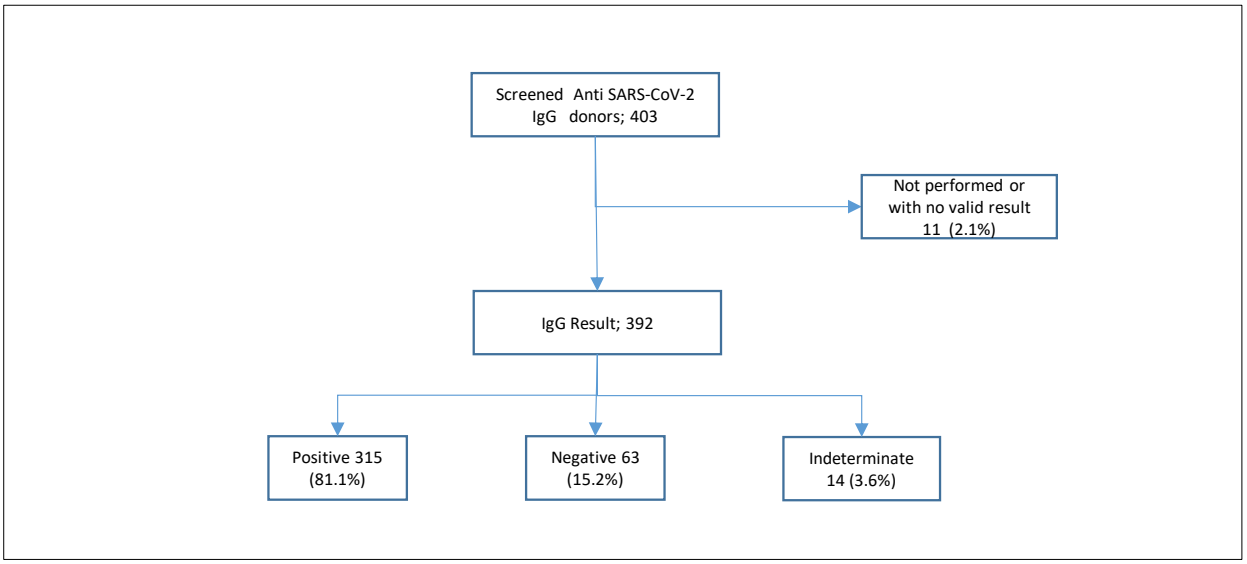



Figure S3. Correlation of results obtained from the two assays used for anti SARS-CoV-2 IgG anti Spike protein in serum samples of CP donors, Euroimmun assay and VITROS ORTHO clinical assay.

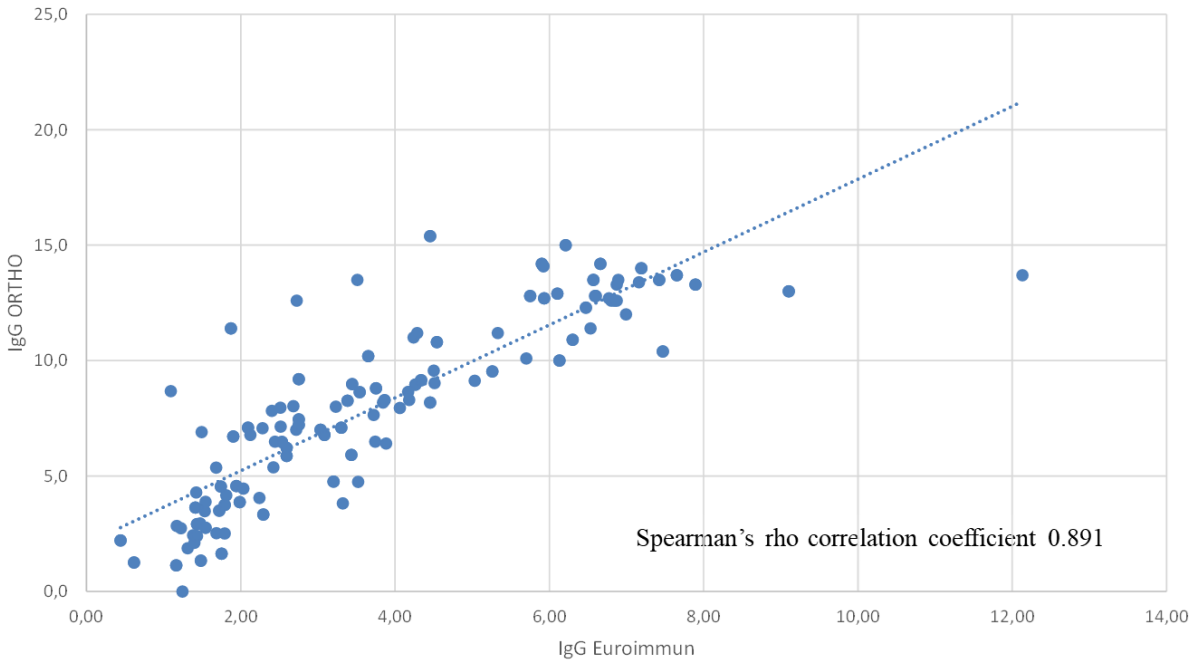


Figure S4. Time to discharge by groups.

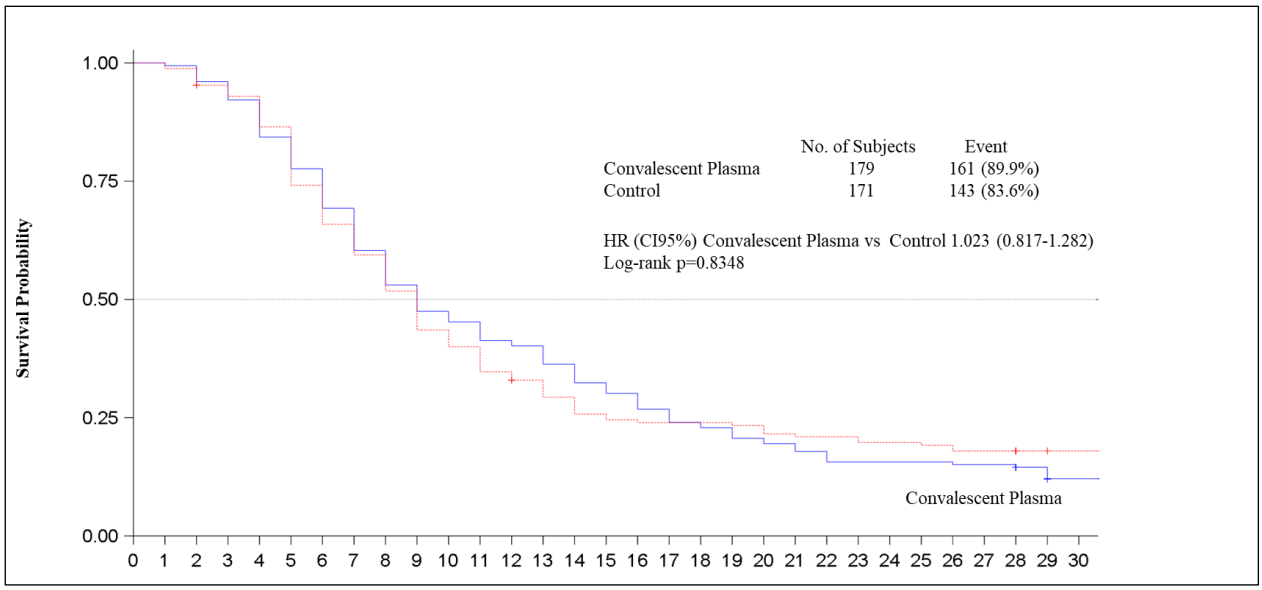



Figure S5. Time to first worsening in the COVID ordinal scale by group

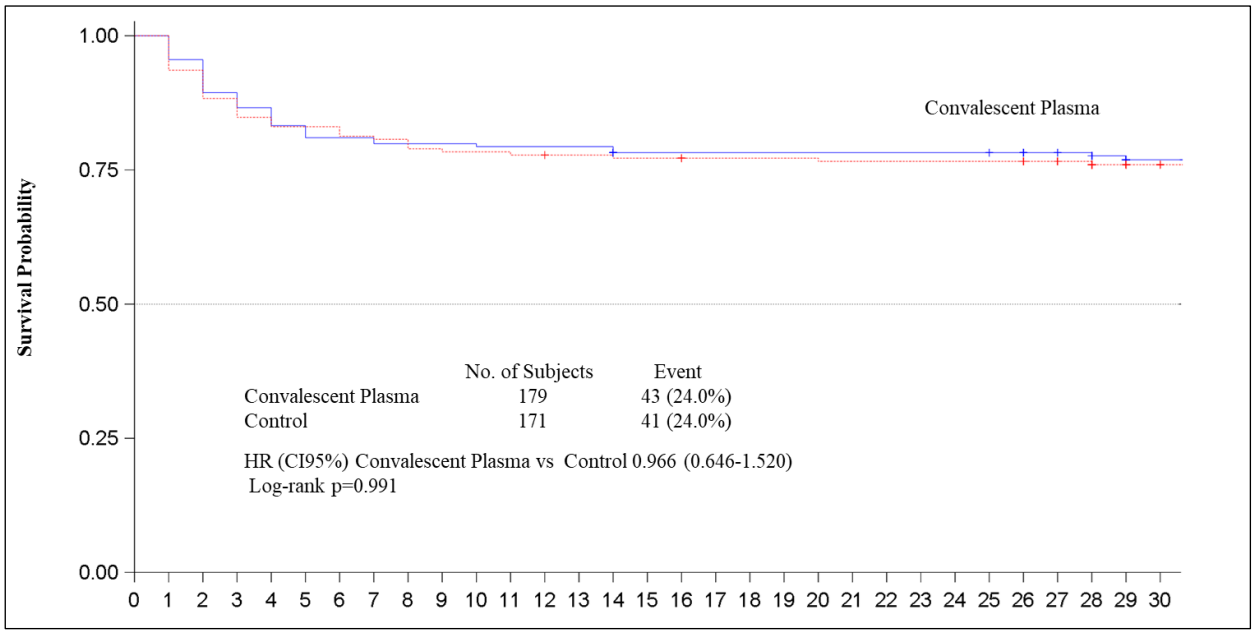


Figure S6. Time to first improvement of one category by group

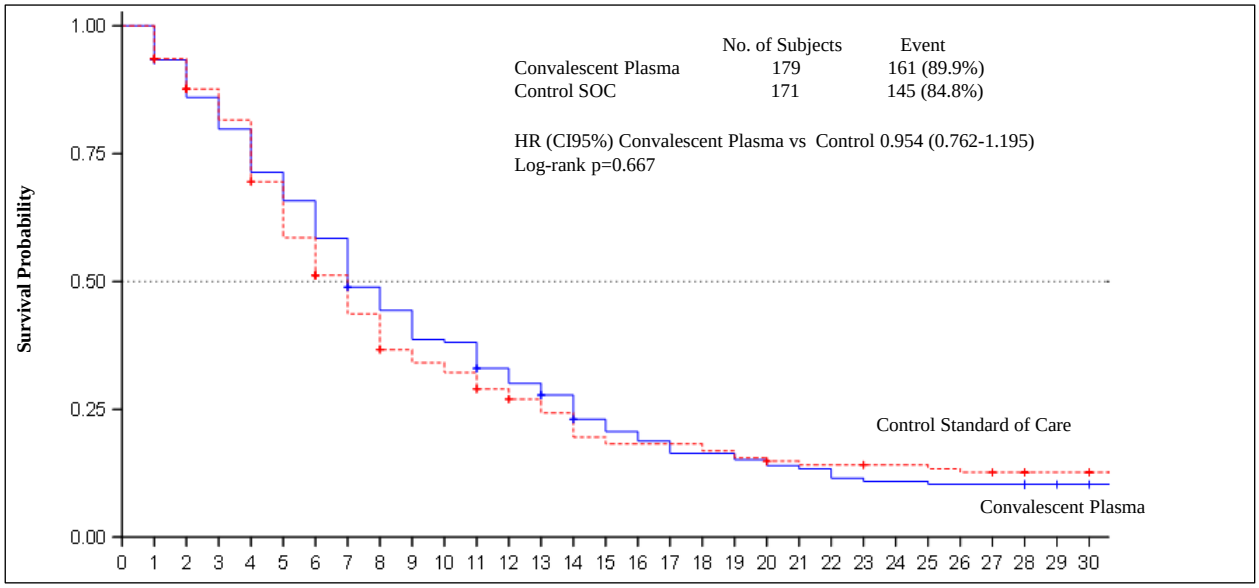
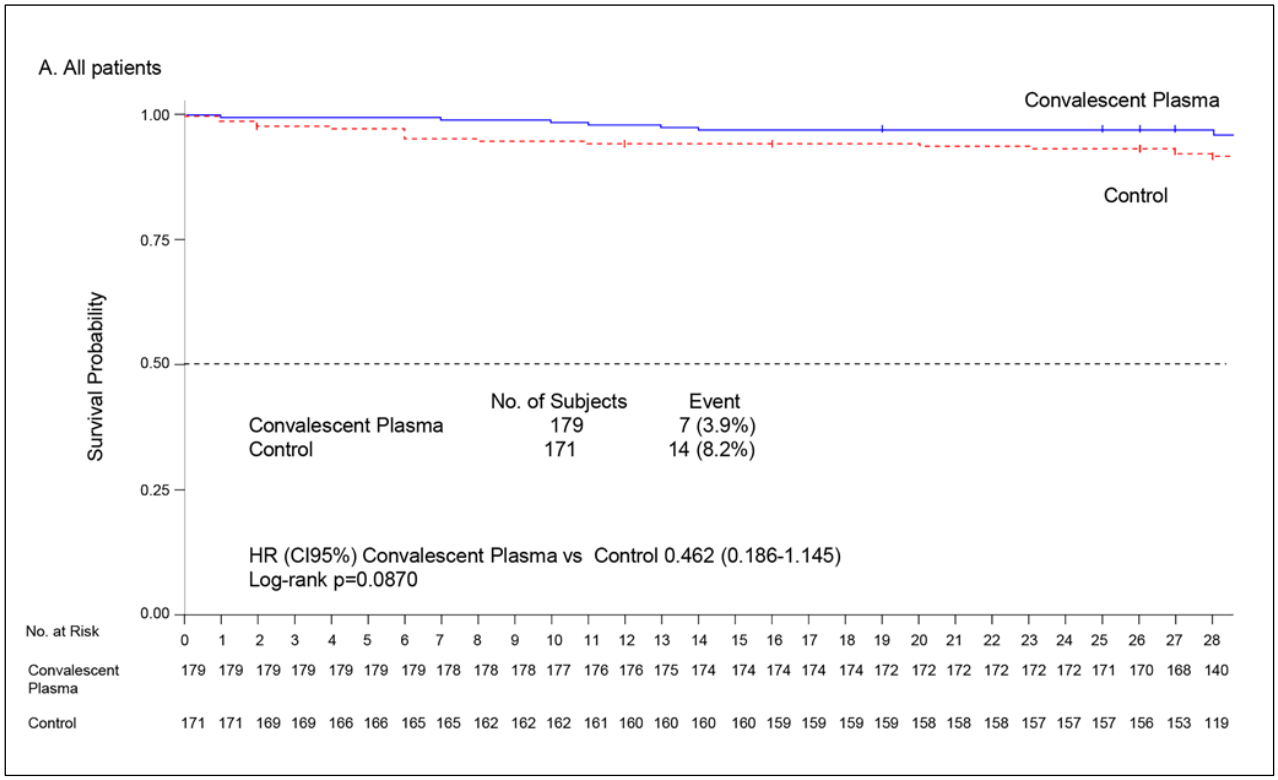
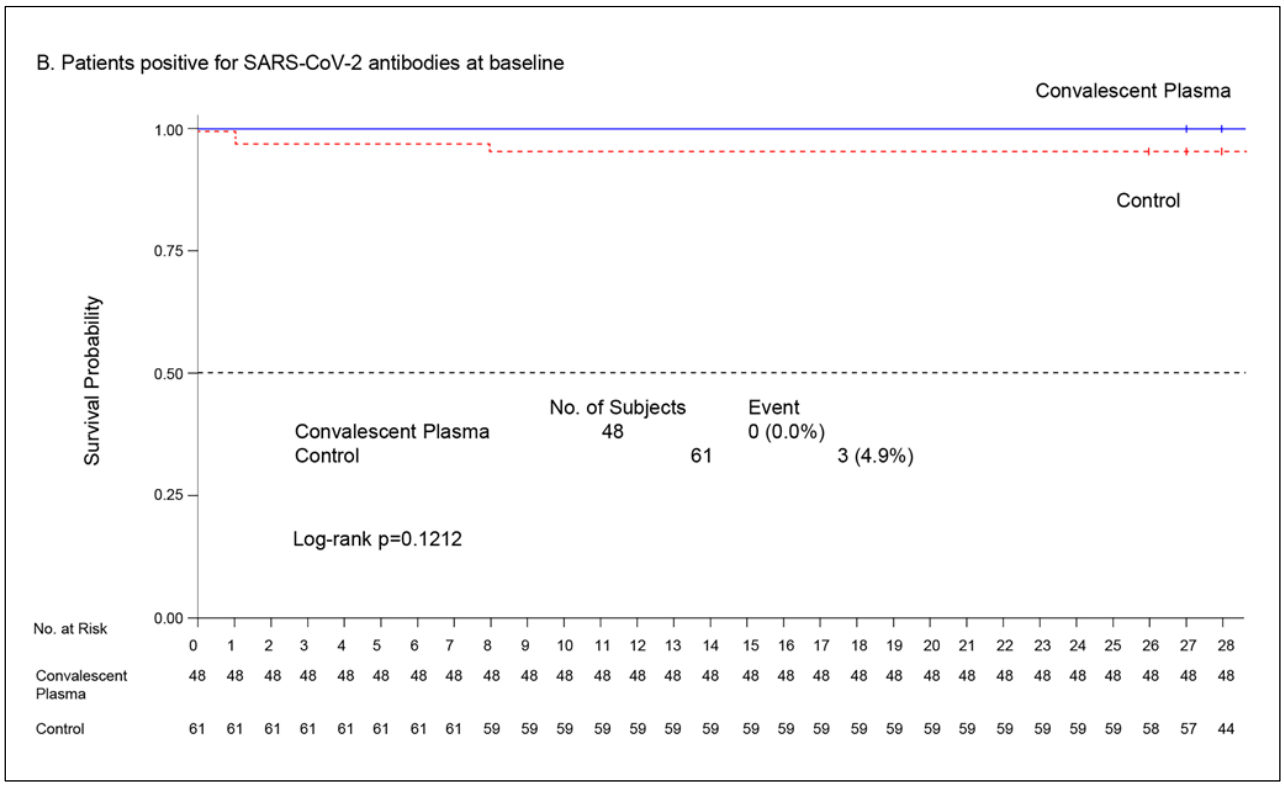
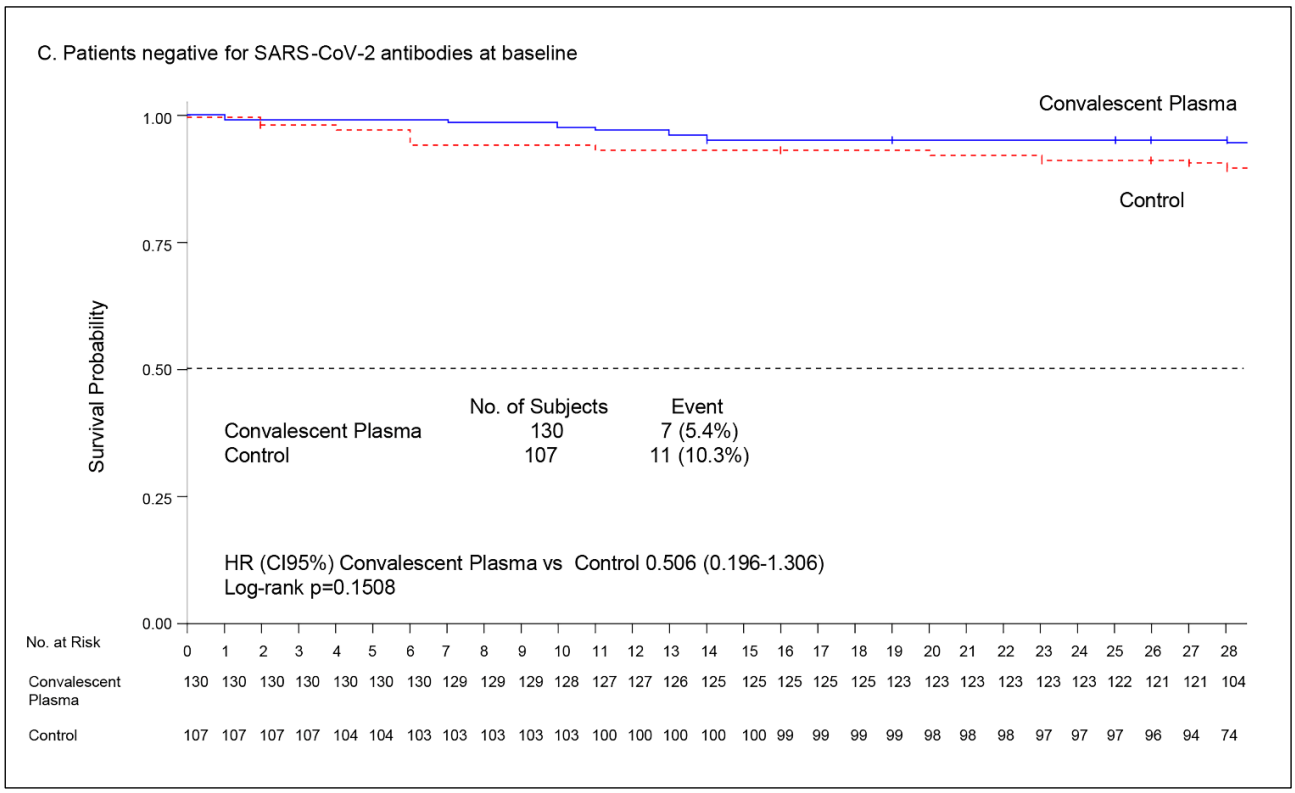



Figure S7. Overall survival by group. A) All patients; B) patients positive for SARS-CoV-2 antibodies at baseline C) patients negative for SARS-CoV-2 antibodies at baseline.




B. Patients positive for SARS-CoV-2 antibodies at baseline
Convalescent Plasma
Control
Survival Probability
1.00
0.75
0.50
0.25
0.00
No. of Subjects
Event
Convalescent Plasma 48 0 (0.0%)
Control 61 3 (4.9%)
Log-rank p=0.1212
No. at Risk
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Convalescent Plasma 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48 48
Control 61 61 61 61 61 61 61 61 59 59 59 59 59 59 59 59 59 59 59 59 59 59 59 59 59 58 57 44


C. Patients negative for SARS-CoV-2 antibodies at baseline
Convalescent Plasma
Control
Survival Probability
1.00
0.75
0.50
0.25
0.00
No. of Subjects
Event
Convalescent Plasma 130 7 (5.4%)
Control 107 11 (10.3%)
HR (CI95%) Convalescent Plasma vs Control 0.506 (0.196-1.306)
Log-rank p=0.1508
No. at Risk
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Convalescent Plasma 130 130 130 130 130 130 130 129 129 129 128 127 127 126 125 125 125 125 125 123 123 123 123 123 123 122 121 121 104
Control 107 107 107 107 104 104 103 103 103 103 103 100 100 100 100 100 99 99 99 99 98 98 98 97 97 97 96 94 74

# Multi-center, Randomized Clinical Trial of Convalescent Plasma Therapy Versus Standard of Care for the Treatment of COVID-19 in Hospitalized Patients

| | |
|---|---|
| Sponsor Code: | ConPlas-19 |
| Version: | v3.1 |
| Date: | 30/09/2020 |
| Sponsor: | Fundación para la Investigación Biomédica del Hospital Universitario Puerta de Hierro Majadahonda |
| CT Number: | NCT04345523 |
| Funded by: | Instituto de Salud Carlos III |

**CONFIDENTIAL**

**PROTOCOL APPROVAL SIGNATURES**

**Sponsor Representative:**

*Name:* Cristina Avendaño Solá

*Position:* Project Co-Coordinator

*Date*: 30/09/2020

*Signature*:

**Principal Investigators:**

| | | |
|---|---|---|
| *Name*: | Cristina Avendaño Solà | Rafael F. Duarte Palomino |
| *Date*: | 30/09/2020 | 30/09/2020 |
| Signature: | | |

**Clinical Trial National Coordinator:**

*Name*: Antonio Ramos Martínez

*Date*: 30/09/2020

Signature:

**Clinical Site Principal Investigator:**

*Name:*

*Clinical site:*

*Date*:

*Signature*:

**KEY TRIAL CONTACTS**

| | |
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| | |
|---|---|
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| Statistical Analysis | Ferrán Torres, MD, PhD<br>Servicio de Farmacología Clínica. Hospital Clínic Barcelona.<br>E-mail: Ferran.Torres@uab.cat |
| Project Manager<br><br>and<br><br>Monitoring | Ana Velasco Iglesias, MSc, PhD<br>Tel: +34 91 191 7867<br>E-mail: ana.velasco@scren.es<br><br>Alvaro Veiga<br>Tel: +34 91 191 6669<br>E-mail: alvaro.veiga@scren.es<br>SCReN, IIS Puerta de Hierro - Segovia de Arana<br>C/ Joaquín Rodrigo 2. 28222. Majadahonda. Madrid |

## COMMITTEES AND TRIAL ORGANIZATION

| | |
|---|---|
| **Steering Committee** | Dr. Rafael Duarte<br>Servicio de Hematología y Hemoterapia. Hospital Universitario Puerta de Hierro Majadahonda<br><br>Dr. Antonio Ramos<br>Departamento de Medicina Interna. Unidad de Enfermedades Infecciosas. Hospital Universitario Puerta de Hierro Majadahonda<br><br>Dr. José Luis Bueno<br>Unidad de Hemoterapia No transfusional y Aféresis terapéutica. Servicio de Hematología y Hemoterapia. Hospital Universitario Puerta de Hierro Majadahonda<br><br>Dr. Elena Múñez<br>Departamento de Medicina Interna. Unidad de Enfermedades Infecciosas. Hospital Universitario Puerta de Hierro Majadahonda<br><br>Dr. Cristina Avendaño Solà<br>Servicio de Farmacología Clínica. Hospital Universitario Puerta de Hierro Majadahonda<br><br>Dr. Belén Ruiz-Antorán<br>Servicio de Farmacología Clínica. Hospital Universitario Puerta de Hierro Majadahonda |

| | |
|---|---|
| | Dr. Rosa Malo de Molina<br>Servicio de Neumología. Hospital Universitario Puerta de Hierro Majadahonda<br><br>Dr. Ferrán Torres<br>Servicio de Farmacología Clínica. Hospital Clínic Barcelona. Medical Statistics core facility - IDIBAPS.<br><br>Dr. Inmaculada Casas Flecha<br>Unidad de Gripe y Virus Respiratorios, Laboratorio de Referencia de Gripe. Centro Nacional de Microbiología, Instituto de Salud Carlos III |
| **Data Safety Monitoring Board (DSMB)** | Dr. Arantxa Sancho López<br>Servicio de Farmacología Clínica.<br>Hospital Universitario Puerta de Hierro Majadahonda<br><br>Dr. Emilio Ojeda<br>Servicio de Hematología y Hemoterapia.<br>Hospital Universitario Puerta de Hierro Majadahonda<br><br>Dr. José Ríos<br>Unidad de Bioestadística. Universidad Autónoma de Barcelona<br>Medical Statistics core facility - IDIBAPS.<br><br>Dr. Carlos Vilches<br>Inmunogenética – HLA.<br>Hospital Universitario Puerta de Hierro Majadahonda<br><br>Dr. Juan Antonio Vargas<br>Servicio de Medicina Interna.<br>Hospital Universitario Puerta de Hierro Majadahonda |

**Steering Committee (SC)**

Composed by the coordinators and the sponsor representative. The SC will supervise the trial conduct and is responsible for the scientific validity of the study. The SC is responsible for the decision to make recommendations about including adding or removing treatment arms.

**Data Safety and Monitoring Board (DSMB)**

A Data Safety Monitoring Board (DSMB), independent from the clinical trial, will be appointed to perform an independent supervision of the safety aspects of the trial. The DSMB will have 5 members including at least one expert in clinical trial methodology and statistics, and one infectious diseases specialist independent from the study team. The DSMB will be established before the start of the trial and will follow the procedures established in the "DSMB Charter".

The DSMB will review safety data after first 20 subjects are entered into the trial and when 20%, 40%, 60% and 80% of the patients have reached the 15 days follow up. Ad hoc reviews will be undertaken at any time if there are other specific safety concerns. The study will not stop enrollment awaiting these DSMB reviews, though the DSMB may recommend temporary or permanent cessation of enrollment based on their safety reviews.

The DSMB will be also in charge to review the efficacy and futility analysis which will be conducted when 20%, 40%, 60% and 80% of the patients have been assessed the principal endpoint. Statistical stopping boundaries are predefined and described in this working protocol (see section 11.2), and the study may be stopped prematurely if either the efficacy or the futility of the boundaries are crossed. Although the DSMB will review data in an unblinded manner, the date of the SAP closure will be set before the first unblinded review so that the study will maintain the integrity and will avoid any operational bias. Any potential analysis amendment will be traced and justified, if applicable. The study followed the regulatory recommendations regarding the functions and procedures of these committees[1].

1 CHMP/EWP/5872/03 Corr. Guideline on Data Monitoring Committees. URL: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-data-monitoring-committees_en.pdf, last access: 20-Mar-2020.

**TABLE OF CONTENTS**

# 1 TRIAL SUMMARY

| | |
|---|---|
| **Study Title** | **Multi-center, Randomized Clinical Trial of Convalescent Plasma Therapy Versus Standard of Care for the Treatment of COVID-19 in Hospitalized Patients** |
| **CT Number** | NCT04345523 |
| **Rationale and Justification Summary** | Convalescent plasma (CP) from infected patients who have developed an immune response is **likely to be an option for the treatment of patients with a variety of severe acute respiratory infections (SARI) of viral etiology**. This would include patients in the most recent epidemics with coronaviruses, SARS1 in 2003 and MERS in 2012, and **potentially as well patients in the current COVID-19 pandemic**. Despite suggesting safety and potential efficacy, the evidence available has a major limitation of being based on **predominantly low-quality uncontrolled studies**. Here we present a summary of the **rationale and justification to carry out a multicenter, randomized clinical trial of CP therapy in COVID-19 hospitalized patients**.<br><br>- **Passive Immunotherapy of viral SARI with CP:** Passive immunotherapy involves the administration of antibodies against a given agent to a susceptible individual for the purpose of preventing or treating an infectious disease caused by that agent. CP has been historically used in outbreaks of poliomyelitis, measles, mumps, influenza (1918 H1N1 and 2009-2010 H1N1), 2013 Ebola. In addition, although less readily available and requiring more complex manufacturing than CP, conventional and hyperimmune immunoglobulins are used in clinical practice in a number of infections such as respiratory syncytial virus, hepatitis B and others.<br>▪ Currently, the **only source of antibody available for immediate use** against SARS-CoV-2 is human CP.<br>▪ In addition, as more individuals contract COVID-19 and recover, the **number of potential donors will continue to increase** in all areas where COVID-19 epidemic is present.<br><br>- **Use of CP against coronavirus diseases:** The experience with SARI caused by coronavirus is rather recent in a number of epidemics in the twenty-first century:<br>▪ Human CP was used in patients from both SARS-1 in 2003 and MERS in 2012. Overall, the experience showed that **CP is safe and likely to reduce mortality in patients with SARI by coronaviruses**. Of note, the largest study with 80 patients with SARS in Hong-Kong in 2003, and subsequent meta-analyses, showed that **earlier administration after symptom onset is more effective**, in particular before day 14, before seroconversion in patients who remain PCR positive.<br>▪ The success of CP therapy may be limited by the fact that **not all patients who recover from viral disease develop neutralizing antibodies**. (e.g. 12% of SARS-1 patients do not). Thus, antibody content should be tested in convalescent patients to select adequate donors.<br>▪ Finally, in the current SARS-CoV-2 epidemic, China has recently communicated in the media that **245 COVID-19 patients have received CP**, starting with the first patient on February 9th, and with preliminary reports of improvement in clinical indicators and symptoms [http://www.xinhuanet.com/english/2020-02/28/c_138828177.htm]. |

- **Balancing benefit and potential risks of CP in COVID-19 patients:** The efficacy and safety of the therapeutic use of CP in COVID-19 patients cannot be inferred without carrying out a controlled clinical trial. In the current scenario of low-quality uncontrolled studies, treatment appears to be safe and well tolerated, and to associate with improved outcomes. However, a number of potential risks must be considered in the planning of this trial:
  - With modern blood banking techniques, the **risks of transfusion would be low**.
  - In patients with pulmonary disease as the ones we will include in this trial, a particular risk of the use of human plasma is the **development of TRALI** (transfusion-related acute lung injury), which should be considered in the criteria to select CP donors.
  - There is a potential risk that virus-specific IgG, despite its likely contribution to viral clearance, could also be involved in the severe lung inflammatory damage seen in a minority of infected patients. This process, named **antibody-dependent enhancement of disease** (ADE), has been normally seen at later stages of infection in critical patients in intensive care units, and in Asian COVID-19 patients who may have been exposed before and had antibodies against other coronaviruses. Of note, l**arge anecdotal evidence from CP use in 245 patients with COVID-19 in China suggest it is safe**. However, this is in a non-controlled fashion and without a full scientific report as yet.

Hence, for the design of our trial, we **exclude critical patients with more advanced lung involvement** on mechanical ventilation, who are overall less likely to respond to passive immunotherapy and more likely to develop ADE, and propose an **earlier intervention with CP in the course of the disease** based on severity and time from admission to hospital and onset of symptoms.

In summary, historical and current anecdotal data suggest that the use of **CP is safe in coronavirus infection** and the high severity and mortality of COVID-19 suggests that the **benefits of its use in those with early disease clearly outweigh the risks** to be tested in a clinical trial.

From this background and rationale, we aim to focus this study on **hospitalized patients with severe COVID-19 who still have an early form of the disease**, rather than as a last resort in patients with critical illness who require mechanical ventilation in whom it may be less effective and more prone to immune-mediated adverse-events. In the midst of a worldwide pandemic of SARS-CoV-2 and COVID-19, for which we currently have no available vaccines or drugs, CP represents a potential therapeutic option with a **favorable benefit/risk balance for these patients**. CP could be **rapidly available in any areas where there are sufficient numbers of patients** who have recovered. Its potential efficacy and safety should be tested in a randomized, controlled study, as recommended by the WHO R&D Blueprint and Master Protocol for COVID-19 Therapeutic Trials, from which we can obtain **strong evidence to inform decisions worldwide**.

**Key References:**


- Arabi YM, et al. Feasibility of using convalescent plasma immunotherapy for MERS-CoV infection, Saudi Arabia. Emerging Infect Dis. 2016;22 (9):1554–1561. PMID: 27532807.
- Casadevall A and Pirofski LA. The convalescent sera option for containing COVID-19. J Clin Invest. 2020 Mar 13; PMID: 32167489.

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| | - Cheng Y, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. Eur J Clin Microbiol Infect Dis. 2005;24(1):44–46. PMID: 15616839.<br>- Fu Y, et al. Understanding SARS-CoV-2-Mediated Inflammatory Responses: From Mechanisms to Potential Therapeutic Tools. Virologica Sinica. 2020 Mar 3; PMID: 32125642.<br>- Mair-Jenkins J, et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. J Infec Dis. 2015;211:80-90. PMID: 25030060.<br>- Tetro JA. Is COVID-19 receiving ADE from other coronaviruses? Microbes Infect. 2020;22(2):72-73. PMID: 32092539.<br>- Zhang JS, et al. A serological survey on neutralizing antibody titer of SARS convalescent sera. J Med Virol. 2005;77(2):147–150. PMID: 16121363. |
| **Trial Design** | A multi-center, randomized, clinical trial with two arms to study the efficacy and safety of passive immunotherapy with CP compared to a control of standard of care (SOC).<br>All trial participants will receive SOC:<br>- Treatment arm: Pathogen-reduced CP from patients recovered from COVID-19, whom, for the purpose of this trial, are herein designated as donors.<br>- Control arm: SOC for COVID-19.<br><br>Randomization among the two arms will be 1:1 and will be stratified per center.<br>Of note, in the current status of a worldwide pandemic for which we have no approved vaccines or drugs, for the purpose of this trial SOC would also accept any drugs that are being used in clinical practice (e.g. remdesivir, corticosteroids, tocilizumab, etc.), other than those used as part of another clinical trial.<br><br>The study is planned with a sequential design. Interim analyses: comprehensive safety data monitoring analyses will be conducted when 20%, 40%, 60% and 80% of patients, or at the discretionary DSMB criteria when needed. A DSMB charter will be set before the trial initiation where criteria for prematurely stopping the trial due to safety issues will be set. Interim analyses will be predefined upfront based on the DSMB recommendations. |
| **Participant centers** | 32 hospitals of regions in Spain with a high incidence of COVID-19.<br>Laboratorio de Virus Respiratorios, Laboratorio de Serología and AIDS Immunopathology Unit. Centro Nacional de Microbiología. Instituto de Salud Carlos III (ISCIII).<br>Transfusion Centers. |
| **Trial Participants** | Hospitalized adult (≥18 years old) patients with severe COVID-19 not requiring mechanical ventilation (invasive or non-invasive). |
| **Planned Sample Size** | 278 patients (1:1 ratio for CP: Control arm). Also, approximately 140-200 CP donors. |
| **Follow-up duration** | 60 days after randomization. |
| **Trial Period** | 12 months in total |
| **Primary Objective** | To evaluate the clinical efficacy of CP plasma versus SOC |

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| **Secondary Objective** | - To evaluate effects of CP on secondary efficacy endpoints, including all-cause mortality and respiratory deterioration with progression to mechanical ventilation, as compared to standard of care.<br>- To evaluate safety of CP as compared to standard of care. |
| **Exploratory Objective** | - To evaluate the virologic efficacy of CP as compared to the SOC.<br>- To evaluate the serologic conversion of COVID-19 patients.<br>- To evaluate COVID-19 antibody titers in donor CP and impact on outcomes.<br>- Impact of patient virologic state and seroconversion at the time of infusion on outcomes.<br>- To study neutralizing antibodies against COVID-19 in donor and patients and their impact with the efficacy of CP and clinical outcomes<br>- To evaluate feasibility of a model of CP timely procurement and supply in the Spanish National Health System. |
| **Primary Endpoint** | Proportion of patients in categories 5, 6 or 7 of the 7-point ordinal scale at day 15.<br>Ordinal scale:<br>1. Not hospitalized, no limitations on activities.<br>2. Not hospitalized, limitation on activities.<br>3. Hospitalized, not requiring supplemental oxygen.<br>4. Hospitalized, requiring supplemental oxygen.<br>5. Hospitalized, on non-invasive ventilation or high flow oxygen devices.<br>6. Hospitalized, on invasive mechanical ventilation or ECMO.<br>7. Death. |
| **Secondary Endpoints** | <u>Clinical Severity</u><br>- Ordinal scale (see 7-point ordinal scale above):<br>o Status at day 30<br>o Time to category 5, 6 or 7 of the ordinal scale.<br>o Time to an improvement of one category from admission on the ordinal scale.<br>o Time to first deterioration<br>o Mean change in the ranking on an ordinal scale from baseline to days 3, 5, 8, 11, 15, 29 and 60.<br>- Ordinal scale of 11 points:<br>o Mean change in the ranking on an ordinal scale from baseline to days 3, 5, 8, 11, 15, 29 and 60<br>o Status at day 15 and 30<br>o Ordinal scale of 11 points (Clinical progression scale) :<br>0. Uninfected ; no viral RNA detected.<br>1. Asymptomatic; viral RNA detected, limitation on activities.<br>2. Symptomatic; independent<br>3. Symptomatic; assistance needed<br>4. Hospitalized, no oxygen therapy.<br>5. Hospitalized, oxygen by mask or nasal prongs6. Oxygen by mask or nasal prongs<br>7. Intubation and mechanical ventilation, pO2/FiO2 ≥150 or SpO2/FiO2 ≥200<br>8. Mechanical ventilation pO2/FIO2 <150 (SpO2/FiO2 <200) or vasopressors<br>9. Mechanical ventilation pO2/FiO2 <150 and vasopressors, dialysis, or ECMO<br>10. Dead |

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| **Secondary Endpoints** | - Mortality at 15, 29 and 60 days.<br>- Oxygenation free days in the first 28 days (day 29).<br>- Ventilator free days in the first 28 days (day 29).<br>- Duration of hospitalization (days).<br>- Incidence of thrombotic arterial events<br>- Incidence of thrombotic venous events<br>- Rate of rehospitalizations.<br>- Serum level of CRP, lymphocyte count, LDH, D Dimer, IL-6, CPK, troponine, platelets, coagulation tests at baseline and days 3, 5, 8, 11, 15, 29 and 60.<br><br><u>Safety</u> through day 60<br>- Infusion-related adverse events<br>- Cumulative incidence of serious adverse events (SAEs)<br>- Cumulative incidence of Grade 3 and 4 adverse events (AEs).<br><br><u>Virology and Immunology endpoints</u><br>- Qualitative and quantitative PCR for SARS-CoV-2 in naso/oropharyngeal swabs at baseline and at discharge.<br>- Qualitative and quantitative PCR for SARS-CoV-2 in blood at baseline and on days 3, 5, 8, 11, 15, 29 and 60 (while hospitalization).<br>- Quantitative total antibodies at baseline and on days 8, 15, 29 and 60 (while hospitalization).<br>- Neutralizing titer in patients at baseline.<br>- Assessment of the neutralizing antibody activity against SARS-CoV-2 in the sera from donors using viral pseudotypes.<br>- Assessment of neutralizing antibodies against SARS-CoV-2 in the sera from donors using VMNT-ID50 |
| **Main Inclusion Criteria** | 1. Written informed consent prior to performing study procedures. Witnessed oral consent will be accepted in order to avoid paper handling. Written consent by patient or representatives will be obtained as soon as possible.<br>2. Male or female adult patient ≥18 years of age at time of enrolment.<br>3. Has laboratory-confirmed SARS-CoV-2 infection as determined by PCR test in naso/oropharyngeal swabs or any other relevant specimen obtained in the ongoing COVID-19 symptomatic period. Alternative tests (i.e rapid antigenic tests) are also acceptable as laboratory confirmation if their adequate specificity has been accepted by the sponsor<br>4. Patients requiring hospitalization for COVID-19 without mechanical ventilation (invasive or non-invasive) or high flow oxygen devices and at least one of the following:<br>▪ Radiographic evidence of pulmonary infiltrates by imaging (chest x-ray, CT scan, etc.), OR<br>▪ Clinical assessment (evidence of rales/crackles on exam) AND SpO2 ≤ 94% on room air that requires supplemental oxygen.<br>5. No more than 7 days between the onset of symptoms (fever or cough) and treatment administration day. |

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| **Main Exclusion Criteria** | 1. Requiring mechanical ventilation (invasive or non-invasive) or high flow oxygen devices at screening.<br>2. More than 7 days since symptoms (fever or cough).<br>3. Participation in any other clinical trial of an experimental treatment for COVID-19.<br>4. In the opinion of the clinical team, progression to death is imminent and inevitable within the next 24 hours, irrespective of the provision of treatments.<br>5. Any incompatibility or allergy to the administration of human plasma.<br>6. Stage 4 severe chronic kidney disease or requiring dialysis (i.e. eGFR <30). |
| **Investigational Product** | Convalescent plasma (CP) from patients recovered from COVID-19. |
| **Dose** | Patients will receive a single unit of plasma (250-300 mL; CP) after randomization on day 1. |
| **Control arm** | Standard of Care |
| **Convalescent Plasma Donor Selection** | - Convalescent donor selection and plasma production will be coordinated by the trial's WP #2 and Steering Committee. Logistic differences should adapt to the structure at a regional level:<br>o Convalescent COVID-19 donor identification and recruitment will be carried out in collaboration among *Servicios de Transfusión Hospitalarios, Centros de Transfusión, Departamentos de Salud Pública* and *Consejerías de Sanidad*.<br>o Donor assessment, plasma collection, inactivation and production will be carried out in collaboration between *Servicios de Transfusión Hospitalarios* and *Centros de Transfusión*.<br>A common protocol for CP production will be shared and followed by all participating centers. As a general guidance for this sinopsis, preliminary criteria for patients recovered from COVID-19 to be selected as CP donors would be:<br><br>- Inclusion criteria:<br>▪ Willing and able to provide written informed consent.<br>▪ Fulfilling all the current requirements to be a plasma apheresis donor according to our regulations for donation of blood products (European Guidelines and RD 1088/2005 in Spain).<br>▪ Absence of COVID-19 symptoms within the last 14 days.<br>▪ Anti SARS-CoV-2 antibodies detectable in peripheral blood.<br>≥18 years of age at time of donation.<br>▪ Weight >50 kg and good vein access are standard criteria, for which exceptions could be considered according to the criteria of the blood bank and hematologist.<br><br>- Exclusion criteria:<br>▪ Plasmapheresis in the previous seven days.<br>▪ Whole blood donation in the previous 30 days.<br>▪ Donation of more than 25 liters of plasma in the previous 12 months.<br>▪ With the intention to minimize the risk of TRALI (transfusion related acute lung injury), an additional exclusion criteria would be for previously transfused donors, and women currently or previously pregnant, including miscarriages. |

# 2 INTRODUCTION

## 2.1 Study Rationale

COVID-19 is a respiratory disease caused by a novel coronavirus (SARS-CoV-2) and causes substantial morbidity and mortality. There is currently no vaccine to prevent COVID-19 or infection with SARS-CoV-2 or therapeutic agent to treat COVID-19. This clinical trial is designed to evaluate passive immunotherapy with Convalescent plasma for the treatment of adult patients hospitalized with COVID-19.

## 2.2 Background

Convalescent plasma (CP) from infected patients who have developed an immune response is **likely to be an option for the treatment of patients with a variety of severe acute respiratory infections (SARI) of viral etiology**. This would include patients in the most recent epidemics with coronaviruses, SARS1 in 2003 and MERS in 2012, and **potentially as well patients in the current COVID-19 pandemic**. Despite suggesting safety and potential efficacy, the evidence available has a major limitation of being based on **predominantly low-quality uncontrolled studies**. Here we present a summary of the **rationale and justification to carry out a multicenter, adaptive, randomized clinical trial of CP therapy in COVID-19 hospitalized patients**.

**Passive Immunotherapy of viral SARI with CP:** Passive immunotherapy involves the administration of antibodies against a given agent to a susceptible individual for the purpose of preventing or treating an infectious disease caused by that agent. CP has been historically used in outbreaks of poliomyelitis, measles, mumps, influenza (1918 H1N1 and 2009-2010 H1N1), 2013 Ebola. In addition, although less readily available and requiring more complex manufacturing than CP, conventional and hyperimmune immunoglobulins are used in clinical practice in a number of infections such as respiratory syncytial virus, hepatitis B and others.

- Currently, the **only source of antibody available for immediate use** against SARS-CoV-2 is human CP.
- In addition, as more individuals contract COVID-19 and recover, the **number of potential donors will continue to increase** in all areas where COVID-19 epidemic is present.

**Use of CP against coronavirus diseases:** The experience with SARI caused by coronavirus is rather recent in a number of epidemics in the twenty-first century:

- Human CP was used in patients from both SARS-1 in 2003 and MERS in 2012. Overall, the experience showed that **CP is safe and likely to reduce mortality in patients with SARI by coronaviruses**. Of note, the largest study with 80 patients with SARS in Hong-Kong in 2003, and subsequent meta-analyses, showed that **earlier administration after symptom onset**

**is more effective**, in particular before day 14, before seroconversion in patients who remain PCR positive.

- The success of CP therapy may be limited by the fact that **not all patients who recover from viral disease develop neutralizing antibodies**. As many as 12% of patients with SARS do not develop neutralizing antibodies. Thus, they should be tested to select adequate donors.
- Finally, in the current SARS-CoV-2 epidemic, China has recently communicated in the media that **245 COVID-19 patients have received CP**, starting with the first patient on February 9th, and with preliminary reports of improvement in clinical indicators and symptoms [http://www.xinhuanet.com/english/2020-02/28/c_138828177.htm].

**Balancing benefit and potential risks of CP in COVID-19 patients:** The efficacy and safety of the therapeutic use of CP in COVID-19 patients **cannot be inferred without carrying out a controlled clinical trial**. In the current scenario of low-quality uncontrolled studies, treatment appears to be safe and well tolerated, and to associate with improved outcomes. However, a number of potential risks must be considered in the planning of this trial:

- With modern blood banking techniques, the **risks of transfusion would be low**.
- In patients with pulmonary disease as the ones we will include in this trial, a particular risk of the use of human plasma is the **development of TRALI** (transfusion-related acute lung injury), which should be considered in the criteria to select CP donors.
- The theoretical risk of **antibody-dependent enhancement of disease** (ADE), has been suggested in Asian COVID-19 patients who may have been exposed before to other coronaviruses. However, early use of CP and selection of donors with antibodies against SARS-CoV-2 make the risk of ADE very unlikely in our proposal. It will of course be analyzed, but anecdotal evidence from CP use in 245 patients with COVID-19 in China suggest it is safe. It has been speculated that appearance of virus-specific IgG, despite its likely contribution to virus clearance, could also be involved in the severe lung inflammatory damage seen in a minority of infected patients. In this situation, administration of convalescent plasma could be potentially harmful and potentiate ADE. This potential risk would be minimized selecting patients in whom seroconversion has not yet taken place and are not suffering from severe lung infiltration/inflammation. Of note, large anecdotal evidence from CP use in 245 patients with COVID-19 in China suggests that it is safe. However, this is in a non-controlled fashion and without a full scientific report as yet.

Hence, for the design of our trial, we **excluded critical patients with more advanced lung involvement** on mechanical ventilation, who are overall less likely to respond to passive immunotherapy and more likely to develop ADE, and **propose an earlier intervention with CP in the course of the disease** based on severity and time from admission to hospital and onset of symptoms.

In summary, the historical and current anecdotal data suggest that the use of **CP is safe in coronavirus infection**, and the high severity and mortality of COVID-19 suggests that the **benefits of its use in those with early disease clearly outweigh the risks**.

From this background and rationale, we aim to focus this study on **hospitalized patients still with an early form of COVID-19**, rather than as a last resort in patients with critical illness who require mechanical ventilation in whom it may be less effective and more prone to immune-mediated adverse-events. In the midst of a worldwide pandemic of SARS-CoV-2 and COVID-19, for which we currently have no available vaccines or drugs, CP represents a potential therapeutic option with a **favorable benefit/risk balance for these patients**. CP could be **rapidly available in any areas where there are sufficient numbers of patients** who have recovered. Its potential efficacy and safety should be tested in a randomized, controlled study, as recommended by the WHO R&D Blueprint and Master Protocol for COVID-19 Therapeutic Trials, from which we can obtain **strong evidence to inform decisions worldwide**.

## 3 OBJETIVES AND ENDPOINTS

The overall objective of the study is to evaluate the clinical efficacy and safety of Convalescent plasma versus SOC under a sequential design.

| **PRIMARY** | |
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| **OBJECTIVES** | **ENDPOINTS (OUTCOME MEASURES)** |
| **To evaluate the clinical efficacy of Convalescent plasma (CP) versus SOC, under a sequential design.**<br>• Proportion of patients in categories 5, 6 or 7 (of the 7-point ordinal scale) at day 15 | Ordinal scale:<br>1. Not hospitalized, no limitations on activities.<br>2. Not hospitalized, limitation on activities.<br>3. Hospitalized, not requiring supplemental oxygen.<br>4. Hospitalized, requiring supplemental oxygen.<br>5. Hospitalized, on non-invasive ventilation or high flow oxygen devices.<br>6. Hospitalized, on invasive mechanical ventilation or ECMO[2].<br>7. Death. |

[2] Extracorporeal membrane oxygenation

| SECONDARY | |
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| **OBJECTIVES** | **ENDPOINTS (OUTCOME MEASURES)** |
| **To evaluate effects of CP on secondary efficacy endpoints, including all-cause mortality and respiratory deterioration with progression to mechanical ventilation, as compared to standard of care as assessed by:**<br><br>**Clinical Severity**<br>• Ordinal scale:<br>o Status at day 29 and 60<br>o Time to get categories 5, 6 or 7 in the 7-points ordinal scale in the 29 days of follow up<br>o Time to an improvement of one category from admission using an ordinal scale.<br>o Time to first deterioration<br>o Mean change in the ranking on an ordinal scale from baseline to days 3, 5, 8, 11, 15, 29 and 60.<br>• Ordinal scale of 11 points:<br>o Status at days 15, 29 and 60<br>o Mean change in the ranking on an ordinal scale from baseline to days 3, 5, 8, 11, 15, 29 and 60.<br>• Incidence of thrombotic arterial events<br>• Incidence of thrombotic venous events | • Ordinal outcome assessed daily while hospitalized and on day 15 and 60. |
| • Oxygenation:<br>o Oxygenation free days in the first 28 days (to day 29).<br>o Mechanical Ventilation<br><br>• Ventilator free days in the first 28 days (to day 29).<br><br>• Hospitalization:<br>o Duration of hospitalization (days)<br>o Rate of rehospitalizations. | • Duration of supplemental oxygen (if applicable).<br><br>• Duration of mechanical ventilation (if applicable.<br><br>• Duration of hospitalization. |

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| • Mortality<br>o 15-day mortality<br>o 29-day mortality<br>o 60-day mortality | • Date and cause of death (if applicable). |
| Serum level of CRP, lymphocyte count, LDH, D Dimer, IL-6, CPK troponine, platelets, coagulation tests at baseline and days 3, 5, 8, 11, 15 and 29 | • White cell count, CRP, D-Dimer, LDH, ferritin, CPK troponine, platelets, and coagulation test on days 3, 5, 8, 11 (while hospitalized); and Day 15 and 29 (if still hospitalized). |
| **To evaluate safety of CP as compared to SOC**<br><br>Safety through day 60<br>• Cumulative incidence of serious adverse events (SAEs)<br>• Cumulative incidence of Grade 3 and 4 adverse events (AEs).<br>• Infusion-related adverse reactions | • Serious adverse events (SAEs).<br>• Grade 3 or 4 Adverse events (AEs).<br>• Infusion-related adverse reactions. |
| **EXPLORATORY** | |
| **OBJECTIVES** | **ENDPOINTS (OUTCOME MEASURES)** |
| • To evaluate the virologic efficacy of CP as compared to the SOC.<br>• To evaluate the serologic conversion of COVID-19 patients.<br>• To evaluate COVID-19 antibody titers in donor CP and impact on outcomes.<br>• Impact of patient virologic state and seroconversion on outcomes.<br>• To study neutralizing antibodies against COVID-19 in donor and patients and their impact with the efficacy of CP and clinical outcomes<br>• To evaluate feasibility of a model of CP timely procurement and supply in the Spanish National Health System. | • Qualitative and quantitative PCR for SARS-CoV-2 in NP/OP swabs at baseline and at discharge.<br>•Qualitative and quantitative PCR for SARS-CoV-2 in blood on Days 3, 5, 8, 11, 15, 29 and 60 (while hospitalized) until two of them are negative consecutively.<br>• Quantitative total antibody levels at baseline and at days 8, 15, 29 and 60 (while hospitalized).<br>• Quantitative total antibodies and neutralizing titer in patients at baseline.<br>• Assessment of the neutralizing antibody activity against SARS-CoV-2 in the sera from donors using viral pseudotypes.<br>• Assessment of neutralizing antibodies against SARS-CoV-2 in the sera from donors using VMNT-ID50 |

# 4 TRIAL DESIGN

## 4.1 Overall Design

This study is a multi-center, randomized, clinical trial with two arms to study the efficacy and safety of passive immunotherapy with CP compared to a control of standard of care (SOC) in hospitalized adult patients diagnosed with COVID-19.

The study is a multi-centre trial that will be conducted in up to 25 sites globally.

Randomization among the three arms will be 1:1

All trial participants will receive SOC:

- Treatment arm: Inactivated CP from patients recovered from COVID-19, whom, for the purpose of this trial, are herein designated as donors.
- Control arm: SOC for COVID-19.

Of note, in the current status of a worldwide pandemic for which we have no approved vaccines or drugs, for the purpose of this trial SOC would also accept any drugs that are being used in clinical practice (e.g.,remdesivir, corticosteroids, tocilizumab, etc.), other than those used as part of another clinical trial.

Randomization will be stratified by site.

Subjects will be assessed daily while hospitalized. Follow-up is for 60 days. Discharged patients before the end of follow up will be regularly phone called or asked to attend study visits. All subjects will undergo a series of efficacy, safety, and laboratory assessments. Naso/ oropharyngeal (NP/OP) swabs will be obtained on baseline and at discharge in all patients. Blood samples for PCR will be obtained on baseline and day 3, in all patients. While hospitalized, blood samples on days 3, 5, 8, 11, 15, 29 and 60 will be obtained as far as there is a positive result. If there are 2 consecutive negative PCR results, no more blood samples for PCR will be needed. Blood samples for serology will be obtained on baseline in all patients and, while hospitalized, on days 8, 15, 29 and 60.

A global independent data and safety monitoring board (DSMB) is proposed to monitor interim data to make recommendations about early study closure or changes to conduct.

Interim analyses for comprehensive efficacy and safety data monitoring analyses will be conducted when 20%, 40%, 60% and 80% of patients have been recruited, or at the discretionary DSMB criteria when needed. A DSMB charter will be set before the trial initiation.

The trial will be designed with intention to provide reliable evidence about the efficacy and safety of convalescent plasma therapy in addition to standard-of-care. The efficacy and safety of this experimental regimen will be assessed through comparison with the standard-of-care control arm.

### 4.2 Scientific Rationale for Study Design

This study utilizes a design that maximizes efficiency in identifying a safe and efficacious therapeutic agent for COVID-19 during the current outbreak by the implementation of a sequential design with 4 interim analyses (IA) for efficacy and futility, as well as a preplanned sample size re-estimation (see section 11 for more details). As the study will be a multicenter, randomized controlled study, it will be possible to acquire rigorous data about the safety and efficacy of investigational therapeutic agents for COVID-19 that will lead to generalizable evidence.

Randomization is essential for establishing efficacy of these new therapy. Also, collecting clinical and virologic data on enrolled patients using a standardized timeline and collection instruments provide valuable information about the clinical course of and morbidities associated with severe COVID-19 in a diverse group of hospitalized adult patients.

### 4.3 Study settings

The trial will be conducted in 32 clinical investigational sites (see Appendix 1) including additional centers from other regions in Spain with a high incidence of COVID-19.

Donor assessment, plasma collection, inactivation and production will be carried out by Servicios de Transfusión Hospitalarios and Centros Regionales de Transfusión and coordinated by the trial's WP #2 and Steering Committee (see Appendix 1 and 2 for details). Logistic differences should adapt to the structure at a regional level.

### 4.4 Duration of Study Participation

Duration of the recruitment: 4 months.

Duration of the follow-up and treatment for each patient: 60 days

Total study duration (enrolment + follow-up + analysis): 12 months.

## 5 STUDY POPULATION

### 5.1 Inclusion Criteria

To be eligible to enter the study candidates must satisfy all of the following criteria:

- Written informed consent prior to performing study procedures. Witnessed oral consent will be accepted in order to avoid paper handling. Written consent by patient or representatives will be obtained as soon as possible.
- Male or female adult patient ≥18 years of age at time of enrolment.
- Has laboratory-confirmed SARS-CoV-2 infection as determined by PCR test in naso/oropharyngeal swabs or any other relevant specimen obtained in the ongoing COVID-19 symptomatic period. Alternative tests (i.e rapid antigenic tests) are also acceptable as laboratory confirmation if their adequate specificity has been accepted by the sponsor.

- Patients requiring hospitalization for COVID-19 without mechanical ventilation (invasive or non-invasive) or high flow oxygen devices, and at least one of the following:
    - Radiographic evidence of pulmonary infiltrates by imaging (chest x-ray, CT scan, etc.), OR
    - Clinical assessment (evidence of rales/crackles on exam) AND SpO2 ≤ 94% on room air that requires supplemental oxygen.
- No more than 7 days between the onset of symptoms (fever or cough) and treatment administration day.

### 5.2 Exclusion Criteria

- Requiring mechanical ventilation (invasive or non-invasive) or high flow oxygen devices at screening.
- More than 7 days since symptoms (fever or cough).
- Participation in any other clinical trial of an experimental treatment for COVID-19.
- In the opinion of the clinical team, progression to death is imminent and inevitable within the next 24 hours, irrespective of the provision of treatments.
- Any incompatibility or allergy to the administration of human plasma.
- Stage 4 severe chronic kidney disease or requiring dialysis (i.e. eGFR <30).

Patients fulfilling all the inclusion criteria and not presenting any of the exclusion criteria at time of trial screening will be invited to participate in the trial.

## 6 STUDY PRODUCT

### 6.1 Investigational Therapeutic

Study Product Description

Convalescent donor selection and plasma production will be coordinated by the trial's WP #2 and Steering Committee. Logistic differences should adapt to the structure at a regional level:

- Convalescent COVID-19 donor identification and recruitment will be carried out in collaboration among *Servicios de Transfusión Hospitalarios, Centros de Transfusión, Departamentos de Salud Pública* and *Consejerías de Sanidad*.
- Donor assessment, plasma collection, inactivation and production will be carried out in collaboration between *Servicios de Transfusión Hospitalarios* and *Centros de Transfusión*.

A common protocol for CP production will be shared and followed by all participating centers.

Criteria for patients recovered from COVID-19 to be selected as CP donors would be:

- Inclusion criteria:

  - Willing and able to provide written informed consent.
  - Fulfilling all the current requirements to be a plasma apheresis donor according to our regulations for donation of blood products (European Guidelines and RD 1088/2005 in Spain).
  - Absence of COVID-19 symptoms within the last 14 days.
  - Anti SARS-CoV-2 antibodies detectable in peripheral blood.
  - ≥18 years of age at time of donation.
  - Weight >50 kg and good vein access are standard criteria, for which exceptions could be considered according to the criteria of the blood bank and hematologist.

- Exclusion criteria:

  - Plasmapheresis in the previous seven days.
  - Whole blood donation in the previous 30 days.
  - Donation of more than 25 liters of plasma in the previous 12 months.

With the intention to minimize the risk of TRALI (transfusion related acute lung injury), an additional relative exclusion criteria would be for previously transfused donors and women currently or previously pregnant, including miscarriages.

Dosing and Administration.

Patients in the treatment arm will receive a single unit of CP (250-300 mL) after randomization on day 1 under control by the haematologist of the hospital.

Route administration: Intravenous.

## 6.2 Preparation/Handling/Storage/Accountability

Standard procedures in place for healthy plasma donors in transfusion centers will be followed. In particular:

- Written informed consent
- The collection day, convalescent donors (CD) will be again screened as an altruistic plasmapheresis donors, performed a finger hemoglobin test, blood pressure and cardiac rate.
- A single identification number (SIN) according to ISBT128 codification will be assigned to each plasmapheresis donation.
- Convalescent donors will undergo an apheresis process using any apheresis machine validated to collect plasma , and a single use apheresis kit to obtain 600 ml of leuko-depleted plasma (< 1 x $10^6$ leukocytes per bag) according to local procedures.
- Convalescent donors may receive a 500 ml saline infusion through the inline apheresis vein access, after the plasma collection, according to local procedures (not mandatory).
- Plasma units can be kept at room temperature (20ºC), until 8h before the pathogen reduction process (inactivation). If not feasible, units must be frozen to perform a further pathogen reduction procedure.
- The 600 ml collection will be separated into two bags containing 300 ml, to be further pathogen reduced (inactivation), or whole inactivated, if the system allows to perform the pathogen reduction process with that volume
- If plasma has been previously frozen, it will be thawed, inactivated and refrozen before 2 hours.
- The inactivation process will be done using any pathogen reduction (inactivation) system validated for transfusional plasma, (i.e Methilene Blue, Amotosalen, Rivoflavin…). The procedure will be performed according to local procedures.
- All the plasma transference between different bags will be done using a closed system and preferably using a sterile tubing welder.

## 6.3 Formulation, Appearance, Packaging, and Labelling

- CP units will be preferably identified and labeled according to ISBT128 requirements.
- The following ISBT codes are recommended:

  - E9743 = Apheresis CONVALESCENT PLASMA|NS/XX/<=-25C|COVID-19
  - E9744 = Apheresis CONVALESCENT PLASMA|NS/XX/<=-25C|Methylene blue-treated|COVID-19
  - E9745 = Apheresis CONVALESCENT PLASMA|NS/XX/<=-25C|Psoralen-treated|COVID-19
  - E9746 = Apheresis CONVALESCENT PLASMA|NS/XX/<=-25C|Riboflavin-treated|COVID-19

- As the collected product will be usually spliced into two aliquots; the product will include a SIN subcode as 01 and 02.
- Pathogen reduced 300 ml bags will be labeled using the primary SIN number followed by a subset code (01 and 02).

### 6.4 Product Storage and Stability

- Inactivated plasma bags will be frozen below -25ºC, and stored and shipping following European guidelines for blood components storage. (36 months below -25ºC or 3 months below -18ºC). For transport, plasma bags must be kept frozen. No special containers are needed if plasma units are kept frozen at the delivery.
- All the procedure data will be recorded using the defined CRF for this trial. Also, the blood bank software in each hospital Transfusion Service will be used for recording the plasma units transfused.
- The plasmas will be labelled with a code and will be only used for this research project or other related COVID-research projects.

## 7 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

This open-label trial will use blind randomization of patients in a 1:1 ratio to CP or SOC through a centralized system embedded in the eCRF (Oracle Clinical). Randomization will be stratified by site and the randomization procedure will be described in a SOP.

### 7.1 Study Intervention Compliance

The study product will be administered by a member of the clinical research team. The single identification number of the plasma unit will be recorded in the patient clinical record and case report form (eCRF) as well as the time of administration and incidences.

### 7.2 Concomitant Therapy

All concomitant therapies will be permitted. No restriction is established regarding concomitant treatment.

Only clinical relevant concomitant treatment related to COVID-19 will be recorded for the purposes of the trial. The list of medications will be assessed only from 7 days prior to enrolment to end of study.

### 7.3 Rescue Medicine

Not Applicable

### 7.4 Non-Research Standard of Care

Not Applicable

# 8 STUDY INTERVENTION DISCONTINUATION AND SUBJECT DISCONTINUATION/WITHDRAWAL

## 8.1 Study Halting for Safety

There are no pre-specified stopping rules. Instead, there will be a close oversight by the protocol team and frequent DSMB reviews.

## 8.2 Withdrawal from Randomized Treatment or from the Study

Patients are free to withdraw from participation in the study at any time. Patients are 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. In the case of a patients becoming lost to follow-up, attempts to contact the patient be made and documented in the patient's medical records.

## 8.3 Discontinuation of Study Drug

Not Applicable.

## 8.4 Withdrawal of Patients from the Study

A patient may be removed from the study treatment for the following reasons; however, whenever possible the patient should be followed regardless of their protocol adherence as per the efficacy and safety evaluations:

- Patient withdraws consent or requests discontinuation from the study for any reason
- Termination of the study
- Lost to follow-up.

Patients, who withdraw from this study or are lost to follow-up after signing the informed consent form (ICF) and administration of the study product, will not be replaced. The reason for patient discontinuation from the study will be recorded on the appropriate case report form.

# 9 ASSESSMENTS AND PROCEDURES

## 9.1 Screening Procedures

After the informed consent, all the following assessments are performed to determine eligibility requirements as specified in the inclusion and exclusion criteria:

- Positive SARS-CoV-2 test result. Laboratory-confirmed SARS-CoV-2 infection as determined by PCR test in naso/oropharyngeal swabs or any other relevant. Both local (hospital) or central (ISCIII) laboratory results are acceptable for inclusion, although a basal sample for central laboratory will be always collected. Alternative tests (i.e rapid antigenic tests) are also acceptable as laboratory confirmation if their adequate specificity has been accepted by the sponsor.
- Focused medical history, including the following information:
    - Day of onset of COVID-19 symptoms (fever and/or other symptoms).
    - History of chronic medical conditions related to inclusion and exclusion criteria and known risk factors for COVID-19.
- Review recent radiographic imaging, maximum 7 days old (x-ray or CT scan).
- Confirm the period since hospitalization due to COVID.

The overall eligibility of the subject to participate in the study will be assessed once all screening values are available. The screening process can be suspended prior to complete assessment at any time if exclusions are identified by the study team. Study subjects who qualify will be immediately randomized and treatment should be administered the same day of randomization.

## 9.2 Visit 1 (Treatment)

This visit can be done at the same time as the screening visit.

**RANDOMIZATION**

Participants will be randomly assigned to:

- Treatment arm: Inactivated CP from patients recovered from COVID-19,

- Control arm: SOC for COVID-19.

The participants will be assigned using a central randomization service implemented in the eCRF. Randomization codes will be provided by the CRO and will be charged into the eCRF as to maintain the treatment concealment.

**PROCEDURES PRE-ADMINISTRATION**

The following assessments are performed before administration of therapy:

- Ordinal scale.
- Vital signs including SpO2 and Tª.
- Targeted physical exam focused on lung auscultation.

- Naso/Oropharyngeal swab.
- Blood for PCR SARS-CoV-2.
- Blood samples (ABO group, hematology, chemistry, CRP, LDH, ferritin, IL-6, coagulation, D-dimer, CPK, platelets and troponine).
- Blood for antibodies determination.
- Review medications and therapies related to COVID-19 (SOC).

### 9.3 Efficacy Assessments (follow-up visits)

At each study day while hospitalized, the following measure of clinical support should be assessed:

- Hospitalization.
- Oxygen requirement.
- Oxygen supply device: nasal device, mask with reservoir bag.
- Non-invasive mechanical ventilation (via mask) or high flow oxigen.
- Mechanical ventilator requirement (via endotracheal tube or tracheostomy tub).
- ECMO requirement.
- SpO2.
- TºC.
- Death.

### 9.4 Viral and Immunology Shedding

NP/OP swabs will be collected on baseline and at discharge.

Blood samples for PCR will be collected on baseline and day 3, in all patients. While hospitalization, PCR on days 5, 8, 11, 15, 29 and 60 will be also be performed, unless two consecutive PCRs are negative.

Bloods samples for serology will be collected on baseline and at discharge, in all patients. Additionally, while hospitalization, blood samples on days 8, 15, 29 and 60 will be collected.

### 9.5 Safety Assessments

During the entire follow-up, until the end of the study, the adverse events and complications.

- Physical examination: A symptom-directed (targeted) physical examination will be performed to evaluate for any possible adverse event. No physical exam is needed for routine visits.

- Clinical laboratory evaluations: Blood will be collected at the following time points: days 3, 5, 8, 11, 15, 29 and 60.

Clinical laboratory parameters include CRP, lymphocyte, neutrophils, LDH, D-Dimer, ferritin, IL-6, coagulation test, CPK, platelets and troponine.

This testing will be performed at each clinical trial site in real time.

## 9.6 Schedule of Procedures

**Table 1. Study timetable of ConPlas-19 clinical trial**

| **VISITS** | **Screening[1]** | **Baseline[1]** | **Follow-Up VISITs[4]** | **Day15[4]** | **Day29[4]** | **Day60[4] (End of study)** |
|---|---|---|---|---|---|---|
| **Day +/- Window** | **−3 to 1** | **1** | Daily until hospital discharge | **± 2** | **± 3** | **± 3** |
| **ASSESSMENTS/PROCEDURES** | | | | | | |
| Informed consent | X | | | | | |
| Inclusion and exclusion criteria | X | X | | | | |
| Demographics & Medical History | X | | | | | |
| SARS-CoV-2 PCR or antigen test | X[2] | | | | | |
| Rx Thorax | X[7] | | | | | |
| Randomization | | X | | | | |
| Administration Convalescent Plasma | | X | | | | |
| Clinical data collection | | X | Daily until hospital discharge | X | X | X |
| Vital signs: $SpO_2$ /Tª | X | X | Daily until hospital discharge | X | X | X |
| Oxygen requirement | | X | Daily until hospital discharge | X | X | |
| Mechanical ventilator requirement | | X | Daily until hospital discharge | X | X | |
| Mortality | | | Daily until hospital discharge | X | X | |
| Concomitant medication (Only related with COVID-19) | | X | Daily until hospital discharge | X | X | X |
| AE /SAE (eCRF reporting) | | Daily until hospital discharge | | X | X | X |
| Blood samples (ABO group) | | X | | | | |
| Routine blood samples (hematology and chemistry)[3] | | X | (3, 5, 8 and 11 days)[5] | X[5] | X[5] | X[5] |
| Blood for PCR SARS-CoV-2 [6] | | X | (3, 5, 8 and 11days)[5] | X[5] | X[5] | X[5] |
| Blood for antibodies determination | | X | (8, 15, 29 and 60 days)[5] | | | |
| Naso/Oropharyngeal swab | | X[2] | At discharge | | | |

1. This visit can be done at the same time as the screening visit
2. Positive PCR or COVID-19 antigen test (accepted by the sponsor), in naso/oropharyngeal swabs or any other relevant specimen is needed prior to randomization. If inclusion is based on a previous local swab, a new basal swab to be sent to CNM-ISCIII will be needed. If previous SARS-CoV-2 test + is not available and the swab is obtained as a screening procedure, the basal swab could be obtained at the same time, taking into account that the basal swab HAS TO BE SENT to the CNM-ISCIII central lab.
3. Haematology, chemistry, ferritin, CRP, LDH, IL-6, coagulation, D-dimer, CPK, platelets and troponine.
4. These visits may be conducted by phone. In this case, blood samples and vital signs will not be available.
5. Only during hospitalization.
6. If two consecutive negative results are obtained, no more PCR tests needed.
7. XR obtained during the ongoing COVID-19 symptomatic period (maximum 7 days old).

### 9.7 Rehospitalization Substudy

Some COVID-19 patients can experience disease recrudescence approximately 3-6 weeks after the initial onset of symptoms and clinical improvement, which consists in a clinical, analytical and / or radiological deterioration of variable severity.

This disease recrudescence could be related to different causes (e.g., viral reactivation, reinfection, nosocomial superinfection, inflammatory response, thromboembolic disease…), several of which could coexist in the same patient.

There is a clear need to better characterize these recrudescence events, as well as to characterize the impact that they might have on the patient's plasma immunity pattern.

Those patients that have been discharged from the hospital and who require a new hospital admission within 60 days from their initial COVID-19 hospitalization, will be included in the Con-Plas "Rehospitalization Substudy".

The objective of the Rehospitalization Substudy is to describe the clinical, analytical, radiological and microbiological features and frequency of late recrudescence in ConPlas COVID-19 patients, occuring no later than 6 weeks after the randomization in the main ConPlas-19 study.

See Appendix 3 for detailed information about the protocol and procedures of the Rehospitalization Substudy.

## 10 PHARMACOVIGILANCE/HEMOVIGILANCE

### 10.1 Definition of Adverse Event (AE)

AE means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention related. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational) product.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre- existing medical condition increases, it should be recorded as an AE.

Given the nature of severity of the underlying illness, subjects will have many symptoms and abnormalities in vitals and laboratory. Only Grade 3 and 4 AEs will be captured as AEs in this trial, according to the following classification:

- Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.
- Severe (Grade 4): Events that are potentially life threatening.

## 10.2 Definition of Serious Adverse Event (SAE)

An SAE is defined as "An AE or suspected adverse reaction is considered serious if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening AE,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- or a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

"Life-threatening" refers to an AE that at occurrence represents an immediate risk of death to a subject. An event that may cause death if it occurs in a more severe form is not considered life-threatening. Similarly, a hospital admission for an elective procedure is not considered a SAE.

All SAEs, will be assessed for severity and relationship to study intervention.

All SAEs will be recorded

## 10.3 Adverse events of Special Interest

Investigators will be instructed to actively monitor the occurrence of:

- TRALI, *Transfusion-related acute lung injury*
- ADE, A*ntibody-dependent enhancement of infection.*
- TACO, T*ransfusion-associated cardiac overload.*

### 10.4 Relationship to Study Intervention

For each reported adverse reaction, the PI or designee must assess the relationship of the event to the study product using the following guideline:

- Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate aetiology has been established.

### 10.5 Hemovigilance

The adverse events related to the CP will be evaluated by a trained Hemovigilance nurse or physician, who will document and record any adverse event that could be related to the CP transfusion along the 24h after the end of the research plasma transfusion. This is defined as an Active 24h quarantine Hemovigilance Program (HEMACUA program).

Adverse event reporting will record three single fields according to the hemovigilance rules:

1. Type of event (Events are defined to be concordant to the EU and SHOT definitions, (https://www.shotuk.org)
2. Severity
3. Imputability

## 11 STATISTICS AND DATA ANALYSIS

### 11.1 General Remarks

The statistical analysis will be carried out in accordance with the principles specified in the International Conference on Harmonization (ICH) Topic E9 (CPMP / ICH / 363/96)[3]. A detailed Statistical Analysis Plan (SAP) agreed upon by the CT Executive Board and the Project Statistician will be available early during the recruitment phase. This SAP will follow the general regulatory recommendations given in the ICHE9 guidance, as well as other specific guidance on methodological and statistical issues[4]. Also, it will stick to the recommendations given by the consensus documents of the scientific journals[5,6] to improve reliability and value of medical

3 CPMP/ICH/363/96. ICH E9 Statistical Principles for Clinical Trials. URL: http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500002928, last access: 20-Mar-2020.

4 EMEA Scientific Guidelines for Human Medicinal Products, Clinical Efficacy and Safety Guidelines, General Guidelines. URL: https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/clinical-efficacy-safety/biostatistics , last access: 20-Mar-2020.

5 Schulz KF, Altman GD, Moher D for the CONSORT Group*. CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomized Trials. Ann Intern Med. 2010;152:726-732.

6 EQUATOR-network (Enhancing the Quality and Transparency of Health Research). URL: http://www.equator-network.org/resource-centre/library-of-health-research-reporting/, last access 20-Mar-2020

research literature by promoting transparent and accurate reporting of clinical research studies.

The SAS System[7] (Release 9.4, or an upgraded version), or equivalent validated statistical software, will be the statistical software used to analyse the data sets.

A summary of the overall approach to statistical analysis is presented hereafter.

### 11.2 Sample size calculation

There are uncertainties on the expected rate of study endpoint. As per the end point of clinical improvement recommended by the WHO R&D Blueprint expert group[8], in a recent negative trial[9] the rates for the standard of care were:

| | Description | Rate in SOC % |
|---|---|---|
| 1 | Not hospitalized, no limitations on activities | 0 |
| 2 | Not hospitalized, limitation on activities | 28 |
| 3 | Hospitalized, not requiring supplemental oxygen | 24 |
| 4 | Hospitalized, requiring supplemental oxygen | 20 |
| 5 | Hospitalized, on non-invasive ventilation or high flow oxygen devices | 6 |
| 6 | Hospitalized, on invasive mechanical ventilation or ECMO | 5 |
| 7 | Death | 17 |

The final rates for a standard of care group in our setting are expected to be inferior to those observed in that study, with internal data suggesting figures ≈20% when grouping categories from 4 to 7. Given the high uncertainties two strategies will be put in place in this study: (a) a series of futility and efficacy interim analyses and (b) a sample size re-estimation when 60% of endpoints are assessed.

Therefore, we propose an open-labelled, standard of care controlled, randomised with a 1:1 ratio, clinical trial with stopping boundaries for efficacy and futility at 20%, 40%, 60 and 80% of the final sample size. The statistical design including the sample size and stopping have been calculated using the East validated software v6.5 by Cytel Inc.[10]. The stopping boundaries for

7 SAS version 9.4 software, SAS Institute Inc., Cary, NC, URL: http://www.sas.com/, last access: 20-Mar-2020

8 Coronavirus disease (Covid-19) R&D. Geneva: World Health Organization (http://www .who .int/ blueprint/ priority -diseases/key -action/novel -coronavirus/en/ ).

9 Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Li H, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Wang J, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020 Mar 18. doi: 10.1056/NEJMoa2001282. Epub ahead of print. PMID: 32187464.

10 East 6.4 (2016). Statistical software for the design, simulation and monitoring clinical trials. Cytel Inc., Cambridge MA.

superiority and inferiority have been calculated using the Rho family Spending functions with (□=7) and shown below:

| Look # | Info. Fraction | p-values | |
|---|---|---|---|
| | | Efficacy Boundary | Futility Boundary |
| 1 | 20% | 0.0000003 | 0.9995467 |
| 2 | 40% | 0.0000413 | 0.9480048 |
| 3 | 60% | 0.0006785 | 0.6436939 |
| 4 | 80% | 0.0051563 | 0.2154427 |
| 5 | 100% | 0.0243397 | 0.0243397 |

With this design, 278 patients (139 per arm) will be required assuming 20% rate in the control group and an absolute reduction of 10% (10% rate in experimental group), with 80% statistical power a 2.5% one-sided alpha level (5% two-sided).

With regards to the final sample size, it is predefined that a sample size recalculation will be put in place when 60% of the patients with assessed events are available and the 3rd analysis are conducted. Please refer to section on multiplicity adjustments with regards to the type-I error control due to this potential adaptation. The detailed methodology will be described in the SAP. No withdrawal rates are considered to compensate the sample size since all patients will count in the analysis conducted under the intention to treat principle.

### 11.3 Data Review (DR)

The Data Review (DR) will be performed before lock of database. Data will be examined for compliance with the trial protocol by the monitor and the data manager. Deviations will be sent to the project statistician to plan listings for the Data Review (DR). The objective is to carry out the population selection and definition of the final study populations as well as a preliminary assessment of the quality of the trial data.

### 11.4 Analysis populations

There will the following analysis populations for this study:

1) Full Analysis Set (FAS): All patients who are randomized into the study will be included in the FAS population.

2) Per Protocol Population: Per protocol (PP) patient sets will be defined as those patients included in the FAS set without major protocol deviations that might impact the study's main assessments. These deviations will be assessed during the data review prior to database lock.

3) The Safety population is defined as all randomized participants who received the investigational product.

The precise reasons for excluding participants from each population will be fully defined and documented independently of the randomization codes during the data blinder review and before data lock.

### 11.5 Randomisation Procedure

Randomisation codes were produced by means of the PROC PLAN of the SAS system, with a 1:1 ratio of assignment between both arms, stratifying by centre, blocks multiple of 2 elements. The randomisation schedule will be released to the manufacturer site, will be managed from the eCRF in a concealed manner.

### 11.6 Descriptive analysis

A descriptive analysis will be performed for all parameters overall and by arm at every study time-point. Categorical parameters will be presented by means of frequencies and percentages. Continuous parameters will be summarized by means of the appropriate descriptive statistics (mean ± standard deviation or median and interquartile range).

The efficacy and safety endpoints will be descriptively compared between study arms. Changes from baseline, when applicable, will also be summarized by study arm.

### 11.7 Inferential analysis

No inferential analysis will be performed for the baseline comparability. The inferential analyses will be limited to the efficacy variables, and the adverse events.

### 11.8 Primary endpoint

The proportion of patients with failure, defined as categories 5, 6 or 7 of the 7-point ordinal scale at day 15, will be estimated using a log-binomial regression model including stratification variables. In the unexpected event that the model does not fit, the Poisson regression model with long-link and robust variance estimator will be used instead[11,12,13,14,15].

11 Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and differences. Am J Epidemiol. 2005 Aug 1;162(3):199-200. Epub 2005 Jun 29.

12 Wacholder S. Binomial regression in GLIM: estimating risk ratios and risk differences. Am J Epidemiol 1986;123: 174-84.

13 Greenland S. Model-based estimation of relative risks and other epidemiologic measures in studies of common outcomes and in case-control studies. Am J Epidemiol 2004; 160:301-5.

14 Huber PJ. The behavior of maximum likelihood estimates under non-standard conditions. In: Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability Vol 1. Berkeley, CA: University of California Press, 1967:221-33.

15 Zou G. A modified Poisson regression approach to prospective studies with binary data. Am J Epidemiol 2004;159:702-6.

### 11.8.1 Secondary endpoints and safety outcomes

**Binary outcomes**

Binary efficacy and safety outcomes will be analysed as described for the primary endpoint.

**Shift outcomes**

The shift analysis of OMS proposed ordinal scale endpoint will be analysed using the proportional odds model[16] and the stratification variables. The common odds ratio can also be interpreted as the average shift over the total ordinal outcome scale caused by the treatment under study[17,18,19]. The stratified non-parametric van Elteren test[20], using modified ridit scores which is as a direct extension of the extension of the Wilcoxon's rank-sum test for 2-samples, will be calculated as a sensitivity analysis to compare the scale as an ordinal rather than a binary outcome, without assuming proportional odds[21,22]

The median of the absolute values the 95% confidence interval (95%CI) will calculated using the Hodges-Lehmann methods (i.e. median of all cross differences between treatments based on the Mann-Whitney distribution)[23,24].

**Continuous outcomes**

Continuous variables will be analysed using Mixed Models[25], including in the model the baseline measurement, the stratification variables, treatment as well as the interaction between treatment and time, declaring time as categorical. The variance-covariance matrix will be fixed initially as unstructured. If this analysis fails to converge, the following structures will be tested in the following order until convergence: AR(1) (Auto- Regressive first order), Toeplitz and CS (Compound Symmetry). Contrasts between study groups will be performed by time-point. The treatment effect will be estimated through adjusted means –Least Square Means (LSMeans) – its standard error – Standard Error of Mean (SEM)- and its 95%CI. Differences between treatments will be estimated through the differences between LSMeans, SEM and 95%CI.

16 Agresti, A. (2002) Categorical Data Analysis, Second Edition. Hoboken, New Jersey: John Wiley & Sons, Inc.

17 McHugh GS, Butcher I, Steyerberg EW, Marmarou A, Lu J, Lingsma HF, Weir J, Maas AI, Murray GD.A simulation study evaluating approaches to the analysis of ordinal outcome data in randomized controlled trials in traumatic brain injury: results from the IMPACT Project. Clin Trials. 2010 Feb;7(1):44-57. doi: 10.1177/1740774509356580.

18 Saver JL: Novel end point analytic techniques and interpreting shifts across the entire range of outcome scales in acute stroke trials. Stroke 2007, 38:3055-3062.

19 Valenta Z, Pitha J, Poledne R: Proportional odds logistic regression– effective means of dealing with limited uncertainty in dichotomizing clinical outcomes. Stat Med 2006, 25:4227-4234.

20 van Elteren PH. On the combination of independent two-sample tests of Wilcoxon. Bulletin of the International Statistical Institute, 1960;37:351-361.

21 Stokes ME, Davis CS, Koch GG. Categorical data analysis using the SAS system. 2nd ed. Cary, NC: SAS Institute, 2000.

22 Koch GG, Edwards S. Clinical efficacy trials with ordinal data. In: Peace KK, ed. Biopharmaceutical statistics for drug development. New York: Marcel Dekker, 1988:403-57.

23 Hollander M, Wolfe DA. Nonparametric statistical methods. New York: Wiley, 1973

24 Hodges JL, Lehmann EL. Estimates of location based on rank tests. The Annals of Mathematical Statistics 1963; 34:598-611

25 Verbeke G, Molenberghs G. Linear Mixed Models for longitudinal Data. 1st Ed., New York: Springer-Verlag; 2000.

**Survival endpoints**

The survival function as well as the median [95% confidence interval -95%CI-] time to event will be estimated by means of the Kaplan-Meier method. Group comparisons will be done using the (stratified) log-rank test and the (stratified) hazard ratios -HR- (95%CI) were taken from the Cox model.[26]

### 11.8.2 General strategy for the rest of variables

The rest of variables will be analysed according to the following strategy: The Fisher's exact test used for categorical variables, the t-test for Gaussian distributed variables and, for non-Gaussian continuous or ordinal variables, non-parametric methods (Mann-Whitney test).

## 11.9 Multiplicity adjustments and interim analysis

The analysis will follow the principles specified in the ICHE9[27], the regulatory guidelines on multiplicity issues [28,29].

Please refer to the sample size calculation section for the interim analysis boundaries. To maintain the overall type I error in the context of a sample size adjustment, the method of Cui, Hung and Wang (CHW, 1999[30]) will be used.

## 11.10 Study Estimand and Handling of Missing Data

The handling of missing data will follow the principles specified in the ICH-E9 and the CPMP/EWP/1776/99 Rev1. Guideline on Missing Data in confirmatory trials Guidelines[31].

As per the ICH E9(R1) (*Addendum on estimands and sensitivity analysis in clinical trials EMA/CHMP/ICH/436221/2017*)[32], the plan for the assessment of the Primary endpoint (PEP) will be fully described in the SAP. In principle, the rate of missing data is estimated to be very low due to the type of endpoint, easily available with a fast-clinical assessment, so no impact is expected in the primary analysis. In any case, a very conservative strategy will be implemented consisting of imputing any missing data or other binary efficacy secondary outcomes will be

26 Therneau T, Grambsch P. Modeling Survival Data: Extending the Cox Model (Statistics for Biology and Health). Springer-Verlag New York Inc.; Edición: 1st ed. 2000.

27 CPMP/ICH/363/96. ICH E9 Statistical Principles for Clinical Trials. URL: http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500002928, last access: 20-Mar-2020.

28 CPMP/EWP/908/99. Points to Consider on Multiplicity issues in Clinical Trials. URL: https://www.ema.europa.eu/documents/scientific-guideline/draft-guideline-multiplicity-issues-clinical-trials_en.pdf , last access: 20-Mar-2020.

29 EMA/CHMP/44762/2017. Draft Guideline on multiplicity issues in clinical trials. URL: https://www.ema.europa.eu/documents/scientific-guideline/draft-guideline-multiplicity-issues-clinical-trials_en.pdf, last access: 20-Mar-2020.

30 Cui L, Hung HM, Wang SJ. Modification of sample size in group sequential clinical trials. Biometrics 1999; 55:853--857

31 CPMP/EWP/1776/99 Rev1. Guideline on Missing Data in confirmatory trials. URL: https://www.ema.europa.eu/documents/scientific-guideline/guideline-missing-data-confirmatory-clinical-trials_en.pdf, last access: 20-Mar-2020.

32 EMA/CHMP/ICH/436221/2017. ICH E9(R1) (Addendum on estimands and sensitivity analysis in clinical trials. URL: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e9-r1-addendum-estimands-sensitivity-analysis-clinical-trials-guideline-statistical-principles_en.pdf, last Access 20-03-2020

considered to failures, irrespectively to the reason for missingness. With regards to the continuous variables, mixed models[33,34,35] are robust to the presence of missing at random (MAR) and conducts the analysis with all participants despite the presence of missingness. Of note, this method calculates the estimations based on the variance- covariance structure but without any formal imputations.

No formal imputations will be performed for the rest of variables and the analyses will be based on the Available Data Only (ADO) approach.

### 11.11 Subgroup analysis

Subgroup analyses will be performed to assess the impact on efficacy of two different key factors: (a) the level of neutralizing antibodies in the administered plasma and (b) timing of the disease i.e. early or late stages . In case of any post-hoc subgroup analysis, they will be justified and identified as data-driven and, they will follow the principles and regulatory recommendations[36].

The following strategy will be conducted before splitting the analysis into subgroups:

1. Test of the overall treatment effect
2. Test of the treatment-by-subgroup interaction at the 10% level of significance
3. Test of the treatment effect in each subgroup category

If the three criteria are met, then the subgroup analysis will be given the maximal level of evidence for this analysis. However, this subgroup analysis is predefined as exploratory and the interpretation should be taken with caution. If any of the criterion are not meet, the chances of type I error increase are higher and this will have an impact in the interpretation.

## 12 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

### 12.1 Regulatory, Ethical, and Study Oversight Considerations

This study will be conducted in conformity with the principles set forth in the declaration of Helsinki.

A Research Ethics Committee (REC) will review and approve this protocol, associated informed consent documents, prior to the recruitment, screening, and enrolment of subjects.

Any amendments to the protocol or consent materials will be approved by the REC before they are implemented.

33 Verbeke G, Molenberghs G. Linear Mixed Models for longitudinal Data. New York: Springer- Verlag, 2000

34 Brown H, Prescott R. Applied Mixed Models in Medicine. New York: J. Wiley & Sons, 1999.

35 Molenberghs G, Kenward MG. Missing data in clinical Studies. Chichester, West Susex: John Wiley & Sons, Ltd., 2007

36 EMA/CHMP/EWP/117211/10. Guideline on the investigation of subgroups in confirmatory clinical trials (Draft). URL: https://www.ema.europa.eu/documents/scientific-guideline/guideline-investigation-subgroups-confirmatory-clinical-trials_en.pdf, last access: 20-Mar-2020.

### 12.2 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Investigators will obtain the subject's informed consent in accordance with the Ley 14/2007 de Investigación Biomédica and the internationally accepted guidances.

Subjects will receive a concise and focused presentation of key information about the clinical trial, orally. Due to paper handling limitation in COVID wards, oral witnessed consent will be accepted before entering into the trial. Written consent form will be obtained from the patient himself or acceptable representatives as soon as possible. The key information about the study will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

### 12.3 Study Termination and Closure

This study may be prematurely terminated if there is sufficient reasonable cause, including but not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Results of interim analysis
- Insufficient compliance to protocol requirements
- Data that is not sufficiently complete and/or not evaluable
- Regulatory authorities

If the study is prematurely terminated, the site PI will inform study subjects and the REC as applicable.

The sponsor will notify regulatory authorities as applicable.

### 12.4 Confidentiality and Privacy

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover clinical information relating to subjects, test results of biological samples and all other information generated during participation in the study. No identifiable information concerning subjects in the study will be released to any unauthorized third party. Subject confidentiality will be maintained when study results are published or discussed in conferences.

The study monitor, other authorized representatives of the sponsor, representatives of the REC, and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the subjects in this study. The clinical study site will permit access to such records.

All source records including electronic data will be stored in secured systems.

All study data and research specimens that leave the site (including any electronic transmission of data) will be identified only by a coded number that is linked to a subject through a code key maintained at the clinical site.

## 12.5 Safety Oversight

Protocol team oversight

The protocol team will review pools of AE data daily to ensure there no significant number of unexpected AEs (AEs that do not fit with the known course of COVID-19). If there are a significant number of unexpected AEs, the DSMB will be asked to review safety data in an ad hoc meeting.

Data Safety Monitoring Committee

Safety oversight will be conducted by a DSMB that is an independent group of experts that monitors subject safety and advises the sponsor. The DSMB members will be independent of study personnel participating in this trial and should not have scientific, financial or other conflict of interest related to this trial.

The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial. The DSMB should be as broadly informed as possible regarding emerging evidence from related studies as well as from the conduct of this trial. The DSMB will operate under the guidelines of a charter that will be written at the organizational meeting of the DSMB.

The DSMB will conduct the following reviews:

- After first 20 subjects are included.
- Ad hoc meeting if any specific safety concern arises.
- A final review meeting after final clinical database lock, to review the cumulative unblinded safety data for this trial.

The study will not stop enrolment awaiting these DSMB reviews, though the DSMB may recommend temporary or permanent cessation of enrolment based on their safety reviews.

Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by the sponsor. The DSMB may receive data in aggregate and presented by treatment arm. The DSMB may also be provided with expected and observed rates of the expected AEs in an unblinded fashion and may request the treatment assignment be unblinded for an individual subject if required for safety assessment. The DSMB will review grouped and unblinded data in the closed session only. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study interventions (as applicable), and to continue, modify, or terminate this trial.

## 12.6 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial subjects are protected, that the reported trial data are accurate, complete, and verifiable. Clinical Monitoring also ensures conduct of the trial is in compliance with the currently approved protocol/ amendment(s), ICH, GCP, and with applicable regulatory requirement(s) and sponsor requirements.

Monitoring for this study will be performed by the sponsor. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, CRFs, ICFs, medical and laboratory reports, site study intervention storage records, training records, and protocol and GCP compliance.

The monitoring visits can be made online.

### 12.7 Publication

Following completion of the study, the results will be published in a scientific journal. Nevertheless, due to the critical need of results during the current epidemic COVID-19 crisis, preliminary results will be released by the sponsor to the Health Authorities.

## 13 APPENDICES

Appendix 1. Participating Investigation sites

Appendix 2. Regional Transfusion Centers

Appendix 3. Rehospitalization Substudy protocol

## Appendix 1. Participating Investigation sites (v30_09_2020)

| SITE # | PRINCIPAL INVESTIGATORS (Co-IP) | CLINICAL SITE | CITY | CONTACT |
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| 3 | Dr. Javier Martínez Sanz | H. U. Ramón y Cajal | Madrid | javier.jmas@gmail.com |
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| | Dra. Gabriela Ene | | | gsene@bst.cat |
| 11 | Dra. Dolores Colunga | H. U. de Asturias | Oviedo | lolacolunga@yahoo.es |
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| 12 | Dra. María del Carmen Fariñas | H. U. Marqués de Valdecilla | Santander | mcarmen.farinas@scsalud.es |
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| 13 | Dr. Carlos Jesús Dueñas Gutierrez | H. Clínico U. de Valladolid | Valladolid | jduenas@saludcastillayleon.es |
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| 28 | Dr. Antoni Castro | H. Dr Josep Trueta | Girona | acastro.girona.ics@gencat.cat |
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## Appendix 2. Regional Transfusion Centers (v30_09_2020)

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| PRINCIPAL INVESTIGATORS | Regional Transfusion Centers | CITY | CONTACT |
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**Appendix 3. Rehospitalization Substudy Protocol**

**(Investigador responsable: Dr. José Ramón Paño Pardo)**

## 1. Objetivos

### 1.1. Objetivo general

Describir las frecuencias y características clínicas, analíticas, radiológicas y microbiológicas de las recrudescencias tardías en pacientes con Covid-19.

### 1.2. Objetivos específicos

- Identificar la frecuencia de enfermedad tromboembólica venosa (ETEV) en las recrudescencias tardías de Covid-19
- Cuantificar y caracterizar las infecciones debidas a otros virus respiratorios y las sobreinfecciones bacterianas o fúngicas asociadas en pacientes Covid-19 con recrudescencia tardía.
- Identificar la presencia de SARS-CoV-2 y confirmar su viabilidad en pacientes Covid-19 con recrudescencia tardía.
- Describir la situación inmunológica en pacientes con Covid-19 y recrudescencia tardía.

## 2. Métodos

### 2.1. Diseño

Aplicación de un protocolo diagnóstico en recrudescencias de Covid-19 que requieren ingreso, en una cohorte prospectiva de pacientes con infección por SARS-CoV-2.

### 2.2. Definiciones

- **Reactivación viral**: Cuadro clínico causado por la reactivación de SARS-CoV-2 en el contexto de una infección no curada.
- **Reinfección:** Cuadro clínico causado por una nueva infección por SARS-CoV-2.

### 2.3. Criterios de inclusión

- Edad ≥18 años
- Infección por SARS-CoV-2 confirmada por PCR
- Pacientes Incluidos en el ensayo ConPlas-19, que hayan sido dados de alta hospitalaria
- Precisar un nuevo ingreso entre el alta hospitalaria y las 6 semanas tras la aleatorización en el estudio

- Presentar alguna de las siguientes características:
  1) **Síndrome pseudogripal de ≥24 horas de evolución (2 o más síntomas**): Fiebre, mialgias, cefalea, diarrea, astenia
  2) **Deterioro** respiratorio (2 o más de los siguientes): Aumento de la disnea basal, de la tos, de los requerimientos de O2 o dolor torácico
  3) **Manifestación focal grave de potencial origen infeccioso:** meningitis, encefalitis, mielitis, mielo-meningo-encefalitis, polineuropatía, miocarditis o artritis.

## 3. Protocolo diagnóstico

A los participantes se les aplicará un protocolo diagnóstico para el despistaje de las principales etiologías potencialmente implicadas. **(Anexo I).**

Se registrarán en el CRDe las variables basales, del episodio inicial y del episodio de recrudesencia descritas en el **Anexo II**

### Categorización de los componentes de la recrudescencia

Cada uno de los componentes será categorizado de la siguiente manera:

| Componente | 0 | + | ++ | +++ | ++++ |
|---|---|---|---|---|---|
| **Replicación viral** | Ninguna evidencia | • PCR + en frotis nasofaríngeo<br>• Serología - | PCR + para SARS-CoV-2 en sangre | Cualquier cultivo + para SARS-CoV-2 | Evidencia de efecto citopático en Anatomía Patológica |
| **Inflamación** | • PCR < 10mg/L<br>• Ferritina <300<br>• IL-6 <5 | • PCR 10-50 mg/L<br>• Ferritina 300-500<br>• IL-6: 6-10 | • PCR 51-100 mg/L<br>• Ferritina 501-1000<br>• IL-6:11-40 | • PCR 101-200 mg/L<br>• Ferritina 1001-3000<br>• IL-6:41-100 | • PCR >200 mg/L<br>• Ferritina >3000<br>• IL-6: > 100<br>• Miocarditis |
| **Daño tisular** | • LDH <250 | • LDH 251-350 | • LDH 351-500 | • LDH 501-700 | • LDH > 700 |
| **Infección nosocomial / oportunista** | Ninguna evidencia | • Cultivo de un patógeno bacteriano fúngico sitio no estéril + | | • PCR CMV +<br>• PCR virus respiratorios +<br>• Galactomanano + | • Hemocultivo o cultivo de sitio estéril +<br>• PCR + *M. tuberculosis* o *P. jirovecii* en muestra respiratoria baja |
| **Trombosis** | • DD < 500 | • DD 500-3000 | DD > 3000 | • TEP segmentario o subsegmentario<br>• TVP distal<br>• AIT<br>• SCASEST | • TEP lobar o central<br>• Ictus establecido<br>• SCACEST |

**Tabla 1. Componentes de la recrudescencia**

## Anexo I. Protocolo diagnóstico

El abordaje diagnóstico de los pacientes con deterioro clínico tardío debe adaptarse a la gravedad del paciente y a la existencia de una sospecha diagnóstica alternativa.

- **Evaluación clínica**: historia epidemiológica, focalidad y determinación de gravedad clínica
- **Evaluación analítica**:
  - Hemograma
  - Coagulación básica con DD
  - Bioquímica básica con perfil hepático, CPK, troponina, LDH, ferritina y PCR
  - Inmunología: inmunoglobulinas, poblaciones linfocitarias, IL-6
- **Evaluación radiológica**:
  - Rx simple de tórax
  - Otras pruebas radiológicas según sospecha
- **Evaluación microbiológica**:
  - PCR de SARS-CoV-2 y de múltiples virus respiratorios en frotis nasofaríngeo
  - Aislamiento en cultivos celulares de SARS CoV2 en condiciones BSL3 (y cultivo)
  - PCR de CMV, VEB y SARS-CoV-2 en sangre
  - Serología específica para detección de anticuerpos anti SARS CoV2
  - Cultivos y resto de pruebas microbiológicas en función de la sospecha y focalidad

Los criterios orientativos de gravedad:

| | | Leve | Moderado | Grave |
|---|---|---|---|---|
| **Frecuencia respiratoria en reposo** | | <18 | 18-24 | >24 |
| **SatO2 (aire ambiente)** | **Pacientes SIN comorbilidad cardio-respiratoria** | ≥96% | 93-95% | <93% |
| | **Pacientes CON comorbilidad cardio-respiratoria** | Similar a la basal | Inferior a la basal o < 90% | |
| **Rx tórax** | | Sin cambios o mejoría respecto a la última disponible | Progresión radiológica respecto a la última prueba disponible (misma técnica) | |
| **LDH** | | < 300 | 300-500 | >500 |
| **Otros biomarcadores:**<br>• **PCR**<br>• **CPK Rangos generales**<br>• **Troponina (cualitativa)**<br>• **Ferritina**<br>• **DD**<br>• **IL-6** | | Normales o inferiores respecto a la última determinación disponible | **Aumento <50%** respecto a la última determinación disponible | **Aumento >50%** respecto a la última determinación disponible |

**Tabla 2. Criterios orientativos de gravedad**

A considerar en la evaluación en pacientes con gravedad moderada / grave:

- ✓ **Evaluación de la situación del parénquima pulmonar** con **TC tórax**
- ✓ **Despistaje de ETEV según protocolo local del centro**
- ✓ **Broncoscopia con BAS/BAL**: Valorar de forma individualizada la realización de broncoscopia para BAS y BAL (Cutivo de bacterias, PCR SARS-CoV-2, PCR multiplex virus respiratorios, PCR CMV, PCR *P. jirovecii,* galactomanano, PCR de *M. tuberculosis* y cultivo de micobacterias)

Si la PCR SARS-CoV-2 es positiva (una muestra por paciente) se procesará para:

- ✓ Aislamiento de SARS CoV2 en Cultivo celular de SARS-CoV-2
- ✓ Secuenciación masiva mediante NGS de los virus detectados en las muestras clínicas directamente

## Anexo II. Variables

- **Visita basal del episodio de recrudescencia**
  - Fenotipo clínico: Síndrome pseudogripal, Gastrointestinal, Neumonía, SDRA Neurológico, Miocarditis, Otros
  - Ingreso en UCI: Sí / No
  - Fecha de ingreso en UCI
  - Fecha de alta en UCI
  - Fecha de inicio de los síntomas
  - Fecha de ingreso
  - Registro de síntomas y datos de la exploración
  - Fenotipo clínico
  - Rx tórax al ingreso
    - Infiltrado S/N.
    - Tipo infiltrado
    - Lateralidad (izquierdo / derecho / bilateral)
    - Progresión (sin cambios / progresión / mejoría)
  - Datos analíticos.
  - Muestras enviadas al CNM
  - Muestra otro estudio enviadas por fuera de GIPI(no en ConPlas)
  - Gravedad al ingreso (ver Tabla 1
  - Gravedad escala OMS al ingreso

- **Visita de alta del episodio de recrudescencia**
  - Fecha de alta hospitalaria
  - Motivo de alta hospitalaria
  - Gravedad escala OMS máxima
  - Fecha de gravedad máxima
  - Gravedad escala OMS al alta
  - Ingreso en UCI: Sí / No. Fecha ingreso/alta
    - Fecha de ingreso en UCI
    - IOT: Sí / No. Si Sí, indicar: Fecha de IOT/fecha extubación
  - ETEV documentada en este episodio
  - Coinfección / sobreinfección: Sí/No. Indicar: Síndrome y Microorganismo
  - Componentes de la recrudescencia
  - Priorización de los componentes de la recrudescencia
  - Tratamiento recibido

- **Resultados microbiológicos ISCIII**

## 14 REFERENCES


1. Arabi YM, et al. Feasibility of using convalescent plasma immunotherapy for MERS-CoV infection, Saudi Arabia. Emerging Infect Dis. 2016;22(9):1554–1561. PMID: 27532807.
2. Casadevall A and Pirofski LA. The convalescent sera option for containing COVID-19. J Clin Invest. 2020 Mar 13; PMID: 32167489.
3. Cheng Y, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. Eur J Clin Microbiol Infect Dis. 2005;24(1):44–46. PMID: 15616839.
4. Fu Y, et al. Understanding SARS-CoV-2-Mediated Inflammatory Responses: From Mechanisms to Potential Therapeutic Tools. Virologica Sinica. 2020 Mar 3; PMID: 32125642.
5. Mair-Jenkins J, et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. J Infec Dis. 2015;211:80-90. PMID: 25030060.
6. Tetro JA. Is COVID-19 receiving ADE from other coronaviruses? Microbes Infect. 2020;22(2):72-73. PMID: 32092539.
7. Zhang JS, et al. A serological survey on neutralizing antibody titer of SARS convalescent sera. J Med Virol. 2005;77(2):147–150. PMID: 16121363.
8. CPMP/ICH/363/96. ICH E9 Statistical Principles for Clinical Trials. URL: http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webC ontentId=W C500002928, last access: 09-Dec-2016.
9. CPMP/ICH/363/96. ICH E9 Statistical Principles for Clinical Trials. URL: http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webC ontentId=W C500002928, last access: 09-Dec-2016.
10. EMEA Scientific Guidelines for Human Medicinal Products, Clinical Efficacy and Safety Guidelines, General Guidelines. URL: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_con tent_000602 .jsp&mid=WC0b01ac05807d91a4, last access: 09-Dec-2016.
11. Schulz KF, Altman GD, Moher D for the CONSORT Group*. CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomized Trials. Ann Intern Med. 2010;152:726-732.
12. EQUATOR-network (Enhancing the Quality and Transparency of Health Research). URL: http://www.equator-network.org/resource-centre/library-of-health-research-reporting/, last access 16-Jun-2014
13. EMA/CHMP/EWP/117211/10. Guideline on the investigation of subgroups in confirmatory clinical trials (Draft). URL: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_con tent_001215 .jsp&mid=WC0b01ac05807d91a4, last access: 09-Dec-2016.
14. CPMP/ICH/363/96. ICH E9 Statistical Principles for Clinical Trials. URL: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_con tent_001228 .jsp&mid=WC0b01ac05807d91a4, last access: 09-Dec-2016.
15. CHMP/EWP/5872/03 Corr. Guideline on Data Monitoring Committees.URL: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_con tent_001225 .jsp&mid=WC0b01ac05807d91a4, last access: 09-Dec-2016.