

**Convalescent Plasma Therapy from Recovered Patients to Treat  
Severe SARS-CoV-2 Disease (CONCOVID study)**

**PROTOCOL v 3.1 01 may 2020. Amendement 3**

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**Sponsor : Erasmus MC**

## PROTOCOL SIGNATURE SHEET

Name	Signature	Date
<b>Sponsor or legal representative:</b> <i>Erasmus MC</i>  <b>Head of Department:</b> <i>S. Klein-Nagelvoort Schuit</i>		
<b>Coordinating Investigator/Principal Investigator:</b> <i>Bart Rijnders</i>		

**SITE INVESTIGATOR SIGNATURE PAGE**

Local site name:

1/may/2020

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Signature of site investigator

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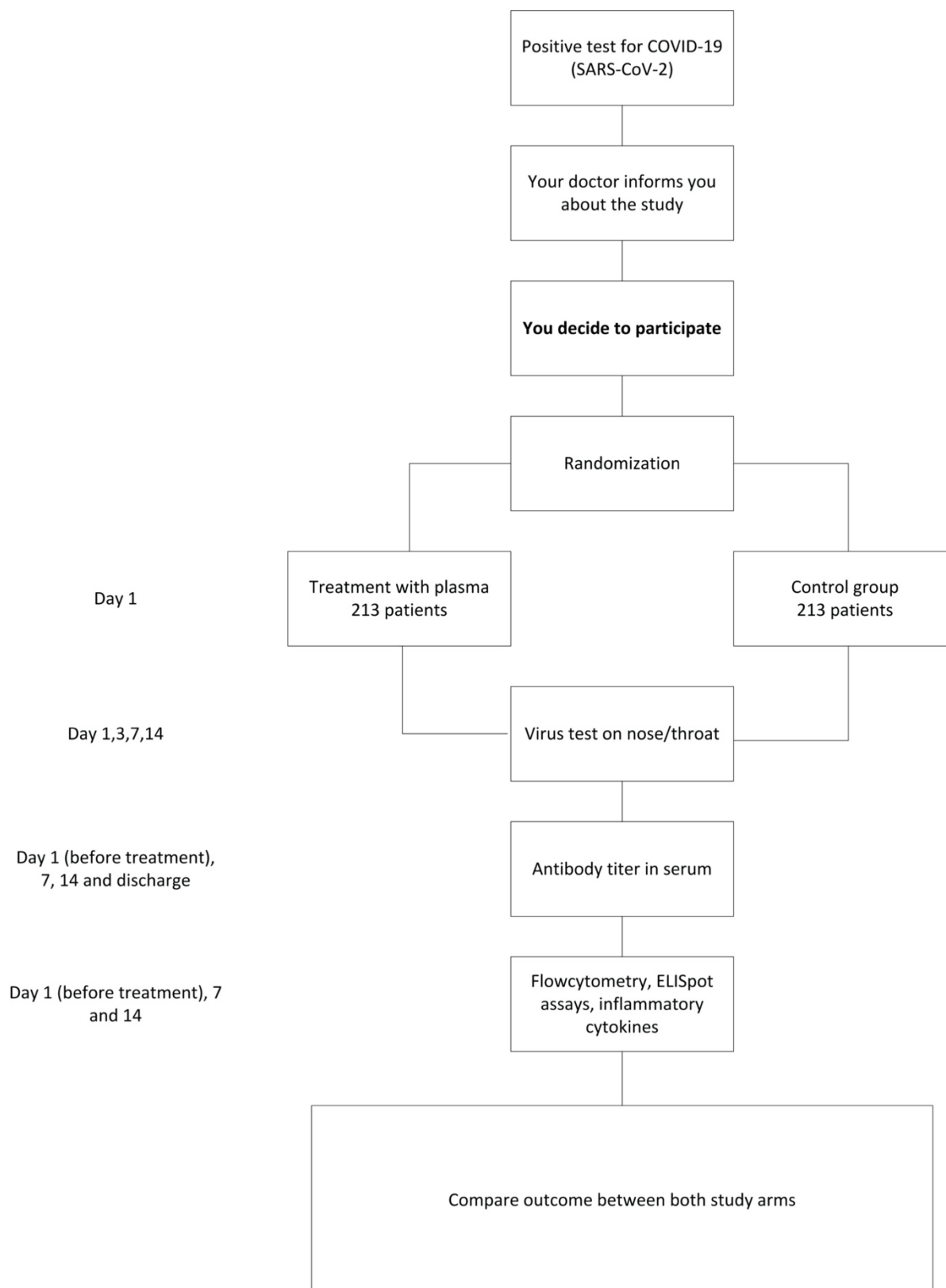
Date

Printed name of site investigator

Bart Rijnders

By my signature, I agree to personally supervise the conduct of this study in my affiliation and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice (2001-20-EG), and local regulations governing the conduct of clinical studies.

# 1 Scheme of study



See table 1 for information on optional long term follow-up study regarding chronic lung damage

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### 3 Synopsis

Rationale	Immunization with immunoglobulins is occasionally used as therapy for the treatment of viral infectious disease. For example CMV disease, VZV disease, rabies and hepatitis B. Neutralizing antibodies have been found against SARS-CoV-2 present in patients who have been infected with SARS-CoV-2. During the 2003 SARS outbreak, convalescent plasma from SARS recovered donors was shown to increase the discharge rate. With no proven effective therapy against COVID-19, this protocol will evaluate the therapeutic potential of therapy with convalescent plasma (conP) from COVID-19 recovered donors.
Study objectives	<p><b>Primary objectives</b></p> <ul style="list-style-type: none"> <li>◆ Decrease overall mortality in patients within COVID disease</li> </ul> <p><b>Secondary (exploratory) objectives</b></p> <ul style="list-style-type: none"> <li>◆ Evaluate the effect of 300ml convP on hospital stay and on the change in WHO disease severity score</li> <li>◆ Evaluate the effect of 300ml convP on mortality in patients admitted to the ICU</li> <li>◆ Evaluate the effect of 300ml plasma therapy on hospital days for patients admitted to the ICU within 24 hours after admission</li> <li>◆ Evaluate the impact of plasma therapy on the decrease in SARS-CoV2 shedding from airways.</li> <li>◆ Evaluate the impact of plasma on long-term lung function</li> </ul>
Study design	This trial is a randomized comparative trial. Patients will be randomized between the infusion of 300mL of convP with standard of care.
Patient population	<p>Patients with PCR confirmed COVID disease, age 18 years or older</p> <p>Donors will be included with a known history of COVID who have been asymptomatic for at least 24 hours.</p>
Intervention	300mL of convP
Duration of treatment	ConvP will be given as a one-time infusion
Duration of follow up	<p>For the primary endpoint: Until discharge or death before day 60, whichever comes first</p> <p>For secondary endpoints (with separate consent) up to 1 year:</p>

Target number of patients	426
Target number of donors	100
Expected duration of accrual	36 months

## Main study endpoints

**Primary endpoints**

- ◆ Overall mortality

**Secondary (exploratory) endpoints**

- ◆ Impact of 300ml convP therapy on hospital days
- ◆ Change of the 8-point WHO COVID19 disease severity scale on day 15
- ◆ Change of the 8-point WHO COVID19 disease severity scale on day 30
- ◆ Change of the 8-point WHO COVID19 disease severity scale on day 15 in the subgroup of patients with a baseline neutralizing antibody titer (PRNT50) <80.
- ◆ Impact of 300ml convP on weaning from oxygen therapy
- ◆ Impact of 300ml convP on overall mortality in patients not admitted to the ICU within 24 hours after admission
- ◆ Impact of 300ml convP on overall mortality in patients admitted to the ICU within 24 hours after admission
- ◆ Difference in the effect of convP on mortality in patients with a duration of symptoms < or ≥ the median duration of symptoms in the study population
- ◆ Impact of 300ml convP therapy on ICU days in hospital days in patients admitted to the ICU within 24 hours after admission
- ◆ Impact of plasma therapy on the decrease in SARS-CoV2 shedding from airways.
- ◆ Impact of CTL and NK cell immunity on the likelihood of being protected from immune serum transfer.
- ◆ Safety of convP therapy
- ◆ Impact of plasma therapy on risk of long-term structural lung damage and lung function



Benefit and nature and extent of the burden and risks associated with participation	Benefits of this study may include shorter stay in hospital and a decrease in mortality. The risks of plasma infusion are comparable to risks associated with regular bloodtransfusions. These include transfusion reactions, transfusion related acute lung injury and transmission of (unkown) transmittable diseases. Maximal precautions will be taken against these risks comparable to precautions taken by Sanquin.
Planned interim analysis and DSMB	An interim-analysis for efficacy will be perfored on the primary endpoint when 50% of patients have been randomised and have been followed-up for at least 15 days, with the use of a predefined stopping rule as described in the protocol. The interim analysis will be performed by an independent statistician an a DSMB will be installed

#### 4 Investigators and study administrative structure

Responsibility	Name	Affiliation/Address
Sponsor	Erasmus MC	
Representative of Sponsor	S. Klein-Nagelvoort Schuit	
Principal Investigator	<i>Bart Rijnders</i>	<i>Erasmus MC</i>
Co-investigators	<i>Arvind Gharbharan</i>	<i>Erasmus MC</i>
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	<i>Peter te Boekhorst</i>	
	<i>Rik Endeman</i>	
	<i>Casper Rokx</i>	
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	<i>Dorine de Vries</i>	
	<i>Els van Nood</i>	
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	<i>Karin Schurink</i>	
	<i>Annelies Verbon</i>	
	<i>Menno van der Eerden</i>	
	<i>Jelle Miedema</i>	
Coordinating investigator	<i>Bart Rijnders</i>	Erasmus MC
Protocol writing committee	Bart Rijnders, Arvind Gharbharan, Jeroen van Kampen, Corine Korf-van Kessel, Peter te Boekhorst, Marion Koopmans, <i>Peter Katsiki</i> , <i>Yvonne Müller</i> , <i>Casper Rokx</i>	Erasmus MC
	<i>Francis Swaneveld</i> <i>Věra Novotny</i>	Sanquin Blood Supply
Statistician	Gregorios Papageorgiou	Erasmus MC
Monitoring	To be decided	Erasmus MC

Serious (SAEs) notification	Adverse Events	Bart Rijnders, Casper Rokx Arvind Gharbharan, Carlijn Jordans	Erasmus MC
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Study sites: See appendix E

## 5 Introduction and rationale

Passive immunization with immunoglobulins is occasionally used as therapy for the treatment of viral infectious diseases. Immunoglobulins are used for the treatment of CMV disease, and is effective as prophylaxis when given soon after exposure to varicella zoster virus (ref. NVMM, varicella richtlijn, 2010), rabies (ref. PMID22541629), and hepatitis B virus (ref. PMID 25678010)

Neutralizing antibodies against MERS, SARS-CoV-1 and SARS-CoV-2 have been shown to be present in patients previously infected with MERS, SARS-CoV-1 and SARS-CoV-2 respectively (ref. PMID 32150528, PMID 16121363, Okba et al, biorxiv, 2020). During the 2003 SARS outbreak in Hong-Kong, a non-randomized study in hospitalized SARS patients showed that treatment with convalescent plasma (convP) from SARS-recovered donors significantly increased the day 22 discharge rate ( $p < 0.001$ ) and decreased mortality ( $p = 0.05$ ) (ref. PMID 15616839). In a meta-analysis of studies evaluating the effectivity of convalescent plasma therapy for severe H5N1 influenza, a significant reduction in the pooled odds of mortality following treatment was observed. The overall crude case-fatality rate was 16% (54 of 336) among treated patients and 37% (452 of 1219) among controls. The range of absolute risk differences in death was 8% to 26% (pooled risk difference, 21% [95% CI, 15% to 27%]). In a meta-analysis of observational studies performed on the use of convalescent plasma for SARS or severe influenza, an odds ratio for mortality of 0.25 (95% C.I. 0.14-0.45) was observed. At a time when SARS-CoV-2 was not yet known, the authors concluded that convalescent plasma should be studied within the context of a well-designed clinical trial or other formal evaluation, including for treatment of Middle East respiratory syndrome coronavirus CoV infection.

Finally, in an as yet not yet peer-reviewed study in non-human primates rhesus macaques could not be re-infected with SARS-CoV-2 after they had been recovered from a primary infection. (ref. <https://www.biorxiv.org/content/10.1101/2020.03.13.990226v1>).

Although these data may look promising, the role of neutralising antibodies and certainly the role of passive immunization with donor plasma containing these antibodies in the pathway from infection towards cure of the patient infected with SARS-CoV-2 remains to be established.

With no proven effective therapy against COVID, this study will evaluate the safety and efficacy of convalescent plasma from COVID-recovered donors as a treatment for hospitalized patients with symptomatic COVID-19. The study will focus on patients who tested positive for SARS-CoV-2 in the last 96 hours before inclusion.

### 5.1 Immune effects of passive antibody immunotherapy against COVID-19 disease

Passive antibody immunotherapy is assumed to act via viral neutralization and ADCC of infected cells. Such ADCC requires functional NK cells and possibly also macrophages. However, there is ample data suggesting that passive antibody therapy may also augment anti-viral T cell responses and that these are critical to the control of infection. Transferring highly neutralizing SIV-hyperimmune sera or neutralizing antibodies to non-human primates infected with SIV virus led to the increased virus-specific T-cell responses [PMID: 19297503; PMID: 24172905]. Administering anti-RSV neutralizing monoclonal antibodies in mice enhance CD8<sup>+</sup> T-cell responses [PMID: 24990999], while anti-LCMV

antibodies promote CTL responses in mice [PMID: 26805453]. These CTL enhancing effects of passive antibody immunotherapy may be due to stimulation of Dendritic cells (DC) with immune complexes composed of virions while other may be due to antibodies blocking immune suppressing effects of the virus. Therefore, regarding plasma therapy for SARS-CoV-2 as well, the success of passive antibody immunotherapy may depend on the presence of T cells and in particular virus-specific CTL.

It is well established that T-cell immunity, and in particular cytotoxic CD8<sup>+</sup> T cells (CTL), are essential for the clearance of acute viral respiratory infections. Recent data have indicated that the severity of SARS-CoV-2 infection increases with age. This may be due to immune senescence. Immune senescence and the age-related decline in immunity against respiratory infections is well established in humans and mice (PMID: 17197143). In humans that have defects in T-cell immunity associated with aging there is an increase in morbidity and mortality due to impaired viral clearance [pmid:22529314; pmid:28320361]. Severe SARS-CoV infection in humans has been previously characterized by a delayed T cell response that is accompanied by delayed viral clearance (PMID: 17374415). From the currently available data, the severity of SARS-CoV-2 infection in patients is clearly correlated with ageing and this most likely is mediated by an impaired CTL response that fails to control viral replication. This impaired CTL immunity against severity of SARS-CoV-2 infection may also affect the efficacy of passive antibody immunotherapy, where one would expect anti-viral T cell immunity synergizes with the neutralizing effect of the antibodies. This would especially be important in the case of immune serum transfer where very large titers of neutralizing antibody may be difficult to achieve in patients.

In this part of the study we will examine the numbers and phenotype of immune cells in peripheral blood. Our hypothesis is that patients with reduced or impaired CTL or NK cell immunity are less likely to be protected or exhibit a delayed effect of immune serum transfer. Also, we postulate that the efficacy of passive antibody transfer reduces with a higher amount of autologous antibody secreting cells.(Nat Med 2020 Thevarajan et al) The goal of the study is to determine parameters that identify patients most likely to benefit from immune serum transfer or identify parameters that predict the most beneficial timing of immune serum transfer.

## **6 Study objectives**

### **6.1 Primary objectives**

- ◆ Decrease in overall mortality in patients within COVID disease

### **6.2 Secondary (exploratory) objectives**

- ◆ Evaluate the effect of 300ml convP on hospital stay and the change in WHO disease severity score
- ◆ Evaluate the effect of 300ml convP on mortality in patients admitted to the ICU
- ◆ Evaluate the effect of 300ml plasma therapy on hospital days for patients admitted to the ICU within 24 hours after admission
- ◆ Evaluate the impact of plasma therapy on the decrease in SARS-CoV2 shedding from airways.
- ◆ Evaluate the safety of plasma therapy
- ◆ Evaluate the impact of plasma on long term lung function

## 7 Study design

The trial is designed as a randomized comparative trial. All eligible patients will be randomized between the infusion of 300 ml of convP or standard of care therapy. The 300ml dosing regimen was based on experience from the 2003 SARS epidemic in which 280 ml infusions were used and the fact that Sanquin collects plasma in bags of 300ml (ref. PMID 15616839).

Details of treatment are given in paragraph 9.

## 8 Study population

### 8.1 Patients: Eligibility for randomization

All patients must be randomized before start of treatment. and must meet all of the following criteria. A total of 426 patients will be included.

#### 8.1.1 Inclusion criteria

- ◆ Patients with PCR confirmed COVID disease
- ◆ Admitted to the hospital
- ◆ The most recent PCR positive sample is <96hrs old
- ◆ Age at least 18y
- ◆ Written informed consent by patient or legal patient representative

#### 8.1.2 Exclusion criteria

- ◆ Participation in another intervention trial on the treatment of COVID-19 that falls under the Dutch law human research (WMO) and in which individual patients are randomized to different treatment options
- ◆ Know IgA deficiency
- ◆ Invasive ventilation for already >96 hours

### 8.2 Donors

Only voluntary, non-remunerated donors are accepted. The medical assessment of the donor is in accordance with the existing guidelines of Sanquin Blood Supply. Donors will have been screened for transfusion-transmitted diseases through the use of the uniform donor questionnaire and tested negative for:

- Hepatitis B: HBsAg, anti-HBc en HBV-DNA (NAT)
- Hepatitis C: anti-HCV en HCV-RNA (NAT)
- Hepatitis E: HEV-RNA (NAT)
- HIV: anti-HIV-1/2/(O) en HIV-RNA (NAT)
- Syphilis: anti-TP
- HTLV I/II: Anti-HTLV I/II

Donors will be asked to fill in a questionnaire (appendix A) regarding clinical symptoms of COVID, first and last day of symptoms, underlying medical diseases,

### **8.3 Eligibility for plasma donation (criteria used by Sanquin)**

#### **8.3.1 Inclusion criteria**

- ◆ A history of COVID infection that was documented by PCR
- ◆ Known ABO-Resus(D) blood group
- ◆ A screening for irregular antibodies with a titer  $\leq 1:32$
- ◆ Asymptomatic for at least 14 days
- ◆ Written informed consent regarding the plasmapheresis procedure
- ◆ Tested negative for HIV, HBV, HCV, HEV, HTLV and syphilis

#### **8.3.2 Exclusion criteria**

- ◆ Age  $<18$  years or age  $>65$  years (80 in donors who were already registered as a donor at sanquin before the age of 65 years)
- ◆ Weight  $<50$ kg
- ◆ Medical history of heart failure
- ◆ History of transfusion with red blood cells, platelets or plasma after 01-01-1980
- ◆ History of organ- or tissue transplant
- ◆ A cumulative stay in the United Kingdom of  $\geq 6$  months in the period between 01-01-1980 and 31-12-1996
- ◆ A history of i.v. drug use
- ◆ Insulin dependant diabetes
- ◆ An underlying severe chronic illness (i.e. history of heart failure, cancer or stroke)
- ◆ Tested positive for HLA- or HNA-antibodies

## **9 Treatment**

### **9.1 Intervention: infusion of plasma**

Infusion of plasma retrieved from donors with a history of PCR proven symptomatic COVID (see 8.2 for details regarding donors). Furthermore, only plasma from donors in whom a sufficiently high neutralizing antibody titer can be demonstrated will be used (see 9.2.1)

Patients will be randomized with the use of blocks with variable length (2 or 4) into a group of

- 213 patients that will receive the standard of care plus 300 ml of convP
- 213 patients that will receive the standard of care therapy

Plasma will be administered according to the Erasmus MC KIS protocol regarding the use of blood products (see appendix D)

Patients who, according to the treating physician have not improved on day 5 can be given a second 300ml plasma unit but only after approval from the sponsor and after a new SARS-CoV-2 PCR was done preceding the administration of the second plasma unit. .

### **9.1.1 Assessment of immuneresponse during plasma treatment**

The department of immunology will examine the numbers and phenotype of immune cells in peripheral blood. Specifically, we will determine by multicolor flow cytometry the numbers, phenotype and activation state of T cells, B cells and innate cells (NK cells, monocytes, DC) in blood of patients. We will also examine the SARS-CoV-2 specific CD8<sup>+</sup> T cell response using immunodominant peptides that are shared between SARS-CoV and SARS-CoV-2. These peptides are selected on the basis that they have been validated to elicit CTL responses in SARS-CoV patients. Using peptide-loaded HLA class I tetramers with flow cytometry, and ELISpot assays we will quantitate and characterize the CTL response against SARS-CoV-2. We will measure inflammatory cytokines in plasma. We will correlate these T cell responses, immune cell changes and inflammatory cytokines with clinical outcome and other immunological parameters determined in this protocol. This part of the study should be considered exploratory.

### **9.1.2 Assessment of antibody titer measurement in serum**

The department of virology will evaluate the dynamics of antibodies against SARS-CoV-2, using an S1 ELISA.

## **9.2 Plasma retrieval, administration and determination of SARS-CoV2 antibodies**

Plasma will be retrieved from donors at Sanquin Blood Supply according to their standard procedures for collection of fresh frozen plasma. Each donation 600 ml plasma will be collected in 2 bags of 300ml each. The donor will be asked (but is not obliged) to give plasma 4 times with a one-week interval. Plasma will be stored at minus 25 degrees Celsius or colder. There is no quarantine period before release of the product. Neither will there be a pathogen reduction procedure.

Anti-COVID-19 plasma has its own product code: E9740 = Apheresis CONVALESCENT PLASMA|Citrate/XX/≤-18°C|COVID-19. The two products of each donation will receive product codes E9740VA0 and E9740VB0 and will be labelled according to ISBT128 (see appendix B).

Before delivery of the anti-COVID-19 plasma to Erasmus MC by Sanquin Blood Supply an informed consent regarding a magisterial blood product ('Bewustzijnsverklaring magistraal bloedproduct') will be signed by the ordering physician (see appendix F)

At the end of the plasmaferesis procedure Sanquin Blood Supply collects 8 ml blood from the donor for Erasmus MC to perform an antibody test (see below).

The department of virology at Erasmus MC will screen plasma donors for the presence of neutralizing antibodies against SARS-CoV-2 using a plaque reduction neutralization assay (gold standard for detection of neutralizing antibodies) as well as with an S1 ELISA. To examine the long-term humoral and cellular immunity against SARS-CoV-2 donors who consent will be asked to come to Erasmus MC to collect serum and cells from blood on several occasions in the 12 months following plasma donation.



Plasma will be labeled according to appendix B. Every plasma unit will have a unique identification number, a so-called EIN (Eenheid Identificatie Nummer), by which the product can always be traced back to the donor. When plasma is administered, this number will be registered in the patient file.

### **9.2.1 Assessment of SARS-CoV2 neutralizing antibodies in donor serum**

The department of virology will screen plasma donors for the presence of neutralizing antibodies against SARS-CoV-2 using a plaque reduction neutralization assay (gold standard for detection of neutralizing antibodies) as well as an S1 ELISA. Only plasma from patients with detectable neutralizing antibodies in serum with at least a titer of PRNT50 of 1/80 or higher. Furthermore, the plasma unit with the highest antibody titer available at the time of inclusion of the patient will be used. Plasma with an antibody titer <1/80 will not be used for this study but can be used by Sanquin for other purposes.

## **10 Study procedures**

### **10.1 Time of clinical evaluations**

- ◆ Screening
- ◆ Baseline
- ◆ Day 3,5, 7,10, 14 (only if still admitted in the hospital)
- ◆ At discharge or death or day 60 whichever comes first
- ◆ Optional after additional consent: Six weeks and 3 months lung function and structural lung damage evaluation.
- ◆

### **10.2 Required investigations**

Required investigations at entry, during treatment and during follow up:

	Screening D1	Baseline D1 (preceding plasma administration)	D2	D3	D4	D5	D6	D7 +2d	D10	D11-13	D14 +2d	D15	D30	Discharge or death	Lung function and low dose CT 6 weeks after discharge <sup>(l)</sup>	Lung function and low dose CT 3 months after discharge <sup>(l)</sup>
Eligibility check	x	x														
Informed consent	x															
Plasma administration		x														
Transcutaneous O2 saturation, O2 therapy (l/min), body temperature		x	x	x	x	x	x	x	x	x	x			x		
Medical history		x														
COVID disease Scale (0-8)		x										x	x			
COVID progression scale (0-10)		x										x	x			
ICU patients: SOFA and APACHE II		x														
Patient details and baseline characteristics		x														
Register the use of any antiviral agents given against COVID since diagnosis		x												x		
Lab results registration <sup>(+)</sup>		x						x			x					
Nasal or Nasopharyngeal swab <sup>(*)</sup>		x		x				x			x					
Register hospital and ICU days														x		
Survival status registration														x		
Immune response evaluation <sup>(#)</sup>		x						x			x					
Antibody titer measurement <sup>(@)</sup>		x						x			x			x		
Register thromboembolic events <sup>(^)</sup>		x												x	x	x
CT lungs <sup>(l)</sup>															x	(x)
Lung function test <sup>(l)</sup>															x	

**Legend:**

(\*) for SARS-CoV2 PCR testing. The sample should be stored at minus 20 or minus 70-80 (if available) and will be tested at a later point in time at Erasmus MC. For testing, the same technique should be used at all time (e.g. nasal or nasopharyngeal). If testing capacity becomes limited for any reason please skip the day 14 and discharge sampling days first and finally the baseline and day 3 test.

Immune response evaluation and antibody titer measurement is for selected sites only

(#) Day 1: 4 natrium heparine tubes of 9mL each, 1 EDTA tube of 6mL, 1 serum tube of 6mL

(#) Day 7: 4 natrium heparine tubes of 9mL each, 1 EDTA tube of 6mL, 1 serum tube of 6mL

(#) Day 14: 1 EDTA tube of 6mL, 1 serum tube of 6mL.

(@) One serum tube of 6mL

(+) The following lab results are registered in the eCRF at these timepoints but only if they have been measured as per standard of care; Highest CRP, ALAT, ferritine and lowest GFR and thrombocytes all in the previous 7 days

(!) Long term pulmonology evaluation is optional for the patient and at selected sides only.

Low dose CT and lung function is done 6 weeks after discharge and if abnormal again 3 months after discharge.

If the lung function or CT is still abnormal at month 3, the pulmonologist will decide if further follow-up is needed with lung function testing and CT outside the context of the study. In Erasmus MC, the standard of care for long term follow-up is at 6 and 12 months after discharge with lung function and in combination with a low dose CT if abnormalities were observed on the month 3 CT.

(^) Whether or not post-discharge radiologically documented thrombotic events occurred will be questioned at the week 6 and month 3 post discharge visit,

### 10.3 Specification of required investigations

#### Medical history

- Sex and age at hospital admission
- Date of first day of illness of SARS-CoV-2 infection
- Underlying medical illness at the time of first day of SARS-CoV-2 disease
- Concomittant medication used at the time of first day of SARS-CoV-2 disease

#### Physical examination

- Lowest measured oxygen saturation when breathing room air

#### Hematology

- Absolute Neutrophil count (ANC), Absolute lymphocyte count. These have to be done on baseline but results already available from a timepoint no later than 48hrs preceding baseline can be used instead.

#### Blood chemistry

- Total bilirubine, LDH, CRP. These have to be done on baseline but results already available from a timepoint no later than 48hrs preceding baseline can be used instead.
- 

## 11 Withdrawal of patients or premature termination of the study

### 11.1 Withdrawal of individual patients from protocol treatment

Patients should be withdrawn from protocol treatment if any of the following criteria for withdrawal are met:

- ♦ Potentially life-threatening transfusion reaction during plasma infusion.

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can also decide to withdraw a patient from protocol treatment for urgent medical reasons. Patients who are withdrawn from protocol treatment will receive medical care according to local practice

### 11.2 Follow up of patients withdrawn from protocol treatment

Patients who are withdrawn from treatment for other reasons than death will be followed as described in 10.2. SAE information will be collected as described in 12.3.

### 11.3 Withdrawal of informed consent

If a patient states that he or she withdraws their consent to participate in the trial, the investigator should attempt to verify the patient's intent and record this in the patients medical file:

- The patient can refuse further treatment and/or procedures according to protocol, while still consenting with further follow up data collection.

- The patient can refuse further treatment and/or procedures according to protocol and withdraw consent for further follow up data collection.

#### **11.4 Premature termination of the study**

The sponsor may decide to terminate the study prematurely based on the following criteria:

- ♦ There is evidence of an unacceptable risk for study patients (i.e. safety issue);
- ♦ There is reason to conclude that continuation of the study cannot serve a scientific purpose

The sponsor will promptly notify all concerned investigators, the ethics committee(s) and the regulatory authorities of the decision to terminate the study. The sponsor will provide information regarding the timelines of study termination and instructions regarding treatment and data collection of enrolled patients.

## **12 Safety**

The first 10 patients (4 to 6 plasma and 4 to 6 controls depending on the exact allocation by the block randomization) will be patients who are not mechanically ventilated. The start of recruitment of patients who are mechanically ventilated will not begin before the data of these first 10 patients have been reviewed by the DSMB and a recommendation of the DSMB has been given regarding further enrollment of patients into the study. This will include the SAE described below (12.2.2), convP related AE and all venous and arterial thromboembolic events that were observed.

After 10 mechanically ventilated patients have been treated with plasma, the DSMB will be asked to review the safety data of these 10 patients and advice upon further enrollment of mechanically ventilated patients into the study.

From there on, the DSMB will receive safety data after every 10 additional included patients (ventilated or not) until 50 patients have been included. From there on the DSMB will receive updates of the (S)AE after every 25 inclusions unless the DSMB prefers to receive data after every 10 patients.

To make it as likely as possible that viral replication is still part of the clinical disease entity, only patients with a positive SARS-CoV-2 PCR not older than 96 hours will be included.

### **12.1 Temporary halt for reasons of subject safety**

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

## 12.2 AEs and SAEs

### 12.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention].

However, given the setting of a large outbreak, the registration of AE is not feasible and will not serve the safety of the patients. However, all AE at least possibly related to the plasma transfusion will be registered.

### 12.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The registration of SAE will be limited to the following:

- ◆ Death
- ◆ Life threatening transfusion reactions

Serious Adverse Events (SAEs) will be reported from moment of plasma infusion according to protocol until 60 days following infusion.

The investigator will report the SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

## 12.3 Follow-up of adverse events

All SAEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

## 12.4 Data Safety Monitoring Board (DSMB) / Safety Committee]

A data safety monitoring board will be implemented, review the available study data regarding SAEs observed during the trial, review the safety of the participants on a regular basis (see 12 above) and recommend the study team regarding the further conduct of the study.

A pre-planned interim analysis for efficacy will be conducted after 50% (n=213) of the patients have been randomized and have been followed-up for at least 15 days. The interim analysis will be performed by an independent statistician blinded for the treatment allocation. The statistician will report the results to the independent DSMB. The O'Brien-Fleming boundary will be used to and if mortality in the intervention arm is lower than the control arm with a p-value of 0.0052 or lower, study discontinuation will be discussed with the DSMB.

A complete DSMB charter will be submitted to the institutional review board before the 25<sup>th</sup> patients is included. The DSMB will consist of a biostatistician, an infectious diseases specialist and a medical ethicist.

## 13 Endpoints

### 13.1 Primary endpoints

- ◆ Overall mortality until discharge from the hospital or a maximum of 60 days after admission whichever comes first

### 13.2 Secondary (exploratory) endpoints

- ◆ Impact of 300ml convP therapy on hospital days
- ◆ Change in 8-point WHO COVID19 disease severity scale on day 15
- ◆ Change in the 8-point WHO COVID19 disease severity scale on day 30
- ◆ Change in the 8-point WHO COVID19 disease severity scale on day 15 in the subgroup of patients with a baseline neutralizing antibody titer (PRNT50) <80.
- ◆ Impact of 300ml convP on weaning from oxygen therapy
- ◆ Impact of 300ml convP on overall mortality in patients not admitted to the ICU within 24 hours after admission
- ◆ Impact of 300ml convP on overall mortality in patients admitted to the ICU within 24 hours after admission
- ◆ Difference in the effect of convP on mortality in patients with a duration of symptoms < or ≥ the median duration of symptoms in the study population
- ◆ Impact of 300ml convP therapy on ICU days in hospital days in patients admitted to the ICU within 24 hours after admission
- ◆ Impact of plasma therapy on the decrease in SARS-CoV2 shedding from airways.
- ◆ Impact of CTL and NK cell immunity on the likelihood of being protected from immune serum transfer.
- ◆ Safety of convP therapy
- ◆ Impact of plasma therapy on risk of long-term structural lung damage and lung function

## 14 Statistical considerations

### 14.1 Patient numbers and power considerations

We estimate that the overall mortality of patients admitted to the hospital for COVID is 20%. This is based on an overall mortality of 50% in patients admitted to the ICU and 10% for those admitted to a general ward.

Using this anticipated mortality rate of 20% for the control group and an anticipated effect on overall mortality of 50% (i.e. from 20% to 10%) and with a control to intervention ratio of 1:1, the sample size per study arm needs to be 213 patients for the study to have 80% power with a global alpha level of 0.05 and adjusted alpha level for the primary endpoint of 0.0480, which takes 1 interim analyses into account (using the O'Brien-Fleming boundary method for alpha-spending). Power and sample size calculations were done with R (version 3.6.3). We used a simulation-based approach with 10000 replications from a univariable logistic regression and then compared the marginal estimates of mortality between the two study arms using a Wald test.

### 14.2 Statistical analysis

At the time of writing of this protocol, the available data suggested that some clinical or laboratory values may be associated with poor outcome (mortality). Of these, the following can be expected to be measured in almost all patients in the Netherlands and Belgium and will be taken into account in the statistical analysis: Age and sex at admission, in ICU or not at the time of inclusion in the study, the highest value measured of CRP, FI02, total bilirubine and the lowest absolute lymphocyte count measured. For all these variables values will be used that were measured between hospital admission and before plasma transfusion (or the time of randomization plus hours).

Several experimental therapies, although without any document clinical efficacy so far, are being used in a substantial number of patients across the world and also in the Netherlands. The most important of these are hydroxychloroquine or chloroquine and lopinavir/ritonavir and remdesivir. Although, the current standard of care does not recommend any of these therapies, this may change in the future. To this end, the statistical analysis will follow the intention to treat principle to retain the power of 80%. If other as yet unknown variables are described in the medical literature, we may decide to include them as well by submitting an amendment to this protocol.

#### 14.2.1 Primary endpoint analysis

1. The primary endpoint is overall mortality until discharge from the hospital or a maximum of 60 days after admission whichever comes first. For this primary endpoint analysis, the mortality in the 300ml convP group will be compared with the control arm. A multivariable logistic regression model will be used to estimate the effect of plasma therapy on overall mortality. The factors that will be included in this analysis are sex, age at admission, type of department on admission, CRP, absolute lymphocyte count, total bilirubine and FI02. A 2-sided Wald test on the odds ratio of the treatment effect between the two arms as estimated by multivariable covariate-adjusted logistic regression analysis will be used



to assess whether convP reduces mortality at a global alpha level of 5% and an adjusted alpha for one interim analysis of 4.80%.

### **14.2.2 Secondary endpoint analysis**

As these outcomes are exploratory, no correction for multiple testing is performed.

1. Impact of 300ml convP therapy on hospital days. For this outcome both a proportional hazards model for the subdistribution of hospital discharge as proposed by Fine and Gray (1999) and a cause-specific hazard analysis will be used, both accounting for death as a competing risk. Using both analyses will allow assessing the impact of 300ml convP therapy on both absolute and relative risk.
2. Odds difference of being improved according to the 8 point WHO ordinal scale between the 300ml convP and the control groups after 15 days of treatment. For this analysis a proportional odds logistic regression model will be used. The following factors will be included in the model: Age and sex at admission, in ICU or not at the time of inclusion in the study, the highest value measured of CRP, absolute lymphocyte count, total bilirubine and the lowest oxygen saturation measured when the patient was breathing room air. Based on this model, the hypothesis that the treatment to control odds ratio is equal to one will be tested. A Chi-square score test will be used to assess the validity of the proportional odds assumption. According to the result of this test the assumption of proportional odds will be relaxed if necessary, by using a partial proportional or non-proportional odds model.
3. Odds difference of being improved according to the 8 point WHO ordinal scale between the 300ml convP and the control groups after 30 days of treatment. For this analysis a proportional odds logistic regression model will be used. The following factors will be included in the model: Age and sex at admission, in ICU or not at the time of inclusion in the study, the highest value measured of CRP, absolute lymphocyte count, total bilirubine and the lowest oxygen saturation measured when the patient was breathing room air. Based on this model, the hypothesis that the treatment to control odds ratio is equal to one will be tested. A Chi-square score test will be used to assess the validity of the proportional odds assumption. According to the result of this test the assumption of proportional odds will be relaxed if necessary, by using a partial proportional or non-proportional odds model.
4. Odds difference of being improved according to the 8 point WHO ordinal scale between the 300ml convP and the control groups after 15 days of treatment in the subgroup of patients with a baseline neutralizing antibody titer (PRNT50) <80. For this analysis a proportional odds logistic regression model will be used. The following factors will be included in the model: Age and sex at admission, in ICU or not at the time of inclusion in the study, the highest value measured of CRP, absolute lymphocyte count, total bilirubine and the lowest oxygen saturation measured when the patient was breathing room air. Based on this model, the hypothesis that the treatment to control odds ratio is equal to one will be tested. A Chi-square score test will be used to assess the validity of the proportional odds assumption. According to the result of this test the assumption of proportional odds will be relaxed if necessary, by using a partial proportional or non-proportional odds model.

5. Impact of 300ml convP on time to weaning from oxygen therapy from admission. Only patients on oxygen therapy at the time of inclusion will be included in this analysis. A patient will be considered weaned from oxygen therapy when the patient did not receive oxygen for at least 24 hours. For this outcome both a proportional hazards model for the subdistribution of hospital discharge as proposed by Fine and Gray (1999) and a cause-specific hazard analysis will be used, both accounting for death as a competing risk. Using both analyses will allow assessing the impact of 300ml convP therapy on both absolute and relative risk.
6. Impact of 300ml convP on overall mortality in patients not admitted to the ICU within 24 hours after admission. For this analysis a multivariable logistic regression model will be used similar to the analysis of the primary endpoint. The factors that will be included in this analysis are sex, age at admission, type of department on admission, CRP, absolute lymphocyte count, total bilirubine and oxygen saturation at room air.
7. Impact of 300ml convP on overall mortality in patients admitted to the ICU within 24 hours after admission. For this analysis a multivariable logistic regression model will be used similar to the analysis of the primary endpoint. The factors that will be included in this analysis are sex, age at admission, type of department on admission, CRP, absolute lymphocyte count, total bilirubine and oxygen saturation at room air.
8. Difference in the effect of convP on mortality in patients with a duration of symptoms  $<$  or  $\geq$  the median duration of symptoms in the study population. For this analysis a multivariable logistic regression model will be used similar to the analysis of the primary endpoint. The factors that will be included in this analysis are sex, age at admission, type of department on admission, CRP, absolute lymphocyte count, total bilirubine and oxygen saturation at room air.
9. Impact of 300ml convP therapy on ICU days in hospital days in patients admitted to the ICU within 24 hours after admission. For this outcome both a proportional hazards model for the subdistribution of hospital discharge as proposed by Fine and Gray (1999) and a cause-specific hazard analysis will be used, both accounting for death as a competing risk. Using both analyses will allow assessing the impact of 300ml convP therapy on both absolute and relative risk.
10. Impact of plasma therapy on the rate of decrease in SARS-CoV2 shedding from airways. For this purpose, airway samples will be taken according to schedule in chapter 10 and the decline in the number of RNA copies detected by PCR will be evaluated and compared with the controls. For this outcome a marginal regression model for repeated measures adjusted for sex, age at admission, type of department on admission, CRP, absolute lymphocyte count, total bilirubine and oxygen saturation at room air. An unstructured variance-covariance matrix will be used to account for the correlation between the serial measurements. An interaction effect between time and study arm will be used to capture differences in the rate of decrease in SARS-CoV2 RNA shedding from airways between the two study arms. A Likelihood ratio test for the interaction term will be used at the alpha level of 5% to determine whether the rate of decrease differs between study arms.

11. Impact of the PRNT50 titer on the rate of decrease in SARS-CoV2 RNA shedding from airways. For this purpose, airway samples will be taken according to schedule in chapter 10 and the decline in the number of RNA copies detected by PCR will be evaluated and compared with the controls. For this outcome a marginal regression model for repeated measures adjusted for sex, age at admission, type of department on admission, CRP, absolute lymphocyte count, total bilirubine and oxygen saturation at room air. An unstructured variance-covariance matrix will be used to account for the correlation between the serial measurements. An interaction effect between time and antibody titer will be used to capture differences in the rate of decrease in SARS-CoV2 RNA shedding from airways for each unit change in antibody titer. A Likelihood ratio test for the interaction term will be used at the alpha level of 5% to determine whether the rate of decrease depends on the PRNT50 titer. From those hospitals that use viral transport medium for airway sampling, the viral transport medium will be submitted for viral culture. The impact of convP therapy on the chances of detecting replication competent (=culturable) virus will be evaluated by comparing the proportion of culture positive samples in patients in the convP group versus controls using a chi-square test.
12. Exploratory immunological analyses. This includes the impact of CTL or NK cell immunity on the likelihood of being protected from immune serum transfer, the extend and duration of acquired humoral and cellular immunity in covid19 patients. Note that all immunological endpoints are exploratory so the results of these analyses should be interpreted with this in mind.
13. As we will only register mortality as SAE and AE at least possibly related to convP therapy, the use of inferential statistical analyses in which these (S)AE are compared is not needed. Indeed, mortality is already the primary endpoint and the control group will not receive any plasma so plasma related AE will not be observed in the control group. Because a high incidence of thromboembolic events has recently been described in COVID-19 infected patients, thromboembolic events observed in patients will be provided to the DSMB every time they receive the (S)AE interim data.
14. Impact of plasma therapy on risk of long-term structural lung damage and lung function. As a quantitative measure of lung function, forced vital capacity (FVC) and lung diffusion (DLCOc) will be used. For structural lung damage a continuous quantative measure by a validated automated functional respiratory imaging (FRI) to evaluate the patient's airway and lung geometry (Fluidda®, ref <https://www.fluidda.com/publications/>). Descriptive statistics will be used first to describe these endpoints in the entire study population. After that, a comparison between the convP and control arm will be made at 6 weeks and 3 months post-discharge for lung function (FVC as % predicted of normal for age and DLCOc as % predicted of normal for age) with the use of a T-test or Wilcoxon rank sum tests as appropriate. Differences in structural lung damage (FRI) will be compared at 6 weeks and 3 months post-discharge between groups with T-tests, Wilcoxon rank sum tests as appropriate.

### 14.3 Interim efficacy and safety analysis

An interim-analysis for efficacy will be performed on the primary endpoint when 50% (n=213) of the patients have been randomised and followed-up for at least 15 days. The stopping rule is described in the next section. The safety of the study will be monitored by the DSMB (see above) who will receive

updates on (S)AE at predefined points in time. The DSMB will recommend the sponsor regarding further enrollment of patients.

#### **14.4 Stopping rules**

The study will be discontinued at the time of the interim analysis when a reduction in overall mortality is observed in the intervention arm compared with the control with a p value  $<0.0088$ . Instead of the 60-day mortality endpoint, we will use the 15-day mortality endpoint for the interim analysis to allow for an earlier interim analysis. A multivariable logistic regression model will be used to estimate the effect of plasma therapy on overall mortality. The following factors will be included in this analysis; age at admission, type of department admission, total bilirubine and oxygen saturation at room air. A 2-sided Wald test on the odds ratio of the treatment effect between the two arms as estimated by multivariable covariate-adjusted logistic regression analysis will be used to assess whether convP reduces mortality at a global alpha level of 0.0052.

### **15 Registration and randomization**

#### **15.1 Regulatory documentation**

Required regulatory and administrative documents must be provided to the sponsor before enrolment of the first patient. This will always include an ethics committee approval for the investigational site. Each investigational site will be notified when all requirements are met and enrolment can start.

#### **15.2 Registration and randomization**

Eligible patients should be registered before start of treatment. Patients need to be registered in the electronic case record form and by sending an email to [conccovid.inclusion@erasmusmc.nl](mailto:conccovid.inclusion@erasmusmc.nl) with the patient study ID as provided by the eCRF.

All eligibility criteria will be checked with a checklist at the time of randomization. Patients will be randomized without stratification with the use of an online randomization system using blocks of 4 and 2 in ALEA. Each patient will be given a unique patient study number (a sequence number by order of enrolment in the trial). Patient study number and result of randomization will be given immediately by the online registration database and confirmed by email.

### **16 Data collection and quality assurance**

#### **16.1 Case Report Forms**

Data will be collected on electronic case report forms (CRF, openclinica) to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected on the CRF are derived from the protocol and will include at least:

- ◆ Inclusion and exclusion criteria;
- ◆ Baseline status of patient including medical history and stage of disease;
- ◆ Timing and dosage of protocol treatment;
- ◆ Any other parameters necessary to evaluate the study endpoints;
- ◆ Survival status of patient;

Each CRF page will be identified by a trial number, and a combination of patient study number (assigned at registration) and hospital name.

The e-CRF will be completed on site by the investigator or sub-investigator or an authorized staff member. All CRF entries must be based on source documents.

## **16.2 Data quality assurance**

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study centers, review of protocol procedures with the investigator before the study, and site visits by the sponsor.

Data collected on the CRF will be verified for accuracy. If necessary, queries will be sent to the investigational site to clarify the data on the CRF. The investigator should answer data queries within the specified timeline.

## **16.3 Monitoring**

The sponsor will perform on-site monitoring visits to verify that the rights and well-being of patients are protected, the reported trial data are accurate, complete, and verifiable from source documents and the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement(s). Monitoring visits will take place according to the study specific monitoring plan.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. The sponsor expects that during monitoring visits the relevant investigational staff will be available, the source documentation will be available and a suitable environment will be provided for review of study-related documents.

## **16.4 Audits and inspections**

In accordance with regulatory guidelines, audits may be carried out for this study. The investigator is required to facilitate an audit by means of a site visit.

These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Patient privacy must, however, be respected.

Similar auditing procedures may also be conducted by agents of any regulatory body reviewing the results of this study. The investigator should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

# **17 Ethics**

## **17.1 Accredited Ethics Committee**

An accredited ethics committee will approve the study protocol and any substantial amendment.

## 17.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the ICH-GCP Guidelines, the EU Clinical Trial Directive (2001/20/EG), and applicable regulatory requirements. The site investigator is responsible for the proper conduct of the study at the study site.

## 17.3 Patient information and consent

In general, patients presenting at the participating hospital with the diagnosis under study and possibly qualifying for participation will be informed about the trial by the treating physician and asked if they are interested to participate.

Written informed consent of patients is required before enrolment in the trial and before any study related procedure takes place. ICH-GCP and other applicable regulations must be followed in informing the patient and obtaining consent. It should be taken into consideration if the patient is capable of giving informed consent. Before informed consent may be obtained, the patient should be given ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the patient.

There is no set time limit for the patient to make a decision. The investigator should inform each patient if there is a specific reason why he/she must decide within a limited time frame, for example if patients condition necessitates start of treatment or if the trial is scheduled to close for enrolment.

The content of the patient information letter, informed consent form and any other written information to be provided to patients will be in compliance with ICH-GCP, GDPR and other applicable regulations and should be approved by the ethics committee in advance of use.

The patient information letter, informed consent form and any other written information to be provided to patients will be revised whenever important new information becomes available that may be relevant to the patient's consent. Any substantially revised informed consent form and written information should be approved by the ethics committee in advance of use. The patient should be informed in a timely manner if new information becomes available that might be relevant to the patient's willingness to continue participation in the trial. The communication of this information should be documented.

Specific to this study protocol, patients admitted with COVID-19 in one of the participating hospitals will be informed about the study by the infectious diseases physician who is involved in patient care of COVID patients. If the patient is interested, a member of the study team will visit the patient to inform him/her about the study. In case the patient is unable to give informed consent (e.g. in the ICU on ventilatory support), the appropriate relative will be contacted and informed about the study and to give written informed consent.

Plasma donors will be recruited on a voluntary basis. When a person is considered to be a possible donor he or she will be referred to the site of Sanquin Blood Supply for this purpose ([www.sanquin.nl/coronahulp](http://www.sanquin.nl/coronahulp)) where they can sign themselves up as a potential plasmadonor for anti-COVID-19 plasma. Plasma donors will be asked to read and sign a written informed consent before donating plasma at Sanquin Blood Supply.

**17.4 Benefits and risks assessment.**

No additional blood samples will be taken. No additional visits are required. 1 to 5 additional nasopharyngeal swabs will be taken. There will be no additional blood samples taken

The risk of plasma infusion is comparable to the risk associated with blood transfusions. These include transfusion reactions, transfusion related acute lung injury (TRALI) and the transmission of as yet unknown infectious or other transmittable diseases. The precautions as taken by the Sanquin Blood Supply regarding the prevention of infectious and non-infectious complications of blood product transfusion are taken in this study. These include, matching the donor and recipient for blood group, testing for infectious agents as well as testing for irregular antibodies and when indicated HLA- and HNA-antibody testing in the donor.

**17.5 Trial insurance**

Prior to the start of the trial, the sponsor will ensure that adequate insurance for patients is in place covering losses due to death or injury resulting from the trial, in accordance with applicable laws and regulations in each country where the trial is conducted. The sponsor will take out an insurance policy or delegate this responsibility to a national co-sponsor. Proof of insurance will be submitted to the ethics committee.

In addition, the sponsor will ensure that adequate insurance is in place for both investigator(s) and sponsor to cover liability pertaining to death or injury resulting from the trial, in accordance with applicable laws and regulations in each country where the trial is conducted.

**17.6 Incentives**

Plasma donors will receive a bol.com voucher to compensate for travel costs to the plasma donation site and to Erasmus MC for PBMC donation with a maximum value of 50 euro per donor.

**18 Administrative aspects and publication****18.1 Handling and storage of data and documents**

Data and documents will be controlled and processed conform the EU General Data Protection Regulation (GDPR) and the Dutch Act on Implementation of the General Data Protection Regulation. (in Dutch: Uitvoeringswet AVG, UAVG).

**18.1.1 Patient confidentiality**

Each patient is assigned a unique patient study number at enrolment. In trial documents the patient's identity is coded by patient study number as assigned at enrolment.

The site investigator will keep a subject enrolment and identification log that contains the key to the code, i.e. a record of the personal identification data linked to each patient study number. This record is filed at the investigational site and should only be accessed by the investigator and the supporting hospital staff, and by representatives of the sponsor or a regulatory agency for the purpose of monitoring visits or audits and inspections.

Patients confidentiality will be ensured in compliance with EU regulation and the Dutch Act on Implementation of the General Data Protection Regulation.

### **18.1.2 Filing of essential documents**

Essential Documents are those documents that permit evaluation of the conduct of a trial and the quality of the data produced. The essential documents may be subject to, and should be available for, audit by the sponsor's auditor and inspection by the regulatory authority(ies)

The investigator should file all essential documents relevant to the conduct of the trial on site. The sponsor will file all essential documents relevant to the overall conduct of the trial. Essential documents should be filed in such a manner that they are protected from accidental loss and can be easily retrieved for review.

### **18.1.3 Record retention**

Essential documents should be retained for 25 years after the end of the trial. They should be destroyed after this time, unless a longer record retention period is required by site specific regulations.

Source documents (i.e. medical records) of patients should be retained for at least 15 years after the end of the trial described in section 18.4. Record retention and destruction after this time is subject to the site's guidelines regarding medical records.

### **18.1.4 Storage and sharing of data**

Electronic patient data collected in the e-CRF will be stored at the sponsor for 25 years.

The data collected by questionnaires filled in by the consenting donors will be sorted for 25 years.

Encoded data may be shared with other study groups for research purposes. If data are sent to countries outside de EU, patients confidentiality will be ensured at an equal level of EU regulation and the Dutch Act on Implementation of the General Data Protection Regulation.

### **18.1.5 Storage of samples**

Biological samples should only be stored for the purpose of additional research if the patient has given consent. If no informed consent was obtained, samples should be destroyed after the patient has completed all protocol treatment and procedures.

Storage of biological samples on site is subject to the site's guidelines.

Samples that are shipped to another facility (e.g. a central laboratory) for a purpose as described in this protocol or for additional scientific research, should be stripped from any identifying information and labeled with a code (trial name or number and patient study number as assigned at enrolment).

## **18.2 Amendments**

A 'substantial amendment' is defined as an amendment to the terms of the ethics committee application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the patients of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be submitted to the ethics committee and to the competent authority.

Non-substantial amendments will not be submitted, but will be recorded and filed by the sponsor.



### **18.3 Annual progress report**

The sponsor will submit a summary of the progress of the trial to the accredited ethics committee once a year. The first report is sent one year after the first approval date of the trial. Subsequent reports are sent annually until end of trial. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

### **18.4 Temporary halt and (prematurely) end of trial report**

The sponsor will notify the accredited ethics committee and the competent authority of the end of the trial within a period of 90 days. The end of the trial is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited ethics committee and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the trial, the sponsor will submit an end of trial report with the results of the study, including any publications/abstracts of the study, to the accredited ethics committee and the competent authority.

### **18.5 Publication policy**

Trial results will always be submitted for publication in a peer reviewed scientific journal regardless of the outcome of the trial – unless the trial was terminated prematurely and did not yield sufficient data for a publication.

## **19 STRUCTURED RISK ANALYSIS**

### **19.1 Potential issues of concern**

The only concern is the concern that exists for the use of human blood products and a possible (although as yet undocumented) increased risk of transfusion acute lung injury (TRALI). No other concerns are in place

a. Level of knowledge about mechanism of action

See introduction

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism.

See introduction

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

No

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Unknown

e. Analysis of potential effect

See introduction

f. Pharmacokinetic considerations

Unknown for SARS-Cov-2 antibodies at this time

g. Study population

SARS-Cov-2 infected patients in the hospital

h. Interaction with other products

None

i. Predictability of effect

Not predictable at this time

j. Can effects be managed?

Transfusion reactions are managed with standard transfusion reaction management protocols

## **19.2 Synthesis**

All standard blood product safety measures are in place except for the 4-month quarantine period that is normally adhered to. The overall risk of a single allogeneic plasma transfusion is low. To reduce this risk further, we will only use plasma from male donors who have no history of blood transfusion, or female donors / donors with a history of blood transfusion before 01-01-1980 when tested negative for HLA- and HNA-antibodies. Given the mortality of the disease under study the risk is acceptable in our opinion.

**References**

These are included in the text. When a pubmed number is given this refers to the pubmed study nummer at <https://www.ncbi.nlm.nih.gov/pubmed/>








**20 Glossary of abbreviations**

ADCC	Antibody-dependent cell-mediated cytotoxicity
AE	Adverse Event
convP	Convalescent plasma
CA	Competent Authority
CRF	Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data Safety and Monitoring Board
ECG	Electrocardiogram
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IMP	Investigational Medicinal Product
LDH	Lactate Dehydrogenase
METC	Medical Ethical Review Committee
NYHA	New York Heart Association
OS	Overall Survival
PB	Peripheral Blood
PRNT50	The concentration of serum to reduce the number of plaques by 50% compared to the serum free virus
SAE	Serious Adverse Event
SC	Subcutaneous
SD	Stable Disease
SUSAR	Suspected Unexpected Serious Adverse Reaction
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen

**A. Questionair that will be used for donors**

See the separate pdf document with the name “ConCoViD donor questionnaire version 2.1 1 May 2020”. The questionnaire is an online questionnaire in gemstracker, the software tool provided by Erasmus MC for this purpose (<https://gemstracker.org>)

**B. Labeling of plasma**

 N0011 20 200876  3	 0600
 0200780830	<b>A</b> <b>Rh-D negatief</b>
Afg.op 18 Mrt 2020 08:30  E9740VA0 AF PLASMA anti-COVID-19 vers bevroren	 Exp.dat. 0220772359 18 Mrt 2022 23:59  N0000 Geen bijzonder kenmerk
Bevat 6% v/v NaCitraat Volume ca 310 mL Leukocyten < 1 x 10 <sup>6</sup> /E Bewaren bij -25°C of lager Zie <a href="http://www.sanquin.nl/bloedwijzer">www.sanquin.nl/bloedwijzer</a>	

**C. Common Terminology Criteria for Adverse Events**

The grading of adverse events will be done using the NCI Common Terminology Criteria for Adverse Events, CTCAE version *<see current version>*.

**D. Erasmus MC KIS protocol plasma toedienen 19 maart 2020**

Zie apart document

**E. Study sites**

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Ziekenhuis Alrijne

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## F. Bewustzijnsverklaring magistraal bloedproduct'

	TG08.004.F.BB / versie 001	Status: Geldig
	Bewustzijnsverklaring magistraal bloedproduct COVID-19	Geldig: 31-mrt-2020 Pagina: 1 van 1

Dit product wordt aangevraagd in het kader van de klinische studie:	
Datum aanvraag:	
Ziekenhuis:	Telefoonnummer:
Afdeling:	Email-adres:
Aanvragend arts:	Seinnummer:

<p>Geachte collega,</p> <p>Voor bovengenoemde patiënt vraagt u convalescent plasma COVID-19 aan. Convalescent plasma COVID-19 is geen standaard bloedproduct, maar een magistraal bloedproduct. Onderzoek naar de effectiviteit en veiligheid van dit product is tot op heden niet verricht.</p> <p>U verklaart hierbij dat:</p> <ul style="list-style-type: none"> <li>• U zich ervan bewust bent dat het een magistraal bloedproduct betreft</li> <li>• U zich ervan bewust bent dat bij dit product geen quarantaine periode is gehanteerd</li> <li>• U zich ervan bewust bent dat dit product afkomstig kan zijn van een vrouwelijke donor of een donor die voor 1980 een bloedtransfusie heeft gehad, echter uitsluitend wanneer deze donoren negatief getest zijn op HLA- en HNA-antistoffen</li> <li>• U de verantwoordelijkheid draagt voor de behandeling van uw patiënt met dit product en dat Sanquin geen aansprakelijkheid aanvaardt voor toepassing van dit product</li> <li>• U Sanquin zult vrijwaren voor alle aanspraken van de patiënt en derden ingeval van schade ontstaan door toediening van dit product</li> <li>• U dit product niet aan andere patiënten zult verstrekken dan de patiënt genoemd in deze bewustzijnsverklaring</li> <li>• U dit product zult toedienen in het kader van een METC goedgekeurde studie</li> <li>• U alle aan u bekend geworden bijwerkingen die tijdens of na toediening van dit product worden geconstateerd zult melden aan Sanquin, op geanonimiseerde wijze en zodanig dat de privacy van de betrokken patiënt zal zijn gewaarborgd.</li> </ul> <p>Als u akkoord gaat met de hier genoemde voorwaarden verzoeken wij u vriendelijk deze bewustzijnsverklaring ingevuld en ondertekend te mailen naar: <a href="mailto:anti-COVID-19@sanquin.nl">anti-COVID-19@sanquin.nl</a></p>
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<b>Voor akkoord Behandelend Arts</b>		
Datum:	Naam:	Handtekening: