ANCILLARY RESEARCH STUDY PROTOCOL

The impact of COVID-19 treatment on the type, strength and duration of antibody and cellular immune responses in SARS-CoV-2 patients in sub-Saharan Africa

ANTICOV-IMMUNO
Version 4.0 16/11/2020

Principal investigator	*Please complete*
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Scientific Lead Signatures

I have read and approved this protocol. My signature, in conjunction with the signatures of the Investigators, confirms the agreement of the Sponsor, Scientific lead and Investigator that the ancillary research study will be conducted in accordance with the protocol and all applicable laws and regulations, including, but not limited to, the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP), the ethical principles that have their origins in the Declaration of Helsinki and applicable privacy laws.

Signature of the Sponsor's Medically Responsible Person

The signatory agrees to the content of the final ancillary research study protocol as presented.

Name: ....   Role: ....

Date: ....   Signature: ....

Signature of the Scientific lead

The signatory agrees to the content of the final ancillary research study protocol as presented.

Name: ....   Role: ....

Date: ....   Signature: ....

Name: ....   Role: ....

Date: ....   Signature: ....

Signature of Principal Investigator

I have read this protocol and agree that it contains all information necessary to carry out the study. I will conduct the study as described herein.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of the study. I will discuss this material with them to ensure they are fully informed regarding the conduct of the study.

I will use only the Informed Consent Form approved by the Sponsor or its representative and will fulfil all responsibilities for submitting pertinent information to the Ethics Committee (EC) responsible for the study if required by national law.

I agree that the Sponsor or its representatives shall have access to any source documents from which case report form information may have been generated.

I agree that the study will be conducted in accordance with all applicable laws and regulations, including, but not limited to, the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP), the ethical principles that have their origins in the Declaration of Helsinki, and applicable privacy laws.

Nothing in this document is intended to limit the authority of a physician to provide emergency medical care under applicable regulations.

Name:

Affiliation:

Date: _____   Signature: _____

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LIST OF ABBREVIATIONS

Ab	Antibody
ADR	European Agreement on International Carriage of Dangerous Goods
Ag	Antigen
COVID-19	coronavirus disease 2019
CYP3A	cytochrome P450 3A4
D	day
EC	Ethics committee
eCRF	electronic case report form
GCP	Good clinical practices
HH	Household
HIV	human immunodeficiency virus
IATA	International Air Transport Association
Ig	Immunoglobulin
ICF	Informed Consent Form
IC50	Half maximal inhibitory concentration
M	month
MFI	Mean Flourescence Intensity
PBMC	Peripheral Blood Mononuclear cells
PCR	Polymerase chain reaction
RDT	Rapid diagnostic test
RR	respiratory rate
SARS-CoV-2	severe acquired respiratory syndrome - coronavirus 2
SFC	Spot forming cells
SD	Standard deviation
SpO2	blood oxygen saturation level
TB	Tuberculosis
WHO	World Health Organisation

1. INTRODUCTION

1.1 Background and Rationale

A key immunological question that underlies the current COVID-19 pandemic is the mechanism by which the human immune system is able to develop protection against the acute infection with the SARS-CoV-2 virus. This is important for the progression towards deconfinement strategies and vaccination campaigns as we need to know how many individuals have developed natural immunity, how durable it is, how protective it is, and how it is impacted by providing early antiviral treatments.

In terms of the antibody response, during an acute viral infection, immunoglobulins of different types (IgM, IgA and IgGs) are induced several days after the peak of viral load (1). Antibody levels are initially low and gradually increase over a period of weeks. IgM develops first followed by IgG that is considered the main protective isotype responsible for long-lasting immunity. IgA responses are particularly important for mucosal immunity and appear to be detected before IgG for SARS-coronavirus (1–3). An antibody response requires the presence of antigen in order to expand B cells specific for a maximum number of epitopes within the antigen. As the virus is cleared, B cells undergo affinity maturation, which leads to genetic modifications in the B cell that select and improve antibody affinity and often are accompanied by more efficient neutralization responses. The exact amount and duration of viral replication necessary to ensure a long-lasting immune response is unknown, but there are many examples showing that lower viral replication in the acute phase either due to treatment, lower inoculation dose, or vaccination, is associated with a lower antibody response (4–7).

Early treatment of viral infections can have immediate therapeutic effects but there is a risk of blunting the immune response and thus reducing the memory response to potential reinfection. In HIV infection, if antiretroviral treatment is initiated during acute HIV infection, it has been demonstrated that the antibody response develops but can wane very quickly to low or undetectable levels (5–7). In Ebola and SARS viral infections, asymptomatic or mild infection has been associated with lower antibody titers as compared to symptomatic or severe infection (8–10). Similarly, available data show that severe cases of COVID-19 have higher antibody levels than mild cases (1,11) and asymptomatic SARS-coronavirus infections seem to elicit even lower levels (3,8).

In addition to the strong focus on the humoral immune system in most studies, in particular serological testing and neutralizing antibodies, the role of cellular immunity against the current (SARS-Cov-2) and prior coronavirus outbreaks (MERS and SARS) is often partially neglected. First reports have observed that COVID-19 does not always results in detectable antibodies, let alone with neutralizing/protective properties, in particular in young adults (12,13). This indicates a key role for both the humoral and cellular immunity in resolving the disease (14). Likewise, research to date showed a severe lymphopenia and an exhausted T-cell phenotype in severe COVID-19 patients (15). In combination with its critical role in virus clearance and activation of antibody-producing B cells, T cells are argued to be important moderators of the COVID-19 disease. This is in line with prior knowledge from other coronaviruses, where it is known that long-lasting virus-specific memory cytotoxic T-cells (lasting >6 years) can be key for optimal protection (16). Similar to antibody levels, it remains unkown how this is affected by providing early antiviral treatments (e.g. impaired antigen presentation).

The longevity of antibody and cellular SARS-CoV-2-specific responses is yet to be characterized due to the early days of the pandemic. In the previous SARS outbreak, approximately 90% possessed strongly neutralizing antibodies, and 50% of convalescent patients were positive for T cell responses (17,18). Although similar findings are to be expected and being investigated in severe hospitalized cases of

COVID-19 patients, it remains unclear how durable immunity is in ambulant or asymptomatically infected patients, and how this would be affected by early antiviral treatment.

Therefore, embedded within the master clinical trial, a highly warranted study on the development and duration of humoral and cellular immune responses to SARS-CoV-2 in mild to asymptomatically infected patients will be performed in several key countries, aiming to identify the impact of early antiviral treatment on immunity, and immune correlates and duration of protection. Although similar studies are ongoing in Europe, US and Asia, upcoming findings are likely not to be extrapolated to sub-Saharan Africa due to different genetic backgrounds (e.g. HLA-dependent epitope presentation) and a large range of different exposures and comorbidities (e.g. malnutrition, helminths, HIV, latent TB, malaria, other viruses) and infection histories that severely impact immune responses, which has been previously exhibited in numerous vaccine efficacy and immunoepidemiologic studies (19–21).

Our initiative will thus provide the first evidence-based insights in types of immune responses that may be (partially) responsible for virus clearance or disease progression in sub-Saharan Africa and assess how this is affected by early antiviral treatment. In addition, the duration of these responses will be studied to inform on susceptibility to reinfection, possibility of herd immunity or the need of mass vaccination strategies. Hence, this study may serve as a benchmark for SARS-CoV-2 deconfinement and vaccine strategies in sub-Saharan Africa.

2. STUDY OBJECTIVES

Our main aim is to assess the impact of (effective) COVID-19 treatment on the strength and duration of SARS-CoV-2-specific antibody and cellular immunity in mild / moderate COVID-19 patients across sub-Saharan Africa.

2.1 Primary objectives
1. To evaluate whether (effective) treatment of mild SARS-CoV-2 infected patients impacts the generation and strength of antibody and cellular immune responses to the infection.
2. To evaluate whether (effective) treatment of mild SARS-CoV-2 infected patients impacts the longevity of antibody and cellular immune responses to the infection.

2.2 Secondary objectives
1) To compare the antibody and cellular immune responses between mild patients and asymptomatically infected household contacts.
2) To assess the impact of treatment on avidity and neutralizing capacity of antibody responses.
3) To assess the association of antibody and cellular immune responses with reinfection during a 12 month follow-up period based on passive and active case detection, and to assess the impact of treatment on correlates of protection.
4) To assess the impact of highly prevalent comorbidities (HIV, TB, malaria, helminth infections, and malnutrition) on the strength and type of SARS-CoV-2 antibody and cellular response.
5) To assess the prognostic value of baseline antibody and cellular characteristics for treatment and patient outcomes.
6) To assess antibody responses to other coronaviruses and their association with the strength and duration of SARS-CoV-2 response and patient outcome.

2.3 Exploratory objectives

1. To assess the impact of treatment on fine epitope specificity of SARS-CoV-2 antibodies evaluated with peptide arrays and/or Luminex.
2. To obtain human monoclonal antibodies with neutralizing capacity.
3. To assess the impact of treatment on the diversity of the SARS-CoV-2 T cell receptor repertoire.

2.4 Primary Endpoints

1) Seroconversion for SARS-CoV-2 IgM, IgA and IgG by treatment arm and patient outcome
2) Quantitative levels of antibodies (IgM, IgG, IgA, IgE) specific for SARS-CoV-2 at day [d]0, d7, d21, month [M]1, M3, M6 and M12.
3) Frequency of SARS-CoV-2-specific effector/memory CD4+ and CD8+ T cells (d0, d21, M3, M6, M12)
4) Proportions of (poly)functional SARS-CoV-2-specific T cells (d0, d21, M3, M6, M12)
5) Inhibition of viral replication by cellular immunity (d0, d21, M3, M6, M12)

2.5 Secondary endpoints

1) Epitope specificity of IgM, IgA and IgG isotypes targeting SARS-CoV-2
2) Avidity index and neutralization activity (% neutralization or IC50) in selected participants and time points based on antibody levels and kinetics
3) Quantitative levels of antibodies (IgM, IgG, IgA, IgE) against prevalent infections such as malaria and helminths, antibody levels against other respiratory infections, and medical history of TB, HIV and other known comorbidities (e.g. malnutrition).
4) Quantitative levels and seropositivity of antibodies (e.g. IgM, IgG, IgA, IgE) against coronaviruses circulating in participating countries.
5) Quantitative levels of autoantibodies (e.g. antibodies against IFN).
6) Quantitative levels of proinflammatory and cellular markers (cytokines/chemokines/Acute phase proteins/growth factors) in plasma.
7) Positivity for SARS-CoV-2 in saliva (PCR/RDT)

2.6 Exploratory endpoints

1) Frequencies of fine B cell epitopes of all viral proteins recognized by antibodies
2) Neutralizing activity of the monoclonal antibodies
3) Clonal size/diversity and convergence of TCR repertoire of SARS-CoV-2-specific cells

3. STUDY DESIGN

The study will be embedded within the "An open-label, multicentre, randomised, adaptive trial of the safety and efficacy of several therapies, including antiviral therapies, versus control in mild / moderate cases of COVID-19" (ANTICOV) Study and follow the outlined study design in the master protocol.

Up to 800 mild patients (200 in each treatment arm) will be recruited from the master study. In addition, 200 asymptomatically infected patients will be recruited among households in the epidemiological ancillary study as an additional comparison arm, if conducted at the same sites.

Additional study schedule for patients recruited from the master clinical trial

In addition to the master study protocol, the following adjustments are to be noted to the study schedule and design (see Figure 1):

Research study protocol: Ancillary immunological study to AntiCOV study

- Patients will only be recruited in a limited number of countries fully equipped for the planned analyses (outlined in section 4.1)
- Patients will have the option to separately consent for this ancillary study
- Patients will need to perform 4 additional on-site visits (M1, M3, M6, M12)
- Blood sampling will be performed on-site during the master clinical study and the additional follow-up visits as outlined below. On time points requiring only plasma collection, up to 200 μL of finger-prick blood will be collected. On all other timepoints, up to 30mL of peripheral blood will be collected at each timepoint by venipuncture (outlined in section 5.3).
- In addition, a saliva sample will be collected in the follow-up period for detection of SARS-CoV-2 viral load and reinfection (M1, M3, M6, M12).
- Additional patient clinical data will be collected on CRFs similar to those of the master protocol and added to the database (outlined in section 5.2).

On-site visits	MASTER protocol			Immuno study			
	D0	D7	D21	M1	M3	M6	M12
Additional ICF	X						
Plasma collection (antibody)	X	X	X	X	X	X	X
PBMC collection (cellular)	X		X		X	X	X
Saliva collection (reinfection)				X	X	X	X
Patient clinical data				X	X	X	X

Figure 1: Additional study schedule for mild / moderate COVID-19 patients included in the master study (n=800). PBMC: peripheral blood mononuclear cells.

<u>Additional study schedule for household members recruited from the epidemiological ancillary study</u>

Data on antibody and cellular immune responses will also be collected on an asymptomatically infected population for comparison reasons (secondary objective 1) and which will be analyzed as an additional arm in the study. This will be facilitated via the conduct of the epidemiological ancillary study where household (HH) contacts of any index case are recruited. Confirmed asymptomatic SARS-CoV-2 positive subjects can be enrolled, and the following adjustments are to be noted to the study schedule and design of the epidemiological ancillary study (outlined in Figure 2):

- PCR-confirmed asymptomatically infected HH participants will have the option to separately consent for this ancillary study at or after the end of the follow-up period of the epidemiological study.
- Participants will need to perform 4 additional visits at the hospital or health centre (M1(b), M3, M6, M12).
- Blood sampling will be performed during these visits as outlined below. Up to 30mL of peripheral blood will be collected at each timepoint by venipuncture (outlined in section 5.3).
- In addition, a saliva sample will be collected in the follow-up period (M1b, M3, M6, M12) for detection of SARS-CoV-2 viral load and reinfection.
- Additional patient clinical data will be collected on CRFs similar to those of the master protocol and added to the database.

Visits	Household study			Immuno study			
	D2	D14	M1	M1(b)**	M3	M6	M12
Additional ICF				X			
Plasma collection (antibody)	X*	X*	X*	X	X	X	X
PBMC collection (cellular)				X	X	X	X
Saliva/swab collection (reinfection)	X*	X*	X*	X	X	X	X
Patient clinical data	X*	X*	X*	X	X	X	X

Figure 2: Additional study schedule for asymptomatically infected household contacts included in the epidemiological ancillary study (n=200). PBMC: peripheral blood mononuclear cells.
* Planned and budgeted within epidemiological ancillary study.
** As soon as possible after M1, to account for delayed identification of asymptomatic status.

It is of note that even if the IPs are ineffective for the master study endpoints, they may still exert immunomodulatory capacities and affect the generation and longevity of SARS-CoV-2-specific antibody and the cellular immune responses. Likewise, comparisons between asymptomatic and mild/moderate patients remains highly valuable for vaccination and deconfinement strategies.

4. PARTICIPANTS, POPULATION & SELECTION

4.1 Study Setting, Population and Sampling Strategy

Mild/moderate COVID-19 patients will be recruited from the master study and randomized following the adaptive randomization outlined in the master protocol. Asymptomatically infected patients will be recruited among household contacts in the epidemiological ancillary study.

In order to be eligible, study participants must meet the in- and exclusion criteria as outlined in the master protocol or epidemiological ancillary study.

For participants of the master study in particular, the following additional inclusion criteria are maintained for mild/moderate patients:

1. Able and willing to provide consent for the immunological ancillary study
2. Able and willing to perform all study visits for a duration of 12 months after treatment start

For the epidemiological ancillary study in particular, the following additional inclusion criteria are maintained for household contacts:

1. Able and willing to provide consent for the immunological ancillary study
2. SARS-CoV-2 PCR/RDT-confirmed during the follow-up period of the epidemiological ancillary study (D2-M1)
3. Not having or had any symptoms compatible with COVID-19 during the follow-up period of the epidemiological ancillary study (D2-M1)
4. Being above legal age
5. Able and willing to perform all study visits for a duration of 11 months.

Patients will be recruited in approximately six to eight selected countries/sites among Ethiopia, DRC, Mozambique, Ghana, Equatorial Guinea, Kenya, Sudan, and Cameroon, depending on patient load and capacity at start of the ANTICOV trial. Study sites not fully equipped to conduct PBMC isolation and cryopreservation (cf. cellular immunity part), could still be selected to incorporate the antibody part without cellular immunity evaluations.

4.2 Sample size

The ancillary study aims to include 200 mild COVID-19 patients per treatment arm, with a total of 800 patients. This would allow the study of maximum 4 treatment arms following the adaptive design outlined in the master protocol. The ancillary study also aims to include 200 SARS-CoV-2 asymptomatically infected household contacts as a 'fifth arm' (comparison mild disease vs asymptomatic group), rendering a total maximum of 1000 patients.

Assuming a mean Mean Fluorescence Intensity (MFI) in antibody levels of 14000 (SD±6250) in the paracetamol arm, this would allow to detect of difference of 1755 MFI in antibody levels between treatment arms at D21 with 80% power at 5% significance level. Likewise for the cellular measurements, assuming a mean log(spot forming cells [SFC]/million PBMC) of 4.6 (SD±0.6) in the paracetamol arm, this would allow to detect of a relative difference of 16% in SFC/million PBMC (reduction in mean from 100 to 84) between treatment arms at D21 with 80% power at 5% significance level.

4.3 Discontinuation/Withdrawal Criteria

In addition to the rules described in the master protocol, SARS-CoV-2 asymptomatically infected patients among the household contacts developing mild/moderate disease will be discontinued and referred to the master study.

4.4 Lost to follow-up

A participant will be considered lost to follow-up if s/he can no longer be contacted by the study personnel.

Before the patient can be considered as lost to follow-up, the study personnel must make every effort to re-establish contact with the patient or relatives as soon as possible to advise him/her of the importance of continuing in the study and to ascertain whether or not s/he wishes to and/or should continue in the study. During the participant's planned study period, participants will be contacted by phone or visited at home if either not completing the questionnaire or not attending a planned visit, at least 3 times on different days. These contact attempts will be documented in a note in the Investigator Site File. Information on patient status, i.e. alive or dead, hospitalised, should be collected.

If all attempts to re-establish contact with the patient are unsuccessful, s/he will be considered to have withdrawn from the study and the primary reason for loss to follow-up will be recorded in the eCRF.

5. PROCEDURES

5.1 Obtaining informed consent

We will obtain informed consent from mild/moderate COVID-19 patients at the same time when they consent to participate in the master study (D0). For asymptomatic household contacts of the epidemiological ancillary study, we will obtain informed consent at the first visit in the hospital/health

centre (M1b), but patients can already be approached during the household visits of the follow-up period in the epidemiological ancillary study if infection status is known.

The informed consent procedure will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, etc. Participants will be informed that participation in the study is completely voluntary. They will also be informed that they may withdraw from the study at any time without any negative consequences. Patients will have the option to separately consent for and withdraw from this ancillary study.

No participant may be enrolled in the study and no study sample collection will take place until the study investigator or designee has obtained the informed consent. All informed consent procedures will be conducted by qualified staff members identified by the principal investigator and done in the language chosen by the participant. Participant Information Sheets and consent forms will be provided to the study participants for review. The informed consent form will be provided in the local language or a language understood by the patients. The participants will be given enough time to consider whether or not to participate in the study.

Participants will be informed that their samples may be analyzed and stored in other countries if centralized measurements are preferred.

Participants will be asked orally and in writing on the informed consent form to consent, as an optional tick box in the ICF, for long term storage of samples pertaining to the study (max 25 years after final study report, in some countries depending on the country/institutional legislation or guidelines it may be for a shorter duration and this will be specified in country specific ICFs) for future research related to infectious diseases, with the exception of genetic testing. Future testing may include repeat and confirmatory testing to arrive at a proper diagnosis, or additional research. Before samples will be used or shared for secondary research, approval from the sponsor and the ethics committee will need to be obtained. If proposed secondary research would be outside the scope of infectious diseases, the participants may be re-contacted and asked for IC as per IRB/EC advice. If a participant refused long-term storage, his/her samples will be destroyed after the submission of the final study report (end of the study).

The link between biological specimens and personal identifiers of the donors will at all times be secured by data management, and will not be shared/used in future pseudo-anonymized analyses unless patient safety is compromised.

5.2 Data collection
Relevant D0 to D21 patient data will be collected as part of the eCRF of the master protocol with the adjustments mentioned below, and continued to be collected in the additional immunology study specific visits (M1-M12).

<u>D0 until D21 (for mild/moderate patients)</u>:

No additional data will be collected than those demographic and clinical data, including medical history, described in the master protocol (described in 6.2.1 – master protocol), expect with a larger focus on infection history of HIV, TB, malaria, and malnutrition.

<u>M1/M1b until M12</u>:

The data listed below will be further collected in mild/moderate COVID-19 patients (from master study) or asymptomatic patients (from epidemiological study):

- Physical examination (Section 6.3.1.7 of Master Protocol
- Collection of vital signs (Section 6.3.3.2 of Master protocol)
- Collection of weight
- Update on any reported illness or comorbidities and concomitant medications as described in section 6.2.1 of the master protocol, including the outcomes of any illness episodes (hospitalization, outpatient visits) since last visit
- Any medical event due to participation in the study
- More detailed follow-up information on HIV, TB, malaria, and malnutrition
- Questionnaire for COVID-19 history including:
    o Collection of symptoms compatible with COVID-19 (see Section 6.3.1.4 of master protocol) since last visit
    o If experienced any symptoms, start and end date of symptoms
    o diagnosis of COVID-19 since last visit
    o contact with a person with confirmed or probable COVID-19 (if known)

5.3 Laboratory procedures

Blood samples will be collected in up to 3x 10 mL heparinized blood tubes, followed by isolation and liquid nitrogen cryopreservation of Peripheral Blood Mononuclear cells (PBMC) within 4-6 hours of collection. After the centrifugation step, plasma is concurrently collected, aliquoted and stored at -80°C or at a minimum of -20°C. The volume of 30 mL of blood is required to ensure sufficient cell numbers in the planned cellular assays (see section below) to be able to evaluate the SARS-CoV-2-specific T cell responses, including low-abundant antigen-specific T cells.

In study sites or at timepoints requiring only antibody evaluations, up to 200 μL of finger-prick blood samples will be collected with a heparin microtainer, followed by centrifugation within 6 hours of collection. Plasma will be aliquoted and subsequently stored at -80°C or at a minimum of -20°C.

Procedures will be standardized with Standard Operating Procedures (SOPs) for all sites.

Samples will be biobanked at each respective site according the local biobanking laws, unless requested by the site to be centrally biobanked at ISGlobal or ITM, in which case biobanking will adhere to Belgian or Spanish biobanking laws.

Planned assays

Assays for the antibody measurements will be performed in-country or centralized in case infrastructure and capacity to perform the assays is lacking or for quality monitoring purposes. Assays for the cellular measurements will be performed in a centralized lab to avoid inherent variation due

to different flow cytometers and settings. Local researchers will always have the possibility to travel to the centralized labs to learn the techniques and to personally process/analyze the samples of the researcher's site. For the exploratory objectives, requiring specialized labs, samples will be shipped to partners within the consortium once the necessary funding has been acquired. Material Transfer agreements (MTA) will be fully signed and approved upfront by all involved organizations before shipment of any samples to other centers/countries and submitted to EC depending on the countries' requirements. Samples will be shipped in accordance with postal regulations, such as IATA (International Air Transport Association) and ADR (European Agreement on International Carriage of Dangerous Goods), with official couriers.

Antibody responses:
- Primary objectives: MFI levels of IgM, IgG and IgA against 5 or more SARS-CoV-2 antigens, including RBD, will be measured by a multiplex Luminex assay. Seropositivity will be determined using a threshold of antibody levels estimated with negative controls (pre-pandemic if possible) and/or using finite mixture models. We will collaborate with the different consortium partners, FIND and international reference standards (e.g. NIBSC) to establish a collection of adequate pre-pandemic biological samples to be used as control samples. The use of these biobanked control samples will be part of a separate study or amendment and submitted to all relevant ECs once identified. Levels (MFI) of IgM, IgG and IgA against other coronaviruses (229E, HKU1, NL63 and OC43) will be measured by Luminex in a separate (secondary objectives) or the same SARS-CoV-2 Luminex panel. Levels of IgE, IgA and IgG subclasses (IgA1, IgA2,IgG1, IgG2, IgG3 and IgG4) against SARS-CoV-2 antigens may also be assessed in secondary analyses if relevant data on their role in the immune response to SARS-CoV-2 becomes available in European, US and Asian ongoing studies.
- Secondary objectives: Past/current exposure to infections (e.g. helminths, malaria) will be assessed by antibody serology using the Luminex assay and combined with all known clinical data on coinfections to study the impact of comorbidities. Autoantibodies will be measured by serology in a separate or in the same SARS-CoV-2 Luminex panel as above. Avidity will be assessed in Luminex assays using a chaotropic agent. Neutralization assays with SARS-CoV-2 will be performed using a plaque reduction neutralization test or similar in a centralized lab.
- Exploratory objectives: The fine epitope specificity of anti-SARS-CoV-2 IgM, IgG and IgA response will be analyzed with peptide microarrays and/or Luminex in a centralized lab.

Cellular responses:
- Primary and secondary objectives: The frequency, phenotype and (poly)functionality of SARS-CoV-2-specific CD4+ and CD8+ T cells will be assessed by *ex vivo* stimulation of thawed PBMCs with SARS-CoV-2 peptide pools followed by multi-colour flow cytometric evaluations or enzyme-linked immune absorbent spot (ELIspot) assays (performed in a centralized lab). For flow cytometry, the panels will be targeted on CD4:8 subsets, effector/memory differentiation, effector production (e.g. IFN-y, IL-2, IL-4, IL-13, IL-6, IL-17, CD107a) and activation induced or exhaustion markers (OX40, CD137, PD1). Positivity will be determined by comparing responses to non-stimulated samples and negative blood donor controls. If required, after magnetic cell enrichment, highly sensitive IFN-y and perforin Elispot assays will be used to define the frequency of low-abundant antigen-specific CD4+ and CD8+ T cells. Likewise, viral inhibition assays will be performed to determine the cells direct ability to inhibit viral replication. As vital knowledge on effector functions is being generated in similar studies across Europe, US and Asia, the literature will be continuously reviewed, and selected markers updated accordingly.

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> The patient's inflammatory status will be monitored in plasma samples by Luminex and/or ELISA.

- Exploratory objectives: PBMCs will be biobanked to perform additional SARS-CoV-2-specific TCR repertoire analyses, single cell RNAseq analyses and development of human monoclonal antibodies from participants with antibodies with high neutralizing capacity (performed in centralized lab).

Detection of SARS-CoV-2 infections**:**

- Secondary objective: Direct detection of SARS-CoV-2 will be performed by PCR/RDT in saliva samples in a quantitative or qualitative assay.

Quality assurance and quality control (QC/QA)

Before analysis of study samples, all assays and labs performing the antibody and cellular assays will undergo a qualification process after which a report will be shared with the consortium and submitted to all required ECs. The Luminex antibody assay has been developed and optimized at ISGlobal and will be adapted for use on the Luminex MagPix machine available at the participating sites. It will be transferred to the sites with proper training and site qualification. For antibody and cellular assays SOPs, QA/QC, monitoring plans and control samples will be used to ensure high-quality data. In addition to the Sponsor's monitoring visits, regular laboratory monitoring activities will be performed at each participating site concerning PBMC sample quality (cell yield/viability). For Luminex antibody assays, positive control samples (pool of samples) and negative samples will be used in all sites for QA/QC and to ensure comparability. Weekly assay reports will be evaluated for QA/QC purposes.

Biobanking, Material Transfer agreement and availability of materials

Samples will be biobanked at the respective sites and monitored by detailed inventory lists and temperature logs at site, unless central biobanking at ISGlobal/ITM is requested by the site by which relevant biobanking laws will be stipulated in the respective Material Transfer Agreement.

Besides local measurements, some samples will be sent to ITM and ISGlobal to perform centralized immunological assays as stipulated above. In this case, left-over materials can be biobanked for delayed QC at those institutions and will adhere to local biobanking legislation. All sample shipments will be preceded by a Material Transfer Agreement (aligned with country regulatory requirements) that will describe the sharing of data and derived results. The latter includes that all data and results and all intellectual property rights in the data and results derived from the study will be the property of the Sponsor who may utilise them in various ways, such as for submission to regulatory authorities or shared with research institutions through IDDO in the first instance and other mechanisms as needed. The Sponsor grants Recipient and Provider of materials a royalty-free non-exclusive license to use the Results, including any resulting Intellectual Property, for further non-commercial research and education. We do not expect unsolicited findings that require to be disclosed.

Any party from the consortium may request access to the materials for future research. Access to materials will be regulated by the general access principles and after the Consortium JSC agreement including the scientific leads of both ancillary studies. Future studies on biobanked samples will be separately approved by the Ethics Committee, according to local legislation.

5.4 Safety assessments

Since the specific study procedures entail minimal risk and no drugs will be administered as part of this ancillary study, no reportable adverse events are expected from finger prick, venous blood collection and respiratory/saliva sample collection, and no pharmacovigilance will be performed.

6. STATISTICAL METHODS

6.1 Statistical analysis

6.3.1 Analysis populations

We will analyze the data both using Intention-to-Treat and Per-Protocol approaches, with Intention-to-Treat as primary approach. In the Intention-to-Treat analysis, all patients will be analyzed according to their randomized allocation, even in case they receive another treatment regimen, show protocol violations prior to or during the study, or are lost-to follow. In the per-protocol analysis only patients who receive study drug as planned, have complete follow-up and follow the protocol as planned are included.

6.3.2 Baseline characteristics

Patients in each treatment group, pooled and by site, will be described with respect to baseline characteristics. The clinical importance of any imbalance will be noted though statistical tests of significance will not be undertaken.

6.3.3 Primary Analysis

Time between onset of symptoms and positive antibody response will be compared between treatment arms using Cox proportional hazards models. Antibody levels (MFI) at day 21 will be compared between treatment arms, using linear regression adjusted for site and other study variables (sex, age, etc). Log transformed T-cell frequencies at day 21 will be compared between treatment arms, using linear regression adjusted for site and other study variables. Linear mixed effects models will be used to model the kinetics of antibody levels and log transformed T-cell levels over time. Longevity will be compared between treatment arms using Cox proportional hazards models.

6.3.4 Secondary and exploratory analysis

Antibody and cellular responses will be compared between mild patients and asymptomatically infected household contacts using linear mixed effects models. Association between avidity and neutralizing capacity of antibody responses and treatment will be estimated using mixed effects models. A joint model for the time to reinfection will be fitted with antibody and cellular responses as time-varying independent variables. Association of antibody and cellular responses with comorbidities will be estimated using linear mixed effects models. A logistic regression model will be fitted with treatment outcome as dependent variable and baseline antibody and cellular characteristics as independent variables.

6.3.5 Subgroup analyses

No Subgroup analyses are planned.

6.3.6 Multiplicity and Missing Data

No multiplicity adjustments are needed. For each objective, the proportion of missing information and potential biases due to missing data will be assessed. The analyses will be based on available data, but the adequacy of imputation methods may be considered. Sensitivity analyses will be performed to assess the impact of missing data on the results. The handling of missing data will be specified in the SAP.

7. DATA MANAGEMENT

The ancillary study will adhere to the data management procedures of the master protocol (section 9 in master protocol).

7.1 Data collection

Investigational sites will be provided with access to electronic case report forms (eCRFs) in which to record protocol-specified data for patients in the study. Data will be entered in the forms by authorised personnel at the investigational centre and should be verifiable against source documents. Some data items may be entered directly into the eCRF and, in which cases, the entry in the eCRF will be considered as the source data.

Separate patient logs will also be kept of all patients recruited in this ancillary study. In all protocol-specified data, patients will be identified only by the coded numbers from the master protocol in order to maintain confidentiality and ensuring easy patient identification across studies. The exceptions are SARS-CoV-2 testing results, which may be subject to local and/or state reporting requirements, potentially on a named basis.

The Investigator will be responsible for reviewing all data and entries in the reporting forms and will verify that the information is true and correct. Data cleaning will be performed on an ongoing basis. Queries may be generated by the Study Monitor or Data Management personnel to request clarification of data items or provision of missing data. Queries will be sent to the investigational site electronically. The Investigator is responsible for the review and approval of all responses to queries.

The collected respiratory and blood samples are planned to be stored for a maximum of 25 years after study completion to be used for future scientific research. In some countries depending on the country/institutional legislation or guidelines it may be for a shorter duration, as documented in country specific ICFs. Such future studies will be separately approved by the Ethics Committee in advance, according to local legislation. Participants will have the option to separately consent for the long-term storage of their samples by means of a tick box in the ICFs. If participants do not agree, their biological samples will be discarded at the end of the study.

Biological samples can be sent for analysis and storage to ISGlobal and ITM as indicated above. In no case, personal identification data will be shared, and local regulations will be followed with regard to shipment and biobanking.

7.2 Data Handling on Sites

The Investigator must maintain adequate and accurate records at the investigational centre to ensure that the conduct of the study is fully documented and that study data can subsequently be checked.

These documents include the Investigator Site File, source documents for study data, patient logs and results of assays.

Investigator Site File

The Investigator Site File will contain the protocol and any protocol amendments, a paper copy of the eCRF, query forms, ethics committee and regulatory approval with correspondence, sample of informed consent form, staff curriculum vitae and authorisation forms and any other appropriate documents, file notes and correspondence as appropriate.

The Investigator Site File will be stored safely and securely so as to ensure that the documents are readily available upon request from the regulatory authorities.

Source Documents

The Investigator must maintain source documents for possible review and/or audit by the Sponsor and/or regulatory authorities. Source documents may include patient PCR test result, original laboratory reports, signed ICFs, Luminex raw data, PBMC cell counts and viability data.

Participants' Logs

Participants Logs will be maintained trhough the study and an additional Log for the immunology study will be kept.

7.3 Data Processing

Processing of study data will be performed in accordance with standard operating procedures and the Data Management Plan for the study, using a validated database.

7.4 Missing Data

For the analyses of missing data, see Section 6.3.6.

7.5 Study Monitoring

In addition to the clinical monitoring visits by the sponsor, regular laboratory monitoring activities will be performed at each participating site concerning PBMC sample quality (cell yield/viability) and Luminex antibody assays. For the latter, positive control samples (pool of samples) and negative samples will be used in all sites for QA/QC and to ensure comparability. Weekly assay reports will be evaluated for QA/QC purposes.
The frequency of the on-site laboratory monitoring visits and data checks will be decided by the Immunology Scientific co-leads and Sponsors and defined in the Monitoring Plan.

The Investigator agrees to allow the monitors direct access to all study-related documents.

7.6 Data Archiving

The study documents will be archived according to local regulations and in accordance with the maximum period of time permitted by the investigational centre. Where the archiving procedures do not meet the minimum timelines required by the Sponsor, i.e. 25 years, alternative arrangements must be made to ensure that the study documents are archived for the required period. The Investigator or head of the medical institution will notify the Sponsor of any change in the arrangements for archiving,

e.g. relocation or transfer of ownership. The study documents are not to be destroyed without the Sponsor's approval.

7.7 Audit and Inspection

To ensure compliance with ICH GCP E6 and local GCP requirements if applicable, a member of the Sponsor's quality assurance team, or a designated contract research organisation, may arrange to perform an audit to assess the conduct of the study at the investigational centre and the handling of the study documents originating there. The Investigator/institution will be informed of the audit findings.

In addition, inspections by regulatory authority and ethics committee representatives are possible. The Investigator should notify the Sponsor immediately of any such inspection.

The Investigator/institution agrees to allow the auditor or inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any issues. Audits and inspections may occur at any time before, during or after completion of the study.

8. ETHICAL AND LEGAL ASPECTS

The ancillary study will adhere to the legal/ethical aspects of the master protocol (section 10 to 12 in master protocol).

8.1 Investigator and Other Study Personnel

All study personnel not specifically referred to in this protocol are identified in a separate list of study personnel. The list will be updated as needed. An abbreviated version with personnel relevant for the centre will be available in the Investigator Site File of the investigational centre.

Whenever the term 'Investigator' is used in the protocol, it may refer to either the Principal Investigator at the investigational centre or an appropriately qualified, trained and delegated individual in the investigational centre.

The Principal Investigator must sign the protocol signature page and must receive all required external approvals, i.e. regulatory authorities, ethics committee, Sponsor, before patient recruitment may begin at the investigational centre. Likewise, all amendments to the protocol must be signed by the Principal Investigator and must have received all required external approvals before coming into effect at the investigational centre.

8.2 Infectious risk

All site staff must take into consideration National Guidance including relevant Government Directives aimed at mitigating the spread of COVID-19 disease. Sponsors will provide Personnel Protection Equipment to clinical staff and patients. In all study procedures that involve physical contact with the participants, additional measures should be taken to factor in participants' opinions and concerns and safeguard against penalties for refusal of participation/ continuation. For visits to the hospital, the sponsors will ensure that the mode of transportation will not expose others to a risk of infection. The ICF contains a section regarding infectious risks and transportation.

8.3 Funding of the Study

The study will be funded by the Sponsors. The study is being conducted by a consortium of partners who may support the study by providing services or study materials, free of charge.

8.4 Costs for Patients

The patients' travels costs to and from the investigational centre should be covered depending on the particular practices in the centre. Missed days of work due to participation in the study may be compensated, depending on local procedures and if permitted by the regulatory authorities and/or EC. This will be clearly stipulated in the country-specific ICF. Food during the quarantine period in hospitals or governmental facilities and any overnight stays at the investigational centre will be provided free of charge to the patient.

During the D0-D21 period, any medication that is required during the study will be provided free of charge. In the event of AEs, reasonable and necessary medical treatment will be provided free of charge by the Investigator at the investigational centre as stipulated in the master protocol. If the condition requires treatment at another hospital and is a direct result of participation in the study, the cost will be paid by the Sponsor.

During the immunological observational follow-up period M1-M12, all study related procedures and tests will be done for free and patients will be referred to national care programs in case of SARS-CoV-2 reinfection or deterioration of health condition.

8.5 Risks and benefits

The collection of up to 200 μL of blood by finger prick, 30 ml of blood by venipuncture or the collection of saliva has minimal risks for the participant with temporary pain at the vein puncture site, but the quantity of blood is too little to affect wellbeing of the patient. The participant can contact at any time the study team for any concern.

There are no large benefits to the participant, outside a close health monitoring during the follow-up period of the study and possible reassessment of SARS-CoV-2 reinfection. In addition, they will receive proper advice on how to protect themselves against SARS-CoV-2 reinfection. However, results will provide valuable data on the induction and maintenance of immune responses against SARS-CoV-2, needed for informing control strategies and development of vaccines against SARS-CoV-2 across sub-Saharan Africa.

All results from SARS-CoV-2 serological testing will not be automatically communicated to the participants because 1) serological testing will be batched therefore often delayed, possibly for several months, therefore of no clinical relevance 2) it remains unclear if a positive or negative individual result equates to protection or not and therefore the result per se has no clinical implications. The information could potentially be misinterpreted and lead to harmful behavior (e.g. asked to care for COVID-19 cases because they are thought to be immune). We will communicate serologic results to all participants that request the results at the end of the study or at the end of the sample analysis as per ICF or according to National Guidelines.

8.6 Ethical and Legal Conduct of the Study

Investigational centres have been selected to ensure that patients will have appropriate rescue management in the event of disease progression.

The procedures set out in this protocol, pertaining to the conduct, evaluation and documentation of the study, are designed to ensure that the Sponsor and Investigator abide by GCP guidelines and the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in accordance with applicable local laws and regulations.

Documented approval from the regulatory authorities and ethics committee will be obtained before the start of the study, in accordance with GCP, local laws and regulations. When necessary, an

extension, amendment or renewal of the approval from the regulatory authorities must be obtained and forwarded to the Sponsor. The responsible unit (e.g., the regulatory authorities, head of the investigational centre/medical institution) must, upon request, provide the Sponsor with a list of the members of the ethics committees involved in the vote and a statement to confirm that the ethics committees are organised and operate according to GCP and applicable laws and regulations.

Strict adherence to all specifications laid down in the protocol is required for all aspects of study conduct. The Investigator may not depart from the procedures described in the protocol. Any deviations from the protocol must be explained and documented by the Investigator.

8.7 Patient Information and Informed Consent
All relevant information on the study will be summarised in a patient information document and informed consent form, provided by the Sponsor or the investigational centre. Patients will be included in the study only after written informed consent is obtained from the patient (section 5.1).

8.8 Publication Policy and Use of Study Data
The immunology study is an ancillary study of the clinical trial that will be registered with a recognised clinical study registry, e.g. www.clinicaltrials.gov, Pan African Clinical Trials Registry.

All data and results and all intellectual property rights in the data and results derived from the study will be the property of the Sponsor who may utilise them in various ways, such as for submission to regulatory authorities or shared with research institutions through IDDO in the first instance and other mechanisms as needed. Access to data will be regulated by an Independent Data Access Committee following the general governance principles of IDDO and in line with the Policy Statement by the WHO in the context of Public Health Emergency.

The final Immunology Report will be submitted to the ethics committee and regulatory authorities, if required.

The Sponsor encourages the communication and/or publication of the results, in accordance with the terms of the Clinical Trial Agreement.

Any reliable interim findings will be disseminated rapidly by the consortium of partner conducting the study and will be published in the names of the partners of the consortium.

8.9 Insurance
The Sponsor will cover any claim made by a patient for damage resulting directly from the study and will also indemnify the Investigator against any liability resulting from such. Coverage of damage to the patient and indemnification of the Investigator will not apply if the damages to the patient result from negligence or intentional misconduct by the Investigator.

8.10 Confidentiality and Protection of Privacy

All records identifying the patient will be kept confidential and, to the extent permitted by the applicable laws and regulations, will not be made publicly available.

Patient names will not be supplied to the Sponsor. Names will not be entered in the eCRF or any document or digital file supplied to the Sponsor. The patient identification number and randomisation number will be used as identification and, if the patient's name appears on any other document or file it will be redacted before a copy of the document is supplied to the Sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. As part of the informed consent process, the patients will be informed in writing that representatives of the Sponsor or regulatory authorities may inspect their medical records to verify the information collected, and that

all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the participant's identity will remain confidential.

The Investigator will maintain master patient log to enable patients to be identified.

8.11 Incidental findings
In case of a postive result of the SARS-CoV-2 saliva PCR during study follow-up the participant will be contacted by the study personnel and national guidelines for COVID-19 diagnosed cases will be followed. We do not expect additional unsolicited findings that require to be disclosed.

9. REFERENCES

1. Ma H, Zeng W, He H, Zhao D, Yang Y, Jiang D, et al. COVID-19 diagnosis and study of serum SARS-CoV-2 specific IgA, IgM and IgG by a quantitative and sensitive immunoassay. medRxiv. 2020;

2. Hsueh P-R, Huang L-M, Chen P-J, Kao C-L, Yang P-C. Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. Clin Microbiol Infect. 2004 Dec;10(12):1062–6.

3. Garcia-Basteiro AL, Moncunill G, Tortajada M, Vidal M, Guinovart C, Jimenez A, et al. Seroprevalence of antibodies against SARS-CoV-2 among health care workers in a large Spanish reference hospital. medRxiv. 2020 May 2;2020.04.27.20082289.

4. Marois I, Cloutier A, Garneau É, Richter M V. Initial infectious dose dictates the innate, adaptive, and memory responses to influenza in the respiratory tract. J Leukoc Biol. 2012 Jul;92(1):107–21.

5. Hare CB, Pappalardo BL, Busch MP, Karlsson AC, Phelps BH, Alexander SS, et al. Seroreversion in Subjects Receiving Antiretroviral Therapy during Acute/Early HIV Infection. Clin Infect Dis. 2006 Mar 1;42(5):700–8.

6. Connick E. Incomplete Antibody Evolution and Seroreversion after Treatment of Primary HIV Type 1 Infection: What Is the Clinical Significance? Clin Infect Dis. 2005 Mar 15;40(6):874–5.

7. Manak MM, Jagodzinski LL, Shutt A, Malia JA, Leos M, Ouellette J, et al. Decreased Seroreactivity in Individuals Initiating Antiretroviral Therapy during Acute HIV Infection. Loeffelholz MJ, editor. J Clin Microbiol. 2019 Jun 19;57(10).

8. Wilder-Smith A, Teleman MD, Heng BH, Earnest A, Ling AE, Leo YS. Asymptomatic SARS Coronavirus Infection among Healthcare Workers, Singapore. Emerg Infect Dis. 2005 Jul;11(7):1142–5.

9. Becquart P, Mahlakõiv T, Nkoghe D, Leroy EM. Identification of Continuous Human B-Cell Epitopes in the VP35, VP40, Nucleoprotein and Glycoprotein of Ebola Virus. Kuhn JH, editor. PLoS One. 2014 Jun 10;9(6):e96360.

10. Hoff NA, Mukadi P, Doshi RH, Bramble MS, Lu K, Gadoth A, et al. Serologic Markers for Ebolavirus Among Healthcare Workers in the Democratic Republic of the Congo. J Infect Dis. 2019 Jan 29;219(4):517–25.

11. Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020 Apr 29;1–4.

12. Tan W, Lu Y, Zhang J, Wang J, Dan Y, Tan Z, et al. Viral Kinetics and Antibody Responses in Patients with COVID-19. medRxiv. 2020;

13. Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv. 2020 Apr 20;2020.03.30.20047365.

14. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Rydyznski Moderbacher C, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and

unexposed individuals. Cell. 2020;

15. Zheng H-Y, Zhang M, Yang C-X, Zhang N, Wang X-C, Yang X-P, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. Cell Mol Immunol. 2020 May 17;17(5):541–3.

16. Channappanavar R, Fett C, Zhao J, Meyerholz DK, Perlman S. Virus-Specific Memory CD8 T Cells Provide Substantial Protection from Lethal Severe Acute Respiratory Syndrome Coronavirus Infection. J Virol. 2014 Oct 1;88(19):11034–44.

17. Li CK, Wu H, Yan H, Ma S, Wang L, Zhang M, et al. T Cell Responses to Whole SARS Coronavirus in Humans. J Immunol. 2008 Oct 15;181(8):5490–500.

18. Huang AT, Garcia-Carreras B, Hitchings MDT, Yang B, Katzelnick L, Rattigan SM, et al. A systematic review of antibody mediated immunity to coronaviruses: antibody kinetics, correlates of protection, and association of antibody responses with severity of disease. medRxiv. 2020 Apr 17;2020.04.14.20065771.

19. Kollmann TR. Variation between populations in the innate immune response to vaccine adjuvants. Front Immunol. 2013 Apr 2;4(APR):81.

20. Quach H, Rotival M, Pothlichet J, Loh YHE, Dannemann M, Zidane N, et al. Genetic Adaptation and Neandertal Admixture Shaped the Immune System of Human Populations. Cell. 2016 Oct 20;167(3):643-656.e17.

21. Haralambieva IH, Salk HM, Lambert ND, Ovsyannikova IG, Kennedy RB, Warner ND, et al. Associations between race, sex and immune response variations to rubella vaccination in two independent cohorts. Vaccine. 2014 Apr 7;32(17):1946–53.