PROJECT:

PHASE IV STUDY TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF THE COVID-19 ADSORBED (INACTIVATED) VACCINE OF THE INSTITUTO BUTANTAN IN IMMUNOCOMPROMISED HOSTS

Researchers:

Ana Marli Christovam Sartori (Principal Investigator) [1,2,3], Amanda Nazareth Lara [1,2], Camila Cristina Martini Rodrigues [1,2], Camila Picone [2], Marta Heloisa Lopes [1,2], Lígia Câmera Pierrotti [2], Edson Abdala [3,10], Karim Yaqub Ibrahim [2,10], Sílvia Figueiredo Costa [1], Luiz Sergio Azevedo [4] , Elias David-Neto [4] , William Carlos Nahas [4], Fabiana Agena [4], Alice Tung Wan Song [5], Luiz Augusto Carneiro D'Albuquerque [5], Fernando Bacal [6], Tânia Mara Varejão Strabelli [7], Silvia Vidal Campos [8], Vanderson Geraldo Rocha [9], Alberto José da Silva Duarte [11], Gil Benard [12], Paula Keiko Sato [13], Maria Isabel de Moraes Pinto [14], Carolina Sanchez Aranda [15], Maria Teresa de Sande e Lemos Ramos Ascensão Terreri [16], Alexander Precioso [17], Vanessa Infante[17], Patricia Emília Braga [17], Juliana Yukari Kodaira Viscondi [17], Sandra Coccuzzo Sampaio Vessoni[18], Isabelle Valle dos Anjos [18], Monique da Rocha Queiroz Lima [18], Jonatas William Silva [18], Guilherme Rabelo Coelho [18]

Participating Institutions

1. Centro de Referência para Imunobiológicos Especiais do Hospital das Clínicas da Faculdade de Medicina da USP (HC-FMUSP), São Paulo, Brazil
2. Divisão de Clínica de Moléstias Infecciosas e Parasitárias, HC-FMUSP, São Paulo, Brazil
3. Departamento de Moléstias Infecciosas e Parasitárias da Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil
4. Serviço de Transplante Renal (STR) do HC-FMUSP, São Paulo, Brazil
5. Divisão de Transplante de Fígado e Órgãos do Aparelho Digestivo, HC-FMUSP, São Paulo, Brazil

6. Núcleo de Transplantes do Instituto do Coração (INCOR), HCFMUSP, São Paulo, Brazil
7. Subcomissão de Controle de Infecção Hospitalar do INCOR, HCFMUSP, São Paulo, Brazil
8. Serviço de Pneumologia Unidade de Transplante de Pulmão, HCFMUSP, São Paulo, Brazil
9. Serviço de Hematologia, Hemoterapia e Terapia Celular, Divisão de Clínica Médica I, HC-FMUSP, São Paulo, Brazil
10. Instituto do Câncer do Estado e São Paulo (ICESP), São Paulo, Brazil
11. Divisão de Laboratório Central (DLC), HCFMUSP, São Paulo, Brazil
12. Laboratório de Micologia Médica (LIM-53) da Divisão de Clínica Dermatológica, HC / Instituto de Medicina Tropical da FMUSP, São Paulo, Brazil
13. Laboratório de Investigação Médica – Imunologia (LIM-48) da Divisão de Clínica de Moléstias Infecciosas e Parasitárias, HCFMUSP, São Paulo, Brazil
14. Laboratório de Pesquisas, Disciplina de Infectologia Pediátrica, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil
15. Disciplina de Alergia, Imunologia Clínica e Reumatologia do Departamento de Pediatria da Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil
16. Centro de Farmacovigilância, Segurança Clínica e Gestão de Risco, Instituto Butantan, São Paulo, Brazil
17. Laboratório Estratégico de Diagnóstico Molecular / Sorologia, Instituto Butantan, São Paulo, Brazil

ABSTRACT

In March 2021, Brazil reached more than 12 million confirmed cases and more than 300 thousand deaths related to COVID-19, becoming the epicenter of the pandemic in the world. A huge world scientific and financial investment allowed the rapid development of COVID-19 vaccines. Seven vaccines have been licensed or approved for emergency use, so far. Four of them have been approved for use in Brazil by the National Regulatory Agency (ANVISA): the inactivated virus COVID adsorbed vaccine (CoronaVac), produced by Sinovac / Instituto Butantan, two non-replicating viral vector vaccines, the one produced by Oxford / AstraZeneca / Instituto BioManguinhos and another produced by Janssen, and the Pfizer RNAm vaccine. Data on the safety, immunogenicity and efficacy of COVID-19 vaccines in immunocompromised persons are scarce. The objective of this study is to evaluate the safety and immunogenicity of the inactivated virus COVID vaccine (CoronaVac) in non-HIV/AIDS immunocompromised people.

Methods: This open-label, non-randomized, phase IV clinical trial will include 1,370 participants aged 18 to 59 years, of which 600 solid organs transplant recipients (kidney [n=300], liver [100], heart [100] and lung [100]); 100 patients with chronic kidney disease; 50 patients with liver cirrhosis before organ transplantation; 100 hematopoietic stem cell transplant recipients; 300 cancer patients, 100 people with innate immunity errors and 20 patients with immune-mediated rheumatological disorders; 100 healthy people of the same age group (comparator group) will also be included. All participants will receive two doses of the CoronaVac with 28-day interval between doses. Vaccination will be carried out at the HC-FMUSP Reference Center for Special Immunobiologicals. Participants clinical data will be retrieved from the medical records. Local and systemic, solicited and unsolicited, adverse events will be monitored, within 7 days after each dose of the vaccine, through a diary recorded by the participant and telephone contact. Serious adverse events and adverse events of special interest (EAIE) will be monitored throughout the study period. The immunogenicity of the vaccine will be assessed by measuring IgG antibodies against the nucleocapsid (N), the S1 and S2 portions of the spike (S) protein, and the SARS-CoV2 spike protein receptor-binding domain (RBD). Serology will be performed at the Strategic Laboratory for Molecular / Serology Diagnosis of the Instituto Butantan. Five blood samples will be collected for

serology: pre-vaccination (M0), before the 2nd vaccine dose (M1), 28 days after the 2nd vaccine dose (M2), six months (M7) and 12 months after completing vaccination (M13). Participants will be monitored for disease by SARS-CoV2 during the entire 13-month follow-up, through monthly contact by phone or electronic message. In case of symptoms of COVID, the participants will be seen at the clinic where they are followed and, if necessary, referred to the laboratory for nasopharyngeal sample collection for PCR-COVID. In case of positive PCR, part of the sample will be saved for subsequent genetic sequencing.

Key words:

COVID-19; Vaccines; Immunogenicity, Vaccine; Safety; Adverse events; Immunocompromised hosts; Transplant recipients; Chronic kidney disease; Liver cirrhosis; Neoplasms; Primary immunodeficiency diseases

1. Introduction

In December 2019, an outbreak of pneumonia started in Wuhan, Hubei province, central China. Most of the initial cases appeared to have been in contact with a local seafood market, but later inter-human transmission was demonstrated (1). The disease appeared to be caused by viruses, according to clinical and laboratory criteria, such as leukopenia and lymphopenia, in addition to pulmonary infiltrates that did not improve with antibiotics. A new coronavirus, later named SARS-CoV-2, was isolated from some samples of bronchoalveolar lavage fluid from Chinese patients (1, 2). Attempts to contain the virus were made, such as large-scale testing, cases and cities isolation, contact tracking and quarantine, and travel restrictions. But, the measures were not enough to prevent the virus from reaching Europe and North America and establishing community transmission on these continents in the late 2019 / begin of 2020 (3).

The first case of COVID-19 was detected in Brazil on February 25, 2020, in São Paulo state. After emerging in large urban centers, the virus spread quickly and sustainably to municipalities with smaller populations. The lack of adequate containment measures, such as lockdown, difficulties in surveillance and restricted and unequal access to testing and diagnosis contributed to the rapid virus spread in the whole country (4). In addition, more than 100 international virus introductions have been identified in Brazil (5).

More than 128 million confirmed cases and more than 2.8 million deaths from COVID-19 were reported in the world up to March 2021. The Americas region is the hardest hit with almost half of the reported cases and deaths. The United States and Brazil have the greatest number of cases and deaths in the region and in the world. More than 12 million confirmed cases and more than 310,000 deaths were reported in Brazil up to March 2021 [https://covid.saude.gov.br/]. The numbers of COVID-19 new cases and deaths have been decreasing worldwide, in the first months of 2021, but Brazil registered an important increase in the number of cases and deaths, experiencing its worst moment in the pandemic, so far. In March 2021, Brazil became the epicenter of the COVID-19 pandemic in the world, posing a risk to the countries in the region (6).

SARS-CoV2 is a beta-coronavirus. Its nucleotide sequence has high similarity (88 to 96%) with bat coronaviruses, from which it probably originated, through a jump between species, with an intermediate host being a possibility (7). It also shows 79% similarity to SARS-CoV that caused an epidemic of severe acute respiratory syndrome (SARS), initially identified in China in November 2002, and spread to 29 countries, with more than 8,000 cases up to May 2004; and with MERS-CoV (Middle East respiratory syndrome-related coronavirus), identified in Saudi Arabia in June 2012, which caused a SARS outbreak with more than 2,500 cases and high case-fatality (approximately 34%) in 24 countries.

Coronaviruses are transmitted by respiratory droplets. Like SARS-CoV and MERS-CoV, SARS-CoV2 uses the angiotensin-converting enzyme receptor (ACE-2) to enter the host cell, however SARS-CoV-2 has a greater affinity for ACE-2 being more transmissible. SARS-CoV2 basic reproduction rate (R0) varies from 2 to 3, according to the containment measures.

After an incubation period of 4 to 5 days (varying from 1 to 14 days), COVID-19 is clinically manifested with fever, headache, myalgia, fatigue and cough, which may progress to more severe conditions, with dyspnea or difficulty to breath. Clinical worsening with SARS generally occurs in the second week of the disease (8 to 12 days after the symptoms onset) (8). Extrapulmonary manifestations are frequent, mainly loss of smell and taste, intestinal manifestations (diarrhea, nausea and vomiting) and thromboembolism, in addition to cardiac, renal, neurological and skin involvement (9).

Population-based studies showed 30% to 40% of infections are asymptomatic. Of the symptomatic cases, 81% have mild disease; 14% have moderate to severe disease, requiring hospitalization and oxygen support; and 5% have critical illness, with respiratory failure, shock and multiple organ dysfunction, requiring intensive care and advanced ventilatory support. The overall case-fatality is about 2.3% (8, 10).

Several factors are associated to the disease greater severity, mainly advanced age. An analysis of hospitalized confirmed COVID-19 cases aged ≥20 years that occurred in the first five months of the pandemic in Brazil, the overall case-fatality was 38%; increasing to 80% among those who needed mechanical ventilation (11). Case-fatality

increased with age, from 12% among patients aged 20 to 39 years to 66% in those aged more than 80 years. Comorbidities, such as diabetes, heart disease and obesity, among others, contribute to a greater severity of cases and death (12).

The role of immunosuppression as a risk factor for COVID-19 severity is unclear. Cancer and recent cancer therapy, including immunotherapy and tyrosine kinase inhibitors, seem to lead to an increased severity and death, as compared to persons without cancer (13). There are some evidence of increased disease severity and mortality among solid organ transplant recipients. Patients using biological therapies do not appear to be at an increased risk for serious illness (13). The ideal management of these patients, including changes in immunosuppressive regimens, is unknown (13). In the United States, a retrospective study involving 2,121 individuals, of whom 108 immunosuppressed, found no significant differences in the need of mechanical ventilation, case-fatality and length of hospital stay between persons who were immunosuppressed or not (14). It is also unclear whether HIV leads to an increased risk of severe COVID-19. An observational study conducted in Madrid evaluated 63 persons living with HIV, and found no association between the disease severity and HIV-related factors. Age and comorbidities were the main determinants of COVID-19 severity (15).

In Brazil, COVID-19 mortality was greater in the North (50%) than in the South (31%), being influenced by the health system regional disparities (11). The Brazilian universal public health system (*Sistema Único de Saúde, SUS*) was essential during the pandemic. However, regional disparities, such as differences in the number of available hospital beds and healthcare workers training, and the health system failure to rapidly respond to the increasing number of cases led to its collapse. The imbalance between the speed at which the disease spread and the pace at which the health system expanded led to the health system disrupt in Ceará state, in the Northeast of the country, at the beginning of the pandemic (16). In addition to regional disparities, social inequalities also impacted the disease mortality. A study in São Paulo city showed that black, low-educated and poor people have greater risk of dying for Covid, “exposing structural inequities in the Brazilian society that were not addressed by the government's response to COVID-19” [Ribeiro KB et al. Int J Epidemiol. 2021; doi: 10.1093/ije/dyab022].

Since the late 2020, new variants of SARS-CoV2 have been described. Currently, there are three variants of concern (VOC): B.1.1.7 (first described in England), B.1.351 (described in South Africa) and P.1 (initially recognized in Japan among travelers from Brazil) (17-19). All three variants have mutations in the spike viral protein (S), which has 2 subunits, S1 and S2. The S1 subunit contains the receptor binding domain (RBD) portion that binds to the ACE2 receptor, allowing SARS-CoV2 to enter the cell. All three variants have been described as more transmissible and may cause overload on health systems.

P.1 variant was identified in 42% of COVID-19 confirmed cases in Manaus, Amazonas state, in December 2020, when the city had an increase of cases and deaths (19). Two months before, a study with blood donors had shown SARS-CoV2 seroprevalence rate of 76%, above the theoretical threshold of herd immunity (20). Even so, a large outbreak occurred in January 2021, with health system collapse, raising the hypothesis that the P.1 variant could escape the immune response induced by previous infections. In February 2021, the P.1 variant had already been detected in twelve Brazilian states: Amazonas, Bahia, Espírito Santo, Pará, Paraíba, São Paulo, Roraima, Ceará, Piauí, Santa Catarina, Rio de Janeiro and Rio Grande do Sul, causing great concern. P.1 variant was identified as the predominant one in Araraquara, São Paulo state, that recorded a huge increase in the number of new cases, hospitalizations and deaths, with 100% occupancy of the ICU beds, in February 2021 (21). A large increase of cases with health system breakdown also occurred in Porto Alegre, in Rio Grande do Sul state, in March 2021, concomitant to P.1 variant community transmission. Recent studies have shown the P.1 variant is associated with greater viral load and greater transmissibility (22). The plasma of COVID-19 convalescents had been demonstrated to have reduced neutralization capacity against the P.1 variant (six times lower than against the B strain that circulated in Brazil in 2020), suggesting P.1 variant can escape the neutralizing antibodies generated in response to SARS-CoV-2 variants that have previously circulated (23). The second wave of COVID in Brazil is associated with greater severity and an increase in hospitalizations and deaths of young adults (30 to 50 years old). P.1 variant also carries the risk of reducing the vaccines efficiency in the pandemic control.

1.1. Immune response to SARS-CoV2

Most immunocompetent people develop an adaptive immune response after SARS-CoV-2 infection, with B and T cell response, and antibodies against nucleocapsid (N) proteins, spike protein (S) and the RBD (receptor binding domain). RBD is the main target of neutralizing antibodies (24). IgM, IgG and IgA anti-S antibodies can be detected two to three weeks after infection. Approximately 5 to 10% of the infected persons do not develop detectable IgG antibodies (24).

Endemic seasonal coronavirus infection induce short-lived protective immunity (25). Antibodies against SARS-CoV-1 and MERS-CoV are detected for two to three years after infection (26). Anti-SARS-CoV-2 antibodies duration after natural infection is not well known, but IgG antibodies, including IgG anti-S and anti-N, persist for several months in most people (27). People with more severe COVID19 disease seem to develop a more robust antibody response, with higher IgM, IgG and IgA titers and longer antibody persistence (24, 27). There is evidence that SARS-CoV-2 infection may result in some level of protection against SARS-CoV-2 reinfection, but the duration of clinical protection has not yet been determined.

1.2. COVID-19 Vaccines

Great international scientific efforts and financial investment were made to rapidly develop effective and safe COVID vaccines. Different technologies have been used, and in less than six months, several vaccine candidates had reached the clinical stage. Traditional platforms (inactivated virus vaccines, live attenuated viruses, or recombinant proteins) were used, as well as new platforms, such as viral vectors (adenovirus) replicating or not, nucleic acids (DNA and RNA), and virus-like particles (VLP). COVID vaccines development benefited from the previous SARS-CoV-1 and MERS-CoV vaccines development, which had reached clinical phase 1 and were then discontinued (28), and also from Ebola, Zika and Chikungunya vaccines development (29).

From December 2020, preliminary results of phase 3 trials have allowed several vaccines emergency use license and vaccination introduction in many countries around

the world. Four vaccines were approved by the Brazilian Regulatory Agency (*Agência Nacional de Vigilância Sanitária, ANVISA*) for use in Brazil: the inactivated virus vaccine (CoronaVac) developed by Sinovac Life Science Co., which has a technology transfer agreement with the Instituto Butantan[https://www.gov.br/anvisa/pt-br/assuntos/paf/coronavirus/vacinas-uso-emergencial], and the non-replicating viral vector (adenovirus 26) vaccine with the protein S gene, from Janssen, were approved for emergency use [https://agenciabrasil.ebc.com.br/saude/noticia/2021-03/anvisa-aprova-autorizacao-para-uso-emergencial-da-vacina-da-janssen]. And the non-replicating viral chimp vector vaccine (ChAdOx), developed by Oxford University in partnership with AstraZeneca, which has a technology transfer agreement with BioManguinhos, and the Pfizer / BioNTech vaccine, consisted of RNAm encoding the RBD portion of the spike protein wrapped in a lipid nanoparticle, was granted with definitive registration [https://www.gov.br/anvisa/pt-br/assuntos/noticias-anvisa/2021/informe-a-populacao-brasileira].

A concern regarding the COVID-19 vaccines is the theoretical risk of respiratory antibody-dependent enhancement disease in vaccinees, in case of antibody titers decay and loss of protection over time. This concern is based on challenge animal models after immunization with SARS-CoV-1 and MERS-CoV vaccines. The vaccinated animals that were experimentally exposed to the respective coronavirus showed an inflammatory reaction in the lung parenchyma with eosinophils predominance (30). Another concern is the vaccines' ability to induce protection against new viral variants.

1.3. Adsorbed COVID-19 vaccine (inactivated) – CoronaVac

The adsorbed inactivated COVID-19 vaccine is derived from the new coronavirus SARS-CoV-2, strain CZ02, isolated in China, grown in African green monkey kidney cells (Vero Cells), followed by inactivation, concentration, purification, and aluminum-hydroxide adsorption. It contains inactivated SARS-CoV-2 viral particles, aluminum hydroxide (adjuvant), disodium hydrogen phosphate, sodium dihydrogen phosphate and sodium chloride. The vaccine is preservative-free.

1.3.1. CoronaVac Clinical Trials

Two phase I / II trials were developed in China, involving healthy young adults aged 18 to 59 years and healthy adults over 60 years:

1. Phase I / II randomized, double-blind, placebo-controlled clinical trial to assess the vaccine safety and immunogenicity in 144 healthy adults (Phase I) and 600 healthy adults (Phase II) aged from 18 to 59 years, randomized to receive two vaccine doses, with medium (3µg, equivalent to 600 SU) and high (6µg, equivalent to 1200 SU) doses, in an emergency (Day 0, 14) or routine (Day 0, 28) schedule, or placebo (31).

   Safety Results: In phase 1, the incidence of adverse reactions in the emergency schedule cohort was 29% in the 3ug group, 38% in the 6µg group and 8% in the placebo group. In the routine schedule cohort, these features were 13%, 17% and 13%, respectively. In the phase 2 trial, the incidence of adverse reactions in the emergency schedule cohort was 33% among participants in the 3µg group, 35% in the 6µg group and 22% in the placebo group; and in the routine schedule cohort, they were 19%, 19% and 18%, respectively. The most frequent local and systemic adverse reactions were pain at the injection site and weakness (31).

   Immunogenicity Results: In phase 1, neutralizing antibody seroconversion 14 days after emergency schedule vaccination was observed in 46% of participants in the 3µg group, 50% in the 6 µg group and 0% in the placebo group. After routine schedule, seroconversion was observed in 83%, 79% and 4%, respectively. In phase 2, with the emergency regimen, neutralizing antibody seroconversion was observed in 92% of the 3 µg group, 98% of the 6 µg group and 3% of the placebo group; while with routine regimen, seroconversion occurred in 97% , 100% and 0%, respectively (31).

2. A randomized, double-blind, placebo-controlled Phase 1/2 study to assess the vaccine safety and immunogenicity in 72 healthy adults (Phase 1) and 350 participants (Phase 2) aged over 60 years assigned to receive 2 vaccine doses (in different dosages: 1.5µg, 3µg or 6µg) or placebo on a routine schedule (Day 0, 28) (32).

Safety Results: In both phases, within 28 days after vaccination, 20% of participants in the 1.5µg group, 20% in the 3µg group, 22% in the 6µg group and 21% in the placebo group had some adverse reaction. All adverse reactions were mild or moderate. Injection site pain (9% of participants) was the most frequent event. There were eight serious adverse events, all considered unrelated to vaccination (32).

Immunogenicity Results: In phase 1, seroconversion after the second dose was 100% (95%CI, 85.8–100%), in the 3µg group, and 95.7% (95%CI, 78.1–99, 9%), in the 6µg group. In phase 2, seroconversion was 90.7% (95%CI, 83.1–95.7%), in the 1.5µg group; 98% (95%CI, 92.8–99.8%), in the 3µg group and 99% (95%CI, 94.5–100%), in the 6µg group. No antibody responses was detected in the placebo groups (32).

Table 1 summarizes the phase I / II trials immunogenicity results. In young adults, the routine regimen, with a 28 day-interval between the two doses, resulted in a better immune response (greater seroconversion and higher neutralizing antibody titers). Considering safety, immunogenicity and production capacity, the 3µg dose was chosen to be evaluated in phase 3 studies (31).

Table 1. Neutralizing antibody seroconversion rates in adults aged ≥18 years, 28 days after the 2nd dose of inactivated virus COVID vaccine (CoronaVac) observed in phase I/II trials

Group	Emergency schedule (0, 14 days)			Routine schedule (0, 28 days)		
	N	Seroconversion rate (%) (95%CI)	GMT (95%CI)	N	Seroconversion rate (%) (95%CI)	GMT (95%CI)
Adults aged 18 to 59 years	118	92.37 (86.01- 96.45)	27.6 (22.7- 33.5)	117	97.44 (92.69- 99.47)	44.1 (37.2- 52.2)
Adults aged ≥ 60 years				98	97.96 (92.82- 99.75)	42.2 (35.2- 50.6)

Source: CoronaVac package insert (available at: https://vacinacovid.butantan.gov.br/bulas)

Phase 3 clinical trials with CoronaVac vaccine are being conducted in China, Brazil, Indonesia, Turkey, Malaysia, Philippines and Chile (33). Results of the preliminary analysis of the phase 3 study carried out in Turkey (disclosed in March, 2021) showed vaccine efficacy of 83.5% in preventing symptomatic PCR-confirmed COVID-19 cases [https://asia.nikkei.com/Spotlight/Coronavirus/Sinovac-s-COVID-19-shot-is-83-effective-not-91-Turkey-says]. Indonesia phase 3 trial preliminary analysis (released in January, 2021) showed vaccine efficacy of 65.3% [https://www.wsj.com/articles/indonesia-is-first-to-approve-sinovac-vaccine-outside-china-11610372933].

In Brazil, phase 3 trial included 11,469 healthcare workers aged >18 years: 5,738 participants were randomized to receive the vaccine and 5,731 received placebo. The preliminary analysis included 4,653 participants in the vaccinated group and 4,589 participants in the placebo group; 186 and 176 participants aged ≥60 years in each group. Vaccine efficacy was 50.39% (95%CI, 35.26 - 61.98%) in preventing COVID-19 symptomatic cases (WHO score ≥2) and 77.96% (95%CI 49.15–90.44%) in preventing COVID-19 cases that require healthcare (WHO score ≥ 3) (10). Moderate (WHO score 4 and 5) and severe (WHO score 6) disease were observed only in the placebo group (6 and 1 cases, respectively) (34). The number of COVID-19 cases in persons aged ≥60 years was too small to draw any conclusions on vaccine efficacy in this population: there were 3 cases in the placebo arm and 2 cases in the vaccine arm [Source: Vaccine Package insert. Available at: https://vacinacovid.butantan.gov.br/bulas].

The adsorbed (inactivated) vaccine was safe in the studied groups. Local and systemic adverse reactions observed up to 7 days after completing the vaccination schedule were similar in young adults (18-59 years old) and elderly (≥60 years old). The most frequent local and systemic adverse reactions were mild to moderate pain at the injection site and mild to moderate headache. To date, no case of COVID-19 exacerbation as a result of vaccination has been reported. No serious adverse events (SAE) were observed in phases 1/2. In phase 3, only one SAE was reported, however, the blinding code was not break, the definitive causal relationship has not been established (34).

According to available data, the adsorbed (inactivated) COVID-19 vaccine was proved to be well tolerated and to have a safety profile similar to other vaccines in use. The vaccine safety profile has not been established in children and under-18 adolescents, pregnant and lactating women, persons with severe comorbidities or immunosuppressed, persons with history of severe allergic reaction or anaphylaxis to the vaccine or vaccine components, and in ethnic groups not included in clinical studies. There is no data on simultaneous administration with other vaccines. In an in vitro neutralization study, sera from vaccinees with the adsorbed (inactivated) COVID-19 vaccine from Sinovac / Instituto Butantan were able to neutralize the P.1 variant, suggesting the vaccine may provide protection against the variant, but further studies are needed [https://agencia.fapesp.br/estudo-preliminar-do-butantan-indica-que-coronavac-neutraliza-as-novas-variantes-do-sars-cov-2/35382/].

2. JUSTIFICATION

Clinical trials safety data are limited for some age groups, persons with comorbidities or taking immunosuppressive drugs, and for adverse events with a long incubation period. Adverse reactions rates may vary considerably between populations, countries and regions due to differences in the frequency of diseases and in health services utilization.

Immunocompromised people may have a lower immune response (lower seroconversion and lower antibody titers) to vaccines and a faster antibody waning, compared to healthy persons of the same age. There are few data on the COVID-19 vaccines licensed or approved for emergency use in immunocompromised person by underlying disease or by immunosuppressive therapy. There are no data on the safety, immunogenicity and efficacy of the adsorbed (inactivated) COVID-19 vaccine produced by Sinovac / Instituto Butantan in immunocompromised persons.

3. OBJECTIVES

3.1. General Objectives

1. Assess CoronaVac vaccine safety in immunocompromised participants
2. Assess CoronaVac vaccine immunogenicity in immunocompromised participants
3. Assess pre-vaccination SARS-CoV-2 seroprevalence in immunocompromised participants

3.2. Specific Objectives

3.2.1. Safety

1. Assess the frequency of solicited and unsolicited adverse reactions within 7 days after each vaccine dose, in each group of immunocompromised participants
2. Assess the frequency of Adverse Events of Special Interest (AESI) in participants who received at least one vaccine dose during the entire study period, in each group of immunocompromised participants
3. Assess SARS-CoV-2 disease occurrence in participants who received at least one vaccine dose within one year after the last vaccine dose received, for each group of immunocompromised participants
4. Characterize SARS-CoV-2 disease, according to the WHO Clinical Progression Score, for each group of immunocompromised participants

3.2.2. Immunogenicity

1. Assess the humoral immune response to vaccination 28 days after each vaccine dose, and six and twelve months after the last vaccine dose, for each group of immunocompromised participants

3.3. Exploratory Objectives

1. Assess humoral immune response to vaccination according to previous exposure to SARS-CoV-2 in participants who received at least one vacine dose, for each group of immunocompromised participants
2. Assess the cellular immune response to SARS-CoV-2 antigens pre-vaccination and 4 weeks after completing vaccination with CoronaVac in a subgroup of participants, for each group of immunocompromised
3. To characterize the SARS-CoV-2 variants in COVID-19 cases among vaccinees

4. METHODS

4.1. Study Design

Open-label, non-randomized, phase IV clinical trial.

4.2. Vaccine

All study participants will receive two doses of the adsorbed (inactivated) COVID-19 vaccine produced by Sinovac / Instituto Butantan (CoronaVac), with 28-day interval between doses, as recommended by the National Immunization Program (PNI). Vaccines will be provided by the Butantan Institute. The vaccine will be administered with a 14-day interval from the administration of other vaccines. During the National Influenza Vaccination Campaign 2021, the administration of the adsorbed vaccine COVID-19 (inactivated) will be prioritized and the influenza vaccine will be scheduled respecting the 14-day minimum interval of between vaccines, as recommended by the PNI (35).

4.3. Participants

Adults aged from 18 to 59 years, belonging to one of the following groups:

1. Solid organs transplant recipients: kidney, liver, heart and lung
2. Chronic kidney disease, pre-transplantation
3. Liver cirrhosis, pre-transplantation

4. Hematopoietic stem cell transplantation
5. Cancer in active therapy
6. Adults with innate immunity errors: diseases with predominant antibody deficiency
7. Immune-mediated rheumatic diseases
8. Healthy people (comparator group)

4.3.1. Inclusion criteria:

1. Solid organs transplant recipients (kidney, liver, heart or lung):
    - at least three months after transplantation;
    - being on immunosuppressive drugs

2. Adults with chronic kidney disease (CKD), on dialysis or not:
    - glomerular filtration rate ≤44 ml / min (categories G3b, G4 and G5 of KDIGO 2012 (36);
    - diagnosis for at least three months;
    - On transplant list

3. Adults with liver cirrhosis on a liver transplant list

4. Hematopoietic stem-cell transplant recipients:
    - Autologous transplant: from 30 days post-transplant;
    - Allogeneic transplantation: total lymphocytes ≥1000 cells/mm^3 and CD4 T cells ≥250 cells/mm^3

5. Cancer patients
    - solid or hematological tumors,
    - Any clinical or surgical oncological therapy in the last six months. The participant may be under treatment at the time of enrollment in the study

6. Adults with innate immunity errors: defects in the antibody production, in particular, variable common immunodeficiency, agammaglobulinemia and Hyper-IGM Syndrome

7. Adults with immune-mediated juvenile rheumatic diseases: systemic lupus erythematosus, dermatomyositis, rheumatoid arthritis or juvenile idiopathic arthritis

8. Healthy adults of the same age (comparator group)

4.3.2. Exclusion criteria:

1. Have previously received any COVID vaccine;
2. History of allergy to any vaccine component;
3. Have received any other vaccine within 14 days before enrollment in the study;
4. Acute illness or fever at the time of enrollment;
5. Behavioral, cognitive or psychiatric disorders that, in the researchers opinion, affects the ability to understand and collaborate with the research protocol requirements;
6. Alcohol or drug addiction;
7. Any other condition that, according to the investigators judgment, may compromise the study procedures

4.3.2.1. Group-specific exclusion criteria:

1. Solid organs transplant recipients:
    - Transplant recipients of more than one organ,
    - Loss of the transplanted organ,
    - Cancer in activity,
    - HIV / AIDS or
    - Any other comorbidity associated with immunocompromise, such as dialysis renal failure

2. Chronic kidney or liver disease:
    - Cancer in activity,
    - HIV / AIDS or
    - Any other comorbidity associated with immunocompromise

3. Hematopoietic stem cell transplantation (HSCT)
    - Cancer in activity,

- HIV / AIDS,
- Graft versus host grade IV disease and / or refractory to corticosteroids, or
- Any other comorbidity associated with immunocompromise

4. Cancer patients:

- Immunobiological therapy,
- Solid organ transplant or HSCT,
- HIV / AIDS, or
- Any other comorbidity associated with immunocompromise

5 . Innate immunity errors:

- Marked lymphopenia with CD4 T cells <200/mm^3

6. Immune-mediated rheumatological disease:

- Cyclophosphamide or pulse therapy with methylprednisolone in the last 30 days

7. Healthy people:

- HIV / AIDS or
- Any other comorbidity associated with immunocompromise

4.4. Recruitment

The transplant recipients will be recruited at the outpatient clinics of the Kidney Transplantation, Liver Transplantation, Heart Transplantation, Lung Transplantation and Hematology (HSCT) of HC-FMUSP. Cancer patients will be recruited at the Cancer Institute of São Paulo State (ICESP). Participants with innate immunity errors will be recruited at the UNIFESP Clinical Immunology Service. Participants with immune-mediated rheumatic diseases will be recruited at the UNIFESP Pediatric Rheumatology Service. Participants in the comparator group (immunocompetent adults) will be recruited among healthcare workers, researchers, students and interns of the HC-FMUSP complex, and among other adults with COVID vaccination recommendation by the National Immunization Program and the Immunization Division of the São Paulo Municipality Secretariat of Health. If the group is not completed with these persons, close contacts of the participants and other healthy adults will also be enrolled.

4.4. Study Outcomes

4.4.1. Primary outcomes

Safety Outcomes:

- Frequency of local and systemic adverse reactions, solicited and unsolicited, up to 7 days after each dose of the vaccine, for each group of participants
- Frequency of serious adverse events (SAE) throughout the study period, for each group of participants
- Frequency of adverse events of special interest (EAIE) throughout the study period, for each group of participants

Immunogenicity outcomes:

- Frequency of seroconversion 28 days after each dose of vaccine, and seropositivity at six and twelve months after the last vaccine dose, for each group of participants. Seroconversion will be defined by the presence of anti-SARS-CoV-2 antibody after vaccination in participants seronegative to SARS-CoV-2 antibody before vaccination ,and / or at least a 4-fold increase in anti-SARS-CoV-2 antibody titers in participants seropositive to SARS-CoV-2 previous to vaccination.
- Anti-SARS-CoV-2 antibody Geometric mean titers (GMT) before and 28 days after each vaccine dose, and six and twelve months after the last vaccine dose, for each group of participants

4.4.2. Secondary Outcomes

Safety Outcomes

- Frequency of virologically-confirmed COVID-19 cases in the participants who received at least one vaccine dose during the entire study period

Immunogenicity Outcomes

- Frequency anti-SARS-CoV-2 antibody seropositivity before, 28 days after the first and second vaccine doses, and six and twelve months after the last vaccine dose

4.4.3. Exploratory Outcomes

- Frequency of seroconversion and anti-SARS-CoV-2 antibody geometric mean titers (GMT) after vaccination according to previous virus exposure, in participants who have received at least one dose of the vaccine, for each group of participants
- Cellular immune response to SARS-CoV-2 antigens, before vaccination and 4 weeks after completing a two-dose schedule vaccination, in a subgroup of participants, for each group of immunocompromised
- Characterization of the SARS-CoV-2 lineage in COVID-19 cases among the vaccinees participating in the study

4.5. Study procedures

1. After checking the inclusion and exclusion criteria, clarifying the study objectives and procedures, and signing the informed consent form, a questionnaire on demographic and clinical characteristics and treatments will be applied and a blood sample (10 mL) for anti-SARS-CoV-2 serology will be collected (Visit 1);
2. Participants will receive the first dose of adsorbed (inactivated) COVID-19 vaccine up to a maximum of 15 days after inclusion in the study;
3. Participants will receive a diary with guidance for recording all adverse events in the first 7 days following vaccination;
4. Participants' hospital records will be consulted to complement clinical and laboratory data (blood count, creatinine clearance, and blood level of tacrolimus or mTOR inhibitors);
5. Four weeks after the first vaccine dose, the participants must return to receive the second vaccine dose (Visit 2). Participants must return the adverse events

diary, which will be checked by the researchers, and a blood sample (10 mL) for SARS-Cov2 serology will be collected before administering the 2nd vaccine dose. Participants will receive a second diary with guidance for recording all adverse events in the first 7 days after the administration of the second dose of the vaccine;

6. Four weeks after the second dose, the participants must return the diary of adverse events after the second dose and collect a blood sample (10mL) for SARS-Cov2 serology (Visit 3);

7. Six and 12 months after the administration of the second vaccine dose (respectively Month 7 and Month 13 of the study), the participants must return to the research center to collect blood samples (10 mL) for SARS-Cov2 serology (Visits 4 and 5);

8. All participants will be monitored for possible adverse events and will be instructed to return to CRIE or to the clinic where they are followed-up in case of moderate / serious adverse events;

9. In case of signs or symptoms suggestive of COVID-19, participants will be instructed to return the research clinic where they will be clinically evaluated and referred to collect nasopharyngeal and oropharyngeal swabs for SARS-CoV2 PCR. Part of the sample must be separated and frozen for further characterization by genetic sequencing, if positive;

10. All participants will be contacted by phone, email or text message (SMS, WhatsApp), 10 to 15 days after each of the two doses of the vaccine to assess local and systemic adverse events, and every 30 days, during the entire study period to assess COVID-19 symptoms and / or diagnosis;

11. Participants' medical records will be reviewed for register of COVID-19 disease during the study period;

12. The follow-up time of the participants in the study will be 12 months from the last vaccine dose.

Table 2. Study procedures

Visit	V1	V1 + 10 (+5) days	V2 (V1 + 28 days)	V2 + 10 (+5) days	V3 = V2 +28 (±7) days	V4 = V2 + 6 months (± 15 days)	V5 = V2 + 12 months (± 30 days)
Study month	M0		M1		M2	M7	M13
Inclusion / exclusion criteria	X						
Consent form	X						
Clinical evaluation	X		X				
Blood sample – Serology (10 mL)	X		X		X	X	X
Blood sample for cell immunity (15 mL)*	X		X				
Vaccination – dose	1st		2nd				
Adverse events diary delivery	X		X				
Adverse events assessment		X	X	X	X	X	X
Adverse events diary return			X		X		

* Cell immunity evaluation will be performed for a sample of the participants

4.6. Vaccine safety assessment

Solicited and unsolicited, local and systemic adverse events will be evaluated immediately after vaccination up to 7 days following each vaccine dose, for all participants. Serious adverse events (SAE) and adverse events of special interest (AESI) will be evaluated after each dose, up to one year after the last vaccine dose, for all participants.

All participants will be asked to register the signs and symptoms that occur within 7 days after each dose of the vaccine, in detail, in a diary to be provided by the researchers. All participants will be asked to bring the completed diary on the next visit so that any additional information is properly added to the e-CRF.

A thermometer will be provided for each participant and they will be asked to check their body temperature once a day and anytime they have fever, recording the result in the participant's diary.

All participants will be contacted by phone, email or text message (SMS, WhatsApp) between the 10th and 15th days after the administration of each dose of the

COVID vaccine and a structured questionnaire will be applied. The purpose of this contact is to reduce the loss of information about adverse events due to the diary loss or non-return and to monitor any sign related to the vaccine safety.

Solicited adverse events will be:

- Local: pain, tenderness, edema, erythema, induration and itching
- Systemic: fever, headache, malaise, fatigue, myalgia, arthralgia, chills, nausea, vomiting, diarrhea, anorexia, skin rash, itching, cough, allergic reactions

Serious adverse event (SAE) will be any event that leads to hospitalization; cause risk of death and require immediate clinical intervention to prevent death; cause significant dysfunction and / or permanent disability; result in congenital anomaly; cause death (37).

Adverse Events of Special Interest (AESI) must be monitored for new vaccines, recent clinical evaluation and emergency approval, as the case of COVID-19 vaccines, which will be used on large scale, right after clinical trials with a small number of volunteers and short follow-up. The following events were defined as AESI by WHO in partnership with Brighton Collaboration (38):

- Enhanced disease associated with the vaccine
- Multisystemic inflammatory syndrome in children
- Severe Acute Respiratory Syndrome
- Acute cardiovascular injury (microangiopathy, heart failure, cardiomyopathy, coronary disease, arrhythmia, myocarditis)
- Coagulation disorder (thromboembolism and hemorrhage)
- Acute renal failure
- Generalized seizure
- Guillain Barré syndrome
- Liver failure
- Anosmia and ageusia
- Pernio-like erythema (spots and plaques of erythematous to purplish color)

- Isolated cutaneous vasculitis
- Erythema multiforme
- Anaphylaxis
- Acute aseptic arthritis
- Meningoencephalitis
- Acute disseminated encephalomyelitis
- Thrombocytopenia

Solicited and unsolicited adverse events will be assessed by the investigators in relation to:

- Severity and intensity
- Causal relationship to the vaccine
- Measures taken
- Evolution of the framework

Classification of the intensity of the solicited and unsolicited adverse events will be made according to Table 3. Classification of the adverse events according to the association with the vaccine will be made according to Table 4.

Adverse reactions after vaccination will be defined as local or systemic adverse events, solicited or unsolicited, and adverse events of special interest that have a certain, probable or possible causal relationship with the vaccine (Table 4)

Table 3. Classification of local and systemic adverse events according to intensity (adapted from FDA, 2007) (39)

Local adverse events				
	Mild (grade 1)	**Moderate (grade 2)**	**Intense (grade 3)**	**Potentially life threatening (grade 4)**
Pain /	Does not interfere with daily activity	Repeated use of non-narcotic pain reliever for> 24 hours / little interference in daily activity	Narcotic pain reliever use or prevents daily activity	Visit to the emergency room or hospitalization
Tenderness	Mild discomfort to the touch	Discomfort with movement	Significant discomfort at rest	Visit to the emergency room or hospitalization
Erythema	2.5 to 5.0 cm	5.1 to 10cm	>10cm	Necrosis or exfoliative dermatitis
Induration / edema	2.5 to 5.0 cm and does not interfere with daily activity	5.1 to 10cm, interferes in daily activity	>10cm or prevents daily activity	Necrosis
Systemic adverse events				
Fever	38.0 to 38.4°C	38.5 to 38.9ºC	39.0 to 40.0ºC	>40ºC
Nauseas / vomiting	Does not interfere with daily activity or $\leq$2 episodes/24 hours	Some interference in daily activity, or >2 episodes/ 24 hours	Prevents daily activity / needs intravenous hydration	Visit to the emergency room or hospitalization
Diarrhea	2 to 3 bowel movements or <400g / 24 hours	4 to 5 bowel movements or 400g to 800 / 24 hours	$\geq$6 bowel movements or >800g / 24 hours or needs intravenous hydration	Visit to the emergency room or hospitalization
Headache	Does not interfere with daily activity	Repeated use of non-narcotic pain reliever for> 24 hours / some interference in daily activity	Narcotic pain reliever use or prevents daily activity	Visit to the emergency room or hospitalization
Other adverse events	Does not interfere with daily activity; causes mild discomfort, is well tolerated and disappears spontaneously	Some interference in daily activity	Prevents daily activity	Potentially fatal. Visit to the emergency room or hospitalization

Table 4. Classification of adverse events according to the causal relationship with the vaccine (40).

Causality term	Assessment criteria*
Certain	• Event or laboratory test abnormality, with plausible time relationship to drug intake • Cannot be explained by disease or other drugs • Response to withdrawal plausible (pharmacologically, pathologically) • Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon) • Rechallenge satisfactory, if necessary
Probable / Likely	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Unlikely to be attributed to disease or other drugs • Response to withdrawal clinically reasonable • Rechallenge not required
Possible	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Could also be explained by disease or other drugs • Information on drug withdrawal may be lacking or unclear
Unlikely	• Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) • Disease or other drugs provide plausible explanations
Conditional / Unclassified	• Event or laboratory test abnormality • More data for proper assessment needed, or • Additional data under examination
Unassessable / Unclassifiable	• Report suggesting an adverse reaction • Cannot be judged because information is insufficient or contradictory • Data cannot be supplemented or verified

*All points should be reasonably complied with

4.6.1. Adverse Events Reporting

All adverse events (AE) that occur in the first 7 days after each vaccine dose and those occurring at any time that have a reasonable causal relationship with the product under investigation must be reported to the Pharmacovigilance, Clinical Safety and Risk Management Center of Instituto Butantan, through the adverse event report form, within up to seven calendar days of knowledge by any member of the study team.

Updates on an adverse event must be recorded as soon as new data are available until its outcome (recovered, in recovery, recovered with sequelae, loss of follow-up, unrecovered or death).

Adverse Events of Special Interest (AESI) and serious AEs must be notified to the Pharmacovigilance, Clinical Safety and Risk Management Center of Instituto Butantan, to the Health Department of São Paulo Municipality, to National Committee of Ethics in Research (*Comissão Nacional de Ética em Pesquisa, CONEP*) and to the National Regulatory Agency (ANVISA), within one calendar day from the date of knowledge by the research staff. The initial notification should not be extended even if the information is incomplete. The notification must be made through a specific form sent by email or telephone call and must be registered, as soon as possible, in the form integrated in the CRF system, referencing the date and time of the first notification.

4.7. Laboratory Methods

4.7.1. Anti-SARS-CoV-2 antibody detection

The following tests will be used:

- Elecsys® anti-SARS-CoV2 test, from Roche Diagnostics - qualitative electrochemiluminescence immunoassay (ECLIA), which detects antibodies against the SARS-CoV2 nucleocapsid (N). The test has high sensitivity (99.5%) and specificity (99.8%) for anti-SARS-CoV2 antibodies (41)
- Elecsys Anti-SARS-CoV-2 S, from Roche Diagnostics - Electrochemiluminescence immunoassay (ECLIA) for quantitative measure of antibodies (including IgG) against the SARS CoV-2 receptor binding domain (RBD). The test has 98.8% sensitivity and 99.9% specificity. Antibody quantification can assist monitoring the antibody response dynamics (42).
- Liaison® SARS-CoV-2 S1 / S2 IgG test, from DiaSorin Inc, Saluggia, Italy. The test uses chemiluminescent immunoassay technology for the quantitative determination of IgG antibodies against SARS-CoV2 S1 and S2 proteins. The test has high sensitivity (97.6%), high specificity (99.8%) and high agreement with the

plate reduction neutralization assay (PRNT), which suggests that it identifies neutralizing antibodies. It does not cross-react with other human coronaviruses circulating in Brazil and in the world (HCoV-OC43, HCoV-229E, HCoV-NL63 and HCoV-HKU) (43).

Blood samples (10 mL) for serology will be collected in tubes with separating gel and clot activator. After homogenization, the samples will be kept at room temperature until transport to the laboratory (within 3 hours). In the laboratory, the tubes will be centrifugated at 1008 × g for 10 minutes to separate serum and clot. The serum will then be aliquoted in 2.0 ml microtubes, which will be frozen. The samples will be kept at -20◦C, at the Medical Research Laboratory / Immunology (LIM-48) at FMUSP, until the transport to the Strategic Laboratory for Molecular / Serology Diagnosis, at Instituto Butantan, where serological tests will be performed (V1, V2 and V3 samples will be analyzed together, after completing V3 blood collection; V4 and V5 samples will be analyzed together, later). The biological samples will be stored until the study finalization.

4.7.2. SARS-CoV2 specific cellular immune response:

A sample of 15 participants from each group of participants will be evaluated for SARS-CoV2 specific cellular immune response. Stable participants (transplanted without rejection in the last year) will be selected. For cellular immune response assessment, 20-30 mL of blood will be collected at baseline (before the first vaccine dose) and at M2 (28 days after the second vaccine dose) for:

- IFN-gamma and TNF production by CD4 and CD8 T cells and CD38 and HLADR activation markers in response to SARS-CoV2 antigens stimulation

For cellular immunity evaluation, blood samples (20-30 mL) will be collected in tubes with heparin. In the Laboratory of Medical Investigation / Immunology (LIM-48), mononuclear cells will be separated by density gradient, with steps of centrifugation and washing of the cells with physiological saline solution. After counting the cells and

determining their viability, the cells will be placed in cryovials and stored in liquid nitrogen cylinders. When samples collections are completed, the cell samples will be thawed and cultured in microplates with the stimulation by SARS-CoV-2 virus proteins. After the incubation time, these cells will be analyzed in a flow cytometer for CD4 (helper T lymphocytes), CD8 (cytotoxic T lymphocytes), CD38 (activation marker), and of the IFN-gamma and TNF-alpha cytokines expression. The samples will be stored until the study completion.

4.8. Sample Size

There are no data on immunogenicity and safety of the adsorbed (inactivated) COVID-19 vaccine in immunocompromised persons. Thus, the study will have a convenience sample. 1,370 participants will be included, distributed as follows:

- 600 transplanted solid organs, of which: 300 kidney transplanted, 100 liver transplanted, 100 heart transplanted and 100 lung transplanted
- 100 patients with dialytic and non-dialytic chronic kidney disease
- 50 patients with liver cirrhosis pre-liver transplantation
- 100 hematopoietic stem cell transplant recipients
- 300 cancer patients
- 100 patients with variable common immunodeficiency
- 20 patients with immune-mediated rheumatological disease
- 100 healthy adults

4.9. Statistical Analysis

The collected data will be inserted in an electronic database built on RedCap.

A detailed statistical analysis plan is attached. Briefly:

4.9.1. Safety analyses:

A descriptive analysis of adverse reactions (AR) occurring in the first 7 days after vaccination will be conducted. Solicited and unsolicited, local and systemic AR will be described according to vaccine dose and participants' group. Adverse events of special interest (AESI) and serious adverse events (SAE) will be described according to vaccine dose and participants' group during the entire study period (13 months). The events will be listed, individually for each participant, and summarized according to frequency, intensity and duration, in tables and graphs according to vaccine dose and participants' group.

4.9.2. Primary immunogenicity analysis:

The primary humoral immune response to vaccination will be assessed by seroconversion rates (%), antibody Geometric Mean Titers (GMT) and post-/pre-vaccination Geometric Mean Ratios (GMR), with respective 95% Confidence Intervals, in blood sample collected 28 days after each vaccine dose. Seroconversion rates (%), antibody GMTs and GMRs will be analyzed for each group of immunocompromised participants separately and will be compared with the results of the group of immunocompetent adults of the same age group. Per protocol and intention to treat analyses will be conducted.

4.9.3. Antibody persistence analysis:

Antibody persistence will be assessed by the proportion of participants who persist seropositive (among those who had seroconverted post-vaccination), antibody GMTs and post- / pre-vaccination GMRs and respective 95% Confidence Intervals, in blood sample collected 6 months and 12 months after the last vaccine dose. The results will be analyzed for each group of immunocompromised participants, separately, and will be compared with the results of the immunocompetent adults group. Per-protocol and intention-to-treat analyses will be conducted

5. ETHICAL ISSUES

The project was submitted for approval to the Ethics Committee for Analysis of Research Projects (CAPPesq) of Hospital das Clínicas, FMUSP and by the National Committee of Ethics in Research (*CONEP*). Participants enrollment and data collection will only be started after the Ethical Committee and Regulatory Agency (ANVISA) approval.

Participants will only be included after signing the Informed Consent Form (ICF). The collection and processing of the participants personal data will be limited to the necessary to meet the objectives of the study. The data will be collected and processed with precautions to guarantee the confidentiality and secrecy of the participants. All study reports will contain aggregated data only and will not identify participants individually.

The biological samples will be used only for the purposes described in this protocol. The samples utilization in other studies must be approved by the Ethics Committees (CAPPesq or CONEP, as appropriate), and the participant will be contacted again to authorize the new use, by signing a new ICF.

5. BUDGET

Item	Value (US$)
Serological tests	132,442.46
Cellular immunity assessment	26,908.76
RT-PCR-SARS-CoV-2	27,932.96
SARS-CoV-2 sequencing	5,446.93
Blood collection and laboratory material	22,010.80
Laboratory equipment	2,767.41
Biological material transport	595.91
Thermometers	4,469.27
Forms printing	2,696.74
Papers publication	5,000.00
TOTAL	**234,740.55**

* US$1 = R$5.37

A detailed budget is attached.

7. FINANCING

The Instituto Butantan will be responsible for supplying 3,000 doses of the adsorbed (inactivated) COVID-19 vaccine for this project.

8. EXECUTION SCHEDULE

	April / May 2021	May to September 2021	October 2021 to June 2022	July to October 2022	November 2022 to June 2023	July to October 2023
Submission of the project for approval by the Research Ethics Committees and the National Regulatory Agency (ANVISA)	X					
Participants enrollment and vaccination		X				
Data collection		X	X	X		
Database building and feeding		X	X	X		
Laboratory analyses			X*		X**	
Statistical analyses			X*		X**	
Paper writing and submission to publication				X*		X**

* Primary immunogenicity (M0, M1, M2) and safety (adverse reactions after vaccination, serious adverse events and adverse events of special interest)

** Immunogenicity: antibody persistence (at M4, M5) and safety (serious adverse events and adverse events of special interest throughout the study)

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