



**Federal University of Minas Gerais
CT Advanced and Innovative Therapies
Clinical Research Unit**

Evaluation of Immunogenicity and Duration of Immunity of the IXCHIQ® Vaccine Against CHIKV

Short title: Evaluation of the Immunogenicity of the IXCHIQ® vaccine

Acronym: IMUNO-CHIQ Protocol Number: 01

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Summary

Study title	Evaluation of Immunogenicity and Duration of Immunity of the IXCHIQ® Vaccine Against CHIKV
Rational	The chikungunya vaccine, developed by the Butantan Institute in partnership with the French-Austrian pharmaceutical company Valneva, called IXCHIQ®, is the first against the disease to be registered in the world and was approved by Anvisa in April 2025 for use in adults in Brazil. IXCHIQ® has a high efficacy rate (98.8% - 100% seroneutralization) and has demonstrated long persistence of antibodies (up to 3 years or more). In February 2026, a pilot vaccination program in Brazil was initiated, but little is known about the cellular immune response of individuals vaccinated with a single dose and whether this cellular response is associated with the intensity of the humoral immune response in different age groups.
Hypothesis	The hypothesis of this study is that IXCHIQ® will induce robust cellular immune response and activation of different compartments of the cellular immune response that are still unknown. The cellular immune response will be similar in adolescents and adults
Location	Clinical Research Unit, Center for Advanced and Innovative Therapies, UFMG Laboratory of Basic and Applied Virology, UFMG
Design	Prospective cohort clinical trial for evaluation of humoral and cellular immunogenicity.
Participants	75 individuals in total followed before, 3, 7, 30, 180 and 720 days after a single dose of the IXCHIQ® vaccine, as recommended in the package insert, being: PART A (Adults) - 50 Subjects aged 18-59 years PART B (Children/adolescents) – 25 subjects aged 12 to 17 years
Criteria Inclusion	<i>Inclusion Criteria:</i> • The participant or guardian understands the procedures and requirements, is willing and able to give informed consent for full participation in the study; • Subjects of both sexes aged between 12 and 59 years.
Exclusion criteria	<i>Exclusion Criteria:</i> • Participants under the age of 12 or 60 years or older • Who have any contraindications raised by TECHNICAL NOTE No. 9/2026-CGFAM/DPNI/SVSA/MS of the Ministry of Health (https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/notas-tecnicas/2026/nota-tecnica-no-9-2026-cgfam-dpni-svsa-ms.pdf - Technical note: Guidelines for monitoring the safety of the Chikungunya vaccine used during the pilot vaccination strategy in Brazil - 2026). • Participants who are not available to undertake the planned study visits; • Participants who do not agree to sign the ICF; • β-HCG positive on the day of vaccination, in the case of women of reproductive potential; • Participants Who are pregnant or planning to become pregnant within the next 3 months after the vaccine dose. • Participants breastfeeding. • The participant with a known or suspected immune system defect that may influence the immune response to the vaccine, such as participants with congenital or acquired immunodeficiency including HIV infection, post-organ transplant status, or immunosuppressive therapy within 4 weeks prior to Visit 1. Immunosuppressive therapy is defined as chronic administration of prednisone (more than 14 days) or equivalent ≥ 2 mg/kg/day within 4 weeks prior to study entry. Note: radiation therapy or

	immunosuppressive cytotoxic drugs/monoclonal antibodies within the previous 3 years; topical and inhaled steroids are allowed. Any clinical instability identified by the treating physician that may put the participant at risk.
Size Sample planned	Total of 75 participants aged 12 to 59 years, with 50 participants between 18 and 59 years old and 25 participants between 12 and 17 years old.
Duration of the Study planned	3 years for the total duration of the study from the first participant recruited.
Interventions	Prospective cohort study of immunogenicity follow-up after vaccination with IXCHIQ.® All participants are vaccinated and no additional intervention will be given to patients in this study.
Primary objective and outcome	<u>Primary Objective:</u> To evaluate the cellular response profile of mononuclear (monocytes, DC, T, B, NK) and polymorphonuclear (Neutrophil) cell subpopulations of individuals of different age groups immunized with IXCHIQ.® Cellular immune response will be assessed before, 3, 7, 30, 180, and 720 days after vaccination; <u>Primary outcome:</u> Number and percentage of the various populations of vaccine-induced mononuclear and polymorphonuclear when evaluated by the flow cytometry technique contrasting age groups. <u>Expected outcome:</u> Detailed description of the innate and memory-induced immune response after vaccination in participants vaccinated as IXCHIQ.® of different age groups; <u>Objective and safety outcome:</u> To assess the incidence of adverse events, in particular adverse events of special interest (Chikungunya Like adverse events – CLARs, carditis, encephalitis, and grade 3 or higher adverse events) up to 60 days after vaccination.
Secondary objectives and outcomes	<u>Secondary Objective 1:</u> Longitudinally evaluate the kinetics of the neutralizing antibody-mediated response to CHIKV of individuals immunized with IXCHIQ.® before, 3, 7, 30, 180, and 720 days after vaccination; Secondary outcome 1: titer of antibodies that are capable of neutralizing a Brazilian strain of Chikungunya and the vaccine strain in a plaque neutralization assay. <u>Secondary Aim 2:</u> To evaluate the profile of soluble mediators (cytokines) and microparticles of individuals immunized with IXCHIQ.® before, 3, 7, 30, 180, and 720 days after vaccination; Secondary outcome 2: levels, in pg/mL, of cytokines, chemokines, and growth factors as well as the percentage of microparticles in the plasma of vaccinated individuals. <u>Secondary Aim 3:</u> To correlate the neutralizing antibody profile with the innate and adaptive immunity cell response of individuals immunized with IXCHIQ.® before, 3, 7, 30, 180, and 720 days after vaccination; Secondary outcome 3: correlation matrix in correlograms or heatmaps of the humoral and cellular response. <u>Secondary Aim 4:</u> To compare cellular immune response and neutralizing antibody profile in adolescents and adults; Secondary outcome 4: Comparative table of cellular and humoral immune responses in adolescents (12-17 years) and adults (18-59 years).

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1. Project Summary

The Chikungunya virus (CHIKV) is an arbovirus transmitted by *Aedes aegypti* and *Aedes albopictus* species. More recently, 154,800 new cases of Chikungunya Fever (CHIKF) were registered, indicating expansion of the disease in the Brazilian territory. Recently, an immunizer was approved by Anvisa in 2025 for use in adults in Brazil. The chikungunya vaccine, developed by the Butantan Institute in partnership with the French-Austrian pharmaceutical company Valneva, called IXCHIQ®, is the first against the disease to be registered in the world. IXCHIQ® has a high efficacy rate (98.8% - 100% seroneutralization in previous studies and application in a single dose. Thus, in 2026, a pilot vaccination program was started in Brazil. ***However, little is known about the cellular immune response and protection correlates induced by vaccination and their duration with a single-dose vaccination protocol in the different age groups of children/adolescents and adults. There is still no information on the response of immune memory T cells that are essential for the stimulation of B lymphocytes that have produced neutralizing antibodies.*** Thus, this project aims to evaluate the kinetics of cellular memory response of adult individuals vaccinated with IXCHIQ.® To this end, a total of 75 participants aged 12 to 59 years will be included in the study who will be evaluated at 7 times, 6 face-to-face visits (baseline or D0, D3, D7, D30, D180 and D720 days after vaccination) and one C1 contact visit (60 days after vaccination). Participants will be subdivided into PART A (Adults) – 50 Subjects aged 18 to 59 years and PART B (Children/adolescents) – 25 subjects aged 12 to 17 years. PRNT analyses for the evaluation of neutralizing antibodies, as well as multiparametric analyses using spectral flow cytometry will be employed. These more refined analyses will allow the evaluation of the cellular response of memory T and B cells, innate and trained immunity cells and unconventional induced after vaccination. In vitro stimulation of peripheral blood cells with vaccine antigens (VLA1553 and other vaccine antigens produced by our group) will be performed for the evaluation of intracytoplasmic cytokine production and activation profile of cell subpopulations. We believe that this study will fill an important gap on the cellular immune response and activation of different compartments of the immune response by the vaccine that are still unknown. The study of new vaccine antigens in vitro will be equally important to provide subsidies for their use as future booster prototypes for the IXCHIQ® vaccine.

2. State of the Art and Justification

Chikungunya virus (CHIKV) is an arbovirus belonging to the alphavirus genus, family *Togaviridae*, transmitted mainly by mosquitoes of the species *Aedes aegypti* and *Aedes albopictus* (ABDELNABI; NEYTS; DELANG. 2015).

The name Chikungunya derives from "kungunyala", a verb from the Maconde language that means "to writhe" and "to bend", and is related to the symptomatology of patients affected by Chikungunya fever (CHIKF). The Maconde language is spoken by the inhabitants of southeastern Tanzania, northern Mozambique and East Africa (LUMSDEN, 1955). In 2004 there was a global outbreak of CHIKV, reaching islands in the Indian Ocean, India, Southeast Asia and China. The first cases in the European region were identified in 2007 in Italy and France. In 2013, there were the first reports of CHIKV infection in the Americas, reaching the entire Caribbean region and Latin America. CHIKV was intensively introduced in new regions across the globe, this outbreak may have been driven by air travel, high population densities and extensive global distribution of vectors (GOULD et al., 2010; SILVA; et al., 2017). In Brazil, the first reports of autochthonous transmission of CHIKV occurred in Amapá and Bahia, in September 2014. It was rapidly spread to all states, caused by the ECSA and Asian lineages (SILVA et al., 2018). In 2014, about 4577 suspected cases of CHKF were recorded affecting five states (SILVA et al., 2018).

More recently, in 2022, 174,517 probable cases were registered, 78.9% higher than the record in 2021. In 2023, until epidemiological week 52, about 154,800 cases of CHIKF HAD BEEN RECORDED (MINISTRY OF HEALTH, 2024). The clinical aspects of CHIKF can begin to appear between the third and seventh day, ranging from one to twelve days. Affecting individuals of different age groups (BANDYOPADHYAY; GOSH, 2010; THIBERVILLE, et al 2013). The vast majority of individuals infected with CHIKV manifest symptoms, according to the Ministry of Health (2017) about 70% of patients are symptomatic. This characteristic is one of the main points to differentiate this infection from other arbovirus infections that do not have such a high rate of symptomatic patients, however, there are patients infected with CHIKV who do not have clinical symptoms and are classified as asymptomatic.

The chronic phase is a matter of great concern for public health and can be characterized when there are joint edemas that persist for more than three months, leading to an arthrotogenic condition, which can persist for months or even years (DA CUNHA; TRINTA, 217). In the chronic stage of the disease, arthralgia and arthritis tend to be bilateral and symmetrical, and may be migratory, with intermittent or constant pain.

Currently, there is still no specific antiviral treatment for symptomatic cases of CHIKV infection, whether in the acute or chronic phase (SIMON et al., 2015). Because there is no specific antiviral against CHICV, different drugs are used for the alternative treatment of the different clinical phases of infection. However, some vaccines are in the process of being authorized for commercialization and have the potential to reduce the impact of CHIKF (BARTHOLOMEEUSEN et al., 2023).

The Chikungunya VLA1553 vaccine was developed as a candidate using live attenuated CHIKV virus, designed for active immunization for the prevention of diseases caused by CHIKV infection. The vaccine candidate is intended to prevent disease caused by CHIKV in populations living in endemic regions, as well as to serve as a prophylactic measure for travelers to epidemic areas or areas at risk of outbreak (ROQUES et al., 2017). The replicating live-attenuated CHIKV contains a large genetically modified deletion of 60 amino acids in the nsP3 gene, which encodes the non-structural protein nsP3 of the replicase complex, leading to attenuation of the virus in vivo. The sequence of the missing nucleotides ranges from amino acid residues 1656 to 1717 of the viral polyprotein P1234. The vaccine is based on the La Reunion viral line (LR2006-OPY1) of the east-central South African genotype produced in Vero cells and purified by centrifugation, ultrafiltration, batch chromatography, and sucrose gradient centrifugation. The new vaccine is designed to protect against all circulating CHIKV genotypes, and potentially against closely related alphaviruses (WRESSNIGG et al., 2020).

In a phase 1 dose-finding study, it was found that the live-attenuated CHIKV vaccine was well tolerated and highly immunogenic with up to 3.2×10^5 TCID₅₀/mL vaccine dose (WRESSNIGG et al., 2020). In a phase 2, randomized, dose-response observer blinded study, these results were confirmed demonstrating high tolerability (solicited adverse events), high safety (unsolicited adverse events), and high immunogenicity with the presence of neutralizing antibodies after VLA1553 administration in healthy children from 1 to 11 years of age in endemic countries (WEISOVÁ et al., 2025).

However, concerns regarding the vaccine have already been reported. On August 22, 2025, the FDA's Center for Biologics Evaluation and Research (FDA) suspended the biologic license of the IXCHIQ vaccine (live chikungunya vaccine) from Valneva, Austria. This vaccine was initially approved by the FDA in November 2023, through the accelerated approval process, for the prevention of chikungunya virus disease (CHIKV) in individuals aged 18 years and older who are at increased risk of CHIKV exposure. CBER's decision is based on serious safety concerns related to the vaccine, which appears to be causing chikungunya-like symptoms in vaccinated individuals. There has been one death from encephalitis directly

attributable to the vaccine (CSF PCR was positive for the vaccine strain of the virus) and more than 20 serious adverse events reported, consistent with chikungunya-like symptoms. Reported serious adverse events included 21 hospitalizations and 3 deaths. In addition, the clinical benefit of the vaccine has not yet been proven in confirmatory clinical studies. The FDA's risk-benefit analysis generally demonstrates that the benefits of the vaccine do not outweigh the risks in most plausible scenarios. For these reasons, the FDA believes that this vaccine is not safe and that its continued administration to the public would pose a health hazard (FDA, 2025).

For the European regulatory agency (EMA), the EMA's safety committee has completed its review of IXCHIQ (a live attenuated chikungunya vaccine), following reports of serious adverse events. The previous temporary restriction on vaccination of people aged 65 and over, which was implemented during the review, will now be lifted. However, the Committee concluded that for people of all ages, the vaccine should only be administered when there is a significant risk of chikungunya infection and after careful analysis of the benefits and risks.

Finally, the safety and immunogenicity of single-dose VLA was evaluated in a double-blind, multicenter, randomized, placebo-controlled, phase 3 study. In this study, 4128 US participants were enrolled and randomized (3093 to VLA1553 and 1035 to placebo). Of these, 358 participants in the VLA1553 group and 133 participants in the placebo group discontinued the study before termination. The per-protocol population for immunogenicity analysis comprised 362 participants (266 in the VLA1553 group and 96 in the placebo group).

After a single vaccination, VLA1553 induced levels of neutralizing antibodies against chikungunya virus in 263 (98.9%) of 266 participants in group VLA1553 (95% CI 96.7–99.8; $p < 0.0001$) 28 days after vaccination, regardless of age. The VLA1553 was generally safe, with an adverse event profile similar to that of other licensed vaccines and equally well tolerated in young and elderly adults. Serious adverse events were reported in 46 (1.5%) of 3082 participants exposed to VLA1553 and in eight (0.8%) of 1033 participants in the placebo group. Only two serious adverse events were considered to be related to VLA1553 treatment (a mild myalgia and an inappropriate antidiuretic hormone secretion syndrome). Both participants recovered completely. The results of this study strongly indicate that the vaccine has high tolerability and antibody-mediated immunogenicity (SCHNEIDER et al., 2023).

In a study of adolescents in Brazil in which we participated, we demonstrated results very similar to those recorded in adults with 98.8% serological conversion after vaccination with IXCHIQ® (BUERGER et al., 2025). The vaccine was generally well tolerated, with the vast majority (2,082 [97%] of 2,155) of adverse events being mild or moderate intensity. Compared

with placebo, participants exposed to VLA1553 had a significantly higher frequency of related adverse events (352 [70%] of 502 vs. 122 [48%] of 252; $p < 0.0001$), mainly headache, injection site pain, myalgia, fever, and fatigue. The study concludes that the vaccine was safe and induced seroprotection titers for up to 12 months in almost all adolescents, with favorable safety data in those who were seropositive for Chikungunya (BUERGER et al., 2025).

Recently, the Butantan Institute in partnership with the French-Austrian pharmaceutical company Valneva started the production of the VLA1553 vaccine that was called IXCHIQ®, which is the first against the disease to be registered in the world. The immunizer was approved by Anvisa in April 2025 for use in adults in Brazil, based on recent studies in Brazil demonstrating safety and high seroconversion in adolescents in Brazil (BUERGER et al., 2025). IXCHIQ® has a high efficacy rate (98.8% - 100% seroneutralization in previous studies and single-dose application. Registered by Anvisa, the vaccine has shown long persistence of antibodies (up to 3 years or more) (MCMAHON et al., 2024) and started, in February 2026, a pilot vaccination program in Brazil. However, little is known about the cellular immune response of vaccinated individuals and the late duration after 3 years of vaccination in the single-dose vaccination protocol.

3. Hypothesis

The hypothesis of this study is that IXCHIQ® will induce robust cellular immune response and activation of different compartments of the cellular immune response that are still unknown. The cellular immune response will be similar in adolescents and adults.

4. Objective

To seek a more complete understanding of the immune response induced by vaccination with IXCHIQ.® To achieve our goal, we will investigate the cellular and humoral immune response in individuals immunized at different times before and after vaccination with IXCHIQ® in different age groups. This knowledge may be useful in the future discovery of correlates of protection in vaccinated studies. In addition, this study may provide important data for the planning of future studies aimed at evaluating lower doses of the vaccine that cause fewer side effects.

Specific objectives, outcomes and expected results

4.1 Primary Objective:

To evaluate the cellular response profile of mononuclear (monocytes, DC, T, B, NK) and polymorphonuclear (Neutrophil) cell subpopulations of individuals of different age groups immunized with IXCHIQ.® Cellular immune response will be assessed **before, 3, 7, 30, 180, and 720** days after vaccination;

Primary outcome: Number and percentage of the various populations of vaccine-induced mononuclear and polymorphonuclear cells when evaluated by the flow cytometry technique contrasting age groups.

Expected outcome: Detailed description of the innate and memory-induced immune response after vaccination in participants vaccinated as IXCHIQ® of different age groups;

Objective and safety outcome: To assess the incidence of adverse events, in particular adverse events of special interest (Chikungunya Like adverse events – CLARs, carditis, encephalitis, and grade 3 or higher adverse events) up to 60 days after vaccination.

4.2 Secondary objectives:

4.2.1 Secondary Objective 1: Longitudinally evaluate the kinetics of the neutralizing antibody-mediated response to CHIKV of individuals immunized with IXCHIQ® before, 3, 7, 30, 180, and 720 days after vaccination;

Secondary outcome 1: titer of antibodies that are capable of neutralizing a Brazilian strain of Chikungunya and the vaccine strain in a plaque neutralization assay.

Expected outcome 1: titer profile of neutralizing antibodies of both the ECSA strain of Chikungunya circulating in Brazil and the vaccine strain and antigens (proteins and peptides) of CHIKV

4.2.2 Secondary objective 2: To evaluate the profile of soluble mediators (cytokines) and microparticles of individuals immunized with IXCHIQ® before, 3, 7, 30, 180, and 720 days after vaccination;

Secondary outcome 2: levels, in pg/mL, of cytokines, chemokines, and growth factors as well as the percentage of microparticles in the plasma of vaccinated individuals.

Expected outcome 2: kinetic profile of cytokines, chemokines and growth factors as well as microparticles throughout vaccination in the different age groups.

4.2.3 Secondary objective 3: To correlate the neutralizing antibody profile with the innate and adaptive immunity cell response of individuals immunized with IXCHIQ® before, 3, 7, 30, 180, and 720 days after vaccination;

Secondary outcome 3: correlation matrix in correlograms or heatmaps of the humoral and cellular response.

Expected outcome 3: description of the associations between cellular response profile and soluble mediators.

4.2.4 Secondary objective 4: To compare cellular immune response and neutralizing antibody profile in adolescents and adults;

Secondary outcome 4: Comparative table of cellular and humoral immune responses in adolescents (12-17 years) and adults (18-59 years).

Expected outcome 4: description of cellular and humoral immune responses in the two age groups of interest.

4.3 Exploratory objectives: Evaluation of the B cell repertoire and identification of vaccine immunodominant epitopes;

Exploratory outcome 1: reactivity of immunodominant epitopes present in the vaccine through peptide microarray and percentages of B lymphocytes reactive to vaccine antigen.

Expected outcome 1: detailed description of the reactivity of immunodominant epitopes present in the vaccine obtained through peptide microarray and kinetic profile of B lymphocytes reactive to vaccine antigen.

5. Methodology

5.1. Study population

This exploratory prospective cohort study, with a convenience sample, will be conducted at the Clinical Research Unit of the CT Therapies of UFMG and will include a study population consisting of individuals previously unvaccinated with the single-dose vaccine consisting of live-attenuated virus (IXCHIQ®) and not infected. The individuals to be included will be recruited within the metropolitan area of Belo Horizonte by the team of the Clinical Research Unit of the CT therapies of UFMG. We will also disseminate it through social media and folders. After applying the consent form, individuals will be included and blood samples obtained before vaccination (baseline – D0) and at 3 , 7, 30, 180 and 720 days after vaccination.

This project will be subdivided into two stages:

PART A , which consists of adult subjects, including a total of 50 volunteer participants aged 18 to 59 years.

PART B consisting of children and adolescents, up to 25 volunteer individuals aged 12 to 17 years.

The Study will last for 36 months or until the end of the follow-up of the last subject. Subjects will be evaluated by the attending physician who will identify those who meet all inclusion and exclusion criteria. This project will be submitted for approval by the appropriate ethics committees and all volunteers, in addition to filling out a data collection form relevant to the research, will sign the informed consent form before any procedure. Children and adolescents will sign the informed consent form and the legal guardian will give informed consent before any procedure.

5.2. Study design:

After obtaining consent and assent, the following visits will be made for PART A and PART B:

- **Visit 1 (V1) or baseline visit:** Assessment of inclusion and exclusion criteria, clinical examination, and sample collection prior to vaccination (Baseline). After collection, the participant will be vaccinated by the IXCHIQ vaccine and will be under observation for a minimum of 30 min.
- **Visit 2 (V2) – 3 days after vaccination:** the participant will be examined by the medical team, active search for symptoms potentially arising from the vaccine will be evaluated and new blood samples will be collected.

- **Visit 3 (V3) – 7 days after vaccination:** the participant will be examined by the medical team, active search for symptoms potentially resulting from the vaccine will be evaluated and new blood samples will be collected.
- **Visit 4 (V4) – 30 days after vaccination:** the participant will be examined by the medical team, active search for symptoms potentially arising from the vaccine will be evaluated and new blood samples will be collected.
- **Telephone Contact (C1) – 60 days after vaccination:** a call will be made to the participant in search of active adverse events potentially associated with the vaccine.
- **Visit 5 (V5) – 180 days after vaccination:** The participant will be examined by the medical team and blood samples will be taken.
- **Visit 6 (V6) – 720 days after vaccination:** The participant will be examined by the medical team and blood samples will be taken.

For PART A, each participant included in this study will be obtained collections of the following blood samples for the performance of complementary laboratory tests at each of the visits envisaged in the study, except for visit 2 (detailed below):

- 27 mL of blood in Heparin for plasma and PBMC (3 tubes of 9 ml);
 - 9 mL of blood to obtain serum (humoral immune response).
- Total: 36 mL

Visit 2:

- 9 mL of blood in Heparin for plasma and total cells (1 tube of 9 ml);
 - 4 mL of blood to obtain serum (humoral immune response).
- Total: 13 mL

For PART B, each participant included in this study will be obtained collections of the following blood samples for the performance of complementary laboratory tests at each of the visits anticipated in the study, except for visit 2 (detailed below):

- 18 mL of blood in Heparin for plasma and PBMC (2 tubes of 9 mL);
 - 4 mL of blood to obtain serum (humoral immune response).
- Total: 22 mL

Visit 2:

- 9 mL of blood in Heparin for plasma and total cells (1 tube of 9 ml);

- 4 mL of blood to obtain serum (humoral immune response).

Total: 13 mL

5.3. Eligibility Screening and Assessment

Individuals of both sexes from and who do not have the contraindications in the package insert and defined by the Ministry of Health will be selected (TECHNICAL NOTE No. 9/2026-CGFAM/DPNI/SVSA/MS - <https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/notas-tecnicas/2026/nota-tecnica-no-9-2026-cgfam-dpni-svsa-ms.pdf>).

Inclusion Criteria:

- The participant or guardian understands the procedures and requirements, is willing and able to give informed consent for full participation in the study;
- For PART A, adults of both sexes aged between 18 and 59 years and according to the indication of the Ministry of Health (<https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/notas-tecnicas/2026/nota-tecnica-no-9-2026-cgfam-dpni-svsa-ms.pdf> - Technical note: Guidelines for monitoring the safety of the chikungunya vaccine used during the pilot vaccination strategy in Brazil - 2026).
- For PART B, children/adolescents aged 12 to 17 years of both sexes.

Exclusion Criteria:

- Participants under the age of 12 or 60 years or older
- Who have any contraindication raised by TECHNICAL NOTE No. 9/2026-CGFAM/DPNI/SVSA/MS of the Ministry of Health.
- Participants who are not available to undertake the planned study visits;
- Participants who do not agree to sign the ICF;
- Participants who have had any live vaccine or other vaccine in the last 30 or 15 days, respectively.
- B-HCG positive on the day of vaccination, in the case of women of reproductive potential;
- Participants Who are pregnant or planning to become pregnant within the next 3 months after the vaccine dose.
- Participants breastfeeding.

- The participant with a known or suspected immune system defect that may influence the immune response to the vaccine, such as participants with congenital or acquired immunodeficiency, including HIV infection, post-organ transplant status, or immunosuppressive therapy within 4 weeks prior to Visit 1. Immunosuppressive therapy is defined as the chronic administration of prednisone (more than 14 days) or equivalent ≥ 2 mg/kg/day within 4 weeks prior to study entry. Note: radiation therapy or immunosuppressive cytotoxic drugs/monoclonal antibodies within the previous 3 years; topical and inhaled glucocorticoids are allowed.
- Any clinical instability identified by the treating physician that may put the participant at risk.

5.4. Study site

The study will be the responsibility of and will be carried out at the Clinical Research Unit of the Center for Advanced and Innovative Therapies of UFMG located at Rua Carlos Pinheiro Chagas 20, Bairro Jardim Montanhês, Belo Horizonte. All members of the team of the Clinical Research unit of the Center for Therapies of UFMG must have certificates of suitability for each function (nurse, clinical physician, etc.) they perform. All support laboratory and research staff that handle samples and biological data are trained and certified for good clinical practice [e.g., Collaborative Institutional Training Initiative (CITI) Program], as required by good clinical research practices. In addition, all staff will be trained annually in detailed SOPs that cover all items of clinical research, including patient confidentiality and privacy.

5.5. Informed consent

Written versions of the Informed Consent Forms (ICF) will be presented to participants detailing the exact nature of the study, what this will involve for the participant, and any risks involved in their participation. Each participant will sign the ICF corresponding to the cohort in which they will be enrolled. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason, without prejudice to future care and without obligation to provide the reason for withdrawal. The participant will be given as much time as necessary to weigh the information, with the opportunity to question the investigator or other independent parties to decide whether they will participate. The application of the ICF will then be documented by a signature of the participant with the date and signature of the person who submitted it and obtained consent. The person who obtained consent must be appropriately

qualified and experienced and have been delegated to do so by the principal investigator (PI) of the site. One copy of the signed ICF will be given to the participant, the other signed copy will be filed at the research site.

The IXCHIQ vaccine is approved for clinical use in Brazil, but is not recommended for use in pregnant women. During this study, for female participants, as it is a live attenuated virus vaccine, every care will be taken to avoid exposure of pregnant women to the vaccine. Therefore, all female participants will be considered to be of childbearing potential, except for those who have a documented history of hysterectomy, tubal ligation, or are menopausal (12 months of amenorrhea after their last menstrual period).

Women of reproductive age with pregnancy confirmed by β -hCG test (performed at admission), breastfeeding and/or manifesting intention to have sexual practices with reproductive potential without the use of contraception in the three months following vaccination will be excluded from the study. For female participants of childbearing potential, the study physician will assess the need to start using a contraceptive method before receiving the vaccine. Female participants who are at risk of becoming pregnant will be advised to use highly effective contraception for 6 months after receiving the vaccine. Contraceptive methods will not be mandatory if the volunteer declares that she is exempt from the risk of becoming pregnant, either by not engaging in sexual practices or by exercising them in a non-reproductive way, during the entire study period.

5.6. Sample Size

Previous studies have shown that the IXCHIQ vaccine induces seroconversion in about 99% of vaccinated individuals for a period of more than 1 year (Schneider et al., 2023). As there are no studies evaluating the efficacy of the vaccine in inducing cellular immune response, we will use a convenience sample and follow a group of 75 vaccinated individuals. The larger sample in adults will be necessary to assist in the parameters to be studied in children/adolescents. After analyzing the initial parameters of the study, especially the response of IFN- γ of memory T lymphocytes to Chikungunya antigens, the number of individuals in the study will be reassessed, especially the number of children. The study will characterize the cellular immune response and evaluate whether the mean/median of the molecular parameters associates with clinical parameters and with the mean/median of the humoral immunity parameters (neutralizing antibody titers). The parameters will be evaluated longitudinally.

5.7 Sample Processing: Blood Collection and Isolation of Peripheral Blood Mononuclear Cells (PBMC)

In total, at each visit, blood will be collected in sterile vacuum system tubes to carry out the analyses provided for in the project (volumes detailed in item 5.2 above). The sodium heparin anticoagulant will be used to study total cells or obtain peripheral blood mononuclear cells (PBMC) for flow cytometry assays. Tubes with clot activator to obtain serum will be used to evaluate neutralizing antibodies and response to cytokines, chemokines and growth factors.

The isolation of peripheral blood mononuclear cells (PBMC) from vaccinated individuals will be performed using a Ficoll-Paque gradient with a density of 1,078 g/mL (Cytivia). In general, sodium heparin tubes containing the collected blood will be centrifuged at 3000 rpm for 10 minutes at 20°C to remove the plasma and obtain aliquots to be deposited in the project's biorepository. The next step will be the dilution of the blood with the same volume of incomplete RPMI medium (Gibco – ThermoFisher Scientific) in a 1:1 ratio in a 50 mL falcon tube. Then, the diluted blood was homogenized and applied slowly with the aid of a serological pipette over the Ficoll-Paque solution in a 1:2 ratio, approximately 15 mL of Ficoll-Paque to approximately 30mL of whole blood. After this step, a 40-minute centrifugation will be performed at 500 x g at room temperature (T.A) with acceleration 5 and deceleration 1, which will allow the formation of a PBMC ring on the Ficoll-Paque layer. This cell ring should then be carefully removed with the aid of a 3 mL Pasteur pipette and transferred to a new 50 mL falcon tube. Then, two washes will be performed to remove the excess Ficoll-Paque.

Cell counting will be performed on the automated cell counter Countess 3 (Thermo Fisher Scientific) and viability values, total cells, live cells and dead cells will be noted. For cell concentration counting, live cell data will be used and PBMC freezing will be at a concentration of 5×10^6 cells in 1 mL per cryovial, considering 30% cell loss after thawing. The cells will then be resuspended in cryopreservation solution containing 90% SFB (Sigma-Aldrich) and 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich), kept in a -80°C *overnight* freezer and later transferred to liquid nitrogen until use. Additionally, approximately 5×10^5 cells from each study volunteer will be cryopreserved separately to be used in the identification of HLA-A*02+ subjects.

5.8. Immunophenotypic and functional analysis of memory T cells

The performance of immunophenotypic and functional analysis of cells will be performed as recently described with modifications (REIS et al., 2025). Approximately 1×10^6 of PBMC isolates first thawed at 37°C will be used. Then, the samples in the cryovials will be transferred

to a 96-well plate with a "U" bottom and stimulated with CHIKV antigens for 48 to 96h. Then, the cells will be labeled with T and B cell activation antibody mix, NK.

After incubation, the plate should be incubated for 30 minutes. After the incubation time, 150 μ L of 1x PBS will be added to the wells and the volume will be transferred to 5mL polystyrene tubes for reading in flow cytometry. The tubes will be kept in a refrigerator wrapped in aluminum foil until the moment of reading, which will be performed on the same day.

5.9. In vitro stimulation with vaccine and CHIKV antigens

First, the PBMC samples stored in the cryovials should be thawed rapidly in a water bath at 37°C. The PBMC will then be adjusted to a concentration of 1×10^7 cells/mL in 10% SFB RPMI medium with antibiotic solution and then 50 μ L of this cell suspension will be added to a 96-well U-bottom plate. The volume of the wells will be completed to 200 μ L and the cells will be incubated in an incubator at 37°C and 5% CO₂ until the next day. After incubation, vaccine antigen stimulation (VLA1553-301) and other vaccine prototypes at concentrations of 1, 2.5 and 5 micrograms/mL will be added to the cultures. There will be control (CC) culture wells in the absence of antigens and there will also be an "unlabeled" well with absence of stimulus. As a functionality control (positive control) for the cell activation study, cultures will be performed in the presence of nonspecific PMA stimulus (final concentration 20ng/mL) (Forbol 12-myristate 13-acetate, Sigma-Aldrich) and ionomycin PMA (final concentration 1microg/mL) (Sigma-Aldrich).

After the addition of the stimuli, the plate must be incubated for 68 hours to perform ELISPOT according to the manufacturer's instructions. Part of the cells will be sent to the analysis of intracytoplasmic cytokines and for this, Brefeldin A (BFA-A) will be added, soon after the addition of PMA to obtain a final concentration in the well of 10 μ g/mL. Thus, 20 μ L of BFA solution (100 μ g/mL) will be added to each well of the 96-well plate and the plate will be incubated for 4 hours in the incubator at 37° C 5% CO₂.

Fifteen minutes before the end of the 4 hours of incubation of the plate, EDTA will be added to obtain a final concentration in the well of 2 mM and the plate will be stored again in the oven. After incubation, the oven plate will be centrifuged at 1500 rpm for 5 minutes at 4°C to remove the culture supernatant. The culture supernatants will then be transferred to Eppendorfs or mirror plate duly identified and subsequently stored at -20°C.

The next steps consist of carefully resuspending the cells in the vortex, adding 100 μ L of PBS 1X, and joining the wells in duplicate to make a single marking. Then the plate will be centrifuged at 1500 rpm for 5 minutes at 4°C and supernatant will be discarded. Finally, the

cells will be resuspended in 20µL of the Live/Dead dye solution for use, carefully homogenizing with subsequent incubation for 15 minutes in the dark at room temperature. Then, add 100 µL of PBS-W buffer and incubate at 5 minutes before proceeding to the addition of the surface antibody mix.

After the addition of the surface antibody mix, incubation will be done for 30 minutes in the dark at 4°C. Then, 100 µL of PBS-W will be added for washing with subsequent centrifugation at 1500 rpm for 5 minutes at 4°C. The supernatant will then be discarded and the cells carefully resuspended in the vortex. The cells will be fixed with 100 µL of 4.2% PFA diluted in 1x PBS and incubated on ice for 20 minutes. After 20 minutes, the cells will be centrifuged again at 1500 rpm for 5 minutes at 4°C and 100 µL of PBS-P will be added. After 5 minutes of incubation, the cells will be homogenized again and incubated for another 5 minutes. Finally, the plates will be centrifuged at 1500 rpm for 5 minutes at 4°C, the supernatant will be discarded and the cells will be carefully resuspended in the vortex.

As a next step, the antibody mix for intracytoplasmic cytokine labeling will be added. After the addition of the intracytoplasmic cytokine antibody mix, the samples will be carefully homogenized in the vortex and incubated for 30 minutes in the dark on ice. After incubation, add 100 uL of PBS-P buffer and centrifuge at 1500 rpm for 5 minutes at 4°C. Then, the supernatant will be discarded and the cells will be carefully resuspended in the vortex. Subsequently, 200 uL of PBS-W buffer will be added and homogenized in the vortex carefully. Finally, the cells will be centrifuged at 1500 rpm for 5 minutes at 4°C, the supernatant will be discarded and carefully resuspended in the vortex with 200 uL of PBS 1X pH:7.2-7.4 for the reading.

Immunophenotyping will be performed by the BD Symphony equipment (BD Biosciences) and the data will be acquired by the Diva software (BD Biosciences) in the flow cytometry platform of the Institute of Biological Sciences of UFMG. All cytometry data will be analyzed using the FlowJo v.10.6.2 software (BD Biosciences).

5.10. Assessment of soluble factors and microparticles

The evaluation of the soluble factors present in the serum samples of the patients included in this project will be carried out using methodologies similar to the *LuminexPlex Panel* (Bio-Rad Laboratories, Inc. or Thermo Fisher). The magnetic spheres of the assay are covered by monoclonal antibodies specific to immune mediators: such as chemokines, proinflammatory cytokines, regulators and growth factors. The reading will be done in the Bio-Plex 200 device (Bio-Rad Laboratories, Inc). The results will be expressed in pg/mL for all mediators tested.

For the detection of microparticles (PM), samples of plasma obtained in heparin anticoagulant will be subjected to low-rotation centrifugation. The PM-rich sediment will be labeled for identification by universal marker with Annexin V. The cytometer will be calibrated based on the front and side dispersion of fluorescent microbeads of defined size – 0.7 to 0.9 μm (SPHERO™ Amino Fluorescent Particles- Spherotech Inc. Libertyville, Illinois, US) for the identification of the size of the PMs. The phenotypic characterization of the PMs and the determination of their cellular origin will be done by incubating 100 μL of the PM-rich plasma with protocol-specific antibodies.

5.11. Detection of circulating antibodies, immunoglobulin epitope repertoire, BCR sequencing and Plaque Reducing Neutralizing Titers (PRNT) against CHIKV and vaccine virus.

Initially, the measurement of anti-CHIKV IgG antibodies will be performed using commercially available ELISA kits and following the manufacturer's recommendation. In the relevant groups, we will also perform the measurement of antibodies to DENV. To perform the seroneutralization test (PRNT), the serum samples of interest will be incubated at 56 °C/30 min, for inactivation of the complement system. In 96-well plates, the samples will be subjected to serial dilution in a 1:1 ratio (1 volume of the serum sample + 1 volume of MEM medium). In the first row of the plate, corresponding to the first dilution of the serum (1:10), 15 μL of serum will be added to 135 μL of MEM containing 1% SFB, in six consecutive wells. In the subsequent wells, 75 μL of medium will be added, which will be used to perform serial dilutions up to 1:320. After the preparation of the dilutions, 75 μL of the viral suspension will be inoculated into each well, which will double the final dilution of the serum; thus, the initial dilution of 1:10 will change to 1:20 after the addition of the virus, continuing until the final dilution of 1:640. The plate containing serum and virus will be incubated for 1 h at 37 °C in an incubator. Then, 100 μL of the serum + virus suspension will be inoculated into the respective wells of a 24-well plate containing Vero CCL-81 cells and the adsorption step will be performed for 1 h. After this period, the inoculum will be removed, 700 μL of carboxymethylcellulose (1% CMC) will be added, and the plates will be incubated for 48 h. Subsequently, they will be fixed with 3.7% formaldehyde and stained with 1% violet crystal. At the end, the number of PFUs of the tested samples will be counted and compared to the viral control, and the results will be expressed in reduction percentage graphs. The CHIKV virus, ECSA genotype (BHI3762/H804917) and vaccine virus will be used, in the amount of 80 PFU/well. As a control, a non-reactive serum will be used in association with the same viral dilution, constituting the viral control of the

experiment. To evaluate the immunoglobulin repertoire, the PepperPrint System will be used according to the manufacturer's description.

5.12. Characterization of plasma cells and virus-specific memory B cells

For analysis of the B-cell-mediated immune response, PBMCs obtained from vaccinated participants as previously described, will be distributed in sterile culture microplates at the concentration of 1×10^6 cells and stimulated ex-vivo with viral antigens.

After stimulation, plasma cells and specific memory B cells will be identified and quantified by flow cytometry using specific cell surface markers, including CD19 and CD27, among others. Subsequently, these memory B cells will be isolated individually by cell screening, allowing the achievement of antigen-specific monoclonal B cells. The repertoire and clonality of the B cells will be analyzed by B cell receptor sequencing (BCR). For monoclonal antibody production, the monoclonal B cells will be expanded in vitro and the DNA of the isolated cells will be sequenced to characterize the immunoglobulin repertoire.

6. Discontinuation and withdrawal of study participants

Participants may choose to stop study assessments. Participants may also withdraw their consent, meaning they wish to withdraw from the study altogether. In the event of withdrawal from active follow-up, the following options for a staggered withdrawal from the study would apply

- a) Patients withdraw from the study but allow data and samples obtained up to the point of withdrawal to be retained for use in study analysis. No other data or samples will be collected after withdrawal,
- b) Patients withdraw from active follow-up and further communication, but allow study staff to continue accessing their medical records and any relevant data that is recorded as part of the routine standard of care; i.e., blood results, etc.
- c) Patients withdraw but do not allow the collected data and samples to be used. In this case, the participant must make this request in writing, sign and date.

In addition, the Investigator may exclude the study participant at any time in case of significant protocol deviation that would impair the main study objectives; non-compliance with study requirements; ineligibility (arising during the study or retrospectively having been neglected at screening), pregnancy, serious adverse event resulting in the inability to comply with study requirements. The reason for discontinuation and/or withdrawal will be recorded on

the Case Report Form. Consenting participants may rescind their consent until completion of the study. Unless requested, data and samples collected prior to the withdrawal date will be retained in the study database and analysis.

7. SECURITY REPORT

7.1. Definitions

Adverse Event (AE)	Any untoward medical occurrence in a patient to whom the investigational product has been administered, including occurrences that are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An undesirable and unintended response in a patient to a PSI that is related to any dose administered to that patient. It means that a causal relationship between a PSI and an EA is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by the qualified medical professional to have a reasonable suspicion of a causal relationship to the PSI's qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death; • it is life-threatening; • requires hospitalization or prolongation of existing hospitalization; • results in persistent or significant disability/incapacity; • It consists of a congenital anomaly or birth defect. Other 'major medical events' may also be considered serious if they endanger the patient or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "severe" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically could have caused death if it had been more severe. Hospitalization is defined as a formal, unplanned hospitalization, even if hospitalization is a precautionary measure for continuous observation. The patient must be hospitalized for at least 24h; a short stay of several hours to receive treatment is not considered hospitalization. If a patient is admitted overnight or longer for social/economic or isolation reasons and is clinically stable, this does not constitute an SAE. Other examples of visits to a hospital facility that are not considered hospitalization are: emergency department visits, outpatient surgery, pre-planned or elective procedures for a pre-existing condition (provided that this condition has not worsened during experimental treatment or anticipated due to worsening symptoms), and for the purpose of this study, being hospitalized for isolation of Influenza.
Serious Adverse Reaction (SAR)	An adverse event that is serious and, in the opinion of the reporting investigator, is believed to be reasonably likely to be due to one of the study treatments, based on the information provided.

NB: to avoid confusion or misunderstanding about the difference between the terms "serious" grade 3 EA, the following note of clarification is provided: "Serious" is often used to describe the intensity of a specific event, which may be of relatively minor medical importance. EAG is the regulatory definition provided above.

Any pregnancy that occurs during the clinical trial and the outcome of the pregnancy must be recorded and followed for congenital abnormalities or birth defects, in which case it would fall under the definition of AGA.

Special attention should be given to some events – which we will call adverse events of special interest – up to 60 days after vaccination, which are:

- Chikungunya Like adverse Events (CLARs) - A systemic adverse reaction is officially classified as CLAR if the patient has fever (axillary temperature $\geq 37.8^{\circ}\text{C}$) within 60 days of vaccination, associated with at least one of the following classic Chikungunya symptoms: severe joint pain (arthralgia) or joint swelling (arthritis), muscle pain (myalgia), severe headache, rash, fatigue, or general malaise.
- Neurological events (such as encephalitis, encephalopathy, or mental confusion).
- Cardiac events (tachyarrhythmia or pericardial effusion).

7.2. Causality

The relationship of each adverse event to the study drug must be determined by a medically qualified individual according to the following definitions:

Definitely related:	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
Probably related:	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.
Possibly related:	There is some evidence to suggest a causal relationship (e.g., because the event occurs within a reasonable amount of time after administration of the study drug). However, the influence of other factors may have contributed to the event (e.g., patient's condition, other concomitant treatments).
Probably unrelated:	There is little evidence to suggest the existence of a causal relationship (e.g., the event did not occur within a reasonable time after administration of the study drug) or other reasonable explanation for the event (e.g., the patient's clinical condition, other concomitant treatment).
Unrelated:	There is no evidence of any causal relationship.

7.3. Procedures for recording adverse events

The severity of adverse events will be assessed following the Common Terminology Criteria for Adverse Events (CTCAE) v6.0. AEs occurring in participants since inclusion and

during study participation that are observed by the investigator or reported by the investigator will be recorded in the CRF, whether or not assigned to the study vaccine.

The following information will be recorded: description, start date and end date, severity, assessment of relationship to study drug, other suspected cause, and action taken. Follow-up information should be provided as needed. AEs considered related to the study drug (vaccine) as judged by a qualified medical investigator will be followed until resolution or the event is deemed stable. All of these cases will be properly reported and discussed with the company producing the PSI as needed. Occurrences or abnormalities may be considered to be related to the participant's medical history or considered to be not clinically significant. In these circumstances, the occurrence should not be recorded as an AE.

Special attention should be taken in relation to a non-clinically significant event so that an AE is not missed. Physiologically expected events such as menstruation, occasional cramps, self-limited fatigue after exercise, can be classified in this way, but if you observe a worsening of these events in relation to baseline, this is done.

7.4. Serious adverse event reporting procedures

All SAEs detected by the site investigator are to be reported to the CEP as soon as possible after knowledge of the EAG. Other follow-up reports are to be submitted if necessary until the EAG resolves, is deemed stable/permanent, or results in death. A final status should be determined for any ongoing SAEs at the end date of the study.

8. Data Management and Record Keeping

Clinical study data will be recorded in CRFs and entered into a password-protected database by the local study PI, nurse researcher, or designee. The study database will be built on the REDCap system, a clinical data management system that complies with ICH GCP and FDA regulation, title 21 of the Code of Federal Regulations Part 11. It will be hosted on a secure server with restricted access.

No information about the study or data will be disclosed to unauthorized third parties without the prior written approval of the sponsor. All research activities will be conducted in as private an environment as possible. The study participant's contact information will be stored securely at UFMG for internal use during the study. Paper forms will be stored in locked files in locked rooms. Participants will be identified only by an identification number in all study documentation and in the electronic database.

Electronic information will be stored in encrypted, password-protected software. At the

end of the study, all records will continue to be kept in a secure location for the period determined by the review ZIP code, institutional policies, or sponsor requirements. The study will comply with the General Data Protection Law (LGPD), which requires that personal data not be kept as identifiable data for longer than necessary for the purposes in question.

9. Graphical and statistical analysis

After obtaining the quantitative data, the statistical and graphical analyses will be made using the GraphPad Prism v.8.0 software (GraphPad Software, Inc.) to compare the different parameters obtained during the project.

The study analyses will be conducted in accordance with relevant regulations and standard operating procedures. The study will be conducted in compliance with this protocol, the International Harmonization Conference (ICH) Guidelines for Good Clinical Practice (GCP), and any applicable regulatory requirement. Validation of the data will be performed to identify errors or discrepancies and thus ensure the integrity, validity, and accuracy of the data.

Integer or continuous variables will be expressed as mean and standard deviation or median and interquartile range. Categorical variables will be presented with absolute and relative frequencies. Normality of variables will be tested with the Shapiro-Wilk test while equality of variance will be analyzed with the F or Bartlett test. Mean or median differences will be evaluated by Wilcoxon, Kruskal-Wallis tests, T-test or analysis of variance considering the number of groups and assumptions of each test. Associations between categorical variables will be tested by Fisher's exact test or Pearson's chi-square. Pearson's or Spearman's correlation coefficients will be estimated to explore the existence of linear association between continuous or integer variables. Generalized linear and linear models will be used for multivariate structures and data with temporal or spatial relationships will be explored with random effects models or generalized estimation equations. Estimates of time to occurrence of an event will be made using non-parametric Cox models or equivalent. The significance level will be 5%.

First, the Shapiro-Wilk normality test will be performed to verify if the data have a Gaussian distribution. Then, the comparative analysis between groups will be done by the ANOVA test if the data present a parametric distribution and comparisons of more than one group, followed by the Holm-Sidak test for multiple comparisons. For non-parametric data, the Kruskal-Wallis test will be used followed by the Dunn post-test for multiple comparisons. For paired data, the Wilcoxon test or the mixed effects model (REML) will be used to compare the control and samples after stimulation. Finally, analysis and graphing by interaction networks

will be performed, using the Cytoscape 3.10.1 software (Cytoscape Open Consortium, <https://cytoscape.org/>). Clinical and laboratory data will also be used for correlation analyses.

10. Project Risks and Ethical Considerations

The risks associated with participation in the research involve the usual risk of vaccination with an approved vaccine, possible discomfort in the clinical examination and in obtaining blood samples, in addition to the loss of confidentiality of the data. To minimize these risks, follow-up and examinations will be performed by experienced clinicians and blood collection will be performed by staff trained in the process and able to deal with the adverse effects associated with the procedure (lipothymia and hematoma, in particular). The Investigator will ensure that this study is conducted in accordance with the current revision of the Declaration of Helsinki. The Investigator will ensure that this study is conducted in accordance with relevant regulations and Good Clinical Practice (ICH GCP).

The protocol and informed consent form will be submitted to the local ethics committees for written approval. The Investigator will submit and, where necessary, obtain approval from the above parties for all changes to the original approved documents. The study team will ensure that the anonymity of participants is maintained. Participants will be identified only by a participant identification number in all study documents and in any electronic database, with the exception of the CRF, where the participant's initials may be added. The name and any other identifying details will NOT be included in any electronic study data files. All documents will be stored securely and accessible only by study staff, the Human Research Ethics Committee, and any external study auditors. All documents will be stored securely and accessible only by study staff and authorized personnel. The study will comply with the General Data Protection Law (LGPD), which requires that personal data not be kept as identifiable data for longer than necessary for the purposes in question

All assistance will be provided to the participant in case there are any health issues arising from their participation in the study. Women who are pregnant, actively trying to conceive, or breastfeeding will be excluded from this study as it is not known if the vaccine will have additional benefits that outweigh any risks associated with pregnancy/breastfeeding.

11. Benefits

Participation in the study may eventually benefit the research subject. In the event that clinical problems are identified, the subject will be referred for workup and/or therapy in the health care system. Although an individual patient may not benefit personally, this study should

aid in the development of new vaccines for CHIKV. Participants will be reimbursed for costs associated with travel to the study site and food.

12. Quality Control

The study will be conducted in accordance with the relevant regulations and standard operating procedures of the Clinical Research Unit of the Center for Advanced and Innovative Therapies at UFMG.

The study will be conducted in compliance with this protocol, the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP), and any applicable regulatory requirement. Monitoring will be conducted locally and supervised with the assistance of the to ensure compliance with the study protocol and applicable guidelines and regulations. Biological samples will be processed and stored in accordance with the Research Center's SOPs.

Data validation will be carried out to identify errors or discrepancies and thus ensure the integrity, validity and accuracy of the data.

13. Counterpart resources

This project will receive counterpart from the CT Advanced and Innovative Therapies and projects already previously approved by our group. This project was approved by the FAPEMIG 2026 Universal Notice (APQ-03199-26). The vaccine doses will be donated by the Valneva Company and the Butantan Institute. New resources will be actively sought for the development of all stages of the process.

14. Ownership of data

The data generated in this study belong to the study group as a whole. The final database will be shared between the site PI and key members of the research team. As vaccine donors, the data will be shared with the Valneva Company and the Butantan Institute. This data will be sent in anonymized form.

The database may be shared with researchers not directly involved in this study after the publication of the main article. The criteria for authorship will be consistent with international guidelines (<http://www.icmje.org/#autor>). The investigators will be involved in reviewing the drafts of the manuscripts, abstracts, press releases, and any other publications arising from the study. Authorship will be determined according to the guidelines of the International Committee of Medical Journal Editors (ICMJE) and other collaborators will be acknowledged. The results of the study will be summarized in lay language, both in English and in the language(s) commonly spoken at the study sites, and disseminated to key stakeholders, user communities, and patients.

15. Participant Confidentiality

The confidentiality and privacy of the participants will be strictly kept confidential by the investigators and their staff. This confidentiality extends to cover tests with biological samples in addition to the clinical information of the participants. Therefore, the study protocol, documentation, data, and all other information generated will be kept in strict confidentiality. The study team will ensure that the anonymity of the participants is maintained. Participants will be identified only by an identification number in all study documents and in any electronic databases.

No information about the study or the data will be disclosed to unauthorized third parties without the prior written approval of the sponsor. All research activities will be conducted in as private an environment as possible. The study monitor, other authorized representatives of the sponsor, representatives of the IRB, the regulatory agencies, or the pharmaceutical company providing the study product may inspect all documents and records that must be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) and pharmacy records of participants in this study. The clinical study center will allow access to these records.

16. Schedule of activities

Procedures	Visit 01 Inclusion D0	Visit 02 D3±1	Visit 03 D7±1	Visit 04 D30±3	Contact Now Phone. D60±5	Visit 05 D180±7	Visit 06 D720±14
Informed consent							
Eligibility Assessment							
Demographics, Medical history, and physical examination							
Review of clinical data after vaccination and physical examination							
Review of adverse events of special interest							
Blood Collection							
Heparin (PBMC and plasma) PART A: 27 mL ^A PART B: 18 mL ^A							
TSS (serum) PART A: 9 mL ^{to} PART B: 4 mL ^b							
a- Heparinized blood will be used to obtain PBMC and plasma to study the cellular immune response and microparticles. For visit 2, 1 tube of 9 mL heparin will be collected for both groups. b- Blood in TSS will be used to obtain serum and evaluate soluble mediators and circulating antibodies. For visit 2, 1 4 mL serum tube will be collected for both groups.							

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