CLINICAL STUDY PROTOCOL

Protocol Title: A Phase 1, First-in-human, Randomized, Observer-blind, Parallel design, Controlled, Dose Level and Schedule-finding Study to Evaluate the Safety, Reactogenicity, and Immunogenicity of a Self-Amplifying mRNA Pandemic Influenza Vaccine (ARCT-2304) When Administered to Healthy Adults.

Short Title: Safety and Immunogenicity Study of Self-Amplifying RNA Pandemic Influenza Vaccine in Adults.

Protocol Number:	ARCT-2304-01
Vaccine(s):	Investigational ARCT-2304 Pandemic Influenza Vaccine (H5N1) Flucelvax Trivalent® (Influenza vaccine, surface antigen, inactivated, prepared in cell cultures) Fluad Trivalent® (Influenza vaccine, surface antigen, inactivated, adjuvanted) Placebo
Study Phase:	Phase 1
Sponsor Name:	Arcturus Therapeutics, Inc.
Legal Registered Address:	10628 Science Center Dr #250 San Diego, CA 92121
Version:	1.0
Date of Protocol:	29 August 2024

SPONSOR SIGNATORY

Protocol accepted and approved by:

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Date

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event Of Special Interest
ANCOVA	Analysis Of Covariance
A2M	α2-macroglobulin
CCAC	Central Cardiac Adjudication Committee
CDC	Centers For Disease Control And Prevention
BMI	Body Mass Index
CI	Confidence Interval
CIOMS	Council For International Organizations Of Medical Sciences
CK-MB	Creatine kinase-myocardial band
CONSORT	Consolidated Standards Of Reporting Trials
COVID-19	Coronavirus Disease-19
CRF	Case Report Form
CSR	Clinical Study Report
D	Day
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FDA	US Food And Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GMFR	Geometric Mean Fold Rise
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
GMR	Geometric Mean Ratio
HA	Hemagglutinin
HCP	Health Care Provider
HIV	Human Immunodeficiency Virus
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HRT	Hormone Replacement Therapy
IB	Investigator's Brochure
ICF	Informed Consent Form

ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
I.M.	Intramuscular
IP-10	interferon gamma induced protein
IRB	Institutional Review Board
IRT	Interactive Response Technology
IUD	Intrauterine Device
IUS	Intrauterine Hormone-Releasing System
LL	Lower Limit
LLoQ	Lower Limit Of Quantitation
LNP	Lipid nanoparticle
MAAE	Medically-Attended Adverse Event
MedDRA	Medical Dictionary For Regulatory Activities
mRNA	Messenger ribonucleic acid
PC	Phone Contact
PPS	Per Protocol Set
PT	Preferred Term
RdRP	RNA-dependent RNA polymerase
SAE	Serious Adverse Event
SAF	Safety Set
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SCR	Seroconversion Rate
SD	Standard Deviation
SoA	Schedule Of Assessments
SOC	System Organ Class
sa-mRNA	Self-amplifying messenger RNA
SUSAR	Suspected Unexpected Serious Adverse Reaction
Vac	Vaccination
VEEV	Venezuelan equine encephalitis alphavirus

1 PROTOCOL SYNOPSIS

Sponsor: Arcturus Therapeutics, Inc. 10628 Science Center Dr #250 San Diego, CA 92121	**Protocol number:** ARCT-2304-01	**ClinicalTrials.gov:** TBD **EudraCT:** TBD	**Date:** 29 August 2024
Title of Study: A Phase 1, First-in-human, Randomized, Observer-blind, Parallel design, Controlled, Dose Level and Schedule-finding Study to Evaluate the Safety, Reactogenicity, and Immunogenicity of a Self-Amplifying mRNA Pandemic Influenza Vaccine (ARCT-2304), when administered to Healthy Adults			
Publication (reference): Not applicable.			
Duration of Participation: Approximately 9 months for each participant.		**Clinical Phase:** Phase 1	

Background and Rationale

Infections with the highly pathogenic influenza A/H5N1 viruses have been associated with poultry outbreaks and infections in humans in many countries. Human infections with A/H5N1 virus have been reported in 23 countries since 1997, resulting in severe pneumonia and death in about 50% of cases (CDC 2024).

Multiple studies have established the viral hemagglutinin (HA) surface glycoprotein as the major determinant of H5N1 virulence. HA mediates host-specific virus binding to cells, and mutations that allow efficient binding to viral receptors on mammalian cells are critical (although not sufficient) for H5N1 transmissibility among mammals. In addition, the HA gene of highly pathogenic avian H5N1 influenza viruses has reassorted with the neuraminidase (NA) and other viral genes originating from different avian influenza viruses, giving rise to novel viruses of the H5 subtypes (Neumann 2015).

It is generally accepted that new pandemics will occur in the future, but when, where, and how they will spread is difficult to predict. As demonstrated by COVID-19, a pandemic can have significant health, economic, and social consequences. An influenza pandemic will occur when an influenza virus emerges with the ability to cause sustained human-to-human transmission, and the human population has little to no immunity against the virus. With the continuous growth of global travel, a pandemic can spread rapidly. Whether currently circulating avian, swine, or other influenza viruses will result in a future pandemic is unknown. However, the diversity of zoonotic influenza viruses that have caused human infections necessitates strengthened surveillance in both animal and human populations, thorough investigation of every zoonotic infection, and pandemic preparedness planning (WHO 2024).

Developing an effective and safe vaccine to respond rapidly in a pandemic setting is a vital public health measure to reduce the spread of infection and associated morbidity and mortality of pandemic influenza (WHO 2024). With the success of COVID-19 messenger RNA (mRNA) vaccines, newly created mRNA vaccines against other infectious diseases are beginning to emerge. Influenza vaccines developed using a self-amplifying messenger RNA (sa-mRNA) platform technology may offer broader and longer protection than currently available influenza vaccines, a higher level of cross-neutralization of heterologous influenza strains, and an improved tolerability profile, and may serve as a rapid and flexible response to influenza (Vogel 2018; Blakney 2021; Pollock 2022; Low 2002).

The investigational pandemic influenza vaccine ARCT-2304 is a vaccine containing a lipid nanoparticle (LNP) and sa-mRNA replicons. The vaccine contains nucleotide sequences encoding Venezuelan equine encephalitis alphavirus (VEEV) nonstructural proteins 1, 2, 3, and 4 (nsP1-4) and nucleotide sequences encoding the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins from pandemic A/H5N1 virus. The VEEV nsPs, when assembled in vivo, form an RNA-dependent RNA polymerase (RdRP) enzyme that makes copies of the target mRNAs encoding for the HA and NA glycoproteins, resulting in high levels of HA and NA antigen expression. Conventional mRNA influenza vaccines do not have such an alphavirus replication machinery and encode only the gene of interest (Bloom 2021).

The LNP delivery system used in ARCT-2304 is similar to the LNP used for the Sponsor's COVID-19 (ARCT-154) and seasonal influenza (ARCT-2138) vaccines. Overall, more than 19,000 adult participants have received at least one dose of ARCT-154 as a primary vaccination series or booster dose. ARCT-154 vaccine has an acceptable safety profile with no major safety concerns identified and received marketing authorization by the

Pharmaceuticals and Medical Devices Agency in Japan in November 2023. In addition, the Sponsor's seasonal quadrivalent influenza vaccine, ARCT-2138, aims to induce antibodies against HA and NA surface glycoproteins of seasonal influenza strains. The Sponsor has initiated a Phase 1 study of ARCT-2138 in early 2024 and is testing multiple dose levels of the vaccine (from [redacted]) in young and older adults (Study ARCT-2138-01).

The purpose of the ARCT-2304-01 Phase 1 study is to evaluate the safety, reactogenicity, and immunogenicity of an influenza pandemic A/H5N1 vaccine (ARCT-2304) in approximately 200 healthy participants (120 participants in age group 18-59 years and 80 participants in age group 60-80 years). Participants will be randomized to receive either one of the 3 dose levels of investigational vaccine, administered as a 2-dose vaccination series (on Days 1, 29 or Days 1, 57) or control vaccine.

The study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and applicable regulatory requirement(s).

Primary Objectives	Endpoints	Estimands
1. To evaluate the safety and reactogenicity of different dose levels of ARCT-2304	Local and systemic AEs reported within 7 days after each vaccination. Unsolicited AEs reported within 28 days after each vaccination. Any laboratory abnormalities on Days 1, 8, 29, 36 and 57 (for D1,29 cohort) or Days 1, 8, 57, 64 and 85 (for D1,57 cohort). Any clinically significant abnormal vital signs on Days 1, 8, 29, 36 and 57 (for D1,29 cohort) or Days 1, 8, 57, 64 and 85 (for D1,57 cohort) Serious Adverse Events (SAEs), Medically Attended AEs (MAAEs), AEs of Special Interest (AESIs), and AEs leading to early termination reported from Day 1 up to Day 57 (for D1,29 cohort) and Day 1 up to Day 85 (for D1,57 cohort) in: • ARCT-2304 recipients (by dose level) • Control/placebo vaccine recipients	Number and percentage of participants with: • Local and systemic solicited AEs • Unsolicited AEs • Any treatment-emergent ≥ Grade 3 abnormal clinically significant laboratory values • Any clinically significant abnormal vital signs • SAEs, MAAEs, AESIs, and AEs leading to early termination from study. by Safety Set
2. To describe the hemagglutination inhibition (HAI) antibody responses against the HA glycoprotein through 28 days after the second vaccination	Serum HAI antibody titers against the H5N1 HA glycoprotein on Days 1, 29, 36 and 57 (for D1,29 cohort) and on Days 1, 57, 64 and 85 (for D1,57 cohort), in: • ARCT-2304 recipients (by dose level) • Historical control vaccine recipients[a]	• Geometric Mean Titers (GMTs) • Geometric Mean Fold Rise (GMFRs) • Seroconversion Rates (SCRs) • Proportions of participants with HAI titer ≥1:10, 1:20, 1:40, 1:80, 1:160, 1:320 by PPS
3. To describe the neuraminidase enzyme-linked lectin assay (ELLA) antibody responses against the NA glycoprotein, through 28 days after the second vaccination	Serum ELLA antibody titers against the H5N1 NA glycoprotein on Days 1, 29, 36, and 57 (for D1,29 cohort) and on Days 1, 57, 64 and 85 (for D1,57 cohort), in: • ARCT-2304 recipients (by dose level)	• GMTs • GMFRs • SCRs • Proportions of participants with NA titer ≥1:10, 1:20, 1:40, 1:80, 1:160, 1:320 by PPS

Secondary Objectives		
1. To evaluate the safety of different dose levels of ARCT-2304 through the entire study period	SAEs, MAAEs, AESIs, and AEs leading to early termination from the study, from Day 1 through Day 240, in: • ARCT-2304 recipients (by dose level) • Control/placebo vaccine recipients	Number and percentage of participants with: • SAEs; MAAEs; AESIs; and AEs leading to early termination from study. by Safety Set
2. To describe the persistence of humoral immune response, following ARCT-2304, as measured by HAI and ELLA assays, throughout the entire study period	Serum HAI and ELLA antibody titers against the HA and the NA glycoproteins on Days 1, 29, 36, 57, and 240 (for D1,29 cohort) and Days 1, 57, 64, 85, and 240 (for D1,57 cohort), in: • ARCT-2304 recipients (by dose level)	• GMTs • GMFRs • SCRs • Proportions of participants with antibody titer ≥ 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320 by PPS
3. To describe serum neutralizing (MN) antibody titer against the HA glycoprotein through 28 days after the second vaccination	Serum MN antibody titers against the HA glycoprotein on Days 1, 29, and 57 (for D1,29 cohort) and Days 1, 57, and 85 (for D1,57 cohort), in: • ARCT-2304 recipients (by dose level) • Historical control vaccine recipients[a]	• GMTs • GMFRs • SCRs • Proportions of participants with antibody titer ≥LLOQ, 1:40, 1:80, 1:160 and 1:320 by PPS
Exploratory Objectives		
1. To describe HA and NA-specific CD4+ and CD8+ T-cell responses elicited by the different dose levels of ARCT-2304[b]	Expression of activation markers and subset-specific markers by ELISpot following HA and NA peptide pool stimulation on Days 1, 8, 29 and 36 (for D1,29 cohort) and on Days 1, 8, 57 and 64 (for D1,57 cohort) in: • ARCT-2304 recipients (by dose level)	• Frequency of T-cells responding to HA and NA influenza vaccine antigens by assessing cells expressing markers such as interferon-gamma (IFN-γ), interleukin 13 (IL-13), IL-4, and others (e.g. RNA sequencing). by PPS

Abbreviations: AE, adverse event; AESI, adverse event of special interest; GMFR, fold rise in serum GMT post-vaccination compared to pre-vaccination; ELLA, enzyme-linked lectin assay; GMT, geometric mean titer; HA, hemagglutination; HAI, hemagglutination inhibition; LLOQ, lower limit of quantitation; MAAE, medically attended AE; MN, microneutralization; NA, neuraminidase; PPS, per protocol set; SAE, serious adverse event; SCR, seroconversion rate.

SCR is the percentage of participants with either a pre-vaccination antibody titer < LLOQ and a post-vaccination antibody titer ≥ 4 x LLOQ, or a pre-vaccination antibody titer ≥LLOQ and a ≥4-fold increase in post-vaccination antibody titer.

a Historical control vaccine recipients: Day 1 and Day 43 samples from a previous study performed with an MF59-adjuvanted A/H5N1 vaccine (A/Indonesia/05/2005) will be used as the comparator for the primary immunogenicity objective 2 and secondary immunogenicity objective 3.

b Testing for CD4+ and CD8+ T-cell response in D1,57 cohort samples might be performed as future research evaluation.

Study Design: A Phase 1, first-in-human, randomized, observer-blind, parallel design, controlled, dose level and schedule-finding study to evaluate the safety, reactogenicity, and immunogenicity of a self-amplifying mRNA pandemic influenza (H5N1) vaccine (ARCT-2304), when administered as a 2-dose vaccination series to healthy adults.

The study design is provided in Figure 1. The Schedule of Activities (SoA) is provided in Table 1 and Table 2.

Number of Planned Participants: Approximately 200 evaluable participants (120 participants in age group 18-59 years and 80 participants in age group 60-80 years) are planned to be enrolled in this study; 150 participants receiving ARCT-2304 (different dose levels) and 50 participants receiving the control vaccine.

Drop-Out Replacement: Any participant who withdraws/discontinues after randomization but prior to the study vaccination will be replaced.

Vaccination schedule: All participants will receive two intramuscular (IM) doses of either one of the three dose levels of the ARCT-2304 vaccine or control/placebo vaccine and will follow one of the two different vaccination schedules (Days 1, 29 or Days 1, 57), according to Table 1.

Table 1 Study Cohorts and Treatment Arms

Cohort	Treatment Arm	Schedule of Vaccine Administration	Total Enrolled	Enrolled by Age Group	
				Age 18 to ≤ 59 yrs	Age 60 to ≤ 80 yrs
D1,29	1,5 µg ARCT-2304	Day 1, Day 29	25	15	10
	5 µg ARCT-2304	Day 1, Day 29	25	15	10
	12 µg ARCT-2304	Day 1, Day 29	25	15	10
	Control vaccine/placebo	Day 1, Day 29	25	15	10
D1,57	1,5 µg ARCT-2304	Day 1, Day 57	25	15	10
	5 µg ARCT-2304	Day 1, Day 57	25	15	10
	12 µg ARCT-2304	Day 1, Day 57	25	15	10
	Control vaccine/placebo	Day 1, Day 57	25	15	10

Investigational Vaccine: ARCT-2304

Control: A licensed age-appropriate seasonal influenza vaccine (first vaccination) and placebo (second vaccination).

Randomization: An Interactive Response Technology (IRT) system will be used in the study. Participants will be randomized into two cohorts according to the vaccination schedule (Days 1, 29 or Days 1, 57). Within each cohort, participants will be randomized to one of 4 groups (1,5 µg, 5 µg or 12 µg of ARCT-2304 or control vaccine) in a 1:1:1:1 ratio. Randomization will be performed separately for each age group, 18-59 and 60-80 years of age.

Phases of the study: As ARCT-2304 will be administered for the first time to humans in this study, safety precautions, including staggered enrolment (Figure 2), will be taken. The study will be enrolled in four sequential phases:

1. Lead-in Cohort 1 (18-49 years): enrolment will start with 8 participants, 18-49 years of age, that will be assigned to the Days 1, 29 vaccination cohort and will be randomized in a 1:1:1:1 ratio to receive one of the three dose levels of ARCT-2304 or control/placebo vaccine.

   Data Safety Monitoring Board (DSMB) review will be performed as soon as 7-day safety and reactogenicity data are available after the first vaccination for this first group of participants. The favorable outcome of reactogenicity and safety assessment by the DSMB will permit the expansion of recruitment of study participants in age group 18-59 years of age.

   The second planned DSMB review will be performed as soon as 7-days safety and reactogenicity data are available after the second vaccination for this first group of participants (Lead-in Cohort 1). The safety review will include the totality of safety data (including all available safety of participants in the expansion

age group 18-59 years. The favorable outcome of reactogenicity and safety assessment by the DSMB will permit the initiation of second vaccination in the expansion age group 18-59 years and recruitment of lead-in participants in the 60-80 years age group.

2. Expansion age group 18-59 years: As soon as the DSMB authorizes the expansion in age group 18-59 years, recruitment will be initiated. The participants will be randomized into one of two vaccination schedule cohorts (Days 1, 29, or Days 1, 57) and into one of the 4 treatment arms (three dose levels of ARCT-2304 or control/placebo vaccine) in a 1:1:1:1 ratio.
3. Lead-in Cohort 2 (60-80 years): Recruitment will start after authorization by DSMB (see above). Eight participants in the 60 – 80 years of age group will be enrolled, assigned to the Days 1, 29 vaccination cohort, and will be randomized to one of the 4 treatment arms, similar to the lead-in cohort 1 (18-59 years). DSMB will review all available data as soon as 7-day safety and reactogenicity data after the first vaccination is available. A favorable outcome from the DSMB will permit the expansion of recruitment in age group 60-80 years.
4. Expansion age group 60-80 years: As soon as the DSMB authorizes the expansion for age group 60-80 years, recruitment will be initiated. The participants will be randomized into one of two vaccination schedule cohorts (Days 1, 29, or Days 1, 57) and into one of the 4 treatment arms (three dose levels of ARCT-2304 or control/placebo vaccine) in a 1:1:1:1 ratio. Following post-Dose 2 assessment of Lead-in Cohort 2, DSMB will make a decision regarding administration of the second dose in the expansion age group 60-80 years.

Besides the four scheduled DSMB reviews as indicated above, the DSMB will perform periodic reviews of safety and reactogenicity data as soon as 7-day post-Dose 1 data are available for e.g. the first 80 and 120 recruited participants. The safety review will include the totality of safety data (including post-Dose 2 results) at each time point. In case pre-specified pause rules are met or any safety concerns are raised, enrolment/vaccination will be paused; the DSMB will inform the sponsor and will provide recommendations regarding study continuation, study modification, or study termination to the sponsor.

Study periods: The study has a screening period (Day -28 to Day 1), a vaccination period (Days 1-57 for the Days 1, 29 cohort and Days 1-85 for the Days 1, 57 cohort), and a follow-up period from Day 57 (or 85) to Day 240.

Study visits: Seven (7) study visits, including a screening visit and 6 study visits on Days 1, 8, 29, 36, 57, and 240, in Days 1, 29 cohort and on Days 1, 8, 57, 64, 85 and 240 in Days 1, 57 cohort.

Safety calls: Two safety calls (Days 120 and 180) will be conducted in the Days 1, 29 cohort, and three safety calls (Days 29, 120, and 180) will be performed in the Days 1, 57 cohort to collect any medically attended AEs (MAAEs), serious AEs (SAEs), AEs of special interest (AESIs), concomitant medications, and any vaccinations. For the Lead-in participants, four additional safety phone calls will be performed 2 days and 4 days after each study vaccination (Days 2 and 4 and Days 30 and 32 for the Day 1, 29 Cohort and Days 2 and 4 and Days 58 and 60 for the Day 1, Day 57 Cohort).

Blood samples: Seven (7) blood draws at the screening visit and on Days 1, 8, 29, 36, 57, and 240 for the Days 1, 29 cohort and on Days 1, 8, 57, 64, 85, and 240 for the Days 1, 57 cohort, will be obtained from all participants.

Blinding: Observer-blind study.

Data collection: electronic Case Reporting Form (eCRF) and electronic Diary (eDiary).

Duration of the study/participant participation: Approximately 9 months for each participant.

Study Population and Age Categories

Study Population: Healthy participants or individuals with stable pre-existing medical conditions 18 to 59 and 60 to 80 years of age who have not received influenza vaccination for at least 3 months prior to enrolment.

Age categories: The study population is split into two age categories: n=120 participants 18 to 59 years of age and n=80 participants 60 to 80 years of age.

Inclusion and Exclusion Criteria

Abbreviated inclusion and exclusion criteria are provided below; the full list of inclusion and exclusion criteria is included in Section 5.0 of the protocol:

Main Inclusion Criteria:

- Individuals are male or female adults 18-80 years of age.
- Healthy participants or participants with pre-existing stable medical conditions. Stable medical condition is defined as an existing disease that has not required a significant change in treatment or hospitalization/ emergency room visit for worsening within 3 months prior to Day 1 and participant has full capacity of daily activity.
- Individuals of childbearing potential must be willing to adhere to contraceptive requirements.

Main Exclusion Criteria:

Participants are excluded from the study if any of the following criteria apply:

- Individuals with acute medical conditions or febrile illness, including body temperature ≥100.4°F (≥38.0°C measured by any method) within 3 days prior to randomization.
- Individuals with a known history of severe hypersensitivity reactions, including anaphylaxis, or other significant adverse reactions to any mRNA vaccine, influenza vaccine, or excipients.
- Individuals with a history of myocarditis, pericarditis, myopericarditis or cardiomyopathy.
- Individuals who received any influenza vaccine within 3 months prior to first vaccine administration or plan to receive an influenza vaccine during the study period.
- Individuals who have received mRNA vaccination within 60 days before the first vaccine administration.
- Individuals who have received or plan to receive an A/H5N1 influenza vaccine and/or individuals who had substantial exposure to or direct contact with poultry, wild birds live cattle or raw milk.

Study Vaccine and Control Vaccines Description

Depending on the dose level or the vaccination schedule assigned in the study, participants will receive two 0.5 or 0.25 mL intramuscular (IM) doses into the deltoid muscle of the nondominant arm.

Investigational vaccine: ARCT-2304

For the ARCT-2304 vaccine, the drug substance is formulated in a mixture with lipids and excipients. Together they form a lipid nanoparticle (sa-mRNA-encapsulated LNP called ARCT-2304 drug product).

The drug substance is a bicistronic construct encoding for the non-structural proteins 1–4 (nsP1-4) from the Venezuelan equine encephalitis alphavirus (VEEV) and the full-length, membrane-bound hemagglutinin HA and neuraminidase (NA) glycoproteins from the A/H5N1 influenza virus.

Additional information regarding the preparation of different dose levels of study vaccine, vaccine storage and handling will be provided in the Pharmacy Manual.

Control for safety:

First dose

For participants 18-59 years of age: Flucelvax Trivalent® (Influenza vaccine, surface antigen, inactivated, prepared in cell cultures): a total of 45 µg hemagglutinin (HA) comprising 15 µg each of the World Health Organization (WHO)-recommended influenza strains against A/H1N1, A/H3N2, and one B strain, 0.5-mL dose.

For participants 60-80 years of age: Fluad Trivalent® (Influenza vaccine, surface antigen, inactivated, adjuvanted): a total of 45 µg hemagglutinin (HA) comprising 15 µg each of the World Health Organization (WHO)-recommended influenza strains against A/H1N1, A/H3N2, and one B strain, and MF59C.1 adjuvant, a squalene-based oil-in-water emulsion; 0.5-mL dose.

Second dose

All participants: Placebo (0.9% saline solution will be used as placebo) 0.5-mL dose.

Control for immunogenicity: Historical control samples from a previous study performed with an MF59-adjuvanted A/H5N1 vaccine (A/Indonesia/05/2005).

Immunogenicity assessments

The measures of immunogenicity used in this study are standard, i.e., widely used and generally recognized as reliable, accurate, and relevant (able to describe the quality and extent of the immune response).

The following assays will be used to assess antibody-mediated immune responses:

- A hemagglutination inhibition (HAI) assay for measuring hemagglutinin (HA)-specific antibodies against A/H5N1 influenza virus strain.
- An enzyme-linked lectin (ELLA) assay for measuring influenza neuraminidase (NA) inhibition antibody titers against A/H5N1 influenza virus strain.
- A virus microneutralization (MN) for detecting functional HA-specific antibodies against A/H5N1 influenza virus strain.

The measures of immunogenicity used in this study include GMTs, GMFRs, SCRs, and proportions of participants with antibody titers above pre-specified thresholds as assessed by binding and neutralization assays and have been deemed appropriate to describe the immune response against the A/H5N1 influenza virus.

Cell-mediated immune response to A (H5N1) influenza virus by ELISpot will be assessed on Days 1, 8, 29, and 36 for the Days 1, 29 cohort and may be assessed on Days 1, 8, 57 and 64 for the Days 1, 57 cohort (in a subset of samples collected for each cohort) using frequency of T cells responding to HA and NA influenza vaccine antigens by assessing cells expressing markers such as interferon-gamma (IFN-γ), interleukin 13 (IL-13), IL-4, and others (e.g. RNA sequencing).

Safety assessments

- All participants will be monitored for at least 60 minutes after study vaccination for any immediate post-vaccination AEs with appropriate medical treatment readily available (if needed).
- Solicited local reactions and systemic AEs will be recorded daily for 7 consecutive days after each study vaccination using an electronic diary card (eDiary):
  - Local reactions: injection site pain, erythema and swelling.
  - Systemic AEs: fatigue, headache, myalgia, arthralgia, nausea, dizziness, chills, and fever (derived from measured body temperature ≥100.4 °F [≥38.0 °C]).
- Any unsolicited AEs will be collected within 28 days after each study vaccination.
- Any medically attended AEs (MAAEs), serious AEs (SAEs), AEs of special interest (AESIs), and AEs leading to early study termination and any pregnancies will be reported during the entire study period. These data will be captured by interviewing the participants during the site visits and phone calls and by reviewing available medical records.
- Safety laboratory values that have a toxicity score of Grade 1 or greater will be evaluated by the investigator and classified as “abnormal clinically significant (CS)”, or “abnormal not clinically significant (NCS).”

Sample Size: The sample size is not based on formal power calculations, as previous clinical study data are unavailable, and clinically meaningful group differences have yet to be established. Hence, the number of proposed participants will be sufficient to provide a descriptive summary of the safety and immunogenicity of ARCT-2304.

Approximately 200 participants will be randomly assigned to receive ARCT-2304 or the control/placebo vaccine. Approximately 50 participants per study group (30 participants in 18-59 years age group and 20 in 60-80 years age group) will receive each dose level of ARCT-2304 or the control/placebo vaccine.

Statistical Considerations

Statistical Hypotheses and Hypothesis Testing: No formal hypotheses will be tested in this study.

Safety Evaluation: The sample size is based on clinical considerations to provide sufficient safety information to analyze the primary safety objective. With a sample size of n=150 participants who receive ARCT-2304, the probability of observing at least 1 AE is 98% and 78% if the true event rate is 2.5% and 1.0%, respectively. With a sample size of n=50 per dose level, the probability of observing at least 1 AE is 92% and 72%, if the true event rate is 5.0% and 2.5%, respectively.

Main analysis sets:

Safety Analysis Set: Includes all participants who received at least one dose of a study vaccine and who provide any evaluable post-vaccination reactogenicity and/or safety data.

Per Protocol Analysis Set (PPS): Includes all participants who received the assigned study vaccine and who have no protocol deviations impacting the analysis of immunogenicity data for the time period summarized as evaluated by the blinded Sponsor Medical Monitor.

Immunogenicity Evaluation:

Values below the lower limit of quantitation (LLOQ) for each assay will be set at LLOQ (50% of LLOQ for GMT calculation).

GMTs: Antibody titers will be logarithmically transformed (base 10). For each study group, GMTs and GMFRs of the HA-specific antibodies, NA-inhibition antibodies, and microneutralization antibodies (if applicable), along with their associated 95% CIs, will be computed by exponentiation of the corresponding log-transformed means and 95% CIs.

SCRs are defined as proportions of participants with a ≥4-fold increase in serum antibody titer for respective vaccine antigens from baseline (or from LLOQ if baseline titer <LLOQ) and proportion of participants with antibody titer ≥ the pre-specified thresholds will be presented for each study group together with their 2-sided 95% Clopper-Pearson CIs.

Further details regarding the statistical methods and analysis will be specified in the statistical section of the Study Protocol and the Statistical Analysis Plan (SAP).

Interim Analyses and Analysis Timing: No Interim analyses are planned for this study, however two descriptive statistical analyses may be performed stepwise as follows:

1. An analysis including all safety, reactogenicity, and immunogenicity data associated with primary endpoints will be conducted on cleaned data (for participants 18-59 years and 60-80 years combined or separately). The results will be reported on a study group level only. Individual listings can be generated without information on the participant's study group. Access to participant-level information about study groups will be restricted.
2. A final analysis of safety and immunogenicity data associated with primary, secondary, and available exploratory endpoints will be performed as soon as the data is cleaned and locked. The results of this analysis will be presented in a final clinical study report. Individual data listings with information on treatment allocation on the participant level will be generated and presented.

An additional analysis of safety and immunogenicity data collected may be performed in order to meet company's business decision. Further details will be provided in the Statistical Analysis Plan.

Data Safety Monitoring Board and Central Cardiac Adjudication Committee.

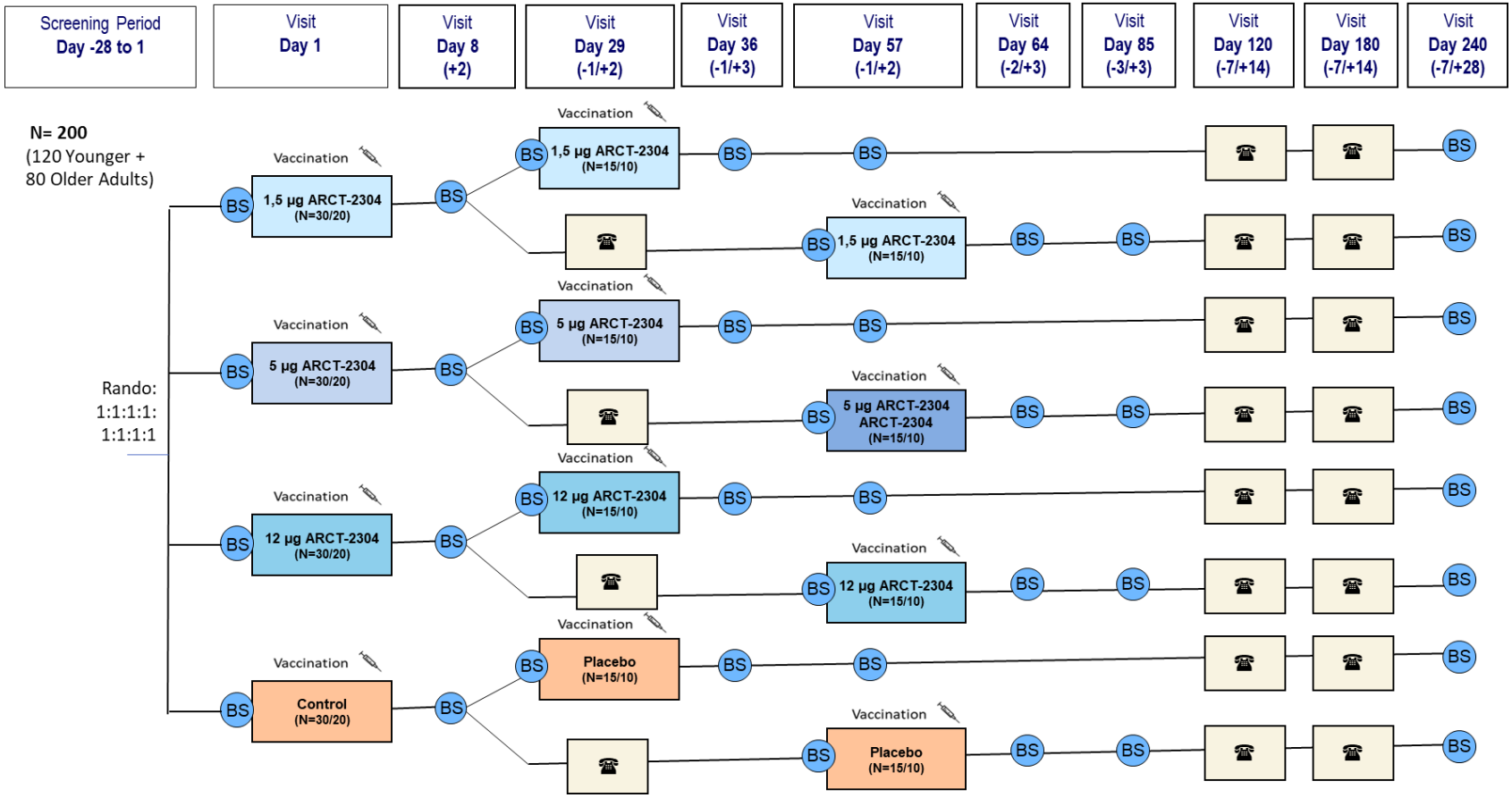
The study will include the use of an independent DSMB and a Central Cardiac Adjudication Committee.

An independent, external DSMB will be established to evaluate participant safety throughout the study at a cohort and participant level, as appropriate. The DSMB will perform i) planned reviews of all available safety and reactogenicity data and ii) reviews of all available safety and reactogenicity data if a pause rule is met and study vaccination is paused or if any safety concern is raised by the PI or Sponsor. The DSMB will make recommendations regarding study continuation, study design modification, and/or study termination. The responsibilities, roles, and procedures of the DSMB are described in the DSMB Charter.

A central cardiac adjudication committee may meet to evaluate ECGs and assess any potential myocarditis/pericarditis cases. Further details are provided in the cardiac adjudication committee charter.

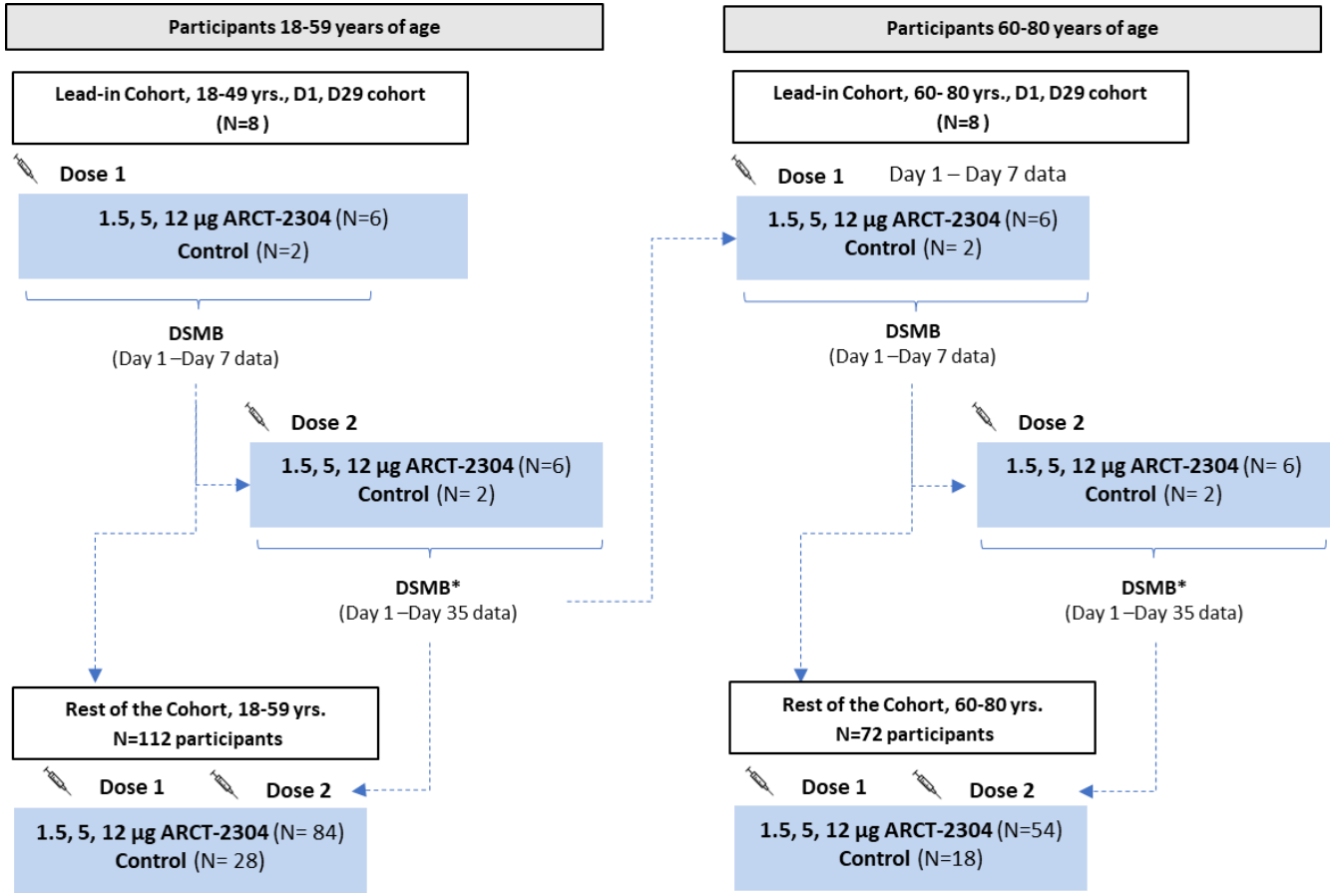
1.1 Study Design

Figure 1 Overall design of study ARCT-2304-01



BS: Blood Sample (for immunogenicity evaluation); Rando: Randomization; Control = seasonal influenza vaccine
Note: Control vaccine for participants 18-59 years of age is Flucelvax Trivalent®; control vaccine for participants 60 – 80 years of age is Fluad Trivalent®.

Figure 2 Staggered Enrolment Plan



* Will include also all available safety data from the rest of cohort.

1.2 Schedule of Activities

Table 2 Schedule of Activities (SoA) for Days 1,29 cohort.

Study Period	**Screening[a] (Day -28 – Day 1)**	**Treatment Period (Day 1 – Day 29)**			**Follow-up Period (Day 30 – Day 240)**					
Visit Type	**Screening Visit[a]**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**	**Phone Call**	**Clinic Visit**	**Unscheduled Visit**
Visit Number	**-**	**V1**	**V2**	**V3**	**V4**	**V5**	**-**	**-**	**V6**	**-**
Study Day	**Day -28 to Day 1**	**Day 1**	**Day 8**	**Day 29**	**Day 36**	**Day 57**	**Day 120**	**Day 180**	**Day 240**	**NA**
Visit Window (Days)	**-28**	**-**	**+2**	**-1 /+2**	**-1 /+3**	**-3/+3**	**-7/+14**	**-7/+14**	**-7/+28**	**NA**
Study Event										
Informed Consent	X									
Demographics Data	X									
Medical History (including prior medication/ vaccinations)	X									
Physical Examination[b]	X	X*	X	X*	X	X			X	X
Vital Sign Measurement[c]	X	X*	X	X*	X	X			X	X
Electrocardiography (ECG)	X[d]									(X)
Blood for Screening Testing[e]	X									
Blood Sample for Safety Laboratory Tests[f]	X	X*	X	X*	X					(X)
Pregnancy Testing[g]	X	X*		X*						(X)

Table 2 Schedule of Activities (SoA) for Days 1,29 cohort.

Study Period	**Screening[a] (Day -28 – Day 1)**	**Treatment Period (Day 1 – Day 29)**			**Follow-up Period (Day 30 – Day 240)**					
Visit Type	**Screening Visit[a]**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**	**Phone Call**	**Clinic Visit**	**Unscheduled Visit**
Visit Number	**-**	**V1**	**V2**	**V3**	**V4**	**V5**	**-**	**-**	**V6**	**-**
Study Day	**Day -28 to Day 1**	**Day 1**	**Day 8**	**Day 29**	**Day 36**	**Day 57**	**Day 120**	**Day 180**	**Day 240**	**NA**
Visit Window (Days)	**-28**	**-**	**+2**	**-1 /+2**	**-1 /+3**	**-3/+3**	**-7/+14**	**-7/+14**	**-7/+28**	**NA**
Study Event										
Inclusion/Exclusion	X	X								
Randomization		X*								
Blood Sample for Antibody-mediated Immunogenicity[h]		X*		X*	X	X			X	
Blood Sample for Cell-mediated Immunogenicity[i]		X*	X	X*	X					
Study Vaccination[j]		X		X						
Post-Vaccination Safety Assessment		X		X						
Distribution of eDiary and Training		X								
Diary compliance check		D2 (±1) and D5 (±1)[m]		D30 (±1) and D33 (±1)[m]						
eDiary Review			X		X					
Follow-up Safety Call[k]							X	X		

Table 2 Schedule of Activities (SoA) for Days 1,29 cohort.

Study Period	**Screening[a] (Day -28 – Day 1)**	**Treatment Period (Day 1 – Day 29)**			**Follow-up Period (Day 30 – Day 240)**					
Visit Type	**Screening Visit[a]**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**	**Phone Call**	**Clinic Visit**	**Unscheduled Visit**
Visit Number	**-**	**V1**	**V2**	**V3**	**V4**	**V5**	**-**	**-**	**V6**	**-**
Study Day	**Day -28 to Day 1**	**Day 1**	**Day 8**	**Day 29**	**Day 36**	**Day 57**	**Day 120**	**Day 180**	**Day 240**	**NA**
Visit Window (Days)	**-28**	**-**	**+2**	**-1 /+2**	**-1 /+3**	**-3/+3**	**-7/+14**	**-7/+14**	**-7/+28**	**NA**
Study Event										
Assessment of Unsolicited AEs[l]		X	X	X	X	X				
Review of Safety Laboratory Results[m]		X	X	X	X					
Assessment of AEs with Medically Attended Visits[n]		← During the entire study period →								
Assessment of SAEs[n]		← During the entire study period →								
Assessment of AESIs[n]		← During the entire study period →								
Recording of Concomitant Medications and Vaccinations		← During the entire study period →								
Study Completion									X	
Early Termination		If participant terminates the study early, the Study Completion Procedures (except sample for antibody-mediated immunogenicity) should be completed								

Table 3 Schedule of Activities (SoA) for Days 1,57 cohort.

Study Period	**Screening[a] (Day -28 – Day 1)**	**Treatment Period (Day 1 – Day 57)**				**Follow-up Period (Day 58 – Day 240)**					
Visit Type	**Screening Visit[a]**	**Clinic Visit**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**	**Phone Call**	**Clinic Visit**	**Unscheduled Visit**
Visit Number	-	V1	V2	-	V3	V4	V5	-	-	V6	-
Study Day	Day -28 to Day 1	Day 1	Day 8	Day 29	Day 57	Day 64	Day 85	Day 120	Day 180	Day 240	NA
Visit Window (Days)	-28	-	+2	-1 /+2	-1 /+2	-1 /+3	-3 /+3	-7/ +14	-7 /+14	-7/+28	NA
Study Event											
Informed Consent	X										
Demographics Data	X										
Medical History (including prior medication/ vaccinations)	X										
Physical Examination[b]	X	X*	X		X*	X	X			X	X
Vital Sign Measurement[c]	X	X*	X		X*	X	X			X	X
Electrocardiography (ECG)	X[d]										(X)
Blood for Screening Testing[e]	X										
Blood Sample for Safety Laboratory Tests[f]	X	X*	X		X*	X					(X)
Pregnancy Testing[g]	X	X*			X*						(X)
Inclusion/Exclusion[a]	X	X									

Table 3 Schedule of Activities (SoA) for Days 1,57 cohort.

Study Period	**Screening[a] (Day -28 – Day 1)**	**Treatment Period (Day 1 – Day 57)**				**Follow-up Period (Day 58 – Day 240)**					
Visit Type	**Screening Visit[a]**	**Clinic Visit**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**	**Phone Call**	**Clinic Visit**	**Unscheduled Visit**
Visit Number	**-**	**V1**	**V2**	**-**	**V3**	**V4**	**V5**	**-**	**-**	**V6**	**-**
Study Day	**Day -28 to Day 1**	**Day 1**	**Day 8**	**Day 29**	**Day 57**	**Day 64**	**Day 85**	**Day 120**	**Day 180**	**Day 240**	**NA**
Visit Window (Days)	**-28**	**-**	**+2**	**-1 /+2**	**-1 /+2**	**-1 /+3**	**-3 /+3**	**-7/ +14**	**-7 /+14**	**-7/+28**	**NA**
Study Event											
Randomization		X*									
Blood Sample for Antibody-mediated Immunogenicity[h]		X*			X*	X	X			X	
Blood Sample for Cell-mediated Immunogenicity[i]		X*	X		X*	X					
Study Vaccination[j]		X			X						
Post-vaccination Safety Assessment		X			X						
Distribution of eDiary and Training		X									
Diary compliance check		D2 (±1) and D5 (±1)[o]			D58 (±1) and D61 (±1)[o]						
eDiary Review			X			X					
Follow-up Safety Call[k]				X				X	X		

Table 3 Schedule of Activities (SoA) for Days 1,57 cohort.

Study Period	**Screening[a] (Day -28 – Day 1)**	**Treatment Period (Day 1 – Day 57)**				**Follow-up Period (Day 58 – Day 240)**					
Visit Type	**Screening Visit[a]**	**Clinic Visit**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**	**Phone Call**	**Clinic Visit**	**Unscheduled Visit**
Visit Number	-	V1	V2	-	V3	V4	V5	-	-	V6	-
Study Day	Day -28 to Day 1	Day 1	Day 8	Day 29	Day 57	Day 64	Day 85	Day 120	Day 180	Day 240	NA
Visit Window (Days)	-28	-	+2	-1 /+2	-1 /+2	-1 /+3	-3 /+3	-7/ +14	-7 /+14	-7/+28	NA
Study Event											
Assessment of Unsolicited AEs[l]		X	X	X	X	X	X				
Review of Safety Laboratory Results[m]		X	X		X	X					
Assessment of AEs with Medically Attended Visits[n]			← During the entire study period →								
Assessment of SAEs[n]			← During the entire study period →								
Assessment of AESIs[n]			← During the entire study period →								
Recording of Concomitant Medications and Vaccinations			← During the entire study period →								
Study Completion										X	
Early Termination			If participant terminates the study early, the Study Completion Procedures (except sample for antibody-mediated immunogenicity) should be completed								

* Procedures that should be completed before study vaccination.

a The following procedures will be conducted at the screening visit: informed consent process; collection of demographic data; medical history and concomitant medications; full physical examination including vital signs, ECG, pregnancy test (if applicable); collection of blood samples for screening and safety laboratory tests; and assessment of inclusion/exclusion criteria (this can be done either at screening visit or on Day 1).

b Physical examination: a full physical examination, including height and weight, will be performed at Screening; symptom-directed physical examinations will be performed at all other scheduled time points. Interim physical examinations will be performed at the discretion of the Investigator.

c Vital sign measurements: systolic and diastolic blood pressures, heart rate, respiratory rate, body temperature (by any method), and heart rate. Vital signs will be collected once, before dose administration and at least 60 minutes after administration of each dose (before participants are discharged from the clinic). Vital signs should be measured in a seated position after the participant has rested comfortably for at least 5 minutes.

d ECG will be performed at screening visit in all participants to identify potential asymptomatic myocarditis and pericarditis cases. In participants with signs or cardiac symptoms suggestive of myocarditis and/or pericarditis, diagnostic evaluation should be performed according to applicable guidelines on Myocarditis and Pericarditis after mRNA vaccines, including laboratory safety measurements (troponin-I, Troponin-T, creatine kinase myocardial band (CK-MB)) with ECG.

e Screening laboratory testing will include the following:
Serology: hepatitis B virus surface antigen, hepatitis C virus antibody, human immunodeficiency virus type 1 and 2 antibodies
Follicle-stimulating hormone level may be measured to confirm menopausal status at the discretion of the Investigator, β-human chorionic gonadotropin (WOCBP).

f Safety Laboratory tests should be included prior to the study vaccination (when applicable) and include:
Hematology: Complete blood count: red blood cells (RBC), hemoglobin, hematocrit, total and differential white blood cells (WBC) and platelet count
Blood Chemistry: alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, urea, blood urea nitrogen (BUN), creatinine, random glucose, total bilirubin and total protein. Coagulation: partial thromboplastin time and prothrombin time. Blood samples for cardiac enzymes will be collected on Day 1 prior to vaccination (baseline) for all participants. The testing of baseline and post-vaccination samples for cardiac enzymes (Troponin-I, Troponin-T, CK-MB) might be performed based on the investigator's or Sponsor's discretion.

g A pregnancy test (β-human chorionic gonadotropin) will be performed on all female participants of childbearing potential at Screening (serum test); urine test can be used before the study vaccination (urine or serum pregnancy test at the discretion of the Investigator).

h For all participants, blood samples will be collected for the analysis of antibody-mediated immunogenicity.

i For a subset of participants in the D1,29 cohort and D1,57, blood samples will be collected for the analysis of cell-mediated immunogenicity. Testing for CD4+ and CD8+ T-cell response in D1,57 cohort samples might be performed as future research evaluation.

j Participants will receive intramuscular ARCT-2304 or a licensed seasonal influenza vaccine and placebo according to randomization. All participants will remain at the study site for at least 60 minutes after administration of each dose, for safety monitoring.

k Trained healthcare providers will call all participants to collect information relating to any medically attended AEs, AEs leading to study withdrawal, SAEs, AESIs, and information on concomitant medications associated with those events. For the Lead-in participants, four additional safety phone calls will be performed (on Day 2 and Day 4 after each vaccination). This call will follow a script and will be made by trained healthcare personnel.

l Unsolicited AEs will be collected from study vaccination (Day 1) to Day 57 (Days 1, 29 cohort) or to Day 85 (Days 1, 57 cohort).

m Laboratory abnormalities should be assessed as soon as the laboratory reports are available. Additional tests might be conducted in case of any Grade ≥3 or clinically significant laboratory abnormalities.

n Medically attended AEs, SAEs, AESIs, and AEs leading to withdrawal from the study will be collected from Day 1 to the study end.

o Compliance checks for eDiary completion will be performed by site staff on Day 2 (±1), Day 5 (±1), Day 30 (±1) and Day 33 (±1) (D1, D29 Cohort) and on Day 2 (±1), Day 5 (±1), Day 58 (±1) and Day 61 (±1) (D1, D57 Cohort) after vaccination. The site will perform a check to verify that the participant has

completed the eDiary. Participants will be contacted by telephone if the eDiary is incomplete. Compliance checks will be documented in the source but not included in the CRF.

2 INTRODUCTION

The purpose of this first-in-human study aims to assess the safety, reactogenicity, and immunogenicity of three dose levels of Arcturus' pandemic influenza vaccine (ARCT-2304), encoding A/H5N1 virus strain, in healthy adults.

2.1 Background

Infections with the highly pathogenic influenza A/H5N1 viruses have been associated with poultry outbreaks and infections in humans in many countries. Human infections with A/H5N1 virus have been reported in 23 countries since 1997, resulting in severe pneumonia and death in about 50% of cases (CDC 2024).

Multiple studies have established the viral hemagglutinin (HA) surface glycoprotein as the major determinant of H5N1 virulence. HA mediates host-specific virus binding to cells, and mutations that allow efficient binding to viral receptors on mammalian cells are critical (although not sufficient) for H5N1 transmissibility among mammals. In addition, the HA gene of highly pathogenic avian H5N1 influenza viruses has reassorted with the neuraminidase (NA) and other viral genes originating from different avian influenza viruses, giving rise to novel viruses of the H5 subtypes (Neumann 2015).

It is generally accepted that new pandemics will occur in the future, but when, where, and how they will spread is difficult to predict. As demonstrated by COVID-19, a pandemic can have significant health, economic, and social consequences. An influenza pandemic will occur when an influenza virus emerges with the ability to cause sustained human-to-human transmission, and the human population has little to no immunity against the virus. With the continuous growth of global travel, a pandemic can spread rapidly. Whether currently circulating avian, swine, or other influenza viruses will result in a future pandemic is unknown. However, the diversity of zoonotic influenza viruses that have caused human infections necessitates strengthened surveillance in both animal and human populations, thorough investigation of every zoonotic infection, and pandemic preparedness planning (WHO 2024).

Developing an effective and safe vaccine to respond rapidly in a pandemic setting is a vital public health measure to reduce the spread of infection and associated morbidity and mortality of pandemic influenza (WHO 2024). With the success of COVID-19 messenger RNA (mRNA) vaccines, newly created mRNA vaccines against other infectious diseases are beginning to emerge. Influenza vaccines developed using a self-amplifying messenger (sa-mRNA) platform technology may offer broader and longer protection than currently available influenza vaccines, a higher level of cross-neutralization of heterologous influenza strains, and an improved tolerability profile, and may serve as a rapid and flexible response to influenza (Vogel 2018; Blakney 2021; Pollock 2022; Low 2002).

The investigational pandemic influenza vaccine ARCT-2304 is a vaccine containing a lipid nanoparticle (LNP) and sa-mRNA replicons. The vaccine contains nucleotide sequences encoding Venezuelan equine encephalitis alphavirus (VEEV) nonstructural proteins 1, 2, 3, and 4 (nsP1-4) and nucleotide sequences encoding the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins from pandemic A/H5N1 virus. The VEEV nsPs, when assembled in vivo,

form an RNA-dependent RNA polymerase (RdRP) enzyme that makes copies of the target mRNAs encoding for the HA and NA glycoproteins, resulting in high levels of HA and NA antigen expression. Conventional mRNA influenza vaccines do not have such an alphavirus replication machinery and encode only the gene of interest (Bloom 2021).

The LNP delivery system used in ARCT-2304 is similar to the LNP used for the Sponsor's COVID-19 (ARCT-154) and seasonal influenza (ARCT-2138) vaccines. Overall, more than 19,000 adult participants have received at least one dose of ARCT-154 as a primary vaccination series or booster dose. ARCT-154 vaccine has an acceptable safety profile with no major safety concerns identified and received marketing authorization by the Pharmaceuticals and Medical Devices Agency in Japan in November 2023. In addition, the Sponsor's seasonal quadrivalent influenza vaccine, ARCT-2138, aims to induce antibodies against HA and NA surface glycoproteins of seasonal influenza strains. The Sponsor has initiated a Phase 1 study of ARCT-2138 in early 2024 and is testing multiple dose levels of the vaccine (from [redacted]) in young and older adults (Study ARCT-2138-01).

The purpose of the ARCT-2304-01 Phase 1 study is to evaluate the safety, reactogenicity, and immunogenicity of an influenza pandemic A/H5N1 vaccine (ARCT-2304) in approximately 200 healthy participants (120 participants in age group 18-59 years and 80 participants in age group 60-80 years). Participants will be randomized to receive either one of the 3 dose levels of investigational vaccine, administered as a 2-dose vaccination series (on Days 1, 29 or Days 1, 57) or control/placebo vaccine.

The study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and applicable regulatory requirement(s).

2.1.1 [redacted]

[redacted]

[redacted]

2.1.2 Clinical Studies

No clinical studies in humans have been conducted to date with the candidate vaccine, ARCT-2304. However, the Sponsor has initiated several clinical trials to evaluate the safety and immunogenicity of prophylactic self-amplifying mRNA COVID-19 vaccines (ARCT-021, ARCT-154, ARCT-165, ARCT-2301, ARCT-2303) that have been developed using the same technology platform.

The Phase 1/2 dose-finding, safety, and immunogenicity study results of the prototype COVID-19 vaccine (ARCT-021-01; NCT04480957) demonstrated that ARCT-021 was well tolerated at doses ≤7.5 µg. Administration of 10 µg dose was associated with a higher rate of post-vaccination AEs. However, the frequency and the nature of reported solicited and unsolicited AEs in this dose level allow the continuation of the assessment. (Low 2002).

In the pivotal study ARCT-154-01 (NCT05012943), two 5 µg doses of the ARCT-154 vaccine were immunogenic against the ancestral SARS-CoV-2 strain and induced cross-neutralizing antibodies against other lineages of the SARS-CoV-2 virus. Vaccine efficacy against COVID-19 of any severity was 56.6% (95% CI: 48.7-63.3), and efficacy against severe COVID-19 was 95.3% (95% 60.5-98.9). More than 17,000 participants were randomized and exposed in this study, with no safety concerns identified (Ho 2024).

In Phase 3 clinical study in Japan (ARCT-154-J01), a booster dose of ARCT-154 (5 µg) elicited a noninferior immune response against the Wuhan-Hu-1 strain and superior immune response against Omicron BA.4/5 subvariant compared with the authorized mRNA vaccine (Comirnaty). Six months after vaccination, GMTs and SRRs of neutralizing antibodies against SARS-CoV-2 (Wuhan-Hu-1 and Omicron BA.4/5 strains) remained higher in ARCT-154 recipients than in Comirnaty recipients, leading to a GMT ratio (ARCT-154/Comirnaty) against the ancestral strain and Omicron BA.4/5 of 2.21 and 2.26, respectively. The reactogenicity and safety profiles of both vaccines were similar, with no safety concerns raised (Oda 2024a, Oda 2024b).

[redacted]

[redacted]

Overall, the available clinical data from Arcturus COVID-19 vaccines (ARCT-021, ARCT-154, ARCT-165) and the quadrivalent seasonal influenza vaccine (ARCT-2138) support the favorable benefit/risk profile of sa-mRNA vaccine and the development of the ARCT-2304 pandemic influenza candidate vaccine.

The investigator's brochure (IB) includes further details.

2.2 Study Rationale

Developing an effective and safe vaccine to respond rapidly to new pandemic influenza virus is a vital public health measure to reduce the spread of infection and associated morbidity and mortality of pandemic influenza (WHO 2024). With the success of COVID-19 mRNA vaccines, newly created messenger ribonucleic acid (mRNA) vaccines against other infectious diseases are beginning to emerge. Influenza vaccines developed using a self-amplifying messenger (sa-mRNA) platform technology may offer broader and longer protection than currently available pandemic influenza vaccines, a higher level of cross-neutralization of heterologous influenza strains, an improved tolerability profile and may serve as a rapid and flexible response to influenza (Vogel 2018; Blakney 2021; Pollock 2022; Low 2002).

Arcturus has developed a candidate sa-mRNA vaccine, ARCT-2304, a synthetically manufactured construct, produced from the portions of published VEEV TC-83 sequence. The vaccine includes a drug substance containing nucleotide sequences encoding Venezuelan equine encephalitis alphavirus (VEEV) non-structural proteins 1, 2, 3, and 4 (nsP1-4) and nucleotide sequences encoding the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins. The VEEV nsPs, when assembled in vivo, for an RNA-de-pendent RNA polymerase (RdRP) enzyme that makes copies of the target mRNAs encoding for the HA and NA glycoproteins, resulting in high levels of HA and NA antigen expression. Conventional mRNA influenza vaccines do not have such an alphavirus replication machinery and encode only the gene of interest (Bloom 2021).

Arcturus' ARCT-2304 vaccine aims to induce antibodies against two surface glycoproteins of influenza, HA and NA. Classically, protection against influenza is recognized to be related to anti-HA antibodies, and currently licensed vaccines are designed to induce neutralizing antibodies against HA. NA is a major target for influenza antivirals but has not yet played a prominent role in vaccine development (Krammer 2018). Arcturus pandemic influenza vaccine aims to induce antibodies against these two surface glycoproteins of influenza to improve influenza disease protection. With the selection of both HA and NA (bicistronic), Arcturus' investigational ARCT-2304 vaccine aims to induce a robust immune response, both humoral and cell-mediated, to improve protection against pandemic influenza disease.

The purpose of this Phase 1, first-in-human (FIH), randomized, observer-blind, parallel design, controlled, dose level and schedule-finding study to evaluate the safety, reactogenicity, and immunogenicity of ARCT-2304, a self-amplifying mRNA pandemic influenza vaccine when administered to healthy adults.

The primary objective of this study is to assess the safety and immunogenicity of 3 dose levels of ARCT-2304 (1,5 µg, 5 µg and 12 µg), administered as two doses schedule on Day 1 the first dose and on Day 29 or Day 57 the second dose in healthy adults, in comparison with a licensed influenza vaccine administered as first dose and placebo as second dose, through 28 days after each vaccination. The secondary objectives are to evaluate longer-term safety and persistence of immune response through 6 months after the second vaccination.

2.2.1 Scientific Rationale for the Study Design

The design, conduct, and analysis of this study comply with international and regional standards for clinical research in humans and for investigating the efficacy, immunogenicity, and safety of investigational influenza vaccines.

This phase 1 first-in-human study is designed to understand the viability of different dose levels of ARCT-2304 vaccine and the sa-mRNA/LNP platform by evaluating the safety, reactogenicity, and immunogenicity of an ARCT-2304 vaccine encoding for both HA and NA of the A/H5N1 influenza virus in healthy adults.

The study is a first in human, controlled, observer-blind, parallel design study. On Day 1, participants will be randomized to receive either one of the 3 intramuscular dose levels of the investigational vaccine, administered as a 2-dose vaccination series (on Days 1, 29 or Days 1, 57) or a control cell-culture inactivated influenza vaccine (adults 18 – 59 years of age) or adjuvanted inactivated influenza vaccine (adults 60 – 80 years of age) in the arm. The control group will receive placebo as a second vaccination. Since no licensed adjuvanted pandemic vaccine was available as comparator for this study, age-appropriate seasonal influenza vaccines will be used as comparator for safety assessments. For immunogenicity comparison, historical samples from a previous study (V89P1, Keitel 2010) performed with an MF59-adjuvanted A/H5N1 vaccine (A/Indonesia/05/2005 strain) will be utilized.

The study has a screening period (Day -28 to Day 1), a vaccination period (Days 1-57 for the Days 1, 29 cohort and Days 1-85 for the Days 1, 57 cohort), and a follow-up period from Day 57 (or 85) to Day 240.

A Data Safety Monitoring Board (DSMB) will perform planned safety evaluations during the study period, detect pre-specified pause rules and identify any potential safety concerns.

See Section 8 for more details.

2.2.2 Justification for Dose and Dose Regimen

In the pivotal efficacy, immunogenicity, and safety study ARCT-154-01 (study with Arcturus COVID-19 vaccine ARCT-154), ARCT-154 vaccine at a dose of 5 µg, administered twice 28 days apart, elicited a robust immune response and induced protection against COVID-19 disease in adults 18 years and above. More than 19,000 participants were exposed in this study, and no safety concerns were identified.

Considering results from Arcturus COVID-19 vaccine studies, preliminary results from Arcturus Phase 1 seasonal influenza study and GLP repeat-dose toxicology study conducted with ARCT-2304 vaccine, the dose levels of 1.5 µg (lowest possible dose to be administered considering vaccine presentation), 5 µg (same dose as used for Arcturus COVID-19 vaccine) and 12 µg (highest dose level with favorable tolerability profile, identified in the Phase 1 seasonal influenza study) are planned to be evaluated in this study.

This study will evaluate two dosing regimens to generate critical data on potential vaccination schedules in a pandemic. By assessing dosing on Day 1 and Day 29 versus Day 1 and Day 57, we aim to provide decision-makers with the necessary evidence to identify the most effective schedule to respond quickly and efficiently to a pandemic. This approach could help reduce the spread of infection and decrease the morbidity and mortality associated with pandemic influenza.

2.3 Benefit/Risk Assessment

2.3.1 Risk Assessment

The following section outlines the risk assessment and mitigation strategy for this study protocol (Table 4 and Table 5):

Table 4 Important Risks associated with ARCT-2304 and Mitigation Strategy

Important Risks	Data/Rationale for Risk	Mitigation Strategy
Potential Risks		
Hypersensitivity including severe reactions such as anaphylaxis	Acute hypersensitivity reactions, such as an anaphylactic event, may occur with any vaccine administration. These are serious, but rare, occurrences estimated in the range of 1 to 10 cases per million of vaccinations, depending on the vaccine studied (Rüggeberg 2007). Based on the experience with other mRNA vaccines, there is a possibility of hypersensitivity reactions including	The onset of vaccine related allergic symptoms is typically within a few minutes. To be able to treat a participant with a serious allergic reaction to the vaccination, the participant will need to remain under observation (visibly followed; no specific procedure) at the study center for at least 60 minutes post-vaccination with appropriate medical

Table 4 Important Risks associated with ARCT-2304 and Mitigation Strategy

Important Risks	Data/Rationale for Risk	Mitigation Strategy
	anaphylaxis. In the phase 1/2/3 ARCT-154-01 study, twelve cases of hypersensitivity were reported in >17,000 study participants. The events reported were mostly mild or moderate in intensity and few severe events were reported as well. The events were treated with standard clinical care, including antihistamines and steroids.	treatment readily available, in case it is needed. Individuals who have a history of severe adverse reaction associated with any mRNA vaccine or severe allergic reaction (e.g., anaphylaxis) to any component of the study vaccines, will be excluded.
Myocarditis, pericarditis and myopericarditis	Myocarditis and pericarditis have been reported in greatest numbers in males under the age of 40 years following a second dose of mRNA COVID-19 vaccines, but cases have been reported in older males and in females as well, and also following other doses. The observed risk is highest in males 12 to 17 years of age. While some cases require intensive care support, available data from short-term follow-up suggest that symptoms resolve in most individuals with conservative management. Information is not yet available about potential long-term sequelae. No cases of myocarditis or pericarditis have been observed to date with COVID-19 (ARCT-021, ARCT-154, ARCT-165) or Influenza (ARCT-2138) Arcturus vaccines.	During the informed consent process, the participants enrolling in the study will be informed of this potential risk and the need to attend the study center if they are unwell. Myocarditis and pericarditis are AESIs and will be collected during the entire study period. All reported cases of myocarditis and pericarditis will be reviewed by medical monitors (blinded review), DSMB (unblinded review). In addition, a Central Cardiac Adjudication Committee (CCAC) may meet to evaluate ECGs, cardiac enzyme results and other relevant data (blinded review). DSMB and CCAC will make recommendations as appropriate.
Other Considerations		
Local and systemic reactions	[redacted]	

Abbreviations: AE, adverse event; AESI: adverse event of special interest; CCAC: Central Cardiac Adjudication Committee; DSMB, data safety monitoring board; I.M., intramuscular; SAE, serious adverse event.

Table 5 Study Procedures Related Risks

Venipuncture (blood sampling)	Pain or bruising at the site where blood is drawn.	Blood samples will be collected by trained medical personnel; participants will remain under medical observation after venipuncture to complete study-specific procedures.
Syncope	Syncope (fainting) can occur following or even before any blood draws as a psychogenic or vasovagal response to the needle injection.	All participants will remain under observation, post venipuncture, through completion of the applicable study visit.

2.3.2 Benefit Assessment

Participants will be exposed to ARCT-2304 or a licensed influenza vaccine and placebo. The study participants may benefit directly from participation in this study if the vaccinations induce a protective immune response against pandemic or seasonal influenza when and if these participants are subsequently exposed to these viruses. Participants will gain some information about their general health, a result of the screening history, examination, blood tests, and urine tests (if applicable). Participants found to have a previously undiagnosed condition considered to require further medical attention will be referred appropriately for further investigation and treatment, with their permission. It is hoped that their contribution to this study will further support the successful development of a safe and effective vaccine against pandemic influenza and provide knowledge about pandemic influenza infection and the mechanism of protection.

2.3.3 Overall Benefit/Risk Conclusion

To date, the safety, immunogenicity, and efficacy of the ARCT-2304 vaccine in humans is unknown. Considering the measures that will be implemented to minimize the risks to participants in this study, the potential and/or known risks identified in association with the ARCT-2304 vaccine is justified by the anticipated benefits for humans to prevent pandemic influenza.

The sponsor will immediately notify the principal investigator, the DSMB and the CCAC if any additional significant new safety or toxicology information becomes available during the study.

This study will be performed in compliance with the protocol, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) and applicable regulatory requirements. Aspects of the study concerned with the investigational medicinal product (ARCT-2304) will meet the requirements of Good Manufacturing Practice (GMP).

More detailed information about the known and expected benefits and risks, and the reasonably expected AEs of the ARCT-2304 vaccine can be found in the IB.

3 OBJECTIVES, ENDPOINTS AND ESTIMANDS

The study objectives, endpoints and estimands are listed in Table 6.

Table 6 Objectives, Endpoints and Estimands for ARCT-2304-01

Primary Objectives	Endpoints	Estimands
1. To evaluate the safety and reactogenicity of different dose levels of ARCT-2304	Local and systemic AEs reported within 7 days after each vaccination. Unsolicited AEs reported within 28 days after each vaccination. Any laboratory abnormalities on Days 1, 8, 29, 36 and 57 (for D1,29 cohort) or Days 1, 8, 57, 64 and 85 (for D1,57 cohort) Any clinically significant abnormal vital signs on Days 1, 8, 29, 36 and 57 (for D1,29 cohort) or Days 1, 8, 57, 64 and 85 (for D1,57 cohort) Serious Adverse Events (SAEs), Medically Attended AEs (MAAEs), AEs of Special Interest (AESIs), and AEs leading to early termination reported from Day 1 up to Day 57 (for D1,29 cohort dule) and Day 1 up to Day 85 (for D1,57 cohort) in: • ARCT-2304 recipients (by dose level) • Control/placebo vaccine recipients	Number and percentage of participants with: • Local and systemic solicited AEs • Unsolicited AEs • Any treatment-emergent ≥ Grade 3 abnormal clinically significant laboratory values • Any clinically significant abnormal vital signs • SAEs, MAAEs, AESIs, and AEs leading to early termination from study. by Safety Set
2. To describe the hemagglutination inhibition (HAI) antibody responses against the HA glycoprotein through 28 days after the second vaccination	Serum HAI antibody titers against the H5N1 HA glycoprotein on Days 1, 29, 36 and 57 (for D1,29 cohort) and on Days 1, 57, 64 and 85 (for D1,57 cohort), in: • ARCT-2304 recipients (by dose level) • Historical control vaccine recipients[a]	• Geometric Mean Titers (GMTs) • Geometric Mean Fold Rise (GMFRs) • Seroconversion Rates (SCRs) • Proportions of participants with HAI titer ≥1:10, 1:20, 1:40, 1:80, 1:160, 1:320 by PPS
3. To describe the neuraminidase enzyme-linked lectin assay (ELLA) antibody responses against the NA glycoprotein, through 28 days after the second vaccination	Serum ELLA antibody titers against the H5N1 NA glycoprotein on Days 1, 29, 36, and 57 (for D1,29 cohort) and on Days 1, 57, 64, and 85 (for D1,57 cohort), in: • ARCT-2304 recipients (by dose level)	• GMTs • GMFRs • SCRs • Proportions of participants with NA titer ≥1:10, 1:20, 1:40, 1:80, 1:160, 1:320 by PPS

Table 6 Objectives, Endpoints and Estimands for ARCT-2304-01

Secondary Objectives		
1. To evaluate the safety of different dose levels of ARCT-2304 through the entire study period	SAEs, MAAEs, AESIs, and AEs leading to early termination from the study, from Day 1 through Day 240, in: • ARCT-2304 recipients (by dose level) • Control/placebo vaccine recipients	Number and percentage of participants with: • SAEs; MAAEs; AESIs; and AEs leading to early termination from study. by Safety Set
2. To describe the persistence of humoral immune response, following ARCT-2304, as measured by HAI and ELLA assays, throughout the entire study period	Serum HAI and ELLA antibody titers against the HA and the NA glycoproteins on Days 1, 29, 36, 57, and 240 (for D1,29 cohort) and Days 1, 57, 64, 85, and 240 (for D1,57 cohort), in: • ARCT-2304 recipients (by dose level)	• GMTs • GMFRs • SCRs • Proportions of participants with antibody titer ≥ 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320 by PPS
3. To describe serum neutralizing (MN) antibody titer against the HA glycoprotein through 28 days after the second vaccination	Serum MN antibody titers against the HA glycoprotein on Days 1, 29, and 57 (for D1,29 cohort) and Days 1, 57, and 85 (for D1,57 cohort), in: • ARCT-2304 recipients (by dose level) • Historical control vaccine recipients[a]	• GMTs • GMFRs • SCRs • Proportions of participants with antibody titer ≥LLOQ, 1:40, 1:80, 1:160 and 1:320 by PPS
Exploratory Objectives		
1. To describe HA and NA-specific CD4+ and CD8+ T-cell responses elicited by the different dose levels of ARCT-2304	Expression of activation markers and subset-specific markers by ELISpot following HA and NA peptide pool stimulation on Days 1, 8, 29 and 36 (for D1,29 cohort) and on Days 1, 8, 57 and 64 (for D1,57 cohort) in: • ARCT-2304 recipients (by dose level)	• Frequency of T-cells responding to HA and NA influenza vaccine antigens by assessing cells expressing markers such as interferon-gamma (IFN-γ), interleukin 13 (IL-13), IL-4, and others (e.g. RNA sequencing). by PPS

Abbreviations: AE, adverse event; AESI, adverse event of special interest; GMFR, fold rise in serum GMT post-vaccination compared to pre-vaccination; ELLA, enzyme-linked lectin assay; GMT, geometric mean titer; HA, hemagglutination; HAI, hemagglutination inhibition; LLOQ, lower limit of quantitation; MAAE, medically attended AE; MN, microneutralization; NA, neuraminidase; PPS, per protocol set; SAE, serious adverse event; SCR, seroconversion rate.

SCR is the percentage of participants with either a pre-vaccination antibody titer < LLOQ and a post-vaccination antibody titer ≥ 4 x LLOQ, or a pre-vaccination antibody titer ≥LLOQ and a ≥4-fold increase in post-vaccination antibody titer.

a Historical control vaccine recipients: Day 1 and Day 43 samples from a previous study performed with an MF59-adjuvanted A/H5N1 vaccine (A/Indonesia/05/2005) will be used as the comparator for the primary immunogenicity objective 2 and secondary immunogenicity objective 3.

b Testing for CD4+ and CD8+ T-cell response in D1,57 cohort samples might be performed as future research evaluation.

4 STUDY DESIGN

This is a phase 1, first-in-human, randomized, observer-blind, parallel design, controlled, dose level and schedule-finding study to evaluate the safety, reactogenicity, and immunogenicity of the ARCT-2304 vaccine, when administered as a 2-dose vaccination series to healthy adults.

4.1 Overall Design

Approximately 200 evaluable participants are planned to be enrolled in this study; 150 participants receiving ARCT-2304 (different dose levels) and 50 participants receiving the control/placebo vaccine(s).

Study groups and vaccination schedule:

Investigational Vaccine: The study will use the investigational ARCT-2304 vaccine (Table 8).

Control for safety: The study will use an age-appropriate comparator influenza vaccine for the control group (first vaccination) and placebo (second vaccination). A licensed cell-culture based inactivated influenza vaccine (Flucelvax Trivalent®) for adults aged 18 – 59 years of age, an adjuvanted inactivated influenza vaccine (Fluad Trivalent®) for adults aged 60 – 80 years of age, and 0.9% saline for placebo.

Control for immunogenicity: Historical control samples from a previous study performed with A/H5N1 vaccine (A/Indonesia/05/2005).

Phases of the study: As ARCT-2304 will be administered for the first time to humans in this study a staggered enrolment approach will be taken as safety precaution (Figure 2). The study will be enrolled in four sequential phases:

1. Lead-in Cohort 1 (18-49 years): enrolment will start with 8 participants, 18-49 years of age, that will be assigned to the Days 1, 29 vaccination cohort and will be randomized in a 1:1:1:1 ratio to receive one of the three dose levels of ARCT-2304 or control/placebo vaccine. Data Safety Monitoring Board (DSMB) review will be performed as soon as 7-day safety and reactogenicity data are available after the first vaccination for this first group of participants. The favorable outcome of reactogenicity and safety assessment by the DSMB will permit the expansion of recruitment of study participants in age group 18-59 years of age. The second planned DSMB review will be performed as soon as 7-days safety and reactogenicity data are available after the second vaccination for this first group of participants (Lead-in Cohort 1). The safety review will include the totality of safety data (including all available safety of participants in the expansion age group 18-59 years. The favorable outcome of reactogenicity and safety assessment by the DSMB will permit the initiation of second vaccination in the expansion age group 18-59 years and recruitment of lead-in participants in the 60-80 years age group.
2. Expansion age group 18-59 years: As soon as the DSMB authorizes the expansion in age group 18-59 years, recruitment will be initiated. The participants will be randomized into one of two vaccination schedule cohorts (Days 1, 29, or Days 1, 57) and into one of the 4 treatment arms (three dose levels of ARCT-2304 or control/placebo vaccine) in a 1:1:1:1 ratio.

3. Lead-in Cohort 2 (60-80 years) : Recruitment will start after authorization by DSMB (see above). Eight participants in the 60 – 80 years of age group will be enrolled, assigned to the Days 1, 29 vaccination cohort, and will be randomized to one of the 4 treatment arms, similar to the lead-in cohort 1 (18-59 years). DSMB will review all available data as soon as 7-day safety and reactogenicity data after the first vaccination is available. A favorable outcome from the DSMB will permit the expansion of recruitment in age group 60-80 years.
4. Expansion age group 60-80 years: As soon as the DSMB authorizes the expansion for age group 60-80 years, recruitment will be initiated. The participants will be randomized into one of two vaccination schedule cohorts (Days 1, 29, or Days 1, 57) and into one of the 4 treatment arms (three dose levels of ARCT-2304 or control/placebo vaccine) in a 1:1:1:1 ratio. Following post-Dose 2 assessment of Lead-in Cohort 2, DSMB will make a decision regarding administration of the second dose in the expansion age group 60-80 years.

Besides the four scheduled DSMB reviews as indicated above, the DSMB will perform periodic reviews of safety and reactogenicity data as soon as 7-day post-Dose 1 data are available for e.g. the first 80 and 120 recruited participants. The safety review will include the totality of safety data (including post-Dose 2 results) at each time point. In case pre-specified pause rules are met or any safety concerns are raised, enrolment/vaccination will be paused; the DSMB will review available safety information and will provide recommendations regarding study continuation, study modification, or study termination.

Drop-Out Replacement: Any participant who withdraws/discontinues after randomization but prior to the first study vaccination will be replaced. Any participant from the Lead-in 1 or 2 who drops out within the first 7 days (from Day 1 to Day 7) of the study will be replaced to ensure eight evaluable participants are included in each Lead-in Cohort. Replacement of participants who withdraw/discontinue post dose(s) will be at the discretion of the Sponsor.

Randomization: An Interactive Response Technology (IRT) will be used in the study. Participants will be randomized into two cohorts according to the vaccination schedule (Days 1, 29 or Days 1, 57). Within each cohort, participants will be randomized to one of 4 groups (1,5 µg, 5 µg or 12 µg of ARCT-2304, or control/placebo vaccine) in a 1:1:1:1 ratio. Randomization will be performed separately for each age group, 18-59 and 60-80 years of age.

Vaccination schedule: All participants will receive two intramuscular (IM) doses of either one of the three dose levels of the ARCT-2304 vaccine or control vaccine (first vaccination) and placebo (second vaccination) and will follow one of the two different vaccination schedules (Days 1, 29 or Days 1, 57), according to Table 7.

Table 7 Study Cohorts and Treatment Arms

Cohort	Treatment Arm	Schedule of Vaccine Administration	Total Enrolled	Enrolled by Age Group	
				Age 18 to ≤ 59 yrs	Age 60 to ≤ 80 yrs
D1,29	1,5 µg ARCT-2304	Day 1, Day 29	25	15	10
	5 µg ARCT-2304	Day 1, Day 29	25	15	10
	12 µg ARCT-2304	Day 1, Day 29	25	15	10

	Control/placebo vaccine	Day 1, Day 29	25	15	10
D1,57	1,5 µg ARCT-2304	Day 1, Day 57	25	15	10
	5 µg ARCT-2304	Day 1, Day 57	25	15	10
	12 µg of ARCT-2304	Day 1, Day 57	25	15	10
	Control/placebo vaccine	Day 1, Day 57	25	15	10

Data collection: electronic Case Reporting Form (eCRF) and electronic Diary (eDiary).

Blinding: Observer-blind study.

Study periods: The study has a screening period (Day -28 to Day 1), a vaccination period (Days 1-57 for the Days 1, 29 cohort and Days 1-85 for the Days 1, 57 cohort), and a follow-up period from Day 57 (or 85) to Day 240.

Study visits: Seven (7) study visits, including a screening visit and 6 study visits on Days 1, 8, 29, 36, 57, and 240, in Days 1, 29 cohort and on Days 1, 8, 57, 64, 85 and 240 in Days 1, 57 cohort.

Safety call: Two safety calls (Days 120 and 180) will be conducted in the Days 1, 29 cohort, and three safety calls (Days 29, 120, and 180) will be performed in the Days 1, 57 cohort to collect any medically attended AEs (MAAEs), serious AEs (SAEs), AEs of special interest (AESIs), concomitant medications, and any vaccinations. For the Lead-in participants, four additional safety phone calls will be performed on Day 2 and Day 4 after each study vaccination.

Number of blood samples: Seven (7) blood draws at the screening visit and on Days 1, 8, 29, 36, 57, and 240 for the Days 1, 29 cohort and on Days 1, 8, 57, 64, 85, and 240 for the Days 1, 57 cohort, will be obtained from all participants.

Duration of the study/participant participation: Approximately 9 months for each participant.

The study design is provided in Figure 1. The schedule of assessments (SoA) is provided in Table 2 and Table 3.

4.2 End of Study Definition

The end of the study is defined as the date of the last visit of the last participant in the study or last scheduled procedure shown in the SoA (Table 2 and Table 3) for the last participant in the study. The duration of participation will be approximately 9 months for each participant.

4.3 Criteria for Suspension of the Clinical Study (Pause Rules)

Pausing Rules for this study are pre-specified. If any of the events listed in Table 8 is identified or any safety concern is raised, study enrolment and study vaccination will be paused. Pause rules 1-3 will be assessed on a blinded manner by the Investigators, CRO and sponsor representatives during the entire study period. Pause rules 4-7 will be assessed by a dedicated unblinded medical monitor from sponsor/CRO.

If an investigator detects the occurrence of one of the pause rules 1-3, he/she should immediately put the enrolment and the vaccination on hold in his/her clinical site, inform CRO's Medical Monitor and sponsor and enter the respective data in the eCRF. All other procedures relating to safety, reactogenicity, and immunogenicity assessment should continue. It is the Sponsor's responsibility to pause the enrolment and/or the vaccination at all sites.

The pause rules 4-7 will be regularly assessed by an unblinded medical monitor based on the actual number of exposed subjects with available results. If a pause rule is met or any safety concern is identified, the unblinded medical monitor should put the enrolment and vaccination on hold and request an ad-hoc DSMB meeting for data review. The DSMB will provide recommendations regarding study continuation, study modification, or study termination to the sponsor.

The sponsor will review all data and DSMB recommendations and will decide whether to stop permanently, modify, or continue the conduct of the study. The sponsor's decision regarding further conduct of the study will be documented and provided in writing to the investigators, the independent ethics committees (IECs), and regulatory authorities.

Table 8 Pause Rules

Pause Rule	Medical Event	Number of Participants
1	Any SAE or Grade 4 AE assessed by the PI as related[a] to the study vaccine during the entire study period	≥1 participant in the study
2	Any case of myocarditis, pericarditis or myopericarditis within 14 days post-vaccination regardless of causality assessment and for any case that occurs within 6 weeks postvaccination that is at least possibility related to study vaccination.	≥1 participant in the study
3	Any severe systemic hypersensitivity, such as anaphylaxis, assessed by the PI as related[a] to the study vaccine within 24 hours after each study vaccination.	≥1 participant in the study
4	Any severe unsolicited AE[b] assessed by the PI as related[a] to the study vaccine within 28 days post-vaccination	≥25% and ≥2 participants within a dose level and age category
5	Any severe (Grade 3 or greater) solicited[c] local AE lasting more than 48 hours, within 7 days post-each vaccination	≥25% and ≥2 participants within a dose level and age category
6	Any severe (Grade 3 or greater) solicited[c] systemic AE lasting more than 48 hours, within 7 days post-each vaccination	≥25% and ≥2 participants within a dose level and age category
7	Any severe (Grade 3 or greater) laboratory abnormality assessed by the PI as related[a] to the study vaccine.	≥25% and ≥2 participants within a dose level and age category

Notes: Grading of laboratory parameters will be based on the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (CBER 2007).

a Related is defined as an event that is at least possibly, or probably related to the investigational vaccine.

b Is a same or similar AE as defined by Preferred Terms based on the Medical Dictionary for Regulatory Activities (MedDRA) coding.

c Solicited adverse events are those events that are reported by the subject in the eDiary.

5 STUDY POPULATION

Participants will be eligible to be included in the study if all of the criteria listed in Section 5.1 and none of the criteria listed in Section 5.2 apply.

5.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Individuals are male or female adults 18-80 years of age.
2. Healthy participants or participants with pre-existing stable medical conditions. Stable medical condition is defined as an existing disease that has not required a significant change in treatment or hospitalization/emergency room visit for worsening within 3 months prior to Day 1 and participant has full capacity of daily activity.
3. Participant must freely provide documented informed consent prior to study procedures being performed.
4. Individuals must agree to comply with all study visits and procedures (including blood tests, diary completion and willingness to be available for planned telephone contacts and unscheduled clinic visits, if required).
5. Individuals of childbearing potential must be willing to adhere to contraceptive requirements (Appendix 1).

5.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Individuals with acute medical conditions or febrile illness, including body temperature ≥100.4°F (≥38.0°C; measured by any method) within 3 days prior to randomization. These individuals may be offered the opportunity to enter the study after fever and illness have stabilized. Participants with suspected or confirmed influenza should be excluded and referred for medical care. Rescreening will be permitted for individuals who are presented with suspected influenza if another diagnosis is confirmed.
2. Individuals with any medical, neurological, or psychiatric condition that, in the opinion of the investigator, could place the participant at an unacceptable risk of injury or render the participant unable to comply with all study procedures and achieve successful completion of the trial.
3. Individuals with a known history of severe hypersensitivity reactions, including anaphylaxis, or other significant adverse reactions to any mRNA vaccine, influenza vaccine, or excipients.
4. Individuals who have a positive pregnancy test at the Screening visit or Day 1 or who intend to become pregnant or breastfeed during the study.
5. Individuals with a history of myocarditis, pericarditis, myopericarditis or cardiomyopathy.
6. Individuals with a history of Guillain-Barré syndrome, encephalomyelitis, or transverse myelitis.
7. Individuals with a known bleeding disorder that would, in the opinion of the investigator, contraindicate intramuscular (I.M.) injection.

8. Individuals with a history of congenital or acquired immunodeficiency.
9. Individuals who have received immunomodulatory, immunostimulatory, or immunosuppressant drugs, including interferon and cytotoxic drugs, within 3 months of first vaccine administration or who plan to receive them during the study.
10. Individuals requiring systemic corticosteroids exceeding 10 mg/day of prednisone equivalent for ≥10 days within 30 days of first vaccine administration. The use of topical, ophthalmic, inhaled, intranasal, and intra-articular steroid preparations will be permitted.
11. Individuals who have received immunoglobulins and/or any blood or blood products within the 3 months before first vaccine administration or plan to receive such products at any time during the study.
12. Individuals with an immunosuppressive or immunodeficient state, asplenia, or recurrent severe infections.
13. Individuals with a documented history of chronic infection including HIV, HBV, HCV, or who are currently known to have active tuberculosis.
14. Individuals with chronic illness that, in the opinion of the Investigator, are at a stage where it might interfere with trial participation or interpretation of study results.
15. Individuals receiving treatment with another investigational drug, biological agent, or device within 28 days of first vaccine administration, or 5 half-lives of the investigational drug, whichever is longer; or are currently enrolled in or plan to participate in another clinical trial with an investigational agent during the study period.
16. Individuals who received any influenza vaccine within 3 months prior to first vaccine administration or plan to receive an influenza vaccine during the study period.
17. Individuals who have received any other licensed vaccines within 14 days prior to first vaccine administration or who are planning to receive any vaccine up to 14 days after the last study vaccination.
18. Individuals who have received mRNA vaccination within 60 days before first vaccine administration.
19. Individuals who have received or plan to receive an A/H5N1 influenza vaccine and/or individuals who had substantial exposure to or direct contact with poultry, wild birds, cattle or raw milk.
20. Individuals with safety laboratory test results (hematology, biochemistry and coagulation) with a toxicity score of Grade ≥ 2 at screening (refer to the laboratory manual for laboratory-specific normal ranges and associated toxicity grades). The inclusion of participants with non-clinically significant (NCS) Grade 1 laboratory abnormalities is allowed at the investigator's discretion.
21. Individuals who are investigator site staff members, employees of the Sponsor or the Clinical Research Organization directly involved in the conduct of the study, or site staff members otherwise supervised by the investigator or immediate family members of any of the previously mentioned individuals.

5.3 Contraindications for the Second Vaccination

The following events constitute absolute contraindications to further administration of study vaccines.

If any of these events occur during the study, the participant must not receive additional doses of vaccine, but should be encouraged to continue study participation for safety at the discretion of the investigator:

- Anaphylaxis or systemic hypersensitivity reaction following the administration of vaccine.
- Any SAE judged to be related to study vaccination.
- Pregnancy.
- Any adverse event that, in the opinion of the investigator, poses an additional risk to the participant if he/she continues to participate in the study.
- If a participant does have a laboratory Grade 2 or greater toxicity score following the administration of the first dose that has not returned to baseline, the Sponsor or Sponsor designee must be contacted to agree on continued dosing.

The following events constitute contraindications to administration of study vaccine at certain points in time, and if any of these events occur at the time scheduled for vaccination, the participant may be vaccinated at a later date, preferably within the time window specified in the protocol (Table 2 and Table 3), or the participant may be withdrawn at the discretion of the investigator:

- Acute moderate or severe infection with or without fever at the time of vaccination.
- Fever, defined as temperature ≥38.0°C (100.4°F; measured by any method) at the time of vaccination.

Participants with a minor illness without fever, as assessed by the investigator, can be administered study vaccines.

5.4 Prior and Concomitant Medications

The use of prior and concomitant medications must be recorded in the electronic case report form (eCRF).

- Prior medications, vaccines, and blood products that are described in the exclusion criteria (Section 5.2) are to be recorded on the eCRF, if they were administered to any enrolled individual. It includes:
  - Receipt of any investigational product within 28 days prior to first vaccine administration (or 5 half-lives of the investigational product, if longer);
  - Immunomodulatory, Immunostimulatory, or Immunosuppressive therapy, including interferon and cytotoxic agents, within 3 months prior to first vaccine administration;
  - Systemic corticosteroids exceeding 10 mg/day of prednisone equivalent for ≥10 days within 30 days prior to first vaccine administration;
  - Receipt of any influenza vaccine within 3 months prior to first vaccine administration. Note: receipt of any A/H5N1 vaccine is not allowed;
  - Receipt of any licensed vaccines within 14 days prior to first vaccine administration;
  - Receipt of mRNA vaccination within 60 days before first vaccine administration;
  - Intravenous immunoglobulins and/or any blood or blood products within 3 months prior to first vaccine administration.

- Concomitant medications include medications and vaccines taken by/administered to the participant at and after enrolment and must be documented on the eCRF. The following are considered concomitant medications:
  - All medications and vaccines administered from Day 1 (day of vaccination) up to 28 days after each study vaccination;
  - All medications associated with SAEs, adverse events of special interest (AESIs), medically attended AEs (MAAEs), and AEs leading to early study termination, during the entire study period (from Visit 1 to study completion);
  - All medications administered for treatment of influenza during the entire study period (from Day 1 to study completion);
  - Any licensed or investigational influenza vaccine (other than the study vaccines), during the entire study period (from Day 1 to study completion);
- Any investigational and non-licensed medications (other than the study vaccines) during the entire study period (from Day 1 to study completion).
- Any medication that meets the reporting criteria (including over the counter or prescription medicines, and/or herbal supplements) must be recorded on the eCRF along with:
  - Reason for use;
  - Dates of administration including start and end dates;
  - Dosage information including dose and frequency.

The use of antipyretics (e.g., paracetamol) will be allowed as treatment for fever and pain or other post-vaccination reactions; participants should not be encouraged to use antipyretics prophylactically. The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

5.5 Prohibited Medications

The use of an excluded medication/therapy is a protocol violation and must be recorded in the eCRF. The use of an excluded medication does not require the withdrawal of a participant from the study.

The following medications are prohibited:

- Receipt of any licensed or investigational influenza vaccine within 3 months prior to first vaccine administration or during the study period;
- Receipt of A/H5N1 vaccine prior to or during the study period;
- Receipt of any licensed vaccines within 14 days prior to first vaccine administration or up to 14 days after the study vaccination;
- Receipt of any investigational product within 28 days prior to first vaccine administration (or 5 half-lives of the investigational product, if longer), or during the study period;
- Immunomodulatory, Immunostimulatory, or Immunosuppressive therapy, including interferon and cytotoxic agents, within 3 months prior to first vaccine administration or during the study period;

- Systemic corticosteroids exceeding 10 mg/day of prednisone equivalent for ≥10 days within 30 days prior to first vaccine administration or during the study period;
- Intravenous immunoglobulins and/or any blood products within 3 months prior to first vaccine administration or during the study period.
- Receipt of any mRNA vaccines within 60 days prior to first vaccine administration.

5.6 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but do not meet all eligibility criteria and are not subsequently randomly assigned to a study group. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. The reason for screen failure must be documented in the Screening and Enrolment log.

A participant who does not meet the eligibility criteria can be rescreened only once. Participants who are rescreened will receive a new participant number. The informed consent process should be repeated if rescreening occurs ≥ 14 days from previous ICF signature date.

6 STUDY VACCINES

All study vaccines associated with this study are to be stored separately from other vaccines and medications in a secure location under appropriate storage conditions with temperature monitoring. A clinical label will be affixed to study vaccine containers in accordance with local regulatory requirements. All vaccines associated with this study must be checked for expiration date prior to use. Expired study vaccines must not be administered to participants.

6.1 Study and Control Vaccines

6.1.1 Study Vaccine

The details about investigational ARCT-2304 vaccine are presented in Table 9. The instructions regarding ARCT-2304 vaccine preparation and administration are provided in the Pharmacy Manual. Refer to the IB for further information about the ARCT-2304 vaccine.

Table 9 Study Vaccine Details –ARCT-2304

Intervention Name	ARCT-2304 vaccine
Formulation	ARCT-2304 Drug Product (DP) contains one Drug Substance (DS): mRNA-2320 (A/Indonesia/05/2005 mRNA) encoding for HA and NA of Indonesia strain. mRNA construct encapsulated in the lipid nanoparticle
Presentation	Multi-dose vial, lyophilized powder to be reconstituted
Manufacturer	Designated manufacturer: Arcturus Therapeutics, Inc.
Route of Administration	IM in the deltoid region of the upper arm
Volume (after preparation)	0.5 and 0.25 mL
Content/vial	mRNA-2320: 320 µg
IMP	Yes
Packaging	The study vaccines will be packaged and labeled according to GMP and local regulations. The study vaccine will not be packed in individual participant kits; one kit may be used for multiple participants.

Abbreviations: GMP, Good Manufacturing Practice; HA, hemagglutinin, IM, intramuscular; IMP, investigational medicinal product, NA, neuraminidase.

Warnings and Precautions

Only participants enrolled in the study may receive study vaccine and only authorized study staff may supply or administer study vaccine. As there is limited experience with the administration of the study vaccine to humans, all effects cannot be reliably predicted. Availability of facilities and staff familiar with the treatment of anaphylaxis and resuscitation and the treatment of other medical emergencies will be ensured.

6.1.2 Control Vaccine

Control for safety:

Flucelvax Trivalent® is a surface antigen, inactivated influenza vaccine, prepared in cell cultures, and indicated for active immunization of persons aged 6 months or older for the prevention of influenza disease. Each 0.5 mL dose, in sterile phosphate buffered saline, is a suspension of a total of 45 µg hemagglutinin (HA). HA antigens are derived from the three influenza strains (A/H1N1, A/H3N2 and 1 influenza type B strain, 15 µg each) recommended by the World Health Organization (WHO) for trivalent vaccines for the respective season when it was manufactured.

Fluad Trivalent® is a surface antigen, inactivated adjuvanted influenza vaccine, indicated for active immunization of persons aged 65 years or older for the prevention of influenza disease. Each 0.5 mL dose of vaccine contains nominally 15 µg of HA of each of the 2 influenza type A strains and 1 influenza type B strains for a total of 45 µg of HA in the vaccine and MF59C.1 adjuvant, a squalene-based oil-in-water emulsion. The strain composition is that recommended by the WHO for trivalent vaccines for the respective season when it was manufactured.

Placebo – 0.9% saline solution, 0.5 mL dose.

Control for immunogenicity:

Historical control samples from a previous clinical study (study V89P1) performed with an MF-59 adjuvanted A/H5N1 vaccine (A/Indonesia/05/2005). Random selection of Day 1 and Day 43 samples from participants who were dosed with two doses (21 days apart) of 7.5 µg A/H5N1 + 100% MF59 or 15 µg A/H5N1 + 100% MF59 (Keitel 2010). The vaccine contained H5N1 influenza virus surface antigens (hemagglutinin and neuraminidase) of Madlin-Darby canine kidney (MDCK)-based A/Indonesia/5/2005 strain.

6.2 Vaccine Preparation, Handling, Storage and Accountability

6.2.1 Vaccine Preparation

ARCT-2304 study vaccine will be prepared in accordance with instructions provided in the Pharmacy Manual.

Flucelvax Trivalent® presentation is a 0.5 mL dose in a pre-filled syringe so no further preparation is required.

Fluad Trivalent® presentation is a 0.5 mL dose in a pre-filled syringe so no further preparation is required.

6.2.2 Vaccine Handling

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for the study vaccine received and any discrepancies are reported and resolved before use of the study intervention. Only participants enrolled in the study may receive study intervention and only authorized unblinded site staff may supply or administer study intervention.

6.2.3 Vaccine Storage

ARCT-2304 vaccine should be stored at -20°C ± 5°C (-4°F to 41°F).

Flucelvax Trivalent® and Fluad Trivalent® vaccines should be stored at 2°C to 8°C (36°F to 46°F) Protected from light and should not be frozen.

Study vaccine must be stored in a secure, temperature controlled and monitored (manual or automated recording) area in accordance with the labeled storage conditions with access limited to the unblinded authorized site staff. At a minimum, daily minimum and maximum temperatures for all site storage locations must be documented and available upon request. Data for non-working days must indicate the minimum and maximum since previously documented for all site storage locations upon return to business.

Any excursions from the study vaccine storage conditions should be reported to Arcturus and the CRO upon discovery along with any actions taken. Once an excursion is identified, the study vaccine must be quarantined and not used until Arcturus provides permission to use the study intervention. Specific details regarding the definition of an excursion and information the site should report for each excursion will be provided to the site in the Pharmacy Manual.

Upon identification of a product complaint, Arcturus should be notified within 1 business day of discovery as described in the Pharmacy Manual.

Study vaccine temperature storage requirements and monitoring procedures are included within the Pharmacy Manual.

6.2.4 Vaccine Accountability

The study staff is required to document the receipt, dispensing, and return/destruction of study vaccine supplies provided by the sponsor. The study site must destroy or return for destruction all unused frozen vials of study vaccine to the Sponsor or designee. Vials of study vaccine should be destroyed by the study site or delegate only after drug accountability has been done by the unblinded study monitor. Quarantining, destruction, or return of study vaccine must be documented and in accordance with guidance provided by the Pharmacy Manual as well as local institutional policies.

6.2.5 Vaccine Administration

Study vaccine will be administered by intramuscular (IM) injection to the deltoid muscle by a qualified, GCP-trained health care provider (HCP) who will not be involved in assessments of any study endpoints. Study vaccine preparation and administration should be performed in an area outside of the view of blinded team members. Unblinded team members will not otherwise participate in other study-related procedures or assessments of the participant post dose administration.

Standard vaccination practices must be observed, and vaccine must not be injected into blood vessels. Appropriate medication and other supportive measures for management of an acute hypersensitivity reaction should be available in accordance with local guidelines for standard immunization practices.

Study vaccine administration details will be recorded in source documents and on the case report form (CRF). Reasons for departure from the expected dispensing regimen must also be recorded.

6.2.6 Errors in Study Vaccine Administration

Study vaccine errors (including overdose, underdose, and administration error) must be documented as protocol deviations. A brief description should be provided of the deviation, including whether the participant was symptomatic (list symptoms) or asymptomatic, and whether the event was accidental or intentional.

Dosing details should be captured on the appropriate source documents and on the eCRF.

An overdose is the accidental or intentional administration of a study vaccine in an amount higher than the dose being studied. An overdose or incorrect administration of study vaccine is not itself an AE, but it may result in an AE. If the participant receives a dose of study vaccine that exceeds protocol specifications and the participant is symptomatic, the symptom(s) should be documented as an AE (Section 9).

Should an overdose occur, the Investigator or designee should contact the Sponsor or designee within 24 hours.

6.2.7 Management of Overdose

There are no known treatments for potential overdose of study vaccine. Unless there is a user error leading to failure to administer any volume of study vaccine, “catch-up” study vaccine administrations will not be performed. In case of medication errors or an overdose, it is recommended that the participant be monitored for any signs or symptoms of adverse reactions and appropriate symptomatic treatment be provided immediately.

6.3 Measures to Minimize Bias: Randomization and Blinding

This study will be observer-blind.

After signing the informed consent form (ICF), each participant will be given a number (Subject ID) according to the screening order. On Day 1, following confirmation of eligibility, a randomization number will be assigned, and participants will be allocated to treatment using an interactive response technology (IRT).

An unblinded dosing teams, not involved with study participant’s evaluation, will prepare and administer the study vaccine doses. The study vaccine syringe will be opacified/masked to avoid unblinding of the participant. The administration of the study vaccine will also be performed in an area outside of the view of blinded team members to ensure that the other blinded staff members do not become unblinded. The investigative study center personnel, as well as the sponsor personnel involved in the monitoring or conduct of the study, will be blinded to the study vaccine code.

The treatment code should be broken only if the investigator/physician in charge of the participant feels that the case cannot be treated without knowing the identity of the study vaccine.

Except in cases of medical necessity, a participant's treatment should not be unblinded without the approval of the sponsor. If unblinding should occur (by either accidental unblinding or emergency unblinding) before completion of the study, the investigator must promptly contact the sponsor or designee and document the date and reason that the blind was broken in the source documentation and CRF. Instructions regarding emergency unblinding will be provided to the investigator.

6.4 Vaccine Compliance

The investigator records all injections of investigational vaccine/comparator/placebo given to the participant.

The prescribed dosage, timing, and mode of administration may not be changed. Any departures from the intended regimen must be recorded in the eCRFs.

All dose administration will be performed at the study center by appropriately trained staff.

The clinical staff is responsible for the ongoing safety and wellbeing of the participants while they are in the study center. There is a physician on-site during normal business hours and medical advice is available by phone 24 hours a day. In addition, if necessary, the clinical staff can contact further on-call physicians or public emergencies services in the event of a serious medical event.

6.5 Treatment after the End of the Study

The sponsor will not provide any additional care to participants after they leave the study.

7 PARTICIPANT WITHDRAWAL FROM THE STUDY

7.1 Discontinuation of Study Treatment

A "withdrawal" from the study vaccine refers to any participant who does not receive the complete treatment, i.e., when no further planned dose is administered from the date of withdrawal. A participant withdrawn from the study vaccine may continue further study procedures (safety or immunogenicity) if planned in the study protocol, as deemed appropriate by the investigator.

Primary reason relative to premature discontinuation of the study vaccine/control will be documented in the eCRF:

- AE(s).
- Death
- Withdrawal of consent (Participant decision, not due to an AE).
- Pregnancy: If a female participant becomes pregnant (such participants will be followed up as described in Section 9.1.13 and Appendix 1).
- Lost to follow-up.
- Study terminated by Sponsor.
- Other (specify).

Contraindication for second vaccination are criteria for delay of the second vaccination are described in Section 5.3.

If a participant who does not meet enrolment criteria is inadvertently enrolled, the Sponsor or Sponsor designee must be contacted. In general, if there are no safety concerns identified, that participant may continue in the study with all study procedures at the investigator's discretion based on benefit/risk assessment and clinical judgement. If continuing in the study, this should be reported as a protocol deviation.

7.2 Participant Withdrawal from the Study

A participant may withdraw from the study at any time at his/her own request, or may be withdrawn, at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons.

If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the study center study records and inform sponsor's representative.

See the SoA (Table 2 and Table 3) for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

Primary reason relative to premature discontinuation from the study will be documented in the eCRF:

- AE(s).
- Death
- Withdrawal of consent (Participant decision, not due to an AE).
- Pregnancy: If a female participant becomes pregnant (such participants will be followed up as described in Section 9.1.13 and Appendix 1).
- Lost to follow-up.
- Study terminated by Sponsor.
- Other (specify).

7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study center.

If a participant fails to return to the clinic for a required study visit, the study site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.

Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

8 STUDY ASSESSMENTS AND PROCEDURES

The following sections describe the study procedures and data to be collected. All procedures must be performed by qualified and trained staff.

The SoA is provided in Table 2 and Table 3.

8.1 Informed Consent

Informed consent must be obtained before any protocol-directed procedures are performed.

The requirements of the informed consent are described in Appendix 1.

8.2 Demographics and Baseline Data

Demographic information to be collected about the participant will include age/year of birth (if applicable), sex, race and ethnicity.

The subject's medical history should be obtained by interviewing the subject or by reviewing the subject's medical records and any pre-existing conditions or signs and/or symptoms present in a subject prior to the study vaccination should be recorded on the Medical History page of the eCRF.

Medication history (including medications, blood products, and vaccines) is to be collected as prior and concomitant medications. The use of concomitant medications does not require withdrawal of participant from the study.

Antipyretics and/or analgesic medications taken within 24 hours prior each vaccination and the reason for their use (prophylaxis versus treatment) must be documented.

Prior medications should be collected as specified in Section 5.3.

Refer to Section 5.4 for details on prohibited medications:

This data must be written in the source documents.

8.3 Physical Examination and Vital Signs

Physical examinations must be performed by a qualified health professional in accordance with local medical practice and regulations and as listed within the site responsibility delegation log.

"Qualified health care practitioner" refers to any licensed health care professional who is permitted by institutional policy to perform physical examinations.

A complete physical examination will be performed at screening visit and may include the examination of the following: general appearance, weight, and height, abdomen; head and neck; eyes, ears, nose, throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid, lymph nodes, and neurological.

A complete physical examination might be repeated prior to enrolment in the study on Day 1 if deemed necessary by the investigator.

Symptom-directed physical examination should be performed at all other scheduled time points. Interim physical examinations might be performed at the discretion of the Investigator.

Systolic/diastolic blood pressure, heart rate, respiratory rate, and body temperature (any method) will be measured at Screening and each following clinic visit. On the days of study vaccination, vital signs evaluations will be measured before vaccination and at least 60 minutes after vaccination of each dose (before participants are discharged from the clinic). Vital signs including body temperature should be measured in a seated position after the participant has rested comfortably for at least 5 minutes.

This data will be written in the source document and any abnormal values or events must be documented in the eCRF AE form (if occurring after vaccination) or eCRF Medical History form (if occurring prior to vaccination). Treatment of any abnormality must be performed according to local medical practice and outside this study or by referral to an appropriate healthcare provider.

8.4 ECG/Cardiac Enzymes Test

A baseline 12-lead ECG will be performed at recruitment on all study participants to identify potential asymptomatic myocarditis and pericarditis cases.

In case of suspected myocarditis or pericarditis cases during the study period, detailed evaluation, including ECG, cardiac enzyme testing, and other relevant data (including laboratory safety measurements, troponin-I, Troponin-T, CK-MB) should be evaluated.

Additional ECG evaluation at unscheduled visits is at the investigator's discretion.

Blood samples for cardiac enzymes will be collected on Day 1 prior to vaccination (baseline) in all participants. The testing of baseline and post-vaccination samples for cardiac enzymes (Troponin-I, Troponin-T, CK-MB) may be performed at the investigator's or Sponsor's discretion.

ECG and the ECG reading should be a part of the source documentation. Any abnormal values must be documented, and an impact of abnormal findings on the subject's eligibility for recruitment should be considered.

8.5 Pregnancy Testing

A pregnancy test (β-human chorionic gonadotropin) will be performed on all female participants of childbearing potential at Screening (serum test). Urine or serum pregnancy test will be performed prior to each study vaccination (urine or serum pregnancy test at the discretion of the Investigator or in accordance with institutional policy or local requirements) (see Section 9.1.13). A positive pregnancy test should be recorded in Electronic Data Capture (EDC), reported to the Sponsor as per Section 9.1.13, and will prevent study vaccination.

8.6 Inclusion and Exclusion Criteria

Only participants who have a signed informed consent form (ICF), and satisfy all of the inclusion (Section 5.1) and exclusion (Section 5.2) criteria are eligible for randomization and to enter the study.

If the participant is ineligible for randomization, the investigator should record the primary reason for failure on the participant screening and enrolment log (Section 5.6).

8.7 Randomization

An Interactive Response Technology (IRT) will be used in the study.

Participants will be randomized into two cohorts according to the vaccination schedule (Days 1, 29 or Days 1, 57). Within each cohort, participants will be randomized to one of 4 groups (1,5 µg, 5 µg or 12 µg of ARCT-2304 or control/placebo vaccine) in a 1:1:1:1 ratio. Randomization will be performed separately for each age group, 18-59 and 60-80 years of age.

The randomization specification and schedule will be approved by the sponsor's study statistician, or designee.

8.8 Vaccination

All participants will be monitored for at least 60 minutes after study injection for any immediate adverse reactions.

The instructions regarding study vaccine preparation and administration are provided in Section 6, the Pharmacy Manual and package insert document for control vaccines.

8.9 Immunogenicity Assessments

The measures of immunogenicity used in this study are standard, i.e., widely used and generally recognized as reliable, accurate, and relevant (able to describe the quality and extent of the immune response).

Antibody-Mediated Immune Response

Five (5) blood samples will be collected before (Day 1) and after vaccination on Days 29, 36, 57, and 240 for the Days 1, 29 cohort and on Days 57, 64, 85, and 240 for the Days 1, 57 cohort.

These blood samples will be used to assess the immune response to the ARCT-2304 vaccine (Table 9) using:

- A hemagglutination inhibition (HAI) assay for measuring hemagglutinin (HA)-specific antibodies against A/H5N1 influenza virus strain.
- An enzyme-linked lectin (ELLA) assay for measuring influenza neuraminidase (NA) inhibition antibody titers against A/H5N1 influenza virus strain.
- A virus microneutralization (MN) for detecting functional HA-specific antibodies against A/H5N1 influenza virus.

The results of HAI and ELLA will be analyzed as:

- Geometric mean titer (GMT) on Days 1, 29, 36, 57, and 240 (Days 1, 29 cohort) and on Days 1, 57, 64, 85, and 240 (Days 1, 57 cohort);
- Geometric mean-fold rise (GMFR) as increases of the post-vaccination titer over the pre-vaccination titer;
- SCR on Day 29, and 57 (for D1,29 cohort) and Days 57 and 85 (for D1,57 cohort), and as the percentage of participants in a group with either:
  - A pre-vaccination titer below the LLoQ for the respective assay and a post-vaccination titer ≥4 x LLoQ; or

  - A pre-vaccination titer ≥ LLoQ and a ≥4-fold increase in post-vaccination titer.

- Proportion of participants with HAI titer ≥1:10, 1:40, 1:80, 1:160 and 1:320 on Days 1, 29, and 57 (for D1,29 cohort) and Days 1, 57 and 85 (for D1,57 cohort);
- Proportion of participants with NA titer ≥1:10, 1:40, 1:80, 1:160 and 1:320 on Days 1, 29, and 57 (for D1,29 cohort) and Days 1, 57 and 85 (for D1,57 cohort);

The measures of immunogenicity using MN assay will include GMTs, GMFRs, SCRs, and proportions of subjects with antibody titers above pre-specified thresholds as deemed appropriate to describe the immune response against A/H5N1 influenza virus. The detailed information will be presented in the Statistical Analysis Plan.

Cell-Mediated Immune (CMI) Response

Four blood samples will be collected before (Day 1) and after vaccination (Days 8, 29, and 36) from a subset of approximately 10 participants per dose level in the D1,29 cohort and before (Day 1) and after vaccination (Days 8, 57 and 64) from a subset of approximately 10 participants per dose level in the D1,57 cohort. These blood samples will be used to assess the cell-mediated immune response to the ARCT-2304 vaccine in the D1,29 cohort and optional samples testing in the D1,57 cohort using:

- Frequency of T-cells responding to HA and NA influenza vaccine antigens by assessing cells expressing markers such as interferon-gamma (IFN-γ), interleukin 4 (IL-4), interleukin 13 (IL-13), and others (e.g. RNA sequencing).

Table 10 Immunogenicity Assessments for ARCT-2304-01

Assay	Purpose	Sample	Days	Comment
Antibody-Mediated Immune Response				
HAI	Primary/Secondary endpoint	Serum	Days 1, 29, 36, 57, 240 (D1,29 cohort) and on Days 1, 57, 64, 85, 240 (D1,57 cohort)	Quantification of antibody response against the HA glycoprotein All ARCT-2304 vaccinated participants and historical control vaccine recipients
ELLA	Primary/Secondary endpoint	Serum	Days 1, 29, 36, 57, 240 (D1,29 cohort) and on Days 1, 57, 64, 85, 240 (D1,57 cohort)	Quantification of antibody response against the NA glycoprotein All ARCT-2304 vaccinated participants
Neutralization (MN)	Secondary endpoint	Serum	Days 1, 29, 57 (D1,29 cohort) and on Days 1, 57, 85 (D1,57 cohort)	Functional assessment of antibody response against the HA glycoprotein All ARCT-2304 vaccinated participants and historical control vaccine recipients
Cell-Mediated Immune Response				

Table 10 Immunogenicity Assessments for ARCT-2304-01

Assay	Purpose	Sample	Days	Comment
ELISpot (CMI)*	Exploratory endpoint	Whole blood (PBMCs)	Days 1, 8, 29, 36 (D1,29 cohort) and on Days 1, 8, 57 and 64 (D1,57 cohort)	Analysis HA and NA-specific CD4+ and CD8+ T-cell responses Subset

Abbreviations: HAI, hemagglutination inhibition; ELLA, enzyme-linked lectin assay; HA, hemagglutination; NA, neuraminidase; MN, Microneutralization; PBMC, peripheral blood mononuclear cell;
*CMI samples will be collected from a subset of approximately 10 participants per dose level in the D1,29 and D1,57 cohorts.

Planned time points for all immunogenicity assessments are provided in the SoA (Table 2 and Table 3).

8.9.1 Safety Laboratory Testing

Safety laboratory tests will be performed by the local laboratory at screening, Days 1, 8, 29, 36 (D1,29 cohort) and at screening, Days 1, 8, 57, 64 (D1,57 cohort)

The safety laboratory tests are listed in Table 11 and values for laboratory abnormalities are listed in Appendix 4.

Blood samples for cardiac enzymes will be collected on Day 1 prior to vaccination (baseline) for all participants. The testing of baseline and post-vaccination samples for cardiac enzymes (Troponin-I, Troponin-T, CK-MB) might be performed based on the investigator's or Sponsor's discretion.

Table 11 Safety Laboratory Tests

Screening only	Hematology	Blood Chemistry	Coagulation
Hepatitis B virus surface antigen, Hepatitis C virus antibody, Human immunodeficiency virus type 1 and 2 antibodies; Follicle-stimulating hormone (may be measured to confirm menopausal status at the discretion of the investigator) β-human chorionic gonadotropin (WOCBP)	Complete blood count: Red blood cells (RBC) Hemoglobin Hematocrit White blood cells (WBC) total and differential Platelet count	Alanine aminotransferase (ALT) Albumin Alkaline phosphatase Aspartate aminotransferase (AST) Urea Blood urea nitrogen (BUN) Creatinine Random glucose Total bilirubin Total protein	Partial thromboplastin time Prothrombin time

8.9.2 Blood Volumes for Testing

All immunogenicity analyses (humoral and cell-mediated) will be performed at a laboratory designated by the sponsor or the sponsor's designee using standardized and validated procedures.

Safety Laboratory tests will be performed in local laboratories.

The maximum volume of blood taken at any single visit is 69.5 mL (range – 15.0 to 69.5 mL), and the maximum total volume of blood collected during the study period in each study participant is approximately 289.5 mL.

The total blood volume planned to be collected in the study is provided in Table 12.

Table 12 Total Blood Volume

Assessment	Blood volume per sample	Planned number of collections[1]	Total amount of collected blood
Clinical Safety Assessments[2]			
Serology	6 mL	1 (screening)	6 mL
Cardiac enzymes	6 mL	1 (Day 1)	6 mL
Hematology	2 mL	5 (Screening, Days 1, 8, 29, 36 (D1,29 cohort) and Screening, Days 1, 8, 57, 64 (D1,57 cohort))	10 mL
Blood Chemistry	8.5 mL	5 (Screening, Days 1, 8, 29, 36 (D1,29 cohort) and Screening, Days 1, 8, 57, 64 (D1,57 cohort))	42.5 mL
Coagulation	3 mL	5 (Screening, Days 1, 8, 29, 36 (D1,29 cohort) and Screening, Days 1, 8, 57, 64 (D1,57 cohort))	15 mL
Humoral Immunogenicity[3]			
Hemagglutination inhibition (HAI) assay	5 mL	5 (Days 1, 29, 36, 57, 240 (D1,29 cohort) and Days 1, 57, 64, 85, 240 (D1,57 cohort))	25 mL
Enzyme-linked lectin (ELLA) assay	5 mL	5 (Days 1, 29, 36, 57, 240 (D1,29 cohort) and Days 1, 57, 64, 85, 240 (D1,57 cohort))	25 mL
Neutralization (MN assay)	5 mL	3 Days 1, 29, 57 (D1,29 cohort) and Days 1, 57, 85 (D1,57 cohort))	15 mL
Cell-Mediated Immunogenicity[3,4]			
ELISpot	30 mL	4 (Days 1, 8, 29, 36 (D1,29 cohort) 4 (Days 1, 8, 57, 64 (D1,57 cohort)	120 mL
Possible Exploratory testing			
Future testing related to vaccine or pandemic disease	5 mL	5 (Days 1, 29, 36, 57, 240 (D1,29 cohort) and Days 1, 57, 64, 85, 240 (D1,57 cohort))	25 mL
Subject total			**289.5 mL**

Abbreviations: HAI, hemagglutination inhibition; ELLA, enzyme-linked lectin assay; ELISpot, enzyme-linked immunospot assay; MN, Microneutralization

[1] Additional blood collections may be required at the discretion of the Investigator to follow up on abnormal results;

[2] Blood volume for Clinical Safety Assessment may vary depending on laboratory requirements, please refer to the study laboratory manual.

[3]Samples collected from the safety comparator group will not be analyzed for humoral immunogenicity or cell-mediated immunogenicity.

[4]CMI samples will be collected from a subset of approximately 10 participants per dose level in the D1,29 and D1,57 cohorts.

8.9.3 Biological Sample Retention and Destruction

Details of biological sample collection, handling, and analysis are presented in the laboratory manual. Repeat or unscheduled samples may be collected for safety reasons or for technical issues with the samples. Biological samples will be preserved and retained at a central laboratory that was contracted by the sponsor for this purpose for up to but not longer than 20 years or as required by applicable law. The sponsor has put into place a system to protect the participant's personal information to ensure optimal confidentiality and defined standard processes for sample and data collection, storage, analysis, and destruction.

8.10 Safety Assessments

All participants will be monitored for at least 60 minutes after each study vaccination for any immediate AEs with appropriate medical treatment readily available in case it is needed.

- Solicited local reactions and systemic AEs (Section 9.1.9) will be recorded daily within 7 consecutive days after study vaccination using an electronic diary card (eDiary):
  - Local reactions: pain, erythema and swelling at the injection site;
  - Systemic AEs: fatigue, headache, myalgia, arthralgia, nausea, dizziness chills, and fever (derived from measured body temperature ≥100.4 °F [≥38.0 °C]).
- Any unsolicited AEs will be collected within 28 days after each study vaccination (Section 9.1.9);
- Pregnancies will be recorded throughout the study (Section 9.1.13);
- Any SAEs (Section 9.1.2), AESIs (Section 9.1.14), MAAEs (Section 9.1.15) and AEs leading to early study termination, will be reported during the entire study period. These data will be captured by interviewing the participant during the site visits and phone calls and by review of available medical records.

Refer to Section 9 for safety definitions. This section contains details on collection (Section 9.1.7), follow-up (Section 9.1.10) and reporting of AEs (Section 9.1.11).

Planned time points for all safety assessments are provided in the SoA (Table 2 and Table 3).

8.11 Safety Laboratory Testing

Safety laboratory tests will be performed by the designated laboratory(ies) at the Screening Visit, Day 1 (before study vaccination), Day 8, Day 29 (before study vaccination) and Day 36 (D1, D29 Cohort) / Screening Visit, Day 1 (before study vaccination), Day 8, Day 57 (before study vaccination) and Day 64 (D1,57 Cohort). Fasting is not required before the collection of laboratory samples.

The following hematology, serum blood chemistry, and coagulation laboratory assessments will be performed:

- Hematology: Complete blood count: red blood cells (RBC), hemoglobin, hematocrit, total and differential white blood cells (WBC) and platelet count

- Blood Chemistry: alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, urea, blood urea nitrogen (BUN), creatinine, random glucose, total bilirubin and total protein.
- Coagulation: partial thromboplastin time and prothrombin time

Should safety laboratory testing at 7 days post vaccination result in a Grade 2 or greater toxicity score, then repeat testing must be performed within the next 10 days and this may include an unscheduled visit. Should the participant's laboratory test value not return to baseline, then periodic testing may be required until the abnormality is deemed to be associated with a new stable AE or determined to be NCS by the investigator. If a participant does have a laboratory Grade 2 or greater toxicity score following the administration of the first dose that has not returned to baseline, the Sponsor or Sponsor designee must be contacted to agree on continued dosing (see Section 5.3).

Those values that fall within the normal range of the laboratory will automatically be classified as normal. All values that have a toxicity score of Grade 1 or greater will be evaluated by the investigator and classified as 'abnormal clinically significant CS' or 'abnormal not clinically significant NCS' as follows:

- Abnormal clinically significant CS – laboratory abnormality cannot be explained or is judged by the investigator to be potentially clinically significant.
- Abnormal not clinically significant NCS –laboratory abnormality can be explained by a condition that is not related to vaccination and does not increase the risk for an adverse outcome of vaccination

Toxicity grades will be defined based on normal ranges and according to Guidance for Industry – Toxicity Grading Scale for Healthy adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trails: Table for Laboratory abnormalities (Appendix 4). A list of laboratory – specific normal ranges and associated toxicity grades will be provided in the laboratory manual.

All laboratory test values with toxicity score of Grade 3 or greater will be recorded as AE as well Grade 1 or greater abnormal clinically significant test values.

8.12 Schedule of Activities

8.12.1 Screening (Day -28 to Day 1)

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

- Informed consent (Section 8.1).
- Demographics (Section 8.2).
- Medical history and prior medication (Section 8.2).
- Physical examination and vital signs (Section 8.3).
- 12-lead electrocardiogram (Section 8.4)
- Blood sampling for safety testing (Section 8.11)

- Pregnancy test (Section 8.5).
- Inclusion/Exclusion criteria assessment (Section 5.1 and 5.2).

8.12.2 Pre-vaccination Procedures (Day 1 and Day 29 (D1, D29 Cohort) / Day 57 (D1,57 Cohort)

If all Screening procedures are performed prior to Day 1 (and prior the second dose), the following assessments should be repeated prior to dosing: physical examination and vital signs and pregnancy testing.

- Physical examination and vital signs (Section 8.3).
- Blood sampling for safety testing (Section 8.9.1)
- Pregnancy test (Section 8.5)
- Blood sampling for antibody-mediated immunogenicity (Section 8.9).
- Blood sampling for cell-mediated immunogenicity for subset only (Section 8.9).

8.12.3 Vaccination and Post-vaccination Procedures (Day 1 and Day 29 (D1, D29 Cohort) / Day 57 (D1,57 Cohort)

Contraindications, warnings, and precautions to vaccination must be reviewed prior to the study vaccination.

After confirming eligibility (Section 8.6), perform randomization (Section 8.7, only for first dose on Day 1) and subsequent vaccination (Section 8.8).

The following post-vaccination procedures will be performed:

- Participants will be observed for at least 60 minutes after vaccination in case of any adverse reaction. Any AEs reported within this period will be recorded in the eCRF.
- After study vaccine administration, vital signs will be measured (blood pressure, respiratory rate, body temperature, and heart rate) and vital sign measurement should be repeated if clinically indicated.

Careful training of the participant (or the individual who will be performing the measurements) on how to measure local reactions and body temperature, how to complete and how often to complete the eDiary.

eDiary instructions must include the following:

- The participant must understand that timely completion of the eDiary on a daily basis is a critical component to study participation.
- The participant should be instructed on how to complete the eDiary and how to make corrections to erroneous entries.
- Starting on the day of each vaccination, the participant will check approximately 6 hours post- vaccination (minimum of 4 hours post-vaccination) for specific types of reactions at the injection site (solicited local adverse reactions), any specific generalized symptoms

(solicited systemic AEs), body temperature (preferably axillary), any other symptoms or change in the participant's health status, and any medications taken.
- The participant must use the thermometer provided by the site for body temperature measurement. If the participant feels unusually hot or cold during the day, the participant should check body temperature several times and record the highest body temperature observed that day on the eDiary.
- The collection of body temperature, solicited local adverse reactions, and solicited systemic AEs will continue for a total of 7 days on the eDiary.
- Once the eDiary is completed on Day 1, participant should aim to record the data in the eDiary at approximately the same time for the following days.

eDiary will be the only source document allowed for solicited local reactions and systemic AEs (including body temperature measurements).

Note: Any solicited AE that meets any of the following criteria must be entered into participants' source document (see Appendix 2) and also as an AE on the AE CRF:

- Solicited local or systemic AEs leading to a visit to a healthcare provider (MAAE, Section 9.1.15).
- Solicited local or systemic AEs leading to the participant withdrawing from the study or the participant being withdrawn from the study by the investigator (AE leading to withdrawal, see Section 7.1).
- Solicited local or systemic AE that otherwise meets the definition of an SAE (Section 9.1.2).
- Solicited local or systemic AE that continues beyond 7 days post-vaccination.

Compliance checks for eDiary completion will be performed by site staff on Day 2 (±1), Day 5 (±1), Day 30 (±1) and Day 33 (±1) (D1, D29 Cohort) and on Day 2 (±1), Day 5 (±1), Day 58 (±1) and Day 61 (±1) (D1, D57 Cohort) after vaccination. The site will perform a check to verify that the participant has completed the eDiary. Participants will be contacted by telephone if the eDiary is incomplete. Compliance checks will be documented in the source but not included in the CRF.

8.12.4 Non-vaccination Site Visit Procedures (Days 8, 36, 57, 240 [D1,29 cohort] / Days 8, 64, 85, 240 [D1, D57 Cohort]).

Site visit that does NOT include a vaccination will be performed on Days 8, 36, 57, 240 (D1,29 cohort) / Days 8, 64, 85, 240 (D1, D57 Cohort). The following procedures will be completed as planned in the SoA (Table 2 and Table 3):

- Review of eDiary (Day 8 and Day 36 (D1, D29 Cohort) / Day 8 and Day 64 (D1, D57 Cohort);
- Concomitant medications/vaccines collection;
- Symptom-directed physical examination and vital signs.
- Collection of respective blood samples (Days 8, 36, 57, 240 [D1,29 cohort] / Days 8, 64, 85, 240 [D1, D57 Cohort]).

- Assessment of unsolicited AEs (Days 8, 36, 57 [D1,29 cohort] / Days 8, 64, 85 [D1, D57 Cohort])
- Adverse events collection (AEs with medically attended visits, AESIs, SAEs).

The site should schedule the next site visit or other study activity with the participant. As soon as the Safety Laboratory Results are available (Days 8, 36 (D1,29 cohort) / Days 8, 64 (D1,57 cohort)) the results should be reviewed by the investigator or designee.

8.12.5 Phone Contacts (Days 120, 180 [D1,29 Cohort] / Days 29, 120, 180 [D1, D57 Cohort]).

Two (2) safety calls will be made by phone contact (PC) on Days 120 and 180 in the D1, D29 Cohort, and three (3) safety calls on Days 29, 120, and 180 will be performed in the D1, D57 Cohort for collection of any signs and symptoms the participant may have experienced, any new diagnosed medical conditions, and medications taken since the previous contact (e.g. medically attended AEs (MAAEs), serious AEs (SAEs), AEs of special interest (AESIs), concomitant medications, and any vaccinations). For the Lead-in Cohorts, four additional safety phone calls will be performed 2 days and 4 days after each study vaccination (Days 2 and 4 and Days 30 and 32 [Day 1, 29 Cohort] and Days 2 and 4 and Days 58 and 60 [Day 1, Day 57 Cohort]). All calls will follow a script and will be made by trained healthcare personnel.

A trained healthcare professional is defined as any healthcare professional permitted by institutional policy and trained to perform delegated tasks, trained on the study procedure(s), and identified within the site signature and delegation log.

Any safety information collected during the phone contact will be entered into the source documents and reported in the eCRF, as appropriate.

8.12.6 End-of-study (Day 240) or Early Termination (ET)/Last Visit Procedures

The final (end of study) contact will be performed on Day 240. If a participant terminates earlier, the ET visit procedures should be performed at their last study visit, if possible. See the SoA (Table 2 and Table 3) for data to be collected at the end-of-study and an early termination visit. The investigator must complete the end of study CRF page for all participants who received the study vaccine.

8.12.7 Unscheduled visits

Unscheduled visits include visits for possible cardiac events, or evaluation of any adverse event of concern. These visits may be performed in the clinic, by home visit, by hospital visit (if allowed by local policy), or via telemedicine/telephone visit. Required procedures at these visits include AE and concomitant medication collection. Should the visit occur in person, the visit should also include evaluation of vital signs including body temperature, and symptom-directed physical examination.

For participants evaluated for cardiac symptoms, 12-lead electrocardiogram and targeted safety laboratory assessments should be performed. If myocarditis or pericarditis is suspected based on investigator evaluation of the participant, the participant should be referred to a cardiologist, ideally within 24 hours (see Section 9.1.16).

A blood sample might be drawn for additional laboratory safety evaluation, including cardiac enzymes, as per investigator discretion.

Additional safety phone calls may be performed as a follow-up for the evaluation of adverse event(s).

8.12.8 Post-study Procedures

Any SAE that occurs outside of the protocol-specified observation period or after the end of the study but is considered to be caused by the study vaccine will be processed by the sponsor (or delegate) and must be reported to the sponsor.

Any post-study pregnancy-related SAE considered reasonably related to the study vaccine by the investigator will be reported to the sponsor. While the investigator is not obligated to actively seek this information in former participants, he or she may learn of an SAE through routine medical practice.

These SAEs and pregnancy information will be considered as part of the spontaneous reporting towards the investigational study vaccine in order to ensure the safety of all subjects.

No post-study care will be provided.

9 ADVERSE EVENTS

The following sections provide definitions for AEs, the procedures for collecting, following up and reporting to the authorities.

AEs will be reported by the participant (or, when appropriate, by a caregiver) or identified at study visit.

The investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study vaccine or study procedures, or that caused the participant to discontinue the study (see Section 7).

9.1 Definitions

9.1.1 Definition of an Adverse Event

- An AE is any untoward medical occurrence in a participant or participant administered a medicinal product, whether or not considered related to the study vaccine.

  NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.

9.1.1.1 Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., electrocardiogram, radiological scans, or vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study vaccine administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.

9.1.1.2 Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with an underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): The condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

- Pre-existing conditions or signs and/or symptoms present in a participant prior to the first study vaccination. These events will be recorded in the medical history section of the eCRF.

9.1.2 Definition of an SAE

An SAE is defined as any AE that, at any dose:

a. Results in death.
b. Is life-threatening.

The term 'life-threatening' in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization.

In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other seriousness criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity.

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect.
f. Is a medically important event:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

9.1.3 Recording of AEs and SAEs

- When an AE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE information in the eCRF. Each event must be recorded separately.
- It is not acceptable for the investigator to send photocopies of the participant's medical records to sponsor/sponsor's designee in lieu of completion of the relevant eCRF page.
- There may be instances when copies of medical records for certain cases are requested by sponsor/sponsor's designee. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to sponsor/sponsor's designee.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE.

9.1.4 Assessment of Intensity

The investigator will make an assessment of intensity for each AE reported during the study and assign it to 1 of the following categories, based on the US Food and Drug Administration (FDA) Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (CBER 2007):

- Mild (Grade 1): transient with no limitation in normal daily activity.
- Moderate (Grade 2): some limitation in normal daily activity.
- Severe (Grade 3): unable to perform normal daily activity.
- Potentially Life-Threatening (Grade 4): an event in which the participant was at immediate risk of death at the time of the event.
- Fatal (Grade 5): an event with a fatal outcome.

Note: An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

9.1.5 Assessment of Causality

- The investigator is obligated to assess the relationship between study vaccine and each occurrence of each AE. The AE must be characterized as related or unrelated:
  - **Related:** An AE that followed a temporal sequence from administration of a drug (including the course after withdrawal of the drug), or for which possible involvement of the drug could not be ruled out, although factors other than the drug, such as underlying diseases, concurrent medical conditions, concomitant drugs, and concurrent treatments, were also responsible.
  - **Unrelated:** An AE that did not follow a temporal sequence from study administration of a drug and/or that could reasonably be explained by other factors, such as

underlying diseases, concurrent medical conditions, concomitant drugs, and concurrent treatments.

- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study vaccine administration will be considered and investigated.
- The investigator will also consult the IB and/or product information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to sponsor/sponsor's designee. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to sponsor/sponsor's designee.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

9.1.6 Assessment of Outcome of AE

The investigator will assess the outcome of all AEs recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not yet recovered/not yet resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).
- Unknown.

9.1.7 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

Medical occurrences that begin before the study vaccination but after obtaining informed consent will be recorded in the medical history/current medical conditions section of the eCRF, not the AE section. Exacerbation of a chronic or intermittent preexisting condition including either an increase in frequency and/or intensity of the condition after the first vaccination will be considered an AE.

For all participants:

- Any AEs that occur within 60 minutes after study vaccination are to be reported in the eCRF.
- Any AEs that are determined by the investigator (or designee) to be SAEs, AESIs (serious and nonserious), MAAEs, or AEs leading to early termination from the study or study vaccination (regardless of the causal relationship) are to be reported in the eCRF

from the moment of first vaccination until completion of the participant's last study-related procedure, which may include contact for safety follow-up (until the end of the study).

All AESIs and SAEs will be recorded and reported to the sponsor or the sponsor's designee immediately and under no circumstance should this exceed 24 hours after the investigator became aware of it. The investigator will submit any updated data to the sponsor within 24 hours of it being available.

9.1.8 Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing intensity, causality and outcome of AEs and SAEs and the procedures for completing and transmitting AESI/SAE reports are provided in Section 9.1.7, Section 9.1.11 and Section 9.1.14.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

9.1.9 Solicited and Unsolicited Adverse Events

The investigator or designee will review and verify the completed eDiary during discussions with the participant as per the time points detailed in the SoA.

Participants will record solicited AEs and a presence of unsolicited AEs in the eDiary. The investigator or designee will review the eDiary entries and collect the details of the unsolicited AEs with the participant. The investigator or designee will then transcribe the appropriate collected information about unsolicited AEs into the relevant eCRF pages, according to the guidance presented in Section 9.1.7. If the AE meets AESI criteria or SAE criteria, it should also be reported to the sponsor or sponsor's designee (Section 9.1.11).

Solicited local (injection site) reactions:

- Injection site pain.
- Erythema.
- Swelling.

Solicited systemic AEs:

- Fatigue.
- Headache.
- Myalgia.
- Arthralgia.
- Dizziness
- Nausea.
- Chills.
- Fever (≥100.4 °F [≥38.0 °C]).

Severity grading for these AEs is presented in Table 13.

Table 13 Severity Grading for Solicited Local Reactions and Systemic Adverse Events

	Mild	Moderate	Severe
Pain	No interference with daily activities	Interferes with daily activities	Prevents daily activity
Erythema	25-50 mm	51-100 mm	> 100 mm
Swelling	25-50 mm	51-100 mm	> 100 mm
Fatigue	No interference with daily activities	Interferes with daily activities	Prevents daily activity
Headache	No interference with daily activities	Interferes with daily activities	Prevents daily activity
Myalgia	No interference with daily activities	Interferes with daily activities	Prevents daily activity
Arthralgia	No interference with daily activities	Interferes with daily activities	Prevents daily activity
Nausea	No interference with daily activities	Interferes with daily activities	Prevents daily activity
Dizziness	No interference with daily activities	Interferes with daily activities	Prevents daily activity
Chills	No interference with daily activities	Interferes with daily activities	Prevents daily activity
Fever (°F°C)	100.4 – 101.1 (38.0-38.4)	101.2 – 102.0 (38.5-38.9)	≥102.1 (≥39.0)

Note: Based on 1.Food and Drug Administration (FDA) Guidance Document: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

Unsolicited AEs

An unsolicited AE is an AE that is not listed as 'solicited' above. Solicited AE that last for more than 7 days will be considered as unsolicited AEs.

Potential unsolicited AEs may be medically attended (defined as symptoms or illnesses requiring hospitalization, or emergency room visit, or visit to/by a healthcare provider; Section 9.1.15) or were of concern to the participants. In case of such events, participants will be instructed to contact the study center as soon as possible to report the event(s). The detailed information about the reported unsolicited AEs will be collected by the qualified study center personnel during the interview and will be documented in the participant's records.

Unsolicited AEs that are not medically attended or perceived as a concern by participants will be collected during planned safety visits/contacts with the participants and by review of available medical records.

9.1.10 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All AEs/SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Section 9.1.10.

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by sponsor/sponsor's designee to elucidate the nature and/or causality of the AE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other healthcare professionals.
- New or updated information will be recorded in the originally completed eCRF.

- The investigator will submit any updated SAE data to the sponsor/sponsor's designee within 24 hours of receipt of the information.
- All SAEs with the outcomes of 'recovering/resolving' and 'not yet recovered/not yet resolved' will be reviewed at visit Day 240 to confirm the outcome of the event.
- A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study vaccine(s), the investigator will promptly notify the sponsor. Please refer to Section 8.12.7 for details.
- Serious adverse events and adverse events of special interest still ongoing at the end of study must be followed up until resolution or stabilization, or until the event outcome is provided. Reporting may continue after the follow-up visit and database lock.

9.1.11 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study vaccine under clinical investigation are met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of study vaccine under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, institutional review board (IRB)/IEC, and investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

9.1.12 Reporting of AESIs and SAEs

The investigator is responsible for ensuring that all SAEs and AESIs observed by the investigator or reported by the participant that occur after study vaccination until the study completion are recorded on the AE CRF page in EDC and immediately reported to Arcturus Therapeutics on an SAE/AESI Report Form.

All initial and follow-up information should be reported within 24 hours of awareness.

The contact (email address) for AESI/SAE reporting can be found in the instructions of the AESI/SAE Report Form.

The investigator must attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented.

The investigator must complete the SAE/AESI Report form in English and must assess the causal relationship of the event to study vaccine.

New information relating to a previously reported SAE or AESI must be immediately reported to Arcturus Therapeutics.

9.1.13 Pregnancy

Female participants of childbearing potential are to have a urine or blood pregnancy test (at investigator discretion or per local requirements) prior to the study vaccine administration. The study vaccine may only be administered if the pregnancy test is negative.

Note: Pregnancy tests must be performed even if the participant is menstruating at the time of the study visit. Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected. Any female participant who becomes pregnant will be encouraged to remain in the study for safety follow-up until the end of the study.

Details of pregnancies in female participants, for which the date of conception is within the entire study period, should be followed for pregnancy outcomes. Information on delivery and newborn should be reported to the sponsor within 1 month of the end of the pregnancy. Information about newborn health condition may be collected at 6 or 12 months after birth.

Details of pregnancies in female partners of male participants will be collected according to local regulations.

If a pregnancy is reported, the investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined below.

Female participants who become pregnant

- The investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. The investigator will record all positive pregnancy tests in EDC. The information on pregnancy will be recorded on the Pregnancy Report Form and submitted to the sponsor within 24 hours of learning of a participant's pregnancy. The participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant and the neonate, and the information will be forwarded to the Sponsor.
- Any female participant who becomes pregnant while participating in the study will discontinue the study vaccine (if applicable). The participant will be encouraged to remain in the study for safety follow-up until the end of the study.

Reporting of Pregnancy Information

Information will be recorded by the investigator on the appropriate paper Pregnancy Report Form and submitted to the Sponsor/Sponsor's designee within 24 hours of learning of a pregnancy case and upon learning of any follow-up pregnancy information. The contacts (email address) for pregnancy reporting can be found in the instructions page of the paper Pregnancy Report Form.

A paper Pregnancy Report Form will be completed for any pregnancy occurring in participants after vaccination during the entire study period.

While pregnancy itself is not considered to be an AE, any pregnancy complications (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Any post-study pregnancy related SAE considered reasonably related to the study vaccine by the investigator will be reported to the sponsor. While the investigator is not obligated to actively seek this information in former participants, he or she may learn of an SAE through spontaneous reporting.

9.1.14 Adverse Events of Special Interest

AEs potentially associated with mRNA vaccines and influenza vaccines should be reported as AEs of special interest. These events are shown in Appendix 3 (Table 17).

Once any AESI is diagnosed (serious or nonserious), the investigator (or designate) must record the AESI in the relevant eCRF page and report the AESI in an expedited manner, as per Section 9.1.12.

When there is enough evidence to make any of the above diagnoses, the AE must be reported as an AESI. Symptoms, signs, or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as AESIs until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

The DSMB may adjudicate reported AEs that meet criteria of AESIs.

9.1.15 Medically Attended Adverse Events

MAAEs are defined as AEs with medically attended visits including hospital, emergency room, urgent care clinic, or other visits (including phone/telehealth visits) to or from medical personnel for any reason, but do not fulfill seriousness criteria. Routine study visits will not be considered medically attended visits.

9.1.16 Suspected myocarditis and pericarditis cases

All suspected cases of myocarditis, pericarditis, and myopericarditis should be reported to the Sponsor in an expedited manner.

All study participants should be instructed to report to site personnel, as soon as possible, a new onset of chest pain, shortness of breath (at rest or during activity), rapid or irregular heartbeat (arrhythmias), swelling of the legs, ankles, and feet, light-headedness, collapse, and fatigue occurring within 6 weeks after vaccination.

All study participants will have a sample collected to test for cardiac enzymes at baseline. Only in case of a suspected myocarditis and pericarditis is identified, these samples will be measured.

If participants present no suspected myocarditis and pericarditis within the first 6 weeks post each vaccination, samples will be destroyed.

The investigator must perform the initial assessment of the study participant with these symptoms/signs and refer individuals with suspected myocarditis and pericarditis to a cardiologist for evaluation and management. A questionnaire will be provided to the investigators for assessment of suspected cases. A 12-lead ECG and cardiac enzyme testing should be performed in all subjects with suspected myocarditis and pericarditis. Additional evaluations (such as echocardiography, cardiac MRI) might be performed based on the cardiologist's recommendations.

A central cardiac adjudication committee will assess all suspected cases of myocarditis and pericarditis (as determined by investigator and cardiologist) according to the Brighton Collaboration case definition (Sexson Tejtel et al, 2021) within 1 week of the initial reporting.

Cases of myocarditis and/or pericarditis will be followed until resolution of abnormal test findings and symptoms.

Cases of myocarditis and/or pericarditis occurring in temporal association with vaccination will be considered potentially related, unexpected, and serious, and are subject to expedited reported requirements as outlined in Section 9.1.11.

9.2 Treatment of Overdose

Not applicable.

9.3 Genetics

Genetics are not evaluated in this study.

10 STATISTICAL CONSIDERATIONS

10.1 Statistical Hypotheses

There are no formal statistical hypotheses in this phase 1 clinical trial.

10.2 Sample Size Determination

The sample size is not based on formal power calculations, and it is based on clinical considerations to provide sufficient safety information to analyze the primary safety objective. With a sample size of n=150 participants who receive ARCT-2304, the probability of observing at least 1 AE is 98% and 78% if the true event rate is 2.5% and 1.0%, respectively. With a sample size of n=50 per dose level, the probability of observing at least 1 AE is 92% and 72%, if the true event rate is 5.0% and 2.5%, respectively.

A sample size of up to 200 participants of study who received either ARCT-2304 vaccine or placebo will provide evaluable and sufficient pre- and post-vaccination information to analyze the primary and secondary immunogenicity objectives.

10.3 Populations for Analyses

The analysis sets are defined in Table 14.

Table 14 Analysis Sets

Analysis set	Description
Enrolled set	All screened participants who provided informed consent, and received a participant ID, and were randomized.
Exposed set / Intent to Treat (ITT) Analysis Set	All participants who received at least one dose of study vaccine. The ITT Analysis Set will be analyzed according to the vaccine assigned.
Modified ITT Analysis Set (mITT)	All participants who are randomized, received at least one dose of study vaccine and provided at least one post-baseline immunogenicity assessment. The mITT will be analyzed according to vaccine assigned.
Per-protocol (PPS) set	Participants in the mITT who correctly received the assigned protocol-required dose of study vaccine and no protocol deviations impacting the analysis of immunogenicity data.
Historical Control set	Random Samples (sample size approximately 30-40 study participants) from the Per Protocol set from the study V89P1. Study arms 7.5 micrograms and 15 micrograms with full dose (100%) of MF59; Day 1 and Day 43 samples
Safety analysis set (SAF)	All participants in the Exposed Set who provide any evaluable post-vaccination reactogenicity and/or safety data. Should there be an error in administration where the actual study vaccine that the participant received was different than the one to which he/she was assigned, the Safety Analysis Set will be analyzed according to the vaccine actually received.

10.4 Statistical Analyses

For this study, statistical analysis will be primarily descriptive, and 95% confidence interval (CI) may be provided whenever applicable. No formal multiple comparison adjustments will be employed for multiple safety, immunogenicity, or exploratory endpoints. Nominal p-values and

CIs may be computed for immunogenicity data analyses without controlling for multiplicity. This section summarizes the planned statistical analysis strategy and procedures for the study. The details of statistical analysis will be provided in the SAP, which will be finalized before the first analysis. If, after the study has begun, but prior to any unblinding, changes are made to primary and/or key secondary objectives/hypotheses or the statistical methods related to those hypotheses, then the protocol will be amended. Changes to other secondary or exploratory analyses made after the protocol has been finalized, along with an explanation as to when and why they occurred, will be listed in the SAP or CSR for the study. Ad hoc exploratory analyses, if any, will be clearly identified in the CSR.

The final database lock will occur when all participants have completed the study.

All personnel involved in the analyses of the study will remain blinded until the final analysis and protocol deviations are identified.

10.4.1 Analysis of Demographic and Baseline Characteristics

Disposition, demographics, and baseline characteristics, including medical history, will be summarized. Descriptive statistics (mean, SD, median, minimum, and maximum) for age, height, weight, and BMI at enrolment will be calculated overall and by study group. Distributions of participants by sex, age, and ethnic origin (race, ethnicity) will be summarized overall, and by study group. The SAF population will be used for all analyses of disposition, demographics, and baseline characteristics. A separate summary may include the number of participants enrolled but not treated, if applicable.

10.4.2 Immunogenicity Analyses

Analyses will be conducted using the Per Protocol Set (PPS) (immunogenicity) set as defined for the specific assay under evaluation. Immunogenicity endpoints are described in Section 3 and will be summarized, by study group and time point, using descriptive statistics. The kinetics of humoral immune response following vaccination will be evaluated. Descriptive immunogenicity analysis including GMT, GMFR, proportion of participants with antibody titer ≥pre-specified thresholds and SCR will be produced for each study group (i.e. investigational arms and historical control group) at all available time points. Immunogenicity analyses will not be conducted for the control vaccine group from the study.

Geometric mean (GM) will be calculated as the mean of the antibody results after the data are log-transformed and then antilog the log-mean to present the results on the original scale. If calculated, the two-sided 95% CI will be obtained by taking the log transformation of the antibody results and calculated based on t-distribution; then antilog the confidence limit.

GMFR analysis will include subjects with antibody results available at both baseline (prior to dose) and post-vaccination. It will be calculated as the mean of the difference after log transformed results (post baseline minus baseline) and exponentiating the mean. If calculated, two-sided 95% CI will be obtained by taking log transform of the antibody results and calculated the 95% CI based on t-distribution for the mean difference after the data are log-transformed, then antilog the confidence limit. The statistical analyses for GMTs and GMFRs are unadjusted but additional analyses may be conducted using an analysis of covariance (ANCOVA) model

with the dose level as a factor and baseline antibody level as a covariate if baseline antibody levels are dissimilar by study group or as a sensitivity analysis.

The percentage of subjects with seroconversion will be summarized and the 95% CI by the Clopper-Pearson method (Clopper et al., 1934) may be presented.

Seroconversion is defined as binary variable for subjects with non-missing values at pre-vaccination and post-vaccination as:

= 1, if there is a post-vaccination titer >=4xLLoQ for pre-vaccination titer <LLoQ

= 1, if there is at least 4-fold increase (post-vaccination) from pre-vaccination titer ≥LLoQ

= 0, otherwise

The LLoQ will be defined before SAP finalization/database lock for the analysis.

An alternative SCR is also defined if there is at least 4-fold increase (post vaccination) from pre-vaccination titer when titer <LLoQ are imputed as LLoQ/2.

10.4.3 Safety Analyses

Safety, reactogenicity, and safety laboratory assessments will be evaluated in the Safety analysis set (SAF). Participants will be summarized according to the study vaccine that was received. Descriptive statistics will be provided for each solicited AE and for each study group. Local and systemic AEs from Day 1 through Day 7 after vaccination will be presented by severity. Descriptive summary statistics will include counts and percentages of participants with the indicated endpoint. Descriptive statistics will also be provided for abnormal hematology and chemistry laboratory values following vaccination. Unsolicited AEs (including MAAEs, AESIs, SAEs, and AEs leading to discontinuation/withdrawal) will be categorized according to MedDRA terms. Descriptive summary statistics (counts, percentages) will be provided for any unsolicited AE event in each study group. Missing solicited AE diary and AEs data will not be imputed. The methods for the safety statistical analyses of AEs are described in Table 15.

Table 15 Safety Analyses

Statistical Analysis Methods
Reactogenicity (solicited local and systemic AEs): • Percentages of participants experiencing each solicited AE will be summarized for each symptom by any (severity), maximum severity (mild, moderate or severe, except fever) during 7 days after each vaccination by study group. For the definition of severity grades, refer to Table 13. • Duration and onset of events will be summarized by study group descriptively by mean, SD etc. • Injection-site erythema and swelling will be summarized according to categories based on linear measurements. • Use of antipyretics and analgesics will be summarized by frequency and percentage of participants reporting use. • Body temperature will be summarized by 0.5°C increments from 38.0°C up to ≥40°C. In addition, fever will be summarized according to "mild", "moderate" or "severe" categorization. **Unsolicited AEs, SAEs, MAAEs, and AESIs:** • Percentages of participants reporting at least one AE, related AE, SAE, MAAE, AESI within 28 days after each study vaccination will be summarized by study group. • Percentages of participants reporting at least one SAE, MAAE, AESI and AE leading to the study termination will be summarized by study group for the entire study period. • For each group and for each hematology, chemistry, and coagulation parameter, the following will be performed: – The percentage of subjects who have hematology, chemistry, and coagulation results below or above the laboratory normal ranges will be tabulated by time point. – The maximum grading from pre-vaccination up to 28 days post vaccination will be tabulated for each vaccination. Grades will be based on the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". • Percentages of participants reported any AEs within 60 minutes after study vaccination will be summarized by study group. AEs will be coded using the most recent version of MedDRA. AEs will be presented by SOC and PT by study group. When applicable the Clopper-Pearson method will be used to calculate the CIs. Missing data will not be imputed.

Abbreviations: AE, adverse event; AESI, adverse event of special interest; CI, confidence interval; MAAE, medically attended adverse event; MedDRA, Medical Dictionary for Regulatory Activities; PT, preferred term; SAE, serious adverse event; SAF, safety analysis set; SD, standard deviation; SOC, system organ class.

10.5 Interim Analyses and Analysis Timing

10.5.1 Interim Analysis

No Interim Analyses will be conducted in this study.

10.5.2 Final Analysis

Two descriptive statistical analyses may be performed stepwise as follows:

A primary analysis including all safety, reactogenicity, and immunogenicity data associated with primary endpoints will be conducted on cleaned data (for participants 18-59 years and

60-80 years combined or separately). The results will be reported on a study group level only. Individual listings may be generated without information on the participant's study group. Access to participant-level information about study groups will be restricted.

A final analysis of safety and immunogenicity data associated with primary, secondary, and available exploratory endpoints will be performed as soon as the data is cleaned and locked. The results of this analysis will be presented in a final clinical study report. Individual data listings with information on treatment allocation on the participant level will be generated and presented.

An additional analysis of safety and immunogenicity data collected may be performed in order to meet company's business decision.

Further details regarding the analysis will be provided in the SAP.

10.6 Central Cardiac Adjudication Committee

The study will include the use of an independent Central Cardiac Adjudication Committee.

A central cardiac adjudication committee may meet to assess any potential myocarditis/pericarditis cases (see Section 9.1.16). Further details are provided in the cardiac adjudication committee charter.

10.7 Data Safety Monitoring Board

An independent, external DSMB will be established to evaluate participant safety throughout the study at a cohort and participant level, as appropriate. The DSMB will perform i) planned reviews of all available safety and reactogenicity data and ii) reviews of all available safety and reactogenicity data if a pause rule is met and study vaccination is paused or if any safety concern is raised by the principal investigator (PI) or sponsor. The DSMB will make recommendations regarding study continuation, study design modification, and/or study termination. The responsibilities, roles, and procedures of the DSMB are described in the DSMB Charter.

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12 APPENDICES

APPENDIX 1 CONTRACEPTIVE GUIDANCE[11]

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are not considered as women of childbearing potential

1. Premenarchal
2. Premenopausal female with 1 of the following:
    a. Documented hysterectomy
    b. Documented bilateral salpingectomy
    c. Documented bilateral oophorectomy

Note: Documentation can come from the study center personnel's review of the participant's medical records, medical examination, or medical history interview.

3. Postmenopausal female:
    a. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
    b. Females on HRT and whose menopausal status is in doubt will be required to use 1 of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.

Female Participant Reproductive Inclusion Criteria

A female participant is eligible to participate if she is not pregnant or breastfeeding and at least 1 of the following conditions applies:

- Is not a WOCBP (see definitions above).

OR

- Is a WOCBP and uses a highly effective contraceptive method as described below during the intervention period (for a minimum of 60 days after the last dose of study intervention; or as required by local regulations). The Investigator should evaluate the effectiveness of the contraceptive method in relationship to the first dose of study intervention.

The Investigator is responsible for review of medical history, menstrual history, and recent sexual activity to decrease the risk for inclusion of a woman with an early undetected pregnancy.

Contraception Guidance

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception that can achieve a failure rate of less than 1% per year when used consistently and correctly.[11]

Such methods include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - Oral
  - Intravaginal
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - Oral
  - Injectable[1]
  - Implantable[2]
- Intrauterine device (IUD)[2]
- Intrauterine hormone-releasing system (IUS)[2]
- Bilateral tubal occlusion[2]
- Vasectomized partner[2,3]
- Sexual abstinence[4]

[1] Combination contraceptive injectables are allowed if licensed in the respective countries and have a failure rate <1% per year.

[2] Contraception methods that in the context of this guidance are considered to have low user dependency.

[3] Vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomized partner has received medical assessment of the surgical success.

[4] In the context of this guidance, sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the participant.

APPENDIX 2 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
  - Applicable ICH GCP Guidelines.
  - Applicable laws and regulations.
- The protocol, protocol amendments, ICF and, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC and regulatory authority approval, when applicable, before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to participants.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.
  - Providing oversight of the conduct of the study at the study center and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.
- After reading the protocol, each investigator will sign the protocol signature page and send a copy of the signed page to the sponsor or representative (Appendix 5). The study will not start at any study center at which the investigator has not signed the protocol.

Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Insurance

The sponsor will provide insurance in accordance with local guidelines and requirements as a minimum for the participants in this study. The terms of the insurance will be kept in the study files.

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.
- Participants or their legally authorized representative must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.
- The medical records must include a statement that written informed consent was obtained before the participant was entered in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants or their legally authorized representative must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

A participant who is rescreened is not required to sign another ICF if the rescreening occurs within 14 days from the previous ICF signature date.

Participant card

The investigator (or designee) must provide a "participant card" to each participant. In an emergency situation, this card serves to inform the responsible attending physician that the participant is in a clinical study and that relevant information may be obtained by contacting the investigator. The address and telephone number of the main contact for information about the clinical study must appear on the card. Participants must be instructed to keep participant cards in their possession at all times during the study duration.

Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by clinical quality assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Administrative Structure

The administrative structure for this study is presented in Table 16.

Table 16 Study Administrative Structure

Function	Responsible Organization
Study Operations Management	CRO
Medical Monitoring	CRO/study center
Site Monitoring	CRO
Study Master File	CRO
Randomization Code	CRO
Data Management	CRO, and third party
Clinical Supply Management	Sponsor, CRO, and third party
Quality Assurance Auditing	Sponsor, CRO
Biostatistics	Sponsor, CRO
Medical Writing	Sponsor, CRO
Laboratory Assessments	Third parties
Data Safety Monitoring Board (see Section 10.7)	Sponsor, CRO
Central Cardiac Adjudication Committee	Sponsor, CRO

Abbreviation: CRO, contract research organization

Dissemination of Clinical Study Data

The results of the study should be reported within 1 year from the end of the clinical study. Irrespective of the outcome, the sponsor will submit to the regulatory authority database a summary of the results of the clinical study within 1 year from the end of the clinical study. It shall be accompanied by a summary written in a manner that is understandable to laypersons.

Data Quality Assurance

- All participant data relating to the study will be recorded on eCRFs unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized study center personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local

regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Source Documents

The investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the study center's participants. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., via an audit trail).

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's study center.
- Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Study and Study Center Closure

The sponsor designee reserves the right to close the study center or terminate the study at any time for any reason at the sole discretion of the sponsor. Study centers will be closed upon study completion. A study center is considered closed when all required documents and study supplies have been collected and a study center closure visit has been performed. The investigator may initiate study center closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Reasons for the early closure of a study center by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of participants by the investigator.
- Discontinuation of further study vaccine development.

Publication Policy

The data generated by this study are confidential information of the sponsor. The sponsor will make the results of the study publicly available. The publication policy with respect to the investigator and study center will be set forth in the Clinical Trial Agreement.

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support

publication of multicenter studies only in their entirety and not as individual study center data. In this case, a coordinating investigator will be designated by mutual agreement.

- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

APPENDIX 3 ADVERSE EVENTS OF SPECIAL INTEREST

Table 17 List of Adverse Events of Special Interest

Myocarditis / pericarditis[2]
Anaphylaxis [1,2]
Thrombocytopenia [1,2]
Generalized convulsion [1,2]
Acute disseminated encephalomyelitis [1]
Acute peripheral facial paralysis (Bell's Palsy)[1]
Guillain Barre Syndrome [1]

Abbreviations: AESI, adverse event of special interest;
[1] Proven association with immunization encompassing several different vaccines, including influenza vaccines
[2] Proven association with a vaccine that could theoretically be true for mRNA vaccines as signal was recognized and validated during COVID-19 mass campaigns.

APPENDIX 4 SAFETY LABORATORY ABNORMALITIES VALUES

The safety laboratory abnormalities values presented in Table 18 serve as guidelines and are dependent upon institutional normal parameters. Normal reference ranges in SI units (CBER Table for Laboratory Abnormalities in SI Units, CBER 2007) are provided in a Study-Specific Laboratory Manual.

Table 18 Values for Laboratory Abnormalities

Serum Chemistry	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)*
Sodium – hyponatremia (mEq/L)	132 – 134	130 – 131	125 – 129	<125
Sodium – hypernatremia (mEq/L)	144 – 145	146 – 147	148 – 150	>150
Potassium – hyperkalemia (mEq/L)	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	>5.6
Potassium – hypokalemia (mEq/L)	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	<3.1
Glucose – hypoglycemia (mg/dL)	65 – 69	55 – 64	45 – 54	<45
Glucose – hyperglycemia Fasting (mg/dL) Random (mg/dL)	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood urea nitrogen (mg/dL)	23 – 26	27 – 31	>31	Requires dialysis
Creatinine (mg/dL)	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	>2.5 or requires dialysis
Calcium – hypocalcemia (mg/dL)	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	<7.0
Calcium – hypercalcemia (mg/dL)	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	>12.0
Magnesium – hypomagnesemia (mg/dL)	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	<0.9
Phosphorous – hypophosphatemia (mg/dL)	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	<1.6
Albumin – hypoalbuminemia (g/dL)	2.8 – 3.1	2.5 – 2.7	<2.5	–
Total Protein – hypoproteinemia (g/dL)	5.5 – 6.0	5.0 – 5.4	<5.0	–
Alkaline phosphate; increase by factor	1.1 – 2.0 × ULN	2.1 – 3.0 × ULN	3.1 – 10 × ULN	>10 × ULN
Liver function tests –ALT and AST; increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 × ULN	5.1 – 10 × ULN	>10 × ULN
Bilirubin – when accompanied by any increase in liver function test; increase by factor	1.1 – 1.25 × ULN	1.26 – 1.5 × ULN	1.51 – 1.75 × ULN	>1.75 × ULN
Bilirubin – when liver function test is normal; increase by factor	1.1 – 1.5 × ULN	1.6 – 2.0 × ULN	2.0 – 3.0 × ULN	>3.0 × ULN
Cholesterol	201 – 210	211 – 225	>226	–
Pancreatic enzymes – amylase and lipase	1.1 – 1.5 × ULN	1.6 – 2.0 × ULN	2.1 – 5.0 × ULN	>5.0 × ULN

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of the normal range.

Table 18 Values for Laboratory Abnormalities

* The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as potentially life threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125 – 129 mEq/L) should be recorded as a Grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

Source: Food and Drug Administration (FDA) Guidance for industry – Toxicity grading scale for heathy adult and adolescent volunteers enrolled in preventative vaccine clinical trials; Tables for laboratory abnormalities (CBER 2007).

APPENDIX 5 INVESTIGATOR SIGNATURE

PROTOCOL TITLE: A Phase 1, First-in-human, Randomized, Observer-blind, Parallel design, Controlled, Dose Level and Schedule-finding Study to Evaluate the Safety, Reactogenicity, and Immunogenicity of a Self-Amplifying mRNA Pandemic Influenza Vaccine (ARCT-2304) When Administered to Healthy Adults.

PROTOCOL NO: ARCT-2304-01

VERSION: Protocol Version 1.0 (29 August 2024)

This protocol is a confidential communication of the sponsor. I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and the applicable laws and regulations. Acceptance of this document constitutes my agreement that no unpublished information contained herein will be published or disclosed without prior written approval from the sponsor.

Instructions to the Investigator: Please SIGN and DATE this signature page. PRINT your name, title, and the name of the study center in which the study will be conducted. Return the signed copy to the CRO.	
I have read this protocol in its entirety and agree to conduct the study accordingly:	
Signature of the Investigator:	
Date:	
Printed Name:	
Investigator Title:	
Name/Address of Study Center:	