NCT04024228

Immunogenicity and Safety of a High-Dose Quadrivalent Influenza Vaccine Administered by the Intramuscular Route in Subjects 60 Years of Age and Older

Phase III, randomized, modified double-blind, active-controlled, multi-center study evaluating the immunogenicity and safety of high-dose quadrivalent influenza vaccine (QIV HD) in healthy subjects 60 years of age and older in Europe

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	QHD00011
Development Phase:	Phase III
Sponsor:	Sanofi Pasteur 14 Espace Henry Vallée, 69007 Lyon, France
Investigational Product(s):	High-Dose Influenza Vaccine (split virion, inactivated), Quadrivalent (QIV-HD)
Form / Route:	Liquid / Intramuscular
Indication For This Study:	Single dose for individuals 60 years of age and older
Version and Date of the SAP core body part:	Version 2.0 dated 18 March 2020

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List of Abbreviations

AE	adverse event
AESI	adverse event of special interest
ANCOVA	analysis of covariance
AR	adverse reaction
BL	blood sample
CI	confidence interval
CRB	case report book
CSR	clinical study report
D	day
Df	degree of freedom
dil	dilution
eCRF	electronic case report form
EDC	electronic data capture
EIA	enzyme immunosorbent assay
ELLA	enzyme-linked lectin assay
FAS	full analysis set
FDA	Food and Drug Administration
GBS	Guillain-Barré syndrome
GM	geometric mean
GMT	geometric mean titer
GMTR	geometric mean titer ratio
HA	hemagglutinin
HAI	hemagglutination inhibition
ICF	informed consent form
IM	intramuscular
IRB	institutional review board
IRT	interactive response technology
LLOQ	lower limit of quantification
MCAR	missing completely at random
MD	missing data
MedDRA	Medical Dictionary for Regulatory Activities
µg	microgram
mL	milliliters

NA	neuraminidase
NM	non-measurable
PPAS	per-protocol analysis set
PT	preferred term
QIV-HD	high-dose quadrivalent influenza vaccine
RCDC	reverse cumulative distribution curve
SAE	serious adverse event
SafAS	safety analysis set
SAP	statistical analysis plan
SMQ	standardized MedDRA query
SN	seroneutralization
SOC	system organ class (primary)
TIV-HD	high-dose trivalent influenza vaccine
TLF	table(s), listing(s), and figure(s)
ULOQ	upper limit of quantification
US	United States
V	visit
WHO	World Health Organization

1 Introduction

Influenza is a highly contagious, acute viral respiratory disease caused by influenza A subtype and type B viruses and is typically characterized by the rapid onset of fever, myalgia, sore throat, and nonproductive cough. Influenza can also cause severe malaise lasting for several days. Influenza virus types A and B belong to the genus Orthomyxoviridae and are characterized as enveloped, negative strand, segmented ribonucleic acid (RNA) viruses. The viral envelope contains 2 virus coded glycoproteins, hemagglutinin (HA) and neuraminidase (NA), which form spikes on the viral surface and are key antigens in the host response in both natural infection and vaccination.

Traditional trivalent and quadrivalent inactivated influenza vaccines (TIV and QIV) administered by the intramuscular (IM) route contain a standard dose (SD) of 15 µg HA of each of the virus strains (one A/H1N1 strain, one A/H3N2 strain and one B strain for TIV and 2 B strains [B Yamagata lineage and B Victoria lineage for QIV]) with a total of 45 µg and 60 µg of HA antigen per dose, respectively.

However, the effectiveness of the influenza vaccine in preventing or attenuating illness depends in part on the age, underlying conditions, and immune competence of the vaccine recipient and on the similarity between the virus strains present in the vaccine and the strains circulating in the community.

While federal health agencies in European countries recommend that elderly persons receive influenza vaccination, the results of a European seasonal influenza vaccination survey, reported by the European Centre for Disease Prevention and Control, reveal that the recommended ages for influenza vaccination of the elderly vary from country to country. During the 2014-2015 influenza season, all 30 participating countries recommended seasonal influenza vaccination for people in the "older" age groups, but the actual age specified as "older" differed between countries. The recommended age of "older" adults who should be vaccinated against influenza was ≥ 65 years of age in 18 countries. A cutoff of ≥ 60 years was reported by 6 countries (Germany, Greece, Hungary, Iceland, the Netherlands, and Portugal). Slovakia recommended vaccination of persons 59 years of age and older. Malta and Poland recommended vaccination of persons 55 years of age and older. Three countries (Austria, Belgium, and Ireland) recommended vaccination of those who are 50 years of age and older.

Clinical data generated with TIV-HD or QIV-HD support an indication in persons starting at 65 years of age and older. To extend the age indication downwards to 60 to 64 years of age in Europe, the QHD00011 study is being proposed to collect and analyze immunogenicity and safety data in the 60 to 64 years of age population. In addition, as no data comparing the QIV-HD to the QIV-SD standard of care licensed in Europe have been generated to date, a group of subjects 65 years of age and older is also proposed to be evaluated in QHD00011.

QHD00011 will be a Phase III, randomized, modified double-blind , active-controlled, multi-center trial to assess the immunogenicity and safety of the QIV-HD in approximately 1540 healthy subjects 60 years of age and older in Europe. The goal of this study is to show that vaccination with QIV-HD induces an immune response (as assessed by hemagglutination inhibition [HAI] GMTs) that is superior to responses induced by the QIV-SD for the 4 virus

strains at 28 days post-vaccination in subjects 60 to 64 years of age and in subjects 65 years of age and older.

2 Trial Objectives

2.1 Primary Objective

Immunogenicity

To demonstrate that QIV-HD induces an immune response that is superior to the responses induced by QIV-SD for all 4 virus strains 28 days post-vaccination in subjects 60 to 64 years of age and in subjects 65 years of age and older.

2.2 Secondary Objectives

Immunogenicity

To further describe the immune response induced by QIV-HD and QIV-SD in all subjects by age group, in pooled age groups, and by vaccine group (QIV-HD; QIV-SD).

Safety

To describe the safety profile of all subjects by age group, in pooled age groups, and by vaccine group (QIV-HD; QIV-SD).

2.3 Observational Objectives

Immunogenicity

- To describe the immune response 28 days after vaccination by virus seroneutralization (SN) measurement method for at least 50% of subjects in each age group (60 to 64 years of age; 65 years of age and older) and in pooled age group by vaccine group (QIV-HD; QIV-SD).
- To describe the anti-NA immune response in at least 50 subjects in each age group and in pooled age group by vaccine group (QIV-HD; QIV-SD).

Blood samples from at least 50% of subjects in each age group and each vaccine group will be analyzed for SN. And among these subjects, blood samples from at least 50 subjects in each age group and each vaccine group will be analyzed for NA.

3 Description of the Overall Trial Design and Plan

3.1 Trial Design

QHD00011 will be a Phase III, randomized, modified double-blind, active-controlled, multi-center study to assess the immunogenicity and safety of the QIV-HD in approximately 1540

healthy subjects 60 years of age and older in Europe. The goal of this study is to show that vaccination with one dose of QIV-HD induces an immune response (as assessed by HAI GMTs) that is superior to responses induced by one dose of QIV-SD for the 4 virus strains at 28 days post-vaccination in subjects 60 to 64 years of age and in subjects 65 years of age and older.

In each age group, eligible subjects will be randomized in a 1:1 ratio to receive a single IM injection of either QIV-HD or QIV-SD at Day (D)0 as follows:

1) QIV-HD; stratified by age (60 to 64 years of age; 65 years of age and older)
2) QIV-SD; stratified by age (60 to 64 years of age; 65 years of age and older)

Table 3.1: Randomization of subjects by vaccine group (QIV-HD; QIV-SD)

	QIV-HD		QIV-SD	
	60 to 64 years of age	**≥65 years of age**	**60 to 64 years of age**	**≥65 years of age**
Subjects to be Randomized per Age Group	385	385	385	385
Subjects to be Randomized per Vaccine Group	770		770	

All subjects will provide a pre-vaccination (baseline) blood sample at D0 and a post-vaccination blood sample at Visit (V) 02 (D28 [+7 days]) for HAI testing. A randomized subset of these subjects (observational subset) will be used for SN and NA testing.

Solicited injection site and systemic reactions will be collected up to 7 days after vaccination, and unsolicited adverse events (AEs) will be collected up to V02, which is the active phase of the trial (V01 to V02 [D0-D28]). Serious adverse events (SAEs) and adverse events of special interest (AESIs[1]) will be collected throughout the trial (D0 through approximately D180 [6-month follow-up period]).

Interactive response technology (IRT) will be used to randomly assign subjects to one of the 2 study groups and to assign subject numbers in each of the groups. In addition, IRT will be used to randomly assign subjects to the observational subsets. Electronic data capture (EDC) will be used for the collection of data.

3.2 Trial Plan

The study plan is summarized in the Table of Study Procedures in the Study Protocol.

[1] **Note:** AESIs will be captured as SAEs. These include new onset of GBS, encephalitis / myelitis (including transverse myelitis), Bell's palsy, optic neuritis, and brachial neuritis.

Vaccination

All eligible subjects will be randomized to receive a single injection of either the QIV-HD vaccine or QIV-SD at V01 (D0).

Blood Sampling

All subjects will provide a pre-vaccination blood sample at V01 (D0) and a post-vaccination blood sample at V02 (D28 [+ 7 days]).

Collection of Safety Data

Subjects will be asked to notify the site immediately about any potential SAEs at any time during the study.

All subjects will be observed for 30 minutes after vaccination, and any unsolicited systemic AEs occurring during that time will be recorded as immediate unsolicited systemic AEs in the Case Report Book (CRB).

Subjects will record information about solicited reactions (D0-D7), unsolicited AEs (D0-V02), SAEs (D0-V02), and AESIs (D0-V02) in a diary card (DC).

Staff will review the D0 to V02 safety data with subjects at V02.

Subjects will continue to collect information on SAEs and AESIs in a memory aid (from V02-D180). Staff will contact subjects by telephone at D180 (+14 days) post-vaccination to review the memory aid and to identify the occurrence of any SAEs and AESIs that had not yet been reported.

Table 3.2: Study procedures

Phase III Study, 2 Visits, 1 Telephone Call, 1 Vaccination, 2 Blood Samples, Approximately 180 Days Duration Per Subject

Visit (V)/Contact	**V01**	**V02**	**D180 Safety Follow-up Call**
Study timelines (day [D])	D0	D28	D180
Time windows (days)	Not applicable	[+7 days]	[+14 days]
Informed consent	X		
Inclusion/exclusion criteria	X		
Collection of demographic data*	X		
Urine pregnancy test (if applicable)	X		
Medical history	X		
History of seasonal influenza vaccination/influenza infection (diagnosis laboratory confirmed) in the previous 3 years	X		
Collection of reportable concomitant medications	X	X	
Physical examination†	X	X	
Contacting interactive response technology (IRT)	X		
Randomization/allocation of subject number and unique treatment number using IRT	X		
Blood sampling (BL), 10 mL‡	BL0001§	BL0002	
Vaccination	X		
Immediate surveillance (30 minutes)	X		
Diary card (DC) provided**	X		
Collection of solicited injection site and systemic reactions	D0-D7		
Collection of unsolicited adverse events (AEs)	D0-V2		
DC reviewed and collected		X††	
Memory aid provided‡‡		X	
Study termination record for the active phase of the trial		X	
Follow-up telephone call			X§§
Memory aid reviewed			X
Study termination for the 6-month safety follow-up			X
Collection of serious adverse events (SAEs) and adverse events of special interest (AESIs)***	To be reported at any time during the study		

Abbreviations: AE, adverse event; AESI, adverse event of special interest; BL, blood sampling; D, day; DC, diary card; FDA, Food and Drug Administration; GBS, Guillain-Barré Syndrome; ICF, informed consent form; SAE, serious adverse event; US, United States; V, visit.

* To comply with US FDA expectations, Sponsors are to enroll participants who reflect the demographics for clinically relevant populations with regard to age, gender, race, and ethnicity as described in https://www.fda.gov/downloads/regulatoryinformation/guidances/ucm126396.pdf. Please note that ethnicity will not be collected during this study.

† Targeted physical examination based on medical history will be performed at V01. Targeted physical examination may also be performed at V02, as necessary.

‡ If the D0 blood sample cannot be obtained, the subject should be given the opportunity to return to the study site for another attempt, as long as the study is still enrolling subjects and as long as the subject continues to remain eligible for the trial and that includes reviewing Inclusion/Exclusion criteria, medical history, and ICF process. All attempts should be made to obtain a blood sample; however, if the attempts are unsuccessful, the subject should not be vaccinated and should be discontinued from the study.

§ Collection of the first blood sample (BL0001) is to occur before vaccination.

** Subjects will use this diary card to record information about solicited reactions from D0 to D7, as well as unsolicited AEs, SAEs, and AESIs from D0 to V02 after vaccination.

†† Staff will collect the diary card at V02, and review any solicited reactions ongoing at V02, unsolicited AEs, concomitant medications, SAEs, and AESIs.

‡‡ Subjects will use this memory aid to collect information on SAEs and AESIs from V02 to the D180 Safety Follow-up Call.

§§ During the D180 telephone call, staff will review the memory aid to identify the occurrence of any SAEs and AESIs that had not yet been reported.

*** AESIs will have the same detailed information collected as SAEs. These include new onset of Guillain-Barré Syndrome (GBS), encephalitis/myelitis (including transverse myelitis), Bell's Palsy, optic neuritis, and brachial neuritis.

4 Endpoints and Assessment Methods

4.1 Primary Endpoints and Assessment Methods

See Section 9.1 of the protocol.

4.2 Secondary Endpoints and Assessment Methods

See Section 9.2 of the protocol.

4.3 Observational Endpoints and Assessment Methods

See Section 9.3 of the protocol.

4.4 Derived Endpoints: Calculation Methods

4.4.1 Safety

4.4.1.1 Solicited Reactions

4.4.1.1.1 Daily Intensity

All daily records for solicited reactions will be derived into daily intensity according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing.

For measurable injection site reactions (Erythema/Swelling/Induration/Bruising):

- Grade 1: ≥ 25 to ≤ 50 mm
- Grade 2: ≥ 51 to ≤ 100 mm
- Grade 3: > 100 mm

For measurable systemic reactions (Fever):

- Grade 1: ≥ 38.0°C to ≤ 38.4°C, or ≥ 100.4°F to ≤ 101.1°F
- Grade 2: ≥ 38.5°C to ≤ 38.9°C, or ≥ 101.2°F to ≤ 102.0°F
- Grade 3: ≥ 39.0°C or ≥ 102.1°F

For the derivation of daily intensities the following sequential steps will be applied:

Solicited reactions (except Fever/Pyrexia) with an investigator occurrence recorded as “No” and with all daily records missing then all daily intensities will be derived as “None”.

For non-measurable solicited reactions, daily intensities will correspond to daily records reported in the clinical database. For measurable solicited reactions the daily measurements reported in the clinical database will be converted based upon the intensity scales defined in the protocol; this assumes a reaction that is too large to measure (non-measurable, "NM") is Grade 3. Note the intensity could be considered "None" (not a reaction) in the analysis despite being considered a reaction by the investigator (eg, swelling measurement > 0 mm but < 25 mm in adults).

Note: The maximum intensity on the ongoing period is derived from the record of the maximum intensity/measurement after the end of the solicited period following the rule described above.

4.4.1.1.2 Maximum Intensity

Maximum intensity is derived from the daily intensities computed as described in Section 4.4.1.1.1 and is calculated as the maximum of the daily intensities over the period considered.

The Grade of intensity is applied following the rules described in the Section 9.2.1.3.2 of the protocol.

4.4.1.1.3 Occurrence

Occurrence is derived from the maximum intensity on the period considered:

- None: No occurrence
- Grade 1, Grade 2, or Grade 3: Occurrence
- Missing: Missing occurrence

Subjects with at least one non-missing occurrence for a specific endpoint will be included in the analysis. Conversely, those without a non-missing occurrence will not be included in the analysis of the endpoint.

4.4.1.1.4 Time of Onset

Time of onset is derived from the daily intensities computed as described in Section 4.4.1.1.1. It corresponds to the first day with intensity of Grade 1, Grade 2, or Grade 3.

Note: If a reaction is not continuous (ie, reaction occurs over 2 separate periods of time intervened by at least one daily intensity Missing or None) then the time of onset is the first day of the first occurrence.

Table 4.1: Categories for time of onset

Injection Site Reactions (D0-D7)	Systemic Reactions (D0-D7)
D0-D3	D0-D3
D4-D7	D4-D7

4.4.1.1.5 Number of Days of Occurrence

Number of days of occurrence over the period considered is derived from the daily intensities computed as described in Section 4.4.1.1.1. It corresponds to the number of days with daily intensities of Grade 1, Grade 2, or Grade 3. Number of days of occurrence on the solicited period with a specified intensity may also be derived.

Table 4.2: Categories for number of days of occurrence during the solicited period

Injection Site Reactions (D0-D7)	Systemic Reactions (D0-D7)
1-3 days	1-3 days
4-7 days	4-7 days
8 days	8 days

4.4.1.1.6 Overall Number of Days of Occurrence

If a reaction is ongoing at the end of the solicited period, then the overall number of days of occurrence is derived from the daily intensities and the stop date of the reaction after the end of the solicited period. The overall number of days of occurrence is:

- (stop date - vaccination date) + (number of days of occurrence within the solicited period) − length of the solicited period + 1.

If the stop date is missing or incomplete (containing missing data [MD]), the overall number of days of occurrence will be considered as Missing.

Table 4.3: Categories for overall number of days of occurrence

Injection Site Reactions	Systemic Reactions
1-3 days	1-3 days
4-7 days	4-7 days
≥ 8 days	≥ 8 days
Missing	Missing

4.4.1.1.7 Ongoing

Ongoing is derived from the last daily intensity of the solicited period computed as described in Section 4.4.1.1.1 and the maximum intensity on the ongoing period. The investigator's ongoing flag is not used because the measurement would determine the ongoing status of the reaction.

Note: a reaction could be derived as not ongoing for the analysis despite being considered as ongoing by the investigator (eg, when the maximum measurement after D7 is > 0 mm but < 25 mm). If the last daily intensity of the solicited period is at least Grade 1 and maximum intensity on the ongoing period is also at least Grade 1, then the reaction is considered ongoing. In any other cases the reaction will not be considered as ongoing.

4.4.1.2 Unsolicited AEs

4.4.1.2.1 Occurrence

An observation will be considered an event if it has at least a verbatim term and is not a Grade 0 intensity event. Grade 0 events should be included in the listing "Unsolicited (non-serious) adverse events not included in the safety analysis."

4.4.1.2.2 Intensity

Intensity for unsolicited AEs will be derived according to the following classification:
None, Grade 1, Grade 2, Grade 3, or Missing.

If the unsolicited AE is measurable and its preferred term is part of the list of solicited reactions, then the measurement is derived based upon and following the same rule than the intensity scales defined in the protocol for that measurable injection site or systemic reaction.

Note the intensity could be considered "None" (not a reaction) in the analysis despite being considered a reaction by the investigator (eg, swelling measurement > 0 mm but < 25 mm in adults). Intensity for the other unsolicited non-serious AEs will correspond to the value reported in the electronic case report form (eCRF).

The maximum intensity corresponds to the highest intensity for a unique term.

4.4.1.2.3 Time of Onset

Time of onset is derived from the start date of the unsolicited AE provided in the clinical database and the date of last vaccination:

- Start date of the unsolicited AE – date of vaccination.

The time of onset should be considered as missing only if one or both of the dates are missing or partially missing.

The unsolicited non-serious AEs will be analyzed "Within 28 days", which corresponds to AEs with a time of onset between 0 and 28 days after vaccination or missing. An AE with missing time of onset will be considered to have occurred just after the vaccination indicated by the visit number, so will be included in these tables.

Note: Unsolicited AEs that occurred before vaccination (negative time of onset) or with a time of onset higher than defined above (> 28 days) will not be included in analysis, but will be listed separately.

Time of onset will be displayed as follows:

- D0-D3
- D4-D7
- D8-D14
- ≥ D15
- Missing

SAEs will be analyzed throughout the study using the following periods:

- During post-Dose period (ie, within 28 days after the injection)
- During the 6-month follow-up period (ie, from 29 days after the injection until the last subject contact)
- During the study (ie, all SAEs that occurred during the study)

An SAE with missing time of onset will be considered to have occurred just after the vaccination indicated by the visit number, so will be included in these tables.

4.4.1.2.4 Duration

Duration is derived from the start and stop dates of the unsolicited AE provided in the clinical database:

- Stop date of unsolicited AE - start date of unsolicited AE + 1.

The duration should be considered as missing only if one or both of the start and stop dates of the unsolicited AE is missing or partially missing.

Duration will be displayed by period as following:

- 1-3 days
- 4-7 days
- 8-14 days
- ≥ 15 days
- Missing

4.4.1.2.5 Seriousness (SAE)

No derivation or imputation was done. This information was at least listed and eventually could be summarized as collected.

4.4.1.2.6 Outcome (SAE)

No derivation or imputation was done. This information was at least listed and eventually could be summarized as collected.

4.4.1.3 Other Safety Endpoints

4.4.1.3.1 Action Taken

This information will be summarized as collected, including missing observations. No derivation or imputation will be done.

4.4.1.3.2 Causality

This information will be summarized as collected. An adverse reaction (AR) is defined as an unsolicited non-serious AE or an SAE with causality to the vaccine. Missing causality (relationship) will be handled as described in Section 5.3.1.2.

4.4.1.3.3 AEs Leading to Study Discontinuation

A flag is available in the clinical database for all AEs in order to identify AEs leading to discontinuation.

In general, the items that are counted are:

- Disposition table: A subject who has, on the termination form, the reason for early termination "adverse event" is checked.
- Safety overview table: A subject who has either on the termination form, the reason for early termination "adverse event" is checked or lists an AE on an AE page (solicited, unsolicited, or SAE) that has "Reaction Leading to Termination" or "Event Leading to Termination" or "Serious Adverse Event Leading to Termination" checked that is at least Grade 1 and is within the time period indicated. Note: If the Grade is below 1, the AE will be excluded from the list of AEs leading to study discontinuation.
- System organ class (SOC)/Preferred term (PT) table: An event (solicited, unsolicited, or SAE) that has "Cause Study Termination" or "Caused Study Discontinuation" checked that is at least Grade 1 and is within the time period indicated.

4.4.1.3.4 AEs of Special Interest (AESIs)

AESIs will be collected throughout the study (from the inclusion until the D180 safety follow-up contact after vaccination). AESIs are to be reported as SAEs. Each AESI will be retrieved using the following (as shown in Table 4.4):

Table 4.4: AESI PTs

AESI	PT
Guillain-Barré Syndrome (GBS)	Chronic inflammatory demyelinating polyradiculoneuropathy Demyelinating polyneuropathy Guillain-Barré syndrome Miller Fisher syndrome
Bell's Palsy	Facial paralysis Facial paresis
Encephalitis/Myelitis (including Transverse Myelitis)	Myelitis Myelitis transverse Encephalitis is using the narrow Standardized MedDRA Query (SMQ) term "Noninfectious encephalitis" (Medical Dictionary for Regulatory Activities [MedDRA 22.1])
Optic Neuritis	Optic neuritis Optic neuropathy
Brachial Neuritis	Radiculitis brachial Brachial plexopathy Neuralgic amyotrophy

4.4.1.3.5 Subjects with at-risk condition

At-risk subjects with stable chronic illness are eligible for inclusion unless they could interfere with trial conduct or completion. Subjects with specific chronic diseases such as chronic respiratory, heart, renal, metabolic or hematological disorders will be identified by medical review through reported Medical History.

4.4.2 Immunogenicity

4.4.2.1 Computed Values for Analysis

In order to appropriately manage extreme values (< lower limit of quantification [LLOQ] and ≥ upper limit of quantification [ULOQ]) for analysis purposes, the following computational rule is applied to the values provided in the clinical database for each BL drawn:

- If a value is < LLOQ, then use the computed value LLOQ/2
- If a value is ≥ LLOQ and < ULOQ (or ≤ ULOQ), then use the value
- If a value is ≥ ULOQ (or > ULOQ), then use the computed value ULOQ

Duplicate records (per subject, antigen, and method) were recorded for HAI titration. A geometric mean of duplicate was applied in order to obtain a unique value for the statistical analysis. Unique records were recorded for SN and enzyme-linked lectin assay (ELLA) titration.

4.4.2.2 Fold-rise

The derived endpoint fold-rise is driven by both baseline and post-baseline computed values which are computed as described in Section 4.4.2.1. The computed value for fold-rise is:

- Computed value = Post-vaccination computed value / Baseline computed value.

For HAI assay, if the computed value is ≥ 4-fold rise, then the derived 4-fold rise indicator will be "Yes" for that test, otherwise the corresponding indicators will be "No".

For SN and ELLA assays, if the computed value is ≥ 2-fold rise or 4-fold rise, then the derived corresponding 2-fold rise or 4-fold rise indicator will be "Yes" for that test, otherwise the corresponding indicators will be "No".

Note: If baseline or post-baseline is missing, then the fold-rise is missing.

4.4.2.3 Seroconversion

Seroconversion is defined for HAI assay as either

- A computed value < 10 [1/dil] at D0 and post-injection computed value ≥ 40 [1/dil] at D28,

or

- A computed value ≥ 10 [1/dil] at D0 and a ≥ 4-fold rise in computed titer values [1/dil] at D28 as described in Section 4.4.2.1.

4.4.3 Efficacy

Not applicable.

4.4.4 Derived Other Variables

4.4.4.1 Age for Demographics

The age of a subject in the study was the calendar age in years at the time of inclusion. The age calendar was the age computed automatically in the eCRF, and presented as an integer.

4.4.4.2 Duration of the Study

The duration of the study is computed in days as follows:

- Maximum (Visit dates, Termination date, safety follow-up date) – minimum (V01 date) + 1

The duration of the active phase of the study is computed in days as follows:

- Maximum (latest date of V02, latest date of termination during the active phase) − minimum (V01 date) + 1,

The duration of the D180 safety follow-up phase of the study is computed in days as follows:

- Maximum (date of D180 safety follow-up) − minimum (V02 dates, termination dates during the active phase) + 1.

4.4.4.3 Subject Duration

The duration of a subject participation in the trial, including the D180 safety follow-up, is computed as follows:

- Maximum (visit dates, termination date, safety follow-up date) – V01 date + 1.

The duration of a subject in the active phase of the trial is computed as follows:

- Maximum (V02 dates, termination date) – V01 date + 1.

4.4.4.4 Time Interval

The time interval between 2 visits/vaccinations/blood samples is computed as follows:

Later date – earlier date.

4.4.4.5 Influenza Vaccination during the 3 Past Influenza Seasons (2016-2017, 2017-2018, and since 01SEP2018)

This information will be used as collected. No derivation or imputation will be done.

5 Statistical Methods and Determination of Sample Size

The statistical analyses will be performed under the responsibility of the Sponsor's Biostatistics platform using SAS® Version 9.4 software or later.

The results of the statistical analysis will be available in the final clinical study report (CSR).

For descriptive purposes, the following statistics will be presented:

Table 5.1: Descriptive statistics produced

Baseline characteristics and follow-up description	**Categorical data**	Number of subjects. Percentage of subjects.
	Continuous data	Mean, standard deviation, quartiles, minimum, and maximum.
Clinical safety results	**Categorical data**	Solicited: Number and percentage (95% confidence intervals [CIs]) of subjects. Unsolicited: Number and percentage (95% CIs) of subjects, and number of events.
Immunogenicity results	**Categorical data (seroconversion, cutoff)**	Number and percentage (95% CIs) of subjects.
	Continuous data **(titer / data)**	Log10: Mean and standard deviation. Anti-Log10 (work on Log10 distribution, and anti-Log10 applied): Geometric mean, 95% CI of the geometric mean, quartiles, minimum, and maximum. Graphical representation by Reverse Cumulative Distribution Curve (RCDC).

The CI for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (1), ie, using the inverse of the beta integral with SAS®).

For immunogenicity results, assuming that Log10 transformation of the titers / data follows a normal distribution, at first, the mean and the 95% CI will be calculated on Log10 (titers / data) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom), then antilog transformations will be applied to the results of calculations, in order to provide GMs and their 95% CI.

5.1 Statistical Methods

For the purposes of the statistical methods section, the 4 virus strains in the QIV-HD trial groups and the QIV-SD trial groups will be labeled as follows:

- A/(H1N1)-like strain A/H1N1
- A/(H3N2)-like strain A/H3N2
- B/(Victoria Lineage)-like strain B1
- B/(Yamagata Lineage)-like strain B2

5.1.1 Hypotheses and Statistical Methods for Primary Objective

5.1.1.1 Superiority of QIV-HD to QIV-SD

5.1.1.1.1 Hypotheses

A superiority approach will be used to compare post-vaccination GMTs between QIV-HD and QIV-SD groups for each strain and in each age group using a 1-sided test with Type I error rate of 0.025 following the individual hypotheses:

$$H_0^s : \frac{GMT^s_{QIV-HD}}{GMT^s_{QIV-SD}} \leq 1 \Leftrightarrow log_{10}(GMT^s_{QIV-HD}) - log_{10}(GMT^s_{QIV-SD}) \leq 0$$

$$H_A^s : \frac{GMT^s_{QIV-HD}}{GMT^s_{QIV-SD}} > 1 \Leftrightarrow log_{10}(GMT^s_{QIV-HD}) - log_{10}(GMT^s_{QIV-SD}) > 0$$

where

s: strain

If superiority is demonstrated for post-vaccination GMTs for the 4 strains within a same age group, then the immunogenicity of QIV-HD will be considered as superior to QIV-SD in the corresponding age group.

5.1.1.1.2 Statistical Methods

The statistical methodology will be based on the use of the lower bound of the 2-sided 95% CIs of the ratio of post-vaccination GMTs between the QIV-HD and QIV-SD groups. The CIs will be calculated by normal approximation of log-transformed titers for GMTs.

The superiority objective will be achieved if it is demonstrated for the 4 strains in each age groups. Analyses will be performed for both the Full Analysis Set (FAS) and the Per-protocol Analysis Set (PPAS), but the conclusion will be made from PPAS results.

A sensitivity analysis will be performed with adjustment on the pre-vaccination HAI titers. Superiority objective will be analyzed on the PPAS and FAS through an analysis of covariance (ANCOVA) model, using the HAI titers at D0 as a covariate for adjustment, in order to account for the variability linked to the baseline HAI titer and to provide a ”change from baseline” analysis.

5.1.2 Hypotheses and Statistical Methods for Secondary Objectives

5.1.2.1 Immunogenicity

5.1.2.1.1 Hypotheses

No hypotheses will be tested.

5.1.2.1.2 Statistical Methods

For descriptive purposes, the statistics presented in Table 5.1 will be produced. Immunogenicity endpoints will be summarized by age group, in pooled age groups, and by vaccine group with 95% CIs. Assuming that $\log_{10}$ transformation of the data follows a normal distribution, the $\log_{10}$ (data) will be used for the statistical analysis, then antilog transformations will be applied to the results of calculations, in order to provide the results in terms of GMs. The following descriptive statistics will be displayed:

- GMTs for each strain at D0 and D28
- The geometric mean titer ratios (GMTRs): geometric mean of the post-vaccination/pre-vaccination ratio for each strain
- Seroconversion rates at D28 compared to the baseline titers at D0
- Proportion of subjects with titers ≥ 1:40 (1/dilution [dil]) for each strain at D0 and D28
- Reverse cumulative Distribution of titers for each strain at D0 and D28 (eg, RCDCs of the titers)

In addition, immunogenicity data will be displayed using GMTs and seroconversion rates by subgroups of age (< 65 years, 65 to 74 years, 75 to 84 years, ≥75 years, and ≥85 years), sex, race, previous influenza vaccination status (received a seasonal influenza vaccine in the last past influenza season or not), at-risk condition, and baseline seropositivity status (seropositive and seronegative are defined as baseline antibody titer ≥ 1:10 or < 1:10).

5.1.2.2 Safety

5.1.2.2.1 Hypotheses

No hypotheses will be tested.

5.1.2.2.2 Statistical Methods

Safety endpoints will be summarized by age group, in pooled age groups, and by vaccine group. Solicited reactions (solicited injection site and systemic reactions), unsolicited AEs, SAEs, and AESIs will be summarized. The main parameters will be described with 95% CIs (Clopper-Pearson method) (1)

5.1.3 Hypotheses and Statistical Methods for Observational Objectives

5.1.3.1 Immunogenicity by SN Method

5.1.3.1.1 Hypotheses

No hypotheses will be tested.

5.1.3.1.2 Statistical Methods

Neutralizing Ab titers will be measured for each influenza strain with the SN method.

The following endpoints will be described by age group, in pooled age groups, and by vaccine group with 95% CIs:

- Individual neutralization test (NT) Ab titer on D0 and D28
- Individual NT Ab titer ratio (fold rise in serum NT post-vaccination relative to D0) at D28
- Subjects with NT Ab titers ≥ 20 (1/dil), ≥ 40 (1/dil), ≥ 80 (1/dil) at D28
- Fold rise in NT Ab titer [post/pre] ≥ 2 and ≥ 4 at D28
- Detectable NT (NT Ab titer ≥ 10 [1/dil]) at D0 and D28

In addition, the descriptive summary of SN immunogenicity results will also be produced by:

- Serological SN status at baseline (< 10 and ≥ 10 1/dil)
- Previous influenza vaccination status

5.1.3.2 Immunogenicity by ELLA Method

5.1.3.2.1 Hypotheses

No hypotheses will be tested.

5.1.3.2.2 Statistical Methods

Anti-N1and -N2 titers will be measured for the 2 influenza A strains using ELLA.

The following endpoints will be described by age group, in pooled age groups, and by vaccine group with 95% CIs:

- Individual ELLA Ab titer on D0 and D28
- Individual ELLA Ab titer ratio (fold-rise in serum ELLA post-vaccination relative to D0) at D28
- Subjects with ELLA Ab titers ≥ 20 (1/dil), ≥ 40 (1/dil), ≥ 80 (1/dil) at D28
- Fold-rise in ELLA Ab titer [post/pre] ≥ 2 and ≥ 4 at D28
- Detectable ELLA (ELLA Ab titer ≥ 10 [1/dil]) at D0 and D28

5.2 Analysis Sets

Three main analysis sets will be used: the PPAS, the FAS, and the Safety Analysis Set (SafAS).

5.2.1 Full Analysis Set

The FAS is defined as the subset of randomized subjects who received the study vaccine and had a post-vaccination blood sample. Subjects will be analyzed according to the vaccine group to which they were randomized.

For the assessment of the immune response by virus SN and ELLA method, the analysis will be performed on the subjects from the FAS randomized into the respective observational subsets (FAS-SN subset and FAS-NA subset). The FAS-NA subset is a subset of the FAS-SN subset.

5.2.2 Per-Protocol Analysis Set

The PPAS is a subset of the FAS. The subjects presenting with at least one of the following relevant protocol deviations will be excluded from the PPAS:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject did not receive vaccine
- Subject received a vaccine other than the one that he / she was randomized to receive
- Preparation and / or administration of vaccine was not done as per-protocol
- Subject did not provide the post-dose serology sample at V02 in the proper time window (ie, 28 to 35 days after vaccination) or a post-dose serology sample was not drawn at V02
- Subject received medications impacting or that may have an impact on the immune response (Category 2 or 3)

- Any other deviation identified during the study conduct and identified as relevant by the clinical team during data review, ie, indicated as excluding subjects from this analysis set in the manual deviations dataset.

5.2.3 Safety Analysis Set

The SafAS is defined as those subjects who have received the study vaccine and have any safety data available. All subjects will have their safety analyzed according to the vaccine they actually received.

Safety data recorded for a vaccine received out of the protocol design will be excluded from the analysis (and listed separately).

5.2.4 Other Analysis Sets

Enrolled Subjects

Enrolled subjects are subjects for whom a CRB has been created.

Randomized Subjects

A randomized subject is a subject for whom a vaccine group has been allocated.

5.2.5 Populations Used in Analyses

All subjects with data in the CRB will be taken into account in the description of the population (eg, the disposition, the demographic or baseline characteristics).

All immunogenicity analyses from the HAI method for primary and secondary objectives will be performed on both FAS and PPAS.

The safety analyses will be performed on the SafAS.

The observational objective analyses will be performed on subsets of the FAS (FAS-SN and FAS-NA subsets).

5.3 Handling of Missing Data and Outliers

5.3.1 Safety

Generally, no replacement of missing data will be done. Nevertheless, missing relationship will be considered as related at the time of the statistical analysis. No search for outliers will be performed. In all subject listings, partial and missing data will be clearly indicated as missing.

5.3.1.1 Immediate

For unsolicited non-serious systemic AEs, a missing response to the "Immediate" field is assumed to have occurred after the 30-minute surveillance period and will not be imputed.

For SAEs, missing or partially missing elapsed time from last vaccination recorded if within 24 hours will remain missing and not be imputed. Such SAEs will not be considered as immediate.

5.3.1.2 Causality

Missing causality (relationship) for unsolicited non-serious AEs and SAEs will be considered at the time of analysis as related to vaccination.

5.3.1.3 Measurements

Partially missing temperatures will be handled as described in Section 4.4.1.1.1.

5.3.1.4 Intensity

For solicited reactions, missing intensities will be handled as described in Section 4.4.1.1.1. For unsolicited non-serious AEs, missing intensities will remain missing and will not be imputed.

5.3.1.5 Start Date and Stop Date

Missing or partially missing start dates for unsolicited AEs will remain missing and not be imputed. If either the start or stop date is missing or partially missing, the time of onset will be considered to be missing. Nevertheless unsolicited AEs with missing time of onset will be included in analyses according to the visit collected.

Missing or partially missing stop dates for AEs (solicited reactions and unsolicited AEs) will remain missing and not be imputed.

5.3.1.6 Action Taken

Missing actions taken will remain missing and not be imputed.

5.3.2 Immunogenicity

LLOQ and ULOQ management will be performed as described in Section 4.4.1.3.5. No test or search for outliers will be performed.

No replacement will be done for missing values. Based on the previous QIV-HD and QIV-SD trials in this population, the amount of missing immunogenicity data is expected to be ≤ 5% in this trial. Usually in vaccine trials, it seems generally reasonable to assume missing immunogenicity data are missing completely at random (MCAR) (2). Indeed, it is highly unexpected that the dropout (or any other reason for missing data) could be linked to the immune

response of the subject. Therefore, confirming the results of the PPAS for the primary analysis with the FAS would be satisfactory in terms of sensitivity analysis.

5.4 Interim / Preliminary Analysis

No formal interim analyses are planned.

The statistical analysis will be performed in at least two steps:

- First analysis on immunogenicity and safety results obtained on data collected within the 28 days following the vaccination (from D0 to D28). The study blind will be broken at that time. If SN and ELLA exploratory results are not available at the same time as HAI, the first analysis will focus on HAI data to address the primary and secondary study objectives and the exploratory immunogenicity objectives will be addressed in further analysis steps.
- Final analysis after the 6-month data have been collected.

After the first database lock and unblinding, the unblinded data and interim analysis results will be kept at the sponsor level and will not be available to the study site staff, study site monitors or laboratories performing the assays until the completion of the last 6-month follow-up. The study sites will remain blinded during the 6-month safety follow-up and through the final database lock so as not to bias any 6-month safety reporting.

No statistical adjustment for the interim analysis is necessary because there are no repeat analyses of the same hypotheses.

5.5 Determination of Sample Size and Power Calculation

A total of approximately 1540 adults 60 years of age and older (770 adults 60 to 64 years of age and 770 adults 65 years of age and older) will be enrolled. This sample size is determined per simulations based on an overall power of 90% for demonstrating the primary objective. The thresholds for superiority are defined as 1 for GMTs. No alpha adjustment is needed. Other assumptions are listed as follows:

- Allocation ratio: 1:1 (QIV-HD versus QIV-SD)
- GMT ratio: 1.5 for all strains
- Standard deviations of log10-transformed titers in QIV-SD group of 0.6 for 2 strains and 0.5 for the other 2 strains
- Attrition rate: 5% in FAS

It should be noted that the power per strain is 97.7% when the standard deviation is 0.6 and 99.7% when the standard deviation is 0.5.

An arbitrary number of subjects, ie, at least 50% of subjects in each age group and each vaccine group will be randomly assigned to the observational subset of subjects for SN testing and at least 50 subjects in each age group and each vaccine group for NA testing as shown in Table 5.2.

Table 5.2: Random assignment of subjects to observational subsets

Vaccine/Assay	Number of subjects 60 to 64 years of age	Number of subjects 65 years of age and older	Total
QIV-HD Group/SN	≥ 193	≥ 193	≥ 386
QIV-SD Group/SN	≥ 193	≥ 193	≥ 386
QIV-HD Group/NA	≥ 50	≥ 50	≥ 100
QIV-SD Group/NA	≥ 50	≥ 50	≥ 100

5.6 Data Review for Statistical Purposes

This trial will not include an early review of safety data (ie, no early safety review of preliminary data occurring at pre-determined milestones defined in the protocol with pause in enrollment). However, it may be interrupted at any time if new data about the investigational product become available, and/or on advice of the Sponsor, the institutional review boards (IRBs), or the governing regulatory authorities in the country where the trial is taking place.

A blind review of the data is anticipated through the data review process led by Data Management before database lock (DB) This review of the data will include a statistical review.

5.7 Changes in the Conduct of the Trial or Planned Analyses

Due to expected delay in availability of exploratory assays results, the first analysis may only address the HAI data for primary and secondary objectives, as well as safety data up to D28. Depending on their availability, SN and ELLA results may be analyzed in a second and/or final analysis. Therefore, the blind may be broken before the SN and ELLA results are released. As these are exploratory objectives, this does not raise any risk on the integrity on the main study conclusions.
In addition, unblinded information will not be communicated to the laboratories involved in these assays so we do not anticipate any risk of bias in these results.

6 References List

1 Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med. 1998;17(8):857-72.

2 Li X, Wang WW, Liu GF, Chan IS. Handling missing data in vaccine clinical trials for immunogenicity and safety evaluation. J Biopharm Stat. 2011;21(2):294-310.

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