NCT Number: NCT03282240

Safety and Immunogenicity of High-Dose Quadrivalent Influenza Vaccine Administered by Intramuscular Route in Subjects Aged 65 Years and Older

Phase III, randomized, modified double-blind, active-controlled, multi-center trial evaluating the safety and immunogenicity of QIV-HD in healthy subjects aged 65 years and older in the United States

Supplementary Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	QHD00013
Development Phase:	Phase III
Sponsor:	Sanofi Pasteur Inc. Discovery Drive, Swiftwater, PA 18370-0187, USA
Investigational Product(s):	High-Dose Influenza Vaccine Quadrivalent, (Zonal Purified, Split Virus) 2017–2018 Strains (QIV-HD)
Form / Route:	Liquid / Intramuscular
Indication For This Study:	Single dose for individuals aged 65 years and older
Version and Date of the Supplementary SAP:	Version 1.0, 18Apr2018

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List of Abbreviations

BL	blood sample
CI	confidence interval
DB	database lock
dil	dilution
ELLA	enzyme-linked lectin assay
GCI	global clinical immunology
GM	geometric mean
GMT	geometric mean titer
GMTR	geometric mean titer ratio
LLOQ	lower limit of quantification
NA	neuraminidase
NI	neuraminidase inhibiting
OD	optical density
OPD	o-Phenylenediamine dihydrochloride
PNA	peanut-agglutinin
QIV-HD	high-dose quadrivalent influenza vaccine
RCDC	reverse cumulative distribution curve
SAP	statistical analysis plan
ULOQ	upper limit of quantification
V	visit

1 Introduction

See Section 1 of the statistical analysis plan (SAP) core body. This document outlines the SAP of an additional immunogenicity assessment method (anti-neuraminidase antibody titration by enzyme-linked lectin assay [ELLA]).

1.1 Additional Immunogenicity Assessment Method

Anti-neuraminidase Antibody Titration by Enzyme-Linked Lectin Assay
Assays will be performed at an external testing laboratory (Q2 Solutions) under Global Clinical Immunology (GCI) supervision.
The ELLA measures neuraminidase inhibiting (NI) antibody by quantifying neuraminidase (NA) enzymatic activity using peanut-agglutinin (PNA) to bind to terminal galactose moieties that are exposed after enzymatic cleavage. Serial dilution of serum samples, quality control serum and a pre-determined amount of virus is added into duplicate wells of a fetuin-coated 96-well plate and incubated overnight. The following day, peroxidase conjugated PNA is added to the washed plate and incubated, followed by washing and color development with o-Phenylenediamine dihydrochloride (OPD) substrate. The absence of color indicates inhibition of NA activity due to the presence of NA-specific inhibiting antibodies. The titer of each determination is the reciprocal of the last dilution with an optical density (OD) equal to or less than the midpoint between the mean OD of the virus only control wells and the mean OD of the background wells on each plate. The lower limit of quantitation (LLOQ) is set at the reciprocal of the lowest dilution used in the assay, i.e., 10 (1/dil). Titers below this level will be reported as < 10 (1/dil).

2 Trial Objectives

See Section 2 of the SAP core body.

3 Description of the Overall Trial Design and Plan

See Section 3 of the SAP core body.

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

Not Applicable.

4.4 Derived Endpoints: Calculation Methods

4.4.1 Safety

See Section 4.4.1 of the SAP core body.

4.4.2 Immunogenicity

4.4.2.1 Computed Values for Analysis

In order to appropriately manage extreme values (< 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 blood sample (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

For immunogenicity values from ELLA, each BL will provide two values on each strain on each time point (duplicates). In order to appropriately manage replicate values for analysis purposes, the individual geometric mean (GM) of all values will be computed for each BL after managing extreme values as described above.

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 ELLA, 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.

4.4.3 Efficacy

Not applicable.

4.4.4 Derived Other Variables

See Section 4.4.4 of the SAP core body.

5 Statistical Methods and Determination of Sample Size

See Section 5 of the SAP core body for the same analysis plan. Subsections are deleted if they are not applicable for immunogenicity analyses by ELLA.

5.1 Statistical Methods

For ELLA, only 2 virus strains have available immunogenicity lab test results as follows:

- A/Michigan/45/2015 (H1N1) strain
- A/Hong Kong/4801/2014 (H3N2) strain

5.1.1 Hypotheses and Statistical Methods for ELLA

5.1.1.1 Hypotheses

No hypotheses will be tested for ELLA.

5.1.1.2 Statistical Methods

5.1.1.2.1 Immunogenicity by ELLA

For descriptive purposes, the statistics presented on Table 5.1 of the SAP core body will be produced.

Immunogenicity in terms of geometric mean titers (GMTs), 2-fold rise and 4-fold rise will be summarized along with their 95% confidence intervals (CIs) for post-vaccination immunogenicity (visit 02 (V02)). The normal approximation of log-transformed titers will be applied to calculate the 95% CIs for GMTs. The 95% CIs will be calculated using Clopper-Pearson method (1) for percentages. The geometric mean titer ratios (GMTRs) will be calculated for post-vaccination immunogenicity (V02) over the baseline immunogenicity (V01) with the corresponding 95% CIs (assuming normal approximation of log-transformed values). Reverse cumulative distribution curves (RCDCs) against each strain will be performed for baseline (V01) and post-vaccination immunogenicity (V02). Additional parameters may be displayed as appropriate.

Statistical details of methods used for above analyses are the same as in Section 5.1.2.2 of the SAP core body.

5.2 Analysis Sets

See Section 5.2 of the SAP core body for the definitions of analysis sets.

5.2.1 Populations Used in Analyses

The immunogenicity analyses by ELLA will be performed on the Expanded Immunogenicity Subset.

5.3 Handling of Missing Data and Outliers

5.3.1 Immunogenicity

LLOQ and ULOQ management will be performed as described in Section 4.4.2 in the SAP core body. No test or search for outliers will be performed.

5.4 Interim / Preliminary Analysis

Analysis of immunogenicity results from ELLA will be performed after the 2nd database lock (DB).

6 References List

1 Newcombe R.G., Two-sided confidence intervals for the single proportion: comparison of seven methods, Statistics in Medicine, (1998) 17, 857-872