CLINICAL STUDY REPORT

Study Title	**Immunogenicity, molecular profiling and safety of a marketed quadrivalent influenza vaccine (Vaxigrip Tetra®) administered by the intramuscular route in participants 60 years of age and older**
Product	Vaxigrip Tetra®
Protocol Number	INCENTIVE-QIV-1-EU
EudraCT Number	2021-003307-18
Clinical Phase	IV
Clinical Indication	Influenza immunization
Study Description	Phase IV, non-randomized vaccine trial of approximately 1-month duration for each recruited participant after vaccination. The intervention was a single dose of Vaxigrip Tetra® intramuscular injection into the deltoid muscle on Day 0 with follow-up visits on Days 3, 7 and 28.
First participant enrolled	12 October 2021
Last participant completed	02 February 2022
Principal Investigator	Ilse De Coster, MD, PhD
Sponsor	University of Antwerp
Sponsor representative	Pierre Van Damme, MD, PhD University of Antwerp Campus Drie Eiken, Drie Eikenstraat 663 2650 Antwerpen (Edegem) Belgium
Good Clinical practices Compliance	This study was performed in compliance with International Council for Harmonization GCP, including the archiving of essential documents as well as the ethical principles of the Declaration of Helsinki.
Study report version and date	Final CSR version 1.0, 18-DEC-2024

4 SYNOPSIS

Sponsor: University of Antwerp	
Product: Vaxigrip Tetra®	
Study Title: Immunogenicity, molecular profiling and safety of a marketed quadrivalent influenza vaccine (Vaxigrip Tetra®) administered by the intramuscular route in participants 60 years of age and older	
Principal investigator: Dr Ilse De Coster	
Study Centre: Centre for the Evaluation of vaccination Vaccine & Infectious Disease Institute Faculty of Medicine and Health Sciences Campus Drie Eiken – Vaccinopolis Drie Eikenstraat 663-2650 Antwerp (Edegem) Belgium	
Publication (s) (Reference): None at the time of this report	
Study period: 12 October 2021 (First subject first visit)- 02 February2022 (Last subject last visit)	**Phase of development:** IV

Background and rationale:

Influenza causes a heavy health and economic toll as it cycles recurrently between the human population and an animal reservoir. Understanding human susceptibility to the influenza virus is essential for reducing and possibly eliminating disease burden.

Seasonal flu vaccines must be given annually, with effectiveness varying between 10 and 60%, while failing to adequately protect the most vulnerable - infants, elderly, individuals with co-morbidities and the developing world populations. This is due in large part to the recent recognition that in addition to antigen discovery and vaccine platform optimization, the influenza vaccine problem is primarily a human immunology problem, rooted in our lack of understanding of how to generate broadly protective, long-lasting immunity, in everyone.

The INCENTIVE (Indo-European Consortium for Next Generation Influenza vaccine Innovation) project is funded by the European Union's Horizon 2020 research and innovation program and the Department of Biotechnology (DBT), Govt. of India. The highly integrated INCENTIVE consortium comprises 19 institutions representing a true partnership between Indian and European/United States of America (US) groups that addresses the global health and economic challenge posed by influenza infections, to reduce the worldwide burden resulting from outbreaks. INCENTIVE's strategic goals are to provide seminal knowledge on the underlying mechanisms of poor responsiveness to influenza vaccines in vulnerable individuals and advance the development of two next generation universal influenza vaccines. This study in elderly (parallel study in India and Europe) is part of a first step in this INCENTIVE project with phase IV studies in elderly, infants and children. The aim was to assess immunogenicity in 3 different age groups with the same quadrivalent commercially available influenza vaccine. Immunogenicity evaluation included the traditional immunogenicity as well as more detailed analysis of the immune profile, including in depth analysis of antibodies and their effector functions and cell-mediated immunity, with the goal to identify correlates of responsiveness across populations in EU and India, predicting responses versus non-responses and the quality of responses to influenza vaccines across populations and according to gender. The identification of common or unique determinants of vaccine responses could provide essential guidance to the development of universal influenza vaccine protecting diverse populations.

Objectives and endpoints:	
Objectives	**Endpoints**
Primary	
To measure the level of immune response (HAI titres) of a single intramuscular dose of the quadrivalent inactivated influenza vaccine (Vaxigrip Tetra®) in healthy participants aged 60 years or above	• HAI antibody titres at D0 and D28 • Proportion of participants with HAI titres ≥ 40 (1/dilution) at D28 • HAI antibody titres fold increase between D0 and D28 • Proportion of participants with seroconversion (titre < 10 [1/dilution] at D0 and post-vaccination titre ≥ 40 [1/dilution] at D28, or titre ≥ 10 [1/dilution] at D0 and a ≥ 4-fold increase in titre [1/dilution] at D28 • Proportion of high and low responders (HAI titres <40 (1/dilution) at D28)
Exploratory	
To measure the levels, avidity, biophysical characteristics and functionality of influenza-specific antibodies induced by the vaccine	**a.** Neutralizing Ab titres will be measured for each vaccine strain with the microneutralization (MN) assay. The analyses will be performed on blood samples obtained on D0 and D28. • Individual MN Ab titre at D0 and D28 • Detectable MN (MN Ab titre ≥ 10 [1/dilution]) at D0, D28 • Proportion of participants with MN Ab titres ≥ 20 (1/dilution), ≥ 40 (1/dilution), ≥ 80 (1/dilution) at D28 • Individual MN Ab titre fold-increase D28 post-vaccination relative to D0 • Fold-increase in MN Ab titre [D28/D0] ≥ 2 and ≥ 4 **b.** Anti-Haemagglutinin (HA) and Neuraminidase (NA) antibody titres to vaccine strain and antibody avidity. • Individual HA and NA Ab titres at D0 and D28 • Detectable HA and NA Ab titre ≥ 10 [1/dilution]) at D0 and D28 • Proportion of participants with HA and NA Ab titres ≥ 20 (1/dilution), ≥ 40 (1/dilution), ≥ 80 (1/dilution) at D28 • Individual HA and NA Ab titre ratio (D28/ D0) • Fold-increase in HA and NA Ab titre [post/pre] ≥ 2 and ≥ 4 at D28 • Avidity index of HA and NA Ab at D0 and D28 **c.** Level (mean fluorescence intensity) and avidity (avidity index) of influenza-specific antibody isotypes at D0 and D28 **d.** Level of influenza-specific antibody isotypes triggering Fc-dependent effector functions (proportion of activated cells, phagocytic score or mean fluorescence intensity) at D0 and D28 **e**. Proportions of influenza-specific peripheral blood T cells with effector or regulatory phenotypes at D0, D7 and D28 **f.** Proportions of peripheral blood B cells with effector or regulatory phenotypes at D0, D7 and D28 **g.** Proportions influenza-specific IgG Fc expressing individual glycans at D0 and D28

	h. Level of binding (mean fluorescence intensity) of Fc receptors and complement by influenza-specific antibodies at D0 and D28 **i.** Level of cytokines (pg/ml) and mRNA (arbitrary units) induced by microbial products in an ex vivo whole blood assay at D0. **j.** Number (n per microliter) and proportions of immune cell subsets in peripheral blood at D0. **k.** Level of expression of peripheral blood cell mRNA, plasma metabolites and plasma proteins (arbitrary units) at D0 and D3

Study Design:

This was a Phase IV, open-label vaccine trial with the quadrivalent vaccine Vaxigrip Tetra®, marketed by Sanofi Pasteur. In this ambulatory study, 50 healthy elderly participants aged 60 years and older were enrolled in Belgium. In India, a similar (separate) study with the same study design was conducted in 100 participants (CTRI/2020/09/027913). Participants with well-controlled comorbidities were included. The vaccine was given on Day 0 with a postvaccination observation period of 30 minutes. Subsequently, subjects came for follow up on Days 3, 7 and 28. SAEs related to study procedures or vaccination have been collected until the end of study.

This is a population in which the complications of influenza are relevant in terms of morbidity and mortality, and therefore the vaccine is recommended in developed countries as part of their national program. There is no such recommendation in India. This is also a population in which an adequate immune response may or may not be mounted. Therefore, the development of the immune response was further characterized. Titers of influenza-specific antibodies were measured and their biophysical and functional profile will be analyzed to assess the magnitude and quality of vaccine responses. In parallel, profiling of immune cells and plasma will be performed to identify predictors of vaccine responses.

In this CSR, results of the primary objectives are presented. Results of exploratory objectives will be presented in a CSR amendment as soon as results become available.

Number of Participants (Planned and Analyzed):

Planned and Analyzed: 50 participants

- Screened:76
- Vaccinated: 50
- Completed study per protocol: 49
- Early withdrawals: 0

Main Criteria for Inclusion and Exclusion

Main Inclusion Criteria:

Eligible participants must meet all of the below criteria at the time of enrollment:

- Male or female of non-child bearing potential 60 years and above at the time of study.
- Provide written informed consent.
- The participant is willing to comply with study protocol requirements, including availability for all scheduled visits of the study.
- Subjects are healthy or with well-controlled pre-existing medical conditions by the opinion of the investigator.

Main exclusion criteria:

Participants meeting any of the below criteria at the time of enrolment were ineligible to participate in the trial:

- Acute illness, at the time of study vaccine administration
- Recorded fever (for eligibility purpose defined as a body temperature greater than 37.5°C) within 3 days prior to study vaccine administration

- Not willing to refrain from physical exercise during 48 hours prior to vaccination
- History of influenza vaccine administration during the past 6 months and during study participation.
- Current or previous, laboratory confirmed case of influenza during the past 6 months, based on anamnesis or medical file (if available) at screening visit.
- Household contact with and/or intimate exposure to an individual with any laboratory confirmed influenza infection during the past 6 months prior to vaccination.
- History of severe allergic reactions after previous vaccinations or hypersensitivity to any study vaccine component
- Previous history of Guillain Barre Syndrome.
- Any confirmed or suspected condition with impaired/altered function of immune system (e.g. immunodeficient or autoimmune conditions).
- Having tested positive for Human Immuno-deficiency Virus (HIV), Hepatitis B or Hepatitis C on the blood tests of the screening visit
- Chronic administration (defined as more than 14 days) of immunosuppressant or other immune-modifying drugs within three months prior to the study vaccination or planned use throughout the study period. (For corticosteroids, this means prednisone, or equivalent, ≥ 0.5 mg/kg per day. Inhaled, intranasal and topical steroids were allowed.
- Participants were also excluded based on:
    - Prior/concomitant therapy
    - Other exclusion criteria

For detailed inclusion/exclusion criteria, refer to the main body of the CSR.

Test product, Dose and Mode of Administration, batch Number(s):
Vaxigrip Tetra®(Quadrivalent Influenza Vaccine, season 2021 – 2022) is an inactivated quadrivalent influenza vaccine indicated for the prevention of influenza disease caused by influenza types A and B viruses contained in the vaccine.

All subjects received a single dose (0.5 ml) on Day 0 by intramuscular injection into the deltoid muscle.

Batch number: V3H342V

Duration of Treatment:
Participants were vaccinated once, on Day 0.

Statistical methods:
Safety Analyses:
The safety analysis was conducted on the total vaccinated cohort (TVC). Medical history and SAEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) (version 27.0 or later). The frequency count and percentage of participants having an event were summarized by system organ class and preferred term. Concomitant medication was coded using the ATC Index, version 2024. The frequency counts and percentage of participants using the medications were summarized by substance name and combination levels within the ATC Index (first – fourth level). Participant-wise data listing is provided for both the medical history and the concomitant medication.

The laboratory investigations, vital signs and physical examinations were categorized as normal or abnormal. For abnormal findings, the results were indicated as either clinically not significant (CNS) or clinically significant (CS) by one of the investigators. The results were summarized using frequency counts and percentage of participants belonging to a specific category.

Immunogenicity Analyses:
The analysis of the primary endpoint was conducted on the according-to-protocol (ATP) population and on the TVC. Geometric mean titres (GMTs), mean geometric increases (MGIs), seroprotection rates and seroconversion rates at D28 as compared to D0 were calculated with their respective 95% and 98.75% confidence interval for HAI titres against influenza strains included and not included in the Vaxigrip Tetra

vaccine (2021 – 2022). In addition, GMTs and MGIs were also estimated using linear mixed models with a random intercept for each subject and time as a fixed effect.

As the primary endpoint data for India has not yet been finalized, the analyses comparing Belgium and India have not yet been performed. The exploratory endpoint analyses have not yet been performed at the time of this CSR and will be covered in a CSR Addendum.

Summary and conclusions:

Disposition and Demography:

A total of 76 subjects were enrolled and screened. From these, 50 participants were randomized and subsequentially vaccinated in the study. All 50 vaccinated subjects completed the study. The mean age and body mass index of the total vaccinated cohort were 65 years and 27.2 kg/m^2, respectively. All study participants were of Caucasian origin.

Safety Results:

No serious adverse events were reported in this study.

Immunogenicity results:

The following results were reported for the primary endpoint analyses:

- The D28 GMTs ranged between 69.5 and 180.5 (for the A/Victoria strain and the B/Phuket strain, respectively).
- The MGIs ranged between 2.2 and 5.8 (for the B/Phuket strain and the A/Victoria strain, respectively).
- The proportion of participants with HAI titres ≥ 40 at D28, considered to be a 'high responder' in the protocol, was over 60% for all 4 strains included in the vaccine. However, 76% of the participants already had pre-vaccination HAI titres ≥ 40 for the B/Phuket strain at D0.
- The proportion of participants with seroconversion, defined as pre-vaccination HAI titres < 10 and post-vaccination titres ≥ 40 or as pre-vaccination HAI titres ≥ 10 and a ≥ 4-fold increase in HAI titre post-vaccination, ranged between 27% and 55% (for the B/Phuket and A/Victoria strain, respectively). Notably, the Committee for Medicinal Products for Human use (CHMP) seroconversion criterium of 30% (EMA) was not met for the B/Phuket strain.

Conclusion:

Significant increases in HAI antibodies were elicited to each of the vaccine viruses between D0 and D28 after vaccination with Vaxigrip Tetra®, although a large proportion of the study population was already protected against B/Phuket.

The results of this study with the Vaxigrip Tetra® (2021 – 2022) vaccine are consistent with the CHMP licensure criteria for HAI-based immunogenicity analyses in adult subjects aged 60 years and above for the A/Victoria strain, the A/Tasmania strain and the B/Washington strain. Although the strains were not included in the vaccine, the criteria were also met for the strains A/California/07/2019 and A/Brisbane/02/2018, indicating cross protection for these influenza strains.

Date and version of Report: Final CSR version 1.0, 18-DEC-2024