# Effect of High-Dose Quadrivalent Influenza Vaccine (Efluelda®) versus Standard-Dose (QIV-SD), in subjects 65 years of age and older on innate immunity, including gene expression.

# INFLUOMICS

## Abstract of Clinical Study Report Version 1.0 of 2023-05-17

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| **Name of test drug/investigational product** | EFLUELDA |
| **Indication studied** | Influenza |
| **Brief study design / Development phase of study** | Phase IV, randomized, open-label, active-controlled, multi-center study comparing the immune response induced by QIV-HD vaccine (intervention) and QIV-SD vaccine (control). |
| **Sponsor** | Centre Hospitalier Annecy Genevois (CHANGE) |
| **Internal Study Code** | CHANGE : 21-05<br>SANOFI PASTEUR : QHD00029 |
| **EudraCT** | 2021-004573-32 |
| **ClinicalTrials.gov Identifier** | NCT05154383 |
| **Coordinating Investigator** | Dr Cécile JANSSEN |
| **Study initiation date** | First patient enrolled : 2021/11/15 |
| **Date of early study termination** | *Not applicable* |
| **Study completion date** | Last patient completed : 2022/07/21 |
| **Date of Clinical Study Report** | 2023/04/17 |
| **GCP statement** | This study was performed in compliance with ICH Good Clinical Practise (GCP) including the archiving of essential documents |

## VERSION HISTORY

| Version | Date | Reason for amendment |
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| *Abstract V1.0* | *2023-05-17* | |

## ABSTRACT

**Name Of Sponsor**
Centre Hospitalier Annecy Genevois

**Name Of Finished Product**
EFLUELDA®

**Name Of Active Ingredient(s)**
- A/Victoria/2570/2019 (H1N1) pdm09-like strain
- A/Cambodia/e0826360/2020 (H3N2)-like strain
- B/Washington/02/2019 (B/Victoria lineage)-like strain
B/Phuket/3073/2013 (B/Yamagata lineage)-like strain

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| **Full study title** | Effect of High-Dose Quadrivalent Influenza Vaccine (Efluelda®) versus Standard-Dose (QIV-SD), in subjects 65 years of age and older on innate immunity, including gene expression. |
| **Study Acronym** | INFLUOMICS |
| **Name of Product / Active Ingredient (if applicable)** | EFLUELDA® |
| **Phase of development (if applicable)** | Phase IV |
| **Sponsor** | Centre Hospitalier Annecy Genevois (CHANGE) |
| **Coordinating Investigator / Study Centre** | Dr Cécile JANSSEN – Centre Hospitalier Annecy Genevois |
| **Other Principal Investigtator / Study Centres** | Dr Olivier ROGEAUX – Centre Hospitalier Métropole Savoie Chambéry |
| **Studied period (year)** | Date of first enrolment: 2021/11/15<br>Date of last completed: 2022/07/21 |
| **Scientific justification** | Influenza is a viral respiratory infection and respiratory complications are most common. The consequences associated with infection are broader and include the exacerbation of chronic underlying conditions, which can increase the risk of influenza-associated morbidity and mortality.<br>Vaccination against influenza is the primary method for preventing influenza and its complications. It has been shown to be effective in reducing influenza-associated morbidity and mortality in groups at increased risk for influenza-related complications such as infants and young children, and persons 50 years of age and older. Of note, immune responses to the vaccine are lower among seniors than in young healthy adults. |

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| | Strategies to improve immune responses induced by the vaccine in elderly could substantially reduce influenza-associated morbidity and mortality. One could increase the dose of HA (Haemagglutinin) in inactivated vaccines. Previous studies evaluating hemagglutination inhibition (HAI) antibodies induced by different influenza vaccines showed a dose-response effect between higher doses of HA per strain and immune response.<br>Efluelda® (Sanofi Pasteur Quadrivalent Influenza Vaccine High-Dose Quadrivalent vaccine, QIV-HD) was developed to improve protection among the elderly through the use of higher antigen content. Efluelda® remains the only influenza vaccine to have demonstrated superior efficacy against lab-confirmed illness relative to the standard-dose vaccine in a large randomized controlled trial among adults 65 years of age and older. These results were shown consistent over many consecutive seasons.<br>In addition to the clinical benefits, the immunological responses were improved, compared to standard dose vaccines. In addition to higher HAI titers, higher neutralization and anti-neuraminidase titers were found with the split Virus Influenza trivalent – High Dose vaccine (IIV3-HD) than with IIV3-SD (standard dose). However, the role of cell-mediated immunity in host protection remains unclear.<br>It has been proposed that innate immunity at the initiation phase of immune responses (hours post vaccination) would program the amplitude and quality of the adaptive immunity (weeks after vaccination).<br><br>We hypothesize that higher dose of antigen can increase the intensity and the quality of innate immunity, as the adaptive humoral and cellular responses.<br>In this study, we aim at evaluating innate and adaptive immunity following QIV-HD vaccination compared to QIV-SD vaccination in people 65 years of age and older.<br>We will investigate modifications of early blood molecular (transcriptome) and cellular (blood phenotyping) signatures within the first 24 hours following vaccination. We will also investigate the association between early gene signature and late influenza-specific humoral immune responses weeks/months after vaccination. |
| **Main objective** | The objective was to evaluate the effect of QIV-HD and QIV-SD vaccines in subjects 65 years of age and older on:<br>- the early systemic innate immune response through transcriptomic analysis i.e. innate gene signature including interferon signaling pathways,<br>- innate cells including antigen presenting and inflammatory cells,<br>- gene signature associate with adaptive immune response before and after the influenza vaccination, humoral immunity i.e. HI titers, at different time points. |
| **Endpoint** | Outcomes were measured in each subject. Measures were reported by allocated arm: QIV-HD arm or QIV-SD arm.<br><br>*Humoral immune responses*<br>We measured and reported the evolution of:<br>• HAI titers obtained on D0, D21, D90 and D210<br>• Individual HAI titer ratio D21/D0, D90/D0 and D210/D0<br>• Subjects with titers ≥ 40 at D21, D90 and D210 (seroprotection)<br>• Seroconversion: titer < 10 at D0 and post-vaccination titer ≥ 40 at D21, D90 and D210 or titer ≥ 10 at D0 and a ≥ 4-fold increase in titer at D21, D90 and D210 |

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| | Humoral response results were reported in HAI titers for each of the 4 antigens at D0, D21, D90 and D210 time.<br><br>*Transcriptomic*<br>Transcriptomic profiles of blood cells (microarrays) were performed to measure early systemic innate immune response. Samples were collected seven days prior vaccine injection (D-7, first baseline), the day of vaccine injection (D0, second baseline) to assess the stability of biomarkers, and one day after vaccine injection (D+1).<br><br>*Innate cellular phenotyping*<br>Innate cellular phenotyping was performed using 30 surface markers deciphering lineage cells monocytes, neutrophils, NK and antigen-presenting cells. The technology used was based on Cytek® Aurora spectral cytometry (Cytek, France). These data will be integrated in final innate gene signature analysis.<br><br>*Gene signature*<br>The innate immune response was assessed at baselines (D-7 and D0) and D+1 after vaccination. We compared gene expression at D+1 to baselines, by studying the transcriptional profile of the whole blood cells by microarrays. |
| **Methodology** | Phase IV, randomized, open-label, active-controlled, multi-center study comparing the immune response induced by QIV-HD vaccine (intervention) and QIV-SD vaccine (control). |
| **Study population** | Number of patients planned: 60 randomized and evaluable subjects.<br><br>Number of patients analyzed: 59<br><br>Cellular innate immunity: 28 subjects received QIV-SD vaccine and 31 subjects who received QIV-HD vaccine<br><br>Gene expression: 28 subjects received QIV-SD vaccine and 31 subjects who received QIV-HD vaccine |
| **Inclusion criteria** | 1. Aged 65 years or older, the day of inclusion;<br>2. Have signed and dated Informed Consent Form;<br>3. Able and willing to attend all scheduled visits, and to comply with study procedures;<br>4. Covered by French health insurance. |
| **Non-Inclusion criteria** | 1. Any vaccine injection (including COVID-19 vaccine) in the 4 weeks preceding study inclusion;<br>2. Plan to receive any vaccine (including COVID-19 vaccine) in the 24 hours following study inclusion;<br>3. Already vaccinated against influenza for 2021-2022 season;<br>4. Hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccines used in the study or to a vaccine containing any of the same substances;<br>5. HIV infection;<br>6. Active Hepatitis B, or active Hepatitis C;<br>7. Previous Guillain Barré syndrome;<br>8. Ongoing immunosuppressive treatment or active immunodeficiency;<br>9. Receipt of immune globulins, blood or blood-derived products in the past 3 months;<br>10. Thrombocytopenia or bleeding disorder, receipt of anticoagulants contraindicating IM vaccination based on investigator's judgment; |

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| | 11. Influenza-like illness symptoms, including COVID-19, within 4 weeks before study inclusion;<br>12. Persons referred to in articles L1121-6 and L1121-8 of Public Health Code (persons deprived of liberty by judicial or administrative decision and persons subject to a legal protection measure: guardianship or curatorship). |
| **Exclusion criteria** | 1. Any subject presenting with influenza-like illness symptoms, including COVID-19 between inclusion (visit 1) and randomization (visit 2);<br>2. Subject unable to attempt visit at Day 0 (visit 2) and Day 1 (visit 3);<br>3. Blood sample at Day 0 (visit 2) impossible to obtain;<br>4. Receipt of vaccine injection or equivalent between inclusion (visit 1) and randomization (visit 2), other than those allowed and planned in the study. This includes non-study dose of 2021–2022 influenza vaccine, blood-derived immune globulins, blood, or blood-derived products.<br>5. Any unexpected event relevant to the physician in charge, after discussion with the coordinating investigator and the sponsor<br>These subjects will not be randomized and not followed after the second visit. Study team will propose influenza vaccination outside of the study, as per standard of care. |
| **Test product, dose, mode of administration, batch number** | Efluelda®:<br>- Therapeutic class: Quadrivalent Influenza Vaccine (split virion, inactivated), High-Dose (QIV-HD), used with its manufacturer's commercial labeling and packaging.<br>- Mode of administration: Suspension for injection in pre-filled syringe. IM, injected into the upper arm (deltoid area).<br>- Dose: Each 0.7mL dose contains 60 µg hemagglutinin (HA) of each influenza strain. Strains were determined based on World Health Organization (WHO) and European Union (EU) recommendations for the 2021-2022 Northern Hemisphere (NH) influenza season.<br>- Batch number: UT503AC<br>- Exp date: June 2022 |
| **Duration of treatment** | 1 single injection |
| **Comparator or associated treatment, dose, mode of administration, batch number** | InfluvacTetra®:<br>- Therapeutic class: Quadrivalent Influenza Vaccine (split virion, inactivated), Standard-Dose (QIV-SD), used with its manufacturer's commercial labeling and packaging.<br>- Mode of administration: Suspension for injection in pre-filled syringe. IM, injected into the upper arm (deltoid area).<br>- Dose: Each 0.5mL dose contains 15 µg hemagglutinin (HA) of each influenza strain. Strains were determined based on World Health Organization (WHO) and European Union (EU) recommendations for the 2021-2022 Northern Hemisphere (NH) influenza season.<br>- Batch number: 236<br>- Exp date: August 2022 |
| **Criteria for evaluation** | **Efficacy**<br>Effect of QIV-HD and QIV-SD vaccines was evaluated biologically through assessment of:<br>- humoral immunity *i.e.* HI (Haemagglutinin inhibition) titers, at different time points.<br>- the early systemic innate immune response: transcriptomic analysis *i.e.* innate gene signature including interferon signaling pathways,<br>- innate cells including antigen presenting and inflammatory cells,<br>- gene signature associate with adaptive immune response before and after the influenza vaccination. |

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| | **Safety**<br>To assess safety, the evolution of the following was considered:<br><br>***Medical History***<br>Prior to enrollment, participants were assessed for pre-existing conditions.<br><br>***Physical Examinations***<br>At each visit, the Investigator or a designee performed a history-directed physical examination.<br><br>***Vital Signs***<br>At each visit, oral - temperature (before vaccine injection at Visit 2) was systematically collected by the investigator on the source document. |
| **Statistical methods** | Immunogenicity and transcriptomic analyses were provided for Full Analysis Set (FAS) and Per Protocol Set (PP). Safety analysis was provided for Safety Set (SS).<br><br>**<u>Immunogenicity</u>**<br><br>Parameters were presented by vaccine group, and against flu strain, with their 95%<br><br>Confidence Interval (95%CI) for D21, D90, and D210:<br><br>1. Geometric Mean (GM) of titers on D0 and (D21, D90, D210) with and without log transformation<br>2. Distribution of titers against flu<br>3. Rate of subjects with titer ≥ 1:10 on D0 and (D21, D90, D210)<br>4. Rate of subjects with titer ≥ 1:40 on D0 and (D21, D90, D210) - Seroprotection<br>5. Seroconversion or significant increase rate from D0 to (D21, D90, D210)<br>6. GM of titer ratio (D21, D90, D210)/D0 (immunological response):<br>a. Individual evolution of ratios<br>b. Distribution of evolution of ratios<br>c. Mean evolution of ratios<br>7. Evolution of (log) antibody titers over time<br><br>The 95% CIs were computed using the normal approximate method for GMs, and the exact binomial distribution for percentages (Clopper-Pearson's method). Outliers were managed using standard procedure for GMT estimation. Missing data was not imputed.<br><br>**<u>Transcriptomic analysis</u>**<br><br>Transcriptomic data were analyzed using the "limma package".<br>Raw data were analyzed assuming a linear model fit with a moderated t-test by empirical Bayes statistics. To decrease the influence of outlier samples, we adjusted the linear model by estimated relative quality weights of each array using the "*arrayWeights"* function from the limma package. We applied linear regression models using function "*lm*" from the stats package.<br><br>Comparisons of interest were computed through statistical contrasts, using the "emmeans" package. We moderated *p*-values using the empirical Bayes method using the "eBayes" function from the limma package. We filtered out genes using the I/NI method to declare them non-informative. We run sensitivity analysis using machine learning (*i.e.* Model-based Gene Set Analysis MGSA) to further propose hypothesis linking biological pathways to cellular function. The model |

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| | assumes that some gene sets are active and that all genes member of active sets are themselves active. Only sets with at least 10 genes and at most 1000 were considered.<br>*P-values* were corrected in multiple comparisons using the Benjamini and Hochberg's false discovery rate (FDR) controlling procedure.<br><br>Cellular innate analysis by spectral flow cytometry<br><br>We normalized data from each sample using the Cytek software (Spectroflo® 3.0.0). A quality control procedure consisting in checking number of cell events and marker signal in comparison with an internal control was then performed. Doublets were discarded and dead cell removed to keep only CD45+ cells. We identified innate cell populations based on lineage population into major clusters: neutrophil, basophil, monocyte, NK, T, B and dendritic cell populations. Each cell population percentage is plotted against the total number of CD45+ cells in blood. Fold change of cell population abundance was compared between average of baselines (D-7 and D0) and first day after vaccine injection. One-way ANOVA and Bonferroni's multiple comparison tests were used to compare abundance of cell populations across subgroup of subjects (including study arms).<br><br>**Safety**<br>The frequency, the severity, and the causal relationship of adverse events with allocated vaccine were tabulated by system organ class and preferred term according to the current version of the Medical Dictionary for Drug Regulatory Activities (MedDRA) for any potential SAE/AESI assessed during the clinical/biological assessments. |
| **Results** | The innate signature of QIV-HD vaccine is showing higher intensity than QIV-SD vaccine.<br>High-dose QIV induces higher HAI titers compared to QIV-SD vaccine, 21 days after vaccination, without sustain effect on HAI titers level until 210 days. The safety profile of both vaccines is similar. |