Statistical Analysis Plan

Study alias & e-track number(s): Flu CC-SUIV-AS03-001 (201598)

Detailed Title:	An exploratory, retrospective laboratory evaluation, using specimens from completed clinical trials, of the humoral immune response to the ***hemagglutinin*** stalk domain and other influenza A virus protein epitopes in adults 18-64 years of age and children 6-35 months of age, following administration of GSK Biologicals' adjuvanted or unadjuvanted H5N1, H1N1pdm09, ***H7N9*** and H9N2 pandemic influenza vaccines ***or following a non-adjuvanted seasonal quadrivalent inactivated influenza vaccine***
SAP version	***Amendment 1 (Version 1 : 30-Jun-2015)***
SAP date	***20 January 2017***
Scope:	All data pertaining to the above study.
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The complete statistical analysis plan and results presentation is divided into 2 parts: the first part detailing the analyses to be performed (known as SAP, current document) and a second part, annex (-es) (called TFL) describing the flow and format of tables, figures and listings to be annexed to the SR.

One TFl is developed for each analysis. Following are the TFLs available for tables, figures and listing to be generated in this study:

Corresponding TFLs	**Applicable time point and analysis to be performed**
TFL_H5N1_ELISA	Demog and Immuno analysis for ***anti- H1 stalk*** ELISA test ***performed at MSSM*** for Adult H5N1 cohort
TFL_ANTI_H1 ANALYSIS	Demog and Immuno analysis for anti-H1 ***stalk*** ELISA test ***performed at Neomed***, ***anti-H1 stalk MN and CMI*** for studies Q-PAN H1N1-019, CC-PAN-H5N1-001, Q-PAN H9N2-001, Q-PAN H5N1-AS03-021

Additional TFLs will be developed for future analyses. Analyses will be performed in sequence based on the availability of the different test results.

LIST OF ABBREVIATIONS

ATP	According-To-Protocol
CI	Confidence Interval
EL.U/ml	ELISA unit per milliliter
ELISA	Enzyme-linked immunosorbent assay
GMT	Geometric mean antibody titre
GSK	GlaxoSmithKline
LL	Lower Limit of the confidence interval
N.A.	Not Applicable
SAP	Statistical Analysis Plan
SD	Standard Deviation
SR	Study Report
SYN	Synopsis
TFL	Tables Figures and Listing template annexed to SAP
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
30-JUN-2015	Version 1	Amendment 1 Final - 10-MAR-2015
20-JAN-2017	***Amendment 1*** ***Changes from the previous version:-*** ***1. Addition of new study cohorts and groups*** ***2. Changes in the study design, objective, endpoints and analysis to be performed per Protocol amendment 2.*** ***3. The ATP cohort for immunogenicity analysis has been removed.*** ***4. The correlation analysis will be performed including all timepoints together***	***Amendment 2 Final – 01-SEP-2016***

2. STUDY DESIGN

Experimental design: This retrospective study is designed to assess immunogenicity (in terms of the humoral immune response to the H1 hemagglutinin stalk domain and other influenza A virus protein epitopes), ***by ELISA and microneutralization (MN) assay,*** of H5N1, H1N1pdm09, H9N2, adjuvanted or unadjuvanted pandemic influenza vaccines (standard adult dose) using archived serum specimens from 3 completed clinical trials with adult subjects ***[Q-Pan H1N1-019 (113536), CC-Pan H5N1-001 (114371), and Q-Pan H9N2-001 (116358)]***. The samples were collected from subjects 18-64 years of age (19-40 years of age for H1N1 study), who had participated in one of the 3 clinical trials and who had been administered 2 doses of the designated investigational vaccine, 21 days apart. Blood specimens were collected from each subject at pre-vaccination (D0), post-dose 1 (D21), post-dose 2 (D42), and 6-12 months after dose 1 (i.e., D182 and, for the CC-Pan-H5N1-001 study only, D385, since this study also had a follow-up blood collection at D385). For each of these 3 studies, a serology sub-cohort will be generated specifying the subjects whose serum samples will be evaluated in serological assays. The sub-cohorts will each be comprised of approximately 60 subjects from each adult study (i.e., approximately 30 subjects administered the adjuvanted standard dose vaccine candidate together with approximately 30 subjects administered the unadjuvanted standard dose vaccine candidate, and matched to the adjuvanted group by age and study center).

The study will also assess immune response to the HA stalk in a group of children 6-35 months of age who had no HI antibodies (titer <10) to H1N1pdm09 before they were

vaccinated with 2 doses of adjuvanted ($AS03_B$) Q-PAN H5N1 vaccine (half adult dose, i.e., 1.9 µg) ***or with placebo***. This will allow an evaluation of the vaccine's potential to elicit anti-H1 HA stalk reactive antibodies in those children who were anti-H1N1 HI negative at baseline (i.e., in the absence of priming due to prior exposure to H1N1). For this pediatric H5N1 cohort, samples will be analyzed from 4 timepoints (D0, D21, D42, and D385) collected from approximately 30 subjects from the adjuvanted vaccine treatment group ***and 30 subjects from the placebo group***.

In addition, serum samples from the adults subjects 18 to 40 years of age enrolled in group C and G of the Q-Pan-005 study will be used to describe the anti HA stalk response following a heterologous booster dose of an adjuvanted pandemic vaccine (i.e. an adjuvanted monovalent A/turkey/Turkey/1/05 (H5N1) vaccine) administered to subjects primed 18 months earlier with an adjuvanted monovalent A/Indonesia/5/05 (H5N1) vaccine. The anti HA stalk response after an homologous booster dose of the adjuvanted monovalent A/turkey/Turkey/1/05 (H5N1) vaccine administered to subjects primed with the same vaccine 12 months earlier will also be described. Samples from the H5N1-012 study will also be used to measure the antibodies directed against the H1 HA stalk domain elicited by an adjuvanted H5N1 vaccine booster dose (A/Vietnam/1194/2004 like or A/Indonesia/5/2005 like) administered 12 months after an homologous or an heterologous adjuvanted priming dose (A/Vietnam/1194/2004) in subjects 18-60 years of age. The analysis will be stratified by age (18-30 years vs 31-60 years) to evaluate the age effect on the immune response. These assessment will be informative for designing the E-SUIV-001 Phase 1 study in which the vaccine schedules are planned to be similar to the ones of the Q-Pan-H5N1-005 and the H5N1-012 study.

To assess the anti HA stalk response that could be observed in subjects potentially exposed to the H1N1pdm09 strain and who are vaccinated with an IIV4, pre-vaccination (Day 0) and post-vaccination (Day 21) serum samples of subjects 18-≤39 years of age who were vaccinated with an IIV4 (Fluarix Quadrivalent, also called D-QIV) in the FLU D-QIV-015 study will also be assessed. The results of this assessment will be informative for the design of the E-SUIV-001 study in which a quadrivalent inactivated influenza vaccine (IIV4) will serve as control.

To evaluate whether a post-vaccination boost in anti-H1 stalk ELISA antibody titers exhibits cross reactivity to diverse influenza A Group 1 subtype viruses, pre-,post-vaccination, and final timepoint (for persistence) samples from all subjects who received an adjuvant system (AS) vaccine will be tested for reactivity with H2 and H18 full length recombinant hemagglutinin proteins. This will also be assessed on the pre- and post-vaccination samples of the FLU D-QIV-015 study cohort.

To evaluate whether a post-vaccination boost in ELISA antibody titers could also result in neutralization of target virus, all samples from subjects who received an adjuvant system (AS) vaccine, will be further analyzed with an anti-H1 stalk domain microneutralization (MN) assay (target virus is cH6/1N5, i.e., any antibody mediated

neutralization is expected to arise from antibody binding to the H1 stalk domain only, because the HA head domain and the NA proteins are "exotic" and most humans have not been exposed to them). To assess the breadth of neutralization effected by anti-H1 stalk ELISA antibodies, samples ***pre-, and post-vaccination*** from ***all*** subjects ***who received an adjuvanted vaccine*** in each study cohort will also be tested in a second MN assay with a reverse genetics (RG) reassortant heterologous HA Group 1 influenza virus (H5N8)***, with an avian-like swine H1N1 virus (A/Swine/Jiangsu/40/2011) and with a H1N1 pdm09 like virus. This will also be assessed on the pre- and post-vaccination samples of the FLU D-QIV-015 study cohort.***

As an exploratory analysis of the influenza A group 2 HA stalk (i.e., H3) reactive response and based on the experience that will be acquired in testing group 1 anti-H1 stalk responses mentioned above, serum samples of subjects vaccinated with a H7N9 adjuvanted or unadjuvanted pandemic vaccine in the Q-Pan-H7N9-AS03-001 study will also be tested for the antibody response by ELISA and microneutralization assay. Furthermore, D0, D42, and Month 12 (for persistence) samples from all subjects who received the adjuvanted vaccine will be tested for reactivity with H4 and H10 full length recombinant hemagglutinin (HA) proteins. This analysis will also assess the performance of the influenza A anti-H3 stalk assays. Approximately 60 subjects (i.e. approximately 30 subjects having received the adjuvanted standard candidate vaccine dose and approximately 30 subjects having received the unadjuvanted standard candidate vaccine dose) will be randomly selected to generate the serology sub-cohort in which both groups will match in terms of age and center.

The microneutralization testing will be done at Icahn School of Medicine at Mount Sinai (ISMMS) and the ELISAs will be performed at Neomed Laboratories. Since Neomed is currently developing and validating ELISAs for this study, any ELISAs already completed at ISMMS may be reanalyzed using the Neomed-validated ELISAs to ensure that serum samples have been tested with the same set of ELISAs to assure consistency of assay results across samples from different studies. Testings will be performed step wise, according to antigen, assay and samples availability at the assigned laboratory. Based on the results of evaluations, the laboratory analyses may be further extended to include additional assays and/or assay types to further assess the anti-influenza virus antibody response elicited by vaccination, provided the additional assays and/or assay types are in compliance with the informed consent granted by subjects in the primary studies with regards to the use of serum samples

- **Study groups:** There will be 15 study groups, as described in Table 1

Table 1 Study groups and epochs foreseen in the study

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples	Age range of enrolled	Study Group (treatment groups	§Number of subjects to be randomly	Number of subjects in ATP-I

will be analyzed	subjects (yrs)	to be sampled)	selected from ATP-I or Persistence cohort	cohort (i.e, up to D42)
Q-Pan H1N1-019 (113536) (A/California/7/2009)	19–40	***Group* E** 15 µg HA (no AS)	20-30	91
		***Group* F** 3.75 µg HA/AS03$_A$	20-30	91
CC-Pan H5N1-001 (114371) (A/Indonesia/5/2005 RG)	18-49	***Group* A** 3.75 µg HA/AS03$_A$	20-30	124
		***Group* B** 15 µg HA (no AS)	20-30	50
Q-Pan H9N2-001 (116358) (A/chicken/Hong Kong/G9/1997 NIBRG-91)	18-64	***Groups* *375_A_VVP and 375_A_VVV** 3.75 µg HA/AS03$_A$	20-30	55
		***Groups* *1500_VVP and 1500_VVV** 15 µg HA (no AS)	20-30	56
Q-Pan H5N1-AS03-021 (114464) (A/Indonesia/5/2005 RG)	6-35 months	***Group A**:*** 1.9 µg HA/AS03$_B$ ***(at Day 0 and Day 21)***	20-30	182
		Group B**: Placebo (at Day 0 and Day 21)	***20-30***	***67***
Q-Pan-005 (110624)	***18-40***	***Group C: 3.8 µg A/Indonesia/5/05 (H5N1) with AS03$_A$ on D0; PBS preserved with 20 ppm thimerosal on Day 182; 3.8 µg A/turkey/Turkey/1/05 (H5N1) with AS03$_A$ on Day 549***	***All (~ 30)***	***N=variable depending on timepoint***
		Group G: PBS preserved with 20 ppm thimerosal on Day 0; 3.8 µg A/turkey/Turkey/1/05 (H5N1) with AS03$_A$ on Days 182 and 549	***All (~ 30)***	***N=variable depending on timepoint***
H5N1-012 (107495)) A/Vietnam/1194/2004-like or A/Indonesia/05/2005-like	***18-60***	***Group VT/VT/12M: Two administrations of the adjuvanted (AS03$_A$) pandemic***	***All (~60)***	***N=variable depending on timepoint for ATP persistency***

		influenza vaccine containing the Vietnam (VT) strain at Day 0 and Month 12		
		Group VT/IN/12M: One administration of the adjuvanted ($AS03_A$) pandemic influenza vaccine containing the Vietnam (VT) strain at Day 0 and one administration of the adjuvanted ($AS03_A$) pandemic vaccine containing the Indonesia (IN) strain at Month 12	*All (~60)*	*N=variable depending on timepoint for ATP persistency*
FLU D-QIV-015 (201251) (A/Christchurch/16/2010 (H1N1)pdm09 A/Texas/50/2012 (H3N2) B/Massachusetts/02/2012 B/Brisbane/60/2008)	*18-≤39*	*15 µg HA (no AS) of each of 4 strains (total 60 µg HA) at Day 0*	*Approximately 30 subjects 18-≤39y from DQIV-IP group*	*47 subjects 18-≤39y (in DQIV-IP group)*
Q-Pan H7N9-AS03-001 (201072) (A/Shanghai/2/2013(H7N9)-RG32A (H7N9))	*18–64*	*Group 1500: 15 µg HA (no AS) at Day 0 and Day 21*	*20-30*	*56*
		Group 375_A 3.75 µg HA/$AS03_A$ at Day 0 and Day 21	*20-30*	*56*

FLU Q-Pan H1N1-019: Group E: = co-administration of 15 µg HA (no AS) A/California vaccine and saline placebo on Day 0 followed by 15 µg HA (no AS) A/California vaccine on Day 21 and TIV on Day 42
FLU Q-Pan H1N1-019: Group F = co-administration of 3.75 µg A/California vaccine adjuvanted with $AS03_A$ and saline placebo on Day 0 followed by 3.75 µg A/California vaccine adjuvanted with $AS03_A$ on Day 21 and TIV on Day 42
FLU CC-Pan H5N1-001: Group A = 3.75 µg HA CC-PAN H5N1 vaccine adjuvanted with $AS03_A$ given at Day 0 and Day 21
FLU CC-Pan H5N1-001: Group B = 15 µg HA (no AS) CC-PAN H5N1 vaccine given at Day 0 and Day 21
FLU Q-Pan H9N2-001: Group 375_A_VVP = 3.75 µg HA H9N2 vaccine antigen adjuvanted with AS03A given at Day 0 and Day 21; saline placebo at Day 182
FLU Q-Pan H9N2-001: Group 375_A_VVV = 3.75 µg HA H9N2 vaccine antigen adjuvanted with $AS03_A$ given at Day 0, Day 21, and Day 182
FLU Q-Pan H9N2-001: Group 1500_VVP = 15 µg HA (no AS) H9N2 vaccine given at Day 0 and Day 21; saline placebo at Day 182
FLU Q-Pan H9N2-001: Group 1500_VVV = 15 µg HA (no AS) H9N2 vaccine given at Day 0, Day 21, and Day 182
FLU D-QIV-IP: ***Subjects in study FLU D-QIV-015 who received D-QIV (Fluarix Quadrivalent) manufactured with an investigational process (IP). D-QIV-IP is the IIV4 manufactured in Dresden (FLU D-QIV) with an optimized manufacturing process. FLU D-QIV IP has demonstrated to be non-inferior in terms of immunogenicity to FLU-QIV manufactured with the previously licensed manufacturing process.***
Details for studies FLU Q-Pan H5N1-AS03-021, FLU Q-Pan-005, and FLU Q-Pan-H7N9-AS03-001 are provided

in the Table.

§ Exact number of subjects per treatment uncertain, but likely to be in this range

* From D0, D21, D42, and D182 time points (no D385)

** From D0, D21, D42, and D385 time points (no D182)

- **Blinding:** Laboratory staff conducting the testing will have knowledge of the subject number, treatment received, and specimen time-point for every specimen tested

- **Randomization:** The primary studies were randomized and the archived serum samples from these studies that will be retrospectively analyzed in the current study will be subjected to a sub-randomization procedure prior to selection for inclusion in the serology analysis.

  The target is to randomly select approximately 30 subjects from each of the 3 adult studies ***(Q-Pan H1N1-019, CC-Pan H5N1-001, and Q-Pan H9N2-001)*** who:: 1) received the adjuvanted standard dose vaccine candidate, and 2) were in the ATP-I and Persistence cohort (depending on the study) of the completed studies, and 3) have valid vaccine homologous HI result available at all required timepoints where HI test is done and MN result available at Day 0 and 21 (for H5N1 and H9N2 studies only). Within each study cohort, after subjects are selected from the adjuvanted (AS) group, subjects who received the unadjuvanted standard dose vaccine candidate will be matched (1:1) by subject age (<30 years and ≥ 30 years) and study center to the selected subjects from the adjuvanted vaccine group. Such matched pairs will then be checked to confirm if they have a sample with adequate volume at every time point. ***Furthermore, subjects assigned to the CMI and MN subset in study H9N2 and subject with available homologous MN result in the CC-H5N1-001 study should be preferentially selected from adjuvant group***. In order to select 3 independent study cohorts of approximately 60 subjects each, allocated 1:1 to an adjuvanted or unadjuvanted formulation, ~40 subjects from the AS group who meet the above criteria in each study (note that criterion #3 for MN result is for the H5N1 and H9N2 studies only), will be randomly selected. However, if fewer than 40 subjects meet the above selection criteria, then all the available subjects will be selected.

  For the pediatric H5N1 study, ~ 40 subjects (6- 35 months of age) belonging to both ATP-I and Persistence cohort (Month 12), who have received the adjuvanted standard dose vaccine, were seronegative for H1N1pdm09 HI at Day 0, and have valid vaccine homologous HI and MN results available at either Day 0, D21, D42 or D385 will be selected. If fewer than 40 subjects meet the above selection criteria, then all the available subjects will be selected. ***Subjects from the placebo group in the 6-35 months of age range who were seronegative for H1N1pdm09 HI at Day 0 and belonging to both ATP-I and Persistence cohorts (Month 12) with blood sample available at required timepoints will be selected***.

For the adult Q-Pan-005 study, all evaluable serum specimens (in the ATP cohort for immunogenicity) from Day 0, 42, 182, 224, 549, 591 and 729 of the 18 to 40 years of age subjects enrolled in group C and G in the Q-Pan-005 (110624) study and for the H5N1-012 study, all evaluable serum samples (in the ATP cohort for immunogenicity) from Day 0, 21, M6, M12, M12+21 days and M18 of the subjects enrolled in the VT/VT/12M and the VT/IN/12M groups will be used (i.e. no random selection for the sub-cohort).

For the adult FLU D-QIV-015 study, approximately 30 samples (in the ATP cohort for immunogenicity) pre-vaccination (Day 0) and post-vaccination (Day 21) from subjects 18-≤ 39 years who received D-QIV-IP, will be assessed. D-QIV-IP is the IIV4 manufactured in Dresden (FLU D-QIV) with an optimized manufacturing process. FLU D-QIV IP has demonstrated to be non-inferior in terms of immunogenicity to FLU-QIV manufactured with the previously licensed manufacturing process.

For exploratory analysis of samples from the adult Q-Pan-H7N9 study: approximately 30 subjects having received the adjuvanted standard H7N9 candidate vaccine dose and approximately 30 subjects having received the unadjuvanted standard candidate vaccine dose will be randomly selected to generate the serology sub-cohort in which both groups will match in terms of age and center. First, the subjects from the adjuvanted group will be selected. These subjects will have had to be included in the ATP-I and persistency cohorts of the primary study and have homologous HI results available for most of the applicable timepoints. Preference will be given to subjects who also have available results for homologous MN. Once these subjects are selected, the non-adjuvanted group will be selected to match the adjuvanted group by age (<30 years and ≥ 30 years) and by center. In order to select the study cohort, allocated 1:1 to the adjuvanted or the unadjuvanted group ~ 40 subjects from the AS group who meet the above criteria will be selected.

Please note that analysis to be performed and table to be presented for the exploratory mice experiment will be presented separately as it will be done by pre-clinical statistical team.

The following group names and description will be used for the statistical analyses:

Group order in tables	Group label in tables	Group definition for footnote
1	H1N1_AS	Q-Pan-H1N1 (A/California/7/2009) 3.75 µg + $AS03_A$ vaccine at D0 and D21 and TIV at D42 in H1N1-019 study
2	H1N1_NAS	Q-Pan-H1N1 (A/California/7/2009) 15 µg vaccine at D0 and D21 and TIV at D42 in H1N1-019 study
3	H5N1_AS	CC-PAN H5N1 (A/Indonesia/5/2005 RG) 3.75 µg +

		$AS03_A$ vaccine at D0 and D21 in H5N1-001 study
4	H5N1_NAS	CC-PAN H5N1 (A/Indonesia/5/2005 RG) 15 µg vaccine at D0 and D21 in H5N1-001 study
5	H9N2_AS	Q-Pan H9N2 (A/chicken/Hong Kong/G9/1997 NIBRG-91) 3.75 µg + $AS03_A$ vaccine at D0 and D21 in H9N2-001 study (375_A_VVP + 375_A_VVV group)
6	H9N2_NAS	Q-Pan H9N2 (A/chicken/Hong Kong/G9/1997 NIBRG-91) 15 µg vaccine at D0 and D21 in H9N2-001 study (1500_VVP + 1500_VVV group)
7	H5N1_PAS	Q-PAN H5N1 (A/Indonesia/5/2005 RG) 1.90 µg + $AS03_B$ vaccine at D0 and D21 in pediatric Q-PAN-H5N1-AS03-021 study
8	***H5N1_PCN***	***Placebo at D0 and D21 in pediatric Q-PAN-H5N1-AS03-021 study***
9	***H7N9_AS***	***Q-PAN H7N9 (A/Shanghai/2/2013(H7N9)-RG32A)3.75 µg + $AS03_A$ vaccine at D0 and D21 in Q-PAN-H7N9-AS03-001 study***
10	***H7N9_NAS***	***Q-PAN H7N9 (A/Shanghai/2/2013(H7N9)-RG32A)15 µg vaccine at D0 and D21 in Q-PAN-H7N9-AS03-001 study***
11	***DQIV_NAS***	***Seasonal 15 µg vaccine at D0 in D-QIV-015 study***
12	***QPAN5_C***	***Q-PAN H5N1 (A/Indonesia/5/05) 3.8 µg + $AS03_A$ on D0, placebo on Day 182; (A/turkey/Turkey/1/05) 3.8 µg + $AS03_A$ on Day 549 in Q-Pan-005 study***
13	***QPAN5_G***	***Placebo on Day 0, 3.8 µg A/turkey/Turkey/1/05 (H5N1) with $AS03_A$ on Days 182 and 549 in Q-Pan-005 study***
14	***H5N1_VT***	***Adjuvanted ($AS03_A$) pandemic Vietnam strain influenza vaccine at Day 0 and Month 12 in H5N1-012 study***
15	***H5N1_IN***	***Adjuvanted ($AS03_A$) pandemic Vietnam strain at Day 0 and adjuvanted ($AS03_A$) pandemic Indonesia influenza strain at Month 12 in H5N1-012 study***

3. OBJECTIVES

3.1. Co-Primary objectives

With respect to samples from the HA Group 1-related studies (i.e., with H1N1, H5N1 and H9N2 pandemic, and IIV4 seasonal, influenza vaccines)

- To describe the anti-H1 stalk ELISA antibody levels:
  - ***In adult subject samples of the CC-Pan H5N1-001, the Q-Pan H1N1-019 and the Q-Pan H9N2-001 study cohorts, at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), Day 182 (D182) and at Day 385 (D385) for the CC-Pan H5N1-001 study cohort, by treatment group (unadjuvanted or adjuvanted vaccine)***
  - In pediatric subject samples ***of the Q-Pan H5N1-AS03-21 study cohort,*** at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), and D385 in adjuvanted vaccine group ***and in the placebo group***
  - ***In adult subject samples of the Q-Pan-005 study cohort (groups C and G) at D0, D42, D182, D224, D549, D591, and D729***
  - ***In adult subject samples of the H5N1-012 study cohort (groups VT/VT/12M and VT/IN/12M) at D0, D21, M6, M12, M12+21 days, and M18***
  - ***In the adult samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***
- To describe the anti-H1 stalk microneutralization (MN) antibody levels:
  - ***In adult subject samples of the CC-Pan H5N1-001, the Q-Pan H1N1-019 and the Q-Pan H9N2-001 study cohort, at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), D182, and at D385 (CC-Pan H5N1-001 study cohort only) from subjects who received an adjuvant system (AS) vaccine.***
  - ***In pediatric subject samples of the Q-Pan H5N1-AS03-21study cohort, at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), and at D385 from subjects who received an adjuvant system (AS) vaccine***
  - ***In the adult samples of the Q-Pan-005 study cohort at D0, D 42, D182, D549, D591 and D729 for group C and at D182, D224, D549, D591 and D729 for group G***
  - ***In adult subject samples of the H5N1-012 study cohort (groups VT/VT/12M and VT/IN/12M) at D0, D21, M6, M12, M12+21 days, and M18***
  - ***In the adult samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***

- To describe the anti-H2 and anti-H18 antibody levels:
  - ***In samples from all subjects who received an adjuvanted vaccine in the CC-Pan H5N1-001, the Q-Pan H1N1-019, the Q-Pan H9N2-001 adult study cohorts and in the Q-Pan H5N1-AS03-21 pediatric study cohort at baseline (D0), post-dose 2 (D42) and final timepoint (for persistence)***
  - ***In adult subject samples of the Q-Pan-005 study cohort at D0, D42, D182, D549, D591, and D729 for group C and at D182, D224, D549, D591, and D729 for group G***
  - ***In adult subject samples of the H5N1-012 study cohort (groups VT/VT/12M and VT/IN/12M) at D0, D21, M12, M12+21 days, and M18***
  - ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***
- To describe the ***vaccine heterosubtypic*** virus MN antibody level
  - ***In baseline (D0) and post-dose 2 (D42) samples from all subjects who received an adjuvanted vaccine in the adult CC-Pan H5N1-001, the Q-Pan H1N1-019, the Q-Pan H9N2-001 and the pediatric Q-Pan H5N1-AS03-21 study cohorts***
  - ***In adult subject samples of the Q-Pan-005 study cohort at D0, D42, D549, and D591 for group C and at D182, D224, D549, and D591 for group G***
  - ***In adult subject samples of the H5N1-012 study cohort (groups VT/VT/12M and VT/IN/12M) at D0, D21, M12, and M12+21 days***
  - ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***

3.2. Secondary objectives

With respect to samples from the HA Group 1-related studies (i.e., with H1N1, H5N1 and H9N2 pandemic, and IIV4 seasonal, influenza vaccines):

- To assess the effect of adjuvant in ***the CC-Pan H5N1-001, the Q-Pan H1N1-019 and the Q-Pan H9N2-001 adult study cohorts*** in terms of the adjusted anti-H1 stalk ELISA GMT ratio (AS group/no AS group) at D21, D42, D182, (for all 3 study ***cohorts***) and D385 (for the ***CC-Pan-H5N1-study cohort***), and in terms of the difference in percentage of subjects (AS group minus no AS group) with a ≥4-fold rise from Day 0 to Day 21 and D42, D182, (for all 3 study ***cohorts***) and D385 (***for the CC-Pan-H5N1-study cohort***)
- ***To describe the baseline seropositivity (SP) by hemagglutination inhibition (HI) assay to the pandemic vaccine homologous virus for all subjects and the baseline SP by HI assay to A/California/7/09 (or a like virus) for subjects in the CC-Pan***

H5N1-001, Q-Pan H9N2-001, Q-Pan-005, and H5N1-012 study cohorts (baseline will be Day 0, but for group G of the Q-Pan-005 study cohort only, baseline will be Day 182)

3.3. Tertiary objective

With respect to samples from the HA Group 1-related studies (i.e., with H1N1, H5N1 and H9N2 pandemic, and IIV4 seasonal, influenza vaccines):

- To describe the anti-N1 NA ELISA antibody levels at D0, D21, D42, and D182 for subjects ***in the Q-PAN-H1N1-019 study cohort***, by treatment group
- To assess the effect of adjuvant in the ***Q-PAN-H1N1-019*** study cohort in terms of the adjusted anti-N1 NA ELISA GMT ratio (AS group/no AS group) at D21, D42, D182, and in terms of the difference in percentage of subjects (AS group minus no AS group) with a ≥4-fold rise from Day 0 to Day 21 and D42, D182.
- To explore the correlation between the level of neutralizing antibody to the H1 stalk with the level of vaccine homologous neutralizing antibody, at Day 0 and 21 in the adult ***Q-Pan-H9N2-001 and CC-Pan-H5N1-001 study cohorts and in the pediatric Q-Pan H5N1-AS03-021*** study cohorts (AS group only)
- To explore the effect of being seropositive by HI test to the vaccine homologous virus at D0 on the MGI (D21/D0) of anti-H1 stalk ELISA antibody in the adult H5N1, H9N2 and H1N1 study cohorts
- To explore the cell mediated immune response to H9N2 vaccine with respect to T cells, B memory cells and plasmablasts reactive with H9N2 and related antigens at Days 0, 7, 21, and 28 in selected vaccine groups ***of the Q-Pan-H9N2-001 study cohort***
- To further characterize the humoral immune response to H9N2 vaccine ***in the Q-Pan-H9N2-001 study cohort*** by ELISA using the purified recombinant viral proteins (H9 HA head domain, H9 full length, N2)

With respect to samples from the HA group 2-related study cohort (H7N9 study) (adult subjects):

- ***To describe the anti-H3 stalk ELISA antibody levels:***
  - ***At baseline Day 0 (D0), post-dose 1 at Day 21 (D21), post-dose 2 at Day 42 (D42), Month 6, and Month 12 by treatment group (unadjuvanted or adjuvanted vaccine), from the primary completed H7N9 study***
- ***To describe the anti-H3 stalk microneutralization (MN) antibody levels (target virus: cH14/3Nx):***
  - ***At baseline Day 0 (D0), post-dose 1 at Day 21 (D21), post-dose 2 at Day 42 (D42), Month 6, and Month 12 for subjects who received adjuvanted vaccine***

- ***To describe the anti-H4 and anti-H10 antibody levels at baseline (D0), post-dose 2 (D42), and Month 12 (for persistency) in all subjects from the H7N9 study cohort who received an adjuvanted vaccine***
- ***To describe the heterosubtypic virus MN antibody level (target virus: H4N8) at baseline (D0) and post-dose 2 (D42) in all subjects who received an adjuvanted vaccine***
- ***To assess the effect of adjuvant in the adult H7N9 study cohort in terms of the adjusted anti-H3 stalk ELISA GMT ratio (AS group/no AS group) at D21, D42, Month 6, and Month 12, and in terms of the difference in percentage of subjects (AS group minus no AS group) with a ≥4-fold rise from Day 0 to Day 21 and D42, Month 6, and Month 12***

Please note that the tertiary objectives related to mice experiment will be presented in separate SAP as it will be performed by pre-clinical statistical team.

4. ENDPOINTS

4.1. Primary endpoints

With respect to samples from the HA Group 1-related studies (i.e., with H1N1, H5N1, and H9N2 pandemic, and IIV4 seasonal, influenza vaccines):

1. Levels of anti-H1 stalk antibody by ELISA for all the subjects in each study cohort. The following aggregate variables will be calculated with 95% CI for each treatment group within each study cohort:
   - For adult subject samples ***from the CC-Pan H5N1-001, Q-Pan H1N1-019, and Q-Pan H9N2-001 study cohorts):***
     - Seropositive rate at Day 0, 21, 42, and 182 (and at Day 385 for H5N1 study cohort only)
     - Geometric mean titer (GMT) at Day 0, 21, 42, and 182 (and at Day 385 for H5N1 study cohort only)
     - Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21 ***and from Day 0 to Day 42***
     - ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 21 and from Day 0 to Day 42***
     - Mean geometric increase (MGI) at Day 21, 42, and 182 (and at Day 385 for H5N1 study cohort only) compared to Day 0
   - For pediatric subject samples ***from the Q-Pan H5N1-AS03-21*** cohort:
     - Seropositive rate at Day 0, 21, 42 and at Day 385

- Geometric mean titer (GMT) at Day 0, 21, 42 and at Day 385
- Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21 ***and from Day 0 to Day 42***
- ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 21 and from Day 0 to Day 42***
- Mean geometric increase (MGI) at Day 21, 42 and at Day 385 compared to Day 0

- ***For adult subject samples from the Q-Pan-005 study cohort, groups C and G:***
  - ***Seropositive rate at Day 0, 42, 182, 224, 549, 591, and at Day 729***
  - ***Geometric mean titer (GMT) at Day 0, 42, 182, 224, 549, 591, and at Day 729***
  - ***Percentage of subjects with a ≥4-fold rise***
    - ***For group C: From Day 0 to Day 42, from Day 549 to Day 591 and from Day 0 to Day 591***
    - ***For group G: From Day 182 to Day 224, from Day 549 to Day 591 and from Day 182 to Day 591***
  - ***Percentage of subjects with a ≥10-fold rise***
    - ***For group C: From Day 0 to Day 42, from Day 549 to Day 591 and from Day 0 to Day 591***
    - ***For group G: From Day 182 to Day 224, from Day 549 to Day 591 and from Day 182 to Day 591***
  - ***Mean geometric increase (MGI) at Day 42, 182, 224, 549, 591, and at Day 729 compared to Day 0 for group C and for Day 224, 549, 591 and Day 791 compared to Day 182 for group G***
- ***For adult subject samples from the H5N1-012 study cohort (groups VT/VT/12M and VT/IN/12M):***
  - ***Seropositivity rate at Day 0, 21, M6, M12, M12+21days and M18***
  - ***GMT at Day 0, 21, M6, M12, M12+21days, and M18***
  - ***Percentage of subjects with a ≥ 4-fold rise from Day 0 to Day 21, from M12 to M12+21days, and from Day 0 to M12+21days***
  - ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 21, from M12 to M12+21days, and from Day 0 to M12+21days***
  - ***MGI at Day 0, 21, M6, M12, M12+21, and M18***
- ***For adult subject samples from the FLU D-QIV-015 study cohort:***

- ***Seropositive rate at Day 0 and 21***
- ***Geometric mean titer (GMT) at Day 0 and 21***
- ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
- ***Mean geometric increase (MGI) at Day 21 compared to Day 0***

2. Levels of anti-H1 stalk antibody by microneutralization (MN) for the subjects who received an adjuvant system (AS) vaccine in each study cohort. The following aggregate variables will be calculated with 95% CI:

- For adult subject samples from the ***CC-Pan H5N1-001, Q-Pan H1N1-019, and Q-Pan H9N2-001 study cohorts***:
  - Seropositive rate at Day 0, 21, 42, and 182 (and at Day 385 for H5N1 study cohort only)
  - Geometric mean titer (GMT) at Day 0, 21, 42, and 182 (and at Day 385 for H5N1 study cohort only)
  - Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21 ***and from Day 0 to Day 42***
  - ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 21 and from Day 0 to Day 42***
  - Mean geometric increase (MGI) at Day 21, 42, and 182 (and at Day 385 for H5N1 study cohort only) compared to Day 0
- For pediatric subject samples from the ***Q-Pan H5N1-AS03-21 study cohort***:
  - Seropositive rate at Day 0, 21, 42 and at Day 385
  - Geometric mean titer (GMT) at Day 0, 21, 42 and at Day 385
  - Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21 ***and from Day 0 to Day 42***
  - ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 21 and from Day 0 to Day 42***
  - Mean geometric increase (MGI) at Day 21, 42 and at Day 385 compared to Day 0
- ***For adult subject samples from the Q-Pan-005 study cohort:***
  - ***Seropositive rate at Day 0, 42, 182, 549, 591 and 729 for group C and at Day 182, 224, 549, 591 and 729 for group G)***
  - ***Geometric mean titer (GMT) at Day 0, 42, 182, 549, 591 and 729 for group C and at Day 182, 224, 549, 591 and 729 for group G***
  - ***Percentage of subjects with a ≥4-fold rise***

- ***For group C: From Day 0 to Day 42, from Day 549 to Day 591and from Day 0 to Day 591***
- ***For group G: From Day 182 to Day 224, from Day 549 to Day 591 and from Day 182 to Day 591***

– ***Percentage of subjects with a ≥10-fold rise***

- ***For group C: From Day 0 to Day 42, from Day 549 to Day 591 and from Day 0 to Day 591***
- ***For group G: From Day 182 to Day 224, from Day 549 to Day 591 and from Day 182 to Day 591***

– ***Mean geometric increase (MGI) at Day 42, 182, 549, 591 and 729 compared to Day 0 for group C and at Day 224, 549, 591 and 729 compared to Day 182 for group G***

- ***For adult subject samples from the H5N1-012 study cohort (groups VT/VT/12M and VT/IN/12M):***
  - ***Seropositivity rate at Day 0, 21, M6, M12, M12+21days, and M18***
  - ***GMT at Day 0, 21, M6, M12, M12+21days, and M18***
  - ***Percentage of subjects with a ≥ 4-fold rise from Day 0 to Day 21, from M12 to M12+21days, and from Day 0 to M12+21days***
  - ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 21, from M12 to M12+21days, and from Day 0 to M12+21days***
  - ***MGI at Day 0, 21, M6, M12, M12+21, and M18***
- ***For adult subject samples from FLU D-QIV-015 cohort:***
  - ***Seropositive rate at Day 0 and 21***
  - ***Geometric mean titer (GMT) at Day 0 and 21***
  - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
  - ***Mean geometric increase (MGI) at Day 21 compared to Day 0***

3. Levels of anti-H2 and anti-H18 antibody by ELISA for the subjects who received an AS vaccine ***in each*** study cohort ***and for the subjects in the FLU D-QIV-015 study cohort***. The following aggregate variables will be calculated with 95% CI:
   - ***For samples from subjects in the adult CC-Pan H5N1-001, Q-Pan H1N1-019, Q-Pan H9N2-001 and pediatric Q-Pan H5N1-AS03-21 study cohorts:***
     – Seropositive rate at Day 0, 42, ***and final timepoint (for persistence) (i.e., Day 182 for the Q-Pan-H1N1-019, Q-PAN-H9N2-001 and Day 385 for the CC-Pan-H5N1 and Q-Pan H5N1-AS03-21 study cohorts)***

  - Geometric mean titer (GMT) at Day 0, 42, ***and final timepoint (for persistence) (i.e., Day 182 for the Q-Pan-H1N1-019, Q-PAN-H9N2-001 and Day 385 for the CC-Pan-H5N1 and Q-Pan H5N1-AS03-21 study cohorts)***
  - Percentage of subjects with a ≥4-fold rise from Day 0 to Day 42
  - Mean geometric increase (MGI) at Day 42 ***and final timepoint (for persistence)*** compared to Day 0 ***(i.e., Day 182 for the Q-Pan-H1N1-019, Q-PAN-H9N2-001 and Day 385 for the CC-Pan-H5N1 and Q-Pan H5N1-AS03-21 study cohorts)***

- ***For adult subject samples from the Q-Pan-005 study cohort:***
  - ***Seropositive rate at Day 0, 42, 549, 591, and 729 for group C and Day 182, 224, 549, 591, and 729 for group G***
  - ***Geometric mean titer (GMT) at Day 0, 42, 549, 591, and 729 for group C and Day 182, 224, 549, 591, and 729 for group G***
  - ***Percentage of subjects with a ≥4-fold rise***
    - ***For group C: From Day 0 to Day 42, from Day 549 to Day 591 and from Day 0 to Day 591***
    - ***For group G: From Day 182 to Day 224, from Day 549 to Day 591 and from Day 182 to Day 591***
  - ***Mean geometric increase (MGI) at Day 42, 549, 591, and 729 compared to Day 0 for group C and Day 224, 549, 591, and 729 compared to Day 182 for group G***
- ***For adult subject samples from the H5N1-012 study cohort (groups VT/VT/12M and VT/IN/12M):***
  - ***Seropositivity rate at Day 0, 21, M12, M12+21days, and M18***
  - ***GMT at Day 0, 21, M12, M12+21days, and M18***
  - ***Percentage of subjects with a ≥ 4-fold rise from Day 0 to Day 21, from M12 to M12+21days, and from Day 0 to M12+21days***
  - ***MGI at Day 0, 21, M12, M12+21, and M18***
- ***For adult subject samples from the FLU D-QIV-015 study cohort:***
  - ***Seropositive rate at Day 0 and 21***
  - ***Geometric mean titer (GMT) at Day 0 and 21***
  - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
  - ***Mean geometric increase (MGI) at Day 21 compared to Day 0***

4. Vaccine-heterosubtypic virus titer by microneutralization (MN) for ***all*** subjects who received an AS vaccine in the ***listed study cohorts***. The following aggregate variables will be calculated with 95% CI:

   - ***For adult subject samples from the CC-Pan H5N1-001, Q-Pan H1N1-019, Q-Pan H9N2-001, and Q-Pan H5N1-AS03-21 study cohorts***:
     - Seropositive rate at Day 0 and Day ***42***
     - Geometric mean titer (GMT) at Day 0 and Day ***42***
     - Percentage of subjects with a ≥4-fold rise from Day 0 to Day ***42***
     - Mean geometric increase (MGI) at Day ***42*** compared to Day 0
   - ***For adult subject samples from the Q-Pan-005 study cohort:***
     - ***Seropositive rate at Day 0, Day 42, Day 549 and Day 591 for group C and Day 182, Day 224, Day 549 and Day 591 for group G***
     - ***Geometric mean titer (GMT) at Day 0, Day 42, Day 549 and Day 591 for group C and Day 182, Day 224, Day 549 and Day 591 for group G***
     - ***Percentage of subjects with a ≥4-fold rise***
       - ***For group C: From Day 0 to Day 42, from Day 549 to Day 591 and from Day 0 to Day 591***
       - ***For group G: From Day 182 to Day 224, from Day 549 to Day 591 and from Day 182 to Day 591***
     - ***Mean geometric increase (MGI) at Day 42, 549,and 591 compared to Day 0 for group C and Day 224, 549, and 591 compared to Day 182 for group G***
   - ***For adult subject samples from the H5N1-012 study cohort (groups VT/VT/12M and VT/IN/12M):***
     - ***Seropositivity rate at Day 0, 21, M1, and M12+21 days***
     - ***GMT at Day 0, 21, M12, and M12+21 days***
     - ***Percentage of subjects with a ≥ 4-fold rise from Day 0 to Day 21, from M12 to M12+21days, and from Day 0 to M12+21days***
     - ***MGI at Day 0, 21, M12, and M12+21***
   - ***For adult subject samples from the FLU D-QIV-015 study cohort:***
     - ***Seropositive rate at Day 0 and 21***
     - ***Geometric mean titer (GMT) at Day 0 and 21***
     - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
     - ***Mean geometric increase (MGI) at Day 21 compared to Day 0***

4.2. Secondary endpoints

With respect to samples from the HA Group 1-related studies (i.e., with H1N1, H5N1, and H9N2 pandemic, and IIV4 seasonal, influenza vaccines):

1. Levels of anti-H1 stalk antibody by ELISA for all the subjects in the adult ***CC-Pan H5N1-001, the Q-Pan H1N1-019 and the Q-Pan H9N2-001*** study cohort. The following aggregate variables will be calculated with 95% CI to assess the effect of adjuvant relative to non-adjuvant in each study cohort at D21, D42, and 182 (and at Day 385 for the ***CC-Pan-H5N1*** study cohort only)
   - Geometric mean titer ratio (AS Group/no AS group ***within each study***)
   - Difference (AS group minus no AS group ***within each study***) of percentage in subjects with a ≥4-fold rise from Day 0
2. Levels of HI antibody to pandemic vaccine homologous virus at Day 0 in all subjects ***in all study cohorts (but Day 182 for group G of Q-Pan-005 study cohort)*** by treatment group and level of HI antibody to A/California/7/09 (or a like virus) ***in subjects from the CC-Pan-H5N1-001, Q-Pan-H9N2-001 and Q-PAN-005 and H5N1-012 study cohorts (Day 182 for group G of Q-Pan-005 and Day 0 for the other subjects)***. The following aggregate variable will be calculated with 95% CI:
   - Seropositive rate at Day 0 ***in all subjects except for group G of the Q-Pan-005 study***
   - ***Seropositive rate at Day 182 for group G of the Q-Pan-005 study cohort***

4.3. Tertiary endpoints

With respect to samples from the HA Group 1-related studies (i.e., with H1N1, H5N1, and H9N2 pandemic, and IIV4 seasonal, influenza vaccines:

- ***Levels of anti-N1 NA antibody by ELISA for subjects in the H1N1 study cohort. The following aggregate variables will be calculated with 95% CI:***
  - ***Seropositive rate at Day 0, 21, 42, 182***
  - ***Geometric mean titer (GMT) at Day 0, 21, 42, 182***
  - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21, Day 42 and Day 182***
  - ***Mean geometric increase (MGI) at Day 21, 42, 182 compared to Day 0***
- ***Levels of anti-N1 NA antibody by ELISA for subjects in the H1N1 study cohort with respect to treatment group. The following aggregate variables will be***

calculated with 95% CI to assess the effect of adjuvant relative to non-adjuvant at D21, D42, and 182

- ***Geometric mean titer ratio (AS Group/no AS group)***
- ***Difference (AS group minus no AS group) of percentage in subjects with a ≥4-fold rise from Day 0***

- Levels of vaccine homologous neutralizing antibody and levels of anti-H1 stalk antibody by microneutralization at Day 0 and 21 for the subjects who received an adjuvant system (AS) vaccine in both the adult and pediatric H5N1 and H9N2 study cohorts. The following aggregate variable will be calculated with 95% CI:
  - Correlation between the level of neutralizing antibody to the H1 stalk with the level of vaccine homologous neutralizing antibody at Day 0 and 21
- Vaccine-homologous virus HI titer at Day 0 and level of anti-H1 stalk antibody by ELISA at Day 0 and Day 21 in the adult H5N1, H9N2 and H1N1 study cohorts. The following aggregate variable will be calculated with 95% CI:
  - Mean geometric increase (MGI) for anti-H1 stalk ELISA at Day 21 compared to Day 0
- Cell Mediated Immunity (CMI) parameters at Day 0, 7, 21, and 28 will be evaluated for subjects in the H9N2 study cohort in terms of frequencies of:
  - Antigen-specific CD4+/CD8+ T Cells identified as CD4/CD8 T-cells producing two or more markers within CD40L, IL-2, TNF-α, IFN-γ upon in vitro stimulation using A/chicken/Hong Kong/G9/1997 (H9N2) split virus, A/California (H1N1) split virus or A/ Uruguay/716/2007 (H3N2) split virus
  - B memory cells reactive with the following antigens: A/chicken/Hong Kong/G9/1997 (H9N2) split virus ,H1 stalk domain presented as a recombinant protein chimeric HA 6/1, H9 globular HA domain presented as a recombinant protein, N2 presented as a recombinant protein if available
  - Plasmablasts reactive with the following antigens: A/chicken/Hong Kong/G9/1997 (H9N2) split virus ,H1 stalk domain presented as a recombinant protein chimeric HA 6/1, H9 globular HA domain presented as a recombinant protein, N2 presented as a recombinant protein if available
- Levels of anti-N2 NA antibody, levels of anti-H9 HA head domain antibody, and levels of anti-full length H9 HA by ELISA (A/chicken/Hong Kong/G9/1997) at Day 0, 21, and 42 for subjects in the H9N2 study cohort. The following aggregate variable will be calculated with 95% CI:
  - Seropositive rate at Day 0, 21, and 42
  - Geometric mean titer (GMT) at Day 0, 21, and 42

- Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21, and Day 42
- Mean geometric increase (MGI) at Day 21 and 42 compared to Day 0

With respect to samples from the HA Group 2-related study (i.e., from adult subjects who received H7N9 vaccine)

1. ***Levels of anti-H3 stalk antibody by ELISA for all subjects. The following aggregate variables will be calculated with 95% CI for each treatment group:***
   - ***Seropositive rate at Day 0, 21, 42, Month 6, and Month 12***
   - ***Geometric mean titer (GMT) at Day 0, 21, 42, Month 6, and Month 12***
   - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21 and from Day 0 to Day 42***
   - ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 21 and from Day 0 to Day 42***
   - ***Mean geometric increase (MGI) at Day 21, 42, Month 6, and Month 12 compared to Day 0***
2. ***Levels of anti-H3 stalk antibody by microneutralization (MN) for the subjects who received an adjuvant system (AS) vaccine. The following aggregate variables will be calculated with 95% CI:***
   - ***Seropositive rate at Day 0, 21, 42, Month 6, and Month 12***
   - ***Geometric mean titer (GMT) at Day 0, 21, 42, Month 6, and Month 12***
   - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21 and from Day 0 to Day 42***
   - ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 21 and from Day 0 to Day 42***
   - ***Mean geometric increase (MGI) at Day 21, 42, Month 6, and Month 12 compared to Day 0***
3. ***Levels of anti-H4 and anti-H10 antibody by ELISA for all subjects who received an AS vaccine. The following aggregate variables will be calculated with 95% CI:***
   - ***Seropositive rate at Day 0, 42, and Month 12 (for persistency)***
   - ***Geometric mean titer (GMT) at Day 0, 42, and Month 12***
   - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 42 and from Day 0 to Month 12***
   - ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 42 and from Day 0 to Month 12***

- ***Mean geometric increase (MGI) at Day 42 and at Month 12 compared to Day 0***

4. ***Vaccine-heterosubtypic virus titer by microneutralization (MN) for all subjects who received an AS vaccine in each study cohort. The following aggregate variables will be calculated with 95% CI:***
   - ***Seropositive rate at Day 0 and Day 42***
   - ***Geometric mean titer (GMT) at Day 0 and Day 42***
   - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 42***
   - ***Percentage of subjects with a ≥10-fold rise from Day 0 to Day 42***
   - ***Mean geometric increase (MGI) at Day 42 compared to Day 0***
5. ***Levels of anti-H3 stalk antibody by ELISA for all subjects. The following aggregate variables will be calculated with 95% CI to assess the effect of adjuvant relative to non-adjuvant at D21, D42, Month 6, and Month 12***
   - ***Geometric mean titer ratio (AS Group/no AS group)***
   - ***Difference (AS group minus no AS group) of percentage in subjects with a ≥4-fold rise from Day 0***

5. STUDY POPULATION

One cohort has been defined for analyses:

5.1.1. Total cohort

The total cohort (TC) for immunogenicity analysis will include all subjects from each study cohort with available results for this study. All analyses will be based on the total cohort. All the subjects selected are from the ATP cohort for immunogenicity of respective retrospective studies.

6. STATISTICAL METHODS

All analyses will be descriptive.

6.1. Analysis of demographics/baseline characteristics

Demographic characteristics (age at first study vaccination in years (months for the children 6-35 months of age), gender, ethnicity and geographical ancestry), and vaccination history, of each study cohort, summarized by treatment group in each study using descriptive statistics:

- Mean, median, standard deviation will be provided for continuous data such as age.
- Percentage of subjects with baseline (D0) seropositivity by HI assay to the pandemic vaccine homologous virus for all subjects
- Percentage of subjects with baseline (D0) seropositivity by HI assay to A/California/7/09 (or a like virus) in the ***CC-Pan-H5N1-001*** and ***Q-Pan-H9N2-001*** study cohort (if data become available)

6.2. Analysis of immunogenicity

6.2.1. Within groups assessment

For each study group in the each ***HA Group 1-related*** study cohort (at each time point at which the tests are done and results are available) by anti-H1 stalk ELISA for all subjects, anti-H1 stalk MN (for subjects who received an adjuvant system (AS) vaccine ***and for subjects in the D-QIV-015 study cohort)***, anti-H2 and anti-H18 full length HA by ELISA (for subjects who received an adjuvant system [AS] vaccine ***and for subjects in the D-QIV-015 study cohort), vaccine hetersubtypic MN (for subjects who received an adjuvant system [AS] vaccine and for subjects in the D-QIV-015 study cohort)***, the following analyses will be performed:

- Seropositivity rates and GMTs for anti-H1 stalk ELISA, anti-H1 stalk MN, vaccine heterologous MN, anti-H2 full length HA ELISA, anti-H18 full length HA ELISA, and anti-N1 NA ELISA (for H1N1 cohort), with exact 95% CI, will be calculated.
- MGI with 95% CI will be tabulated for anti-H1 stalk ELISA, anti-H1 stalk MN, vaccine heterologous MN, anti-H2 full length HA ELISA, anti-H18 full length HA ELISA and anti-N1 NA ELISA (for H1N1 cohort).
- Percentage of subjects with at least 4-fold increase from Day 0 ***to all applicable timepoints per endpoint*** (***from Day 182 for Group G subjects in the Q-Pan-005 study cohort***) with exact 95% CI, will be calculated ***(refer to endpoint)***
- ***Percentage of subjects with at least 10-fold increase from Day 0 to all applicable timepoints per endpoint (from Day 182 for Group G subjects in the Q-Pan-005 study cohort)*** with exact 95% CI, will be calculated ***(refer to endpoint***)
- The distribution of antibody titers for anti-H1 stalk ELISA will be displayed using reverse cumulative distribution curves.
- For AS group, the scatter plot of neutralizing antibody results to the H1 stalk with the vaccine homologous neutralizing antibody results ***at all timepoints*** will be presented to examine the correlation in study cohort H9N2 and both adult and pediatric H5N1 study cohorts. One scatter plot will be developed for each study/product. Furthermore, the correlation between the level of neutralizing antibody to the H1 stalk with the level of vaccine homologous neutralizing antibody ***at all vaccination***

timepoints may be explored using a Deming regression if needed in study cohort H9N2 and both adult and pediatric H5N1 study cohorts. However, the results should be interpreted with cautious due to small sample size and testing result variations in vaccine homologous MN and H1 stalk MN.

- the scatter plot of ELISA antibody results to the H1 stalk [recombinant trimeric cHA (cH6/1N5)] with the microneutralizing antibody results to the H1 stalk [chimeric virus (cH6/1N5)] ***at all timepoints*** will be presented to examine the correlation in all the study cohort. One scatter plot will be developed for each study/product. Furthermore, the correlation between the level of ELISA antibody with the level of microneutralizing antibody to the H1 stalk ***at all vaccination timepoints*** may be explored using a Deming regression. However, the results should be interpreted with cautious due to small sample size and testing result variations in H1 stalk ELISA and H1 stalk MN.
- For seropositive subjects by HI assay to the vaccine homologous virus at D0, MGI at Day 21 compared to Day 0 by anti-H1 stalk ELISA (with 95% CI) will be tabulated in the adult H5N1, H9N2 and H1N1 study cohort.
- CMI summaries will be generated for the H9N2 cohort only. The frequency of the response for B-cell, plasma-cell and T-cell elicited by vaccine components measured in a sub-cohort of subjects at Days 0, 7, 21 and 28 will be described according to the technical specifications provided by R&D (Clinical Data – Information Sheet).
- For H9N2 study cohort only, SP, GMT, MGI, and percentage of subjects with ≥4-fold rise from Day 0 to Day 21 and 42 by anti-N2 NA antibody, anti-H9 HA head domain antibody, and anti-full length H9 HA antibody (with exact 95%CI) will be calculated.

For each study group in HA Group 2 -related study H7N9 cohort (at each time point at which the tests are done and results are available), anti-H3 stalk ELISA for all subjects, anti-H3 stalk MN, anti-H4 HA full length ELISA, anti-H10 HA full length ELISA and vaccine heterologous MN antibody levels (for subjects who received an adjuvant system [AS] vaccine), the following analyses will be performed:

- ***Seropositivity rates and GMTs for anti-H3 stalk ELISA, anti-H3 stalk MN, anti-H4 full length HA ELISA, anti-H10 HA full length ELISA and vaccine heterologous MN, with exact 95% CI, will be calculated.***
- ***MGI with 95% CI will be tabulated for anti-H3 stalk ELISA, anti-H3 stalk MN, anti-H4 HA full length ELISA, anti-H10 HA full length ELISA and vaccine heterologous MN***
- ***Percentage of subjects with at least 4-fold increase from Day 0 to Day 42 for anti-H3 stalk ELISA, anti-H1 stalk MN, anti-H4 full length HA ELISA, anti-H10 full length HA ELISA and vaccine heterologous MN, with exact 95% CI, will be calculated.***

- ***The distribution of antibody titers for anti-H3 stalk ELISA will be displayed using reverse cumulative distribution curves.***
- the scatter plot of ELISA antibody results to the H3 stalk with the microneutralizing antibody results to the H3 stalk at D0 and ***at all timepoints*** will be presented to examine the correlation in all the study cohort. One scatter plot will be developed for each study/product. Furthermore, the correlation between the level of ELISA antibody with the level of microneutralizing antibody to the H3 stalk, ***at all vaccination timepoints*** may be explored using a Deming regression. However, the results should be interpreted with cautious due to small sample size and testing result variations in H3 stalk ELISA and H3 stalk MN.

6.2.2. Between groups assessment

6.2.2.1. For adult H5N1, H9N2, and H1N1 study cohorts

- For each ***Group 1-related*** study and for anti-H1 stalk ELISA results for adult H5N1, H9N2 and H1N1 study cohort, if available:
  - The difference in percentage of subject with at least 4-fold increase at ***all applicable timepoints (D21, D42, D182, and D385) for which data are available*** compared to Day 0 (i.e. adjuvanted group minus non-adjuvanted group ***within each study cohort***, and the asymptotic standardized 95% CI, will be computed for each study cohort, separately.
  - The adjusted GMT ratio (adjuvanted group to non-adjuvanted group ***within each study cohort***) of anti-H1 stalk ELISA antibodies for adjuvanted vaccine over non-adjuvanted vaccine at all timepoints (D21, D42, D182, and D385) for which data are available and the two-sided 95% CI on each GMT ratio will be computed for each study cohort, separately. ANCOVA models on the logarithm10 transformation of the titers, including the vaccine group as fixed effect and age category (<30 years and >=30 years) and the anti-H1 stalk ELISA result at Day 0 as covariates.
- For H1N1 study cohort and for anti-N1 NA ELISA results, if available:
  - The difference in percentage of subject with at least 4-fold increase at all time points (D21, 42 and 182) compared to Day 0 (i.e. adjuvanted group minus non-adjuvanted group, and the asymptotic standardized 95% CI, will be computed.
  - The adjusted GMT ratio (adjuvanted group to non-adjuvanted group) of anti-N1 NA ELISA antibodies for adjuvanted vaccine over non-adjuvanted vaccine at all timepoints (D21, D42 and D182) for which data are available and the two-sided 95% CI on each GMT ratio will be computed. ANCOVA models on the logarithm10 transformation of the titers, including the vaccine group as fixed

effect and age category (<30 years and >=30 years) and the anti-N1 NA ELISA result at Day 0 as covariates.

6.2.2.2. For H7N9 study only

For H7N9 study and for anti-H3 stalk ELISA results, if available:

- ***The difference in percentage of subject with at least 4-fold increase at all applicable timepoints (D21, D42, D182, and D385) for which data are available compared to Day 0 (i.e. adjuvanted group minus non-adjuvanted group, and the asymptotic standardized 95% CI, will be computed for each study cohort, separately.***
- ***The adjusted GMT ratio (adjuvanted group to non-adjuvanted group) of anti-H3 stalk ELISA antibodies for adjuvanted vaccine over non-adjuvanted vaccine at all timepoints (D21, D42, D182, and D385) for which data are available and the two-sided 95% CI on each GMT ratio will be computed. ANCOVA models on the logarithm10 transformation of the titers, including the vaccine group as fixed effect and the anti-H3 stalk ELISA result at Day 0 as covariates.***

6.2.3. Analysis of safety

Not applicable since no subject intervention is involved in this exploratory study concerning retrospective laboratory analyses of archived serum samples.

7. STATISTICAL CALCULATIONS

7.1. Derived and transformed variables

- Immunogenicity
  - For a given subject and given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements
  - The cut-off value is defined by the laboratory before the analysis and is described in Section 5.4.1 (Table 2) of the protocol.
  - A seronegative subject is a subject whose titer is below the cut-off value.
  - A seropositive subject is a subject whose titer is greater than or equal to the cut-off value.
  - The Geometric Mean Titers (GMTs) calculations are performed by taking the anti-log of the mean of the log titer transformations. Antibody titers below the cut-off

of the assay will be given an arbitrary value of half the cut-off for the purpose of GMT calculation.

- The 95% CI for geometric mean titres (GMTs) will be obtained within each group separately. The 95% CI for the mean of log-transformed titre will be first obtained assuming that log-transformed values were normally distributed with unknown variance. The 95% CI for the GMTs will be then obtained by exponential-transformation of the 95% CI for the mean of log-transformed titre.

- Four-fold antibody titer increase is defined as post/pre for seropositive subjects; and post/half of the cut off value for seronegative subjects
- ***Ten-fold antibody titer increase is defined as post/pre for seropositive subjects; and post/half of the cut off value for seronegative subjects***
- All CI computed will be two-sided 95% CI. The exact 95% CIs for a proportion within a group will be based on the method by Clopper [Clopper, 1934*].

8. CONDUCT OF ANALYSES

The planned analysis is descriptive and will be performed for each treatment group in the individual study ***cohort.*** No pooled analysis will be performed.

Any deviation(s) or change(s) from the original statistical plan outlined in the protocol will be described and justified in the final study report.

8.1. Sequence of analyses

Analyses will be performed in sequence based on availability of the different results. Results will be presented in a final study report.

8.2. Statistical considerations for interim analyses

Not applicable since no interim analyses are planned; ***all analyses will be descriptive***

9. CHANGES FROM PLANNED ANALYSES

Not applicable

10. REFERENCES

SAS software will be used to derive the confidence intervals

* Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika*. 1934; 26:404-413

** Robert G. Newcombe, interval estimation for the difference between independent proportions: comparison of eleven methods, *Statist Med.* 1998; 17, 873-890

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