Clinical Study Protocol
Sponsor:
GlaxoSmithKline Biologicals
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Primary Study vaccine and number	GlaxoSmithKline (GSK) Biologicals' candidate supra-seasonal universal influenza vaccine, BIO FLU CC-SUIV-AS03 (GSK3448401A)
eTrack study number and Abbreviated Title	201598 (FLU CC-SUIV-AS03-001)
Date of protocol	Final Version 1: 14 November 2014
Date of protocol amendment	Amendment 1 Final: 10 March 2015 ***Amendment 2 Final: 01 September 2016***
Title	An exploratory, retrospective laboratory evaluation of the humoral immune response in adults and children to the ***hemagglutinin*** stalk domain and other influenza A virus epitopes, after administration of GSK's influenza vaccines
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***
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(Amended 01 September 2016)

GSK Biologicals' protocol template for observational studies and non- interventional studies (i.e., without administration of medicinal products) as described in a research protocol based on the Protocol DS v 14.0

Protocol Amendment 2 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	201598 (FLU CC-SUIV-AS03-001)
Date of protocol amendment	***Amendment 2 Final: 01 September 2016***
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***
Sponsor signatory	Bruce Innis, MD, *Vice President*, ***Senior Vaccine Development Leader***
Signature	_____
Date	_____

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Protocol Amendment 2 Rationale

Amendment number: Amendment 2

Rationale/background for changes:

- Addition of N=30 placebo control subjects from study FLU Q-Pan H5N1-AS03-021 to the pediatric H5N1cohort to assess the impact of H1N1pdm09 virus exposure on anti-H1 stalk titers in subjects over 1 year of follow-up.
- Addition of three adult subject study cohorts (FLU Q-Pan-005, H5N1-012, and FLU D-QIV-015) to enable immunogenicity analyses that will provide context to, and will be informative for, designing the up-coming Phase I study, FLU E-SUIV-001, which will assess the safety and the immune response following one or two sequential doses of the SUIV candidate vaccine:
  - Analysis of serum samples collected from the Q-Pan-005 and the H5N1-012 trials to evaluate the H1 HA stalk domain reactive response following heterologous and homologous booster doses of an H5N1 adjuvanted vaccine in adult subjects. The design of these studies will also allow assessment of the immune response to the H1 HA stalk domain several months (12 months and 18 months) after one dose of the adjuvanted pandemic vaccine. These vaccine schedules are representative of the schedules planned to be used with the GSK candidate monovalent group 1 influenza A SUIV vaccine in the Phase I FLU E-SUIV-001 study.
  - The FLU E-SUIV-001 study will use a non-adjuvanted licensed quadrivalent inactivated influenza vaccine (IIV4) control group. Therefore, in order to be able to predict the response that can be seen in this control group, the stalk-reactive response following the administration of an IIV4 (quadrivalent inactivated influenza vaccine, *Fluarix Quadrivalent*) will also be assessed using archived serum samples from the FLU D-QIV-015 study.
- 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. The target virus (cH8/1N3) proposed to be used in this assay is being replaced by the recently developed (by ISMMS) chimeric cH6/1N5 virus, since cH8/1N3 will be used in the vaccine in the FLU E-SUIV-001 study. Therefore, to assess the anti-H1 stalk neutralizing antibody response, a chimeric virus (cH6/1N5) having an exotic HA head domain will be used.

- An additional timepoint was added (to evaluate persistence of the immune response) for the endpoint related to the assessment of the anti-H2 and the anti-H18 antibody levels by ELISA.
- To assess the breadth of neutralization effected by anti-H1 stalk ELISA antibodies, samples 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 (instead of the H6N3 as initially planned, since unlike H6N3, H5N8 is a current pandemic threat and is, thus, of greater public health significance), with an avian-like swine H1N1 virus (A/Swine/Jiangsu/40/2011) and a H1N1 pdm09 like virus. Previously only a randomly selected subset of samples (from subjects who had a ≥4-fold rise in MN titers (Day 21/Day 0) was planned to be tested.
- Based on the experience that will be acquired in testing anti-H1 stalk responses in the HA group 1-related studies, another study cohort drawn from subjects participating in Q-Pan-H7N9-001 (in which adult subjects were administered adjuvanted or unadjuvanted pandemic influenza H7N9 vaccine) will also be evaluated by ELISA and microneutralization assay for antibody responses to the H3 stalk (i.e., Group 2 hemagglutinin). This analysis will also assess the performance of the anti-H3 stalk ELISA.
- Specify use of D0, D42, and D385 serum specimens from the subjects in the CC-Pan-H5N1-001 adult cohort (adjuvanted vaccine recipients only) to be tested by passive transfer to mice that will be subsequently challenged with two chimeric cH viruses (cH5/3Nx and cH6/1N5; Nx=most likely N4 or N5, to be decided) to assess protective effect of vaccination on weight loss, survival, and lung virus titers.
- Addition of NeoMed-Labs Inc., Quebec, Canada (CRO) as a second laboratory authorized to perform analytical assays in addition to ISMMS. The planned transfer of ELISAs to NeoMed Labs from ISMMS will necessitate the use of a new reference serum pool, the qualification of which for such use may alter the defined assay cut-off.
- The algorithm for testing, as outlined in Protocol Amendment 1, has been revised and no formal stepwise algorithm will be followed. Testings will be done as soon as samples, assays, and antigens are available in the assigned laboratory.

Protocol Amendment 2 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about study-related duties and functions as described in the protocol.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.

Sponsor Information

Sponsor

GlaxoSmithKline Biologicals
Rue de l'institut 89, 1330 Rixensart, Belgium

Sponsor Medical Expert for the Study

Not applicable (retrospective, non-interventional, laboratory study).

Sponsor Study Monitor

Not applicable (retrospective, non-interventional, laboratory study).

Sponsor Study Contact for Reporting of a Serious Adverse Event

Not applicable (retrospective, non-interventional, laboratory study).

SYNOPSIS

Detailed Title (Amended 01 September 2016)

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***

Rationale for the study and study design (Amended 01 September 2016)

- Rationale for the study

FLU CC-SUIV-AS03-001 is the initial study in GSK Biologicals' clinical development plan for a candidate supra-seasonal universal influenza vaccine (SUIV) and is an exploratory, retrospective laboratory study, using archived serum samples, to assess the humoral immune response to the ***influenza A group 1 (H1) and the influenza A group 2 (H3)*** hemagglutinin ***(HA)*** stalk domain and other influenza A virus protein epitopes following administration in adults and children of GSK Biologicals' adjuvanted or unadjuvanted pandemic vaccines. These vaccines can serve as a surrogate for one dose of an inactivated chimeric ***HA***-bearing virus vaccine, because the stalk domain of the vaccines' ***HAs*** shares important epitopes with ***all of the group 1 or the group 2 influenza A HA*** stalk ***domains*** despite ***small*** amino acid sequence differences in the overall ***HA*** stalk domains among the ***circulating viruses***. The results of this study will provide context for and ***be informative for designing the Phase I study, FLU E-SUIV-001***, which will evaluate the ***safety and the immune response following one or two sequential doses of the SUIV candidate vaccine. This upcoming Phase I study will use a non-adjuvanted licensed quadrivalent inactivated influenza vaccine (IIV4) control group. Therefore, in order to be able to predict the response that could be observed in this control group, the stalk-reactive response following the administration of an IIV4 will also be assessed in this retrospective laboratory study***. The data obtained from the current FLU CC-SUIV-AS03-001 study will also support a pre-IND meeting with the FDA's Center for Biologics Evaluation and Research (CBER) by demonstrating that the immunoassays to be deployed in ***the Phase I FLU E-SUIV-001 study*** are fit for purpose, and that the antigen and adjuvant dose proposed for the study are reasonably likely to be acceptably immunogenic.

- Rationale for the study design

The retrospective study is designed to assess humoral immune response to the ***Group 1*** H1 hemagglutinin stalk domain (and other influenza A virus protein epitopes) ***in adult and pediatric subjects, by ELISA and microneutralization (MN) assay, using archived serum samples from***:

- ***Adult subjects who received*** adjuvanted ***(3.75 µg HA adjuvanted with AS03$_A$)*** or unadjuvanted ***(15 µg HA)*** H1N1pdm09, H5N1, and H9N2 pandemic influenza vaccines (standard adult dose) in 3 completed clinical trials ***[Q-Pan H1N1-019 (113536), CC-Pan H5N1-001 (114371), and Q-Pan H9N2-001 (116358)].*** The serum samples were collected at baseline, after 1 and 2 doses (Day 21 and Day 42, respectively), and after extended follow-up at Day 182. In 1 of these 3 ***study*** cohorts, samples were also collected at Day 385 (i.e., ***in CC-Pan H5N1-001 study cohort***, since this study also had a follow-up blood collection time point at Day 385).

  For each of these 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).

- ***A group of pediatric subjects, 6-35 months of age, who received adjuvanted pandemic H5N1 vaccine in the completed Q-Pan H5N1-AS03-021 trial (114464), and who had no HI antibodies [titer <10] to H1N1pdm09 before being vaccinated with 2 doses of AS03$_B$-adjuvanted Q-PAN-H5N1 vaccine (half adult dose, i.e., 1.9 µg HA).*** 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). ***To control for the effects of inter-current H1N1pdm09 virus infection (i.e., to assess the impact of H1N1pdm09 virus transmission) on anti-H1 stalk titers, placebo control serum samples from the same study will also be evaluated.*** 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***.

Additionally, to evaluate the H1 HA stalk domain reactive response following a heterologous booster dose of an adjuvanted H5N1 vaccine (3.8 µg HA adjuvanted with AS03$_A$), samples will be analyzed from adult subjects 18 to 40 years of age who received a single dose of an adjuvanted H5N1 vaccine (monovalent A/turkey/Turkey/1/05 [H5N1]) 18 months after their priming dose with a heterologous adjuvanted H5N1 vaccine (i.e., monovalent A/ Indonesia/5/05 [H5N1]) in the Q-Pan-005 (110624) trial. Similarly, the H1 HA stalk domain reactive response following a homologous booster dose of an H5N1 adjuvanted vaccine administered in subjects primed with one dose of the same vaccine 12 months earlier will also be described using samples from the same study. 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 a homologous or a heterologous adjuvanted priming dose (A/Vietnam/1194/2004) in subjects 18-60 years of age. This assessment will be stratified by age (18-30 years vs 31-60 years) to evaluate the age effect on the immune response. The design of these 2 studies will also allow the assessment of the immune response to the H1 HA stalk domain several months after one dose of the adjuvanted pandemic vaccine. These vaccine schedules are representative of the schedules planned to be used with the GSK candidate monovalent group 1 influenza A SUIV vaccine in the E-SUIV-001 Phase I study.

For these anti-H1 stalk ELISAs, all evaluable serum specimens 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 from Day 0, Day 21, Month 6 (M6),Month (M12), M12+21days and M18 of all subjects (18-60y) from the groups VT/VT/12M and VT/IN/12M will be used (i.e. no random selection for the sub-cohort). Groups VT/VT/12M and VT/IN/12M were comprised of subjects administered A/Vietnam/1194/2004 adjuvanted H5N1 followed 12 months later by, respectively, a homologous (A/Vietnam/1194/2004-like) or heterologous (A/Indonesia/5/2005-like) adjuvanted H5N1 booster dose.

In order to further characterize the influenza A group 1

anti-HA stalk response to support design of the E-SUIV-001 Phase 1 study in which a quadrivalent inactivated influenza vaccine (IIV4) will serve as control, serum specimens will also be analyzed from the pre-vaccination (Day 0) and post-vaccination (Day 21) timepoints of a study (D-QIV-015 (201251)) in which subjects were vaccinated with an IIV4 (i.e., Fluarix Quadrivalent, also called D-QIV). Samples from approximately 30 adult subjects, 18-39 years of age, will be randomly selected for analysis.

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, all D0, D42 samples and samples from the last time point (persistency) from subjects who received an adjuvant system (AS) vaccine will be tested for reactivity by ELISA with H2 and H18 full length recombinant hemagglutinin proteins.

For the Q-Pan-005 study, this will be assessed with the Day 0, 42, 549, 591, and Day 729 samples from group C subjects and with the Day 182, 224, 549, 591, and Day 729 samples from group G subjects. For the H5N1-012 study this will be assessed with the Day 0, Day 21, M12, M12+21days and M18 samples of all subjects in the VT/VT/12M and the VT/IN/12M groups. For the D-QIV-015 study cohort, in which the subjects received an IIV4, the testing will be performed with the Day 0 and the Day 21 samples.

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 will be 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 of this virus are "exotic" and most humans have not been exposed to them).

To assess the breadth of neutralization affected by anti-H1 stalk ELISA antibodies, samples ***at baseline (D0) and D42*** 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 a H1N1 pdm09 like virus.***

For the Q-Pan-005 study, this will be assessed with the Day

0, 42, 549 and 591 samples (from group C subjects), and with the Day 182, 224, 549, and 591 samples (from group G subjects). For the H5N1-012 study, this will be assessed with the Day 0, 21, M12 and M12+21days samples of all the subjects from the VT/VT/12M and VT/IN/12M groups. For the D-QIV-015 study cohort, in which the subjects received an IIV4, the testing will be performed with the Day 0 and Day 21 samples.

As part of the exploratory (tertiary) objectives of this retrospective study, and based on the experience that will be acquired in testing group 1 anti-H1 stalk responses mentioned above, archived serum samples (with sufficient volume for the serology analyses) from the Day 0, 21, 42, Month 6, and 12 time points of a H7N9 study cohort (in which adult subjects were administered 2 doses (21-day interval) of an adjuvanted (3.75 µg HA adjuvanted with AS03$_A$) or unadjuvanted (15 µg HA) pandemic influenza H7N9 vaccine) will also be evaluated for antibody responses to the group 2 HA stalk (i.e., H3), by anti-H3 stalk ELISA.

This analysis will also assess the performance of the influenza A anti-H3 stalk ELISA. Approximately 60 subjects (i.e., approximately 30 subjects who received the adjuvanted vaccine dose, and approximately 30 subjects who received the unadjuvanted vaccine dose) will be randomly selected to generate the serology sub-cohort in which both groups will match in terms of age and center.

As an additional exploratory objective for the H7N9 study cohort to evaluate whether a post-vaccination boost in ELISA antibody titers could also result in neutralization of target virus, samples from subjects who received an adjuvant system (AS) vaccine will be further analyzed with an influenza A group 2 anti-H3 stalk domain (at Day 0, 21, 42 M6 and M12) and heterosubtypic (at Day 0 and Day 42) microneutralization (MN) assays (target viruses: cH14/3Nx and a wild-type Group 2 H4N8 virus, respectively). Furthermore, D0, D42 and M12 samples from subjects who received the adjuvanted vaccine will be tested for reactivity with H4 and H10 full length recombinant hemagglutinin (HA) proteins.

Finally, an exploratory human serum transfer/virus challenge experiment will be conducted in mice to assess whether anti-H5 head antibodies and anti-H1 stalk antibodies are protective in vivo. The protective effect of pooled human sera collected on D42 and D385 from adult

recipients of adjuvanted H5N1 vaccine in study CC-PAN H5N1 will be compared to the effect of pooled serum collected at D0, by transferring each serum pool to BALB/c mice, which will be subsequently challenged with approximately 5LD_{50} of cH5/3Nx (Nx=most likely N4 or N5, to be decided) and cH6/1N5 (or alternative challenge viruses with similar attributes, but more fit for purpose). The extent of protection from viral challenge will be evaluated in terms of the proportion of mice surviving viral challenge, mean weight loss over time, and, in randomly selected subgroups of animals from each of the 3 treatment groups administered the D0, D42, or D385 serum pools, mean lung weight at necropsy and geometric mean lung virus titer following euthanization at 3 and 6 days post-challenge.

Objectives (Amended 01 September 2016)

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):

1. 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 (Day 0), post-dose 1 (Day 21), post-dose 2 (Day 42), 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 (Month 6), M12 (Month 12), M12+21 days, and M18 (Month 18)***
    - ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***
2. 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-AS03-21 study 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 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, M6, M12, M12+21 days, and M18***
- ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***

3. 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***
4. 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***

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):

1. 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 ***within each study cohort***) 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 ***within each study cohort***) 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***)
2. ***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)***

Tertiary 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):

1. ***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***
2. ***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

3. 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 cohort (AS group only)
4. 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
5. 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***
6. 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)

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

1. ***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***
2. ***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***
3. ***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***

4. ***To describe the vaccine heterosubtypic virus MN antibody level (target virus: H4N8) at baseline (D0) and post-dose 2 (D42) in all subjects who received an adjuvanted vaccine***

5. ***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***

Tertiary objectives: Passive transfer/challenge in mice with pooled adult human sera from subjects who received adjuvanted H5N1 vaccine in study CC-Pan-H5N1:

1. ***To assess the in vivo protective effect of H5-head specific antibodies in pooled human serum collected on D42 and D385 from adult recipients of CC-Pan-H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that are subsequently challenged with approximately 5LD50- of cH5/3Nx virus (or an alternative challenge virus with similar attributes but more fit for purpose); Nx=most likely N4 or N5, to be decided. In vivo protection to be evaluated in terms of proportion of mice surviving challenge, mean weight loss, and (in randomly selected subgroups from each treatment group) mean lung weight at necropsy and geometric mean lung virus titer.***

2. ***To assess the in vivo protective effect of H1-stalk specific antibodies in pooled human serum collected on D42 and D385 from adult recipients of CC-Pan-H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that are subsequently challenged with approximately 5LD50- of cH6/1N5 virus (or an alternative challenge virus with similar attributes but more fit for purpose). In vivo protection to be evaluated in terms of proportion of mice surviving challenge, mean weight loss, and (in randomly selected subgroups from each treatment group) mean lung weight at necropsy and geometric mean lung virus titer.***

3. ***If the pathogenicity (expressed as pfu/LD50) of the cH5/3Nx virus and cH6/1N5 virus are comparable, to then describe the effect size of anti-HA stalk antibodies (revealed by cH6/1N5 virus challenge) and anti-HA head antibodies (revealed by cH5/3Nx virus challenge) in***

terms of the relative survival rate (D42 rate/D0 rate) for cH6/1N5 vs relative survival rate for cH5/3Nx

4. ***To describe the post-transfer geometric mean ELISA titer of human IgG to cH5/3Nx and human IgG to cH6/1N5 in blood collected from mice receiving each of 3 serum pools (D0, D42, D385)***
5. ***To explore the association between post-transfer ELISA titer of human IgG to the challenge virus and outcome at D3 and D6***

Study design (Amended 01 September 2016)

- **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 assessments 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-005 and the H5N1-012 studies.

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) ***and with an avian-like swine H1N1 virus (A/Swine/Jiangsu/40/2011) and with a H1N1 pdm 09-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. 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.

Finally, an exploratory human serum transfer/virus challenge experiment will be conducted in mice to assess whether anti-H5 head antibodies and anti-H1 stalk antibodies are protective in vivo. The protective effect of pooled human sera collected on D42 and D385 from adult recipients of adjuvanted H5N1 vaccine in study CC-PAN H5N1 will be compared to the effect of pooled serum collected at D0 by transferring each serum pool to BALB/c mice, which will be subsequently challenged with approximately 5LD$_{50}$ of cH5/3Nx and cH6/1N5 (or alternative challenge viruses with similar attributes, but more fit for purpose);); Nx=most likely N4 or N5, to be decided.The design of this animal experiment is detailed in section 7 of Appendix A of this protocol.

- **Study groups:** There will be *13* study groups, as described in Synopsis Table 1.

Synopsis Table 1 Study groups and epochs foreseen in the study

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	Age range to be considered (yrs)	Study Group (treatment groups to be considered)	§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort	Number of subjects in ATP-I 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:	20-30	91

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	**Age range to be considered (yrs)**	**Study Group (treatment groups to be considered)**	**§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort**	**Number of subjects in ATP-I cohort (i.e, up to D42)**
		3.75 µg HA/AS03$_A$		
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 influenza vaccine containing the Vietnam (VT) strain at Day 0 and Month 12***	***All (~60)***	***N=variable depending on timepoint for ATP persistency***
		Group VT/IN/12M:	***All (~60)***	***N=variable***

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	Age range to be considered (yrs)	Study Group (treatment groups to be considered)	§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort	Number of subjects in ATP-I cohort (i.e, up to D42)
		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		*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*

HA=Hemagglutinin content per vaccine dose; $AS03_A$=Adjuvant system 03_A; AS=Adjuvant system; ATP-I=According to Protocol cohort for Immunogenicity.

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

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

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

CC-Pan H5N1-001: Group B = 15 µg HA (no AS) CC-PAN H5N1 vaccine given at Day 0 and Day 21

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

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

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

Q-Pan H9N2-001: Group 1500_VVV = 15 µg HA (no AS) H9N2 vaccine given at Day 0, Day 21, and Day 182

DQIV-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***

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

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

- **Blinding:** Laboratory staff conducting the testing will

have knowledge of the subject number, treatment received, and specimen time-point for every specimen tested.

- **Type of study:** Self contained retrospective study.

Number of subjects/samples (Amended 01 September 2016)

- 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 cohorts (depending on the study) of the completed studies, and 3) have valid vaccine homologous HI result available at all the required time points where the HI test is done and at Day 0 and 21 (for H5N1 and H9N2 studies only). Within each ***of these*** study cohorts, 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 timepoint. ***Furthermore, subjects assigned to the CMI and MN subset in study H9N2 and subjects with available homologous MN results 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 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 vaccine, were seronegative for H1N1pdm09 HI at Day 0, and have valid vaccine homologous HI and MN results available at either D0, 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.***
- ***The number of samples and mice needed for the exploratory passive serum transfer in mice experiment are indicated in Section 7, Appendix A.***

Endpoints (Amended 01 September 2016):

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 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
     - 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***
     - 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***
     - ***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 and*
  - *from Day 549 to Day 591 and*
  - *from Day 0 to Day 591*
- *For group G:*
  - *from Day 182 to Day 224 and*
  - *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 and*
  - *from Day 549 to Day 591 and*
  - *from Day 0 to Day 591*
- *For group G:*
  - *from Day 182 to Day 224 and*
  - *from Day 549 to Day 591 and*
  - *from Day 182 to Day 591*

– *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*

- ***GMT at Day 0 and 21***
- ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
- ***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 and the subjects in the FLU D-QIV-015 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)
     - 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***
     - 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
     - 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***
     - 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)***
     - ***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 and***
    - ***from Day 549 to Day 591 and***
    - ***from Day 0 to Day 591***
  - ***For group G:***
    - ***from Day 182 to Day 224 and***
    - ***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 and***
    - ***from Day 549 to Day 591 and***
    - ***from Day 0 to Day 591***
  - ***For group G:***
    - ***from Day 182 to Day 224 and***
    - ***from Day 549 to Day 591 and***
    - ***from Day 182 to Day 591***
- ***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 the FLU D-QIV-015 cohort:***
  - ***Seropositive rate at Day 0 and 21***

- ***GMT at Day 0 and 21***
- ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
- ***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)***
     - 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
     - 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***
     - ***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 and***
         - ***from Day 549 to Day 591 and***
         - ***from Day 0 to Day 591***

- ***For group G:***
  - ***from Day 182 to Day 224 and***
  - ***from Day 549 to Day 591 and***
  - ***from Day 182 to Day 591***
- ***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***
  - ***GMT at Day 0 and 21***
  - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
  - ***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 and the subjects in the FLU D-QIV-015 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, Q-Pan H9N2-001, and Q-Pan H5N1-AS03-21 study cohorts:***
     - Seropositive rate at Day 0 and Day ***42***
     - GMT at Day 0 and Day ***42***
     - Percentage of subjects with a ≥4-fold rise from Day 0 to Day ***42***
     - 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***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 182 to Day 591***
  - ***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***
  - ***MGI at Day 21 compared to Day 0***

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 cohorts. 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 D182 (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, 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

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):

1. ***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***
   - ***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***
   - ***MGI at Day 21, 42, 182 compared to Day 0***
2. ***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***

3. 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 adult 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
4. 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:
   - MGI for anti-H1 stalk ELISA at Day 21 compared to Day 0
5. 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
6. 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
- GMT at Day 0, 21, and 42
- Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21, and Day 42
- MGI at Day 21 and 42 compared to Day 0

Tertiary endpoints

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***
   - ***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***
   - ***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***
   - ***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***
   - ***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)

- *GMT at Day 0, 42, and Month 12*
- *Percentage of subjects with a ≥4-fold rise from Day 0 to Day 42*
- *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. The following aggregate variables will be calculated with 95% CI:*
   - *Seropositive rate at Day 0 and Day 42*
   - *GMT at Day 0 and Day 42*
   - *Percentage of subjects with a ≥4-fold rise from Day 0 to Day 42*
   - *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*

Tertiary endpoints

Passive transfer/challenge in mice with pooled adult human sera from subjects who received adjuvanted H5N1 vaccine in the CC-Pan-H5N1 study cohort

1. *The in vivo protective effect of transferring pooled adult human serum (from subjects administered adjuvanted H5N1 vaccine in the CC-Pan-H5N1 study cohort) to mice and subsequently challenging them with cH5/3Nx virus (Nx=most likely N4 or N5, to be decided) will be assessed in terms of the following endpoints:*
   - *survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice/serum pool/time-point*
   - *mean weight loss (change from baseline over 14 days post-challenge) in groups of 25 mice/serum pool/time-point*
   - *lung weight in micrograms (D42 minus D0), (D385 minus D0), within challenge group*
   - *lung virus titer in pfu/microgram (log10 fold change*

(D0 minus D42), (D0-D385), within challenge group

2. ***The in vivo protective effect of transferring pooled adult human serum (from subjects administered adjuvanted H5N1 vaccine) to mice and subsequently challenging them with cH6/1N5 virus will be assessed in terms of the following endpoints:***

    - ***survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice/serum pool/time-point***
    - ***mean weight loss (change from baseline over 14 days post challenge) in groups of 25 mice/serum pool/time-point***
    - ***lung weight in micrograms (D42 minus D0), (D385 minus D0), within challenge group***
    - ***lung virus titer in pfu/microgram (log10 fold change (D0 minus D42), (D0-D385), within challenge group***

3. ***Post-transfer titer of human IgG to cH5/3 by ELISA***
4. ***Post-transfer titer of human IgG to cH6/1 by ELISA***

(Amended 01 September 2016)

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AS	Adjuvant System
$AS03_A$	$AS03_A$ is an Adjuvant System containing α-tocopherol and squalene in an oil and water emulsion (11.86 mg tocopherol in 0.25 mL i.e., 47.44 mg tocopherol per mL)
ATP	According-To-Protocol
ATP-I	According-To-Protocol cohort for Immunogenicity
CBER	Center for Biologics Evaluation and Research
CI	Confidence Interval
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Agency
GCP:	Good Clinical Practice
GMT	Geometric Mean Titer
GSK:	GlaxoSmithKline
HA	Hemagglutinin
HI	Hemagglutinin Inhibition
ICF:	Informed Consent Form
ICH:	International Conference on Harmonisation
IEC:	Independent Ethics Committee
IIV4	Inactivated Influenza Vaccine, Quadrivalent
IRB:	Institutional Review Board
ISMMS	Icahn School of Medicine at Mount Sinai, New York, NY, USA
LL	Lower Limit
MGI	Mean Geometric Increase
MN	Microneutralization
SAP	Statistical Analysis Plan

SP	Seropositivity
SUIV	Supra-seasonal Universal Influenza Vaccine
UL	Upper Limit

GLOSSARY OF TERMS

Blinding:	A procedure in which one or more parties to the trial are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. The level of blinding is maintained throughout the conduct of the trial, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be favourable or when required in case of a serious adverse event. In an open-label study such as this one, no blind is used. Both the investigator and the sponsor know the identity of the treatment associated with the archives serum samples.
Epoch:	An epoch is a self-contained set of consecutive timepoints or a single timepoint from a single protocol. Self-contained means that data collected for all subjects at all timepoints within that epoch allows to draw a complete conclusion to define or precise the targeted label of the product.
eTrack:	GSK's tracking tool for clinical trials.
Investigational vaccine/product: **(Synonym of Investigational Medicinal Product)**	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorisation when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
Randomization:	Process of random attribution of treatment to subjects in order to reduce bias of selection.
Self-contained study:	Study with objectives not linked to the data of another study.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine(s)/product(s) or as a control.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject, identified by a unique number, according to the study randomisation or treatment allocation.

1. INTRODUCTION

1.1. Background

Influenza is an acute, highly contagious, respiratory disease caused by influenza viruses, mainly spread through respiratory droplets. The illness is accompanied by fever and variable degrees of other systemic symptoms, ranging from mild fatigue to respiratory failure and death. Influenza occurs in annual epidemics that are associated with significant morbidity and mortality; these epidemics often involve influenza subtypes H1 and H3 and influenza B viruses. The World Health Organization (WHO) estimates that influenza affects 5% to 15% of the population worldwide annually, with 3 to 5 million cases of severe illness and 250,000 to 500,000 deaths [WHO, 2005]. In the United States alone, influenza is estimated to cause 200,000 excess hospitalizations each year [Tosh, 2010].

The use of inactivated vaccines is the primary means of preventing influenza infection, but vaccine efficacy is dependent on how closely the strains included in the vaccine match the circulating virus. The WHO issues vaccine composition recommendations annually.

The need for accurate annual reformulation of current influenza vaccines stems from the high variability of the antigenic regions in the hemagglutinin (HA) globular head domain, which helps the influenza virus evade the human humoral response, since these regions also serve as the major epitopes for neutralizing antibodies. In contrast, because it is highly conserved and stable, the HA stalk domain offers a potential target as an effective component of an universal influenza vaccine. However, the HA stalk is also less immunogenic than the HA head, presenting a challenge for vaccine development which, could be overcome with the use of an adjuvant system to help provide a robust HA stalk-directed immunity that could induce a broad sero-response to influenza infection, irrespective of the predominant circulating virus strain [Goff, 2013]. Sequential immunization using a series of influenza viruses, each expressing a chimeric hemagglutinin composed of a unique exotic head domain and a conserved stalk domain, is one approach to overcoming the favor-dominance of the HA head [Krammer, 2014].

1.2. Rationale for the study and study design

1.2.1. Rationale for the study

FLU CC-SUIV-AS03-001 is the initial study in GSK Biologicals' clinical development plan for a candidate supra-seasonal universal influenza vaccine (SUIV) and is an exploratory, retrospective laboratory study, using archived serum samples, to assess the humoral immune response to the ***influenza A group 1 (H1) and the influenza A group 2 (H3)*** hemagglutinin ***(HA)*** stalk domain and other influenza A virus protein epitopes following administration in adults and children of GSK Biologicals' adjuvanted or unadjuvanted pandemic vaccines. These vaccines can serve as a surrogate for one dose of an inactivated chimeric ***HA***-bearing virus vaccine, because the stalk domain of the vaccines' ***HAs*** shares important epitopes with ***all of the group 1 or the group 2 influenza***

A HA stalk *domains* despite *small* amino acid sequence differences in the overall *HA* stalk domains among the *circulating viruses*. The results of this study will provide context for and *be informative for designing the Phase I study, FLU E-SUIV-001*, which will evaluate the *safety and the immune response following one or two sequential doses of the SUIV candidate vaccine. This upcoming Phase I study will use a non-adjuvanted licensed quadrivalent inactivated influenza vaccine (IIV4) control group. Therefore, in order to be able to predict the response that can be seen in this control group, the stalk-reactive response following the administration of an IIV4 will also be assessed in this retrospective laboratory study*. The data obtained from the current FLU CC-SUIV-AS03-001 study will also support a pre-IND meeting with the FDA's Center for Biologics Evaluation and Research (CBER) by demonstrating that the immunoassays to be deployed in *the Phase I FLU E-SUIV-001 study* are fit for purpose and that the antigen and adjuvant dose proposed for the study are reasonably likely to be acceptably immunogenic.

1.2.2. Rationale for the study design

The retrospective study is designed to assess humoral immune response to the *Group 1* H1 hemagglutinin stalk domain (and other influenza A virus protein epitopes) *in adult and pediatric subjects, by ELISA and microneutralization (MN) assay, using archived serum samples from*:

- *Adult subjects who received* adjuvanted *(3.75 µg HA adjuvanted with AS03$_A$)* or unadjuvanted *(15 µg HA)* H1N1pdm09, H5N1, and H9N2 pandemic influenza vaccines (standard adult dose) in 3 completed clinical trials *[Q-Pan H1N1-019 (113536), CC-Pan H5N1-001 (114371), and Q-Pan H9N2-001 (116358)]*. The serum samples were collected at baseline, after 1 and 2 doses (Day 21 and Day 42, respectively), and after extended follow-up at Day 182. In 1 of these 3 *study* cohorts, samples were also collected at Day 385 (i.e., *in CC-Pan H5N1-001 study cohort*, since this study also had a follow-up blood collection time point at Day 385).

  For each of these 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).

- *A group of pediatric subjects, 6-35 months of age, who received adjuvanted pandemic H5N1 vaccine in the completed Q-Pan H5N1-AS03-021 trial (114464), and who had no HI antibodies [titer <10] to H1N1pdm09 before being vaccinated with 2 doses of AS03$_B$-adjuvanted Q-PAN-H5N1 vaccine (half adult dose, i.e., 1.9 µg HA).* 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). *To control for the effects of inter-current H1N1pdm09 virus infection (i.e., to assess the impact of H1N1pdm09 virus transmission) on anti-H1 stalk titers, placebo control serum*

samples from the same study will also be evaluated. 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***.

Additionally, to evaluate the H1 HA stalk domain reactive response following a heterologous booster dose of an adjuvanted H5N1 vaccine (3.8 µg HA adjuvanted with AS03$_A$), samples will be analyzed from adult subjects 18 to 40 years of age who received a single dose of an adjuvanted H5N1 vaccine (monovalent A/turkey/Turkey/1/05 [H5N1]) 18 months after their priming dose with a heterologous adjuvanted H5N1 vaccine (i.e., monovalent A/ Indonesia/5/05 [H5N1]) in the Q-Pan-005 (110624) trial. Similarly, the H1 HA stalk domain reactive response following a homologous booster dose of an H5N1 adjuvanted vaccine administered in subjects primed with one dose of the same vaccine 12 months earlier will also be described using samples from the same study. 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 a homologous or a heterologous adjuvanted priming dose (A/Vietnam/1194/2004) in subjects 18-60 years of age. This assessment will be stratified by age (18-30 years vs 31-60 years) to evaluate the age effect on the immune response. The design of these 2 studies will also allow the assessment of the immune response to the H1 HA stalk domain several months after one dose of the adjuvanted pandemic vaccine. These vaccine schedules are representative of the schedules planned to be used with the GSK candidate monovalent group 1 influenza A SUIV vaccine in the E-SUIV-001 Phase I study.

For these anti-H1 stalk ELISAs, all evaluable serum specimens 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 from Day 0, Day 21, Month 6 (M6),Month (M12), M12+21days and M18 of all subjects (18-60y) from the groups VT/VT/12M and VT/IN/12M will be used (i.e. no random selection for the sub-cohort). Groups VT/VT/12M and VT/IN/12M were comprised of subjects administered A/Vietnam/1194/2004 adjuvanted H5N1 followed 12 months later by, respectively, a homologous (A/Vietnam/1194/2004-like) or heterologous (A/Indonesia/5/2005-like) adjuvanted H5N1 booster dose.

In order to further characterize the influenza A group 1 anti-HA stalk response to support design of the E-SUIV-001 Phase 1 study in which a quadrivalent inactivated influenza vaccine (IIV4) will serve as control, serum specimens will also be analyzed from the pre-vaccination (Day 0) and post-vaccination (Day 21) timepoints of a study (D-QIV-015 (201251)) in which subjects were vaccinated with an IIV4 (i.e., Fluarix Quadrivalent, also called D-QIV). Samples from approximately 30 adult subjects, 18-39 years of age, will be randomly selected for analysis.

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, all D0, D42 samples and samples from the last time point (persistency) from subjects who received

an adjuvant system (AS) vaccine will be tested for reactivity by ELISA with H2 and H18 full length recombinant hemagglutinin proteins.

For the Q-Pan-005 study, this will be assessed with the Day 0, 42, 549, 591, and Day 729 samples from group C subjects and with the Day 182, 224, 549, 591, and Day 729 samples from group G subjects. For the H5N1-012 study this will be assessed with the Day 0, Day 21, M12, and M12+21days samples of all subjects in the VT/VT/12M and the VT/IN/12M groups. For the D-QIV-015 study cohort, in which the subjects received an IIV4, the testing will be performed with the Day 0 and the Day 21 samples.

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 will be 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 of this virus 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 ***at baseline (D0) and D42*** 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 a H1N1 pdm09 like virus.***

For the Q-Pan-005 study, this will be assessed with the Day 0, 42, 549 and 591 samples from group C subjects and with the Day 182, 224, 549 and 591 samples from group G subjects. For the H5N1-012 study this will be assessed with the Day 0, Day 21, M12, and M12+21days samples of all subjects in the VT/VT/12M and the VT/IN/12M groups. For the D-QIV-015 study cohort, in which the subjects received an IIV4, the testing will be performed with the Day 0 and Day 21 samples.

As part of the exploratory (tertiary) objectives of this retrospective study, and based on the experience that will be acquired in testing group 1 anti-H1 stalk responses mentioned above, archived serum samples (with sufficient volume for the serology analyses) from the Day 0, 21, 42, Month 6, and 12 time points of a H7N9 study cohort (in which adult subjects were administered 2 doses (21-day interval) of an adjuvanted (3.75 µg HA adjuvanted with AS03$_A$) or unadjuvanted (15 µg HA) pandemic influenza H7N9 vaccine) will also be evaluated for antibody responses to the group 2 HA stalk (i.e., H3), by anti-H3 stalk ELISA.

This analysis will also assess the performance of the influenza A anti-H3 stalk ELISA. Approximately 60 subjects (i.e., approximately 30 subjects who received the adjuvanted vaccine dose, and approximately 30 subjects who received the unadjuvanted vaccine dose) will be randomly selected to generate the serology sub-cohort in which both groups will match in terms of age and center.

As an additional exploratory objective for the H7N9 study cohort to evaluate whether a post-vaccination boost in ELISA antibody titers could also result in neutralization of target virus, samples from subjects who received an adjuvant system (AS) vaccine will be further analyzed with an influenza A group 2 anti-H3 stalk domain (at Day 0, 21, 42 M6 and M12) and heterosubtypic (at Day 0 and Day 42) microneutralization (MN) assays (target viruses: cH14/3Nx and a wild-type Group 2 H4N8 virus, respectively). Furthermore, D0, D42 and M12 samples from subjects who received the adjuvanted vaccine will be tested for reactivity with H4 and H10 full length recombinant hemagglutinin (HA) proteins.

Finally, an exploratory human serum transfer/virus challenge experiment will be conducted in mice to assess whether anti-H5 head antibodies and anti-H1 stalk antibodies are protective in vivo. The protective effect of pooled human sera collected on D42 and D385 from adult recipients of adjuvanted H5N1 vaccine in study CC-PAN H5N1 will be compared to the effect of pooled serum collected at D0, by transferring each serum pool to BALB/c mice, which will be subsequently challenged with approximately $5LD_{50}$ of cH5/3Nx (Nx=most likely N4 or N5, to be decided) and cH6/1N5 (or alternative challenge viruses with similar attributes, but more fit for purpose). The extent of protection from viral challenge will be evaluated in terms of the proportion of mice surviving viral challenge, mean weight loss over time, and, in randomly selected subgroups of animals from each of the 3 treatment groups administered the D0, D42, or D385 serum pools, mean lung weight at necropsy and geometric mean lung virus titer following euthanization at 3 and 6 days post-challenge.

(Amended 01 September 2016)

2. OBJECTIVES

2.1. 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)

1. 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 (Day 0), post-dose 1 (Day 21), post-dose 2 (Day 42), 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 (Month 6), M12 (Month 12), M12+21 days, and M18 (Month 18)***
- ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***

2. 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-AS03-21 study 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 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, M6, M12, M12+21 days, and M18***
   - ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***
3. 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***
4. 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***

Refer to Section 10.1 for the definition of the primary endpoints. **(Amended 01 September 2016)**

2.2. 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):

1. 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***)
2. ***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)***

Refer to Section 10.2 for the definition of the secondary endpoints.

2.3. Tertiary 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):

1. 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
2. ***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***
3. 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 cohort (AS group only)

4. 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
5. 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***
6. 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)

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

1. ***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***
2. ***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***
3. ***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***
4. ***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***
5. ***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***

Tertiary objectives: Passive transfer/challenge in mice with pooled adult human sera from subjects who received adjuvanted H5N1 vaccine in study CC-Pan-H5N1:

1. ***To assess the in vivo protective effect of H5-head specific antibodies in pooled human serum collected on D42 and D385 from adult recipients of CC-Pan-H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that are subsequently challenged with approximately 5LD$_{50}$ of cH5/3Nx virus (or an alternative challenge virus with similar attributes but more fit for purpose); Nx=most likely N4 or N5, to be decided. In vivo protection to be evaluated in terms of proportion of mice surviving challenge, mean weight loss, and (in randomly***

selected subgroups from each treatment group), mean lung weight at necropsy and geometric mean lung virus titer

2. ***To assess the in vivo protective effect of pooled human serum collected on D42 and D385 from adult recipients of CC-Pan-H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that are subsequently challenged with approximately $5LD_{50}$ of cH6/1N5 virus (or an alternative challenge virus with similar attributes but more fit for purpose). In vivo protection to be evaluated in terms of proportion of mice surviving challenge, mean weight loss, and (in randomly selected subgroups from each treatment group), mean lung weight at necropsy and geometric mean lung virus titer***
3. ***If the pathogenicity (expressed as pfu/LD_{50}) of the cH5/3Nx virus and cH6/1N5 virus are comparable, to then describe the effect size of anti-HA stalk antibodies (revealed by cH6/1N5 virus challenge) and anti-HA head antibodies (revealed by cH5/3Nx virus challeng) in terms of the relative survival rate (D42 rate/D0 rate) for cH6/1N5 vs relative survival rate for cH5/3Nx***
4. ***To describe the post-transfer geometric mean ELISA titer of human IgG to cH5/3Nx and human IgG to cH6/1N5 in blood collected from mice receiving each of 3 serum pools (D0, D42, D385)***
5. ***To explore the association between post-transfer ELISA titer of human IgG to the challenge virus and outcome at D3 and D6***

Refer to Section 10.3 for the definition of the tertiary endpoints. **(Amended 01 September 2016)**

3. STUDY DESIGN OVERVIEW

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 assessments 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 studies.

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.

Finally, an exploratory human serum transfer/virus challenge experiment will be conducted in mice to assess whether anti-H5 head antibodies and anti-H1 stalk antibodies are protective in vivo. The protective effect of pooled human sera collected on D42 and D385 from adult recipients of adjuvanted H5N1 vaccine in study CC-PAN H5N1 will be compared to the effect of pooled serum collected at D0 by transferring each serum pool to BALB/c mice, which will be subsequently challenged with approximately 5LD_{50} of cH5/3Nx and cH6/1N5 (or alternative challenge viruses with similar attributes, but more fit for purpose); Nx=most likely N4 or N5, to be decided.The design of this animal experiment is detailed in section 7 of APPENDIX A of this protocol.

(Amended 01 September 2016)

- **Study groups:** There will be *13* study groups, as described in Table 1.

Table 1 Study groups and epochs foreseen in the study (Amended 01 September 2016)

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	Age range to be considered (yrs)	Study Group (treatment groups to be considered)	§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort	Number of subjects in ATP-I 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

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	**Age range to be considered (yrs)**	**Study Group (treatment groups to be considered)**	**§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort**	**Number of subjects in ATP-I cohort (i.e, up to D42)**
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 influenza vaccine containing the Vietnam (VT) strain at Day 0 and Month 12*	*All (~60)*	*N=variable depending on timepoint for ATP persistency*
		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*

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	Age range to be considered (yrs)	Study Group (treatment groups to be considered)	§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort	Number of subjects in ATP-I cohort (i.e, up to D42)
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*

HA=Hemagglutinin content per vaccine dose; AS03$_A$=Adjuvant system 03$_A$; AS=Adjuvant system; ATP-I=According to Protocol cohort for Immunogenicity.

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)

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

- **Type of study:** Self contained retrospective study.

4. STUDY COHORT

4.1. Number of samples

The target is to randomly select approximately 30 subjects from each of the adult and pediatric studies. These 30 subjects from each of the 3 adult studies ***(Q-Pan H1N1-019, CC-Pan H5N1-001, and Q-Pan H9N2-001),*** will be those 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 the required time points where the HI test is done and at Day 0 and 21 (for H5N1, 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 timepoint. ***Furthermore, subjects assigned to the CMI and MN subset in study H9N2 and subjects with available homologous MN results 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 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 cohorts (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 D0, 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 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.

The number of samples and mice needed for the exploratory passive serum transfer in mice experiment are indicated in Section 7, APPENDIX A.

(Amended 01 September 2016)

4.2. Inclusion criteria for enrolment (selection of archived samples)

- Not applicable since no subjects will be actively enrolled in this study; only the sera samples of the subjects who were a part of previously conducted primary trials will be used for testing. However, the archived serum samples of only those subjects who satisfy the following criteria will be included in this study:
- Subjects who were included in the ATP cohort for immunogenicity and Persistence cohort (depending on the study) in the primary studies listed.
- Subjects who had agreed that their blood samples could be used for further research while giving informed consent for any of the primary studies listed.
- Subjects who have sufficient residual sample volume (i.e., ≥0.5 mL) of serum at all time points.
- ***Only applicable for study CC-Pan-H5N1-001, study Q-Pan-H9N2-001 and study H5N1-AS03-021***: subjects with vaccine homologous neutralizing antibody result available at Day 0 and at 21 (25 samples available per group in Study H9N2-001)

4.3. Exclusion criteria for enrolment

Not applicable since no subjects will be actively enrolled in this study; only the serum samples of the subjects who were a part of previously conducted trials will be used for testing.

5. CONDUCT OF THE STUDY

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

Conduct of this study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subjects' informed consent (*Note*: Samples of only those subjects who had agreed that their blood samples could be used for further research while providing consent for their participation in any of the primary studies listed will be included in this study).
- Conduct of the primary studies included, but was not limited to, the following:
- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subjects' (or subjects' parents/legally appointed representatives for the pediatric study) informed consent.

5.2. 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.

For the current exploratory, retrospective study, the target is to select approximately 30 subjects from each of the adult and pediatric studies.

The 30 subjects from each of the 3 ***following*** adult studies ***(Q-Pan H1N1-019, CC-Pan H5N1-001, and Q-Pan H9N2-001),*** will be those 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 the required time points where the HI test is done and at Day 0 and 21 (for H5N1, and H9N2 studies only). Within each ***of these*** study cohorts, 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 subjects with available homologous MN results 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 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.

The number of samples and mice needed for the exploratory passive serum transfer in mice experiment are indicated in Section 7, APPENDIX A.

(Amended 01 September 2016)

5.3. Method of blinding

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

5.4. Biological sample handling and analysis

The archived sera samples collected in the previously conducted pandemic influenza vaccine clinical trials will be tested in this study.

5.4.1. Laboratory assays

Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX B for the address of the clinical laboratories used for sample analysis.

Serological assays for the determination of antibodies against the HA stalk domain and other influenza A virus protein epitopes will be performed by ELISA (Table 2) using standardized procedures in a laboratory designated by GSK Biologicals (i.e., Dept. Of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA ***and/or NeoMed Labs Inc., Quebec, Canada***). The laboratory analyses may be 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.

Table 2 Serum Assays to Assess Humoral Immunity (Amended 01 September 2016)

Antigen/ Virus for Test	Component (Strain or Antigen Description)	Method	Kit / Manufacturer	Unit	§Cut-off	*Laboratory
ELISAs						
cH6/1N5 HA	Recombinant antigen based on A/mallard/Sweden/81/02 head domain with A/Puerto Rico/8/34 H1 stalk domain	Anti-H1 HA stalk ELISA	ISMMS ***or NeoMed Labs*** protocol/assay	ELISA units (EU)	100 EU***/ml***	ISMMS ***or NeoMed Labs***
H2 HA full length	Recombinant antigen based on A/mallard/ Netherlands/5/99 HA	Anti- H2 HA full length ELISA				
H18 HA full length	Recombinant antigen based on A/flat-faced bat/ Peru/033/10 HA	Anti- H18 HA full length ELISA				
N1 NA	Recombinant antigen based on A/California/04/2009 NA	Anti-N1 NA ELISA				
N2 NA	**Recombinant antigen based on A/Chicken/ Hong Kong/G9/1997 NA	Anti-N2 NA ELISA				
H9 HA head domain	**Recombinant antigen based on A/Chicken/ Hong Kong/G9/1997 HA head domain	Anti-H9 HA head domain ELISA				
H9 HA full length	**Recombinant antigen based on A/Chicken/ Hong Kong/G9/1997 HA	Anti- H9 HA full length ELISA				
cH14/3	***Recombinant antigen based on the head domain of A/mallard/Gurjev/263/82 and the stalk domain of A/Perth/16/09***	***Anti-H3 HA stalk ELISA***				
H4 HA full length	***Recombinant antigen based on A/duck/Czech/56***	***Anti- H4 HA full length ELISA***				
H10 HA full length	***Recombinant antigen based on A/mallard/IA/10BM01929 /10***	***Anti-H10 HA full length ELISA***				

Antigen/ Virus for Test	Component (Strain or Antigen Description)	Method	Kit / Manufacturer	Unit	§Cut-off	*Laboratory
Microneutralization (MN) assays						
cH6/1N5 virus	cHA virus based on PR8 for 6 genes with 2 surface proteins: N3 is from A/swine/Missouri/4296424/2006(H2N3), HA head domain is from A/mallard/Sweden/24/2002 (H8N4), HA stalk domain is from A/California/04/09 (H1N1)	***Anti-H1 HA stalk*** MN Assay	ISMMS protocol/assay	1/DIL (IC_{50})	10	ISMMS
H5N8 virus	RG reassortant virus based on PR8 for 6 genes with 2 surface proteins: NA from A/swine/Missouri/4296424/2006 (H2N3), HA from A/mallard/Sweden/81/2002 (H6N1)	***Anti-heterosubtypic HA Group 1 virus*** MN Assay				
H1N1 swine Flu virus	***A/Swine/Jiangsu/40/2011***	***Anti-heterosubtypic HA Group 1 virus MN Assay***				
H1N1 pdm09 virus	***A/California/7/2009-like***	***ϕ Anti-heterosubtypic HA Group 1 virus MN Assay***				
cH14/3NX	***RG reassortant virus based on PR8 for 6 genes with 2 surface proteins: NX (to be determined), cHA with the head domain of A/mallard/Gurjev/263/82 and the stalk domain of A/Perth/16/09***	***Anti-H3 HA stalk MN Assay***				
H4N8 virus	***RG reassortant virus based on PR8 for 6 genes with 2 surface proteins: NA from A/mallard/Sweden/50/0, HA from***	***Anti-heterosubtypic HA Group 2 virus MN Assay***				

Antigen/ Virus for Test	Component (Strain or Antigen Description)	Method	Kit / Manufacturer	Unit	§Cut-off	*Laboratory
	A/duck/Czech/56					
Hemagglutination Inhibition (HI) Assay						
H5N1 virus	Pandemic vaccine (H5N1) homologous virus (A/Indonesia/5/2005)	HI assay	GSK GVCL ***CLS*** protocol/assay (if resources available)	1/DIL	10	***GSK Biologicals
H9N2 virus	Pandemic vaccine homologous (H9N2) virus (A/chicken/Hong Kong/G9/1997)	HI assay	GSK GVCL ***CLS*** protocol/assay (if resources available)	1/DIL	10	***GSK Biologicals
H1N1 virus	Pandemic vaccine (H1N1) homologous virus (A/California/7/2009 (or a like virus))	HI assay	GSK ***CLS*** protocol/assay (if resources available)	1/DIL	10	***GSK Biologicals

*Refer to APPENDIX B for the laboratory addresses. ISMMS= Icahn School of Medicine at Mount Sinai, Dept. Of Microbiology, New York, NY, USA. ***NeoMed Labs = NEOMED-LABS INC., 7171 Frederick-Banting St, Saint-Laurent, Quebec H4S 1Z9, Canada***

**These ELISAs to be performed only on samples from the H9N2 cohort

*** GSK Biologicals laboratory refers to the ***Clinical Laboratory Sciences (CLS)*** in Rixensart, Belgium; Wavre, Belgium; Dresden, Germany

§ Cut-off value may change subject to qualification of new reference serum pool (NeoMed Labs)

ϕ Vaccine homologous H1N1 for DQIV015 and H1N1-019 study samples

Table 3 Cell-Mediated Immunity (CMI) (Amended 01 September 2016)

System	Component	Challenge	Method	Unit	Laboratory*	Priority
PBMCs	T cells stained with probes for various cytokines (IL2, TNFα. IFNɣ, CD40L, etc)	None, H9N2 split, H1N1 split, H3N2 split ***H1 (A/California/7/2009) stalk peptide pool***	T Cell response by ICS assay	Frequencies of cytokine+ CD4+ T cells/Mio of CD4+ T cells	GSK Biologicals**	4
PBMCs	Plasmablasts sorted using HA- SA biotinylated probe-	None	Plasmablast response to HA	Frequencies of antigen-specific plasmablasts/Mio plasmablasts	GSK Biologicals**	3
PBMCs	B cells reactive to "challenge" antigens	None, H9N2 split, H1 stalk domain (cHA 6/1), trimeric H9 Head (H9 globular HA domain), tetrameric N2	B memory cells by ELIspot	Frequencies of antigen-specific memory B cells/Mio memory B Cells	GSK Biologicals**	1
PBMCs	Plasmablasts reactive to "challenge" antigens	None, H9N2 split, H1 stalk domain (cHA 6/1), trimeric H9 Head (H9 globular HA domain), tetrameric N2	Plasmablast cells by ELIspot	Frequencies of antigen-specific plasmablasts/Mio plasmablasts	GSK Biologicals**	2

*Refer to APPENDIX B for the laboratory addresses.

GSK Biologicals laboratory refers to the ***Clinical Laboratory Sciences (CLS)* in Rixensart, Belgium; Wavre, Belgium; Dresden, Germany.

PBMC = Peripheral blood monocytes

5.4.2. Biological samples evaluation

5.4.2.1. Immunological read-outs

Table 4 Planned Immunological Read-Outs: ELISAs (Amended 01 September 2016)

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort AS= Adjuvant System	N tests planned as per algorithm)	Comment
Adult subjects: H1 HA stalk ELISA	Recombinant ***antigen based on A/mallard/Sweden/81/02 head domain with A/Puerto Rico/8/34 H1 stalk domain***	D0, D21, D42, D182 (from all 3 adult studies) and D385 (from adult H5N1 study only), 2 treatment groups (AS and no AS)	30 ***(AS)***	780 (30 samples x 4 timepoints x 3 studies x 2 treatment groups + 30 samples x 1 timepoint x 2 treatment groups x 1 study, H5N1)	For all 3 adult study cohorts (***Q-Pan-H1N1-019, CC-Pan-H5N1-001, and Q-Pan-H9N2-001***)
		D0, D21	***30 (no AS)***	***60 (30 samples x 2 timepoints x 1 treatment grp***	***For adult cohort in study D-QIV-015***
		D0, D42, D182, D224, D549, D591, D729	***60 (n=approx 30 each from Groups C and G)***	***420 (60 samples x 7 timepoints x 1 treatment grp***	***For adult study cohorts (Q-Pan-005), Groups C and G***
		D0, D21, Month 6 (M6),Month 12 (M12), M12+21, Month 18 (M18)	***120 (n=approx 60 each from Groups *VT/VT/12M and VT/*IN/12M)***	***720 (60 samples x 6 timepoints x 2 treatment grp***	***For adult cohort from study H5N1-012, Groups *VT/VT/12M and VT/*IN/12M)***
Pediatric subjects: H1 HA stalk ELISA	Recombinant ***antigen based on A/mallard/Sweden/81/02 head domain with A/Puerto Rico/8/34 H1 stalk domain***	D0, D21, D42, D385 (from pediatric H5N1 study only); ***2*** treatment groups (AS ***and placebo***)	30	***240*** (30 samples x 4 timepoints x 1 study x ***2*** treatment groups, ***including placebo group***)	For pediatric ***Q-Pan-H5N1-AS03-021*** study cohort only
N1 NA ELISA (A/California/***04***/2009)	***Recombinant antigen based on A/California/04/2009 NA (N1)***	D0, D21, D42, D182 (from H1N1 study only; done regardless of anti-H1 stalk ELISA results above), 2	30	240 (30 samples x 4 timepoints x 1 study x 2 treatment groups)	For H1N1 study cohort only**

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort AS= Adjuvant System	N tests planned as per algorithm)	Comment
		treatment groups (AS and no AS)			
H2 HA full length ELISA (A/mallard/Netherlands/ 5/99 HA)	Recombinant trimeric H2 HA full length	D0, D42, ***and final timepoint for persistence***, One treatment group (AS)	30	***360*** (30 samples x ***3*** timepoints x ***4*** studies x 1 treatment group)	For ***3*** adult study cohorts (***CC-Pan-H5N1-001, Q-Pan H1N1-019, andQ-Pan H9N2-001)*** and pediatric cohort (***Q-Pan H5N1-AS03-21***)
		D0, D42, D549, D591, and D729; One treatment group	***30***	***150 (30 samples x 5 timepoints x 1 treatment group)***	***For adult study cohort Q-Pan-005: Group C***
		D182, D224, D549, D591, and D729; One treatment group	***30***	***150 (30 samples x 5 timepoints x 1 treatment group)***	***For adult study cohort Q-Pan-005: Group G***
		D0, D21, Month 12 (M12), M12+21, Month 18 (M18)	***120 (n=approx 60 each from Groups *VT/VT/12M and VT/*IN/12M)***	***720 (60 samples x 6 timepoints x 2 treatment grp***	***For adult cohort from study H5N1-012, Groups *VT/VT/12M and VT/*IN/12M)***
		D0, D21; One treatment group	***30***	***60 (30 samples x 2 timepoints x 1 treatment group)***	***For adult study cohort D-QIV-015***
H18 HA full length ELISA (A/flat-faced bat/Peru/033/10 HA)	Recombinant trimeric H18 HA full length	D0, D42, ***and final timepoint for persistence***, One treatment group (AS)	30	***360*** (30 samples x ***3*** timepoints x ***4*** studies x 1 treatment group)	For ***3*** adult study cohorts (***CC-Pan-H5N1-001, Q-Pan H1N1-019 andQ-Pan H9N2-001)*** and pediatric cohort (***Q-Pan H5N1-AS03-21***)
		D0, D42, D549, D591, and D729; One treatment group	***30***	***150 (30 samples x 5 timepoints x 1 treatment group)***	***For adult study cohort Q-Pan-005: Group C***
		D182, D224, D549, D591, and D729; One treatment	***30***	***150 (30 samples x 5 timepoints x 1 treatment group)***	***For adult study cohort Q-Pan-005: Group G***

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort AS= Adjuvant System	N tests planned as per algorithm)	Comment
		group			
		D0, D21, Month 12 (M12), M12+21, Month 18 (M18)	*120 (n=approx 60 each from Groups *VT/VT/12M and VT/*IN/12M)*	*720 (60 samples x 6 timepoints x 2 treatment grp*	*For adult cohort from study H5N1-012, Groups *VT/VT/12M and VT/*IN/12M)*
		D0, D21; One treatment group	*30*	*60 (30 samples x 2 timepoints x 1 treatment group)*	*For adult study cohort D-QIV-015*
N2 NA ELISA (A/chicken/Hong Kong/G9/1997)	Recombinant tetrameric N2 NA	D0, D21, D42; Two treatment groups (AS and no AS)	30	180 (30 samples x 3 timepoints x 1 study x 2 treatment groups)	For H9N2 study cohort only
H9 HA head domain ELISA (A/chicken/Hong Kong/G9/1997)	Recombinant trimeric H9 HA head domain	D0, D21, D42; Two treatment groups (AS and no AS)	30	180 (30 samples x 3 timepoints x 1 study x 2 treatment groups)	For H9N2 study cohort only
H9 HA full length ELISA (A/chicken/Hong Kong/G9/1997)	Recombinant trimeric H9 HA full length	D0, D21, D42; Two treatment groups (AS and no AS)	30	180 (30 samples x 3 timepoints x 1 study x 2 treatment groups)	For H9N2 study cohort only
H3 HA anti-stalk ELISA (cH14/3)	*Recombinant antigen based on the head domain of A/mallard/Gurjev/263/82and the stalk domain of A/Perth/16/09*	*D0, D21, D42; Month 6, and Month 12. Two treatment groups (AS and no AS)*	*30*	*300 (30 samples x 5 timepoints x 1 study x 2 treatment groups)*	*For H7N9 study cohort only*
H4 HA full length ELISA	*Recombinant antigen based on A/duck/Czech /56*	*D0, D42, Month 12 (persistency) ; One treatment group (AS)*	*30*	*90 (30 samples x 3 timepoints x 1 study x 1 treatment group)*	*For H7N9 study cohort only*
H10 HA full length ELISA	*Recombinant antigen based on A/mallard/IA/ 10BM01929/1 0*	*D0, D42, Month 12 (persistency) ; One treatment group (AS)*	*30*	*90 (30 samples x 3 timepoints x 1 study x 1 treatment group)*	*For H7N9 study cohort only*
Total anticipated number of ELISA tests					*6420*

*Anti-N1 NA testing for H1N1 study cohort only, since only for this cohort was the vaccine NA matching with the ELISA test NA

HI testing by GSK for anti-H1N1 at D0 for subjects in the H5N1 and H9N2 cohorts will be elective based on laboratory capacity

VT= A/Vietnam/1194/2004-like strain and IN = A/Indonesia/5/2005-like

Table 5 Planned Immunological Read-Outs: Microneutralization (MN) assays (Amended 01 September 2016)

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested (AS treatment) per study cohort	N tests planned as per algorithm)	Comment
H1 HA stalk domain MN	Chimeric virus (***cH6/1N5***)	Adult subjects: D0, D21, D42, D182; AS groups only	30	240 (30 samples x 4 timepoints x 2 studies)	For adult ***Q-Pan-H1N1-019*** and ***Q-Pan-H9N2-001*** study cohorts ***(all subjects who received an AS vaccine)***
		Adult subjects: D0, D21, D42, D182, D385; AS group only	30	150 (30 samples x 5 timepoints)	For adult ***CC-Pan-H5N1-001*** study cohort ***(all subjects who received an AS vaccine)***
		Pediatric subjects: D0, D21, D42, D385; AS group only	30	120 (30 samples x 4 timepoints x 1 treatment)	For pediatric ***Q-Pan-H5N1-AS03-001*** study cohort ***(all subjects who received an AS vaccine)***
		Adult subjects: Group C: D0, D42, D182, D549, D591, D729; and Group G: D182, D224, D549, D591, D729	***60 (Approx 30 from each of Groups C and G***	***330 (Group C: 30 samples x 6 timepoints = 180; Group G: 30 samples x 5 timepoints = 150)***	***For adult Q-Pan-005 study cohort, Groups C and G***
		Adult subjects: D0, D21, Month 6 (M6), Month 12 (M12), M12+21 days, Month 18 (M18)	***120 (n=approx 60 each from Groups VT/VT/12M and VT/IN/12M)***	***720 (60 samples x 6 timepoints x 2 treatment groups)***	***For adult cohort from study H5N1-012, Groups VT/VT/12M and VT/IN/12M)***
		Adult subjects, D0, D21 (D-QIV group only, no AS)	***30***	***60 (30 samples x 2 timepoints)***	***For adult D-QIV-015 study cohort***
Heterosubtypic Group I influenza A virus MN	RG reassortant virus (***H5N8***), H1N1 swine Flu (A/Swine/Jiangsu/40/2011) ***virus, and H1N1 pdm09-like virus***	D0, ***D42***, (from all 4 studies, i.e., adult H1N1, H5N1, H9N2, and pediatric	***30***	***720*** (***30*** samples x 2 timepoints x 4 studies ***x 3 viruses***)	For ***all subjects who received an AS vaccine in all*** 4 study cohorts (***adult studies Q-Pan-H1N1-019, CC-Pan-H5N1-001, Q-Pan-H9N2-001, and***

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested (AS treatment) per study cohort	N tests planned as per algorithm)	Comment
		H5N1); AS groups only			*pediatric study Q-Pan-H5N1-AS03-021*)
		Adult subjects: Group C: D0, D42, D549, D591, and Group G: D182, D224, D549, D591	*60 (Groups C and G, n= approx 30 each*	*720 (60 samples x 4 timepoints x 3 viruses)*	*For adult Q-Pan-005 study cohort, Groups C and G*
		Adult subjects: D0, D21, Month 12 (M12), and M12+21 days	*120 (n=approx 60 each from Groups VT/VT/12M and VT/IN/12M)*	*480 (60 samples x 4 timepoints x 2 treatment groups)*	*For adult cohort from study H5N1-012, Groups VT/VT/12M and VT/IN/12M)*
		Adult subjects, D0, D21 (D-QIV group only, no AS)	*30*	*180 (30 samples x 2 timepoints x 3 viruses)*	*For adult D-QIV-015 study cohort*
H3 HA stalk domain MN (for H7N9 samples)	*RG reassortant virus cH14/3NX*	*D0, D21, D42, Month 6, Month 12; AS group only*	*30*	*150 (30 samples x 5 timepoints x 1 study)*	*For adult H7N9 study cohort*
Heterosubtypic Group 2 influenza A virus MN (for H7N9 samples)	*RG reassortant virus H4N8*	*D0, D42; AS group only*	*30*	*60 (30 samples x 2 timepoints x 1 study)*	*For adult H7N9 study cohort*
Homologous virus MN	*Adult H5N1, H9N2*	*D0, D21*	*	*	*For adult studies CC-Pan-H5N1-001, Q-Pan-H9N2-001 and pediatric study Q-Pan-H5N1-AS03-021*
	Pediatric H5N1	*D0, D21*			
Total anticipated number of MN tests					*3930*

* Already performed in primary studies and results are available.

Table 6 Planned Immunological Read-Outs: HI assays

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort	N tests planned as per algorithm)	Comment
HI assay	A/Indonesia/5/2005 ***A/Vietnam/1194/2004-like***	D0 (from the ***CC-Pan-H5N1-001 Q-Pan-005, and H5N1-012 studies***); 2 treatment groups (AS and no AS)	***Not applicable***	***Not applicable***	For the ***CC-Pan-H5N1-001, Q-Pan-005, and H5N1-012*** study cohorts only ***HI assays already done in primary study; samples will not be retested.***
HI assay	A/chicken/Hong Kong/G9/1997	D0 (from the H9N2 study); 2 treatment groups (AS and no AS)	***Not applicable***	***Not applicable***	For the ***Q-Pan-H9N2-001*** study cohort only ***HI assays already done in primary study; samples will not be retested.***
HI assay	A/California/07/2009 (or a like virus)	D0 (from the H5N1 and H9N2 studies); ***D182 for Q-Pan-005 study;*** 2 treatment groups (AS and no AS)	30	***180*** (30 samples x 1 timepoint x ***3*** studies x 2 treatment groups)	For the ***CC-Pan-H5N1-001, Q-Pan-H9N2-001, Q-Pan-005, and H5N1-012*** study cohorts
Total anticipated number of HI tests					**180**

The planned immunological read-outs for the study are summarized in Table 4, Table 5, and Table 6. Based on the results of these evaluations, the laboratory analyses may be extended by mutual agreement between GSK and ISMMS ***(and NeoMed Labs)*** to include additional assays and/or assay types to further assess the intensity and breadth of the anti-influenza virus antibody response elicited by vaccination.

6. STUDY VACCINES AND ADMINISTRATION

6.1. Description of study vaccines

See the table below for the vaccine strains administered in the primary prospective studies.

Table 7 Description of study vaccines (Amended 01 September 2016)

Study no.	Vaccine strain	Start date
Q-Pan H1N1-019 (113536)	A/California/7/2009	28 Oct 2009
CC-Pan H5N1-001 (114371)	A/Indonesia/5/2005 RG	29 Nov 2010
Q-Pan H9N2-001 (116358)	A/chicken/Hong Kong/G9/1997 NIBRG-91	22 Aug 2012
Q-Pan H5N1-AS03-021 (114464)	A/Indonesia/5/2005 RG	07 Mar 2011
Q-Pan-005 (110624)	***A/Indonesia/5/2005 or A/turkey/Turkey/1/05***	***15 Jul 2008***
H5N1-012 (107495)	***A/Indonesia/5/2005-like or A/Vietnam/1194/2004-like***	***05 Feb 2007***
Q-Pan H7N9-AS03-001 (201072)	***A/Shanghai/2/2013(H7N9)-RG32A***	***25 Nov 2013***
D-QIV-015 (201251)	***(A/Christchurch/16/2010 (H1N1)pdm09 A/Texas/50/2012 (H3N2) B/Massachusetts/02/2012 B/Brisbane/60/2008)***	***18 Aug 2014***

6.2. Dosage and administration of study vaccines

The dosage and administration (intramuscular in all cases) of study vaccines in the previously conducted trials from which serum samples will be selected for further laboratory analyses in this study, ***were as follows***:

- ***FLU Q-Pan-H1N1-019 (113536):***
  - ***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***
  - ***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 (114371):***
  - ***Group A = 3.75 µg HA CC-PAN H5N1 vaccine adjuvanted with $AS03_A$ given at Day 0 and Day 21***

- ***Group B = 15 µg HA (no AS) CC-PAN H5N1 vaccine given at Day 0 and Day 21***

- ***FLU Q-Pan H9N2-001 (116358):***
  - ***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***
  - ***Group 375_A_VVV = 3.75 µg HA H9N2 vaccine antigen adjuvanted with $AS03_A$ given at Day 0, Day 21, and Day 182***
  - ***Group 1500_VVP = 15 µg HA (no AS) H9N2 vaccine given at Day 0 and Day 21; saline placebo at Day 182***
  - ***Group 1500_VVV = 15 µg HA (no AS) H9N2 vaccine given at Day 0, Day 21, and Day 182***
- ***FLU Q-Pan H5N1-AS03-021 (114464)***
  - ***Group A: 1.9 µg HA/$AS03_B$ (at Day 0 and Day 21)***
  - ***Group B: Placebo (at Day 0 and Day 21)***
- ***FLU Q-Pan-005 (110624)***
  - ***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***
  - ***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***
- ***H5N1-012 (107495)***
  - ***Group VT/VT/M12: Two doses of A/Vietnam/1194/2004-like H5N1 vaccine adjuvanted ($AS03_A$) at Day 0 and Month 12 (i.e., homologous booster dose at Month 12)***
  - ***Group VT/IN/M12: One dose of A/Vietnam/1194/2004 adjuvanted ($AS03_A$) H5N1 vaccine at Day 0 and one dose of A/Indonesia/5/2005-like H5N1 vaccine adjuvanted ($AS03_A$) at Month 12 (i.e., heterologous booster dose at Month 12)***
- ***FLU D-QIV-015 (201251): 15 µg HA (no AS) of each of 4 strains (total 60 µg HA) at Day 0***
- ***FLU Q-Pan H7N9-AS03-001 (201072)***
  - Group 1500: 15 µg HA (no AS) at Day 0 and Day 21
  - Group 375_A: 3.75 µg HA/$AS03_A$ at Day 0 and Day 21

7. HEALTH ECONOMICS

Not applicable.

8. SAFETY

Not applicable since no subjects will be involved and, therefore, safety will not be assessed in this study.

9. SUBJECT COMPLETION AND WITHDRAWAL

Not applicable since no subjects will be enrolled in this study.

10. STATISTICAL METHODS

10.1. 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 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
  - 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***
- 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***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 182 to Day 591***
  - ***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***
  - ***GMT at Day 0 and 21***
  - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
  - ***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)
  - 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***
  - 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
  - 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***
  - 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)***
  - ***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 and***
      - ***from Day 549 to Day 591 and***

        - ***from Day 0 to Day 591***
    - ***For group G:***
        - ***from Day 182 to Day 224 and***
        - ***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 and***
        - ***from Day 549 to Day 591 and***
        - ***from Day 0 to Day 591***
    - ***For group G:***
        - ***from Day 182 to Day 224 and***
        - ***from Day 549 to Day 591 and***
        - ***from Day 182 to Day 591***
- ***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 the FLU D-QIV-015 cohort:***
    - ***Seropositive rate at Day 0 and 21***
    - ***GMT at Day 0 and 21***
    - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
    - ***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)***
- 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
- 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***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 182 to Day 591***
  - ***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***
  - ***GMT at Day 0 and 21***
  - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***

- ***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***
  - GMT at Day 0 and Day ***42***
  - Percentage of subjects with a ≥4-fold rise from Day 0 to Day ***42***
  - 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***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 182 to Day 591***
  - ***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***
- ***MGI at Day 21 compared to Day 0***

(Amended 01 September 2016)

10.2. 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 cohorts. 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 D182 (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, 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***

10.3. 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):

1. ***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***
- ***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***
- ***MGI at Day 21, 42, 182 compared to Day 0***

2. ***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***

3. 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 adult 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

4. 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:

- MGI for anti-H1 stalk ELISA at Day 21 compared to Day 0

5. 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

6. 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
- GMT at Day 0, 21, and 42
- Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21, and Day 42
- MGI at Day 21 and 42 compared to Day 0

Tertiary endpoints: 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***
   - ***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***
   - ***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***
   - ***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***
   - ***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)***
   - ***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***
   - ***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***
   - ***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***
- ***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***

Tertiary endpoints: Passive transfer/virus challenge in mice with pooled adult human sera from subjects who received adjuvanted H5N1 vaccine the CC-Pan-H5N1 study cohort:

1. ***The in vivo protective effect of transferring pooled adult human serum (from subjects administered adjuvanted H5N1 vaccine in the CC-Pan-H5N1 study cohort) to mice and subsequently challenging them with cH5/3Nx virus (Nx=most likely N4 or N5, to be decided) will be assessed in terms of the following endpoints:***
   - ***survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice/serum pool/time-point***
   - ***mean weight loss (change from baseline over 14 days post challenge) in groups of 25 mice/serum pool/time-point***
   - ***lung weight in micrograms (D42 minus D0), (D385 minus D0), within challenge group***
   - ***lung virus titer in pfu/microgram (log10 fold change (D0 minus D42), (D0-D385), within challenge group***
2. ***The in vivo protective effect of transferring pooled adult human serum (from subjects administered adjuvanted H5N1 vaccine) to mice and subsequently challenging them with cH6/1N5 virus will be assessed in terms of the following endpoints:***
   - ***survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice/serum pool/time-point***
   - ***mean weight loss (change from baseline over 14 days post challenge) in groups of 25 mice/serum pool/time-point***
   - ***lung weight in micrograms (D42 minus D0), (D385 minus D0), within challenge group***
   - ***lung virus titer in pfu/microgram (log10 fold change (D0 minus D42), (D0-D385), within challenge group***
3. ***Post-transfer titer of human IgG to cH5/3 by ELISA***
4. ***Post-transfer titer of human IgG to cH6/1 by ELISA***

(Amended 01 September 2016)

10.4. Determination of sample size

In this study, the primary objective is to assess the humoral immune response by anti-H1 stalk ELISA in each study cohort; anti-H2 and anti-H18 full length ELISA, anti-H1 stalk response by neutralization, and vaccine-heterosubtypic virus response by microneutralization (MN) ***for all subjects who received AS vaccine in each study cohort and for the subjects in the FLU-D-QIV015 study cohort***.

Table 8 presents the 95% confidence intervals (95% CI) for various observed proportions (e.g., seropositivity rate and % of subjects with at least 4-fold antibody titer increase by anti-H1 stalk MN) for 30 subjects in each study group.

Table 8 Illustration of the lower (LL) and upper (UL) limits of the exact 95% CI built around various observed proportions with a sample of 30 subjects

Number of subjects seropositive * or subjects who have >= 4 fold increase** (n)	Observed proportion (%) per group (N=30)	Exact 95% CI	
		LL	UL
20	66.7	47.2	82.7
21	70.0	50.6	85.3
22	73.3	54.1	87.7
23	76.7	57.7	90.1
24	80.0	61.4	92.3
25	83.3	65.3	94.4
26	86.7	69.3	96.2
27	90.0	73.5	97.9
28	93.3	77.9	99.2
29	96.7	82.8	99.9
30	100.0	88.4	100.0

*seropositive= subjects with antibody concentration or titer ≥ assay cut-off
subjects who have >= 4 fold increase = subjects with at least a 4-fold increase in post-vaccination reciprocal titer (from D0 to D21) tested by anti-H1 stalk ***MN*. For seronegative subjects, half of the cut-off of the assay will be considered as pre-vaccination titers.

All subjects from each study cohort with available results will be included in the analysis for this study.

10.5. Derived and transformed data

- The cut-off value is defined by the laboratory before the analysis and is described in Section 5.4.1 (Table 2).

- 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 concentration/titer transformations. Antibody concentrations/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.
- 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***

10.6. Analysis of demographics

Demographic characteristics (age at first study vaccination in years, 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 H9N2 and adult H5N1 studies (if data become available)

10.7. Analysis of immunogenicity

10.7.1. Within groups assessment

For each study group in ***the HA Group 1-related*** study cohorts (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); refer to endpoints.***
- ***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); refer to endpoints.***
- The distribution of antibody titers for anti-H1 stalk ELISA will be displayed using reverse cumulative distribution curves.
- For AS group, correlation between the level of neutralizing antibody to the H1 stalk with the level of vaccine homologous neutralizing antibody, at D0 and at D21 in study cohorts H9N2 and both adult and pediatric H5N1 study cohorts.
- 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 cohorts.
- CMI summaries for the H9N2 cohort only
- 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.***

10.7.2. Between groups assessment

10.7.2.1. For adult H5N1, H9N2, and H1N1 study cohorts)

- For each ***Group 1-related*** study and for anti-H1 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 ***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 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 the anti-N1 NA ELISA result at Day 0 as covariates.

10.7.2.2. 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.***

Further details will be provided in the statistical analysis plan.

10.8. Analysis of safety

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

10.9. Conduct of analyses

The planned analysis is descriptive and will be performed for each treatment group in the individual study ***cohorts***.

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

10.9.1. 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.

10.9.2. Statistical considerations for interim analyses

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

11. ADMINISTRATIVE MATTERS

For a typical clinical study protocol, this section provides guidance to comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality and publications.

This is a non-clinical retrospective study whose objectives pertain to analyses of archived serum samples from previously completed studies and, therefore, no study subjects are involved. Essential GCP training was provided to the ISMMS site staff before study start to ensure appropriate management of samples.

11.1. Case Report Form/Remote Data Entry instructions

Not applicable since no subjects will be involved.

11.2. Study Monitoring by GSK Biologicals

Not applicable since no subjects will be involved.

11.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and

retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

11.4. Quality assurance

Essential GCP training was provided to ISMMS site staff before study start to ensure appropriate management of samples.

11.5. Posting of information on publicly available clinical trial registers and publication policy

Study information from this protocol is not required to be posted unless the study results provide important scientific knowledge or are relevant for patient care.

Summary protocols or plans and results for analyses that are designed to inform the feasibility, conduct, or design of other studies are not required to be posted or submitted for publication unless the results provide important scientific knowledge

Provision of study results to investigators:

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK Biologicals will also provide the investigator with the full summary of the study results.

12. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

13. REFERENCES

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APPENDIX A LABORATORY ASSAYS

1. Immunogenicity Assays: ELISA protocol:

Materials:

Flat-Bottom Immuno Nonsterile 96-Well Plates 4 HBX (*ThermoScientific #3855*)

ELISA coating buffer

5.3 g Na_2CO_3
4.2 g $NaHCO_3$
1L of ddH_2O
pH 9.4

T-PBS

0.1% Tween-20 (Fisher BioReagents)
99.9% PBS pH 7.4 (1X) (gibco)

Blocking solution

3% Goat Serum (gibco)
0.5% Milk Powder
96.5% T-PBS (see above)

Anti-Human IgG (Fab specific)-Peroxidase antibody (*Sigma #A0293*)

Water For Injection (WFI) for Cell Culture (*Gibco*)

SigmaFast OPD (*Sigma #P9187*)

3M HCl

Preparation:

96-well plates are coated with 50 µL of antigen diluted in ELISA coating buffer at a concentration of 2 µg/mL per well and refrigerated at 4°C overnight.

Instructions:

1. Discard antigen and coating buffer with a quick single shake whilst holding the plate up-side-down
2. Add 100 µL of blocking solution to each well and incubate for 1 hour at room temperature
3. Add an additional 100 µL of blocking solution to the first column of wells

4. Add 2 µL of patient serum to the first well of each row (result is a 1:100 dilution in the first well); leave 1 row empty on each plate for use as a blank
5. Mix first row by pipetting up and down and transfer 100 µL to the second column
6. Continue dilution series for all 12 columns and discard the last 100 µL; change tips after each column
7. Incubate plates for 2 hours at room temperature
8. Discard diluted patient sera and wash 3x with 100 µL of T-PBS per well; after 3rd wash smack plate onto paper towel to completely empty wells
9. Add 50 µL of Anti-Human IgG antibody diluted at 1:3000 in blocking solution to each well and incubate for 1 hour at room temperature
10. Discard diluted Anti-Human IgG antibody and wash 3x with 100 µL of T-PBS per well - do not remove 3rd wash before finishing step 11
11. For 20 mL of SigmaFast OPD mix 1 tablet of SigmaFast buffer and 1 tablet of SigmaFast OPD each in 20 mL of water for injection; shake until tablets are completely dissolved
12. Discard 3rd wash, smack plate onto paper towel to completely empty wells and add 100 µL of SigmaFast OPD to each well
13. After 10 minutes, add 50 µL of 3M HCl to each well
14. Read ELISA plates at absorbance of 490 nm

2. Immunogenicity Assays: Microneutralization (MN) Assay protocol:

Day 1: plate MDCK cells

1. Plate 100ul of 1.5-1.8 x10^6 MDCK cells/10ml to each well (so 1.5-1.8 x10^4 cells/well) and incubate at 37C overnight

Day 2: dilute antibodies, incubation of Ab & virus, and infection

Layout (VC=virus only control CC=Uninfected cell control)

1. Dilute the mAb with starting concentration of 200ug/ml. 100ul of the diluted Ab would be needed or each of the first well.
2. Perform 2-fold serial dilutions downwards by transferring 50ul from A→B…H.
3. Dilute virus to 30 to 100 TCID50/50ul (60/200 TCID50/100ul) in infection media
4. Add 50ul diluted virus to each well except CC where media without virus would be added.
5. Incubate the Ab/virus mixture at 37C for 1-2 hrs
6. Remove the MDCK plate from day 1 from incubator and wash twice with 100ul media
7. Transfer 100ul the Ab/virus mixture from step 6 to MDCK plate and incubate at 37C for 1 hr.
8. After adsorpsion, remove the inoculation. Wash once with media and replace with infection media containing appropriate mAb concentrations.
9. Incubate at 37C overnight.

Day 3: fix cells and ELISA

1. Observe CPE @~20-22hrs
2. Wash cells with 200ul of 1xPBS
3. Fix cells with 200ul ice cold 80% acetone and incubate at 4C for 1 hr
4. Wash plate 4 times with wash buffer (1xPBS+0.05% Tween)
5. Block plate with 30 min 5% milk then 30min 3% hydrogen-peroxide

6. Dilute primary Ab in 1xPBS/1%BSA/0.1%tween
7. Discard H_2O_2 and add 50ul primary Ab to each well and incubate at 37C for 1 hour
8. Wash plate 4 times with wash buffer
9. Add 50ul secondary Ab to each well and incubate at 37C for 1 hour
10. Wash plate 4 times with wash buffer
11. Add 200ul OPD substrate to each well, incubate at room temperature for 30 min before reading.

Data Analysis (from the booklet page 15)

1. Calculations are determined for each plate individually
2. Determine virus neutralizing Ab endpoint titer using the equation:

(((AverageOD of VC wells)-AverageOD of CC wells))/2)+(AverageOD of CC well) = X

→ where X = 50% of specific signal. All values below this value are positive for neutralization activity.

3. B-cell ELISPOT:

The B Cell ELISPOT technology allows the quantification of antigen-specific B-memory cells by inducing the differentiation of memory B cells into Ab secreting plasma cells *in vitro* following stimulation with CpG for 5 days. The B ELISPOT protocol used was adapted from the protocol developed by the Lanzavecchia laboratory. Briefly, human PBMC were plated in 6-well dishes at 10^6 cells/ml in 5 ml of complete medium supplemented with 3 µg/ml phosphothioated CpG ODN-7909 -5'-TCG TCG TTT TGT CGT TTT GTC GTT-3' (Coley, MA). Cells were then cultured for 5 days at 37 °C in 6–8% CO2. For the detection of antigen specific responses, 96-well PVDF filter plates were coated overnight with the relevant antigen. For the detection of total IgG-secreting cells, 96-well PVDF plates were coated with goat anti-human Ig (Jackson laboratories, PA USA). Plates were washed and blocked with complete medium for 1 h at 37°C before use. Cultured were washed, plated onto ELISPOT plates (between 5 10exp4 and 20 10exp4 cells per 100 µl), and incubated for 1h at 37°C. For detection we used biotinylated mouse anti-human pan IgG (Jackson laboratories,PA USA) in PBS/0.05% Tween 20/1% FCS, followed by 5 µg/ml horse radish peroxidase-conjugated avidin D (Vector Laboratories, Burlingame, CA), and developed using 3-amino-9-ethylcarbozole (Sigma). Developed plates were counted using an automated ELISPOT image analysis system (Zeiss KS400). Results are expressed as a frequency of antigen-specific antibody producing cell within the total IgG-producing B cell population in PBMC. [Refer to Moris, 2011].

4. Plasma -cell ELISPOT:

In order to detect the specific plasma cells, antibody secreting cells, in the blood sample collected, the same method for B cell Elispot is used but the maturation of the memory B

cells into plasma cells is of course not applied, so that, after thawing and washing steps, the PBMC samples are directly plated on the previously coated PVDF plate.

5. Detection of Plasmablast/B cell by cytometry using HA- SA biotinylated probe:

The detection of specific plasma cells and B cells will be performed by cytometry using recombinant HA-SA biotinylated probe that will be stained by a fluorescent dye. Combining the staining of cells using the HA probe with appropriate phenotyping fluorescent as anti-IgD, anti-CD20/19, anti-CD45, the assay will allow detection in the sample the plasma cells and memory B cells specific to the fluorescent probe as described by *Amanna & Slifka* [Amanna, 2006].

6. T Cell detection by intracellular Cytokine Staining (ICS) assay:

***Ex vivo* short term T-cell re-stimulation assay**

Influenza-specific T-cell responses will be assessed using a previously described method adapted from Maecker *et al.* Peripheral blood mononuclear cells (PBMCs) were stimulated for 2 hours by the relevant antigen in the presence of co-stimulatory antibodies to CD28 and CD49d. Brefeldin A was added for a subsequent 18h incubation to promote intracellular accumulation of cytokines. Cells were stained using fluorochrome-conjugated antibodies before enumeration by flow cytometry. $CD3^+$ cells were typed as specific $CD4^+$ or $CD8^+$ T cells expressing different markers among CD40L, IFN-γ, IL-2, TNF-α, with the background response (no stimulation) subtracted.

References: [Moris, 2011; Couch, 2014; and Maecker, 2000]

7. Serum transfer/ virus challenge experiment in BALB/c mice for CC-Pan-H5N1 vaccination sera (from adult subjects who received adjuvanted H5N1 vaccine)

Exploratory protocol objectives (to be placed in main protocol as well):

1. To assess the protective effect of pooled human serum collected on D42 and D385 from adult recipients of CC-PAN H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that is subsequently challenged with approximately $5LD_{50}$ of **cH5/3Nx virus** (or an alternative challenge virus with similar attributes but more fit for purpose; Nx=most likely N4 or N5, to be decided) , in terms of:

A. proportion surviving challenge

B. mean weight loss over time (AUC)

C. the following 2 endpoints in randomly selected subgroups of animals from each of 3 treatment groups administered the D0, D42, or D385 serum pools; animals will be euthanized at D3 (N=5) and D6 (N=5) post challenge:

I. mean lung weight at necropsy

II. geometric mean lung virus titer (pfu/microgram)

2. To assess the protective effect of pooled human serum collected on D42 and D385 from adult recipients of CC-PAN H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that is subsequently challenged with approximately $5LD_{50}$ of **cH6/1N5 virus** (or an alternative challenge virus with similar attributes but more fit for purpose), in terms of:

A. proportion surviving challenge

B. mean weight loss over time (AUC)

C. the following 2 endpoints in randomly selected subgroups of animals from each of 3 treatment groups administered the D0, D42, or D385 serum pools; animals will be euthanized at D3 (N=5) and D6 (N=5) post challenge:

I. mean lung weight at necropsy

II. geometric mean lung virus titer (pfu/microgram)

3. If the pathogenicity (expressed as pfu/LD_{50}) of the **cH5/3Nx virus** and **cH6/1N5 virus** are comparable, describe the effect size of anti-HA stalk antibodies (revealed by **cH6/1N5 virus** challenge) and anti-HA head antibodies (revealed by **cH5/3Nx virus** challenge) in terms of the relative survival rate (D42 rate/D0 rate) for cH6/1N5 vs relative survival rate for cH5/3Nx.

4. To describe the post transfer geometric mean ELISA titer of human IgG to cH5/3Nx and human IgG to cH6/1N5 in blood collected from mice receiving each of 3 serum pools (D0, D42, D385)

To explore the association between post-transfer ELISA titer of human IgG to the challenge virus and outcome at D3 and D6:

A. proportion surviving challenge,

B. mean weight loss,

C. mean lung weight at necropsy, and

D. geometric mean lung virus titer

Exploratory protocol endpoints

Objective	Endpoint
1A, 2A, 3, 5A	survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice*/serum pool/time-point
1B, 2B, 5B	mean weight loss (change from baseline over 14 days post challenge) in groups of 25 mice*/serum pool/time-point
1C, 2C, 5C	lung weight in microgram (D42 minus D0), (D385 minus D0), within challenge group
1D, 2D, 5D	lung virus titer in pfu/microgram (log10 fold change (D0 minus D42), (D0-D385), within challenge group
4	Post-transfer ELISA titer of human IgG to cH5/3 by ELISA, Post-transfer ELISA titer of human IgG to cH6/1 by ELISA

If sufficient serum volumes are not available, the number of mice can be reduced to as low as 15 mice per time-point and virus

Viruses:

Challenge virus	Surface glycoprotein attributes	Virus-specific protection mediated by
cH5/3NX (or an alternative challenge virus with similar attributes but more fit for purpose)	• Hemagglutinin (HA) head domain matched (or cross-reactive) to vaccine strain – can be **neutralized by head antibodies** induced by H5N1 vaccination • HA stalk domain **not matched** to vaccine strain • Exotic neuraminidase **not matched** to vaccine strain	HA head antibody responses
cH6/1N5 (or an alternative challenge virus with similar attributes but more fit for purpose)	• Exotic HA head domain not matched to vaccine strain • HA stalk domain from H1 – can be neutralized by cross-reactive stalk antibodies induced by H5N1 vaccination • Exotic neuraminidase not matched to vaccine strain	HA stalk antibody responses

Both viruses will be pre-assessed for mouse lethality in LD_{50} experiments (with and without presence of adult human serum pools). The volume and route of inoculation will be 0.05 ml intranasal/intratracheal administered to anesthetized mice.

Serum pools:

Three separate serum pools will be created using all residual serum samples with sufficient volume to furnish an aliquot of an equal volume that were collected on D0, D42,and D385 from the adult CC-Pan-H5N1 cohort (18-49 YOA) which received AS03-adjuvanted H5N1 vaccine.

Passive transfer experimental setup:

- 35 mice per time-point/virus challenge are transferred with a standard amount of undiluted pooled serum (volume to be determined, within the range 150-250ul): (35 mice x 2 virus challenges x 3 timepoints = 210 mice total). However, if sufficient serum volumes are not available, the number of mice can be reduced to as low as 15 mice per time-point and virus.
- 5 hours post serum transfer the mice are sedated and:
  - blood is collected from the periorbital plexus for processing to serum and ELISA determination
  - then challenged with 5xLD50 delivered by the IN/IT route (or a lower dose selected to provide a level of weight-loss and lethality using D0 serum that could be reduced by a 3-10 fold increased level of anti-HA stalk antibodies present in post-vaccination serum).
- Mice are monitored daily for 14 days for weight-loss and are euthanized if they lose >25% of their initial body weight.
- On day 3 and day 6 post-infection 5 mice per time-point/virus are euthanized to assess viral lung titers (30 mice per day/virus, 60 mice total). After extraction, lung weights are recorded as an additional measure of morbidity (i.e., fluid accumulation resulting from the infectious process following challenge will lead to increased lung weight). The method of removing the lungs and trachea en bloc after exsanguinations will be standardized to assure comparability among groups.
- Weighing of the mice and the lungs will be performed in an open fashion. Viral plaques will be counted by a blinded technician.
- Lung suspensions will be made by homogenization in PBS and frozen at -80C for later plaque assay using a standard method.

APPENDIX B CLINICAL LABORATORIES

Table 9 Collaborating laboratories (Amended 01 September 2016)

Laboratory	Address
Dept. of Microbiology, Icahn School of Medicine at Mount Sinai	Dept. of Microbiology Icahn School of Medicine at Mount Sinai New York, NY USA
NEOMED LABS INC.	***7171 Frederick-Banting St, Saint-Laurent, Quebec H4S 1Z9, Canada***

Table 10 GSK Biologicals' laboratories

Laboratory	Address
GSK Biologicals Global Vaccine Clinical Laboratory, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart - Belgium
GSK Biologicals Global Vaccine Clinical Laboratory, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium
GSK Dresden GlaxoSmithKline Biologicals Branch of SmithKline Beecham Pharma GmbH & Co. KG	Zirkusstrasse 40, D-01069 Dresden Germany

APPENDIX C AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals	
Clinical Research & Development	
Protocol Amendment 1	
eTrack study number and Abbreviated Title(s)	201598 (FLU CC-SUIV-AS03-001)
IND number	NA
Amendment number:	Amendment 1
Amendment date:	10 March 2015
Co-ordinating author:	PPD, *Lead Scientific Writer*

Rationale/background for changes:

- Modification of the co-primary objectives/endpoints and study design due to inclusion of archived samples (for immunological laboratory evaluation) from a previously completed pediatric study with subjects 6-35 months of age, Q-Pan H5N1-AS03-021 (114464). This will allow an evaluation of the adjuvanted H5N1 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).
- Addition of a new co-primary objective to describe the anti-H2 and anti-H18 antibody levels at baseline and post-dose 2 in all subjects from each study cohort who received an adjuvanted vaccine. This will facilitate an evaluation of whether a post-vaccination boost in anti-H1 stalk ELISA antibody titers exhibits cross reactivity to diverse influenza A Group 1 subtype viruses.
- Modification of the serology algorithm to reflect the inclusion of the pediatric samples and to improve the efficiency of the microneutralization assays (to measure anti-H1 stalk neutralizing antibody) by replacing the mix of cHA 5/1N3 and cHA9/1N3 challenge viruses in the original protocol with a single cHA 8/1N3 virus.
- The serology tables were updated to provide detailed strain or antigen descriptions.

In this summary, the amended or changed text will be indicated in ***bold italics*** and any deleted text will be indicated in strikethrough (e.g. ~~text~~).

Title	An exploratory, retrospective ***lab*** evaluation of the humoral immune response in adults ***and children*** to the H1 HA stalk domain and other influenza A virus protein epitopes, ***after*** administration of GSK Biologicals' pandemic influenza vaccines
Detailed Title	An exploratory, retrospective laboratory evaluation, using specimens from completed clinical trials, of the humoral immune response to the H1 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, and H9N2 pandemic influenza vaccines
Co-ordinating author	PPD, *Lead Scientific Writer*
Contributing authors	PPD, *Director, Lead CRDL* PPD, *Project Statistician* PPD, *Lead Statistician* PPD, *Study Delivery Lead* PPD, *GVCL Project Manager* PPD, *Senior Manager, Human Cellular Immunology* PPD, *Scientific Data Manager* PPD, *Vice President, Late Clinical Development* *PPD, *Assistant Professor* *PPD, *Professor* * *Dept. of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Detailed Title	An exploratory, retrospective laboratory evaluation, using specimens from completed clinical trials, of the humoral immune response to the H1 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, and H9N2 pandemic influenza vaccines
Rationale for the study and study design	• Rationale for the study FLU CC-SUIV-AS03-001 is the initial study in GSK Biologicals' clinical development plan for a candidate supra-seasonal universal influenza vaccine (SUIV) and is an exploratory, retrospective laboratory study, using archived

serum samples, to assess the humoral immune response to the H1 hemagglutinin stalk domain and other influenza A virus protein epitopes following administration in adults ***and children*** of GSK Biologicals' adjuvanted ***or*** unadjuvanted pandemic vaccines. These vaccines can serve as a surrogate for one dose of an inactivated chimeric hemagglutinin-bearing virus vaccine, because the stalk domain of the vaccines' hemagglutinins share important epitopes with the H1 stalk despite amino acid sequence differences in the overall hemagglutinin stalk domain among the H5, H1, and H9 viruses. The results of this study will provide context for and inform the design of the follow-up study, FLU CC-SUIV-AS03-002, which will evaluate the humoral immune responses to sequential administration of SUIV candidate vaccines to find a vaccine formulation (antigen and adjuvant dose) that has an acceptable reactogenicity and immunogenicity profile. The data obtained from the current FLU CC-SUIV-AS03-001 study will also support a pre-IND meeting with the FDA's Center for Biologics Evaluation and Research (CBER) by demonstrating that the immunoassays to be deployed in study FLU CC-SUIV-AS03-002 are fit for purpose and that the range of antigen and adjuvant doses proposed for study are reasonably likely to be acceptably immunogenic.

- Rationale for the study design

The 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) of H1N1pdm09, H5N1, and H9N2 adjuvanted or unadjuvanted pandemic influenza vaccines (standard adult dose) using serum specimens collected from 3 completed clinical trials ***with adult subjects (H1N1, H5N1, and H9N2)*** at baseline, after 1 and 2 doses (Day 21 and Day 42, respectively), and after extended follow-up at Day 182, and in 1 of 3 cohorts, also at Day 385 (i.e., in H5N1, since this study also had a follow-up blood collection time point at Day 385). Therefore, Day 0, 21, 42, and 182 time point samples from the H1N1, H5N1, and H9N2 studies, as well as Day 385 time point samples from the H5N1 study will be tested. For each study, 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). 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.

A serology testing algorithm will be used to rationalize the serology analyses. In the initial step, an anti-H1 stalk domain ELISA (enzyme-linked immunosorbent assay) will be used to analyze archived samples (with sufficient volume for the serology analyses) from the Day 0, 21, 42, and 182 time points of all 3 ***adult*** study cohorts ***(H1N1, H5N1, and H9N2)***, the D385 time point of the ***adult*** H5N1 study cohort, ***as well as the Day 0, 21, 42, and 385 time points of the pediatric H5N1 study***.

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 cH8/1 N3, 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). ***In addition, 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, all D0 and D42 samples from subjects who received an adjuvant system (AS) vaccine will be tested for reactivity with H2 and H18 full length recombinant hemagglutinin (HA) proteins.*** Finally, a randomly selected subset of samples from subjects in each study cohort who had a ≥4-fold rise in MN titers (Day 21/Day 0) will be tested in a second MN assay with a ***reverse genetics (RG) reassortant*** heterologous HA Group 1 influenza virus (H6N3) to assess the breadth of neutralization effected by anti-H1 stalk ELISA antibodies.

Objectives

Co-Primary:

1. To describe the anti-H1 stalk ELISA antibody levels:
   - For adult subject samples, at baseline Day 0 (D0), post-dose 1 at Day 21 (D21), post-dose 2 at Day 42 (D42), Day 182 (D182) by treatment group (unadjuvanted or adjuvanted vaccine), for each study cohort from the ATP cohorts for Immunogenicity of all 3 primary completed studies (H1N1, H5N1, and H9N2), and also at Day 385 (D385) for the H5N1 study cohort
   - ***For pediatric subject samples, at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), and D385 in adjuvanted vaccine group, from the ATP cohort for Immunogenicity of the primary completed study (pediatric H5N1 study cohort)***
2. To describe the anti-H1 stalk microneutralization (MN) antibody levels:
   - For adult subject samples, at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), D182, and at D385 (H5N1 study cohort only) from subjects who received an adjuvant system (AS) vaccine
   - ***For pediatric subject samples (pediatric H5N1 study cohort), at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), and at D385 from subjects who received an adjuvant system (AS) vaccine***

- ***To describe the anti-H2 and anti-H18 antibody levels at baseline (D0) and post-dose 2 (D42) in all subjects from each study cohort who received an adjuvanted vaccine***

Tertiary

- 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 H9N2 and ***both adult and pediatric*** H5N1 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

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) of H5N1, H1N1pdm09, and H9N2 adjuvanted or unadjuvanted pandemic influenza vaccines (standard adult dose) using archived serum specimens from 3 completed clinical trials. The archived samples were collected from adult subjects, 18-64 years of age (19-40 years of age for H1N1 study), who had participated in one of 3 clinical trials (studies Q-Pan H1N1-019, CC-Pan H5N1-001, and Q-Pan H9N2-001) 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 H5N1 study only, D385, since this study also had a follow-up blood collection time point at D385). For each study, 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). 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.*** The microneutralization testing will be done in a step-wise analysis, initially on only the samples from the ***adult*** H5N1 study cohort. If the results of this analysis warrant it, testing may be expanded to the adult H9 and H1 study cohorts ***as well as to the pediatric H5 cohort***, as per protocol or modified by mutual agreement between GSK and Icahn School of Medicine at Mount Sinai (ISMMS). Based on the results of these evaluations, the laboratory analyses may be further extended to include additional assays and/or assay types to further assess the intensity and breadth of 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 7 study groups, as described in Synopsis Table 1.

Synopsis Table 2 Study groups and epochs foreseen in the study

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	**Age range of enrolled subjects (yrs)**	**Study Group (treatment groups to be sampled)**	**§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort**	**Number of subjects in ATP-I cohort (i.e, up to D42)**
Q-Pan H1N1-019 (113536) (A/California/7/2009)	19–40	**E** 15 µg HA (no AS)	20-30	91
		F 3.75 µg HA/AS03$_A$	20-30	91
CC-Pan H5N1-001 (114371) (A/Indonesia/5/2005 RG)	18-49	**A** 3.75 µg HA/AS03$_A$	20-30	124
		B 15 µg HA (no AS)	20-30	50
Q-Pan H9N2-001 (116358) (A/chicken/Hong Kong/G9/1997 NIBRG-91)	18-64	***375_A_VVP and 375_A_VVV** 3.75 µg HA/AS03$_A$	20-30	55
		***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***	***1.9 µg HA/AS03$_B$***	***20-30***	***182***

HA=Hemagglutinin content per vaccine dose; AS03$_A$=Adjuvant system 03$_A$; AS=Adjuvant system; ATP-I=According to Protocol cohort for Immunogenicity.
Group 375_A_VVP = 3.75 µg HA H9N2 vaccine antigen adjuvanted with AS03A at the Day 0 and Day 21 visits; saline placebo at the Day 182 visit
Group 375_A_VVV = 3.75 µg HA H9N2 vaccine antigen adjuvanted with AS03$_A$ at the Day 0, Day 21, and Day 182 visits
Group 1500_VVP = 15 µg HA H9N2 vaccine at the Day 0 and Day 21 visits; saline placebo at the Day 182 visit
Group 1500_VVV = 15 µg HA H9N2 vaccine at the Day 0, Day 21, and Day 182 visits
§ Exact number of subjects per treatment uncertain, but likely to be in this range
* From D0, D21, D42, and D182 time points (no D385)

Number of subjects/samples

The target is to randomly select approximately 30 subjects ***from each of the adult and pediatric studies***. These 30 subjects from the ***adult*** H5N1, H9N2 and H1N1 studies will be those who: 1) received the adjuvanted standard dose vaccine candidate, and 2) were in the ATP-I ~~or~~ ***and*** Persistence cohort (depending on the study) of the completed studies, and 3) have valid vaccine homologous ~~MN result~~ ***HI result*** available ***at all the required timepoints where the HI test is done and*** 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 subset in study H9N2 should be preferentially selected. 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 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 vaccine, were seronegative for H1N1pdm09 HI at Day 0, and have valid vaccine homologous HI and MN results available at either D0, 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.

Endpoints:

Primary endpoints

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 (H1N1, adult H5N1 and H9N2 cohort):***
   - ***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

- ***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 (H5N1 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***
  - ***Mean geometric increase (MGI) at Day 21, 42 and at Day 385 compared to Day 0***
  - The following aggregate variables will be calculated with 95% CI:

- ***For adult subject samples (H1N1, adult H5N1 and H9N2 cohort)***
  - ***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***
  - ***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 (H5N1 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***
  - ***Mean geometric increase (MGI) at Day 21, 42 and at Day 385 compared to Day 0***

2. ***Levels of anti-H2 and anti-H18 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 and 42***

- ***Geometric mean titer (GMT) at Day 0 and 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***

Secondary endpoints

1. 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 D182 (and at Day 385 for H5N1 study cohort only)
   - 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

- ***Level of HI antibody to pandemic vaccine homologous virus at Day 0 in each study by treatment group and level of HI antibody to A/California/7/09 (or a like virus) at Day 0 in H9N2 and adult H5N1 studies, by treatment group***. The following aggregate variable will be calculated with 95% CI:
  - Seropositive rate at Day 0

Tertiary endpoints

- ***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

1.2.2 Rationale for the study 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) of H5N1, H1N1pdm09, and H9N2 adjuvanted or unadjuvanted pandemic influenza vaccines (standard adult dose) using archived serum specimens from 3 completed clinical trials ***with adult subjects (H1N1, H5N1, and H9N2)*** at baseline,

after 1 and 2 doses (Day 21 and Day 42, respectively), and after extended follow-up at Day 182 and in 1 of 3 study cohorts, also at Day 385 (i.e., in H5N1, since this study also had a follow-up blood collection time point at Day 385). Therefore, Day 0, 21, 42, and 182 time point samples from the H1N1, H5N1, and H9N2 studies, as well as Day 385 time point samples from the H5N1 study will be tested. For each study, 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 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). 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).

A serology testing algorithm will be used to rationalize the serology analyses. In the initial step, an anti-H1 stalk domain ELISA (enzyme-linked immunosorbent assay) will be used to analyze archived samples (with sufficient volume for the serology analyses) Day 0, 21, 42, and 182 time points of all 3 ***adult*** study cohorts ***(H1N1, H5N1, and H9N2)***, the D385 time point of the ***adult*** H5N1 study cohort, ***as well as the Day 0, 21, 42, and 385 time points of the pediatric H5N1 study***. 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 cH8/1 N3, 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). ***Additionally, all subjects who received an adjuvant system (AS) vaccine will have their D0 and D42 samples tested for anti-H2 and anti-H8 ELISA antibody titers in order to assess the breadth of cross-reactivity elicited by vaccination.*** Finally, a randomly selected subset of samples from subjects in each study cohort who had a ≥4-fold rise in anti-H1 stalk MN titers (Day 21/Day 0) will be tested in a second MN assay with a ***reverse genetics (RG) reassortant*** heterologous HA Group 1 influenza virus (H6N3) to assess the breadth of neutralization effected by anti-H1 stalk ELISA antibodies (this test will establish the neutralizing potency of antibodies directed to the H6 stalk domain relative to the H1 stalk domain; a ratio ≥0.5 supports the potential of the FLU CC-SUIV candidate vaccine to elicit broadly protective humoral immune response to influenza A viruses with pandemic potential).

2.1. Co-Primary objectives

- To describe the anti-H1 stalk ELISA antibody levels:
  - For adult subject samples, at baseline Day 0 (D0), post-dose 1 at Day 21 (D21), post-dose 2 at Day 42 (D42), Day 182 (D182) by treatment group (unadjuvanted or adjuvanted vaccine), for each study cohort from the ATP cohorts for Immunogenicity of all 3 primary completed studies (H1N1, H5N1, and H9N2), and also at Day 385 (D385) for the H5N1 study cohort
  - ***For pediatric subject samples, at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), and D385 in adjuvanted vaccine group, from the ATP cohort for Immunogenicity of the primary completed study (pediatric H5N1 study cohort)***
- To describe the anti-H1 stalk microneutralization (MN) antibody levels:
  - For adult subject samples, at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), D182, and at D385 (H5N1 study cohort only) from subjects who received an adjuvant system (AS) vaccine
  - ***For pediatric subject samples (pediatric H5N1 study cohort), at baseline (D0), post-dose 1 (D21), post-dose 2 (D42), and at D385 from subjects who received an adjuvant system (AS) vaccine***
- ***To describe the anti-H2 and anti-H18 antibody levels at baseline (D0) and post-dose 2 (D42) in all subjects from each study cohort who received an adjuvanted vaccine***

Refer to Section 10.1 for the definition of the primary endpoints.

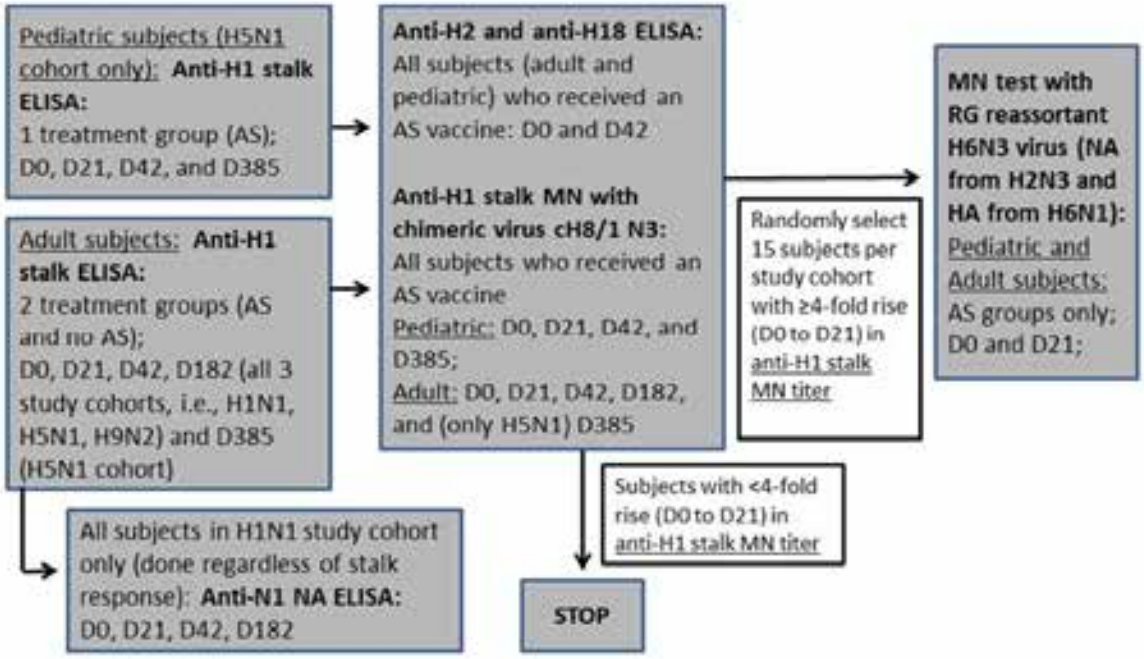
2.3. Tertiary objectives

- 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 H9N2 and ***both adult and pediatric*** H5N1 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

Refer to Section 10.3 for the definition of the tertiary endpoints.

3. STUDY DESIGN OVERVIEW

The serology testing algorithm below summarizes the testing to support the primary and secondary objectives only.



Serology Algorithm specifying the key laboratory analyses and decision points. ELISA= Enzyme-linked immunosorbent assay; MN=Microneutralization assay; AS=Adjuvanted system; ***RG=Reverse genetics;*** HA=Hemagglutinin; NA=Neuroaminidase. Timepoints: D0=Day 0, D21=Day 21, D42=Day 42, D182=Day182, D385=Day 385; H1N1, H5N1, and H9N2 = primary completed studies with archived serum samples.

The microneutralization testing will be done in a step-wise analysis, initially on only the samples from the H5N1 study cohorts. If the results of this analysis warrant it, testing may be expanded to the H9 and H1 study cohorts as per protocol or modified by mutual agreement between GSK and ISMMS.

- **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) of H5N1, H1N1pdm09, and H9N2 adjuvanted or unadjuvanted pandemic influenza vaccines (standard adult dose) using archived serum specimens from 3 completed clinical trials. The archived samples were collected from adult subjects, 18-64 years of age (19-40 years of age for H1N1 study), who had participated in one of 3 clinical trials (studies Q-Pan H1N1-019, CC-Pan H5N1-001, and Q-Pan H9N2-001) 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 H5N1 study only, D385, since this study also had a follow-up blood collection time point at D385). For each study, 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). 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).***

The microneutralization testing will be done in a step-wise analysis, initially on only the samples from the ***adult*** H5N1 study cohort. If the results of this analysis warrant it, testing may be expanded to the H9 and H1 study cohorts ***as well as to the pediatric H5 cohort***, as per protocol or modified by mutual agreement between GSK and Icahn School of Medicine at Mount Sinai (ISMMS). Based on the results of these evaluations, the laboratory analyses may be further extended to include additional assays and/or assay types to further assess the intensity and breadth of 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.

Table 1 Study groups and epochs foreseen in the study

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	**Age range of enrolled subjects**	**Study Group (treatment groups to be sampled)**	**§Number of subjects to be randomly selected from ATP-I or Persistence cohort**	**Number of subjects in ATP-I cohort (i.e, up to D42)**
Q-Pan H1N1-019 (113536) (A/California/7/2009)	19–40 years	**E** 15 µg HA (no AS)	20-30	91
		F 3.75 µg HA/$AS03_A$	20-30	91
CC-Pan H5N1-001 (114371) (A/Indonesia/5/2005 RG)	18-49 years	**A** 3.75 µg HA/$AS03_A$	20-30	124
		B 15 µg HA (no AS)	20-30	50
Q-Pan H9N2-001 (116358) (A/chicken/Hong Kong/G9/1997 NIBRG-91)	18-64 years	***375_A_VVP and 375_A_VVV** 3.75 µg HA/$AS03_A$	20-30	55
		***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***	***1.9 µg HA/$AS03_B$***	***20-30***	***182***

4.1. Number of samples

The target is to randomly select approximately 30 subjects ***from each of the adult and pediatric studies***. These 30 subjects from the ***adult*** H5N1, H9N2 and H1N1 studies will be those who: 1) received the adjuvanted standard dose vaccine candidate, and 2) were in the ATP-I ~~or~~ ***and*** Persistence cohort (depending on the study) of the three completed studies, and 3) have valid vaccine homologous ~~MN result~~ ***HI result*** for ***all the required timepoints where the HI test is done and MN result*** at Day 0 and 21 (for H5N1 and H9N2 studies only).

For the pediatric H5N1 study, ~ 40 subjects (age < 36 months) 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 D0, 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.

5.2. Randomization

For the current exploratory, retrospective study, the target is to randomly select approximately 30 subjects ***from each of the adult and pediatric studies***. These 30 subjects from the ***adult*** H5N1, H9N2 and H1N1 studies will be those 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 three completed studies, and 3) have valid vaccine homologous ~~MN result~~ ***HI result*** available at Day 0 and 21 (for adult H5N1 and H9N2 studies only).

For the pediatric H5N1 study, ~ 40 subjects (age < 36 months) 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.

Table 2 Serum Assays to Assess Humoral Immunity

Antigen/ Virus for Test	Component (Strain or Antigen Description)	Method	Kit / Manufacturer	Unit	Cut-off	*Laboratory
ELISAs						
cH6/1 HA	*Recombinant antigen based on A/mallard/Sweden/81/02 head domain with A/Puerto Rico/8/34 H1 stalk domain*	Anti-H1 HA stalk ELISA	ISMMS protocol/assay	ELISA units (EU)	<100 EU	ISMMS
N1 NA	*Recombinant antigen based on A/California/4/2009 NA*	Anti-N1 NA ELISA	ISMMS protocol/assay	ELISA units (EU)	<100 EU	ISMMS
N2 NA	***Recombinant antigen based on A/Chicken/ Hong Kong/G9/1997 NA*	Anti-N2 NA ELISA	ISMMS protocol/assay	ELISA units (EU)	<100 EU	ISMMS
H9 HA head domain	***Recombinant antigen based on A/Chicken/ Hong Kong/G9/1997 HA head domain*	Anti-H9 HA head domain ELISA	ISMMS protocol/assay	ELISA units (EU)	<100 EU	ISMMS
H9 HA full length	***Recombinant antigen based on A/Chicken/ Hong Kong/G9/1997 HA*	Anti- H9 HA full length ELISA	ISMMS protocol/assay	ELISA units (EU)	<100 EU	ISMMS
H2 HA full length	*Recombinant antigen based on A/mallard/ Netherlands/5/99 HA*	*Anti- H2 HA full length ELISA*	*ISMMS protocol/assay*	*ELISA units (EU)*	*<100 EU*	*ISMMS*
H18 HA full length	*Recombinant antigen based on A/flat-faced bat/ Peru/033/10 HA*	*Anti- H18 HA full length ELISA*	*ISMMS protocol/assay*	*ELISA units (EU)*	*<100 EU*	*ISMMS*
Microneutralization (MN) assays						
	~~cH5/1N3 virus~~	~~MN Assay~~	~~ISMMS protocol/assay~~	~~1/DIL (IC_{50})~~	~~<1:10 1/DIL~~	~~ISMMS~~
	~~cH9/1N3 virus~~	~~MN Assay~~	~~ISMMS protocol/assay~~	~~1/DIL (IC_{50})~~	~~<1:10 1/DIL~~	~~ISMMS~~
cH8/1 N3 virus	*cHA virus based on PR8 for 6 genes with 2 surface proteins: N3 is from A/swine/Missouri/4296424/2006(H2N3), HA head domain is from A/mallard/Sweden/24/2002 (H8N4), HA stalk domain is from*	*MN Assay*	*ISMMS protocol/assay*	*1/DIL (IC_{50})*	*<1:10 1/DIL*	*ISMMS*

Antigen/ Virus for Test	Component (Strain or Antigen Description)	Method	Kit / Manufacturer	Unit	Cut-off	*Laboratory
	A/California/04/09 (H1N1)					
H6N3 virus	***RG reassortant virus based on PR8 for 6 genes with 2 surface proteins: NA from A/swine/Missouri/4296424/2006 (H2N3), HA from A/mallard/Sweden/81/2002 (H6N1)***	MN Assay	ISMMS protocol/assay	1/DIL (IC_{50})	<1:10 1/DIL	ISMMS
Hemagglutination Inhibition (HI) Assay						
H5N1 virus	Pandemic vaccine (H5N1) homologous virus (A/Indonesia/5/2005)	HI assay	GSK GVCL protocol/assay (if resources available)	1/DIL	<1:10 1/DIL	***GSK Biologicals
H9N2 virus	Pandemic vaccine homologous (H9N2) virus (A/chicken/Hong Kong/G9/1997)	HI assay	GSK GVCL protocol/assay (if resources available)	1/DIL	<1:10 1/DIL	***GSK Biologicals
H1N1 virus	Pandemic vaccine (H1N1) homologous virus (A/California/7/2009 (or a like virus))	HI assay	GSK GVCL protocol/assay (if resources available)	1/DIL	<1:10 1/DIL	***GSK Biologicals

*Refer to APPENDIX B for the laboratory address. ISMMS= Icahn School of Medicine at Mount Sinai, Dept. Of Microbiology, New York, NY, USA

**These ELISAs to be performed only on samples from the H9N2 cohort

*** GSK Biologicals laboratory refers to the Global Vaccines Clinical Laboratories (GVCL) in Rixensart, Belgium; Wavre, Belgium; Laval, Canada.

Table 4 Planned Immunological Read-Outs: ELISAs

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort	N tests planned as per algorithm)	Comment
Adult subjects: Anti H1 stalk ELISA (A/California/09/2009)	Recombinant trimeric cHA (H6/1)	D0, D21, D42, D182 (from all 3 ***adult*** studies) and D385 (from ***adult*** H5N1 study only); 2 treatment groups (AS and no AS)	30	**780** (30 samples x 4 timepoints x 3 studies x 2 treatment groups + 30 samples x 1 timepoint x 2 treatment groups x 1 study, H5N1)	All 3 ***adult*** study cohorts ***(H1N1, H5N1, and H9N2)***
Pediatric subjects: Anti-H1 stalk ELISA (A/California/09/2009)	***Recombinant trimeric cHA (H6/1)***	***D0, D21, D42, D385 (from pediatric H5N1 study only); 1 treatment groups (AS)***	***30***	***120 (30 samples x 4 timepoints x 1 study x 1 treatment group)***	***For pediatric H5N1 study cohort only***
Anti-N1 NA ELISA (A/California/09/2009)	Recombinant tetrameric NA (N1)	D0, D21, D42, D182 (from H1N1 study only, done regardless of anti-H1 stalk ELISA results above); 2 treatment groups (AS and no AS)	30	**240** (30 samples x 4 timepoints x 1 study x 2 treatment groups)	For H1N1 study cohort only**
Anti-H2 HA full length ELISA (A/mallard/Netherlands/5/99 HA)	***Recombinant trimeric H2 HA full length***	***D0, D42; One treatment group (AS)***	***30***	***240 (30 samples x 2 timepoints x 4 studies x 1 treatment group)***	***For all 4 study cohorts (adult H1N1, H5N1, H9N2 and pediatric H5N1)***
Anti-H18 HA full length ELISA (A/flat-faced bat/Peru/033/10 HA)	***Recombinant trimeric H18 HA full length***	***D0, D42; One treatment group (AS)***	***30***	***240 (30 samples x 2 timepoints x 4 studies x 1 treatment group)***	***For all 4 study cohorts (adult H1N1, H5N1, H9N2 and pediatric H5N1)***
Anti-N2 NA ELISA (A/chicken/Hong Kong/G9/1997)	Recombinant tetrameric N2 NA	D0, D21, D42; Two treatment groups (AS and no AS)	30	**180** (30 samples x 3 timepoints x 1 study x 2 treatment	For H9N2 study cohort only

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort	N tests planned as per algorithm)	Comment
				groups)	
Anti-H9 HA head domain ELISA (A/chicken/Hong Kong/G9/1997)	Recombinant trimeric H9 HA head domain	D0, D21, D42; Two treatment groups (AS and no AS)	30	**180** (30 samples x 3 timepoints x 1 study x 2 treatment groups)	For H9N2 study cohort only
Anti-H9 HA full length ELISA (A/chicken/Hong Kong/G9/1997)	Recombinant trimeric H9 HA full length	D0, D21, D42; Two treatment groups (AS and no AS)	30	**180** (30 samples x 3 timepoints x 1 study x 2 treatment groups)	For H9N2 study cohort only
Total anticipated number of ELISA tests					~~1560~~***2160***

*Anti-N1 NA testing for H1N1 study cohort only, since only for this cohort was the vaccine NA matching with the ELISA test NA

HI testing by GSK for anti-H1N1 at D0 for subjects in the H5N1 and H9N2 cohorts will be elective based on laboratory capacity

Table 5 Planned Immunological Read-Outs: Microneutralization (MN) assays

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested (AS treatment) per study cohort	N tests planned as per algorithm)	Comment
~~Anti-H1 stalk domain MN~~	~~Chimeric virus (H6/1N3)~~	~~D0, D21, D42, D182 AS groups only~~	~~30~~	~~240 (30 samples x 4 timepoints x 2 studies)~~	~~For H1N1 and H9N2 study cohorts~~
~~Anti-H1 stalk domain MN~~	~~Chimeric virus (H9/1N3)~~	~~D0, D21, D42, D182, and D385, AS group only~~	~~30~~	~~150 (30 samples x 5 timepoints)~~	~~For H5N1 study cohort~~
Anti-H1 stalk domain MN	***Chimeric virus (cH8/1 N3)***	***Adult subjects: D0, D21, D42, D182; AS groups only***	***30***	***240 (30 samples x 4 timepoints x 2 studies)***	***For adult H1N1 and H9N2 study cohorts***
		Adult subjects: D0, D21, D42, D182, D385; AS group only	***30***	***150 (30 samples x 5 timepoints)***	***For adult H5N1 study cohort***
		Pediatric subjects: D0, D21, D42, D385; AS group only	***30***	***120 (30 samples x 4 timepoints)***	***For pediatric H5N1 study cohort***
Heterologous Group I influenza A virus MN	***RG reassortant virus*** (H6N3)	D0, D21, ***(from all 4 studies, i.e., adult H1N1, H5N1, H9N2, and pediatric H5N1)***, AS groups only	15	***120*** (15 samples x 2 timepoints x ***4*** studies)	For all ***4*** study cohorts: randomly selected pairs per study cohort with a ≥4-fold anti-H1 stalk MN titer increase from D0 to D21
Total anticipated number of MN tests					***~~480~~630***

Table 7 Description of study vaccines

Study no.	Vaccine strain	Start date
Q-Pan H1N1-019 (113536)	A/California/7/2009	28 Oct 2009
CC-Pan H5N1-001 (114371)	A/Indonesia/5/2005 RG	29 Nov 2010
Q-Pan H9N2-001 (116358)	A/chicken/Hong Kong/G9/1997 NIBRG-91	22 Aug 2012
Q-Pan H5N1-ASO3-021 (114464)	***A/Indonesia/5/2005 RG***	***07 Mar 2011***

6.2. Dosage and administration of study vaccines

In the previously conducted trials from which serum samples will be selected for further laboratory analyses in this study, ***adult*** subjects were administered two standard adult doses of pandemic influenza vaccine with an interval of 21 days between doses, ***and pediatric subjects were administered two half standard adult doses of pandemic influenza vaccine with an interval of 21 days between doses***.

10. STATISTICAL METHODS

10.1. Primary endpoints

The following aggregate variables will be calculated with 95% CI for each treatment group within each study cohort:

- ***For adult subject samples (H1N1, adult H5N1 and H9N2 cohort):***
  - ***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
  - ***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 (H5N1 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***
  - ***Mean geometric increase (MGI) at Day 21, 42 and at Day 385 compared to Day 0***
  - The following aggregate variables will be calculated with 95% CI:
- ***For adult subject samples (H1N1, adult H5N1 and H9N2 cohort)***

- ***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***
- ***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 (H5N1 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***
  - ***Mean geometric increase (MGI) at Day 21, 42 and at Day 385 compared to Day 0***

1. ***Levels of anti-H2 and anti-H18 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 and 42***
- ***Geometric mean titer (GMT) at Day 0 and 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***

10.2. Secondary endpoints

- ***Levels of anti-H1 stalk antibody by ELISA for all the subjects in the adult H5N1, H9N2, and H1N1 study cohorts***. 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 H5N1 study cohort only)
  - 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
- ***Level of HI antibody to pandemic vaccine homologous virus at Day 0 in each study by treatment group and level of HI antibody to A/California/7/09 (or a like virus) at Day 0 in H9N2 and adult H5N1 studies, by treatment group***. The following aggregate variable will be calculated with 95% CI:
  - Seropositive rate at Day 0

10.3. Tertiary endpoints

- ***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

10.7. Analysis of demographics

Demographic characteristics (age at first study vaccination in years, 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 H9N2 and adult H5N1 studies*** (if data become available)

10.8. Analysis of immunogenicity

10.8.1. Within groups assessment

For each study group in each 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), ***anti-H2 and anti-H18 full length HA by ELISA (for subjects who received an adjuvant system (AS) vaccine),*** vaccine heterologous MN ((subset in subjects who received an adjuvant system (AS) vaccine and have a ≥4-fold antibody titer increase (D21/D0) by anti-H1 stalk MN)), anti-N1 NA (for H1N1 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 (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 (for H1N1 cohort)***.

- Percentage of subjects with at least 4-fold increase from Day 0 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 (for H1N1 cohort), with exact 95% CI, will be calculated.
- The distribution of antibody titers for anti-stalk ELISA will be displayed using reverse cumulative distribution curves.
- ***For AS group, correlation between the level of neutralizing antibody to the H1 stalk with the level of vaccine homologous neutralizing antibody, at D0 and at D21 in study cohorts H9N2 and both adult and pediatric H5N1.***
- ***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 for in adult H5N1, H9N2 and H1N1 study cohort.***
- CMI summaries for the H9N2 cohort only
- 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.

10.10. Conduct of analyses

The planned analysis is descriptive and will be performed for each treatment group in the individual study. However, the analysis will be performed first for the adult H5N1 study and then for the three other study cohorts (i.e., pediatric H5N1, H1N1 and H9N2), pending any necessary change in the sample testing plan. The rationale for selecting the H5N1 adult cohort as the pilot cohort is to assess the acceptability of the analysis plan and the serology algorithm because the H5N1 adult cohort has the lowest baseline vaccine homologous HI titer. Thus, it is expected that the H5N1 vaccine may be most efficient in boosting an anti-H1 stalk response.

The initial analysis will be done for objectives based on ELISA endpoints only. Testing by H8/1 N3 MN will follow as a second step and by H6N3 as a third step when data are available.

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

10.10.1 Sequence of analyses

Analysis will be performed on final and clean data; analysis of data from the ***adult*** H5N1 study will be performed ***first and then for the three other study cohorts (i.e., pediatric H5N1, H1N1 and H9N2),*** pending any necessary change in the sample testing plan. Results will be presented in a final study report.

11. ADMINISTRATIVE MATTERS

For a typical clinical study protocol, this section provides guidance to comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality and publications.

This is a non-clinical retrospective study whose objectives pertain to analyses of archived serum samples from previously completed studies and, therefore, no study subjects are involved. Essential GCP training was provided to the ISMMS site staff before study start to ensure appropriate management of samples.

11.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

11.4. Quality assurance

Essential GCP training was provided to ISMMS site staff before study start to ensure appropriate management of samples.

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment 2

eTrack study number and Abbreviated Title(s)	201598 (FLU CC-SUIV-AS03-001)
IND number	NA
Amendment number:	Amendment 2
Amendment date:	01 September 2016
Co-ordinating author:	PPD, *Lead Scientific Writer*

Rationale/background for changes:

Addition of N=30 placebo control subjects from study FLU Q-Pan H5N1-AS03-021 to the pediatric H5N1cohort to assess the impact of H1N1pdm09 virus exposure on anti-H1 stalk titers in subjects over 1 year of follow-up.

Addition of three adult subject study cohorts (FLU Q-Pan-005, H5N1-012, and FLU D-QIV-015) to enable immunogenicity analyses that will provide context to, and will be informative for, designing the up-coming Phase I study, FLU E-SUIV-001, which will assess the safety and the immune response following one or two sequential doses of the SUIV candidate vaccine:

- Analysis of serum samples collected from the Q-Pan-005 and the H5N1-012 trials to evaluate the H1 HA stalk domain reactive response following heterologous and homologous booster doses of an H5N1 adjuvanted vaccine in adult subjects. The design of these studies will also allow assessment of the immune response to the H1 HA stalk domain several months (12 months and 18 months) after one dose of the adjuvanted pandemic vaccine. These vaccine schedules are representative of the schedules planned to be used with the GSK candidate monovalent group 1 influenza A SUIV vaccine in the Phase I FLU E-SUIV-001 study.
- The FLU E-SUIV-001 study will use a non-adjuvanted licensed quadrivalent inactivated influenza vaccine (IIV4) control group. Therefore, in order to be able to predict the response that can be seen in this control group, the stalk-reactive response following the administration of an IIV4 (quadrivalent inactivated influenza vaccine, *Fluarix Quadrivalent*) will also be assessed using archived serum samples from the FLU D-QIV-015 study.

- 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. The target virus (cH8/1N3) proposed to be used in this assay is being replaced by the recently developed (by ISMMS) chimeric cH6/1N5 virus, since cH8/1N3 will be used in the vaccine in the FLU E-SUIV-001 study. Therefore, to assess the anti-H1 stalk neutralizing antibody response, a chimeric virus (cH6/1N5) having an exotic HA head domain will be used.
- An additional timepoint was added (to evaluate persistence of the immune response) for the endpoint related to the assessment of the anti-H2 and the anti-H18 antibody levels by ELISA.
- To assess the breadth of neutralization effected by anti-H1 stalk ELISA antibodies, samples 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 (instead of the H6N3 as initially planned, since unlike H6N3, H5N8 is a current pandemic threat and is, thus, of greater public health significance), with an avian-like swine H1N1 virus (A/Swine/Jiangsu/40/2011) and a H1N1 pdm09 like virus. Previously only a randomly selected subset of samples (from subjects who had a ≥4-fold rise in MN titers (Day 21/Day 0) was planned to be tested.
- Based on the experience that will be acquired in testing anti-H1 stalk responses in the HA group 1-related studies, another study cohort drawn from subjects participating in Q-Pan-H7N9-001 (in which adult subjects were administered adjuvanted or unadjuvanted pandemic influenza H7N9 vaccine) will also be evaluated by ELISA and microneutralization assay for antibody responses to the H3 stalk (i.e., Group 2 hemagglutinin). This analysis will also assess the performance of the anti-H3 stalk ELISA.
- Specify use of D0, D42, and D385 serum specimens from the subjects in the CC-Pan-H5N1-001 adult cohort (adjuvanted vaccine recipients only) to be tested by passive transfer to mice that will be subsequently challenged with two chimeric cH viruses (cH5/3Nx and cH6/1N5; Nx=most likely N4 or N5, to be decided) to assess protective effect of vaccination on weight loss, survival, and lung virus titers.
- Addition of NeoMed-Labs Inc., Quebec, Canada (CRO) as a second laboratory authorized to perform analytical assays in addition to ISMMS. The planned transfer of ELISAs to NeoMed Labs from ISMMS will necessitate the use of a new reference serum pool, the qualification of which for such use may alter the defined assay cut-off.
- The algorithm for testing, as outlined in Protocol Amendment 1, has been revised and no formal stepwise algorithm will be followed. Testings will be done as soon as samples, assays, and antigens are available in the assigned laboratory.

In this summary, the amended or changed text will be indicated in ***bold italics*** and any deleted text will be indicated in strikethrough (e.g. ~~text~~).

Date of protocol	Final Version 1: 14 November 2014
Date of protocol amendment	Amendment 1 Final: 10 March 2015 ***Amendment 2 Final: 01 September 2016***
Title	An exploratory, retrospective laboratory evaluation of the humoral immune response in adults and children to the ***hemagglutinin*** stalk domain and other influenza A virus epitopes, after administration of GSK's influenza vaccines
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***
Co-ordinating author	PPD, *Lead Scientific Writer*
Contributing authors	PPD, *Clinical Research & Development Lead* PPD, *Project Statistician* PPD, *Lead Statistician* PPD, *Study Delivery Lead* PPD, *Clinical Read-out Lead* PPD, *Senior Manager, Human Cellular Immunology* PPD, *Study Data Manager* PPD, *Oversight Data Manager* PPD, *Vice President,* ***Senior Vaccine Development Leader*** *PPD, *Assistant Professor* *PPD, *Professor* * *Dept. of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

(Amended 01 September 2016)

GSK Biologicals' protocol template for observational studies and non- interventional studies (i.e., without administration of medicinal products) as described in a research protocol based on the Protocol DS v 14.0

SYNOPSIS

Detailed Title (Amended 01 September 2016)

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***

Rationale for the study and study design (Amended 01 September 2016)

- Rationale for the study

FLU CC-SUIV-AS03-001 is the initial study in GSK Biologicals' clinical development plan for a candidate supra-seasonal universal influenza vaccine (SUIV) and is an exploratory, retrospective laboratory study, using archived serum samples, to assess the humoral immune response to the ***influenza A group 1 (H1) and the influenza A group 2 (H3)*** hemagglutinin ***(HA)*** stalk domain and other influenza A virus protein epitopes following administration in adults and children of GSK Biologicals' adjuvanted or unadjuvanted pandemic vaccines. These vaccines can serve as a surrogate for one dose of an inactivated chimeric ***HA***-bearing virus vaccine, because the stalk domain of the vaccines' ***HAs*** shares important epitopes with ***all of the group 1 or the group 2 influenza A HA*** stalk ***domains*** despite ***small*** amino acid sequence differences in the overall ***HA*** stalk domains among the ***circulating viruses***. The results of this study will provide context for and ***be informative for designing the Phase I study, FLU E-SUIV-001***, which will evaluate the ***safety and the immune response following one or two sequential doses of the SUIV candidate vaccine. This upcoming Phase I study will use a non-adjuvanted licensed quadrivalent inactivated influenza vaccine (IIV4) control group. Therefore, in order to be able to predict the response that could be observed in this control group, the stalk-reactive response following the administration of an IIV4 will also be assessed in this retrospective laboratory study***. The data obtained from the current FLU CC-SUIV-AS03-001 study will also support a pre-IND meeting with the FDA's Center for Biologics Evaluation and Research (CBER) by demonstrating that the immunoassays to be deployed in ***the Phase I FLU E-SUIV-001 study*** are fit for purpose, and that the antigen and adjuvant dose proposed for the study are reasonably likely to be acceptably immunogenic.

- Rationale for the study design

The retrospective study is designed to assess humoral immune response to the ***Group 1*** H1 hemagglutinin stalk domain (and other influenza A virus protein epitopes) ***in adult and pediatric subjects, by ELISA and microneutralization (MN) assay, using archived serum samples from***:

- ***Adult subjects who received*** adjuvanted ***(3.75 µg HA adjuvanted with AS03$_A$)*** or unadjuvanted ***(15 µg HA)*** H1N1pdm09, H5N1, and H9N2 pandemic influenza vaccines (standard adult dose) in 3 completed clinical trials ***[Q-Pan H1N1-019 (113536), CC-Pan H5N1-001 (114371), and Q-Pan H9N2-001 (116358)].*** The serum samples were collected at baseline, after 1 and 2 doses (Day 21 and Day 42, respectively), and after extended follow-up at Day 182. In 1 of these 3 ***study*** cohorts, samples were also collected at Day 385 (i.e., ***in CC-Pan H5N1-001 study cohort***, since this study also had a follow-up blood collection time point at Day 385).

  For each of these 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).

- ***A group of pediatric subjects, 6-35 months of age, who received adjuvanted pandemic H5N1 vaccine in the completed Q-Pan H5N1-AS03-021 trial (114464), and who had no HI antibodies [titer <10] to H1N1pdm09 before being vaccinated with 2 doses of AS03$_B$-adjuvanted Q-PAN-H5N1 vaccine (half adult dose, i.e., 1.9 µg HA).*** 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). ***To control for the effects of inter-current H1N1pdm09 virus infection (i.e., to assess the impact of H1N1pdm09 virus transmission) on anti-H1 stalk titers, placebo control serum samples from the same study will also be evaluated.*** 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***.

Additionally, to evaluate the H1 HA stalk domain reactive response following a heterologous booster dose of an adjuvanted H5N1 vaccine (3.8 µg HA adjuvanted with AS03$_A$), samples will be analyzed from adult subjects 18 to 40 years of age who received a single dose of an adjuvanted H5N1 vaccine (monovalent A/turkey/Turkey/1/05 [H5N1]) 18 months after their priming dose with a heterologous adjuvanted H5N1 vaccine (i.e., monovalent A/ Indonesia/5/05 [H5N1]) in the Q-Pan-005 (110624) trial. Similarly, the H1 HA stalk domain reactive response following a homologous booster dose of an H5N1 adjuvanted vaccine administered in subjects primed with one dose of the same vaccine 12 months earlier will also be described using samples from the same study. 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 a homologous or a heterologous adjuvanted priming dose (A/Vietnam/1194/2004) in subjects 18-60 years of age. This assessment will be stratified by age (18-30 years vs 31-60 years) to evaluate the age effect on the immune response. The design of these 2 studies will also allow the assessment of the immune response to the H1 HA stalk domain several months after one dose of the adjuvanted pandemic vaccine. These vaccine schedules are representative of the schedules planned to be used with the GSK candidate monovalent group 1 influenza A SUIV vaccine in the E-SUIV-001 Phase I study.

For these anti-H1 stalk ELISAs, all evaluable serum specimens 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 from Day 0, Day 21, Month 6 (M6),Month (M12), M12+21days and M18 of all subjects (18-60y) from the groups VT/VT/12M and VT/IN/12M will be used (i.e. no random selection for the sub-cohort). Groups VT/VT/12M and VT/IN/12M were comprised of subjects administered A/Vietnam/1194/2004 adjuvanted H5N1 followed 12 months later by, respectively, a homologous (A/Vietnam/1194/2004-like) or heterologous (A/Indonesia/5/2005-like) adjuvanted H5N1 booster dose.

In order to further characterize the influenza A group 1 anti-HA stalk response to support design of the E-SUIV-001

Phase 1 study in which a quadrivalent inactivated influenza vaccine (IIV4) will serve as control, serum specimens will also be analyzed from the pre-vaccination (Day 0) and post-vaccination (Day 21) timepoints of a study (D-QIV-015 (201251)) in which subjects were vaccinated with an IIV4 (i.e., Fluarix Quadrivalent, also called D-QIV). Samples from approximately 30 adult subjects, 18-39 years of age, will be randomly selected for analysis.

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, all D0, D42 samples and samples from the last time point (persistency) from subjects who received an adjuvant system (AS) vaccine will be tested for reactivity by ELISA with H2 and H18 full length recombinant hemagglutinin proteins.

For the Q-Pan-005 study, this will be assessed with the Day 0, 42, 549, 591, and Day 729 samples from group C subjects and with the Day 182, 224, 549, 591, and Day 729 samples from group G subjects. For the H5N1-012 study this will be assessed with the Day 0, Day 21, M12, M12+21days and M18 samples of all subjects in the VT/VT/12M and the VT/IN/12M groups. For the D-QIV-015 study cohort, in which the subjects received an IIV4, the testing will be performed with the Day 0 and the Day 21 samples.

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 will be 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 of this virus are "exotic" and most humans have not been exposed to them).

To assess the breadth of neutralization affected by anti-H1 stalk ELISA antibodies, ~~a randomly selected subset of~~ samples ***at baseline (D0) and D42*** from ***all*** subjects ***who received an adjuvanted vaccine*** in each study cohort ~~who had a ≥4 fold rise in MN titers (Day 21/Day 0)~~ 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 a H1N1 pdm09 like virus.***

For the Q-Pan-005 study, this will be assessed with the Day 0, 42, 549 and 591 samples (from group C subjects), and with the Day 182, 224, 549, and 591 samples (from group G subjects). For the H5N1-012 study, this will be assessed with the Day 0, 21, M12 and M12+21days samples of all the subjects from the VT/VT/12M and VT/IN/12M groups. For the D-QIV-015 study cohort, in which the subjects received an IIV4, the testing will be performed with the Day 0 and Day 21 samples.

As part of the exploratory (tertiary) objectives of this retrospective study, and based on the experience that will be acquired in testing group 1 anti-H1 stalk responses mentioned above, archived serum samples (with sufficient volume for the serology analyses) from the Day 0, 21, 42, Month 6, and 12 time points of a H7N9 study cohort (in which adult subjects were administered 2 doses (21-day interval) of an adjuvanted (3.75 µg HA adjuvanted with AS03$_A$) or unadjuvanted (15 µg HA) pandemic influenza H7N9 vaccine) will also be evaluated for antibody responses to the group 2 HA stalk (i.e., H3), by anti-H3 stalk ELISA.

This analysis will also assess the performance of the influenza A anti-H3 stalk ELISA. Approximately 60 subjects (i.e., approximately 30 subjects who received the adjuvanted vaccine dose, and approximately 30 subjects who received the unadjuvanted vaccine dose) will be randomly selected to generate the serology sub-cohort in which both groups will match in terms of age and center.

As an additional exploratory objective for the H7N9 study cohort to evaluate whether a post-vaccination boost in ELISA antibody titers could also result in neutralization of target virus, samples from subjects who received an adjuvant system (AS) vaccine will be further analyzed with an influenza A group 2 anti-H3 stalk domain (at Day 0, 21, 42 M6 and M12) and heterosubtypic (at Day 0 and Day 42) microneutralization (MN) assays (target viruses: cH14/3Nx and a wild-type Group 2 H4N8 virus, respectively). Furthermore, D0, D42 and M12 samples from subjects who received the adjuvanted vaccine will be tested for reactivity with H4 and H10 full length recombinant hemagglutinin (HA) proteins.

Finally, an exploratory human serum transfer/virus challenge experiment will be conducted in mice to assess whether anti-H5 head antibodies and anti-H1 stalk antibodies are protective in vivo. The protective effect of

pooled human sera collected on D42 and D385 from adult recipients of adjuvanted H5N1 vaccine in study CC-PAN H5N1 will be compared to the effect of pooled serum collected at D0, by transferring each serum pool to BALB/c mice, which will be subsequently challenged with approximately 5LD_{50} of cH5/3Nx (Nx=most likely N4 or N5, to be decided) and cH6/1N5 (or alternative challenge viruses with similar attributes, but more fit for purpose). The extent of protection from viral challenge will be evaluated in terms of the proportion of mice surviving viral challenge, mean weight loss over time, and, in randomly selected subgroups of animals from each of the 3 treatment groups administered the D0, D42, or D385 serum pools, mean lung weight at necropsy and geometric mean lung virus titer following euthanization at 3 and 6 days post-challenge.

~~A serology testing algorithm will be used to rationalize the serology analyses. In the initial step, an anti-H1 stalk domain, ELISA (enzyme linked immunosorbent assay) will be used to analyze archived samples (with sufficient volume for the serology analyses) from the Day 0, 21, 42, and 182 time points of all 3 adult study cohorts (H1N1, H5N1, and H9N2), the D385 time point of the adult H5N1 study cohort, as well as the Day 0, 21, 42, and 385 time points of the pediatric H5N1 study.~~

Objectives (Amended 01 September 2016)

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):

1. 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 (Day 0), post-dose 1 (Day 21), post-dose 2 (Day 42), Day 182 (D182) and at Day 385 (D385) for the CC-Pan H5N1-001 study cohort, by treatment group (unadjuvanted or adjuvanted vaccine)***~~For adult subject samples, at baseline Day 0 (D0), post-dose 1 at Day 21 (D21), post-dose 2 at Day 42 (D42), Day 182 (D182) by treatment group (unadjuvanted or adjuvanted vaccine), for each study cohort from the ATP cohorts for Immunogenicity of all 3 primary completed studies (H1N1, H5N1, and H9N2), and also at Day 385 (D385) for the H5N1 study cohort~~

- 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*** ~~from the ATP cohort for Immunogenicity of the primary completed study (pediatric H5N1 study cohort)~~
- ***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 (Month 6), M12 (Month 12), M12+21 days, and M18 (Month 18)***
- ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***

2. 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-AS03-21 study 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 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, M6, M12, M12+21 days, and M18***
    - ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***
3. 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, M6, M12, M12+21 days, and M18***
- ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***

4. To describe the vaccine heterosubtypic heterologous 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***

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):

1. 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 ***within each study cohort***) 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 ***within each study cohort***) 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)

2. ***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)***

Tertiary 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):

1. ***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***
2. ***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***
3. 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 cohort (AS group only)
4. 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
5. 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***
6. 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)

Tertiary objectives: With respect to samples from the HA

group 2-related study cohort (H7N9 study) (adult subjects):

1. ***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***
2. ***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***
3. ***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***
4. ***To describe the vaccine heterosubtypic virus MN antibody level (target virus: H4N8) at baseline (D0) and post-dose 2 (D42) in all subjects who received an adjuvanted vaccine***
5. ***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***

Tertiary objectives: Passive transfer/challenge in mice with pooled adult human sera from subjects who received adjuvanted H5N1 vaccine in study CC-Pan-H5N1:

1. ***To assess the in vivo protective effect of H5-head specific antibodies in pooled human serum collected on D42 and D385 from adult recipients of CC-Pan-H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that are subsequently challenged with approximately 5LD50-of cH5/3Nx virus (or an alternative challenge virus with similar attributes but more fit for purpose); Nx=most likely N4 or N5, to be decided. In vivo protection to be evaluated in terms of proportion of mice surviving challenge, mean weight loss, and (in randomly selected subgroups from each treatment group) mean lung weight***

at necropsy and geometric mean lung virus titer.

2. ***To assess the in vivo protective effect of H1-stalk specific antibodies in pooled human serum collected on D42 and D385 from adult recipients of CC-Pan-H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that are subsequently challenged with approximately 5LD50- of cH6/1N5 virus (or an alternative challenge virus with similar attributes but more fit for purpose). In vivo protection to be evaluated in terms of proportion of mice surviving challenge, mean weight loss, and (in randomly selected subgroups from each treatment group) mean lung weight at necropsy and geometric mean lung virus titer.***
3. ***If the pathogenicity (expressed as pfu/LD50) of the cH5/3Nx virus and cH6/1N5 virus are comparable, to then describe the effect size of anti-HA stalk antibodies (revealed by cH6/1N5 virus challenge) and anti-HA head antibodies (revealed by cH5/3Nx virus challenge) in terms of the relative survival rate (D42 rate/D0 rate) for cH6/1N5 vs relative survival rate for cH5/3Nx***
4. ***To describe the post-transfer geometric mean ELISA titer of human IgG to cH5/3Nx and human IgG to cH6/1N5 in blood collected from mice receiving each of 3 serum pools (D0, D42, D385)***
5. ***To explore the association between post-transfer ELISA titer of human IgG to the challenge virus and outcome at D3 and D6***

Study design (Amended 01 September 2016)

- **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 ~~(studies Q-Pan H1N1-019, CC-Pan H5N1-001, Q-Pan H9N2-001,~~ 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

assessments 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-005 and the H5N1-012 studies.

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 ~~cH8/1N3~~ 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, ~~a randomly selected subset of~~ samples ***pre- and post-vaccination*** from ***all*** subjects ***who received an adjuvanted vaccine*** in each study cohort ~~who had a ≥4-fold rise in MN titers (Day 21/Day 0)~~ will also be tested in a second MN assay with a reverse genetics (RG) reassortant heterologous HA Group 1 influenza virus (H5N8) ***and with an avian-like swine H1N1 virus (A/Swine/Jiangsu/40/2011). 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 in a step-wise analysis, initially on only the samples from the adult *CC-Pan-H5N1-001* study cohort. If the results of this analysis warrant it, testing may be expanded to the other study cohorts, as per protocol or modified by mutual agreement between GSK and Icahn School of Medicine at Mount Sinai (ISMMS).~~ ***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.*** 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.

Finally, an exploratory human serum transfer/virus challenge experiment will be conducted in mice to assess whether anti-H5 head antibodies and anti-H1 stalk antibodies are protective in vivo. The protective effect of pooled human sera collected on D42 and D385 from adult

recipients of adjuvanted H5N1 vaccine in study CC-PAN H5N1 will be compared to the effect of pooled serum collected at D0 by transferring each serum pool to BALB/c mice, which will be subsequently challenged with approximately 5LD_{50} of cH5/3Nx and cH6/1N5 (or alternative challenge viruses with similar attributes, but more fit for purpose);); Nx=most likely N4 or N5, to be decided.The design of this animal experiment is detailed in section 7 of Appendix A of this protocol.

- **Study groups:** There will be ~~7~~ ***13*** study groups, as described in Synopsis Table 1.

Synopsis Table 1 Study groups and epochs foreseen in the study

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	**Age range to be considered (yrs)**	**Study Group (treatment groups to be considered)**	**§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort**	**Number of subjects in ATP-I 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	~~172~~***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***	***All (~30)***	***N=variable depending on timepoint***

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	Age range to be considered (yrs)	Study Group (treatment groups to be considered)	§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort	Number of subjects in ATP-I cohort (i.e, up to D42)
		Day 549		
		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 influenza vaccine containing the Vietnam (VT) strain at Day 0 and Month 12*	*All (~60)*	*N=variable depending on timepoint for ATP persistency*
		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*

HA=Hemagglutinin content per vaccine dose; AS03$_A$=Adjuvant system 03$_A$; AS=Adjuvant system; ATP-I=According to Protocol cohort for Immunogenicity.

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

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
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
CC-Pan H5N1-001: Group B = 15 µg HA (no AS) CC-PAN H5N1 vaccine given at Day 0 and Day 21
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
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
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
Q-Pan H9N2-001: Group 1500_VVV = 15 µg HA (no AS) H9N2 vaccine given at Day 0, Day 21, and Day 182
DQIV-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***
§ Exact number of subjects per treatment uncertain, but likely to be in this range
* From D0, D21, D42, and D182 time points (no D385)

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

Number of subjects/samples (Amended 01 September 2016)

- 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 cohorts (depending on the study) of the completed studies, and 3) have valid vaccine homologous HI result available at all the required time points where the HI test is done and at Day 0 and 21 (for H5N1 and H9N2 studies only). Within each ***of these*** study cohorts, 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 timepoint. ***Furthermore, subjects assigned to the CMI and MN subset in study H9N2 and subjects with available homologous MN results 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 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 vaccine, were seronegative for H1N1pdm09 HI at Day 0, and have valid vaccine homologous HI and MN results available at either D0, 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.

- ***The number of samples and mice needed for the exploratory passive serum transfer in mice experiment are indicated in Section 7, Appendix A.***

Endpoints (Amended 01 September 2016):

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 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
     - 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***
     - 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

- *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 and*
    - *from Day 549 to Day 591 and*
    - *from Day 0 to Day 591*
  - *For group G:*
    - *from Day 182 to Day 224 and*
    - *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 and*
    - *from Day 549 to Day 591 and*
    - *from Day 0 to Day 591*
  - *For group G:*
    - *from Day 182 to Day 224 and*
    - *from Day 549 to Day 591 and*
    - *from Day 182 to Day 591*
- *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*
  - *GMT at Day 0 and 21*
  - *Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21*
  - *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 and the subjects in the FLU D-QIV-015 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)
  - 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***
  - 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
  - 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***
  - 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)***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***

- ***from Day 182 to Day 224 and***
- ***from Day 549 to Day 591 and***
- ***from Day 182 to Day 591***

– ***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 the FLU D-QIV-015 cohort:***

  – ***Seropositive rate at Day 0 and 21***
  – ***GMT at Day 0 and 21***
  – ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
  – ***MGI at Day 21 compared to Day 0***

3. Levels of anti-H2 and anti-H18 antibody by ELISA for ~~all adult~~ ***the*** subjects who received an AS vaccine ~~in the CC-Pan H5N1-001, the Q-Pan H1N1-019, the Q-Pan H9N2-001 and the Q-Pan H5N1-AS03-21~~ ***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)***
     – 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
     – 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***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 182 to Day 591***
  - ***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***
  - ***GMT at Day 0 and 21***
  - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
  - ***MGI at Day 21 compared to Day 0***

4. Vaccine-heterosubtypic virus titer by microneutralization (MN) for ~~a subset of~~ ***all*** subjects who received an AS vaccine in the ***listed study cohorts and the subjects in the FLU D-QIV-015 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, Q-Pan H9N2-001, and Q-Pan H5N1-AS03-21 study cohorts:

- Seropositive rate at Day 0 and Day ~~21~~ *42*
- GMT at Day 0 and Day ~~21~~ *42*
- Percentage of subjects with a ≥4-fold rise from Day 0 to Day ~~21~~ *42*
- MGI at Day ~~21~~ *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***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 182 to Day 591***
  - ***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***
  - ***MGI at Day 21 compared to Day 0***

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 cohorts. 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 D182 (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)*** ~~in each study~~ by treatment group and level of HI antibody to A/California/7/09 (or a like virus) ~~at Day 0 in H9N2 and adult H5N1 studies, by treatment group~~ ***in subjects from the CC-Pan-H5N1-001, Q-Pan-H9N2-001, 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***

~~1. 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~~

~~2. 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~~

Tertiary endpoints ~~*studies*~~)

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-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***
   - ***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***
   - ***MGI at Day 21, 42, 182 compared to Day 0***
2. ***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***
3. 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 adult 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
4. 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:
   - MGI for anti-H1 stalk ELISA at Day 21 compared to Day 0
5. 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

6. 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
   - GMT at Day 0, 21, and 42
   - Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21, and Day 42
   - MGI at Day 21 and 42 compared to Day 0

Tertiary endpoints

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***
   - ***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***
   - ***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***
   - ***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***

- *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)*
   - *GMT at Day 0, 42, and Month 12*
   - *Percentage of subjects with a ≥4-fold rise from Day 0 to Day 42*
   - *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. The following aggregate variables will be calculated with 95% CI:*
   - *Seropositive rate at Day 0 and Day 42*
   - *GMT at Day 0 and Day 42*
   - *Percentage of subjects with a ≥4-fold rise from Day 0 to Day 42*
   - *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*

Tertiary endpoints

Passive transfer/challenge in mice with pooled adult human sera from subjects who received adjuvanted H5N1 vaccine in the CC-Pan-H5N1 study cohort

1. *The in vivo protective effect of transferring pooled adult human serum (from subjects administered adjuvanted H5N1 vaccine in the CC-Pan-H5N1 study cohort) to mice and subsequently challenging them with cH5/3Nx virus (Nx=most likely N4 or N5, to be decided) will be assessed in terms of the following endpoints:*
   - *survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice/serum pool/time-point*
   - *mean weight loss (change from baseline over 14 days post-challenge) in groups of 25 mice/serum pool/time-point*
   - *lung weight in micrograms (D42 minus D0), (D385 minus D0), within challenge group*

- *lung virus titer in pfu/microgram (log10 fold change (D0 minus D42), (D0-D385), within challenge group*

2. *The in vivo protective effect of transferring pooled adult human serum (from subjects administered adjuvanted H5N1 vaccine) to mice and subsequently challenging them with cH6/1N5 virus will be assessed in terms of the following endpoints:*

    - *survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice/serum pool/time-point*
    - *mean weight loss (change from baseline over 14 days post challenge) in groups of 25 mice/serum pool/time-point*
    - *lung weight in micrograms (D42 minus D0), (D385 minus D0), within challenge group*
    - *lung virus titer in pfu/microgram (log10 fold change (D0 minus D42), (D0-D385), within challenge group*

3. *Post-transfer titer of human IgG to cH5/3 by ELISA*
4. *Post-transfer titer of human IgG to cH6/1 by ELISA*

(Amended 01 September 2016)

LIST OF ABBREVIATIONS

AS	Adjuvant System
$AS03_A$	$AS03_A$ is an Adjuvant System containing α-tocopherol and squalene in an oil and water emulsion (11.86 mg tocopherol in 0.25 mL i.e., 47.44 mg tocopherol per mL)
ATP	According-To-Protocol
ATP-I	According-To-Protocol cohort for Immunogenicity
CBER	Center for Biologics Evaluation and Research
CI	Confidence Interval
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Agency
GCP:	Good Clinical Practice
GMT	Geometric Mean Titer
GSK:	GlaxoSmithKline
HA	Hemagglutinin
HI	Hemagglutinin Inhibition
ICF:	Informed Consent Form
ICH:	International Conference on Harmonisation
IEC:	Independent Ethics Committee
IIV4	***Inactivated Influenza Vaccine, Quadrivalent***
IRB:	Institutional Review Board
ISMMS	Icahn School of Medicine at Mount Sinai, New York, NY, USA
LL	Lower Limit
MGI	Mean Geometric Increase
MN	Microneutralization
SAP	Statistical Analysis Plan

SP	Seropositivity
SUIV	Supra-seasonal Universal Influenza Vaccine
UL	Upper Limit

1. Introduction

1.1 Background

Influenza is an acute, highly contagious, respiratory disease caused by influenza viruses, mainly spread through respiratory droplets. The illness is accompanied by fever and variable degrees of other systemic symptoms, ranging from mild fatigue to respiratory failure and death. Influenza occurs in annual epidemics that are associated with significant morbidity and mortality; these epidemics often involve influenza subtypes H1 and H3 and influenza B viruses. The World Health Organization (WHO) estimates that influenza affects 5% to 15% of the population worldwide annually, with 3 to 5 million cases of severe illness and 250,000 to 500,000 deaths [WHO, 2005]. In the United States alone, influenza is estimated to cause 200,000 excess hospitalizations each year [Tosh, 2010].

The use of inactivated vaccines is the primary means of preventing influenza infection, but vaccine efficacy is dependent on how closely the strains included in the vaccine match the circulating virus. The WHO issues vaccine composition recommendations annually.

The need for accurate annual reformulation of current influenza vaccines stems from the high variability of the antigenic regions in the hemagglutinin (HA) globular head domain, which helps the influenza virus evade the human humoral response, since these regions also serve as the major epitopes for neutralizing antibodies. In contrast, because it is highly conserved and stable, the HA stalk domain offers a potential target as an effective component of an universal influenza vaccine. However, the HA stalk is also less immunogenic than the HA head, presenting a challenge for vaccine development which, could be overcome with the use of an adjuvant system to help provide a robust HA stalk-directed immunity that could induce a broad sero-response to influenza infection, irrespective of the predominant circulating virus strain [Goff, 2013]. Sequential immunization using a series of influenza viruses, each expressing a chimeric hemagglutinin composed of a unique exotic head domain and a conserved stalk domain, is one approach to overcoming the favor-dominance of the HA head [Krammer, 2014].

1.2 Rationale for the study and study design

1.2.1 Rationale for the study

FLU CC-SUIV-AS03-001 is the initial study in GSK Biologicals' clinical development plan for a candidate supra-seasonal universal influenza vaccine (SUIV) and is an exploratory, retrospective laboratory study, using archived serum samples, to assess the humoral immune response to the ***influenza A group 1 (H1) and the influenza A group 2 (H3)*** ~~H1~~ hemagglutinin ***(HA)*** stalk domain and other influenza A virus protein epitopes

following administration in adults and children of GSK Biologicals' adjuvanted or unadjuvanted pandemic vaccines. These vaccines can serve as a surrogate for one dose of an inactivated chimeric ~~hemagglutinin~~ ***HA***-bearing virus vaccine, because the stalk domain of the vaccines' ~~hemagglutinins~~ ***HAs*** shares important epitopes with ***all of the group 1 or the group 2 influenza A HA*** ~~HI~~ stalk ***domains*** despite ***small*** amino acid sequence differences in the overall ~~hemagglutinin~~ ***HA*** stalk domains among the ***circulating viruses***. The results of this study will provide context for and ***be informative for designing the Phase I study, FLU E-SUIV-001***, which will evaluate the ***safety and the immune response following one or two sequential doses of the SUIV candidate vaccine. This upcoming Phase I study will use a non-adjuvanted licensed quadrivalent inactivated influenza vaccine (IIV4) control group. Therefore, in order to be able to predict the response that can be seen in this control group, the stalk-reactive response following the administration of an IIV4 will also be assessed in this retrospective laboratory study***. The data obtained from the current FLU CC-SUIV-AS03-001 study will also support a pre-IND meeting with the FDA's Center for Biologics Evaluation and Research (CBER) by demonstrating that the immunoassays to be deployed in ***the Phase I FLU E-SUIV-001 study*** are fit for purpose and that the antigen and adjuvant dose proposed for the study are reasonably likely to be acceptably immunogenic.

1.2.2 Rationale for the study design

The retrospective study is designed to assess humoral immune response to the ***Group 1*** H1 hemagglutinin stalk domain (and other influenza A virus protein epitopes) ***in adult and pediatric subjects, by ELISA and microneutralization (MN) assay, using archived serum samples from***:

- ***Adult subjects who received*** adjuvanted ***(3.75 µg HA adjuvanted with $AS03_A$)*** or unadjuvanted ***(15 µg HA)*** H1N1pdm09, H5N1, and H9N2 pandemic influenza vaccines (standard adult dose) in 3 completed clinical trials ***[Q-Pan H1N1-019 (113536), CC-Pan H5N1-001 (114371), and Q-Pan H9N2-001 (116358)]***. The serum samples were collected at baseline, after 1 and 2 doses (Day 21 and Day 42, respectively), and after extended follow-up at Day 182. In 1 of these 3 ***study*** cohorts, samples were also collected at Day 385 (i.e., ***in CC-Pan H5N1-001 study cohort***, since this study also had a follow-up blood collection time point at Day 385).

  For each of these 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).

  - ***A group of pediatric subjects, 6-35 months of age, who received adjuvanted pandemic H5N1 vaccine in the completed Q-Pan H5N1-AS03-021 trial (114464), and who had no HI antibodies [titer <10] to H1N1pdm09 before being vaccinated with 2 doses of $AS03_B$-adjuvanted Q-PAN-H5N1 vaccine (half adult dose, i.e., 1.9 µg HA).*** 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). ***To control for the effects of inter-current H1N1pdm09 virus infection (i.e., to assess the impact of H1N1pdm09 virus transmission) on anti-H1 stalk titers, placebo control serum samples from the same study will also be evaluated.*** 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***.

Additionally, to evaluate the H1 HA stalk domain reactive response following a heterologous booster dose of an adjuvanted H5N1 vaccine (3.8 µg HA adjuvanted with AS03$_A$), samples will be analyzed from adult subjects 18 to 40 years of age who received a single dose of an adjuvanted H5N1 vaccine (monovalent A/turkey/Turkey/1/05 [H5N1]) 18 months after their priming dose with a heterologous adjuvanted H5N1 vaccine (i.e., monovalent A/ Indonesia/5/05 [H5N1]) in the Q-Pan-005 (110624) trial. Similarly, the H1 HA stalk domain reactive response following a homologous booster dose of an H5N1 adjuvanted vaccine administered in subjects primed with one dose of the same vaccine 12 months earlier will also be described using samples from the same study. 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 a homologous or a heterologous adjuvanted priming dose (A/Vietnam/1194/2004) in subjects 18-60 years of age. This assessment will be stratified by age (18-30 years vs 31-60 years) to evaluate the age effect on the immune response. The design of these 2 studies will also allow the assessment of the immune response to the H1 HA stalk domain several months after one dose of the adjuvanted pandemic vaccine. These vaccine schedules are representative of the schedules planned to be used with the GSK candidate monovalent group 1 influenza A SUIV vaccine in the E-SUIV-001 Phase I study.

For these anti-H1 stalk ELISAs, all evaluable serum specimens 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 from Day 0, Day 21, Month 6 (M6),Month (M12), M12+21days and M18 of all subjects (18-60y) from the groups VT/VT/12M and VT/IN/12M will be used (i.e. no random selection for the sub-cohort). Groups VT/VT/12M and VT/IN/12M were comprised of subjects administered A/Vietnam/1194/2004 adjuvanted H5N1 followed 12 months later by, respectively, a homologous (A/Vietnam/1194/2004-like) or heterologous (A/Indonesia/5/2005-like) adjuvanted H5N1 booster dose.

In order to further characterize the influenza A group 1 anti-HA stalk response to support design of the E-SUIV-001 Phase 1 study in which a quadrivalent inactivated influenza vaccine (IIV4) will serve as control, serum specimens will also be analyzed from the pre-vaccination (Day 0) and post-vaccination (Day 21) timepoints of a study (D-QIV-015 (201251)) in which subjects were vaccinated with an IIV4 (i.e., Fluarix Quadrivalent, also called D-QIV). Samples from approximately 30 adult subjects, 18-39 years of age, will be randomly selected for analysis.

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, all D0, D42 samples and samples from the last time point (persistency) from subjects who received an adjuvant system (AS) vaccine will be tested for reactivity by ELISA with H2 and H18 full length recombinant hemagglutinin proteins.

For the Q-Pan-005 study, this will be assessed with the Day 0, 42, 549, 591, and Day 729 samples from group C subjects and with the Day 182, 224, 549, 591, and Day 729 samples from group G subjects. For the H5N1-012 study this will be assessed with the Day 0, Day 21, M12, M12+21days and M18 samples of all subjects in the VT/VT/12M and the VT/IN/12M groups. For the D-QIV-015 study cohort, in which the subjects received an IIV4, the testing will be performed with the Day 0 and the Day 21 samples.

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 will be 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 of this virus are "exotic" and most humans have not been exposed to them).

To assess the breadth of neutralization effected by anti-H1 stalk ELISA antibodies, ~~a randomly selected subset of~~ samples ***at baseline (D0) and D42*** from ***all*** subjects ***who received an adjuvanted vaccine*** in each study cohort ~~who had a ≥4-fold rise in MN titers (Day 21/Day 0)~~ 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 a H1N1 pdm09 like virus.***

For the Q-Pan-005 study, this will be assessed with the Day 0, 42, 549 and 591 samples from group C subjects and with the Day 182, 224, 549 and 591 samples from group G subjects. For the H5N1-012 study this will be assessed with the Day 0, Day 21, M12, M12+21days and M18 samples of all subjects in the VT/VT/12M and the VT/IN/12M groups. For the D-QIV-015 study cohort, in which the subjects received an IIV4, the testing will be performed with the Day 0 and Day 21 samples.

As part of the exploratory (tertiary) objectives of this retrospective study, and based on the experience that will be acquired in testing group 1 anti-H1 stalk responses mentioned above, archived serum samples (with sufficient volume for the serology analyses) from the Day 0, 21, 42, Month 6, and 12 time points of a H7N9 study cohort (in which adult subjects were administered 2 doses (21-day interval) of an adjuvanted (3.75 µg HA adjuvanted with AS03$_A$) or unadjuvanted (15 µg HA) pandemic influenza H7N9 vaccine) will also be evaluated for antibody responses to the group 2 HA stalk (i.e., H3), by anti-H3 stalk ELISA.

This analysis will also assess the performance of the influenza A anti-H3 stalk ELISA. Approximately 60 subjects (i.e., approximately 30 subjects who received the adjuvanted vaccine dose, and approximately 30 subjects who received the unadjuvanted vaccine

dose) will be randomly selected to generate the serology sub-cohort in which both groups will match in terms of age and center.

As an additional exploratory objective for the H7N9 study cohort to evaluate whether a post-vaccination boost in ELISA antibody titers could also result in neutralization of target virus, samples from subjects who received an adjuvant system (AS) vaccine will be further analyzed with an influenza A group 2 anti-H3 stalk domain (at Day 0, 21, 42 M6 and M12) and heterosubtypic (at Day 0 and Day 42) microneutralization (MN) assays (target viruses: cH14/3Nx and a wild-type Group 2 H4N8 virus, respectively). Furthermore, D0, D42 and M12 samples from subjects who received the adjuvanted vaccine will be tested for reactivity with H4 and H10 full length recombinant hemagglutinin (HA) proteins.

Finally, an exploratory human serum transfer/virus challenge experiment will be conducted in mice to assess whether anti-H5 head antibodies and anti-H1 stalk antibodies are protective in vivo. The protective effect of pooled human sera collected on D42 and D385 from adult recipients of adjuvanted H5N1 vaccine in study CC-PAN H5N1 will be compared to the effect of pooled serum collected at D0, by transferring each serum pool to BALB/c mice, which will be subsequently challenged with approximately $5LD_{50}$ of cH5/3Nx (Nx=most likely N4 or N5, to be decided) and cH6/1N5 (or alternative challenge viruses with similar attributes, but more fit for purpose). The extent of protection from viral challenge will be evaluated in terms of the proportion of mice surviving viral challenge, mean weight loss over time, and, in randomly selected subgroups of animals from each of the 3 treatment groups administered the D0, D42, or D385 serum pools, mean lung weight at necropsy and geometric mean lung virus titer following euthanization at 3 and 6 days post-challenge.

(Amended 01 September 2016)

~~This retrospective study is designed to assess immunogenicity (in terms of the humoral immune response to the ***Group 1*** H1 hemagglutinin stalk domain and other influenza A virus protein epitopes) of H5N1, H1N1pdm09, and H9N2 adjuvanted or unadjuvanted pandemic influenza vaccines (standard adult dose) using archived serum specimens from 3 completed clinical trials with adult subjects (H1N1, H5N1, and H9N2) at baseline, after 1 and 2 doses (Day 21 and Day 42, respectively), and after extended follow up at Day 182 and in 1 of 3 study cohorts, also at Day 385 (i.e., in H5N1, since this study also had a follow up blood collection time point at Day 385). Therefore, Day 0, 21, 42, and 182 time point samples from the H1N1, H5N1, and H9N2 studies, as well as Day 385 time point samples from the H5N1 study will be tested. ***Additionally, as part of exploratory (tertiary) objectives and based on the experience acquired in testing anti-H1 stalk responses in the first four study cohorts, serum samples from a new H7N9 study cohort (in which adult subjects were administered adjuvanted or unadjuvanted pandemic influenza H7N9 vaccine) will also be evaluated for antibody responses to the H3 stalk (i.e., Group 2 hemagglutinin) by ELISA and microneutralization assay. This analysis will also assess the performance of the anti-H3 stalk ELISA.*** For each study, 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 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). 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). ***To control for the effects of inter-current H1N1pdm09 virus infection (i.e., to assess the impact of H1N1pdm09 virus transmission) on anti-H1 stalk titers, placebo control serum samples from the same study will also be evaluated.*** 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***).~~

~~A serology testing algorithm will be used to rationalize the serology analyses. In the initial step, an anti-H1 stalk domain ELISA (enzyme-linked immunosorbent assay) will be used to analyze archived samples (with sufficient volume for the serology analyses) from the Day 0, 21, 42, and 182 time points of all 3 adult study cohorts (H1N1, H5N1, and H9N2), the D385 time point of the adult H5N1 study cohort, as well as the Day 0, 21, 42, and 385 time points of the pediatric H5N1 study. ***An anti-H3 stalk ELISA will be used to analyze archived samples (with sufficient volume for the serology analyses) from the Day 0, 21, 42, Month 6, and 12 time points of the H7N9 adult study cohort.***~~

~~To evaluate whether a post-vaccination boost in ELISA antibody titers, in the HA group 1 related study cohorts (H1N1, H5N1, and H9N2), 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 cH8/1-N3, 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). Additionally, all subjects who received an adjuvant system (AS) vaccine will have their D0 and D42 samples tested for anti-H2 and anti-H18 ELISA antibody titers in order to assess the breadth of cross-reactivity elicited by vaccination. To assess the breadth of neutralization effected by anti-H1 stalk ELISA antibodies, a randomly selected subset of samples ***at baseline (D0) and D42*** from ***all*** subjects ***who received an adjuvanted vaccine*** in each study cohort who had a ≥4-fold rise in MN titers (Day 21/Day 0) will be tested in a second MN assay with a reverse genetics (RG) reassortant heterologous HA Group 1 influenza virus (H6N3). This test will establish the neutralizing potency of antibodies directed to the H6 stalk domain relative to the H1 stalk domain; a ratio ≥0.5 supports the potential of the FLU-CC-SUIV candidate vaccine to elicit broadly protective humoral immune response to influenza A viruses with pandemic potential.~~

2 Objectives

2.1 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)

1. 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 (Day 0), post-dose 1 (Day 21), post-dose 2 (Day 42), Day 182 (D182) and at Day 385 (D385) for the CC-Pan H5N1-001 study cohort, by treatment group (unadjuvanted or adjuvanted vaccine)***~~For adult subject samples, at baseline Day 0 (D0), post-dose 1 at Day 21 (D21), post-dose 2 at Day 42 (D42), Day 182 (D182) by treatment group (unadjuvanted or adjuvanted vaccine), for each study cohort from the ATP cohorts for Immunogenicity of all 3 primary completed studies (H1N1, H5N1, and H9N2), and also at Day 385 (D385) for the H5N1 study cohort~~
   - 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*** ~~from the ATP cohort for Immunogenicity of the primary completed study (pediatric H5N1 study cohort)~~
   - ***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 (Month 6), M12 (Month 12), M12+21 days, and M18 (Month 18)***
   - ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***

2. 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-AS03-21 study 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 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, M6, M12, M12+21 days, and M18***

- ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***

3. 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, M6, M12, M12+21 days, and M18***
   - ***In adult subject samples of the FLU D-QIV-015 study cohort at baseline (D0) and D21***

4. To describe the ***vaccine heterosubtypic*** ~~heterologous~~ 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***

Refer to Section 10.1 for the definition of the primary endpoints. **(Amended 01 September 2016)**

2.2 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):

1. 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***)
2. ***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)

Refer to Section 10.2 for the definition of the secondary endpoints.

2.3 Tertiary 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):

1. ***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***
2. ***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***
3. 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 cohort (AS group only)
4. 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
5. 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***
6. 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)

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

1. ***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***
2. ***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***

3. ***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***

4. ***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***

5. ***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***

Tertiary objectives: Passive transfer/challenge in mice with pooled adult human sera from subjects who received adjuvanted H5N1 vaccine in study CC-Pan-H5N1:

1. ***To assess the in vivo protective effect of H5-head specific antibodies in pooled human serum collected on D42 and D385 from adult recipients of CC-Pan-H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that are subsequently challenged with approximately 5LD_{50} of cH5/3Nx virus (or an alternative challenge virus with similar attributes but more fit for purpose); Nx=most likely N4 or N5, to be decided. In vivo protection to be evaluated in terms of proportion of mice surviving challenge, mean weight loss, and (in randomly selected subgroups from each treatment group), mean lung weight at necropsy and geometric mean lung virus titer***

2. ***To assess the in vivo protective effect of pooled human serum collected on D42 and D385 from adult recipients of CC-Pan-H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that are subsequently challenged with approximately 5LD_{50} of cH6/1N5 virus (or an alternative challenge virus with similar attributes but more fit for purpose). In vivo protection to be evaluated in terms of proportion of mice surviving challenge, mean weight loss, and (in randomly selected subgroups from each treatment group), mean lung weight at necropsy and geometric mean lung virus titer***

3. ***If the pathogenicity (expressed as pfu/LD_{50}) of the cH5/3Nx virus and cH6/1N5 virus are comparable, to then describe the effect size of anti-HA stalk antibodies (revealed by cH6/1N5 virus challenge) and anti-HA head antibodies (revealed by cH5/3Nx virus challeng) in terms of the relative survival rate (D42 rate/D0 rate) for cH6/1N5 vs relative survival rate for cH5/3Nx***

4. ***To describe the post-transfer geometric mean ELISA titer of human IgG to cH5/3Nx and human IgG to cH6/1N5 in blood collected from mice receiving each of 3 serum pools (D0, D42, D385)***

5. ***To explore the association between post-transfer ELISA titer of human IgG to the challenge virus and outcome at D3 and D6***

Refer to Section 10.3 for the definition of the tertiary endpoints. **(Amended 01 September 2016)**

3 Study Design Overview

~~The serology testing algorithm below summarizes the testing to support the *primary and secondary objectives only*.~~

<<Study design schematic deleted since it is no longer applicable.>>

~~Serology Algorithm specifying the key laboratory analyses and decision points. ELISA= Enzyme linked immunosorbent assay; MN= Microneutralization assay; AS= Adjuvanted system; RG= Reverse genetics; HA= Hemagglutinin; NA= Neuroaminidase. Timepoints: D0= Day 0, D21= Day 21, D42= Day 42, D182= Day182, D385= Day 385. H1N1, H5N1, and H9N2 = primary completed studies with archived serum samples.~~

~~The microneutralization testing will be done in a step wise analysis, initially on only the samples from the H5N1 study cohorts. If the results of this analysis warrant it, testing may be expanded to the H9 and H1 study cohorts as per protocol or modified by mutual agreement between GSK and ISMMS. **(Amended 18 July 2016)**~~

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 ~~(studies Q-Pan H1N1-019, CC-Pan H5N1-001, Q-Pan H9N2-001,~~ 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 assessments 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 studies.

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 ~~cH8/1N3~~ 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, ~~a randomly selected subset of~~ samples pre-, and post- vaccination from all subjects who received an adjuvanted vaccine in each study cohort ~~who had a ≥4 fold rise in MN titers (Day 21/Day 0)~~ 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 in a step-wise analysis, initially on only the samples from the adult *CC-Pan-H5N1-001* study cohort. If the results of this analysis warrant it, testing may be expanded to the other study cohorts, as per protocol or modified by mutual agreement between GSK and Icahn School of Medicine at Mount Sinai (ISMMS).~~ ***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.

Finally, an exploratory human serum transfer/virus challenge experiment will be conducted in mice to assess whether anti-H5 head antibodies and anti-H1 stalk antibodies are protective in vivo. The protective effect of pooled human sera collected on D42 and D385 from adult recipients of adjuvanted H5N1 vaccine in study CC-PAN H5N1 will be compared to the effect of pooled serum collected at D0 by transferring each serum pool to BALB/c mice, which will be subsequently challenged with approximately 5LD_{50} of cH5/3Nx and cH6/1N5 (or alternative challenge viruses with similar attributes, but more fit for purpose); Nx=most likely N4 or N5, to be decided.The design of this animal experiment is detailed in section 7 of Appendix A of this protocol.

(Amended 01 September 2016)

- **Study groups:** There will be ~~7~~ ***13*** study groups, as described in Table 1.

Table 1 Study groups and epochs foreseen in the study (Amended 01 September 2016)

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	**Age range to be considered (yrs)**	**Study Group (treatment groups to be considered)**	**§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort**	**Number of subjects in ATP-I 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	~~172~~ ***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 influenza vaccine***	***All (~60)***	***N=variable depending on timepoint for ATP persistency***

Epoch (Epoch 001: Retrospective laboratory evaluations)				
Primary study from which archived serum samples will be analyzed	Age range to be considered (yrs)	Study Group (treatment groups to be considered)	§Number of subjects per treatment to be randomly selected from ATP-I or Persistence cohort	Number of subjects in ATP-I cohort (i.e, up to D42)
		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*

HA=Hemagglutinin content per vaccine dose; $AS03_A$=Adjuvant system 03_A; AS=Adjuvant system; ATP-I=According to Protocol cohort for Immunogenicity.

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)

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

- **Type of study:** Self contained retrospective study.

4 Study COHORT

4.1 Number of samples

The target is to randomly select approximately 30 subjects from each of the adult and pediatric studies. These 30 subjects from each of the 3 adult ~~H5N1, H9N2, and H1N1, and H7N9~~ studies ***(Q-Pan H1N1-019, CC-Pan H5N1-001, and Q-Pan H9N2-001),*** will be those 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 the required time points where the HI test is done and at Day 0 and 21 (for H5N1, 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 timepoint. ***Furthermore, subjects assigned to the CMI and MN subset in study H9N2 and subjects with available homologous MN results 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 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 (~~age < 36 months~~ ***6-35 months of age***) belonging to both ATP-I and Persistence cohorts (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 D0, 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 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.

The number of samples and mice needed for the exploratory passive serum transfer in mice experiment are indicated in Section 7, Appendix A.

(Amended 01 September 2016)

4.2 Inclusion criteria for enrolment (selection of archived samples)

Not applicable since no subjects will be actively enrolled in this study; only the sera samples of the subjects who were a part of previously conducted primary trials will be used for testing. However, the archived serum samples of only those subjects who satisfy the following criteria will be included in this study:

Subjects who were included in the ATP cohort for immunogenicity and Persistence cohort (depending on the study) in the primary studies listed.

Subjects who had agreed that their blood samples could be used for further research while giving informed consent for any of the primary studies listed.

Subjects who have sufficient residual sample volume (i.e., ≥0.5 mL) of serum at all time points.

Only applicable for study CC-Pan-H5N1-001, study Q-Pan-H9N2-001 and study H5N1-AS03-021: subjects with vaccine homologous neutralizing antibody result available at Day 0 and at 21 (25 samples available per group in Study H9N2-001)

4.3 Exclusion criteria for enrolment

Not applicable since no subjects will be actively enrolled in this study; only the serum samples of the subjects who were a part of previously conducted trials will be used for testing.

5 Conduct of the Study

5.1 Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

Conduct of this study includes, but is not limited to, the following:

Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.

Subjects' informed consent (*Note*: Samples of only those subjects who had agreed that their blood samples could be used for further research while providing consent for their participation in any of the primary studies listed will be included in this study).

Conduct of the primary studies included, but was not limited to, the following:

Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.

Subjects' (or subjects' parents/legally appointed representatives for the pediatric study) informed consent.

5.2 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.

For the current exploratory, retrospective study, the target is to select approximately 30 subjects from each of the adult and pediatric studies.

The 30 subjects from each of the 3 ***following*** adult ~~H5N1, H9N2, and H1N1, *and H7N9*~~ studies ***(Q-Pan H1N1-019, CC-Pan H5N1-001, and Q-Pan H9N2-001),*** will be those

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 the required time points where the HI test is done and at Day 0 and 21 (for H5N1, and H9N2 studies only). Within each ***of these*** study cohorts, 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 subjects with available homologous MN results 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 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 (~~age < 36 months~~ ***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.

The number of samples and mice needed for the exploratory passive serum transfer in mice experiment are indicated in Section 7, Appendix A.

(Amended 01 September 2016)

5.3 Method of blinding

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

5.4 Biological sample handling and analysis

The archived sera samples collected in the previously conducted pandemic influenza vaccine clinical trials will be tested in this study.

5.4.1 Laboratory assays

Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX B for the address of the clinical laboratories used for sample analysis.

Serological assays for the determination of antibodies against the HA stalk domain and other influenza A virus protein epitopes will be performed by ELISA (Table 2) using standardized procedures in a laboratory designated by GSK Biologicals (i.e., Dept. Of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA ***and/or NeoMed Labs Inc., Quebec, Canada***). The laboratory analyses may be 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.

Table 2 Serum Assays to Assess Humoral Immunity (Amended 01 September 2016)

Antigen/ Virus for Test	Component (Strain or Antigen Description)	Method	Kit / Manufacturer	Unit	§Cut-off	*Laboratory
ELISAs						
cH6/1N5 HA	Recombinant antigen based on A/mallard/Sweden/81/02 head domain with	Anti-H1 HA stalk ELISA	ISMMS ***or NeoMed Labs*** protocol/assay	ELISA units (EU)	100 EU/***ml***	ISMMS ***or NeoMed Labs***

Antigen/ Virus for Test	Component (Strain or Antigen Description)	Method	Kit / Manufacturer	Unit	§Cut-off	*Laboratory
	A/Puerto Rico/8/34 H1 stalk domain					
H2 HA full length	Recombinant antigen based on A/mallard/ Netherlands/5/99 HA	Anti- H2 HA full length ELISA				
H18 HA full length	Recombinant antigen based on A/flat-faced bat/ Peru/033/10 HA	Anti- H18 HA full length ELISA				
N1 NA	Recombinant antigen based on A/California/04/2009 NA	Anti-N1 NA ELISA				
N2 NA	**Recombinant antigen based on A/Chicken/ Hong Kong/G9/1997 NA	Anti-N2 NA ELISA				
H9 HA head domain	**Recombinant antigen based on A/Chicken/ Hong Kong/G9/1997 HA head domain	Anti-H9 HA head domain ELISA				
H9 HA full length	**Recombinant antigen based on A/Chicken/ Hong Kong/G9/1997 HA	Anti- H9 HA full length ELISA				
~~*cH14/3 full length*~~	***Recombinant antigen based on the head domain of A/mallard/Gurjev/263/82 and the stalk domain of A/Perth/16/09***	***Anti-H3 HA stalk ELISA***				
H4 HA full length	***Recombinant antigen based on A/duck/Czech/56***	***Anti- H4 HA full length ELISA***				
H10 HA full length	***Recombinant antigen based on A/mallard/IA/10BM01929 /10***	***Anti-H10 HA full length ELISA***				
Microneutralization (MN) assays						
~~cH8/1 N3~~ ***cH6/1N5*** virus	cHA virus based on PR8 for 6 genes with 2 surface proteins: N3 is from A/swine/Missouri/429642 4/2006(H2N3), HA head domain is from A/mallard/Sweden/24/200 2 (H8N4), HA stalk domain is from A/California/04/09 (H1N1)	***Anti-H1 HA stalk*** MN Assay	ISMMS protocol/assay	1/DIL (IC_{50})	10	ISMMS

Antigen/ Virus for Test	Component (Strain or Antigen Description)	Method	Kit / Manufacturer	Unit	§Cut-off	*Laboratory
~~H6N3~~ H5N8 virus	RG reassortant virus based on PR8 for 6 genes with 2 surface proteins: NA from A/swine/Missouri/4296424/2006 (H2N3), HA from A/mallard/Sweden/81/2002 (H6N1)	*Anti-heterosubtypic HA Group 1 virus* MN Assay				
H1N1 swine Flu virus	*A/Swine/Jiangsu/40/2011*	*Anti-heterosubtypic HA Group 1 virus MN Assay*				
H1N1 pdm09 virus	*A/California/7/2009-like*	*ϕ Anti-heterosubtypic HA Group 1 virus MN Assay*				
cH14/3NX	*RG reassortant virus based on PR8 for 6 genes with 2 surface proteins: NX (to be determined), cHA with the head domain of A/mallard/Gurjev/263/82 and the stalk domain of A/Perth/16/09*	*Anti-H3 HA stalk MN Assay*				
H4N8 virus	*RG reassortant virus based on PR8 for 6 genes with 2 surface proteins: NA from A/mallard/Sweden/50/0, HA from A/duck/Czech/56*	*Anti-heterosubtypic HA Group 2 virus MN Assay*				
Hemagglutination Inhibition (HI) Assay						
~~H5N1 virus~~	~~Pandemic vaccine (H5N1) homologous virus (A/Indonesia/5/2005)~~	~~HI assay~~	~~GSK GVCL *CLS* protocol/assay (if resources available)~~	~~1/DIL~~	~~10~~	~~***GSK Biologicals~~
~~H9N2 virus~~	~~Pandemic vaccine homologous (H9N2) virus (A/chicken/Hong~~	~~HI assay~~	~~GSK GVCL *CLS* protocol/assay (if~~	~~1/DIL~~	~~10~~	~~***GSK Biologicals~~

Antigen/ Virus for Test	Component (Strain or Antigen Description)	Method	Kit / Manufacturer	Unit	§Cut-off	*Laboratory
	~~Kong/G9/1997)~~		~~resources available)~~			
H1N1 virus	Pandemic vaccine (H1N1) homologous virus (A/California/7/2009 (or a like virus))	HI assay	GSK ~~GVCL~~ ***CLS*** protocol/assay (if resources available)	1/DIL	10	***GSK Biologicals

*Refer to **APPENDIX B** for the laboratory addresses. ISMMS= Icahn School of Medicine at Mount Sinai, Dept. Of Microbiology, New York, NY, USA. ***NeoMed Labs = NEOMED-LABS INC., 7171 Frederick-Banting St, Saint-Laurent, Quebec H4S 1Z9, Canada***

**These ELISAs to be performed only on samples from the H9N2 cohort

*** GSK Biologicals laboratory refers to the ~~Global Vaccines Clinical Laboratories (GVCL)~~ ***Clinical Laboratory Sciences (CLS)*** in Rixensart, Belgium; Wavre, Belgium; Dresden, Germany

§ Cut-off value may change subject to qualification of new reference serum pool (NeoMed Labs)

ϕ Vaccine homologous H1N1 for DQIV015 and H1N1-019 study samples

Table 3 Cell-Mediated Immunity (CMI) (Amended 01 September 2016)

System	Component	Challenge	Method	Unit	Laboratory*	Priority
PBMCs	T cells stained with probes for various cytokines (IL2, TNFα. IFNγ, CD40L, etc)	None, H9N2 split, H1N1 split, H3N2 split ***H1 (A/California/7/2009) stalk peptide pool***	T Cell response by ICS assay	Frequencies of cytokine+ CD4+ T cells/Mio of CD4+ T cells	GSK Biologicals**	4
PBMCs	Plasmablasts sorted using HA- SA biotinylated probe-	None	Plasmablast response to HA	Frequencies of antigen-specific plasmablasts/Mio plasmablasts	GSK Biologicals**	3
PBMCs	B cells reactive to "challenge" antigens	None, H9N2 split, H1 stalk domain (cHA 6/1), trimeric H9 Head (H9 globular HA domain), tetrameric N2	B memory cells by ELIspot	Frequencies of antigen-specific memory B cells/Mio memory B Cells	GSK Biologicals**	1
PBMCs	Plasmablasts reactive to "challenge" antigens	None, H9N2 split, H1 stalk domain (cHA 6/1), trimeric H9 Head (H9 globular HA domain), tetrameric N2	Plasmablast cells by ELIspot	Frequencies of antigen-specific plasmablasts/Mio plasmablasts	GSK Biologicals**	2

*Refer to **APPENDIX B** for the laboratory addresses.

**GSK Biologicals laboratory refers to the Global Vaccines Clinical Laboratories (GVCL) in Rixensart, Belgium; Wavre, Belgium; Laval, Canada.

PBMC = Peripheral blood monocytes

5.4.2 Biological samples evaluation

5.4.2.1 Immunological read-outs

Table 4 Planned Immunological Read-Outs: ELISAs (Amended 01 September 2016)

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort AS= Adjuvant System	N tests planned as per algorithm)	Comment
Adult subjects: ~~Anti-~~H1 HA stalk ELISA ~~(A/California/09/2009)~~	Recombinant ~~trimeric cHA (H6/1)~~ ***antigen based on A/mallard/Sweden/81/02 head domain with A/Puerto Rico/8/34 H1 stalk domain***	D0, D21, D42, D182 (from all 3 adult studies) and D385 (from adult H5N1 study only); 2 treatment groups (AS and no AS)	30 ***(AS)***	780 (30 samples x 4 timepoints x 3 studies x 2 treatment groups + 30 samples x 1 timepoint x 2 treatment groups x 1 study, H5N1)	For all 3 adult study cohorts (***Q-Pan-H1N1-019, CC-Pan-H5N1-001, and Q-Pan-H9N2-001***)
		D0, D21	***30 (no AS)***	***60 (30 samples x 2 timepoints x 1 treatment grp***	***For adult cohort in study D-QIV-015***
		D0, D42, D182, D224, D549, D591, D729	***60 (n=approx 30 each from Groups C and G)***	***420 (60 samples x 7 timepoints x 1 treatment grp***	***For adult study cohorts (Q-Pan-005), Groups C and G***
		D0, D21, Month 6 (M6),Month 12 (M12), M12+21, Month 18 (M18)	***120 (n=approx 60 each from Groups *VT/VT/12M and VT/*IN/12M)***	***720 (60 samples x 6 timepoints x 2 treatment grp***	***For adult cohort from study H5N1-012, Groups *VT/VT/12M and VT/*IN/12M)***
Pediatric subjects: ~~Anti-~~H1 HA stalk ELISA ~~(A/California/07/2009)~~	Recombinant ~~trimeric cHA (H6/1)~~ ***antigen based on A/mallard/Sweden/81/02 head domain with A/Puerto Rico/8/34 H1 stalk domain***	D0, D21, D42, D385 (from pediatric H5N1 study only); ~~1~~ ***2*** treatment groups (AS ***and placebo***)	30	~~120~~ ***240*** (30 samples x 4 timepoints x 1 study x ~~1~~ ***2*** treatment groups, ***including placebo group***)	For pediatric ***Q-Pan-H5N1-AS03-021*** study cohort only
~~Anti-~~N1 NA ELISA (A/California/~~07~~ ***04***/2009)	~~Recombinant tetrameric NA (N1)~~ ***Recombinant antigen based on A/California/0***	D0, D21, D42, D182 (from H1N1 study only; done regardless of anti-H1 stalk ELISA results	30	240 (30 samples x 4 timepoints x 1 study x 2 treatment groups)	For H1N1 study cohort only**

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort AS= Adjuvant System	N tests planned as per algorithm)	Comment
	4/2009 NA (N1)	above); 2 treatment groups (AS and no AS)			
~~Anti-~~H2 HA full length ELISA (A/mallard/Netherlands/ 5/99 HA)	Recombinant trimeric H2 HA full length	D0, D42, ***and final timepoint for persistence***; One treatment group (AS)	30	~~240~~***360*** (30 samples x ~~2~~ 3 timepoints x 4 studies x 1 treatment group)	For ~~all 4~~ ***3*** adult study cohorts (~~adult H1N1, H5N1, H9N2~~ ***CC-Pan-H5N1-001, Q-Pan H1N1-019, andQ-Pan H9N2-001)*** and pediatric cohort (***Q-Pan H5N1-AS03-21*** ~~H5N1~~)
		D0, D42, D549, D591, and D729; One treatment group	***30***	***150 (30 samples x 5 timepoints x 1 treatment group)***	***For adult study cohort Q-Pan-005: Group C***
		D182, D224, D549, D591, and D729; One treatment group	***30***	***150 (30 samples x 5 timepoints x 1 treatment group)***	***For adult study cohort Q-Pan-005: Group G***
		D0, D21, Month 6 (M6),Month 12 (M12), M12+21, Month 18 (M18)	***120 (n=approx 60 each from Groups *VT/VT/12M and VT/*IN/12M)***	***720 (60 samples x 6 timepoints x 2 treatment grp***	***For adult cohort from study H5N1-012, Groups *VT/VT/12M and VT/*IN/12M)***
		D0, D21; One treatment group	***30***	***60 (30 samples x 2 timepoints x 1 treatment group)***	***For adult study cohort D-QIV-015***
~~Anti-~~H18 HA full length ELISA (A/flat-faced bat/Peru/033/10 HA)	Recombinant trimeric H18 HA full length	D0, D42, ***and final timepoint for persistence***; One treatment group (AS)	30	~~240~~***360*** (30 samples x ~~2~~ 3 timepoints x 3 studies x 1 treatment group)	For ~~all 4~~ ***3*** adult study cohorts (~~adult H1N1, H5N1, H9N2~~ ***CC-Pan-H5N1-001, Q-Pan H1N1-019 andQ-Pan H9N2-001)*** and pediatnc cohort (***Q-Pan H5N1-AS03-21*** ~~H5N1~~)
		D0, D42, D549, D591, and D729; One treatment group	***30***	***150 (30 samples x 5 timepoints x 1 treatment group)***	***For adult study cohort Q-Pan-005: Group C***
		D182, D224,	***30***	***150 (30***	***For adult study cohort***

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort AS= Adjuvant System	N tests planned as per algorithm)	Comment
		D549, D591, and D729; One treatment group		*samples x 5 timepoints x 1 treatment group)*	*Q-Pan-005: Group G*
		D0, D21, Month 6 (M6),Month 12 (M12), M12+21, Month 18 (M18)	*120 (n=approx 60 each from Groups *VT/VT/12M and VT/*IN/12M)*	*720 (60 samples x 6 timepoints x 2 treatment grp*	*For adult cohort from study H5N1-012, Groups *VT/VT/12M and VT/*IN/12M)*
		D0, D21; One treatment group	*30*	*60 (30 samples x 2 timepoints x 1 treatment group)*	*For adult study cohort D-QIV-015*
~~Anti-~~N2 NA ELISA (A/chicken/Hong Kong/G9/1997)	Recombinant tetrameric N2 NA	D0, D21, D42; Two treatment groups (AS and no AS)	30	180 (30 samples x 3 timepoints x 1 study x 2 treatment groups)	For H9N2 study cohort only
~~Anti-~~H9 HA head domain ELISA (A/chicken/Hong Kong/G9/1997)	Recombinant trimeric H9 HA head domain	D0, D21, D42; Two treatment groups (AS and no AS)	30	180 (30 samples x 3 timepoints x 1 study x 2 treatment groups)	For H9N2 study cohort only
~~Anti-~~H9 HA full length ELISA (A/chicken/Hong Kong/G9/1997)	Recombinant trimeric H9 HA full length	D0, D21, D42; Two treatment groups (AS and no AS)	30	180 (30 samples x 3 timepoints x 1 study x 2 treatment groups)	For H9N2 study cohort only
~~Anti-~~H3 HA anti-stalk ~~full length~~ ELISA (cH14/3)	*Recombinant antigen based on the head domain of A/mallard/Gurjev/263/82and the stalk domain of A/Perth/16/09*	*D0, D21, D42; Month 6, and Month 12. Two treatment groups (AS and no AS)*	*30*	*300 (30 samples x 5 timepoints x 1 study x 2 treatment groups)*	*For H7N9 study cohort only*
~~Anti-H4 HA full length ELISA (XX virus/strain)~~	*Recombinant antigen based on A/duck/Czech /56*	*D0, D42, Month 12 (persistency) ; One treatment group (AS)*	*30*	*90 (30 samples x 3 timepoints x 1 study x 1 treatment group)*	*For H7N9 study cohort only*
~~Anti-~~H10 HA full length ELISA ~~(XX virus/strain)~~	*Recombinant antigen based on*	*D0, D42, Month 12 (persistency)*	*30*	*90 (30 samples x 3 timepoints x 1 study x 1*	*For H7N9 study cohort only*

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort AS= Adjuvant System	N tests planned as per algorithm)	Comment
	A/mallard/IA/10BM01929/10	*; One treatment group (AS)*		*treatment group)*	
Total anticipated number of ELISA tests					*6420 ~~4920~~ ~~2580~~ ~~2160~~*

*Anti-N1 NA testing for H1N1 study cohort only, since only for this cohort was the vaccine NA matching with the ELISA test NA

HI testing by GSK for anti-H1N1 at D0 for subjects in the H5N1 and H9N2 cohorts will be elective based on laboratory capacity

VT= A/Vietnam/1194/2004-like strain and IN = A/Indonesia/5/2005-like

Table 4 Planned Immunological Read-Outs: Microneutralization (MN) assays (Amended 01 September 2016)

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested (AS treatment) per study cohort	N tests planned as per algorithm)	Comment
~~Anti-~~H1 HA stalk domain MN	Chimeric virus (~~cH8/1 N3~~ ***cH6/1N5***)	Adult subjects: D0, D21, D42, D182; AS groups only	30	240 (30 samples x 4 timepoints x 2 studies)	For adult ***Q-Pan-H1N1-019*** and ***Q-Pan-H9N2-001*** study cohorts ***(all subjects who received an AS vaccine)***
		Adult subjects: D0, D21, D42, D182, D385; AS group only	30	150 (30 samples x 5 timepoints)	For adult ***CC-Pan-H5N1-001*** study cohort ***(all subjects who received an AS vaccine)***
		Pediatric subjects: D0, D21, D42, D385; AS group only	30	120 (30 samples x 4 timepoints x 1 treatment)	For pediatric ***Q-Pan-H5N1-AS03-001*** study cohort ***(all subjects who received an AS vaccine)***
		Adult subjects: Group C: D0, D42, D182, D549, D591, D729; and Group G: D182, D224, D549, D591, D729	***60 (Approx 30 from each of Groups C and G***	***330 (Group C: 30 samples x 6 timepoints = 180; Group G: 30 samples x 5 timepoints = 150)***	***For adult Q-Pan-005 study cohort, Groups C and G***
		Adult subjects: D0, D21, Month 6 (M6), Month 12 (M12),	***120 (n=approx 60 each from Groups VT/VT/12M and***	***720 (60 samples x 6 timepoints x 2 treatment groups)***	***For adult cohort from study H5N1-012, Groups VT/VT/12M and VT/IN/12M)***

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested (AS treatment) per study cohort	N tests planned as per algorithm)	Comment
		M12+21 days, Month 18 (M18)	***VT/IN/12M)***		
		Adult subjects, D0, D21 (D-QIV group only, no AS)	***30***	***60 (30 samples x 2 timepoints)***	***For adult D-QIV-015 study cohort***
~~Heterologous~~ ***Heterosubtypic*** Group I influenza A virus MN	RG reassortant virus (~~H6N3~~ ***H5N8***), ~~and~~ H1N1 swine Flu (A/Swine/Jiangsu/ 40/2011) ***virus, and H1N1 pdm09-like virus***	D0, ***D42*** ~~D21~~, (from all 4 studies, i.e., adult H1N1, H5N1, H9N2, and pediatric H5N1); AS groups only	***30*** ~~15~~	***720*** ~~240~~ 120 (***30*** ~~15~~ samples x 2 timepoints x 4 studies ***x 3 viruses***)	For ***all subjects who received an AS vaccine in all*** 4 study cohorts (***adult studies Q-Pan-H1N1-019, CC-Pan-H5N1-001, Q-Pan-H9N2-001, and pediatric study Q-Pan-H5N1-AS03-021***) ~~randomly selected pairs per study cohort with a ≥4-fold anti-H1 stalk MN titer increase from D0 to D21~~
		Adult subjects: Group C: D0, D42, D549, D591, and Group G: D182, D224, D549, D591	***60 (Groups C and G, n= approx 30 each***	***720*** ~~240~~ ***(60 samples x 4 timepoints x 3 viruses)***	***For adult Q-Pan-005 study cohort, Groups C and G***
		Adult subjects: D0, D21, Month 12 (M12), and M12+21 days	***120 (n=approx 60 each from Groups VT/VT/12M and VT/IN/12M)***	***480 (60 samples x 4 timepoints x 2 treatment groups)***	***For adult cohort from study H5N1-012, Groups VT/VT/12M and VT/IN/12M)***
		Adult subjects, D0, D21 (D-QIV group only, no AS)	***30***	***180*** ~~60~~ ***(30 samples x 2 timepoints x 3 viruses)***	***For adult D-QIV-015 study cohort***
Anti-H3 HA stalk domain MN (for H7N9 samples)	***RG reassortant virus cH14/3NX***	***D0, D21, D42, Month 6, Month 12; AS group only***	***30***	***150 (30 samples x 5 timepoints x 1 study)***	***For adult H7N9 study cohort***
~~***Heterologous***~~	***RG reassortant***	***D0, D42; AS***	***30***	***60 (30***	***For adult H7N9 study***

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested (AS treatment) per study cohort	N tests planned as per algorithm)	Comment
Heterosubtypic Group 2 Influenza A virus MN (for H7N9 samples)	*virus H4N8*	*group only*		*samples x 2 timepoints x 1 study)*	*cohort*
Homologous virus MN	*Adult H5N1, H9N2*	*D0, D21*	*	*	*For adult studies CC-Pan-H5N1-001, Q-Pan-H9N2-001 and pediatric study Q-Pan-H5N1-AS03-021*
	Pediatric H5N1	*D0, D21*			
Total anticipated number of MN tests					***3930 ~~2730~~ ~~1770~~ ~~630~~***

* Already performed in primary studies and results are available.

Table 6 Planned Immunological Read-Outs: HI assays

Assay type	Virus or Antigen	Time points and Treatment groups	# of subjects tested per treatment (AS vs no AS) per study cohort	N tests planned as per algorithm)	Comment
HI assay	A/Indonesia/5/2005 ***A/Vietnam/1194/2004-like***	D0 (from the ~~H5N1~~ ***CC-Pan-H5N1-001 Q-Pan-005, and H5N1-012 studies***); 2 treatment groups (AS and no AS)	~~30~~ ***Not applicable***	~~60 (30 samples x 1 timepoint x 2 studies x 2 treatment groups)~~ ***Not applicable***	For the ~~H5N1~~ ***CC-Pan-H5N1-001, Q-Pan-005, and H5N1-012*** study cohorts only ***HI assays already done in primary study; samples will not be retested.***
HI assay	A/chicken/Hong Kong/G9/1997	D0 (from the H9N2 study); 2 treatment groups (AS and no AS)	~~30~~ ***Not applicable***	~~60 (30 samples x 1 timepoint x 1 study x 2 treatment groups)~~ ***Not applicable***	For the ~~H9N2~~ ***Q-Pan-H9N2-001*** study cohort only ***HI assays already done in primary study; samples will not be retested.***
HI assay	A/California/07/2009 (or a like virus)	D0 (from the H5N1 and H9N2 studies); ***D182 for Q-Pan-005 study;*** 2 treatment groups (AS and no AS)	30	~~120~~ ***180*** (30 samples x 1 timepoint x ~~2~~ ***3*** studies x 2 treatment groups)	For the ~~H5N1 and H9N2~~ ***CC-Pan-H5N1-001, Q-Pan-H9N2-001, Q-Pan-005, and H5N1-012*** study cohorts
Total anticipated number of HI tests					~~240~~ **180**

The planned immunological read-outs for the study are summarized in Table 4, Table 5, and Table 6. Based on the results of these evaluations, the laboratory analyses may be extended by mutual agreement between GSK and ISMMS ***(and NeoMed Labs)*** to include

additional assays and/or assay types to further assess the intensity and breadth of the anti-influenza virus antibody response elicited by vaccination.

6 Study vaccines and Administration

6.1 Description of study vaccines

See the table below for the vaccine strains administered in the primary prospective studies.

Table 7 Description of study vaccines (Amended 01 September 2016)

Study no.	Vaccine strain	Start date
Q-Pan H1N1-019 (113536)	A/California/7/2009	28 Oct 2009
CC-Pan H5N1-001 (114371)	A/Indonesia/5/2005 RG	29 Nov 2010
Q-Pan H9N2-001 (116358)	A/chicken/Hong Kong/G9/1997 NIBRG-91	22 Aug 2012
Q-Pan H5N1-AS03-021 (114464)	A/Indonesia/5/2005 RG	07 Mar 2011
Q-Pan-005 (110624)	***A/Indonesia/5/2005 or A/turkey/Turkey/1/05***	***15 Jul 2008***
H5N1-012 (107495)	***A/Indonesia/5/2005-like or A/Vietnam/1194/2004-like***	***05 Feb 2007***
Q-Pan H7N9-AS03-001 (201072)	***A/Shanghai/2/2013(H7N9)-RG32A***	***25 Nov 2013***
D-QIV-015 (201251)	***(A/Christchurch/16/2010 (H1N1)pdm09 A/Texas/50/2012 (H3N2) B/Massachusetts/02/2012 B/Brisbane/60/2008)***	***18 Aug 2014***

6.2 Dosage and administration of study vaccines

The dosage and administration (intramuscular in all cases) of study vaccines in the previously conducted trials from which serum samples will be selected for further laboratory analyses in this study, ***were as follows***: ~~adult subjects were administered two standard adult doses of pandemic influenza vaccine with an interval of 21 days between doses, and pediatric subjects were administered two half standard adult doses of pandemic influenza vaccine with an interval of 21 days between doses~~.

- ***FLU Q-Pan-H1N1-019 (113536):***
  - ***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***
  - ***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 (114371):***

- *Group A = 3.75 µg HA CC-PAN H5N1 vaccine adjuvanted with $AS03_A$ given at Day 0 and Day 21*
- *Group B = 15 µg HA (no AS) CC-PAN H5N1 vaccine given at Day 0 and Day 21*

- *FLU Q-Pan H9N2-001 (116358):*
  - *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*
  - *Group 375_A_VVV = 3.75 µg HA H9N2 vaccine antigen adjuvanted with $AS03_A$ given at Day 0, Day 21, and Day 182*
  - *Group 1500_VVP = 15 µg HA (no AS) H9N2 vaccine given at Day 0 and Day 21; saline placebo at Day 182*
  - *Group 1500_VVV = 15 µg HA (no AS) H9N2 vaccine given at Day 0, Day 21, and Day 182*
- *FLU Q-Pan H5N1-AS03-021 (114464)*
  - *Group A: 1.9 µg HA/$AS03_B$ (at Day 0 and Day 21)*
  - *Group B: Placebo (at Day 0 and Day 21)*
- *FLU Q-Pan-005 (110624)*
  - *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*
  - *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*
- *H5N1-012 (107495)*
  - *Group VT/VT/M12: Two doses of A/Vietnam/1194/2004-like H5N1 vaccine adjuvanted ($AS03_A$) at Day 0 and Month 12 (i.e., homologous booster dose at Month 12)*
  - *Group VT/IN/M12: One dose of A/Vietnam/1194/2004 adjuvanted ($AS03_A$) H5N1 vaccine at Day 0 and one dose of A/Indonesia/5/2005-like H5N1 vaccine adjuvanted ($AS03_A$) at Month 12 (i.e., heterologous booster dose at Month 12)*
- *FLU D-QIV-015 (201251): 15 µg HA (no AS) of each of 4 strains (total 60 µg HA) at Day 0*
- *FLU Q-Pan H7N9-AS03-001 (201072)*
  - *Group 1500: 15 µg HA (no AS) at Day 0 and Day 21*
  - *Group 375_A: 3.75 µg HA/$AS03_A$ at Day 0 and Day 21*

7 Health Economics

Not applicable.

8 SAFETY

Not applicable since no subjects will be involved and, therefore, safety will not be assessed in this study.

9 Subject Completion and Withdrawal

Not applicable since no subjects will be enrolled in this study.

10 Statistical methods

10.1 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 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

   – 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***

   – 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***

- *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 and*
    - *from Day 549 to Day 591 and*
    - *from Day 0 to Day 591*
  - *For group G:*
    - *from Day 182 to Day 224 and*
    - *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 and*
    - *from Day 549 to Day 591 and*
    - *from Day 0 to Day 591*
  - *For group G:*
    - *from Day 182 to Day 224 and*
    - *from Day 549 to Day 591 and*
    - *from Day 182 to Day 591*
- *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*
  - *GMT at Day 0 and 21*
  - *Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21*

- ***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)
  - 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***
  - 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
  - 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***
  - 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)***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***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 and***
        - ***from Day 549 to Day 591 and***
        - ***from Day 0 to Day 591***
    - ***For group G:***
        - ***from Day 182 to Day 224 and***
        - ***from Day 549 to Day 591 and***
        - ***from Day 182 to Day 591***
- ***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 the FLU D-QIV-015 cohort:***
    - ***Seropositive rate at Day 0 and 21***
    - ***GMT at Day 0 and 21***
    - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
    - ***MGI at Day 21 compared to Day 0***

3. Levels of anti-H2 and anti-H18 antibody by ELISA for ~~all adult~~ ***the*** subjects who received an AS vaccine ~~in the CC-Pan H5N1-001, the Q-Pan H1N1-019, the Q-Pan H9N2-001 and the Q-Pan H5N1-AS03-21~~ ***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)***

- 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
- 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***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 182 to Day 591***
  - ***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***
  - ***GMT at Day 0 and 21***
  - ***Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21***
  - ***MGI at Day 21 compared to Day 0***

4. Vaccine-heterosubtypic virus titer by microneutralization (MN) for ~~a subset of~~ *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 ~~21~~ *42*
  - GMT at Day 0 and Day ~~21~~ *42*
  - Percentage of subjects with a ≥4-fold rise from Day 0 to Day ~~21~~ *42*
  - MGI at Day ~~21~~ *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***
  - ***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 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 0 to Day 591***
    - ***For group G:***
      - ***from Day 182 to Day 224 and***
      - ***from Day 549 to Day 591 and***
      - ***from Day 182 to Day 591***
  - ***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***

– ***MGI at Day 21 compared to Day 0***

(Amended 01 September 2016)

10.2 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 cohorts. 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 D182 (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)*** ~~in each study~~ by treatment group and level of HI antibody to A/California/7/09 (or a like virus) ~~at Day 0 in H9N2 and adult H5N1 studies, by treatment group~~ ***in subjects from the CC-Pan-H5N1-001, Q-Pan-H9N2-001, 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***

~~1. 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~~

~~2. 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~~

10.3 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):

1. ***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***
- ***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***
- ***MGI at Day 21, 42, 182 compared to Day 0***

2. ***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***

3. 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 adult 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

4. 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:

- MGI for anti-H1 stalk ELISA at Day 21 compared to Day 0

5. 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

6. 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
- GMT at Day 0, 21, and 42
- Percentage of subjects with a ≥4-fold rise from Day 0 to Day 21, and Day 42
- MGI at Day 21 and 42 compared to Day 0

Tertiary endpoints: 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***
   - ***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***
   - ***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***
   - ***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***
   - ***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)***
   - ***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***
   - ***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***
   - ***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***
   - ***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***

Tertiary endpoints: Passive transfer/virus challenge in mice with pooled adult human sera from subjects who received adjuvanted H5N1 vaccine the CC-Pan-H5N1 study cohort:

1. ***The in vivo protective effect of transferring pooled adult human serum (from subjects administered adjuvanted H5N1 vaccine in the CC-Pan-H5N1 study cohort) to mice and subsequently challenging them with <u>cH5/3Nx</u> virus (Nx=most likely N4 or N5, to be decided) will be assessed in terms of the following endpoints:***
   - ***survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice/serum pool/time-point***
   - ***mean weight loss (change from baseline over 14 days post challenge) in groups of 25 mice/serum pool/time-point***
   - ***lung weight in micrograms (D42 minus D0), (D385 minus D0), within challenge group***

- ***lung virus titer in pfu/microgram (log10 fold change (D0 minus D42), (D0-D385), within challenge group***

2. ***The in vivo protective effect of transferring pooled adult human serum (from subjects administered adjuvanted H5N1 vaccine) to mice and subsequently challenging them with cH6/1N5 virus will be assessed in terms of the following endpoints:***

   - ***survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice/serum pool/time-point***
   - ***mean weight loss (change from baseline over 14 days post challenge) in groups of 25 mice/serum pool/time-point***
   - ***lung weight in micrograms (D42 minus D0), (D385 minus D0), within challenge group***
   - ***lung virus titer in pfu/microgram (log10 fold change (D0 minus D42), (D0-D385), within challenge group***

3. ***Post-transfer titer of human IgG to cH5/3 by ELISA***
4. ***Post-transfer titer of human IgG to cH6/1 by ELISA***

(Amended 01 September 2016)

10.4 Determination of sample size

In this study, the primary objective is to assess the humoral immune response by anti-H1 stalk ELISA in each study cohort; anti-H2 and anti-H18 full length ELISA, anti-H1 stalk response by neutralization, and vaccine-heterosubtypic virus response by microneutralization (MN) ***for all subjects who received AS vaccine in each study cohort and for the subjects in the FLU-D-QIV015 study cohort***~~, for a subset of subjects who received an AS vaccine and have a ≥4-fold rise from D0 to D21 by anti-H1 stalk microneutralization in each study cohort~~.

Table 8 presents the 95% confidence intervals (95% CI) for various observed proportions (e.g., seropositivity rate and % of subjects with at least 4-fold antibody titer increase by anti-H1 stalk MN) for 30 subjects in each study group. ~~and 15 subjects in each study AS group~~.

Table 8 Illustration of the lower (LL) and upper (UL) limits of the exact 95% CI built around various observed proportions with a sample of 30 subjects

Number of subjects seropositive * or subjects who have >= 4 fold increase** (n)	Observed proportion (%) per group (N=30)	Exact 95% CI	
		LL	UL
20	66.7	47.2	82.7
21	70.0	50.6	85.3
22	73.3	54.1	87.7
23	76.7	57.7	90.1
24	80.0	61.4	92.3
25	83.3	65.3	94.4
26	86.7	69.3	96.2
27	90.0	73.5	97.9
28	93.3	77.9	99.2
29	96.7	82.8	99.9
30	100.0	88.4	100.0

*seropositive= subjects with antibody concentration or titer ≥ assay cut-off
subjects who have >= 4 fold increase = subjects with at least a 4-fold increase in post-vaccination reciprocal titer (from D0 to D21) tested by anti-H1 stalk ***MN*. For seronegative subjects, half of the cut-off of the assay will be considered as pre-vaccination titers.

~~**Table 9 Illustration of the lower (LL) and upper (UL) limits of the exact 95% CI built around various observed proportions with a sample of 15 subjects**~~

~~Number of subjects seropositive * or subjects who have >= 4 fold increase** (n)~~	~~Observed proportion (%) per group (N=15)~~	~~Exact 95% CI~~	
		~~LL~~	~~UL~~
~~10~~	~~66.7~~	~~38.4~~	~~88.2~~
~~11~~	~~73.3~~	~~44.9~~	~~92.2~~
~~12~~	~~80.0~~	~~51.9~~	~~95.7~~
~~13~~	~~86.7~~	~~59.5~~	~~98.3~~
~~14~~	~~93.3~~	~~68.1~~	~~99.8~~
~~15~~	~~100.0~~	~~78.2~~	~~100.0~~

~~*seropositive= subjects with antibody titer≥1:X at Day 21 tested by heterologous virus MN test~~
~~**subjects who have >= 4 fold increase = subjects with at least a 4-fold increase in post-vaccination reciprocal titer (from D0 to D21) tested by heterologous virus MN. For seronegative subjects, half of the cut-off of the assay will be considered as pre-vaccination titers.~~

10.5 Study cohorts to be analyzed

All subjects from each study cohort with available results will be included in the analysis for this study.

10.6 Derived and transformed data

- The cut-off value is defined by the laboratory before the analysis and is described in Section 5.4.1 (Table 2.
- 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 concentration/titer transformations. Antibody concentrations/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.
- 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***

10.7 Analysis of demographics

Demographic characteristics (age at first study vaccination in years, 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 H9N2 and adult H5N1 studies (if data become available)

10.8 Analysis of immunogenicity

10.8.1 Within groups assessment

For each study group in ~~each~~ ***the HA Group 1-related*** study cohorts (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)*** ~~(subset in subjects who received an adjuvant system [AS] vaccine and have a ≥4-fold antibody titer increase (D21/D0) by anti-H1 stalk MN))~~, ~~anti-N1-NA (for H1N1 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 ~~Day 21~~ all applicable timepoints per endpoint (from Day 182 for Group G subjects in the Q-Pan-005 study cohort); refer to endpoints.*** ~~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.~~
- ***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); refer to endpoints. ~~for anti-H1 stalk ELISA and anti-H1 stalk MN (except for D-QIV-015 cohort), with exact 95% CI, will be calculated.~~***
- The distribution of antibody titers for anti-H1 stalk ELISA will be displayed using reverse cumulative distribution curves.
- For AS group, correlation between the level of neutralizing antibody to the H1 stalk with the level of vaccine homologous neutralizing antibody, at D0 and at D21 in study cohorts H9N2 and both adult and pediatric H5N1 study cohorts.
- 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 cohorts.
- CMI summaries for the H9N2 cohort only
- 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.***

10.8.2 Between groups assessment

10.8.2.1 For adult H5N1, H9N2, and H1N1 study cohorts)

- For each ***Group 1-related*** study and for anti-H1 stalk ELISA results, if available:
  - The difference in percentage of subject with at least 4-fold increase at ***all applicable timepoints*** ~~Day 21~~ ***(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 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 the anti-N1 NA ELISA result at Day 0 as covariates.

10.8.2.2 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.

Further details will be provided in the statistical analysis plan.

10.9 Analysis of safety

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

10.10 Conduct of analyses

The planned analysis is descriptive and will be performed for each treatment group in the individual study ***cohorts***. ~~However, the analysis will be performed first for the adult H5N1 study and then for the three other study cohorts (i.e., pediatric H5N1, H1N1 and H9N2), pending any necessary change in the sample testing plan. The rationale for selecting the H5N1 adult cohort as the pilot cohort is to assess the acceptability of the analysis plan and the serology algorithm because the H5N1 adult cohort has the lowest baseline vaccine homologous HI titer. Thus, it is expected that the H5N1 vaccine may be most efficient in boosting an anti-H1 stalk response.~~

~~The initial analysis will be done for objectives based on ELISA endpoints only. Testing by H8/1 N3 MN will follow as a second step and by H6N3 as a third step when data are available.~~

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

10.10.1 Sequence of analyses

Analyses will be performed in sequence based on availability of the different results. ~~analysis of data from the adult H5N1 study will be performed first and then for the three other study cohorts (i.e., pediatric H5N1, H1N1 and H9N2), pending any necessary change in the sample testing plan.~~ Results will be presented in a final study report.

10.10.2 Statistical considerations for interim analyses

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

11 Administrative Matters

For a typical clinical study protocol, this section provides guidance to comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality and publications.

This is a non-clinical retrospective study whose objectives pertain to analyses of archived serum samples from previously completed studies and, therefore, no study subjects are

involved. Essential GCP training was provided to the ISMMS site staff before study start to ensure appropriate management of samples.

11.1 Case Report Form/Remote Data Entry instructions

Not applicable since no subjects will be involved.

11.2 Study Monitoring by GSK Biologicals

Not applicable since no subjects will be involved.

11.3 Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

11.4 Quality assurance

Essential GCP training was provided to ISMMS site staff before study start to ensure appropriate management of samples.

11.5 Posting of information on publicly available clinical trial registers and publication policy

Study information from this protocol is not required to be posted unless the study results provide important scientific knowledge or are relevant for patient care.

Summary protocols or plans and results for analyses that are designed to inform the feasibility, conduct, or design of other studies are not required to be posted or submitted for publication unless the results provide important scientific knowledge

Provision of study results to investigators:

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK Biologicals will also provide the investigator with the full summary of the study results.

12 Country specific requirements

Not applicable.

APPENDIX A LABORATORY ASSAYS

7. Serum transfer/ virus challenge experiment in BALB/c mice for CC-Pan-H5N1 vaccination sera (from adult subjects who received adjuvanted H5N1 vaccine)

Exploratory protocol objectives (to be placed in main protocol as well):

1. To assess the protective effect of pooled human serum collected on D42 and D385 from adult recipients of CC-PAN H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that is subsequently challenged with approximately $5LD_{50}$ of **cH5/3Nx virus** (or an alternative challenge virus with similar attributes but more fit for purpose; Nx=most likely N4 or N5, to be decided) , in terms of:

A. proportion surviving challenge

B. mean weight loss over time (AUC)

C. the following 2 endpoints in randomly selected subgroups of animals from each of 3 treatment groups administered the D0, D42, or D385 serum pools; animals will be euthanized at D3 (N=5) and D6 (N=5) post challenge:

I. mean lung weight at necropsy

II. geometric mean lung virus titer (pfu/microgram)

2. To assess the protective effect of pooled human serum collected on D42 and D385 from adult recipients of CC-PAN H5N1, compared to the effect of pooled serum collected on D0, when each serum pool is transferred to a group of BALB/c mice that is subsequently challenged with approximately $5LD_{50}$ of **cH6/1N5 virus** (or an alternative challenge virus with similar attributes but more fit for purpose), in terms of:

A. proportion surviving challenge

B. mean weight loss over time (AUC)

C. the following 2 endpoints in randomly selected subgroups of animals from each of 3 treatment groups administered the D0, D42, or D385 serum pools; animals will be euthanized at D3 (N=5) and D6 (N=5) post challenge:

I. mean lung weight at necropsy

II. geometric mean lung virus titer (pfu/microgram)

3. If the pathogenicity (expressed as pfu/LD_{50}) of the **cH5/3Nx virus** and **cH6/1N5 virus** are comparable, describe the effect size of anti-HA stalk antibodies (revealed by **cH6/1N5 virus** challenge) and anti-HA head antibodies (revealed by **cH5/3Nx virus** challenge) in terms of the relative survival rate (D42 rate/D0 rate) for cH6/1N5 vs relative survival rate for cH5/3Nx.

4. To describe the post transfer geometric mean ELISA titer of human IgG to cH5/3Nx and human IgG to cH6/1N5 in blood collected from mice receiving each of 3 serum pools (D0, D42, D385)

To explore the association between post-transfer ELISA titer of human IgG to the challenge virus and outcome at D3 and D6:

A. proportion surviving challenge,

B. mean weight loss,

C. mean lung weight at necropsy, and

D. geometric mean lung virus titer

Exploratory protocol endpoints ~~(to be placed in the main protocol as well)~~

Objective	Endpoint
1A, 2A, 3, 5A	survival over 14 days post-challenge (day of death or euthanasia for weight loss >25% baseline body weight) in groups of 25 mice*/serum pool/time-point
1B, 2B, 5B	mean weight loss (change from baseline over 14 days post challenge) in groups of 25 mice*/serum pool/time-point
1C, 2C, 5C	lung weight in microgram (D42 minus D0), (D385 minus D0), within challenge group
1D, 2D, 5D	lung virus titer in pfu/microgram (log10 fold change (D0 minus D42), (D0-D385), within challenge group
4	Post-transfer ELISA titer of human IgG to cH5/3 by ELISA, Post-transfer ELISA titer of human IgG to cH6/1 by ELISA

If sufficient serum volumes are not available, the number of mice can be reduced to as low as 15 mice per time-point and virus

Viruses:

Challenge virus	Surface glycoprotein attributes	Virus-specific protection mediated by
cH5/3NX (or an alternative challenge virus with similar attributes but more fit for purpose)	• Hemagglutinin (HA) head domain matched (or cross-reactive) to vaccine strain – can be **neutralized by head antibodies** induced by H5N1 vaccination • HA stalk domain **not matched** to vaccine strain • Exotic neuraminidase **not matched** to vaccine strain	HA head antibody responses
cH6/1N5 (or an alternative challenge virus with similar attributes but more fit for purpose)	• Exotic HA head domain not matched to vaccine strain • HA stalk domain from H1 – can be neutralized by cross-reactive stalk antibodies induced by H5N1 vaccination • Exotic neuraminidase not matched to vaccine strain	HA stalk antibody responses

Both viruses will be pre-assessed for mouse lethality in LD_{50} experiments (with and without presence of adult human serum pools). The volume and route of inoculation will be 0.05 ml intranasal/intratracheal administered to anesthetized mice.

Serum pools:

Three separate serum pools will be created using all residual serum samples with sufficient volume to furnish an aliquot of an equal volume that were collected on D0, D42,and D385 from the adult CC-Pan-H5N1 cohort (18-49 YOA) which received AS03-adjuvanted H5N1 vaccine.

Passive transfer experimental setup:

- 35 mice per time-point/virus ***challenge*** are transferred with a standard amount of undiluted pooled serum (volume to be determined, within the range 150-250ul): (***35 mice x 2 virus challenges x 3 timepoints = 210 mice total***). ***However, if sufficient serum volumes are not available, the number of mice can be reduced to as low as 15 mice per time-point and virus.***
- 5 hours post serum transfer the mice are sedated and:
  - blood is collected from the periorbital plexus for processing to serum and ELISA determination
  - then challenged with 5xLD50 delivered by the IN/IT route (or a lower dose selected to provide a level of weight-loss and lethality using D0 serum that could be reduced by a 3-10 fold increased level of anti-HA stalk antibodies present in post-vaccination serum).
- Mice are monitored daily for 14 days for weight-loss and are euthanized if they lose >25% of their initial body weight.
- On day 3 and day 6 post-infection 5 mice per time-point/virus are euthanized to assess viral lung titers (30 mice per day/virus, 60 mice total). After extraction, lung weights are recorded as an additional measure of morbidity (i.e., fluid accumulation resulting from the infectious process following challenge will lead to increased lung weight). The method of removing the lungs and trachea en bloc after exsanguinations will be standardized to assure comparability among groups.
- Weighing of the mice and the lungs will be performed in an open fashion. Viral plaques will be counted by a blinded technician.
- Lung suspensions will be made by homogenization in PBS and frozen at -80C for later plaque assay using a standard method.

APPENDIX B CLINICAL LABORATORIES

Table 10 Collaborating laboratories (Amended 01 September 2016)

Laboratory	Address
Dept. of Microbiology, Icahn School of Medicine at Mount Sinai	Dept. of Microbiology Icahn School of Medicine at Mount Sinai New York, NY USA
NEOMED LABS INC.	***7171 Frederick-Banting St, Saint-Laurent, Quebec H4S 1Z9, Canada***

Table 11 GSK Biologicals' laboratories

Laboratory	Address
GSK Biologicals Global Vaccine Clinical Laboratory, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart - Belgium
GSK Biologicals Global Vaccine Clinical Laboratory, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium
GSK Dresden GlaxoSmithKline Biologicals Branch of SmithKline Beecham Pharma GmbH & Co. KG	Zirkusstrasse 40, D-01069 Dresden Germany

Protocol Amendment 2 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	201598 (FLU CC-SUIV-AS03-001)
Date of protocol amendment	***Amendment 2 Final: 01 September 2016***
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***
Sponsor signatory	Bruce Innis, MD, *Vice President,* ***Senior Vaccine Development Leader***
Signature	PPD
Date	15 SEP 2016

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