Statistical Review and Evaluation-AFLURIA

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1. **EXECUTIVE SUMMARY**

1.1 Conclusions and Recommendations

CSL Limited Inactivated Influenza Virus Vaccine has been shown to be effective in terms of immune responses measured as GMT, seroconversion, and seroresponse, based on the results submitted within the Biologics Licensing Application (BLA), STN 125254 for adults 18 to 65 years of age. Based on the results of one primary Phase III study, CSL Limited's proposed seasonal inactivated trivalent influenza virus vaccine has met the criteria for immune response of inactivated influenza vaccines as per the standards described in the current draft guidance. Furthermore, the results obtained from examining and analyzing supplementary data provided in this submission, including several additional studies, support the sponsor's claim that the primary immunogenicity endpoints have been met. This vaccine does appear to meet safety and tolerability margins based on the results of a Phase III multi-center, dose finding, placebo controlled, double blind, randomized study (Study CSLCT-FLU-05-09/DMID 06-0016 as denoted by the sponsor).

1.2 Brief Overview of Clinical Study

Within this submission, the sponsor provided the results of one Phase III study to support the use of a new seasonal inactivated trivalent influenza virus vaccine currently not approved in the US in Healthy Adults. This study, defined by the sponsor as CLSCT-FLU-05-09/DMID 06-0016 (which we will denote as FLU-05-09), will be summarized and critiqued within this document.

This study is titled, *"A Phase III, Randomized, Double-Blind, Placebo Controlled, Multi-center Study to Evaluate the Immunogenicity, Safety, and Tolerability of CSL Limited Inactivated Influenza Virus Vaccine in Adults ≥ 18 years to <65 years."*

The primary objective of this study was to establish the immunogenicity, safety, and tolerability of CSL Limited's Inactivated Trivalent Flu Vaccine. Indirect inference regarding efficacy was to be based on immune response by comparing several different dosage formats of the CSL Trivalent Flu vaccine (three lots of multi-use vials and one lot of single-use syringes) relative to placebo, approximately 3 weeks post-vaccination. Specifically, primary immunologic responses included seroresponse and seroconversion rates and geometric mean titers at the final exam visit, approximately 21 days post-vaccination. The safety and tolerability

responses of interest include the reactogenicity to the vaccine and the report of AE's and SAE's within ~3 weeks post-vaccination. Within this study, the results of the four dosage formats that were examined and compared to a placebo include: 3 lots of a multiple-dose thimerosal containing vial and one lot of pre-filled single dose thimerosal free syringes. A detailed statistical review of this study is contained within this document.

Data from several additional supplementary Phase II, Phase III, and post-marketing Phase IV studies from various international locations were provided for examination. These studies provided additional data related to immune response, safety, and tolerability of various similar dose formats of CSL Trivalent Influenza vaccine. A brief summary of these additional studies is given in Table 1.2, including: the phase of the study, country in which the study was implemented, the ages of the subjects, and the results of immune response endpoints.

Table 1.2: Summary of studies including immunogenicity results provided in STN 125254

Study ID	Phase	Country	Study Type	Ages	Objective(s)	Total #	Subjects treated w/CSL Product	Pass Sero-Convert	Pass Sero-Protect
CSLCT-NHF-05-09	III	US	Blinded	18-65	Safety, Efficacy (immunogenicity), Lot-to-lot consistency	1359	1089	Yes	Yes
CSLCT-NHF-05-11	III	UK	Blinded	18-60	Safety, Efficacy (immunogenicity)	286	146	Yes	Yes
				60+	Safety, Efficacy (immunogenicity)	120	60	No	No
CSLCT-NHF-05-13	IV	UK	Open Label	18-60	Safety, Efficacy (immunogenicity)	79	79	Yes	Yes
				60+	Safety, Efficacy (immunogenicity)	40	40	No	No
CSLCT-NHF-04-99	IV	UK	Open Label	18-60	Safety, Efficacy (immunogenicity)	83	83	Yes	Yes
				60+	Safety, Efficacy (immunogenicity)	37	37	No	No
CSLCT-NHF-05-15	III	UK	Open Label	65+	Safety, Efficacy (immunogenicity)	275	206	No	Yes

The additional studies (CSLCT-05-11, CSLCT-05-13, CSLCT-NHF-04-99, and CSLCT-NHF-05-15) were not considered pivotal. Thus, only supplementary information related to these studies is contained within this document.

1.3 Statistical Issues and Findings

In Study FLU-05-09, the efficacy, safety, and tolerability of the various dosage formats of the Inactivated Trivalent Flu vaccine proposed by CSL Limited have been demonstrated. The efficacy (based on the current draft guidance for industry guidelines of minimum immune response) of the Inactivated Trivalent Flu vaccine can be inferred, based on all the dosage levels studied, from the immunologic

endpoints consisting of seroconversion rates, geometric mean titers, and seroprotection rates at the 3-week (~21 day) post-vaccination visit of subjects. A summary of the results of these immune responses is shown in Table 1.3.1., utilizing the per protocol analysis group. These results are based on calculations performed by the reviewing statistician.

Table 1.3.1.a: GMT, Seroconversion, and Seroprotection (with 95% CI) ~21 Days after Initial Vaccination of the H1N1 Strain (Per Protocol Analysis Group)

		Treatment Group				
	Acceptable 95% CI Lower Bound	**Placebo (n=264)**	**Lot #1 n=270)**	**Lot #2 (n=275)**	**Lot #3 (n=269)**	**Single dose (n=263)**
GMT	n/a	238 (0,1232)	878 (0,3188)	760 (0,2868)	956 (0,3748)	943 (0,3713)
Sero-Conversion [a]	Lower bound 95% CI: **40%**	1.7% [4/231] (0.8, 4.9)	48.5% [111/238] (42.4, 54.7)	48.4% [114/240] (42.3, 54.4)	49.1% [114/238] (42.9, 55.2)	48.7% [113/231] (42.5, 54.9)
Sero-Protection [b]	Lower bound 95% CI: **70%**	76.2% [176/231] (68.9, 79.8)	96.7% [229/238] (93.8, 98.5)	98.2% [235/240] (95.8, 99.4)	97.4% [228/235] (94.7, 98.8)	98.9% [228/231] 96.7, 99.8)

Note: [a]: Seroconversion is defined as an increase in HI antibody titer of at least 4-fold, with a minimum post-vaccination HI titer of 1:40.
[b]: Seroprotection is defined as a minimum post-vaccination HI titer of 1:40.

Table 1.3.1.b: GMT, Seroconversion, and Seroprotection (with 95% CI) ~21 Days after Initial Vaccination of the H3N1 Strain (Per Protocol Analysis Group)

		Treatment Group				
	Acceptable 95% CI LowerBound	**Placebo (n=264)**	**Lot #1 (n=270)**	**Lot #2 (n=275)**	**Lot #3 (n=269)**	**Single dose(n=263)**
GMT	n/a	256 (0, 1422)	1484 (0, 5704)	1999 (0, 7725)	2161 (0, 10873)	1880 (0, 7446)
Sero-Conversion[a]	Lowerbound of 95% CI: **40%**	0.0% [0/231] n/a	69.3% [162/238] (63.4, 74.7)	71.3% [172/240] (65.5, 76.5)	75.5 [176/238] (69.9, 80.5)	70.0% [157/231] (64.0, 75.4)
Sero-Protection [b]	Lowerbound of 95% CI: **70%**	72.0% [166/231] (66.1, 77.3)	100.0% [238/238] (98.6,100.0)	99.6% [239240] (98.6, 100.0)	100.0% [235/235] (98.6, 100.0)	100.0% [231/231] (98.6, 100.0)

Note: [a] & [b]: Same as described above for Table 1.3.1.a

Table 1.3.1.c: GMT, Seroconversion, and Seroprotection (with 95% CI) ~21 Days after Initial Vaccination of the B Strain (Per Protocol Analysis Group)

	Treatment Group					
	Acceptable 95% CI LowerBound	**Placebo (n=264)**	**Lot #1 (n=270)**	**Lot #2 (n=275)**	**Lot #3 (n=269)**	**Single dose(n=263)**
GMT	n/a	61	422	364	432	418

	Treatment Group					
	Acceptable 95% CI LowerBound	**Placebo (n=264)**	**Lot #1 (n=270)**	**Lot #2 (n=275)**	**Lot #3 (n=269)**	**Single dose(n=263)**
		(0, 263)	(0, 1414)	(0, 1234)	(0, 1560)	(0, 1334)
Sero-Conversion [(a)]	Lowerbound of 95% CI: **40%**	0.4% [1/231] (0.0, 2.1)	71.8% [171/238] (66.1, 77.1)	66.7% [160/240] (62.1, 73.5)	68.4% [159/238] (63.3, 7406)	68.4% [158/231] (64.0, 75.4)
Sero-Protection [(b)]	Lowerbound of 95% CI: **70%**	47.0% [108/231] (40.8, 53.2)	95.4% [227/238] (92.2, 97.7)	93.3% [224/240] (90.3, 96.4)	92.3% [217/235] (89.2, 95.7)	93.9% [217/231] (91.2, 97.1)

Note: [(a)] & [(b)]: Same as described above for Table 1.3.1.a

The results, presented in Table 1.3.1.a-c., illustrate that, based on the immune response at Day 21 post vaccination, this vaccine meets immune response criteria for both seroprotection and seroconversion. Furthermore, trends in GMT values show that this vaccine promotes immune response. In this study, similar safety and tolerability profiles were demonstrated in all dosage groups. In all active treatment groups, no deaths were reported. Furthermore, in this study, no serious adverse events were reported and similar rates of less serious adverse events for all treatment groups (including placebo) were reported. Non-serious local and systemic adverse event rates were also similar between all four dosage treatment groups. In particular, all active treatment dosage groups had similar tolerability profiles of injection site reactions, including: pain, tenderness, redness, and swelling within 7 days of vaccination. Furthermore, all dosage groups had similar tolerability profiles based on the systemic adverse event response rates related (but not limited) to: development of fever, headache, malaise, body aches, and nausea within 7 days of vaccination.

This study, CSL FLU-05-09, was a well-designed, blinded, randomized, placebo-controlled, study in healthy adults 18-65 years of age to determine the immune response, safety, and tolerability of the various dosage formula's for the prevention of seasonal influenza. There were no statistical issues identified in this study. The study was designed to perform several statistical hypothesis tests related to immune response, and the sample size in all treatment groups was adequate to do so. All hypothesis tests were created and implemented appropriately. In this study, the analysis of the data to test these hypotheses yields statistically significant results for immune response. Furthermore based on the safety and tolerability responses, this Trivalent influenza vaccine meets appropriate safety and tolerability standards.

As considered acceptable, according to the Draft Guidance for industry *"Clinical Data Needed to Support Licensure of Seasonal Inactivated Influenza Vaccines"* (May 31, 2007), various immunogenicity responses were collected as "surrogate" endpoints in this study. This study did not collect any clinical response data in support of efficacy claims for this vaccine. This is an acceptable mechanism to study the potential efficacy of a trivalent influenza vaccine. However, limitations exist when utilizing surrogate endpoints to draw conclusions; however, it is

essential that there will be a planned follow-up clinical response study to confirm results found using the results of CSL FLU-05-09.
Based on the results presented in the sponsor's submission and verified by the reviewing statistician, the safety and tolerability of all dosage formats of the influenza vaccine appear to be acceptable. The results of both the single dose syringe and the 3 lots of the multi-use vial vaccine provide support for the claim that this vaccine is immunogenic based on seroprotection, seroresponse, and GMT levels observed within this study. Furthermore, these results indicate that subjects receiving any active treatment vaccine had a positive immune response 3 weeks post-vaccination.

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2. INTRODUCTION

2.1 Overview

This is a BLA submission for CSL's Inactivated Seasonal Trivalent Flu Vaccine. This submission contains a study in which four different dosage formats (3 lots of a multi-use vial and 1 single dose syringe) and a placebo comparator arm are examined and compared. Within this submission, the sponsor proposed to examine the safety, tolerability, and efficacy based on immunogenicity of a currently unapproved in the United States Inactivated Trivalent Flu vaccine. The primary and secondary objectives of this study are described further in Section 3.1.1. To meet these objectives, examinations and comparisons of immune response, safety and tolerability between the four treatment groups and placebo treated group were made.

2.2 Data Sources

The sponsor submitted the results of a single Phase III, multi-center, single country (USA) randomized, double-blind study to support the use of CSL Inactivated Trivalent Flu vaccine for the prevention of influenza in healthy adults. Additionally, the sponsor provided the results of several supportive international studies that support the pivotal Phase III US study. Data sets for all of the studies were submitted electronically and utilized in the review of this study. The location of these datasets is as follows:
Cbsap58\m\EDR Submissions\2007 BLA\DCC414775\
Initially, these files had issues in being located and analyzed; however, these issues were ultimately resolved. Thus, considering the datasets located within this file path, the most recently submitted datasets were found to be adequately documented and well organized.

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3. STATISTICAL EVALUATION

3.1 Evaluation of Efficacy (inferred from immune response)

In addition to several supportive studies, the sponsor submitted the results of one Phase III study to support the use of CSL Trivalent Inactivated Flu vaccine for the prevention of seasonal influenza in healthy adults. This study, CSL-05-09, will be summarized and critiqued within this section.

3.1.1 Study Design

The study of interest, CSL-05-09, is titled "A Phase III, Randomized, Double-Blind, Placebo Controlled, Multi-center Study to Evaluate the Immunogenicity, Safety, and Tolerability of CSL Limited Inactivated Influenza Virus Vaccine in Adults ≥ 18 years to <65 years."

This study was designed to demonstrate the safety, tolerability, and efficacy of the Inactivated Trivalent influenza vaccine compared to placebo. This placebo controlled, randomized, blinded, Phase III study was conducted in 9 sites within the US. The treatment involved a single dose of vaccine (or placebo) with follow-up related to safety and tolerability based on solicited adverse events for 7 days post vaccination and efficacy response (based on immunogenicity endpoints) 21 Days post vaccination.

Objectives of the Study

The primary and secondary objective(s) of this study were:

Primary Objectives:

1. To demonstrate that vaccination with CSL Influenza Vaccine produces an immune response sufficient to meet the Committee for Medicinal Products for Human Use criteria for young adults of 40% seroconversion and 70% seroprotection.
2. **Secondary Objective:**
    1. To demonstrate clinical consistency between three lots of CSL Influenza Vaccine multiple dose via presentation (thimerosal containing)
    2. To demonstrate clinical consistency between three lots of CSL Influenza Vaccine multiple dose via presentation (thimerosal containing) and CSL Influenza Vaccine pre-filled syringe presentation (thimerosal free).
    3. To demonstrate acceptable safety and tolerability of CSL Influenza Vaccine multiple dose via presentation (thimerosal containing) and CSL Influenza Vaccine pre-filled syringe presentation (thimerosal free).

    Endpoints of the Study

    Co-Primary Immunogenicity Endpoints included: seroprotection rates and seroconversion rates to be assessed as follows:

- Seroprotection defined as a minimum post-vaccination HI titer of 1:40.
- Seroconversion defined as an increase in HI antibody titer of at least 4-fold, with a minimum post-vaccination HI titer of 1:40.

The Secondary Immunogenicity Endpoints collected in this study included:

- Geometric Mean Titers, to influenza Hemagglutinin antigens after vaccination of the active treatment arms which were to be utilized in comparison:
- Between the 3 lots of CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing);
- Between the CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing) and pre-filled syringe presentation (thimerosal-free).

The Secondary Safety Endpoints collected in this study included:

- Monitoring Adverse events after vaccination
- Solicited AEs through day 4 (Days 0, 1, 2, 3, 4) following vaccination
- Unsolicited AEs to the Evaluation visit after vaccination (Day 21)
- The proportion of subjects who experienced adverse events.
- The rate, type, frequency, and severity of AEs in the active treatment arms, for the 3 lots of CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing), the CSL Influenza Vaccine pre-filled syringe presentation (thimerosal-free), and Placebo vaccine (thimerosal-containing) were be assessed.
- Monitoring Tolerability of the vaccine based on local and systemic system reactions.

Inclusion/Exclusion and removal of subjects from therapy or assessment criteria

The Protocol specified the following key criteria as requirements for inclusion in the study (note: this list is not comprehensive):

Key Inclusion Criteria:

Subjects were eligible to be included if:

11. Healthy males or non-pregnant females (as indicated by a negative urine or serum pregnancy test immediately prior to vaccination), aged? 18 to <65 years at the time of providing informed consent.
12. Provision of written informed consent to participate in the study and willingness to adhere to all Protocol requirements.
13. Were in good health, as determined by vital signs (heart rate, blood pressure, oral temperature), medical history, and a targeted physical examination based on medical history.
14. Were able to understand and comply with planned study procedures.
15. Females of non-childbearing potential. Or females of childbearing potential were to be abstinent or using adequate contraceptive precautions, and agreed to continue such precautions for 2 months after vaccination.

Key Exclusion Criteria:

Subjects were excluded from the study for any of the following reasons:

16. Known hypersensitivity to a previous dose of influenza vaccine or allergy to eggs, chicken feathers, neomycin, polymyxin, thimerosal, or any components of the study vaccines.
17. Had been vaccinated against influenza in the previous 6 months.
18. Had an underlying medical condition for which influenza vaccination was recommended; chronic heart or lung condition including asthma; metabolic disease; kidney disease; blood disorder (such as sickle cell anemia); or weakened immune system including Human Immunodeficiency Virus (HIV) / Acquired Immunodeficiency Syndrome (AIDS).
19. Had an acute clinically significant pulmonary, cardiovascular, hepatic, or renal functional abnormality.
20. Had known history of Guillain-Barré Syndrome.
21. Had clinical signs of active infection and/or an oral temperature of 38°C (100.4°F). Study entry could be deferred for such individuals at the discretion of the PT.

22. Was unwilling or unable to comply with the study Protocol.
23. Had any condition that, in the opinion of the P1, would prevent the subject from complying with all aspects of the protocol or would put the subject at unnecessary risk.

For a complete listing of both the inclusion or exclusion criteria, please see the Study Protocol.

Time Frame of Collection of Safety and Efficacy Measurements

After meeting eligibility requirements, subjects were enrolled in the study. Upon enrollment, all subjects were to have pre-vaccination blood collected. This blood sample was to be examined to assess baseline antibodies for the three influenza strains included in the CSL trivalent flu vaccine. Subjects were to be followed for 21 days once enrolled and vaccinated. Immunological assessments as well as safety, tolerability, and reactogenicity assessments including subjective assessments (using a daily diary card) were to be performed during this study. The following highlights assessments that were to be performed on the following study days.

Visit 1: Study Day 0

Clinic Visit including (but not limited to)

- Obtaining Informed Consent
- Reviewing Eligibility Criteria
- Collecting and reviewing medical history
- Obtaining recent or history of influenza illness and vaccination history
- Performing targeted physical examination
- Recording concomitant medications
- Obtaining 20 mL blood sample for baseline, anti-hemagglutination antibody titer

Subjects who completed the assessments were eligible for vaccination. Eligible subjects were randomly assigned the next available, unique vaccine pack number and corresponding vaccine.

Vaccination

An unblinded vaccine administrator administered a single dose (0.5 mL) of Study Vaccine into the deltoid region of the arm, by intramuscular injection.

Post Vaccination

Subjects were observed by investigational site personnel for 30 minutes post vaccination in case a rare anaphylactic reaction or other AEs developed. Appropriate medical equipment was readily available in case of an emergency. Subjects were issued with a 5-Day Solicited and local reaction memory aid and were instructed to complete the memory aid on the evening of the vaccination (Day 0), and for every subsequent evening for the following 4 days. Subjects were provided with a digital thermometer and ruler and were instructed to take and record their oral temperature at the same time each evening, in addition to recording any swelling, redness, and/or bruising which may have been present at the site of vaccination. Subjects were also issued with a 21-Day Unsolicited AE memory aid and were instructed to record any Unsolicited AEs. The memory aids were not considered source documents.

An appointment was made for each subject to return to the investigational site for the Follow-up Visit, Visit 2 on Day 5.

Visit 2: Follow-up (acceptable window: Day 5-7 post vaccination)

Clinic Visit including performing the following procedures:

- Reviewing Solicited AE memory aid and transcribing these data into the eCRF.
- Assessing any other AEs.
- Assessing the occurrence of any SAEs.
- Recording of all concomitant medications.
- Reminding subjects to continue to complete the 21-Day Unsolicited AE Memory aid (to be returned at the Exit Evaluation Visit).

Visit 3: Exit Evaluation Visit (acceptable window: Days 21 -24 post vaccination):

Clinic Visit including the following procedures and assessments:

- Review of 21-Day Unsolicited AE memory aid and transcription of these data into the eCRF.
- Assessment of any SAEs.
- Review of health status and recording of any changes since the last visit.
- A brief medical evaluation (including a physical examination if clinically indicated which includes an assessment of erythema, induration, pain, ecchymosis, and tenderness at the injection site).
- Collection of a second 20 mL blood sample for the determination of post-vaccination anti-Hemagglutination antibody titers.
- Recording of all concomitant medications.

In addition to these pre-specified data collection time points, any serious adverse events were to be immediately reported to the clinic and documented.

Description of Treatment Groups

Subjects who met the entry criteria for the study were randomized on Day 0 in a 1:1:1:1:1 ratio to receive one of three lots of Study Vaccine in a multiple-dose vial presentation (thimerosal-containing), a single lot of vaccine in a pre-filled syringe (thimerosal-free) presentation, or Placebo vaccine (thimerosal-containing) in a multiple-dose vial presentation (250 subjects per group for a total of 1250 [up to 1350] subjects). Randomization was stratified according to the age of the subjects (18 to 49 years age range and 50 to 64 years age range). A minimum of 63 subjects in the age range of 50 to 64 years, were required in each group. The stratification by age aimed to ensure a balanced distribution of subjects.

- **Active Treatment Groups:**
- CSL Vaccine Lot #1 for CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing) Lot 1.
- CSL Vaccine Lot #2 for CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing) Lot 2.
- CSL Vaccine Lot #3 for CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing) Lot 3.
- CSL Pre-filled Syringe for CSL Influenza Vaccine pre-filled syringe presentation (thimerosal-free).

- **Multiple-dose Placebo Treatment Group:** Subjects in this treatment group were administered a placebo (thimerosal containing) vaccination.

  To ensure the integrity of the study, blinding of study treatment administered to each subject was performed. With the exception of one unblinded study personnel, who was responsible for the administration of the vaccine, all subjects, investigators, laboratory personnel, study monitors, and other study personnel were blinded as to the allocation of subjects into the four treatment formats or placebo vaccine.

  The use of concomitant vaccine therapies (specifically for influenza) during this study was monitored closely. Receiving any other influenza vaccine would lead to the exclusion of the subject from the per-protocol analysis group.

  Treatment compliance was not an issue with respect to this study. Subjects were administered one dose of vaccine during the study, in which the appropriate clinical materials were administered at the study site by study personnel.

  Efficacy and Safety Measurements Collected During the Study

  The primary efficacy variable for this study was the assessment of immunogenicity response at the Day 21 (3 weeks) post-vaccination visit.

  Safety measurements were gathered during this study during scheduled and unscheduled clinic visits, as well as within the solicited daily observations via the subject daily diary.

  Immunogenicity Measurements

  The primary immunogenicity measurements of this study were the antibody responses to the three influenza strain components. Serum samples were obtained from each subject prior to the study vaccination, as well as approximately 3 weeks post vaccination. As per the others on the CBER review team, the antibody titers for influenza were evaluated by an acceptable assay method.

  The primary endpoints utilized to determine if this Inactivated Trivalent Flu vaccine met the immunogenicity objectives were based on the values for HI assays to measure titer response listed in the FDA/CBER draft guidance for industry, entitled "Clinical Data Needed to Support the Licensure of Trivalent Influenza Vaccines." These criteria are summarized below.

  Immunogenicity response rates (~3 weeks post vaccination)

  Serum HI antibody levels of all subjects were determined in triplicate on serum separated from the whole blood. Pre- and post-vaccination samples were titrated in triplicate, simultaneously within the same assay. This process was repeated three times on the same day so that the titer assigned to each sample was the geometric mean of three independent determinations.

  Co-primary immunogenicity endpoints of seroprotection rate and seroconversion rate were to be assessed as follows:

  44. **Seroprotection** was defined as a minimum post-vaccination HI titer of 1:40.
      - The lower bound of the 95% CI for the percentage of subjects achieving an HI antibody titer ≥ 1:40 should meet or exceed 70%.

45. **Seroconversion** was defined as an increase in HI antibody titer of at least 4-fold, with a minimum post-vaccination HI titer of 1:40.
    - The lower bound of the 95% CI for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 40%.
46. **Geometric mean titers (GMT's)** 3 weeks post vaccination

Patient Responses collected during the Study

In addition to the immunogenicity responses, each subject completed a daily diary to identify and record solicited and unsolicited adverse events. This diary collected daily information about subjects including: body temperatures, injection-site adverse experiences, systemic adverse experiences, and other medications and vaccines given during the period 7 days post vaccination.

Safety Measurements

Safety Measurements were collected throughout the study and were noted within the case report forms. Additionally, safety data were collected within the daily diary. Adverse events were collected from all subjects. These adverse events were to include (but are not limited to):

47. body temperatures,
48. injection-site adverse experiences, and
49. systemic adverse experiences.

Expected and unexpected adverse events were to be tabulated and summarized. Furthermore, comparisons between treatment groups were to be performed to ensure similar safety profiles between the four different dosage formats of CSL's Inactivated Trivalent Flu Vaccine.

3.1.2 Statistical Analysis Plan

Primary Objectives

In the originally submitted protocol, the sponsor indicated that, *"The primary objective of this study was to demonstrate that vaccination with CSL Influenza Vaccine produces an immune response sufficient to meet the Committee for Proprietary Medicinal Products (CPMP) criteria for young adults of 40% seroconversion rate and 70% seroprotection rate."*

Secondary Objectives

The secondary objectives of this study were:

- To demonstrate clinical consistency between 3 lots of CSL Influenza Vaccine multiple-dose vial presentation, containing thimerosal as a preservative.
- To demonstrate clinical consistency between CSL Influenza Vaccine multiple-dose vial presentation containing, thimerosal as a preservative and CSL Influenza Vaccine pre-filled syringe presentation of thimerosal-free vaccine.
- To demonstrate acceptable safety and tolerability of CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing) and CSL Influenza Vaccine pre-filled syringe presentation (thimerosal-free).

Analysis Populations

In the protocol, the sponsor proposed that the primary endpoints of interest were immune responses (based on seroconversion and seroresponse) at the second clinic visit (Day 21 post vaccination). Approximately 1250 (up to 1350) healthy male and female subjects, 18 to <65 years old, were to be enrolled at multiple sites in this study, stratified by age, with approximately 925 subjects

aged 18 to < 50 and approximately 325 aged ≥ 50 to < 65. Within this study, the applicant proposed to examine several pre-specified analysis populations, including three commonly accepted: the Evaluable population, the Per Protocol analysis population, and the Safety analysis population.

The **Evaluable** population is defined as randomized subjects who met the following criteria:

53. Were vaccinated with Study Vaccine on Day 0,
54. Provided both pre- and post-vaccination blood samples, and
55. Were not excluded according to the use of any contraindicated medications.

The **Per Protocol** analysis population is defined as randomized subjects who met the following criteria:

56. Received the appropriate vaccination based on the pre-specified randomization schedule.
57. Had no protocol violations

The **Safety** analysis group is defined as all subjects who received a dose of the Study Vaccine.

Planned Analysis

Safety analysis was carried out on the Safety population. Baseline demographic analyses were carried out using the Evaluable population and the Safety population. The immunogenicity analyses were carried out on the Evaluable Population. If the Per Protocol Population differed from the Evaluable Population, then the immunogenicity analyses were to be repeated on the Per Protocol Population. If results differed substantially, additional comparisons between the groups could be carried out to investigate the source of the differences.

Descriptive statistics were used to present all safety and immunogenicity results: n, mean, standard deviation (SD), median, maximum, and minimum for continuous data, and frequency and percentage for categorical data. Ninety-five percent confidence intervals (CI) were also presented for some imnunogenicity criteria. Geometric means and 95% confidence intervals were presented for the log-transformed immunogenicity parameters. Exact confidence intervals based upon the binomial distribution were calculated for percentages.

All analyses were performed with a significance level of 5% for two-sided tests and 2.5% for one-sided tests.

Statistical Hypothesis

This study is designed to gather critical information on the safety, reactogenicity, and immunogenicity of the investigational trivalent influenza vaccine in healthy adults.

Within this study, the sponsor suggests they planned to examine several primary and secondary objectives related to data collected during the study.

Primary Objectives:

58. To evaluate the immunogenicity of Enzira (CSL trivalent inactived flu vaccine) in both "Adults" and "Older Adults," stratified by individuals less than versus greater than or equal to 50 years of age .

The endpoints associated with these objectives include:

- **Seroprotection** defined as a minimum post-vaccination HI titer of 1:40.
- **Seroconversion** defined as an increase in HI antibody titer of at least 4-fold, with a minimum post-vaccination HI titer of 1:40.

Statistical Analysis to Examine Primary Objectives

Exact binomial based 95% confidence intervals were calculated for the Seroprotection rate and for the Seroconversion rate for each strain. The lower bound of the confidence intervals for seroprotection was to exceed 70% for each strain. Also, the lower bound of the confidence intervals for seroresponse was to exceed 40% for each strain.

To ensure that these results were robust, these analyses were also performed using logistic regression models with lot as a covariate to adjust for potential 'between-lot' differences in the multi-dose vials.

Secondary Objective:

Immunogenicity:

Comparison of the Geometric Mean Titers, to influenza Hemagglutinin antigens after vaccination of the active treatment arms:

- Between the 3 lots of CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing);
- Between the CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing) and pre-filled syringe presentation (thimerosal-free).

Safety

The secondary safety endpoints were defined as the proportion of subjects who experienced adverse events. The rate, type, frequency, and severity of AEs in the active treatment arms, for the 3 lots of CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing), the CSL Influenza Vaccine pre-filled syringe presentation (thimerosal-free), and Placebo vaccine (thimerosal-containing) were to be assessed.

Adverse events were to be monitored after vaccination as follows:

- Solicited AEs through Day 4 (Days 0, 1, 2, 3, 4) following vaccination.
- Unsolicited AEs through the Evaluation Visit after vaccination. Local reactions and systemic symptoms

Safety and tolerability were reported as the proportion of subjects given vaccine (either: the multiple-dose vial presentation, the pre-filled syringe presentation or Placebo), and who experienced the following solicited local or systemic reactions during the 4 days following vaccination:

Local Reactions

- Pain
- Tenderness
- Erythema/Redness
- Induration/Swelling
- Ecchymosis/Bruising

Systemic Reactions

- Fever
- Headache
- Malaise

- Myalgia
- Chills
- Nausea
- Vomiting

Statistical Analysis to Examine Secondary Objectives

Immunogenicity:

Consistency of immune responses across lots was assessed via a linear model of post- vaccination log titers versus lot and presentation (single-dose pre-filled syringe versus multiple-dose vial) with pre-vaccination log titers, vaccination history, age, and gender serving as covariates. Linear contrasts between each pair of the three lots (1 vs 2, 2 vs 3, and 1 vs 3) were presented along with 95% confidence intervals. The confidence intervals for the contrasts were to fall within ±0.4055 ($\log_e$ of 1.5). This corresponds to the ratio of the GMTs falling within 0.667 to 1.5.

Consistency between presentations was assessed via a linear contrast of the single dose presentation versus the multiple-dose presentation, along with 95% confidence interval. Again, the confidence interval was to fall within ±0.4055, corresponding to the ratio falling within 0.667 and 1.5.

Clinical consistency was further investigated by evaluating the co-primary endpoints of seroprotection and seroconversion for each of the 3 lots and for the single dose presentation. Sub-group and covariate analyses of the serological immune responses were performed to investigate the effects of pre-vaccination titer, and age groups (18 to 49 years and 50 to 65 years). The proportion of subjects achieving four-fold or greater increases among those without protective pre-vaccination titers (titers < 1:40) was presented along with 95% confidence intervals.

Safety

Local reactions and systemic systems

The numbers and proportions of subjects experiencing each of the symptoms were presented along with exact 95% confidence intervals, for each dose presentation and the Placebo group. Unsolicited AEs were to be coded by the Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class. For each event type, the vaccine and Placebo groups were compared using a Fisher exact test without correction for multiple comparisons. These analyses were done for all AEs and repeated for SAEs deemed to be associated with the vaccination. This approach was intended to be exploratory. If the number of distinct significance tests was large, it was understood that some statistically significant differences would be observed simply by chance; thus, they should only be considered as a basis for further exploration, not a definitive result. The number of events was also presented within this submission and verified by the reviewing statistician.

Adverse Events

Adverse events and SAEs were summarized separately by vaccine group and overall, by presenting the number and percentage of subjects having any event, having an associated event, having an event in each MedDRA® system organ class and preferred term, having each individual event, and the severity

and relationship to Study Vaccine of each event. Number of events was also presented.
Summaries classifying events according to severity and relationship to Study Vaccine were presented. Associated events were defined as events that were associated with Study Vaccines or with an unknown association. All other information collected (e.g., type) was listed as appropriate.
Only treatment emergent adverse events (commencing after exposure to study treatment) were to be included in the AE and SAE summaries. Non-treatment emergent events (starting prior to exposure to study treatment) were to be included in the subject listings and flagged but not included in the above summaries. Where an AE start date was partially or fully missing, and it was unclear using partial dates as to whether the AE was treatment emergent, it was to be assumed that it was.

Sample Size

This study was adequately powered to satisfy the primary endpoint for each of the 3 influenza strains. To achieve 80% power overall for all 3 strains, the power per strain had to be at least 92.8% per strain, assuming independence in individuals' immune responses to the 3 strains. In practice, the immune response to different strains by individuals tends to be somewhat positively correlated, but this will vary seasonally and by population. A positive correlation would increase the power of the overall test, so considering the case of independence produces conservative sample sizes, i.e., larger than likely needed. The primary objective was achieved if the seroconversion and seroprotection rates for the active vaccines were significantly greater than the CPMP for young adults' benchmark rates of 40% and 70%, respectively. The stratification variables were used to ensure balance by vaccine group and a minimum representation of the older cohort at all sites. These variables were not considered in the primary analysis.
If the true seroconversion rate was at least 45.4%, then with a total sample size of N=1000 the power for this comparison exceeds 93% per strain. If the true seroprotection rate was at least 75%, then with a total sample size of N=1000 the power for this comparison exceeds 93% per strain.
The study was powered for the primary immunogenicity endpoints. Demonstrating consistency of immune response across the three lots of vaccine and across the two presentations (single dose versus multiple-dose vial) was a secondary objective. This comparison was based on the difference in log GMTs between lots and across presentations with pre-vaccination GMT serving as covariate. Analyses of similar recent studies indicated that, with covariate adjustment for log pre-vaccination titers, the standard deviation of log post-vaccination titers range from 0.65 to 1.4 for different strains and cohorts. With n=250 per arm, an alpha=0.05 equivalence test using a delta of ±0.4055 ($\log_e$ of 1.5) has at least 88% power if the standard deviation is 1.4 or less.
The following table supplied by the sponsor and verified by the reviewing statistician provides an indication of the power to detect a significant safety event under the assumption that the true, but unknown, rate for such an event is between 0.01% and 5.00%. For example, there is a 96% chance of

observing an event in the combined vaccine groups (n= 1000) when the underlying rate is 1 in 300 or 0.33%.

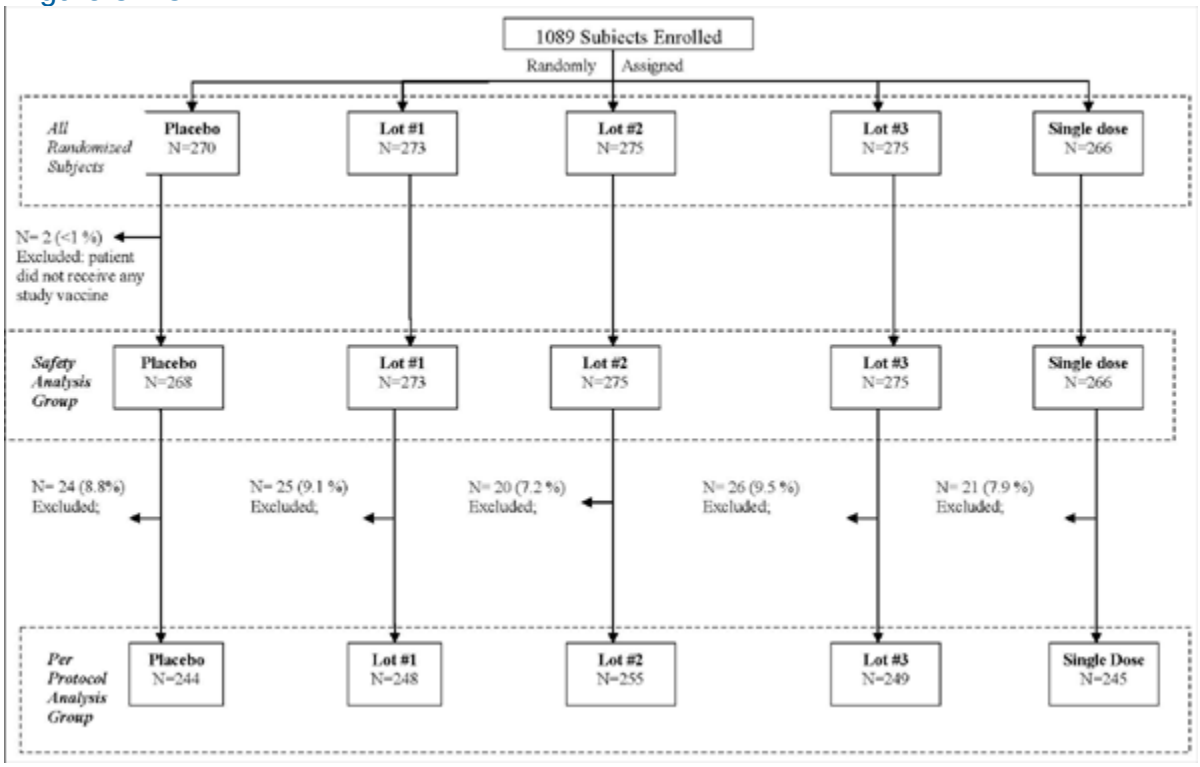
Sample Size	Assumed Event Rates (%)								
	0.01	0.10	0.33	0.50	1.00	2.00	3.00	4.00	5.00
N=250, a single vaccine group	2.47	22.13	56.60	71.44	91.89	99.36	99.95	99.99	99.99
N=1000, all vaccine groups combined	9.52	63.23	96.45	99.33	99.99	99.99	99.99	99.99	99.99

3.1.3 Patient Disposition, Demographics, and Baseline Characteristics

A total of 1359 subjects were randomized and 1357 subjects received either CSL Influenza Vaccine or Placebo, between 12 June 2006 (First Subject First Visit) and Last Subject First Visit 01 August 2006. The Last Subject Last Visit occurred on 25 August 2006. With respect to the CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing), a total of 823 subjects received either Lot 1, 2, or 3 (Lot 1 n=273, Lot 2 n=275, Lot 3 n=275). A total of 266 subjects received the CSL Influenza Vaccine via the pre-filled syringe presentation (thimerosal-free), and 268 subjects received the Placebo multiple-dose presentation (thimerosal-containing). The Safety population consisted of all subjects who received Study Vaccine.
With regard to the Evaluable population: 814 subjects received CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing) (Lot 1 n=270 [98.9% of the safety analysis population], Lot 2 n=275 [100% of the safety analysis population], Lot 3 n=269 [97.8% of the safety analysis population]); 263 subjects (98.9% of the safety analysis population) received CSL Influenza Vaccine via a pre-filled syringe presentation (thimerosal-free), and 264 subjects (97.8% of the safety analysis population) received the Placebo multiple-dose presentation (thimerosal-containing). Percentages are calculated from the Safety population, see Figure 3.1.3.
Included in the Per Protocol population were: 752 subjects who received CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing) (Lot I n=248 [90.8% of the safety analysis population], Lot 2 n=255 [92.7% of the safety analysis population], Lot 3 n=249 [90.5% of the safety analysis population]); 245 subjects (92.1% of the safety analysis population) received CSL Influenza Trivalent Vaccine via a pre-filled syringe presentation (thimerosal-free), and 244 subjects (90.4% of the safety analysis population) received the Placebo multiple-dose presentation (thimerosal-containing).
A total of 1350 subjects (99.5%) completed the study and nine subjects did not. Of the nine subjects, five were lost to Follow-up. Of these five subjects, three received CSL Influenza Vaccine multiple-dose vial presentation (thimerosal-containing) Lot 3, one subject received the CSL Influenza Vaccine pre-filled syringe (thimerosal-free) presentation, and one subject received the Placebo Vaccine (thimerosal-containing). One subject withdrew consent

(Placebo group [thimerosal-containing]), two subjects were randomized and not vaccinated (Placebo [thimerosal-containing]), and one subject (randomized to the CSL Influenza Vaccine pre-filled syringe [thimerosal-free] presentation) did undergo all study assessments but their data could not be source verified. There were no withdrawals due to an AE.
Figure 3.1.3 illustrates the patient disposition in this study, including the Safety, Evaluable, and Per-Protocol analysis groups.

Figure 3.1.3:



Patient Disposition and Analysis Group for Study

Next, demographic and baseline values for select variables based on the safety analysis group are summarized in Table 3.1.3.b. In this study, all subjects received the appropriate clinical material based on the randomization schedule. Thus the safety analysis group and all treated as treated analysis group (containing all subjects receiving any clinical material analyzed by what product was actually received) are identical and are summarized in Table 3.1.3.b.

Table 3.1.3.b: Demographic Characteristics of Subjects included in the Safety Analysis Group (based on subjects receiving the study vaccination)

Baseline Demographics	**Treatment Group**

Categorical Variables	Placebo (n=264)	Lot #1 (n=270)	Lot #2 (n=275)	Lot #3 (n=269)	Single dose (n=263)
Race					
Black/African American	31 (11%)	28 (10%)	35 (13%)	36 (13%)	33 (12%)
Asian	15 (6%)	12 (4%)	14 (5%)	23 (8%)	19 (7%)
American Indian/ Alaskan Native	4(1%)	3(1%)	4(1%)	2(<1%)	1(<1%)
Hawaiian/ Pacific Islander	1 (<1%)	0 (0%)	1 (<1%)	1 (<1%)	0 (0%)
White	216 (80%)	227 (83%)	215 (78%)	210 (76%)	213 (80%)
Gender					
Male	90 (34%)	93 (34%)	103 (37%)	107 (39%)	103 (38%)
Female	178 (66%)	180 (66%)	172 (63%)	168 (61%)	163 (61%)
Continuous Variables	**mean (range in parenthesis)**				
Age (years)	38 (18-64)	38 (18-64)	38 (18-63)	38 (18-64)	38 (18-64)

Based on the demographic and baseline descriptive values shown above and provided in the sponsor's report, the five treatment groups for the safety analysis population appear to be similar. Similar results were observed in the Per Protocol (PP) analysis population.

3.1.4 Efficacy Results

Primary Efficacy Endpoints

The comparisons of immunogenicity based on proportion of subjects that experienced seroprotection and seroconversion (with ≥ 1:40 HI titer and 4-fold increase and ≥ 1:40 HI titer, respectively) approximately three weeks (or Day 21) post vaccination in the Per Protocol analysis group are summarized in Table 3.1.4.a and Table 3.1.4.b, respectively. Included in Table 3.1.4.a are the antibody responses to each of the HI assays for individuals who were initially seronegative at baseline. Included in these tables are the seroprotection and seroconversion rates and 95% CI's for each treatment group.

Table 3.1.4.a : Seroprotection Rate Based on Per Protocol Proportion of Subjects with ≥1:40 HI Titer Post Vaccination (with 95% CI in parenthesis)

	Treatment Group				
Strain	**Placebo (n=264)**	**Lot #1 (n=270)**	**Lot #2 (n=275)**	**Lot #3 (n=269)**	**Single dose (n=263)**
H1N1	76.2% (68.9, 79.8)	96.7% (93.8, 98.5)	98.2% (95.8, 99.4)	97.4% (94.7, 98.8)	98.9% 96.7, 99.8)
H3N1	72.0% (66.1, 77.3)	100.0% (98.6,100.0)	99.6% (98.6, 100.0)	100.0% (98.6, 100.0)	100.0% (98.6, 100.0)
B Strain	47.0% (40.8, 53.2)	95.4% (92.2, 97.7)	93.3% (90.3, 96.4)	92.3% (89.2, 95.7)	93.9% (91.2, 97.1)

Table 3.1.4.b: Seroconversion Rate Based on Per Protocol Proportion of Subjects with ≥1:40 HI Titer Post Vaccination (with 95% CI in parenthesis)

	Treatment Group				
Strain	**Placeb (n=264)**	**Lot #1 (n=270)**	**Lot #2 (n=275)**	**Lot #3 (n=269)**	**Single dose (n=263)**
H1N1	1.7% (0.8, 4.9)	48.5% (42.4, 54.7)	48.4% (42.3, 54.4)	49.1 (42.9, 55.2)	48.7% (42.5, 54.9)

Strain	Treatment Group				
	Placeb (n=264)	**Lot #1 (n=270)**	**Lot #2 (n=275)**	**Lot #3 (n=269)**	**Single dose (n=263)**
H3N1	0.0% n/a	69.3% (63.4, 74.7)	71.3% (65.5, 76.5)	75.5 (69.9, 80.5)	70.0% (64.0, 75.4)
B Strain	0.4% (0.0, 2.1)	71.8% (66.1, 77.1)	66.7% (62.1, 73.5)	68.4% (63.3, 7406)	68.4% (64.0, 75.4)

Considering Table 3.1.4.a and Table 3.1.4.b., which include the seroprotection and seroconversion rates of the placebo vaccine and the various dosage forms of the three different strains represented in CSL's proposed influenza vaccine, it appears that these immunogenicity criteria are met.
Next, the GMT's for influenza antibody titers can be examined. Within Table 3.1.4.c, the GMT values of the antibody titers are listed (with 95% CI in parentheses).

Table 3.1.4.c: Table of Post-vaccination Geometric Mean HI Titers (GMT) based on Per Protocol Analysis Group (with 95% CI in parenthesis)

Strain	Treatment Group				
	Placeb (n=264)	**Lot #1 (n=270)**	**Lot #2 (n=275)**	**Lot #3 (n=269)**	**Single dose (n=263)**
H1N1	238 (0, 1232)	878 (0, 3188)	760 (0, 2868)	956 (0, 3748)	943 (0, 3713)
H3N1	256 (0, 1422)	1484 (0, 5704)	1999 (0, 7725)	2161 (0, 10873)	1880 (0, 7446)
B Strain	61 (0, 263)	422 (0, 1414)	364 (0, 1234)	432 (0, 1560)	418 (0, 1334)

Considering the results presented in Table 3.1.4.c, which include GMT values of the placebo vaccine and the various dosage forms of the seasonal trivalent influenza vaccine, it appears that the vaccine is immunogenic compared to the placebo.

3.2 Evaluation of Safety

A total of 1359 subjects were enrolled in this study. Of these subjects, 2 subjects did not receive any study vaccination. Excluding these subjects, who would contribute no information regarding the safety of this vaccine or the comparator placebo, because there was no subject exposure to study vaccination, a total of 1357 subjects were considered valid for the safety evaluation. Any adverse event which was documented within the case report form was utilized in the tabulation of safety and tolerability. The safety measurements and endpoints of interest included:

- Adverse Events
- Rating of severity of event
- Listing of treatment related Adverse Events
- Deaths

A summary of the safety events are listed in Table 3.2.a.

Table 3.2.a: Results of All Safety Events Attributed to the Vaccine Occurring During the Study Collection Time Period in Each Treatment Group in the CSL Inactivated Trivalent Flu Vaccine Study

Event	Treatment Group				
	Placebo	**Lot #1**	**Lot #2**	**Lot #3**	**Single dose**
Total Subjects in Safety analysis group	**268**	**273**	**275**	**275**	**266**
Survived	268	273	275	275	266
Death	.	.	.	.	.
AE (associated with vaccine)					
Severe	1	.	.	.	.
Moderate	8	9	10	3	7

Event	Treatment Group				
	Placebo	Lot #1	Lot #2	Lot #3	Single dose
Mild	16	30	15	23	20

Note: Based on number of subject(s) experiencing event
In addition to the listing of adverse events, the location of the less serious adverse events can be considered. A summary of the adverse events stratified by systemic and local injection site reactions are listed in Table 3.2.b.

Table 3.2.b: Results of Safety Event Location within 5 Days of Vaccination in the Various Flu Vaccine Dosage Groups in Subjects in the Safety Analysis Group (of Moderate and Severe)

Adverse/Reactogenicity Event	Treatment Group		
	Placebo	All Lots (Multi-Dose)	Single dose
Total Subjects in Safety analysis group	268	823	266
Local injection site reaction			
Erythema/Redness	.	8	1
Induration/Swelling	.	6	3
Bruising	.	6	2
Tenderness	2	24	15

Adverse/Reactogenicity Event	Treatment Group		
	Placebo	All Lots (Multi-Dose)	Single dose
Systemic injection reaction			
Fever	.	1	1
Headache	13	15	7
Chills	2	2	2
Myalgia	3	9	5
Vomit	1	1	1
Nausea	3	4	3

Note: Based on number of subject(s) experiencing event.
Common adverse events are illustrated in Table 3.2.c. Within this table, events which occurred in at least 3 subjects in a treatment group were included. The results in Table 3.2.c illustrate that similar AE rates occurred in the specific vaccination treatment groups.

Table 3.2.c: Counts of Common Adverse Events Occurring from Day 0 to Day 21 Post Initial Vaccination, by Treatment Group, Inactivated Trivalent Flu Vaccine Study

Adverse Event	Treatment Group				
Total Subjects in Safety analysis group	**Placebo**	**Lot #1**	**Lot #2**	**Lot #3**	**Single dose**

Adverse Event	Treatment Group				
Total Subjects in Safety analysis group	**Placebo**	**Lot #1**	**Lot #2**	**Lot #3**	**Single dose**
Diarrhea/Loose Stool	4	5	3	.	1
Sore Throat	.	4	1	4	2
Continued Local Reactogenicity	3	3	4	3	5
Headache	1	2	.	3	1
Bruise at Vaccination Site	.	1	.	5	.

Note: Based on number of subject(s) experiencing event.
Considering Table 3.2.c, the most common adverse events reported were: diarrhea, sore throat, continued local reactogenicity, headache, and bruise at injection site. Many other adverse events were documented but are not listed in this table. Considering the previous table and tables provided by the sponsor, similar rates for adverse events were reported in the various dosage format treatment groups. While these rates may be higher than the placebo comparator arm, it appears that the safety profile of this vaccine is within acceptable limits.
Based on the results presented in Table 3.2.a-c, results reported by the sponsor, and other tolerability and reactogenicity events confirmed by the reviewing statistician and not presented within this review, the tolerability profile of the various dosage formats of Inactivated Trivalent Flu Vaccine are similar. Thus, it appears that the Inactivated Trivalent Flu Vaccine is generally well tolerated.
For a more complete discussion of safety, please see the medical reviewer's comments.

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4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

Several pre-specified and exploratory and subgroup analyses were of interest to the primary medical reviewer. Results of the two primary immunogenicity endpoints (seroconversion and seroprotection) in special populations and subgroups are presented within this section. These subgroups include analysis of immune

response based on stratification of baseline characteristics including gender, race, and age (<50 years of age versus ≥ 50 years of age). An additional subgroup of interest to the medical officer includes the immune response stratified by site and stratified by a revised age cutoff (less than 60 years of age versus greater than or equal to 60 years of age).

4.1 Gender, Race, and Age

This section provides tables and a short synopsis of the immune response to the various inactivated trivalent Flu vaccine dosage formats versus placebo, considering several pre-specified subgroups. Tables 4.1.a- 4.1.f display the seroconversion or seroprotection response rates of the various flu strain antibody titers at the day 21 post-vaccination visit by gender, race, and age (<50 years of age versus ≥ 50 years of age), based on the Per Protocol analysis group.

Table 4.1.a: Percent Seroprotection at Day 21 Post Initial Vaccination in the Per Protocol Analysis Group for the Inactivated Trivalent Flu Vaccine Study, by Gender

Gender	Strain	Treatment				
		Placebo	CSL Lot #1	CSL Lot #2	CSL Lot #3	CSL Syringe
Female	H1N1	47.5	95.5	94.8	92.7	94.4
	H3N2	76.3	98.3	99.4	97	100
	B St	75.1	100	99.4	100	100
Male	H1N1	46	95.7	92.2	93.3	95.1
	H3N2	71.3	93.5	96.1	98.1	97.1
	B St	65.5	100	100	100	100

Table 4.1.b: Percent Seroconversion at Day 21 Post Initial Vaccination in the Per Protocol Analysis Group for the Inactivated Trivalent Flu Vaccine Study, by Gender

Gender	Strain	Treatment				
		Placebo	CSL Lot #1	CSL Lot #2	CSL Lot #3	CSL Syringe
Female	H1N1	.	72.1	70.0	70.1	73.5
	H3N2	1.7	48.0	49.4	47.0	50.6
	B St	.	67.6	73.3	73.8	69.1
Male	H1N1	1.1	72.0	64.1	67.6	65.0
	H3N2	3.4	49.5	46.6	52.4	44.7
	B St	.	73.1	68.0	78.1	69.9

Table 4.1.c: Percent Seroprotection at Day 21 Post Initial Vaccination in the Per Protocol Analysis Group for the Inactivated Trivalent Flu Vaccine Study, by Race

		Treatment				
Race	**Strain**					
		Placebo	**Lot #1**	**Lot #2**	**Lot #3**	**Syringe**

Race	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	B St	25	100	100	100	100
American Indian/Alaskan	H1N1	75	100	100	100	100
	H3N2	75	100	100	100	100
Asian	B St	13	100	85.7	91.3	94.7
H1N1	60	100	92.9	91.3	100	
H3N2	40	100	100	100	100	
	B St	53	93	94.3	91.4	97
Black/African American	H1N1	60	100	97.1	97.1	97
	H3N2	67	100	100	97.1	100
	B St	100	.	100	100	.
Hawaiian/Pacific Islander	H1N1	100	.	100	100	.
	H3N2	100	.	100	100	.
Unknown	B St	33	100	100	100	.

Race	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	H1N1	67	100	100	100	.
	H3N2	100	100	100	100	.
	B St	48	95	94	91.8	94.1
White	H1N1	77	96	98.6	96.6	98.8
	H3N2	74	100	99.5	99	99.8

Table 4.1.d: Percent Seroconversion at Day 21 Post Initial Vaccination in the Per Protocol Analysis Group for the Inactivated Trivalent Flu Vaccine Study, by Race

Race	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	B St	.	33.3	100	100	100
American Indian/Alaskan	H1N1	.	66.7	100	100	100
	H3N2	.	33.3	100	100	100
Asian	B St	.	83.3	42.9	78.3	57.9

Race	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	H1N1	6.7	41.7	42.9	52.2	52.6
	H3N2	.	58.3	50	78.3	57.9
	B St	.	85.7	65.7	62.9	81.8
Black/African American	H1N1	3.3	75	51.4	48.6	69.7
	H3N2	.	82.1	68.6	85.7	90.9
	B St	.	.	.	100	.
Hawaiian/Pacific Islander	H1N1	.	.	.	.	.
	H3N2	.	.	100	100	.
	B St	.	100	83.3	66.7	.
Unknown	H1N1	.	33.3	50	33.3	.
	H3N2	.	66.7	83.3	66.7	.
	B St	0.5	69.8	69.3	67.8	69.2
White						
	H1N1	1.9	45.5	47.4	48.1	44.2

Race	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	H3N2	.	68.9	72.1	72.1	66.8

Table 4.1.e: Percent Seroprotection at Day 21 Post Initial Vaccination in the Per Protocol Analysis Group for the Inactivated Trivalent Flu Vaccine Study, by Age (> 50 years of age versus < 50 years of age)

		Treatment				
Age	Strain	Placebo	CSL Lot #1	CSL Lot #2	CSL Lot #3	CSL Syringe
	H1N1	45	97	96.5	93.8	95.9
Under 50	H3N2	73	98	98.5	97.9	99
	B St	67	100	99.5	100	100
	H1N1	53	93	86.7	90.7	91.4
50+	H3N2	80	93	97.3	96	98.6
	B St	85	100	100	100	100

Table 4.1.f: Percent Seroconversion at Day 21 Post Initial Vaccination in the Per Protocol Analysis Group for the Inactivated Trivalent Flu Vaccine Study, by Age (> 50 years of age versus < 50 years of age)

Age	Strain	Placebo	CSL Lot #1	CSL Lot #2	CSL Lot #3	CSL Syringe

Age	Strain	Placebo	CSL Lot #1	CSL Lot #2	CSL Lot #3	CSL Syringe
	H1N1	0.5	76.3	73	72.2	71.8
Under 50	**H3N2**	2.6	53	54.5	51.5	54.4
	B St	.	72.2	75	76.8	73.8
	H1N1	.	60.8	54.7	61.3	65.7
50+	**H3N2**	1.4	36.5	32	42.7	31.4
	B St	.	62.2	61.3	72	57.1

Considering the results illustrated in Table 4.1.a-f, (the observed % seroconversion and % seroprotection) stratified by gender, race, or age (<50 years of age versus ≥ 50 years of age), it appears that the vaccination has acceptable immunogenicity when compared to placebo. In several cases, the subset analyses do not meet the 40% and 70% criteria for seroconversion and seroprotection, respectively; however, in many of these instances there is a sparse number of subjects in each treatment group subset.

Based on the standards to demonstrate immunogenicity within the guidance document " *Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines,*" the observed results and analyses support the sponsor's claim that this vaccine is immunogenic. These results found in the per protocol analysis group are similar to the results found (but not presented) in the safety and evaluable analysis groups.

The reviewing statistician was able to recreate the sponsor's results based on the various measures of immunogenicity collected and analyzed in this study. Based on the results examined (but not presented in this review), the immune responses measured by other various endpoints and in different analysis groups are supportive of an acceptable level of immune response, within the various pre-specified subgroups, according to the CBER Guidance criteria.

Other Special/Subgroup Populations

Several additional subgroups in this study were identified by the medical officer for further examination in the review of this product. The specific analysis groups to be utilized in the analysis of the data from this study include: the analysis stratified by

center and age, dichotomized by the subject being less than and greater than or equal to 60 years old, based on the per protocol analysis population. Table 4.2.a and b shows the percentage of subjects in the stratification subgroup having positive immune response to the various vaccination formats.

Table 4.2.a: Percent Seroprotection of the Inactivated Trivalent Flu Vaccine, by Center and Dosage Format
(Per Protocol Analysis Group)

		Treatment				
Center	**Strain**					
		Placebo	**Lot #1**	**Lot #2**	**Lot #3**	**Syringe**
	B St	40.9	95.5	91.3	100.0	100.0
Baylor	**H1N1**	72.7	95.5	95.7	100.0	100.0
	H3N2	63.6	100.0	100.0	100.0	100.0
	B St	44.1	97.1	88.9	90.9	88.2
Cincinnati	**H1N1**	79.4	97.1	97.2	97.0	100.0
	H3N2	73.5	100.0	100.0	100.0	100.0
	B St	75.0	89.5	100.0	83.3	100.0
Duke	**H1N1**	93.8	94.7	100.0	94.4	100.0
	H3N2	75.0	100.0	100.0	100.0	100.0
St. Louis	**B St**	46.3	97.6	92.9	97.6	97.6

Center	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	H1N1	68.3	90.5	100.0	100.0	97.6
	H3N2	75.6	100.0	100.0	100.0	100.0
	B St	46.2	100.0	88.9	100.0	96.2
Stanford	H1N1	65.4	100.0	92.6	96.2	100.0
	H3N2	69.2	100.0	100.0	100.0	100.0
	B St	50.0	96.7	100.0	84.4	89.7
U. Iowa	H1N1	75.0	93.3	100.0	96.9	96.6
	H3N2	71.4	100.0	100.0	100.0	100.0
	B St	48.5	97.1	97.1	97.1	100.0
U. Maryland	H1N1	63.6	100.0	100.0	100.0	100.0
	H3N2	72.7	100.0	100.0	100.0	100.0
	B St	38.7	93.8	90.3	93.8	93.5
U. Rochester						
	H1N1	87.1	100.0	96.8	93.8	100.0

Center	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	H3N2	67.7	100.0	100.0	100.0	100.0
	B St	45.5	90.6	96.9	87.5	90.0
Vanderbilt	H1N1	75.8	100.0	100.0	96.9	96.7
	H3N2	75.8	100.0	96.9	100.0	100.0

Table 4.2.b: Percent Seroconversion of the Inactivated Trivalent Flu Vaccine, by Center and Dosage Format
(Per Protocol Analysis Group)

Center	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	B St	.	81.8	82.6	85	72.7
Baylor	H1N1	4.5	63.6	69.6	60	63.6
	H3N2	.	81.8	87	100	72.7
	B St	.	61.8	66.7	63.6	58.8
Cincinnati						
	H1N1	2.9	47.1	44.4	39.4	38.2

Center	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	H3N2	.	61.8	83.3	72.7	70.6
	B St	.	73.7	73.7	66.7	82.4
Duke	H1N1	6.3	52.6	42.1	61.1	52.9
	H3N2	.	63.2	84.2	88.9	64.7
	B St	.	76.2	73.8	76.2	78.6
St. Louis	H1N1	.	45.2	59.5	57.1	57.1
	H3N2	.	61.9	69	69	73.8
	B St	.	77.8	51.9	69.2	73.1
Stanford	H1N1	3.8	40.7	48.1	46.2	46.2
	H3N2	.	66.7	55.6	76.9	50
	B St	.	70	63.3	56.3	75.9
U. Iowa	H1N1	.	56.7	40	46.9	48.3
	H3N2	.	70	73.3	68.8	72.4

Center	Strain	Treatment				
		Placebo	Lot #1	Lot #2	Lot #3	Syringe
	B St	.	73.5	77.1	79.4	82.4
U. Maryland	H1N1	6.1	61.8	54.3	52.9	44.1
	H3N2	.	79.4	68.6	79.4	79.4
	B St	3.2	81.3	61.3	62.5	48.4
U. Rochester	H1N1	.	53.1	45.2	46.9	38.7
	H3N2	.	84.4	74.2	65.6	77.4
	B St	.	56.3	62.5	65.6	63.3
Vanderbilt	H1N1	.	21.9	31.3	37.5	50
	H3N2	.	59.4	53.1	75	56.7

Considering the results in Table 4.2.a and 4.2.b, the seroconversion and seroprotection rates meet the criteria specified in the draft guidance for nearly all centers. Three centers: Rochester , Vanderbilt, and Cincinnati do not explicitly meet the guidance criteria. However, in most cases the immunogenicity results are within 5% of the guidance value. Examining efficacy responses stratified by center is considered a post hoc subset analysis. This post hoc subset analysis can provide information about trends; however, this study is not powered to examine center affects, and the small sample size in the various centers may lead to bias in such results.

Table 4.2.c: Seroprotection of the Inactivated Trivalent Flu Vaccine, by Age (< 60 versus ≥ 60 years of age) and Dosage Format (Per Protocol Analysis Group)

Age	Strain	Treatment				
		Placebo	CSL Lot #1	CSL Lot #2	CSL Lot #3	CSL Syringe
Under 60	H1N1	46	96	94.3	92	94.2
	H3N2	74	97	98.5	97	99
	B St	70	100	99.6	99	99.8
60+	H1N1	50	94	80	94	100
	H3N2	79	94	90	94	93.3
	B St	93	100	100	100	100

Table 4.2.d: Seroconversion of the Inactivated Trivalent Flu Vaccine, by Age (< 60 versus ≥ 60 years of age) and Dosage Format (Per protocol Analysis Group)

Age	Strain	Treatment				
		Placebo	CSL Lot #1	CSL Lot #2	CSL Lot #3	CSL Syringe
Under 60	H1N1	0.4	72	68.7	68.4	70.3
	H3N2	2.4	47.7	49.4	48.4	48.3
	B St	.	69.7	72.1	75.8	70.3
60+	H1N1	.	70.6	50	68.8	66.7
	H3N2	.	58.8	20	50	46.7
	B St	.	64.7	50	56.3	53.3

The results based on the seroconversion and seroprotection rates of the various dosage formats of the trivalent influenza vaccine demonstrate adequate immune response, regardless of the center or age (i.e.., comparing individuals less than 60 years of age to individuals 60 years of age or older). These consistent results combined with other subset analyses of various analysis groups and other subgroups examined, but not reported, provide evidence that this vaccine appears to meet the immune response criteria stated in the FDA draft guidance on clinical data needed to support approval of trivalent flu vaccines.

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5. SUMMARY AND CONCLUSIONS

5.1 Statistical Issues and Collective Evidence

Relevant Protocol Amendments

Following the completion and submission to the agency of the proposed protocol, several minor alterations to the protocol were proposed and implemented. The protocol amendments were minor in nature and did not affect the results and conclusions of this study.

Collective Evidence

The collective body of evidence, included in this submission, is supportive of the sponsor's claim of tolerability and efficacy inferred from immune response to the various dosage formats of the CSL Inactivated Trivalent Flu vaccine. Additionally, considering the various dosage formats of the trivalent vaccine, it appears that immune responses were similar. Furthermore, the overall safety (based on reporting of adverse events) and tolerability of the various dose formats were similar and within acceptable limits. These results and conclusions were confirmed

by the reviewing statistician, utilizing the data sets provided by the sponsor. Based on these results, it appears that the objectives of this study, including:

1. Demonstrating that vaccination with CSL Influenza Vaccine produces an immune response sufficient to meet the Committee for Medicinal Products for Human Use criteria for young adults of 70% SeroProtection and 40% SeroConversion.

have been met.

5.2 General Comments

Proposed label

The label proposed by the sponsor has the following suggestions for label claim:

1. INDICATIONS AND USAGE

- AFLURIA®, Influenza Virus Vaccine, is indicated for active immunization to prevent influenza disease caused by influenza virus types A and B present in the vaccine, in adults 18 years and older.
- For information on treatment in special populations and timing for vaccination, refer to the Advisory Committee on Immunization Practices (ACIP) influenza vaccination recommendations

The clinical studies section includes the following proposed statement:

Three randomized, controlled clinical studies of AFLURIA®* have evaluated the immune responses, (specifically, HI antibody titers) to each virus strain in the vaccine (Studies CSLCT-FLU-05-09, CSLCT-NHF-05-11 and CSLCT-NHF-05-15). In these studies, post-vaccination immunogenicity was evaluated on sera obtained 21 days after administration of AFLURIA®*.

Study CSLCT-FLU-05-09 was a randomized, double-blinded, placebo-controlled, multi-center study performed in the US, in healthy adults ≥ 18 to < 65 years. A total of 1359 subjects were enrolled, with a safety population of 1357 subjects vaccinated (1089 subjects receiving AFLURIA® and 268 receiving a thimerosal-containing placebo). Subjects receiving AFLURIA® were vaccinated with either a thimerosal-containing (one of three lots), or a thimerosal-free vaccine. The evaluable efficacy population consisted of 1341 subjects with complete serological data who had not received any contraindicated medications before post-vaccination immunogenicity assessment. Among the evaluable efficacy population receiving AFLURIA® (1077 subjects), 37.5% were men and 62.5% were women. The mean age of the entire evaluable population recipients of AFLURIA® was 38 years. A total of 73% of the total evaluable population were ≥ 18 to < 50 years and 27% were ≥ 50 to < 65 years.

In study CSLCT-FLU-05-09, the co-primary immunogenicity endpoints assessed were the seroconversion rate and the seroprotection rate. **Seroconversion** was defined as an increase in HI antibody titers of at least 4-fold, with a minimum post-vaccination HI titer of 1:40. **Seroprotection** was defined as a minimum post-vaccination HI titer of 1:40. The pre-specified targets for the two endpoints were for the lower bound of the 2-sided 95% confidence intervals exceeding 40% for seroconversion rate and 70% for seroprotection rate.

In adults ≥ 18 to < 65 years, serum HI antibody responses to AFLURIA® met the pre-specified seroprotection rate and seroconversion rate criteria for all three virus strains (Table 3). Clinical lot-to-lot consistency was demonstrated for thimerosal-

containing and thimerosal-free AFLURIA® vaccine formulations, showing that AFLURIA® formulations are equivalent and interchangeable.

5.3 Conclusions and Recommendations

The Inactivated Trivalent Flu Vaccine developed by CSL Limited has been shown to have positive antibody response based on the GMT's, seroprotection and seroconversion rates observed in the primary clinical study and supporting studies. Additionally, the Inactivated Trivalent Flu Vaccine has been shown to have acceptable tolerability and safety properties based on the results of the blinded study CSL-FLU-05-09. Various post-hoc subset analyses and sensitivity analyses yield similar results and trends, supporting the sponsor's suggestion that this Inactivated Trivalent Flu vaccine should be effective in the prevention of seasonal influenza in healthy adults 18-65 years of age.

Furthermore, based on additional data from all studies submitted within STN 125254, it appears that the label claims proposed by the sponsor, "*AFLURIA® is indicated for active immunization to prevent influenza disease caused by influenza virus types A and B present in the vaccine, in adults 18 years and older. AFLURIA® is not currently indicated for pediatric vaccination,* " may not be supported by the studies and data provided. However, a claim related to subjects aged 18-65 years old appears to be supported, based on the statistical analysis of the sponsor provided studies.

Thus, based on the collective evidence, it is the opinion of this statistical reviewer that the efficacy of this product, both the single use syringe and multi-dose vials, inferred from immune response endpoints has been demonstrated for subjects 18-65 years of age. The safety and tolerability evidenced in this study, examining the various dosage formulations of CSL's Inactivated Trivalent Flu Vaccine and supported by the four supplementary studies provided in this submission are supportive of the vaccine being safe and generally well-tolerated.