DEPARTMENT OF HEALTH & HUMAN SERVICES
FDA/CBER/OVRR/DBPAP

Memorandum

Date: March 25, 2009

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To: File

Subject: Final Review Memo
MenACWY BLA STN 125300/0.0, 125300/0.3, 125300/0.4 and 125300/0.5
Diphtheria and Tetanus Serology

SUMMARY

In support of the BLA for MenACWY Novartis submitted the validation reports for the Tetanus, Diphtheria --b(4)---------- which were used to demonstrate that neither concomitant administration of Tdap vaccine with MenACWY nor administration of Tdap vaccine one month prior to vaccination with MenACWY were inferior to the immune response elicited by Tdap vaccine alone. This review memo focuses specifically on the Diphtheria (D) and Tetanus (T) -b(4)- validation reports provided for supportive study V59P11 and pivotal study V59P18. For both D and T it is considered that antibody levels < 0.01 IU/ml indicate toxin susceptibility, levels between 0.01 and 0.1 IU/ml confer basic protection against toxin-mediated disease and that antibody concentrations ≥ 0.1 IU/ml confer full protection.

The primary immunogenicity objective of phase III trial V59P11 was to demonstrate that the immunogenicity of a single dose of Tdap vaccine separately but concomitantly administered with MenACWY was not inferior to a single injection of Tdap concomitantly given with a saline placebo as determined by the percentage of subjects with anti-D and anti-T responses ≥ 1.0 IU/ml 1 month after vaccination. In addition, a secondary immunogenicity objective in this trial was to compare the immunogenicity of a single dose of Tdap vaccine when given separately but concomitantly with MenACWY to that of a single injection of Tdap vaccine given concomitantly with a saline placebo. To address this objective, D and T immunological responses were defined as: (a) the percentage of subjects with anti-D and anti-T concentrations ≥ 0.1 IU/ml 1 month post vaccination and (b) anti-D and –T -b(4)- geometric mean antibody concentrations (GMCs) at baseline and 1 month post vaccination with the GMC increase (geometric mean ratio, GMR) from baseline to 1 month after vaccination. For this study both D and T -b(4)- were performed at the -b(4)----------. From the serological results obtained from this trial, Novartis concluded that the primary object of the study

was met in that the immune responses to D and T when Tdap was administered concomitantly with MenACWY were not inferior to the responses observed when Tdap was administered concomitantly with a saline placebo.

For the phase III pivotal study V59P18, one of the primary immunogenicity objectives was to demonstrate that the immune response of Tdap given concomitantly with MenACWY was not inferior to the response of Tdap given alone, with the response to Tdap defined as the percentage of subjects with anti-D and anti-T concentrations ≥1.0 IU/ml. In addition, two secondary immunogenicity objectives also involved the assessment of D and T serology. The first of these objectives was to demonstrate that the immune response to Tdap administered alone one month after MenACWY was not inferior to the immune response to Tdap administered alone one month prior to MenACWY while the second objective was to assess the immunogenicity of Tdap administered alone or concomitantly with MenACWY and HPV as measured by anti-D and anti-T GMCs. In this study all D and T serology results were obtained from -b(4)-- performed at --b(4)----------. After analysis of the serological data from this trial, Novartis concluded: (1) that the D and T immune responses to Tdap when administered concomitantly with MenACWY were not inferior to the responses obtained when Tdap was administered alone and (2) that noninferiority of the immune responses to Tdap, as measured by the percentage of subjects with anti-D and anti-T toxin concentrations ≥1.0 IU/ml, was demonstrated for both D and T and the selected endpoints.

REVIEW

To support the serological data obtained from trials V59P11 and V59P18 Novartis provided the validation reports for the D and T -b(4)--- performed at both the Novartis Clinical Serology Laboratories and --b(4)---------- (125300/0 module 5.3.1.4). After initial review of these validation reports, there was considered to be insufficient data/information to adequately assess the validity of the D and T -b(4)-----performed at - b(4)---------- in support of the pivotal trial V59P18. The additional information required for a complete review of the --b(4)---------- D and T -b(4)----- validation reports was conveyed to Novartis via a DI letter dated November 17, 2008 and in response to these comments, Novartis submitted an information amendment to the BLA (125300/0.3) on December 19, 2008. The comments put forth by CBER (in bold) and a summary of the responses provided by Novartis were as follows:

In regards to Clinical Trial V59P18

9. **The diphtheria and tetanus immunogenicity data generated in this trial and submitted in this application were obtained from -b(4)---------performed and analyzed at --b(4)----------. After preliminary review of these validation reports, there is considered to be insufficient information to allow adequate assessment of the validity of each -b(4)----- and the data generated from these assays. Specific comments with regard to the --b(4)---------- diphtheria and tetanus -b(4)------ validation reports are as follows:**

While not inclusive, please provide the following types of information and data in the validation reports:

a The source of the diphtheria and tetanus toxoids used in the assays,

Novartis provided a list of the critical D and T -b(4)----- reagents used in assay validation runs performed from 1994-1998, 2003-2004 as well as those used for assay re-assessment in 2006 (Attachment Q9-1, Tables 3.1, 3.2 and 3.3). This information included the source and concentration of both the D and T -b(4)---- antigens and reference sera.

b A detailed description of how critical reagents were qualified with accompanying data,

To address this comment Novartis provided --b(4)---------- SOP b(4)0003-07 "Qualification of New Reagents and Managing Change for Other Significant Components for -b(4)---- and Other Assays." This SOP, which had been revised in October 2007, detailed how new lots of reagents used in -b(4)---- (*i.e.*, antigen, --b(4)--, secondary antibody, in house reference and control sera) were qualified and indicated the acceptance criteria used in the qualification of each reagent.

c More detailed information on the methods used to calculate the IU/ml of clinical samples with a sample calculation included,

In response to this comment, Novartis referred back to the short description of the calculation of titers (Section 15) in the original D and T validation reports as well as the methodology outlined in the ----b(4)---------- This paper identified the reference line units methodology as the most sensitive means by which to calculate -b(4)---- antibody concentrations and lent support to --b(4)---------- use of this method in the determination of anti-D and anti-T antibody concentrations. In addition, Novartis submitted --b(4)---------- SOP b(4)006-08 "Data Acquisition and Analysis" (Attachment Q1-7) and SOP -b(4)-007-09 "Validation of Serological Results" (Attachment Q1-4) in response to CBER's request for more detailed information on the calculation of antibody concentrations.

SOP -b(4)-006-08, which was last revised in April 2008, outlines the standard procedures used by --b(4)---------- for the acquisition and analysis of -b(4)--- data. This SOP contains information on the software settings used for data analysis, the criteria employed to handle -b(4)--- data (e.g. masking of data points) and a sample calculation of how reference lines were generated and subsequently used to determine D and T antibody concentrations.

SOP b(4)007-09, revised in February 2007, provides the criteria employed by -b(4)---------- for interpretation of the serological data obtained from samples collected during clinical trials. Specifically, this SOP includes: (i) the general assay acceptance criteria; (ii) the acceptance criteria used for --b(4)---- data and (iii) the criteria used to assess individual sample acceptance.

d The concentration of the control low, medium and high positive human serum samples routinely used in the diphtheria and tetanus --b(4)----

To address this comment, Novartis supplied Table 3 in Attachment Q9-2 which contained the current values used for the D and T -b(4)-- reference slopes and controls.

e The specific IU/ml of the diphtheria and tetanus serum samples used to determine precision.

Novartis provided precision data from both the initial assay validation performed between 1994 and 1998 and re-assessment of -b(4)-- validation done in 2006. For the original validation of the D and T ---b(4)---------- examined reproducibility (i.e., a single assay performed by b(4) operators on b(4) different days) and intermediate precision (i.e., single assay performed by b(4)operators on different days). In Attachment Q9-3, Novartis supplied a series of tables with raw precision data that included the concentration (IU/ml) of all serum samples tested. Likewise, the antibody concentrations of the serum samples used to re-assess T -b(4)-- precision were provided by Novartis in Attachment Q1-14 along with a summary of the results obtained (Attachment Q1-14). Re-assessment of the precision of the D-b(4)-- had not been completed at the time of this amendment submission (125300/0.3) so the concentration of serum samples to be used for the evaluation of precision could not be provided.

10. For the determination of parallelism in the tetanus -b(4)--**, the absence of parallelism is considered to occur when the slope of an unknown sample is outside** ----b(4)-- **the predetermined acceptance range of the reference slope. Please provide the acceptance range.**

In -----b(4)---------- SOP b(4)007-09 "Validation of Serological Results" section 5.2.2.6 (Attachment Q1-4) the definition of the acceptance criteria for the standard reference slope was given. Specifically, this criterion was that "--b(4)----------
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To evaluate parallelism between the reference and clinical serum samples, the slope of the clinical sample was compared to the acceptance range of the standard slope. -b(4)-
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----------. In addition, Novartis submitted Table 3 (Attachment Q9-2) that contains the current values for the reference and control slopes used in the D and T -b(4)--

11. For the evaluation of specificity for the diphtheria and tetanus -b(4)--**, an unrelated antigen appears to be included as a control and is the scale of the scale of the Y-axis. Please clarify.**

In the amendment, Novartis supplied a detailed description of the assays used to demonstrate the specificity of the D and T -b(4)--. These assays involved incubation of

different concentrations of either D or T antigens with immune sera from D or T vaccines followed by application of these solutions in the D or T ---b(4)----------
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----------. These were the results seen in both the D and T -b(4)--- (see graphs below) which supported the conclusion, namely that the -b(4)--- showed a high level of specificity.

[b(4)]

12. Please explain why in neither the diphtheria nor tetanus -b(4)---- validation reports has pre-determined acceptance criteria been provided for linearity, precision and accuracy.

Novartis stated that at the time --b(4)---------- originally validated the D and T -b(4)-- (1994-1998), it was not yet common practice to employ pre-set acceptance criteria. However, --b(4)---------- did target a 20% CV acceptance criteria for all -b(4)--- which were in keeping with general standard used by the industry at that time. Novartis also indicated that in the "Guidance for Industry/Bioanalytical Method Validation" issued by the FDA in May 2001 the acceptance criteria for precision was defined as a CV $\leq$ 15% except at the LLOQ where a CV $\leq$ 20% was acceptable while accuracy acceptance criteria was defined as a mean value within 15% of the actual value except at the LLOQ where a 20% deviation was acceptable. In addition, Novartis stated that while neither the FDA or ICH guidelines specified an acceptance criterion for linearity, a $R^2 = 0.99$ was generally considered a good indication of linearity.

13. The assay drift data provided in both the diphtheria and tetanus validation reports were obtained between 1997 and 1998. Please provide more recent data to indicate that the assays still perform in a stable manner.

To address the question of assay drift, Novartis supplied --b(4)---------- control charts for D and T -b(4)-- high, medium and low controls that spanned from 1999 to the present (Attachment Q13-1, see below). Novartis also included the raw data for the reference slopes and controls that were established at the same time as the clinical samples from study V59P18 were being tested. Since reference and control slopes all fell within

acceptance limits Novartis considered these results to be strong evidence to support D and T --b(4)----consistency over time.

[b(4)]

14. For both the diphtheria and tetanus --b(4)---- two dates are indicated for when the validation experimentation was performed: July 1994 – July 1998 and December 2003 – June 4004. Please confirm that the data presented in these reports is from the more recent validation testing.

In response to this comment, Novartis stated that the original D and T -b(4)-- validation occurred between 1994 and 1998 but that in 2003-2004 the validation report was amended due to the inclusion of additional experiments that addressed analytical run length.

Novartis submitted a second information amendment to CBER on January 12, 2009 which provided additional information related to the comments in CBER's deficiency letter dated November 17, 2008. This amendment (125300/0.4) included several documents that directly pertained to either the D or T ----b(4)-- namely: Attachment 1-2 "Report on accuracy re-assessment of Tetanus and ----------b(4)-- Attachment 1-3 "Work plan for the re-assessment of Tetanus -b(4)--"; Attachment 9-1 "Work plan for the re-assessment of Dip and Hib ----b(4)--" and Attachment 9-2 "Report on Dip and Hib -b(4)-- re-evaluation".

After review of the responses provided by Novartis in Amendment 125300/0.3, several additional D and T -b(4)-- validation questions/comments arose. These comments were conveyed to Novartis on January 15, 2009 and on February 6, 2009 Novartis submitted an information amendment (125300/0.5) in responses to these comments. Below are the comments put forth by CBER (in bold) and a summary of the responses provided by Novartis.

Study V59P18 Diphtheria and Tetanus -b(4)-- **Validation**

1. **In neither Attachment Q1-12 (Section 5.6 "Qualifying a new lot of in-house controls" SOP** -b(4)-- **003-07 "Qualification of New Reagents and Managing Change for Other Significant Components for** ---b(4)-- **and Other Assays") nor in Attachment Q9-2 "Reference values and acceptance range for currently used reference slopes and controls for diphtheria and tetanus** ----b(4)-- **are the specific concentrations (IU/ml) of the low, medium and high controls given. Please provide the titers of the low, medium and high control samples currently used in the diphtheria and tetanus** -b(4)--

In response to this comment, Novartis provided attachment Q1-1 which contained the concentration in IU/ml of the reference and the high, medium and low controls used in the current D and T -b(4)-- (see below)

[b(4)]

2. **In Attachment Q1-7 (SOP b(4)006-08 "Data Acquisition and Analysis") there is no indication as to the linear ran of the --b(4)---------- used in the diphtheria and tetanus -b(4)---- Please comment. Additionally in Appendix 3 and 4 of SOP b(4)006-08 (Dip and Tet -b(4)----- Settings) the minimum and maximum b(4) values for the calculation of titers are indicated as --b(4)----- however, in Section 15 "Calculation of Titers" in both the diphtheria and tetanus --b(4)-- validation reports the minimum and maximum b(4) values used for titer calculation are ---b(4)---------- Please comment.**

Novartis indicated that the dynamic range of the --b(4)---------- used was --b(4)--- to -b(4)--- ------ with a measurement range of --b(4)---------- and linearity of b(4) from --b(4)--------- (based on the validation results supplied by the manufacturer). Novartis also acknowledged that the minimum and maximum b(4) values indicated in the original validation summary report were in error. The correct minimum and maximum b(4) values used to calculate antibody titers were ---b(4)---------- values and Novartis indicated that the validation summary reports would be revised to reflect this change.

3. **In response to CBER's question as to the use of pre-determined acceptance criteria (Question #12 in the DI letter), Novartis responded that at the time the** ---b(4)-- **were established and validated at --b(4)---------- (1994-1998) it was not common practice to establish pre-set acceptance criteria. However, Novartis did indicate that a CV of 20% was generally applied to evaluated validation parameters and that --b(4)---------- targeted the 20% CV acceptance criteria for all** ----b(4)-- **Later in their response Novartis stated that "in May 2001, FDA issued a "Guidance for Industry/Bioanalytical Method Validation" that defined acceptance criteria for precision, stating that the coefficient of variation should not exceed 15%, except for the lower limit of quantitation (LLOQ) where a CV of 20% is deemed acceptable and for accuracy, saying that the mean value should be within 15% of the actual value except for the LLOQ where a deviation of 20% is acceptable. Neither FDA nor ICH guidelines specify a general acceptance criterion for linearity but a coefficient of correlation of R2 = 0.99 is generally accepted as indicating good linearity." In the recent re-qualification of the tetanus --b(4)---------- (Attachment Q1-13, "Qualification of --b(4)---------- for Performance of Tetanus & Pertussis Antibody -b(4)--" dated June 27, 2006) the pre-specified acceptance criteria for precision was indicated as -b(4)-- Please confirm and justify the acceptance criteria for precision currently used by ----b(4)---------- for the tetanus** -b(4)--

Novartis acknowledged that the pre-specified acceptance criteria --b(4)---------- used for precision was wider than that recommended in the "Guidance for Industry/Bioanalytical Method Validation" but provided a 2000 paper by --b(4)---------- This publication advocates a higher acceptance limit ----b(4)----------. For this reason, the authors of this paper feel a more lenient target acceptance criteria would be more appropriate.

In addition to the justification Novartis provided for a broader acceptance criteria for precision, Novartis also indicated that the precision results of the T -b(4)---- in the 2006 re-assessment of validation was ≤ 20% (see below and Amendment 125300/0.4 Attachment 1-2 "Report on accuracy re-assessment of Tetanus and Pertussis -b(4)--- Likewise, in the preliminary report of the 2008 re-evaluation of the D -b(4)----, assay

precision was also shown to be ≤ 20% (see below and Amendment 125300/0.4 Attachment 9-2 "Report on Dip and Hib --b(4)--- re-evaluation".)

[b(4)]

4. **In the original validation report no pre-set acceptance criteria was given for accuracy in the tetanus --b(4)--; however, in the 2006 re-qualification report (Attachment Q1-13) and acceptance criteria of -b(4)-- fold-difference between the measured and nominal concentration was specified for accuracy. As indicated above, Novartis also noted that "in May 2001, FDA issued a "Guidance for Industry/Bioanalytical Method Validation" that defined acceptance criteria for precision, stating that the coefficient of variation should not exceed 15%, except for the lower limit of quantitation (LLOQ) where a CV of 20% is deemed acceptable and for accuracy, saying that the mean value should be within 15% of the actual value except for the LLOQ where a deviation of 20% is acceptable." Please comment and justify the use of a -b(4)-- fold difference as the acceptance criteria for accuracy in the tetanus --b(4)--. Have any data on the accuracy of high and medium titer serum samples become available since the 2006 re-qualification of the tetanus --b(4)---? Please comment.**

Novartis acknowledged that an acceptance criterion of -b(4)-- fold difference was used to assess accuracy in the 2006 --b(4)---------- re-qualification report rather than that recommended in the "Guidance for Industry/Bioanalytical Method Validation". To justify the use of the broader acceptance criterion, Novartis referred to the publication by ---b(4)----------

Novartis also provided a 2008 summary of the re-assessment of the accuracy of the T -b(4)---. In this report high, medium and low control samples were assayed for accuracy. From the data provided (see below) both the high and medium samples were within 15% of the actual value and the low control was within 20% of its nominal value. Only the sample that was below the cut-off value of the assay was found to be greater than 20%. Novartis indicated that these data were all within the more stringent accuracy

criterion set forth in the "Guidance for Industry/Bioanalytical Method Validation" and therefore supported the accuracy of the T -b(4)---

[b(4)]

V59P18 Clinical Serology Results

5. **For the Diphtheria immunogenicity results there appears to be a discrepancy between the GMTs recorded for Groups I and III at Day 31 and Group II at Day 61 (Table 14.2.1.31) and the GMT data presented in Table 14.2.1.18 and Table 14.2.1.25. Please comment.**

Novartis indicated that the model used for statistical analysis of the three tables in question involved the establishment of the diphtheria GMT by exponentiation of the least square means of the logarithmically transformed (base10) titers obtained from an analysis of covariance (ANCOVA) which included the log10 transformed pre-vaccination diphtheria titer as the covariate and the vaccine group as a fixed effect. However, the vaccine groups applied to this model were not the same in each table. Specifically, Table 14.2.1.18 included only vaccine Groups I and III in the model, Table 14.2.1.25 Groups II and III and Table 14.2.1.31 all three vaccination groups.

Novartis further noted that the main factor that affected the adjusted GMTs in the tables was the inclusion of vaccination Group II. Group II received MenACWY prior to Tdap but because MenACWY contains a portion of the diphtheria toxin, the pre-Tdap diphtheria titers were higher which in turn affected the LS means calculated by ANCOVA. This factor influenced the GMTs presented in the three tables in question and thus accounted for the perceived discrepancy in the serological responses.

Study V59P11 Diphtheria and Tetanus -b(4)--- Validation

6. **Please provide details on how the IU/ml of clinical sera samples was calculated and include a sample calculation.**

Novartis indicated that in study V59P11, the concentration of anti-D and T antibodies (IU/ml) was calculated against a standard curve generated by four parameter curve fit. Briefly, six standards, whose concentrations were calculated against the D and T international standards, and three dilutions of each clinical sample were run in triplicate and the mean b(4) values for each sample dilution calculated. These results were then analyzed with --b(4)---------- software. After qualitative evaluation of the raw data this program selects one dilution for each clinical sample based on a pre-specified number of criteria. The b(4) value of a sample that meets all criteria is then applied to a

standard reference curve and the antibody concentration (IU/ml) of the sample interpolated with the dilution factor taken into account. The standard curve used in the establishment of anti-D and T antibody concentrations was generated by four parameter logistic analysis of the reference standards which have been qualified against the international D and T standards. In addition to the description of the methodology used to establish the antibody concentrations of clinical samples Novartis also provide a sample calculation used in the T--b(4)-- (Amendment 125300/0.5 AttachmentQ6-1 "Example of Calculation").

7. Please provide information on the qualification of new reagents used in the diphtheria and tetanus --b(4)---

---b(4)--- ---------------------------------

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8. To confirm that the diphtheria and tetanus --b(4)----performed in a stable manner over time please provide chart records for each -b(4)--- that includes the time over which the clinical trial was run (April 2006 to May 2007).

Novartis supplied both the chart records and the raw data for the D and T -b(4)-- which included the period when the clinical samples were tested (see below and Amendment 125300/0.5 Attachment Q8-1 "Diphtheria and Tetanus -b(4)--- Raw Data"). Novartis also noted that aside from one point in the T -b(4)-- were a -b(4)-- was re-tested but not validated all the controls in both the D and T --b(4)-- fell within the predefined range which supported the conclusion that the assays were stable over time.

[b(4)]

RECOMMENDATION:

The information submitted for review of the Diphtheria and Tetanus -b(4)---(125300/0.0 original BLA and 125300/0.4 "Re-assessment of Diphtheria and Tetanus -b(4)-- Validation") as well as Novartis's responses to the comments and questions raised in the review of the material submitted (125300/0.3 and 125300/0.5) are considered adequate. Based on all the information provided, the Diphtheria and Tetanus -b(4)---- used to evaluate the immunogenicity of Tdap given alone or concomitantly with MenACYW in the phase III supportive trial, V59P11, and in V59P18, the pivotal phase III trial are acceptable. There are no outstanding concerns.