Protocol B3451002 Amendment 4

A PHASE 2b, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE SAFETY AND EFFICACY OF *STAPHYLOCOCCUS AUREUS* 4-ANTIGEN VACCINE (SA4Ag) IN ADULTS UNDERGOING ELECTIVE OPEN POSTERIOR INSTRUMENTED SPINAL FUSION PROCEDURES WITH MULTILEVEL INSTRUMENTATION

Statistical Analysis Plan (SAP)

Version: Amendment 5

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LIST OF ABBREVIATIONS

Abbreviation	Term
AE	adverse event
AIDS	acquired immunodeficiency syndrome
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ASA	American Society of Anesthesiologists
BMI	body mass index
BSI	bloodstream infection
CBER	Center for Biologics Evaluation and Research
CC	clonal complex
CCI	Charlson Comorbidity Index
CDC	Centers for Disease Control and Prevention (United States)
CI	confidence interval
ClfA	clumping factor A
cLIA	competitive Luminex immunoassay
CMH	Cochran-Mantel-Haenszel
CP5	capsular polysaccharide serotype 5
CP8	capsular polysaccharide serotype 8
CRF	case report form
CSR	clinical study report
DMC	data monitoring committee
EAC	event adjudication committee
ECG	electrocardiogram
e-diary	electronic diary
FDA	Food and Drug Administration (United States)
GMFR	geometric mean fold rise
GMT	geometric mean titer
ICU	intensive care unit
ISA	invasive *Staphylococcus aureus*
IST	independent statistical team
LLOQ	lower limit of quantitation
LSM	least-squares mean
MAR	missing at random
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
MNAR	missing not at random
MntC	manganese transporter C
MOF	multiple-organ failure
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-sensitive *Staphylococcus aureus*
NHSN	National Health Surveillance Network

Abbreviation	Term
NNB	number needed to benefit
NNH	number needed to harm
NNT	number needed to treat
OPA	opsonophagocytic activity
PDI	protocol-defined infection
PP	per-protocol
P_u	probability that a protocol-defined infection with insufficient microbiological testing to confirm or deny *S aureus* infection is truly an *S aureus* infection
RBC	red blood cell
RCDC	reverse cumulative distribution curve
RR	relative risk
SA4Ag	*Staphylococcus aureus* 4-antigen vaccine
SAE	serious adverse event
SAP	statistical analysis plan
Spa	*Staphylococcus aureus* protein A
SSI	surgical-site infection
ST	sequence type
VE	vaccine efficacy
WGS	whole-genome sequencing

1. AMENDMENTS FROM PREVIOUS VERSION(S)

Document	Version Date	Summary of Changes and Rationale
Amendment 5	22 August 2019	Section 5.4.1 Remove the definition of evaluable immunogenicity population and replace it with the definition of mITT immunogenicity population. Section 5.6.1; Section 5.6.2; Remove the text of "evaluable immunogenicity". Section 6.3.1; Table 5 Update the Charlson Comorbidity Index to correct definition. Section 8.1.3.5 Updated sampling tables to revised number of cases and controls; harmonize/clarify covariates to be used in 2 immunogenicity models; allowed for scheduled visit data to be used in place of unscheduled visits when occurring close to onset of infection. Section 8.2.1 Modified the definition of 'high-enrolling investigator sites'. Section 8.2.3.1; Table 22 Remove the text of "evaluable immunogenicity"; change other text for consistency. Section 8.2.3.5; Table 26 Update analysis population for all analyses. Remove unnecessary graphs.
Amendment 4	27 March 2019	Version to include plans for limited immunogenicity testing following the interim analysis in December 2018. Section 1.1.1.- modified to describe immunogenicity populations. Removed mITT immunogenicity population. Section 6.3.5 – modified to describe immunogenicity endpoints Section 8.1.3.5 – entire section replaced to describe immunogenicity methodology Section 8.1.3.6 – entire section on methodology for colonization/immunogenicity removed Section 8.2.3.5 – entire section replaced to describe immunogenicity analyses Section 8.2.3.6 – entire section on analyses for colonization/immunogenicity removed
Amendment 3	21 February 2018	Version to include changes from protocol amendment 4: Section 2.1: Changed the number of subjects to 6000 subjects with a total target of 48 primary endpoint *S aureus* cases. Section 3: Updated the interim analysis and futility to be performed after accumulating 24 PP adjudicated cases. Updated the outcomes to be reported to the sponsor. Changed the target number of cases to 48.

Document	Version Date	Summary of Changes and Rationale
		Section 4.1: Updated statistical hypotheses to VE ≤20% vs VE >20%. Section 4.2: Updated the conditional power-based futility procedure to be performed after accumulating approximately 10, 15, and 24 cases. Recalculated the information in Table 1 assuming VE=70%. Updated the interim analysis description. Section 5.2: added explanatory text regarding difference in surgery window compared to protocol. Section 5.4.1: harmonized surgery window for immunogenicity and efficacy populations Section 5.6.2: clarified protocol deviations excluding subjects from analysis Section 6.3.1: defined 'baseline' for colonization analyses, to be consistent with protocol. Section 6.3.2: Clarified data handling rules for collection of intraoperative blood loss; moved 'surgeon speciality' to the category where multiple responses are possible. Section 6.3.5: Added immunogenicity endpoints with increases of 2- and 8-fold rises from Visit 1 to postvaccination visits. Section 8.1.1: Updated the null hypothesis and changed the binomial distribution with probability parameter 0.444. Updated the interim analysis and final analysis to be performed on 24 PP cases and 48 PP cases collected. Recalculated information in Table 11. Referenced updated estimate of attack rate. Section 8.1.2.1: Removed the differences between vaccine groups in proportions for Tier 3 AE. Section 8.1.2.3: Updated to compare vaccine groups using the method of Miettinen and Nurminen. Section 8.1.3.5: Added calculation of proportions of subjects with 2-fold and 8-fold rises in titer. Section 8.1.3.6: used protocol definition of baseline for colonization. Section 8.2.1: Updated the subgroup analysis to be performed for all primary and secondary endpoints. Deleted the overrun analysis for subgroups. Changed Table 12 for the exploratory endpoints. Section 8.2.2.1: Updated Tier 2 to Tier 2-3 in Table 16. Section 8.2.3.1: Deleted e-diary completion in Table 20. Section 8.2.3.4: Updated the number of cases for the primary endpoint to 48 cases.
Amendment 2	21 April 2016	Version to include changes from protocol amendment 3:

Document	Version Date	Summary of Changes and Rationale
		Section 2.1: updated surgery description in study design. Section 2.2: updated surgery description in study objectives Section 3: described futility procedure Section 4.2: added details of case totals which would trigger the futility rules Section 5.1: defined restricted mITT population in additional to general mITT population Section 5.2: modified acceptable window for index surgery up to 90 days postvaccination Section 6.1: updated surgery description in endpoints; added exploratory endpoints for postoperative BSI and/or deep incisional or organ/space SSI or superficial SSI, attributable to any pathogen. Section 6.2.3: clarified how information on Grade 4 reactogenicity events will be presented. Section 6.3.2: added collection of surgery information for reoperations/revisions of index surgery; added implant material as a variable. Section 8.1.1: added technical details of futility procedure, including operating characteristics of chosen rule Section 8.1.3.1: added elapsed days between vaccination and index surgery. Section 8.1.3.2: added analysis of requirement for reoperations/revisions of index surgery and implant material. Section 8.2.1: added analysis of postoperative BSI and/or deep incisional or organ/space SSI or superficial SSI, attributable to any pathogen; added analyses of endpoints in the restricted mITT population. Section 8.2.3.1: added analysis of elapsed days between vaccination and index surgery.
Amendment 1	26 October 2015	List of Abbreviations: Added entries for AIDS, ECG, ICU, and SAP, and deleted the entry for FBI, for consistency with the abbreviations used in the document. Section 5.2: Date of birth will be recorded as 01 July for all subjects in Germany because of confidentiality requirements. Therefore, age restrictions for the per-protocol population are expanded slightly for Germany. Section 5.4.1: Expanded the window for baseline immunogenicity sera to up to 7 days before vaccination (per protocol Section 9.3.4.1). The window for the day-of-discharge blood sample is restated as -3 to +3 days around the day of discharge, inclusive (per protocol Sections 6.3.1.3 and 9.3.4.1).

Document	Version Date	Summary of Changes and Rationale
		Section 6.1.3: Additional exploratory endpoints were added as per Amendment 2 to the protocol: the association between the *S aureus* strain colonizing the subject and the *S aureus* strain recovered from the infection was added as an exploratory endpoint, and healthcare utilization data were added as an exploratory endpoint. Also, the exploratory endpoint for antibody thresholds in antibody assays that assess functional activity was revised to omit use of the FBI assay for ClfA, because FBI was removed from the protocol. Section 6.1.4: Given that a subject may progress through worsening infection, the following is added for clarity: "A subject who has 1 infectious episode that worsens, eg, from superficial SSI to deep incisional or organ/space SSI or to BSI, will be counted in all endpoints for which he/she qualifies. However, no subject may be counted more than once in any endpoint analysis." Section 6.2.1: Deaths will be categorized as associated with *S aureus* infection or not associated with *S aureus* infection. Section 6.2.4: Added intraspinal steroids to the list of concomitant products that will be summarized because it was added to the protocol (Section 5.4). Section 6.2.4: Number of days of antibiotic use was added to the safety endpoints, as per protocol Sections 2.4.1 and 9.4.2. Section 6.3.1: Added a source footnote for the ASA scale in Table 5. Section 6.3.3: Number of days in hospital and number of inpatient days in a rehabilitation facility were added to healthcare utilization, as per protocol Section 9.4.2. Section 6.3.4: Per Section 6.3.1.3 of protocol amendment 2, the discharge visit's colonization sample must be not more than 72 hours before discharge. Therefore, if 2 or more samples associated with postsurgery are obtained, only the colonization results from the latest visit will be eligible for analysis. Section 6.3.5: Per Section 6.3.1.3 of protocol amendment 2, the discharge visit's blood sample must not be more than 72 hours before discharge. Therefore, if 2 or more samples associated with postsurgery are obtained, only the assay results from the latest visit will be eligible for analysis. Section 6.3.5: Removed discussion of FBI assay because it was removed from the protocol. Also added that other assays that measure immune response may also be analyzed. Section 8.1.1: Added "The methods for missing-data sensitivity analyses are described in Handling of Missing Values." Section 8.1.1: Added sensitivity analyses for primary and secondary efficacy endpoints per recommendation of the data monitoring committee. These new analyses incorporate follow-up time.

Document	Version Date	Summary of Changes and Rationale
		Section 8.1.1: The formula for estimated efficacy was corrected from "1-hP(1-P)" to "1-hP/(1-P)." Section 8.1.2.1: Clarified text on Tier 2 analyses by noting that Tier 2 confidence intervals will be plotted. Also clarified the analysis of deaths, both overall and those associated with *S aureus* infection, as per protocol Section 9.5. Section 8.1.2.2: Added the text "'Non–microbiologically confirmed infection' will be further split into 'negative' and 'not done.'" The source is email correspondence from Gustavo Dayan dated 11 May 2015. Section 8.1.2.3: Added the statistical test for baseline assessment of fatigue, headache, muscle pain, and joint pain because postbaseline systemic events will be statistically examined. Section 8.1.2.4: Added the summary intervals for intraspinal steroids, as per protocol Section 5.4. Section 8.1.2.4: Added that days of antibiotic use will be summarized over the same 3 intervals as use of antibiotics and systemic steroids. Section 8.1.3.1: added "elapsed days from vaccination to index surgery" to the list of variables, because it is already in Section 6.3.1. Section 8.1.3.2: The text "Statistics will be compiled by fasting status" was changed to "Glucose statistics will be compiled by fasting status" to indicate that not all variables will be so analyzed. Section 8.1.3.2: The paragraph "Vaccine groups will be . . . blood loss" was deleted because the succeeding paragraph has the same details. Section 8.1.3.4: Expanded the window for collection of the baseline colonization swab to up to 7 days before vaccination, per protocol Section 9.3.4.1. Section 8.1.3.5: Expanded the window for collection of baseline immunogenicity sera to up to 7 days before vaccination, per protocol Section 9.3.4.1. Also removed reference to FBI assay because FBI assay was removed from the protocol. Section 8.1.3.6: Per team request, added analysis for examining the possible relationships among colonization at enrollment, postvaccination titers, and PDIs. Also clarified that proposed analyses will be performed separately for CP5/OPA and CP8/OPA. Also added the analysis of maximum titer divided by baseline. Section 8.1.4: Added the phrase "any anatomical site" to specify the colonization status being discussed. Section 8.2: Clarified how days are counted; ie, added the text "The day of surgery will be labeled Day 1. The days before surgery will be labeled Day -1, Day -2, etc."

Document	Version Date	Summary of Changes and Rationale
		Section 8.2.1: For clarity, "high-enrolling sites" and "high–attack rate sites" were changed to "high-enrolling investigator sites" and "high–attack rate investigator sites." Section 8.2.1: Text about sensitivity analyses in Table 10 was removed, as it appears in Table 13. Text about sensitivity analyses in Table 11 was removed, as it appears in Table 13. Also, follow-up time was added to Table 13. Section 8.2.2.1: Table 14 text was changed in 2 places from "Assess safety of SA4Ag until surgery" to "Assess safety of SA4Ag from vaccination until surgery," to clarify the interval. Section 8.2.2.1: Revised Table 14 to clarify that SAEs and deaths will be analyzed over 3 intervals, as described in Section 6.2.1. Section 8.2.2.1: Added text about analyses of events contributing to ISA disease, which are adjudicated for *S aureus* infection, but are not efficacy endpoints, per protocol Sections 2.5 and 7.1.1.2. Section 8.2.2.3: The proportions of subjects with e-diary entries after surgery will be compiled. Per protocol Sections 4.1 and 7.2.1.1, the e-diary need not be completed on Day 10 if that is day of surgery, but for completeness such e-diary data will be summarized. Section 8.2.2.3: The proportions of subjects with surgery on Days 1, 2, 3, etc, after vaccination will be compiled. Per protocol Sections 4.1 and 7.2.1.1, the e-diary need not be completed on Day 10 if that is the day of surgery. For completeness, the distribution of days from vaccination to surgery will be compiled. Section 8.2.2.3: Added a row to Table 16 for baseline assessments of fatigue, headache, muscle pain, and joint pain over the previous month, because postbaseline systemic events will be statistically examined. Section 8.2.2.3: Text was added to Table 16 to clarify that the proportions of local and systemic events are proportions of maximum severity of the event over Days 1-10. Section 8.2.2.4: In Table 17, the statistical test for nonstudy vaccines was changed from CMH to the Fisher exact test, for consistency with Section 8.1.2.4. Also added to Table 17 a row for intraspinal steroids. Section 8.2.2.4: Statistical tests for "days of antibiotic use" were added to Table 17. Section 8.2.3.1: Added "elapsed days from vaccination to index surgery" to the list of variables in Table 18, because it is already in Section 6.3.1. Section 8.2.3.2: A footnote that clarifies which variables are single level, multiple level, and numeric was added to Table 19. Also, a comment was added specifying that glucose variables will be compiled by fasting and nonfasting status. Section 8.2.3.3: All variables specified in Section 6.3.3 were categorized as

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		numeric or categorical in Table 20. Section 8.2.3.3: "Number of rehabilitation/physical therapy visits as an outpatient" added to Table 20 because it is mentioned in Section 6.1.3 and Section 6.3.3. Section 8.2.3.5: Revised text for exploratory analyses now that FBI assay was removed. Section 8.2.3.5: Added that GMT figures will have the footnote "Day 1 = day of vaccination." Section 8.2.3.6: Added a list of endpoints to Table 23 that would be associated with additional analysis described in Section 8.1.3.6, ie, colonization and immunogenicity analyses. Section 9: Added Blackwelder and Kramer references.
Original	23 April 2015	Not applicable (N/A)

2. INTRODUCTION

This statistical analysis plan (SAP) amendment for Study B3451002 is based on protocol amendment 4, dated 05 February 2018. Note: In this document, any text taken directly from the protocol is ***italicized***. Abbreviations are defined at first occurrence in this document.

2.1. Study Design

This is a Phase 2b, multicenter, parallel-group, placebo-controlled, randomized, double-blind study to evaluate Staphylococcus aureus 4-antigen vaccine (SA4Ag) safety and efficacy in the prevention of postoperative S aureus disease in adults aged 18 to <86 years who are undergoing elective open posterior instrumented spinal fusion procedures with multilevel instrumentation.

This is an event-driven study with a total target of 48 primary endpoint *S aureus* cases; it is anticipated that approximately 6,000 subjects will be enrolled globally to accumulate these 48 cases. However, the total enrollment number may vary based on the incidence rate of the primary endpoint, true underlying vaccine efficacy (VE), and potential early study stop for efficacy or futility.

There are 5 scheduled study visits and 1 scheduled telephone contact during 6 to 8 months of subject participation. Study-eligible subjects who provided consent will be randomized in a 1:1 ratio to receive a single dose of SA4Ag or placebo at Visit 1, which occurs 10 to 60 days prior to undergoing elective open posterior instrumented spinal fusion procedures with multilevel instrumentation (index surgical procedure). Visit 2 is a longitudinal visit and is to monitor the index hospital admission period from the day of surgery (Day 1) until the day of discharge. A telephone contact with the subject will occur on Day 21, while postoperative evaluation study visits will occur on Day 42, Day 90, and Day 180 after surgery.

Following the index hospital admission, unscheduled visits will be conducted for assessment of suspected bloodstream infection (BSI) and/or surgical-site infection (SSI) events, and to assess hospitalization(s) subsequent to the index hospital admission.

2.2. Study Objectives

The study objectives and endpoints employ the following definitions:

Protocol-defined infection (PDI): *Postoperative infections that occur following elective spinal surgery, particularly those frequently caused by S aureus, will be prospectively evaluated in this study. PDI clinical and microbiological criteria are based upon Centers for Disease Control and Prevention (CDC) National Health Surveillance Network (NHSN) criteria; complete case definitions* (provided in Appendix 1 of the protocol) *will be applied for case identification and confirmation. Summaries of the PDIs that, when microbiologically confirmed as being due to S aureus, will contribute to study objectives and endpoints are provided below.*

- ***Bloodstream infection:*** *Clinical infection involving a recognized pathogen (eg, S aureus) cultured from 1 or more blood cultures, or a commensal organism cultured from 2 or more blood cultures, whether primary or secondary to infection at another site.*

- ***Surgical-site infection:*** *Infection at a surgical incision, whether involving the primary posterior incision or a secondary (eg, anterior) incision associated with the spinal fusion procedure itself or with the harvesting of autologous bone graft material. SSI is further classified as:*

  - ***Superficial SSI:*** *Infection involves only skin and subcutaneous tissue of the incision.*

  - ***Deep incisional SSI:*** *Infection involves deep soft tissues of the incision (eg, fascial and muscle layers).*

  - ***Organ/space SSI:*** *Infection involves any part of the body, excluding the skin incision, fascia, or muscle layers, that is opened or manipulated during the operative procedure. Specific to spinal fusion surgery,* ***osteomyelitis, vertebral disc space infection, meningitis****, and* ***spinal abscess without meningitis*** *when directly attributable to a surgical incision will be classified as organ/space SSIs. Likewise,* ***intra-abdominal infection****, when directly attributable to a secondary anterior intra-abdominal incision, and* ***joint and bursa infection****, when directly attributable to a surgical incision (eg, harvesting autologous bone), will be considered organ/space SSIs.*

- ***Invasive S aureus (ISA) disease:*** *ISA disease is defined as culture of S aureus from a normally sterile location, with clinical evidence of disease. With the exception of superficial SSI, all microbiologically confirmed S aureus PDIs* (defined in Appendix 1 of the protocol) *will be considered ISA disease.*

2.2.1. Primary Efficacy Objective

- *To assess the efficacy of SA4Ag in the prevention of postoperative S aureus BSI and/or deep incisional or organ/space SSI occurring within 90 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, in adults aged 18 to <86 years.*

2.2.2. Primary Safety Objective

- *To describe the safety and tolerability of a single vaccination of SA4Ag in adults aged 18 to <86 years undergoing elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, by measuring local reactions, systemic events, and adverse events (AEs).*

2.2.3. Secondary Efficacy Objectives

- *To assess the efficacy of SA4Ag in the prevention of postoperative S aureus BSI and/or deep incisional or organ/space SSI occurring within 180 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, in adults 18 to <86 years of age.*

- *To assess the efficacy of SA4Ag in the prevention of postoperative S aureus SSI occurring within 90 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, in adults 18 to <86 years of age.*

- *To assess the efficacy of SA4Ag in the prevention of postoperative S aureus SSI occurring within 180 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, in adults 18 to <86 years of age.*

2.2.4. Exploratory Objectives

- *To assess the efficacy of SA4Ag in the prevention of postoperative ISA disease occurring within 90 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, in adults 18 to <86 years of age.*

- *To assess the efficacy of SA4Ag in the prevention of postoperative ISA disease occurring within 180 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, in adults 18 to <86 years of age.*

- *To assess the efficacy of SA4Ag in the prevention of postoperative S aureus BSI and/or deep incisional or organ/space SSI occurring within 90 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, in adults 18 to <86 years of age, based on baseline S aureus colonization status.*

- *To assess the efficacy of SA4Ag in the prevention of postoperative S aureus BSI and/or deep incisional or organ/space SSI occurring within 180 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, in adults 18 to <86 years of age, based on baseline S aureus colonization status.*

- *To describe the immunogenicity of SA4Ag in adults aged 18 to <86 years undergoing elective open posterior instrumented spinal fusion procedures with multilevel instrumentation.*

- *To describe S aureus colonization in adults aged 18 to <86 years undergoing elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, before and after SA4Ag administration.*

- *To compare healthcare utilization data between vaccine groups.*

3. INTERIM ANALYSES, FINAL ANALYSES, AND UNBLINDING

A conditional power-based futility procedure[1] will be implemented during the earlier stages of primary endpoint case accrual. After accrual of approximately 10, 15, and 24 cases as confirmed by the event adjudication committee (EAC), there will be an assessment of the futility of the study. Only the primary endpoint, defined in Section 6.1.1, will be examined. The sponsor will remain blinded to the case count and will be notified only whether or not the futility threshold has been passed. If the threshold has been passed, then futility may be declared and the study may cease enrollment. Otherwise, enrollment will continue until the next check.

An interim analysis will be performed after accumulating 24 per-protocol (PP) cases that meet the primary efficacy endpoint as confirmed by the EAC. Only the primary endpoint, defined in Section 6.1.1, will be examined. The sponsor will be blinded to the case count, but one of the following outcomes will be reported to the sponsor: (1) lower 99.7% confidence limit on VE exceeds 20%; (2) lower 99.7% confidence limit on VE does not exceed 20%. If the study is not stopped for either futility or efficacy, then the study will continue to the accumulation of 48 cases. Once 48 PP cases have accumulated, SA4Ag will be compared to placebo in analysis of the primary endpoint.

It is anticipated that the immunogenicity analyses will be conducted following database lock. However, an interim immunogenicity analysis may be performed on a subset of subjects prior to the 48th primary endpoint case. The analysis would support regulatory agency interactions for expediting the clinical development of SA4Ag.

The futility checks, the interim efficacy analysis and the interim immunogenicity analysis (if performed) will be compiled by an independent statistical team (IST), in collaboration with the data monitoring committee (DMC).

The sponsor will obtain a snapshot of the database at or near the adjudication of the 48th PP case. After the adjudication, the sponsor will be allowed to unblind the existing snapshot; however, no unblinding information will be communicated to any investigator or subject. The sponsor will then create the unblinded tables, figures, and listings from this unblinded database snapshot. A supplementary clinical study report (CSR) will be produced after all subjects have completed the study.

A database snapshot will also be obtained at or near the adjudication of the 24th PP case. If the study is stopped, then an initial and a supplementary CSR will be created as described above. If the study is not stopped, then no unblinding will occur and the database snapshot will be deleted.

4. HYPOTHESES AND DECISION RULES

4.1. Statistical Hypotheses

SA4Ag will be compared to placebo testing the hypotheses H_0: VE ≤20% vs H_a: VE >20%.

4.2. Statistical Decision Rules

The conditional power-based futility procedure will be performed after accumulating approximately 10, 15, and 24 cases. Only the primary endpoint, defined in Section 6.1.1, will be examined. The power of the study to reject the original null hypothesis, conditional upon the results accumulated so far, will be calculated. Futility may be declared and the study may cease enrollment, if the conditional power falls below 20%. For the planned case totals, the number of vaccine cases which would lead to conditional power below this threshold are shown in Table 1.

Table 1. Conditional Power at Planned Futility Analyses

Total Cases	Minimum SA4Ag Cases for futility	Estimated VE (%)	Conditional Power[a]
10[b]	7	-133% (-1298%, 46.7%)	6.9%
15	9	-50% (-412%, 52.3%)	19.4%
24	11	15.4% (-105%, 66%)	16.1%

VE = vaccine efficacy.

a. Power of the study to detect VE > 20% at the final analysis at 48 cases, conditional upon the cases observed so far, and assuming VE = 70% following the futility check.

b. For 10 total cases, the SA4Ag case criterion and conditional power were calculated before protocol amendment 4 based on the previous study hypothesis.

Conditional power will be evaluated under the assumption that after the futility check, vaccine efficacy is 70%, as in the main study design assumptions. For operational reasons, the futility checks may occur at slightly different case totals from those planned; in that case, the futility rule of less than 20% conditional power will apply.

The interim efficacy analysis will be performed after accumulating 24 PP cases. Only the primary endpoint, defined in Section 6.1.1, will be examined. The probability of the number of vaccine cases will be calculated using the binomial distribution with 24 trials and $\pi = 0.444$ because, under the null hypothesis and 1:1 randomization, the probability that a selected case is from the SA4Ag cohort is 0.444. If the number of vaccine cases is 3 or fewer, then the null hypothesis will be rejected in favor of the vaccine. A 99.7% confidence interval (CI) will be obtained for VE.

Once 48 PP cases have accumulated, SA4Ag will be compared to placebo in the primary endpoint. SA4Ag will initially be compared to placebo, testing the hypothesis H_0: VE ≤20% vs H_a: VE >20%. SA4Ag will be deemed efficacious if it has 14 cases or fewer out of a total of 48 cases (point estimate of VE ≥58.8% and 2-sided lower 95.1% confidence limit >20%).

If study enrollment is stopped for efficacy at the interim analysis, events collected subsequent to the interim analysis will be reported in a supplement in a similar manner to that described by Whitehead[2] for overrunning data. Otherwise, the study will continue to the accumulation of 48 cases. Events collected after the 48th case (or 24th case, if the study ends after the interim analysis) will be analyzed using the method described by Whitehead. This analysis will be considered supplemental. The conclusion from the interim/final analysis will serve as the primary basis for efficacy conclusions.

The interim immunogenicity analysis, if performed, will be limited to group-level statistics and will be conducted by an unblinded team, independent of the sponsor, in order to maintain blinding. All immunogenicity endpoints are defined as exploratory and the study will not be prematurely terminated or altered in any way (eg, no design or operational changes) from the interim immunogenicity analysis; therefore, no type 1 error adjustment is proposed.

5. ANALYSIS SETS

Analysis populations are defined for per-protocol (PP) efficacy, modified intent-to-treat (mITT) efficacy, safety, mITT immunogenicity, and mITT colonization.

5.1. Modified Intent-to-Treat Efficacy Populations

To be included in the mITT efficacy population, a subject must be vaccinated and undergo spinal surgery. Subjects will be assigned to the investigational product to which they were randomized (SA4Ag or placebo).

Subjects who do not undergo spinal surgery following vaccination will not be included in the mITT efficacy population. If any efficacy data are collected in these subjects, it will be listed separately.

A subset of the mITT efficacy population, referred to as the restricted mITT efficacy population, will also exclude subjects with a pre-existing infection, or malignancy, or acute or emergency trauma, at any time from enrollment up to and including the time of the index surgery. This population will be used for selected efficacy analyses only (see Section 8.2.1).

5.2. Per-Protocol Efficacy Population

To be included in the PP efficacy population, a subject must:

- meet all eligibility criteria,
- be vaccinated as randomized,
- undergo the index surgery 9 to 90 days (inclusive) after vaccination (this is expanded from 10 to 60 days as stated in the protocol, which represents a more restricted window with the aim of ensuring the per-protocol population timeframe used in the analyses is respected),
- undergo surgery consistent with study-defined criteria for the index surgery,

- not have the index surgery modified to be a staged procedure on separate days,
- have no major protocol violations before reporting of the suspected *S aureus* infection, and
- have no infection or malignancy identified at the index surgical procedure.

Because of confidentiality requirements in Germany, only year of birth can be provided; therefore, the date of birth will be reported as 01 July of the year of birth. Thus, ordinary age calculations might flag a subject as outside the permitted age range of 18 to <86 years. Subjects from Germany who enroll before 01 July and have a calculated age of 17 years may still be included in the PP efficacy population if all other criteria are fulfilled.

Subjects from Germany who enroll after 01 July and have a calculated age of 86 years may still be included in the PP efficacy population if all other criteria are fulfilled.

Subjects will be assigned to the investigational product to which they were randomized (SA4Ag or placebo).

5.3. Safety Population

All subjects who receive investigational product will be included in the safety population. Subjects will be assigned to the investigational product they actually received.

5.4. Other Populations

5.4.1. mITT immunogenicity population

The mITT immunogenicity population comprises all subjects who were vaccinated and have at least 1 valid and determinate post-vaccination immunogenicity result. Subjects are assigned to the investigational product that they are randomized to.

5.4.2. mITT Colonization Population

The mITT colonization population includes all randomized subjects who had at least 1 valid colonization swab sample result.

5.5. Vaccine Group Misallocations

Subjects who are randomized but receive the wrong investigational product will be included under their randomized vaccine group for mITT efficacy, mITT immunogenicity, and mITT colonization analyses, but will be included in the group for the investigational product they actually received for safety analyses.

Subjects who receive SA4Ag or placebo but do not undergo index surgery will be included in the safety analyses, but will be excluded from all efficacy, immunogenicity, colonization, and healthcare utilization analyses.

5.6. Protocol Deviations

The following protocol deviations will exclude affected subjects from the indicated populations:

5.6.1. Protocol Deviations Occurring Before Randomization

Subjects who do not meet all eligibility criteria will be excluded from the PP efficacy population.

5.6.2. Protocol Deviations Occurring After Randomization

1. Vaccinated subjects who do not undergo the planned index surgery will be included only in the safety population.

2. Nonvaccinated subjects will be excluded from all populations.

3. Subjects will be excluded from the PP efficacy population for any of the following reasons:

    a. Not vaccinated as randomized

    b. Undergoing index surgery 8 days or sooner after vaccination *or* 91 days or later after vaccination

    c. Not meeting 1 or more of the study-defined criteria for the index surgery

    d. Having 1 or more major protocol deviations expected to affect efficacy or immunogenicity, prior to the onset date of suspected *S aureus* infection as determined by a medically qualified clinician at the sponsor

    e. Suspected or confirmed infection or malignancy identified during the index surgical procedure

6. ENDPOINTS AND COVARIATES

6.1. Efficacy Endpoint(s)

All efficacy endpoint events will be evaluated by the EAC. The EAC will determine whether or not the event meets the case definition criteria, including diagnosis and onset date relative to surgery. Confirmed cases will contribute to the efficacy analyses.

Windows described as “within 90 days” (or “within 180 days”) mean that infections with onset occurring on Days 1-90 (or Days 1-180), inclusive, will be included. Day 1 is the day of the index surgery.

6.1.1. Primary Efficacy Endpoint

The number of subjects in each vaccine group with postoperative S aureus BSI and/or deep incisional or organ/space SSI occurring within 90 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, as confirmed by the EAC.

6.1.2. Secondary Efficacy Endpoints

- *The number of subjects in each vaccine group with postoperative S aureus BSI and/or deep incisional or organ/space SSI occurring within 180 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation.*

- *The number of subjects in each vaccine group with postoperative S aureus SSI occurring within 90 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation.*

- *The number of subjects in each vaccine group with postoperative S aureus SSI occurring within 180 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation.*

6.1.3. Exploratory Endpoints

- *The number of subjects in each vaccine group with postoperative ISA disease occurring within 90 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation.*

- *The number of subjects in each vaccine group with postoperative ISA disease occurring within 180 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation.*

- *The number of subjects in each vaccine group with postoperative S aureus BSI and/or deep incisional or organ/space SSI occurring within 90 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, based on baseline S aureus colonization status.*

- *The number of subjects in each vaccine group with postoperative S aureus BSI and/or deep incisional or organ/space SSI occurring within 180 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation, based on baseline S aureus colonization status.*

- The number of subjects in each vaccine group with postoperative BSI and/or deep incisional or organ/space SSI or superficial SSI, attributable to any pathogen, occurring within 90 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation.

- The number of subjects in each vaccine group with postoperative BSI and/or deep incisional or organ/space SSI or superficial SSI, attributable to any pathogen, occurring within 180 days of elective open posterior instrumented spinal fusion procedures with multilevel instrumentation.

- *The proportion of subjects at each time point who achieve specific antibody thresholds in antibody assays that assess functional activity. These may include, for example, opsonophagocytic activity (OPA) assays using an S aureus capsular polysaccharide serotype 5 (CP5)-expressing strain and a capsular polysaccharide serotype 8 (CP8)-expressing strain, and competitive Luminex immunoassays (cLIAs) for clumping factor A (ClfA) and manganese transporter C (MntC). Additional exploratory assays to measure immune responses may also be conducted on all 4 antigens.*

- *The proportion of subjects at each time point who achieve specific antibody thresholds to all 4 antigens both individually and combined using immunological assays.*

- *The number of subjects in each vaccine group determined to be colonized with S aureus on each occasion swab samples are collected.*

- *From subjects with S aureus infection, the association between S aureus strain or strains identified as colonizing the subject and the S aureus strain or strains recovered from the infection.*

- *Healthcare utilization data, including days in hospital, days in an intensive care unit (ICU), discharge disposition, inpatient days in rehabilitation facilities or skilled nursing facility after discharge, number of hospital readmissions and reoperations, days of antibiotic use, and number of rehabilitation/physical therapy outpatient visits.*

6.1.4. Multiple Protocol-Defined Infections from the Same Subject

If a subject has the same PDI more than once in a specified time window, then only the first occurrence will be included in the analysis.

A subject who has 1 infectious episode that worsens, eg, from superficial SSI to deep incisional or organ/space SSI or to BSI, will be counted in all endpoints for which he/she qualifies. However, no subject may be counted more than once in any endpoint analysis.

A subject may be included in more than 1 endpoint in a given time window. For example, a subject with BSI would be counted for both "BSI and/or deep incisional or organ/space SSI" and "ISA disease" endpoints.

A subject with 2 different PDIs in a specified time interval may be included in more than 1 endpoint analysis for that time interval. For example, a subject with superficial SSI within Days 1-90 and ISA disease within Days 91-180 would be included in 2 analyses for the Days 1-180 interval: "SSI occurring within 180 days" and "ISA disease within 180 days."

Statistical methodology for efficacy is described in Section 8.1.1.

6.2. Safety Endpoints

6.2.1. Adverse Events and Serious Adverse Events

AE analyses will be performed for the following intervals:

- From the day of vaccination until the day of surgery
- From the day of vaccination until the Day 42 postoperative evaluation
- From the day of surgery until the Day 42 postoperative evaluation
- From the Day 42 postoperative evaluation until the Day 180 postoperative evaluation (newly diagnosed chronic medical disorders only)

Analyses for serious adverse events (SAEs) and deaths will be performed for the following intervals:

- From vaccination until the day of surgery
- From vaccination until the Day 180 postoperative evaluation
- From the day of surgery until the Day 180 postoperative evaluation

Deaths will be categorized as associated with *S aureus* infection or not associated with *S aureus* infection.

Statistical methodology for AEs is described in Section 8.1.2.1.

6.2.2. Multiple-Organ Failures

Each multiple-organ failure (MOF) will be classified as an AE or SAE and so appear in the appropriate analysis. However, other MOF endpoints will also be examined:

- Number and proportion of subjects with organ system failure by organ (eg, renal, hepatic, pulmonary). By definition of MOF, each subject will be included in analyses of more than 1 organ.
- Number and proportion of subjects who meet the criteria for MOF.
- Among subjects with MOF, the number and proportion associated with PDI, non-PDI (other infection), other (not an infection), or unknown. More than 1 category may be selected.
- Among subjects with MOF that is associated with PDI or non-PDI, whether the infection is microbiologically confirmed *S aureus*, microbiologically confirmed non–*S aureus*, or not microbiologically confirmed.

Statistical methodology for MOF is described in Section 8.1.2.2.

6.2.3. Local Reactions and Systemic Events

Day 1 is defined in this study as the day of the index surgery. References to "Days 1-10" in this section refer to the 10 days following vaccination.

The grading scales for local reactions and systemic events are derived from the Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) guidelines on toxicity grading scales for healthy adult volunteers enrolled in preventive vaccine clinical trials.[3]

Subjects will assess redness, swelling, and pain at the site of vaccination and record the symptoms in the electronic diary (e-diary) for 10 consecutive days starting on the day of vaccination. Redness and swelling will be measured and recorded in caliper units (range: 1 to 21+), and then categorized as mild, moderate, or severe based on the grading scale in Table 2.

Table 2. Local Reaction Grading Scale

	GRADE 1 Mild	GRADE 2 Moderate	GRADE 3[a] Severe	GRADE 4[b]
Redness	5 to 10 caliper units (or measuring device units) 2.5 to 5.0 cm	11 to 20 caliper units (or measuring device units) 5.5 to 10.0 cm	≥21 caliper units (or measuring device units) ≥10.5 cm	Necrosis or exfoliative dermatitis[b]
Swelling	5 to 10 caliper units (or measuring device units) 2.5 to 5.0 cm	11 to 20 caliper units (or measuring device units) 5.5 to 10.0 cm	≥21 caliper units (or measuring device units) ≥10.5 cm	Necrosis[b]
Pain at injection site	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room visit or hospitalization

a. Subjects experiencing local reactions ≥21 caliper units (≥10.5 cm) will telephone the study site. In the event that the subject does not call, the investigator will call the subject.
b. Grade 4 assessment should be made by the investigator and recorded as an AE on the case report form.
Note: If the size of the redness and/or swelling falls between 2 caliper units, the higher caliper unit number will be recorded in the electronic diary (e-diary).

Numeric scores for the local reactions will be: none = 0, mild = 1, moderate = 2, and severe = 3.

Grade 4 will not be collected in the e-diary. Only an investigator is able to classify a subject's local reaction as Grade 4, after physical examination of the subject or telephone contact with the subject. Any such local reactions will be recorded as AE on the CRF. In addition to their inclusion in the general AE analysis, these events will be listed and discussed separately as reactogenicity events.

The derived local reaction variables are:

1. Maximum grade during Days 1-10 after vaccination, for each local reaction.

2. Mild, moderate, or severe response during Days 1-10 after vaccination, for each local reaction.

3. Duration in days, but not for "any local reaction."

4. Onset of the indicated local reaction in days.

5. Onset of "any local reaction" in days.

The mild, moderate, or severe response during Days 1-10 after vaccination is "yes" if present on at least 1 day.

A given local reaction is "yes" for Days 1-10 after vaccination if present on at least 1 day, is "no" if the subject reports any combination of "no" and "missing" on each of Days 1-10 after vaccination, and is "missing" if every day is missing.

"Any local reaction" is "yes" for Days 1-10 after vaccination if at least 1 local reaction is present on at least 1 day, is "no" if the subject reports "no" or missing for every local reaction on each of Days 1-10 after vaccination, and is "missing" if every local reaction on every day is missing.

Duration of local reaction is calculated as the date of the last reported reaction minus the date of the first reported reaction, plus 1. If the local reaction has not resolved by the last date in the e-diary, then the resolution date recorded in the case report form (CRF) will serve as the date of the last reported reaction. If the resolution date is missing from the CRF, then duration will be set to missing. Duration will be missing for subjects without the indicated local reaction. Duration will not be calculated for "any local reaction."

The onset day for a local reaction is the first day of the reaction, even if the reaction later becomes more severe. The onset day for "any local reaction" will be the first day of any local reaction, regardless of severity. Onset day will be missing for subjects without the indicated local reaction.

Local reactions recorded on or after the day of surgery will be excluded from all analyses.

Numeric scores for the systemic events are the same as for local reactions: none = 0, mild = 1, moderate = 2, and severe = 3. The systemic event grading scale is presented in Table 3.

Table 3. Systemic Event Grading Scale

	GRADE 1 Mild	GRADE 2 Moderate	GRADE 3[a] Severe	GRADE 4[b]
Fatigue (Tiredness)	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization
Headache	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization
Vomiting	1 to 2 times in	>2 times in 24 hours	Requires intravenous	Emergency room visit

Table 3. Systemic Event Grading Scale

	GRADE 1 Mild	GRADE 2 Moderate	GRADE 3[a] Severe	GRADE 4[b]
	24 hours		hydration	or hospitalization
Diarrhea	2 to 3 loose stools in 24 hours	4 to 5 loose stools in 24 hours	6 or more loose stools in 24 hours	Emergency room visit or hospitalization
Muscle pain	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization
Joint pain	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization

a. Subjects experiencing severe systemic events will telephone the study site. In the event that the subject does not call, the investigator will call the subject.
b. Grade 4 assessment should be made by the investigator and recorded as an AE on the case report form.

Grade 4 assessments will not be recorded in the e-diary, but will be recorded as AEs on the CRF. Only an investigator is able to classify a subject's systemic event as Grade 4, after physical examination of the subject or telephone contact with the subject. Any such systemic events will be recorded as AE on the CRF. In addition to their inclusion in the general AE analysis, these events will be listed and discussed separately as reactogenicity events.

Four categories are defined for analysis of body temperature (see Table 4).

Table 4. Temperature Categories

Categories
38.0-38.4°C (100.4-101.1°F)
38.5-38.9°C (101.2-102.0°F)
39.0-40.0°C (102.1-104.0°F)
>40.0°C (>104.0°F)

"Any fever" is defined as ≥38.0°C.

Antipyretics or pain medication used to treat symptoms will also be collected in the e-diary. Response values will be "yes" and "no."

The derivations for the systemic event endpoints are the same as for local reactions. "Any systemic event" will include fever ≥38.0°C, but exclude the use of antipyretic or pain medication.

The systemic event endpoints are:

1. Maximum grade during Days 1-10 after vaccination, for each systemic event.

2. Mild, moderate, or severe response during Days 1-10 after vaccination, for each systemic event.

3. Maximum grade during Days 1-10 after vaccination, for "any systemic event."

4. Proportion of subjects with a moderate or severe systemic event on each day.

5. Duration in days, but not for "any systemic event."

6. Onset of the indicated systemic event in days.

7. Onset of "any systemic event" in days.

The endpoints for fever are:

1. Maximum fever category during Days 1-10 after vaccination.

2. Proportion of subjects with any fever category on each day.

3. Duration in days, but only for ≥38.0°C.

4. Onset in days, but only for ≥38.0°C.

Duration and onset will not be calculated for antipyretic or pain medication.

A baseline assessment of fatigue, headache, muscle pain, and joint pain over the previous month will be collected before vaccination. Grading will be none = 0, mild = 1, moderate = 2, and severe = 3. Only 1 baseline assessment per indicated systemic event will be obtained.

Systemic events recorded on or after the day of surgery will be excluded from all analyses.

Statistical methodology for local reactions and systemic events is described in Section 8.1.2.3.

6.2.4. Concomitant Medications, Vaccinations, and Blood Products

The following concomitant medications, vaccinations, and blood products will be summarized:

- Antibiotics, including number of days of antibiotic use.
- Systemic steroids.
- Intraspinal steroids.
- Nonstudy vaccines.
- Blood products, including number of units and type (eg, whole blood, packed red blood cells [RBCs], platelets, and plasma). In addition, whole blood and packed RBCs will be identified as heterologous or autologous donations. Use of cell saver will be analyzed.

Statistical methodology is described in Section 8.1.2.4.

6.3. Other Endpoints

6.3.1. Demographic, Medical History, and Baseline Characteristics

Disposition variables will include enrolled, randomized, vaccinated, completed, and discontinued. Discontinuations will be split into reasons for discontinuation.

The demographic characteristics will include sex, race, racial designation (completed only if race = Asian), ethnicity, and age on the day of vaccination. Age on the day of vaccination will be derived in years ([vaccination date – date of birth + 1]/365.25) and truncated to 0 decimal places. For subjects randomized but not vaccinated, the randomization date will be used instead of the vaccination date in the calculation of age.

Colonization status at baseline (Visit 1 or/and Visit 2 admission sample) will be compiled separately for each anatomical site (nose and throat), as well as "any anatomical site" (meaning both sites combined). The definition of colonization status when combining sites is described in Section 8.1.4.

Body mass index (BMI), vital signs, colonization status at baseline, smoking status, alcohol consumption, and elapsed days from vaccination to index surgery will be summarized.

- BMI will be calculated as (weight [kg]/height [m] squared) and rounded to 1 decimal place.
- Vital signs include temperature (°C or °F), blood pressure (systolic and diastolic), pulse rate, and respiratory rate. All temperatures will be converted to °C.
- Alcohol consumption will be recorded as yes/no. If "yes," then the number of units per week will be recorded.
- Elapsed days from vaccination to the index surgery will be the date of the index surgery minus the date of vaccination, plus 1.

Medical history data will be categorized according to the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by vaccine group.

The Charlson Comorbidity Index[4] (CCI) is a validated prognostic indicator for which factors, individually or in combination, may increase the risk of short-term mortality for subjects enrolled in longitudinal studies. On completion of the physical examination at Visit 1, the investigator will record the presence of each comorbid condition and age cohort as presented in Table 5. The sum of the values for each comorbid condition is added to the subject's value for age to obtain the CCI score. The 10-year mortality probability is calculated as $0.983^{EXP[0.9 \times CCI\ score]}$ (Kastner et al).[5]

Table 5. Charlson Comorbidity Index

Comorbid Condition	Score	Value for Age (on the Date of Assessment)
History of prior myocardial infarction (not ECG changes only)	1	Age <50 years: 0 points
Congestive heart failure	1	Age 50-59 years: 1 point
Peripheral vascular disease	1	Age 60-69 years: 2 points
Cerebrovascular disease	1	Age 70-79 years: 3 points
Dementia	1	Age 80-89 years: 4 points
Diabetes	1	
Mild liver disease	1	
Chronic pulmonary disease	1	
Gastric/duodenal ulcer	1	
Connective tissue disease	1	
Hemiplegia	2	
Moderate or severe renal disease	2	
Diabetes with end-organ damage	2	
Tumor	2	
Leukemia	2	
Lymphoma	2	
Moderate or severe liver disease	3	
Metastatic solid tumor	6	
AIDS	6	

Abbreviations: AIDS = acquired immunodeficiency syndrome; ECG = electrocardiogram.

The anesthesiologist will assess the subject's physical status before surgery using the American Society of Anesthesiologists (ASA) score;[6] see Table 16. Anesthesiologists use the ASA physical status classification system to assess the fitness of surgical patients before undergoing surgery. ASA scores correlate with patient morbidity and postoperative infection risk.

Table 6. American Society of Anesthesiologists (ASA) Physical Status Classification

Score	Description
1	Healthy patient
2	Mild systemic disease with no functional limitation
3	Severe systemic disease with definite functional limitation
4	Severe systemic disease that is a constant threat to life
5	Moribund patient unlikely to survive 24 hours with or without operation

Adapted from American Society of Anesthesiologists. ASA physical status classification system. Available: http://www.asahq.org/Home/For-Members/Clinical-Information/ASA-Physical-Status-Classification-System. 18 Jun 2014.

Statistical methodology for demography is described in Section 8.1.3.1.

6.3.2. Index Surgery and Hospitalization

Surgical characteristics are listed below. Similar characteristics are collected for index surgery reoperation/revision. Variables are classified as categorical (meaning only discrete responses are possible) or numeric. Categorical variables are subdivided into 1 response per subject and multiple responses possible per subject.

One categorical response per subject:

- Any decolonization measures.
- Initial or revisional index surgery.
- *S aureus*/methicillin-resistant *S aureus* (MRSA) carriage evaluation performed.
- Preoperative skin preparation.
- Wound classification.
- Primary incision site.
- Primary skin closure type.
- Graft type.
- Implant material (type and composition)
- Intrawound application of an antibiotic.
- Prophylactic antibiotics administered within 1 hour before incision.
- Prophylactic antibiotics readministered during the index surgical procedure.
- Index surgery modified to be a staged procedure, performed on separate days.
- Evidence of malignancy during the index surgery.
- Evidence of infection during the index surgery.
- Temperature after surgery, using the categories defined in Table 4.
- "Any fever," defined as temperature ≥38.0°C.
- Type of glucose measure (capillary or serum).
- Been in an ICU.
- Location prior to hospital admission.

- Discharge disposition.

Multiple categorical responses possible per subject:

- Indication for surgery.
- Decolonization measure employed.
- Incision site hair removal method.
- Implanted devices.
- Vertebrae fused.
- Specialty of the surgeon, using the categories “orthopedic,” “neurosurgeon,” “orthopedic and general,” “neurosurgeon and general,” and “all others.”

Numeric responses from the subject:

- Intraoperative blood loss (mL) (Where blood loss is unknown, the default value is ’0’ – this will be handled as a missing value in the analysis)
- Number of vertebrae fused.
- Duration of surgery (in minutes), obtained as the time of the last suture minus the time of the first incision, plus 1.
- Duration of hospitalization (numeric: the date of discharge minus the date of admission, plus 1). Missing if the subject was not discharged.
- Number of days the subject was in an ICU (limited to subjects who were in such a unit) associated with the index surgery.
- Glucose values will be collected before the surgery and on the day after surgery. Each value will be identified as fasting (yes/no).

Statistical methodology for surgery and hospitalization is described in Section 8.1.3.2.

6.3.3. Healthcare Utilization

The healthcare utilization endpoints include:

- Location prior to hospital admission.
- Days in hospital.
- Ongoing in hospital (ie, not discharged).

- Discharge disposition:
  - Home self-care
  - Home healthcare
  - Rehabilitation facilities
  - Nursing home/skilled nursing care facility/nursing home
  - Death
  - Other
- Readmission related to complications of the index surgery.
- Proportion of readmissions to hospital.
- Proportion of readmissions to hospital related to complications of the index surgery.
- Proportion of subjects with additional surgery (the denominator will be the number readmitted for complications).
- Proportion in the ICU during readmission (denominator is the number of readmitted subjects).
- Number of days in the ICU, limited to subjects with hospital readmission related to complications for surgery.
- Proportion of subjects discharged from the hospital after readmission (denominator is the number of readmitted subjects).
- Total duration of hospitalization from the index surgery and any readmissions.
- Total days in an ICU from the index surgery and any readmissions.
- Number of rehabilitation/physical therapy visits as an outpatient.
- Inpatient days in rehabilitation facility or skilled nursing facility after discharge.
- Number of service visits (meaning skilled nursing home/skilled nursing facility or rehabilitation facility); discharges from the hospital to either of these will not count as a service visit.

The duration of each hospitalization episode will be the last day minus the first day, plus 1. Duration will be missing for episodes ending as “ongoing.”

Statistical methodology for healthcare utilization is described in Section 8.1.3.3.

6.3.4. Colonization with *Staphylococcus aureus*

If 2 or more samples associated with postsurgery are obtained, only the colonization results from the latest visit will be eligible for analysis.

Four colonization endpoints, based on colonization results from scheduled visits, are specified:

1. The number and proportion of subjects in each vaccine group determined to be colonized with *S aureus* on each scheduled visit where swab samples are collected. Colonization at each visit will be compiled separately for each anatomical site, and for any anatomical site. A subject will be considered positive for "any anatomical site" if either site, or both, is positive.

2. Subject carriage status will be characterized across visits as persistent, indeterminate, intermittent, or noncarrier. Characterizations will be performed by anatomical site and any anatomical site. Characterizations will be based on number of visits where colonization results were obtained, limited to Visits 1, 2 (both the day of surgery and the day of discharge), 4 (Day 42), 5 (Day 90), and 6 (Day 180). Rules for determining carriage status are described next and are also illustrated in Table 7.

    - Persistent: positive at 4 of 5, 5 of 5, 5 of 6, or 6 of 6 visits.
    - Indeterminate: exactly 3 or 4 visits, regardless of combination of positive/negative results.
    - Intermittent: positive at 1 or 2 visits if 2 visits are available, positive at 1 to 3 visits if 5 visits are available, positive at 1 to 4 visits if 6 visits are available.
    - Noncarrier: no positive results at any visit, minimum of 5 visits.

Table 7. Rules for Determining Carriage Status

No. Positive Results							
No. Visits Available	**0**	**1**	**2**	**3**	**4**	**5**	**6**
1	Missing	Missing	N/A	N/A	N/A	N/A	N/A
2	Missing	Intermittent	Intermittent	N/A	N/A	N/A	N/A
3	Indeterminate	Indeterminate	Indeterminate	Indeterminate	N/A	N/A	N/A
4	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	N/A	N/A
5	Noncarrier	Intermittent	Intermittent	Intermittent	Persistent	Persistent	N/A
6	Noncarrier	Intermittent	Intermittent	Intermittent	Intermittent	Persistent	Persistent

Abbreviation: N/A = not applicable.

3. Acquisition is defined as a positive swab at any postsurgery visit, given that the results for the vaccination and presurgery visits were both negative. The proportions of acquisitions will be compiled for each subsequent postsurgery visit and "any postsurgery visit." The denominator at each visit will be the number of subjects with at least 1 nonmissing swab result. Acquisition will be compiled for nose, throat, and "any anatomical site."

4. Clearance is defined as 2 consecutive postbaseline negative swabs, given that all preceding swabs were positive. The proportions of clearances will be compiled for each postbaseline visit. The denominator at each visit will be the number of subjects with at least 1 nonmissing swab result. The analyses will be performed for nose, throat, and "any anatomical site."

Colonization, carriage status, acquisition, and clearance will be compiled for "any *S aureus*," MRSA, and methicillin-sensitive *S aureus* (MSSA).

Capsule type will be identified for each *S aureus* isolate as either CP5 or CP8. A small subset of isolates will be neither capsule type, so these isolates will be considered missing for capsule type. Carriage status, acquisition, and clearance will be obtained for each capsule type. These endpoints will be identified as capsule-type persistence, capsule-type intermittent, capsule-type noncarrier, capsule-type acquisition, and capsule-type clearance. Analyses with these endpoints may be performed.

Other endpoints based on clonal complex (CC) and sequence type (ST) may be explored. *S aureus* protein A (*Spa*) typing and capsule typing may be examined only for PDIs with microbiologically confirmed *S aureus* infection.

Colonization results obtained after a culture-confirmed efficacy endpoint will not be included in deriving characterizations or acquisition. Results from unscheduled visits will not be included in characterizations for persistence, intermittent, etc.

Statistical methodology for colonization is described in Section 8.1.3.4.

6.3.5. Immunogenicity

Titers will be obtained from the OPA assay for CP5 and CP8 and from the cLIA assay for ClfA and MntC. Assay values will be obtained among subjects with adjudication-confirmed *S. aureus*-related SSI and/or BSI and select number of subjects who did not develop adjudication-confirmed *S. aureus* infections at randomization, Visit 2 (both before surgery and after surgery), and Day 90. If 2 or more samples associated with postsurgery are obtained, only the assay results from the latest visit will be eligible for analysis.

Immunogenicity endpoints include the following:

- The proportion of subjects who achieve specific antibody thresholds at each scheduled time point, compiled separately for each antigen/assay. Thresholds are presented in Table 8.

- The proportion of subjects who achieve specific antibody thresholds to all 4 antigens simultaneously at each scheduled time point, limited to CP5/OPA, CP8/OPA, ClfA/cLIA, and MntC/cLIA.
- Geometric mean titer (GMT) at each time point.
- Geometric mean fold rise (GMFR) from Visit 1 to Visit 2 admission.
- Increases of 2-, 4-, 8-, 16-, and 32-fold from Visit 1 to Visit 2 admission.

Table 8. Threshold Values for Assay/Antigens

Antigen	Assay Method	Threshold
ClfA	cLIA	≥4-fold rise from Visit 1
MntC	cLIA	≥4-fold rise from Visit 1
CP5	OPA	≥1000
CP8	OPA	≥2000

Abbreviations: ClfA = clumping factor A; cLIA = competitive Luminex immunoassay;
CP5 = capsular polysaccharide serotype 5; CP8 = capsular polysaccharide serotype 8;
LLOQ = lower limit of quantitation; MntC = manganese transporter C; OPA = opsonophagocytic activity.

Lower limits of quantitation (LLOQs) will be defined in the assay validation report.

The proportions of subjects achieving threshold will be missing for ClfA/cLIA and MntC/cLIA at Visit 1 by definition of its threshold.

Other assays that measure immune response may also be analyzed.

Statistical methodology for immunogenicity is described in Section 8.1.3.5.

6.4. Covariates

No covariates are proposed for the primary and secondary efficacy analyses.

7. HANDLING OF MISSING VALUES

Sensitivity analyses for efficacy will be performed if there are suspected PDIs without sufficient microbiological testing to confirm or deny the PDI as microbiologically confirmed *S aureus*. Only the primary and secondary efficacy endpoints will be so analyzed. The suspected PDI cases that meet clinical criteria but lack sufficient microbiological testing will be assigned as confirmed PDI with probability P_u. The sensitivity analyses will examine a range of P_u values for each vaccine group.

The ranges of P_u values are described in Table 9. Simulations are not required for every value. For example, when $P_u = 0$ in the SA4Ag group and $P_u = 1$ in the placebo group, then every suspect SA4Ag PDI is considered not to be a PDI and every suspect placebo PDI is considered a confirmed PDI. This is the "best-case" sensitivity analysis for SA4Ag.

The missing-at-random (MAR) analysis uses the observed probabilities (P_u) for each vaccine group; SA4Ag's (or placebo's) P_u will be the number of *S aureus* microbiologically confirmed PDIs divided by the number of PDIs with sufficient nonmissing microbiological testing, limited to subjects in the SA4Ag (placebo) group. The missing-not-at-random (MNAR) analyses use a range of P_u values that are selected without reference to observed P_u values.

Table 9. Range of Probabilities for Sensitivity Analyses on Protocol-Defined Infections that Lack Sufficient Microbiological Confirmation

Scenario	P_u = Pr(PDI is microbiologically confirmed *S aureus*, given SA4Ag vaccination and clinical criteria met)	P_u = Pr(PDI is microbiologically confirmed *S aureus*, given placebo vaccination and clinical criteria met)	Comments	Simulations
1	0	1	Best -case analysis	N/A
2	1	0	Worst -case analysis	N/A
3	0	0	Observed; missing PDIs not included	N/A
4	1	1	Every suspect PDI is microbiologically confirmed	N/A
5	Observed Ps[a]	Observed Pp[b]	MAR	2000
6	0	0.5	MNAR	2000
7	0.5	0	MNAR	2000
8	0.5	0.5	MNAR	2000

a. Observed Ps = the number of *Staphylococcus aureus* microbiologically confirmed PDIs divided by the number of PDIs with sufficient nonmissing microbiological testing, limited to subjects receiving SA4Ag.
b. Observed Pp = the number of *S aureus* microbiologically confirmed PDIs divided by the number of PDIs with sufficient nonmissing microbiological testing, limited to subjects receiving placebo.

Abbreviations: MAR = missing at random; MNAR = missing not at random; N/A = not applicable; PDI = protocol-defined infection; Pr = probability.

If both P_u values for SA4Ag and placebo are set to zero, then the resulting VE is a complete case analysis. If the P_u for SA4Ag is 1 and the P_u for placebo is 0, then the resulting VE is worst-case VE. If the P_u for SA4Ag is 0 and the P_u for placebo is 1, then the resulting VE is best-case VE. The distribution of proposed sensitivity analyses is displayed in Table 10.

Table 10. Illustration of Sensitivity Analyses

	P_u for Placebo[a]			
P_u for SA4Ag[a]	**0**	**Observed[b]**	**0.5**	**1**
0	Observed VE		MNAR	Best case for SA4Ag
Observed[b]		MAR		
0.5	MNAR		MNAR	
1	Worst case for SA4Ag			

a. Pu = Pr(PDI is microbiologically confirmed *S aureus*, given clinical criteria met).
b. Observed = the number of microbiologically confirmed PDIs divided by the number of PDIs with sufficient microbiological testing.

Abbreviations: MAR = missing at random; MNAR = missing not at random; PDI = protocol-defined infection; Pr = probability; VE = vaccine efficacy.

The steps in the sensitivity analysis are:

1. Identify suspect PDIs that meet relevant clinical criteria but lack microbiological confirmation because of insufficient microbiological testing.

2. Randomly assign microbiological confirmation = yes to each suspect PDI with probability P_u, depending on vaccine group, as described in Table 9.

3. Concatenate with confirmed PDIs after all suspect PDIs are randomly assigned.

4. Calculate VE and associated CI (see Section 8.1.1). The confidence level will be 95% except for the primary endpoint in the PP efficacy population, where the confidence level will be 95.1%. If the sensitivity analyses are performed after the interim analysis, then the confidence level will be 95% except for the primary endpoint in the PP efficacy population, where the confidence level will be 99.7%.

5. Perform the indicated number of simulations. If the P_u values are combinations of 0 and 1, or if the observed probabilities are used, then exactly 1 iteration is required.

6. Tabulate the proportion of simulations where the lower limit on VE exceeds 0%, 20%, and 30%. The descriptive statistics n, mean, standard deviation, median, minimum, and maximum will also be compiled for both VE and the lower confidence limit.

All realized simulations and VE calculations will be saved for possible submission to regulatory authorities. A separate seed should be used for each MAR and MNAR simulation within each endpoint-population combination.

If the number of suspected PDI cases lacking microbiological confirmation is very small, then all possible enumerations may be substituted for the simulations.

Missing values will not be imputed for any immunogenicity, safety, demographic, or colonization variables. However, immunogenicity values less than LLOQ will be set as 0.5 × LLOQ for the analysis.

Subjects who are vaccinated but do not undergo the index surgery will not have a Day 1 visit. Their last day in the study will be labeled Day -1.

8. STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

8.1. Statistical Methodology

8.1.1. Methodology for Efficacy Endpoints

Vaccine efficacy is defined as VE = 1 - RR, where RR is the relative risk in SA4Ag compared to placebo, ie, the proportion of SA4Ag recipients meeting the primary endpoint relative to the proportion of placebo recipients meeting the primary endpoint. Comparing 2 vaccine groups in number of cases may be restated as a 1-sample test of proportions (Chow et al).[7] Let x = number of SA4Ag cases, y = number of placebo cases, and total cases n=x+y. Under the null hypothesis and given the small incidence of cases (1.4% or less expected in the study), then x has a binomial distribution with probability parameter 0.444 and number of trials n. Given observed values for x and n, and defining p=x/n, then VE = 1-(p/[1-p]). A confidence level will be calculated for p using the Clopper-Pearson method. If the CI for p is (p_L, p_U), then the corresponding CI for VE is (1-[p_U/{1-p_U}], 1-[p_L/{1-p_L}]).

A sequential analysis will be performed on the primary endpoint in the PP efficacy population. Early stopping boundaries are based on a group-sequential design (utilizing single-sample binomial distribution, conditional on the total number of cases in both vaccine groups). The parameters of the group-sequential design were calculated based on 1 interim analysis at 50% of information and O'Brien-Fleming–type α- and β-spending functions. The first-stage probability for rejecting the null hypothesis is 0.0015, and 1-sided probability of rejecting the null hypothesis at the final stage is 0.0245. One-sided probability of 0.0015 corresponds to a 2-sided confidence level of 99.7%, so a 99.7% CI will be calculated for the interim VE estimate. One-sided probability of 0.0245 corresponds to a 2-sided confidence level of 95.1%, so a 95.1% CI will be calculated for the final VE estimate.

The interim analysis will be performed on the first 24 PP cases and the final analysis will be performed on the first 48 PP cases. Although the final analysis is expected to have 48 cases, it is possible that additional potential cases will be present in the adjudication system (entered into the adjudication electronic case report form [eCRF]) on the same day that the 48th per-protocol case is reported to the EAC. These additional cases will be included in the final analysis, if confirmed as cases by the EAC. Therefore, the time order of case notification from the EAC to the sponsor will determine which cases will be included in the analysis. O'Brien-Fleming boundaries will be maintained for decisions regarding VE.

O'Brien-Fleming boundaries will also be maintained if the EAC informs the sponsor of the 25th case (or any further cases) on the same day. It is possible that additional potential cases will be present in the adjudication system. The time order of case notification from the EAC to the sponsor will determine which cases will be included in the interim analysis. Even if more than 24 cases are included in the interim analysis, the final analysis will be performed on 48 cases, unless additional cases are available as described in this section.

The interim analysis will not be revised even if the interim and final case counts do not meet planned values; eg, if the interim analysis is performed with 24 cases, but the final PP analysis has 49 or more cases, the interim analysis will not be revised.

Only the primary endpoint in the PP efficacy population will be subjected to an interim analysis.

The futility analysis will be applied to the primary endpoint only, in the PP efficacy population. After accumulating approximately 10, 15, and 24 primary endpoint cases, the power of the study to reject the original null hypothesis, conditional upon the results accumulated so far, will be calculated. Futility may be declared and the study may cease enrollment, if the conditional power falls below 20%. This will occur if 7 or more vaccine cases are observed out of 10 total cases at the first futility check, or if 9 or more vaccine cases are observed out of 15 total cases at the second futility check, or if 11 or more vaccine cases are observed out of 24 total cases at the third futility check.

The operating characteristics of this futility procedure were explored by simulation. Table 11 displays simulated trial outcomes under a variety of assumptions about the true vaccine efficacy. Under the alternative hypothesis of 70% vaccine efficacy, the futility rule has a low probability of being passed. Conversely, if vaccine efficacy is zero, the chosen futility rule will terminate the study with > 73% probability.

Table 11. Operating Characteristics of Study Design

	Assumed Vaccine Efficacy (%)			
Study Outcome	**0%**	**20%**	**60%**	**70%**
Futility				
at 10 cases	17.0%	9.6%	0.7%	0.2%
at 15 cases	15.7%	9.5%	0.7%	0.2%
at 24 cases	40.9%	34.3%	4.4%	1.0%
at any time	73.6%	53.3%	5.9%	1.4%
Positive Interim Analysis	0.0%	0.1%	5.8%	16.3%
Positive Final Analysis	0.3%	2.2%	54.7%	71.2%
Negative Final Analysis	26.1%	44.4%	33.6%	11.1%
Total Positive Outcomes	0.3%	2.3%	60.5%	87.5%
Total Negative Outcomes	99.7%	97.7%	39.5%	12.5%
Mean no. cases required	26.5	33.0	45.0	43.7

Percentages of study outcomes in 100,000 simulations of the trial under each assumed vaccine efficacy. 'Positive' outcomes are where the null hypothesis that VE ≤20% is rejected at either the interim or final analysis. 'Negative' outcomes are a declaration of futility or failing to reject the null hypothesis at the final analysis. All percentages rounded to 0.1%.

The analyses of the secondary endpoints, the exploratory efficacy endpoints, and the primary endpoint in the mITT efficacy population will be evaluated at a 2-sided alpha level of 0.05. The proportion of SA4Ag cases divided by total cases will be translated into VE for each endpoint. The upper and lower limits of the associated 95% CI on the proportion will be translated to upper and lower 95% confidence limits on VE.

Subgroup analyses will use the same methodology as described above, but will be performed separately at each level of the subgroup. Some levels of subgroups may be combined before analysis. Subgroups without cases in either vaccine group will not be analyzed.

The subgroup analyses on the primary endpoint from the PP efficacy population will be performed on the 24 cases (if the study ends on interim analysis) or 48 cases (if the study ends as planned). More PP cases may accumulate after the 24 or 48 cases as remaining subjects complete enrollment, so the subgroup analyses will also be repeated with the additional cases.

Endpoint definitions include specified microbiological confirmations. Absence of microbiological confirmation excludes the suspected infection from the endpoint analysis. But if the microbiological tests are not performed (because of lack of sample, lost sample, invalid assay, etc), then the PDI will be considered a missing value for endpoint analyses. PDIs adjudicated as noncases in the presence of sufficient and valid microbiological testing will not be considered missing values. Missing-data sensitivity analyses will be performed for the BSI, SSI, and ISA disease endpoints. The methods for missing-data sensitivity analyses are described in Handling of Missing Values.

Another set of sensitivity analyses will incorporate follow-up time in the efficacy calculation. The variable P will be the proportion of SA4Ag cases divided by the number of all cases. The variable h will be ratio of total follow-up time in the placebo group to the total follow-up time in the SA4Ag group. Follow-up time for any subject is the date of study completion (or dropout or case onset, whichever comes first) minus the date of index surgery, plus 1. Then estimated efficacy will be 1-hP/(1-P). The efficacy's CI will be obtained by calculating the CI for P, then substituting in the expression. The CI for P will be obtained using the Clopper-Pearson method. The confidence level will be the same as for the indicated endpoint. The method is described in Blackwelder,[8] substituting follow-up time for number of subjects. Follow-up time sensitivity analyses will be performed for the primary and secondary endpoints in both the mITT and PP efficacy populations.

Number needed to treat (NNT) will be calculated as the reciprocal of the proportion of SA4Ag cases minus the proportion of placebo cases. NNT will be calculated for each endpoint. A 95% CI will be obtained for each NNT by obtaining a 2-sided 95% CI on the difference in proportions using the method of Chan and Zhang,[9] then reciprocating the upper and lower limits.

1. If the difference in proportions and both confidence limits are less than zero, then the results will be presented as NNT = xx.x (xx.x, xx.x), with the negative signs omitted.

2. If the difference in proportions and both confidence limits are greater than zero, then the results will be presented as number needed to harm (NNH) = xx.x (xx.x, xx.x).

3. If the CI brackets zero, then the results will be presented as NNT (if negative) or NNH (if positive) = xx.x (NNT = xx.x, NNH = xx.x), with any negative signs omitted.

4. If the difference in proportions is exactly zero, then the estimated NNT will be presented as NNT = ∞.

Statistical analyses for efficacy are summarized in Section 8.2.1.

8.1.2. Methodology for Safety Endpoints

The denominators for all proportions will be the number of subjects in the vaccine group, except where noted.

All CIs for proportions will be 2-sided 95% intervals, obtained using the Clopper-Pearson method. There will be no adjustments for multiplicity.

8.1.2.1. Adverse Events and Serious Adverse Events

AEs will be summarized using 3-tier methodology.

Tier 1 includes all AEs of special interest. These may be determined by vaccine knowledge, experience with similar vaccines, and results from Phase 1 and 2 studies. For such AEs, incidence proportions, the difference between vaccine groups in proportions, 95% CI on the difference, 2-sided p-value on the difference, and graphical displays of the differences will be compiled. Currently, no Tier 1 events are specified for the *S aureus* studies, as there are no specific AEs considered by Pfizer to have been of sufficient clinical importance to identify them as Tier 1 events during safety reviews performed across the early-phase studies. Tier 1 AEs, if any, will be specified by the sponsor in the safety review plan under separate cover.

Tier 2 includes all AEs with ≥1% incidence in either vaccine group in either the preoperative period or the postoperative (Days 1-42) period. For such AEs, incidence proportions, the differences between vaccine groups in proportions, and 95% CIs on the differences will be compiled.

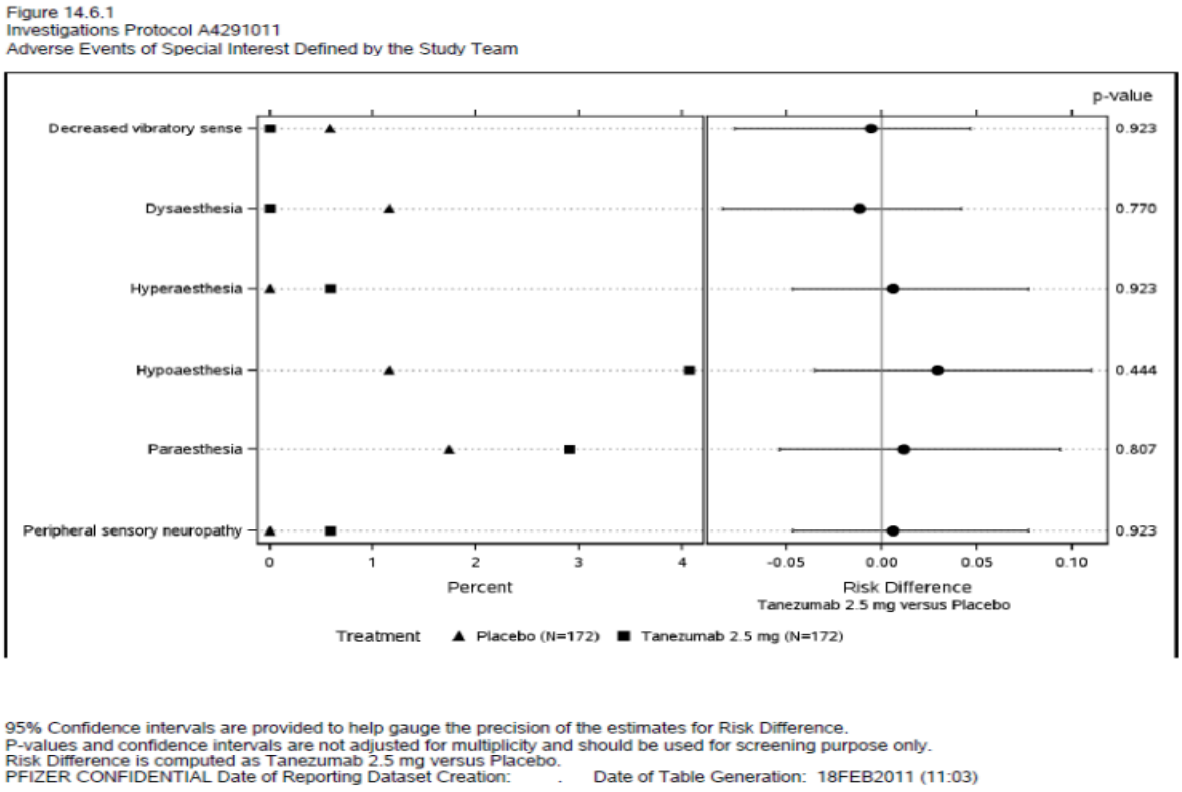
Tier 3 includes all remaining AEs. For such AEs, only incidence proportions will be compiled.

The CIs on the differences between vaccine groups for Tier 1 will be computed using the Chan and Zhang[9] method. The CIs on the differences between vaccine groups for Tier 2 may use the Miettinen and Nurminen[10] method. This method is an approximation and, therefore, computationally faster. The approximation is acceptable for Tier 2 because these analyses are exploratory.

All risk differences will be calculated as SA4Ag minus placebo.

Risk differences will be plotted, along with 95% CIs, for Tier 1. p-Values will be included in the plot. Figure 1 shows an example.

Figure 1. Example of a Figure for Tier 1 Analysis



Tier 2 AE incidences, risk differences, and CIs will also be plotted, but p-values will not be provided.

Tier 1 and Tier 2 AEs will be sorted in order of risk difference between SA4Ag and placebo.

The numbers of subjects and proportions with the indicated AE will be printed for Tier 3 AEs, but there will be no graphs, p-values, or CIs.

Tier 1 through Tier 3 analyses are proposed for subgroups. Any preferred term assigned to a given tier for the analysis of all subjects will be retained in the same tier for the subgroup analyses.

The analyses of newly diagnosed chronic medical disorders from Day 42 until Day 180, SAEs, and deaths will use Tier 2 methods.

For subjects who are vaccinated but do not undergo surgery, all safety data regardless of actual length of follow-up will be included in the interval "the day of vaccination until the day of surgery".

AE rates before surgery: Subjects may be vaccinated 10 to 60 days before the day of surgery, so the recording period for preoperative AEs will vary among subjects. AEs before surgery will be recompiled, expressed as AEs per 100 or per 1000 days. The choice of 100 or 1000 days for rate compilation will be made after blinded data review.

Only AEs that start on or after the day of vaccination and before the day of surgery will be so analyzed. AEs from subjects who are vaccinated but do not have surgery will also be included. The purpose is to adjust for varying time until surgery, although by randomization the time to surgery should be equally distributed between the 2 vaccine groups. Therefore, this is a sensitivity analysis.

The 3-tier methodology will not be used for presurgery AEs, because these AEs will be summarized as rates. The number of days of exposure will be calculated as the date of the index surgery minus the date of vaccination, plus 1, which counts both the day of vaccination and the day of surgery. Exposure will be the discharge day minus the vaccination day, plus 1, for subjects without surgery. Total exposure, in days, will be compiled for both vaccine groups. Counts will be fit to a Poisson regression, with total exposure as an offset. Total exposure will be log transformed.

The proportions of deaths will be compiled by vaccine group, using sample size as the denominators. Vaccine groups will be compared using the Fisher exact test, 2-sided, by preferred term, by system organ class, and with all terms combined. Deaths will be further categorized as associated or not associated with *S aureus* infection, as determined by the EAC. The proportions of deaths associated with *S aureus* will be compiled by vaccine group, but the denominators will be the number of deaths. Vaccine groups will be compared using the Fisher exact test, 2-sided, by preferred term, by system organ class, and with all terms combined.

Statistical analyses for AEs are summarized in Section 8.2.2.1.

8.1.2.2. Methodology for Multiple-Organ Failure

The denominators for organ systems that failed and subjects meeting MOF criteria will be all subjects in the safety population. Vaccine groups will be compared using the Fisher exact test, 2-sided, for each organ system. These p-values will include subjects with 2 or more failed systems, so they should be interpreted cautiously.

Subjects with MOF will be split into 2 categories: “associated with infection” (subdivided as “PDI” or “non-PDI [other infection]”) and “not associated with infection” (subdivided as “other [not an infection]” and “unknown”). The denominators will be all subjects in the safety population in order to obtain the absolute risk of subjects’ having MOF associated with infection. The Fisher exact test, 2-sided, will be used to compare vaccine groups in association with infection.

Subjects with MOF associated with infection will be further split into the categories “PDI” and “non-PDI (other infection).” The denominators will be all subjects in the safety

population. Vaccine groups will be compared in both the PDI and non-PDI subcategories with the Fisher exact test, 2-sided.

The subcategories PDI and non-PDI will each be further split into "microbiologically confirmed *S aureus* infection," "microbiologically confirmed non–*S aureus*," and "non-microbiologically confirmed infection." "Non–microbiologically confirmed infection" will be further split into "negative" and "not done." The denominators will be all subjects in the safety population. Vaccine groups will be compared in each level within each subcategory using the Fisher exact test, 2-sided.

Subjects with MOF not associated with infection will be further split into the categories "other" and "unknown." The denominators will be all subjects in the safety population. Vaccine groups will be compared in the other and unknown subcategories with the Fisher exact test, 2-sided.

Site is not proposed as a stratification factor in any analyses because of the expected sparseness of MOFs.

Statistical analyses are summarized in Section 8.2.2.2.

8.1.2.3. Methodology for Local Reactions and Systemic Events After Vaccination

Binary endpoints (eg, any redness on Days 1-10 after vaccination) will be summarized with proportions by vaccine group. Two-sided 95% CIs will be compiled for all proportions. Vaccine groups will be compared using method of Miettinen and Nurminen.

The number and proportion of subjects with indicated local reaction or systemic event on each day will be compiled by vaccine group, but vaccine groups will not be statistically compared.

Vaccine groups will be compared in duration and onset using the Mann-Whitney test, 2-sided.

Vaccine groups will be compared in baseline assessments of fatigue, headache, muscle pain, and joint pain using the Fisher exact test, 2-sided.

NNH will be calculated for both any local reaction and any systemic event. A 95% CI will be obtained for each NNH by obtaining a 2-sided 95% CI on the difference in proportions using the method of Chan and Zhang,[9] then reciprocating the upper and lower limits. The difference in proportions is defined as SA4Ag minus placebo.

1. If the difference in proportions and both confidence limits are less than zero, then the results will be presented as number needed to benefit (NNB) = xx.x (xx.x, xx.x), with the negative signs omitted.

2. If the difference in proportions and both confidence limits are greater than zero, then the results will be presented as NNH = xx.x (xx.x, xx.x).

3. If the CI brackets zero, then the results will be presented as NNB (if negative) or NNH (if positive) = xx.x (NNH = xx.x, NNH = xx.x), with any negative signs omitted.

Baseline assessments will be summarized by proportion of any response (ie, mild, moderate, or severe) and by proportion that are moderate or severe. Vaccine groups will be compared using the Fisher exact test, 2-sided.

Statistical analyses for local reactions and systemic events are summarized in Section 8.2.2.3.

8.1.2.4. Concomitant Medications, Vaccinations, and Blood Products

Antibiotics and systemic steroids will be summarized over 3 intervals:

1. The day of vaccination until the day before surgery, inclusive
2. The day of surgery until study completion (or dropout or withdrawal), inclusive
3. The day of vaccination until study completion (or dropout or withdrawal), inclusive

Intraspinal steroids will be summarized over 2 intervals:

1. 6 Months before study enrollment until the day before vaccination
2. The day of vaccination until completion of study participation

Nonstudy vaccines will be summarized over 2 intervals:

1. 6 Months before study enrollment until the day before vaccination
2. The day of vaccination until completion of study participation

Subjects will be counted once for each product in each interval. A subject will be included in the specified interval if either the start date or the stop date is contained in the interval. Antibiotics, systemic steroids, intraspinal steroids, and nonstudy vaccines will be compared using the Fisher exact test, 2-sided.

Days of antibiotic use will be summarized over the same 3 intervals as antibiotics and systemic steroids. Any antibiotic uses that start before surgery and end after surgery will be split into 2 segments: the start of use until the day before surgery and the day of surgery to the end of use. The statistical test will be van Elteren, with site as a classification variable.

Use of blood products will be summarized over 3 intervals:

1. The day of vaccination until before surgery
2. Days 1-7 (where Day 1 is the day of surgery)
3. Days 8 or more

Use of blood products will be summarized as follows in each interval:

- Numbers and proportions of subjects receiving at least 1 transfusion will be compiled by vaccine group. The denominator will be the number of subjects in the time interval, ie, whose date of completion, dropout, or withdrawal is on or after the last day of the interval. The statistical test will be the Cochran-Mantel-Haenszel (CMH) test.

- The number of units will be summarized by n, mean, standard deviation, minimum, and maximum. Units will be obtained as the sum of all units received in the indicated interval. Vaccine groups will be compared using the van Elteren test, 2-sided. Only subjects receiving at least 1 transfusion will be included.

- Counts of transfusions per subjects (values 0, 1, 2, etc) will be compiled by vaccine group. Vaccine groups will be compared using the van Elteren test, 2-sided.

- Counts of transfusions will be further split by types of transfusions, also compiled by vaccine group. The types of transfusions will be packed RBCs, platelets, whole blood, plasma, and other. The denominator will be the number of subjects in the time interval. The statistical test will be the CMH test, 2-sided.

- Numbers and proportions of subjects receiving heterologous donation or autologous transfusions will be compiled by vaccine group. The denominator will be the number of subjects receiving packed RBCs or whole blood. The statistical test will be the CMH test, 2-sided.

- Numbers and proportions of subjects using cell saver will be compiled by vaccine group. The statistical test will be the Fisher exact test, 2-sided. Only subjects receiving at least 1 transfusion will be included.

All CMH tests will use site as a stratification factor.

Subjects who have more than 1 transfusion in the specified interval will not be counted more than once for any given category (eg, platelets), but will be counted for each separate category (eg, platelets for one transfusion and whole blood for a different transfusion).

Statistical analyses are summarized in Section 8.2.2.4.

8.1.3. Methodology for Other Endpoints

8.1.3.1. Demographics, Medical History, and Baseline Characteristics

Disposition counts (enrolled, randomized, etc) will be tabulated. Statistical tests will not be employed.

Frequency counts and proportions will be compiled for:

- Sex

- Race
- Racial designation (limited to subjects choosing race = Asian)
- Ethnicity
- Smoking status (current smoker, past smoker, never smoked)
- Age group (18 to <50, 50 to 64, and 65 to <86 years)
- Alcohol use (yes/no)
- Physical examination
- Colonization status on Day 1 (each anatomical site and combined anatomical sites)
- Medical history
- CCI score (0, 1, 2, and ≥3)
- ASA score

The denominators will be the number of subjects in the vaccine group, except the denominators for racial designation, which will be the number of subjects in the vaccine group who choose race = Asian.

The summary statistics n, mean, standard deviation, median, minimum, and maximum will be compiled for:

- Age
- BMI
- Units of alcohol (in units per week, limited to subjects reporting alcohol use)
- ASA score
- CCI score
- Charlson comorbidity 10-year mortality probability
- Vital signs
- Elapsed days from vaccination to index surgery

Medical history will be summarized by preferred term and system organ class. The number and percentage of subjects with at least 1 diagnosis of each preferred term, arranged by system organ class, will be tabulated for each vaccine group. The denominator for the percentages will be the number in the vaccine group or the total sample, respectively, excluding subjects for whom medical history was not collected. "Overall no significant history" will be included as a distinct category.

Physical examination findings will be summarized by the number and percentage of subjects with an "abnormal" score. The denominator will be the number of subjects with either "normal" or "abnormal."

Statistical analyses for demography are summarized in Section 8.2.3.1.

8.1.3.2. Index Surgery and Hospitalization

The number and proportions of responses in each category will be compiled where exactly 1 choice is selected. Vaccine groups will be compared using the CMH test, with site as a stratification factor. "Other" will be retained as a category. This analysis applies to:

- *S aureus*/MRSA carriage evaluation performed
- Preoperative skin preparation
- Index surgery (initial or revisional)
- Wound classification
- Primary incision site
- Primary skin closure type
- Graft type
- Implant material (type and composition)
- Intrawound application of antibiotic
- Prophylactic antibiotics administered within 1 hour before incision
- Prophylactic antibiotics readministered during the index surgical procedure
- Index surgery modified to be a staged procedure, performed on separate days
- Requirement for index surgery reoperation/revision
- Specialty of the surgeon (levels defined in Section 6.3.2)
- Evidence of malignancy during the index surgery

- Evidence of infection during the index surgery
- Capillary or serum glucose value before surgery
- Capillary or serum glucose value after surgery
- Temperature after surgery, using the categories in Table 4
- Any temperature, defined as ≥38.0°C, after surgery
- Been in an ICU
- At least 1 readmission to hospital
- Number of readmissions to hospital

Separate statistics will be compiled for subjects who were readmitted. The denominators will be the number of subjects who were readmitted, except as noted.

- At least 1 readmission was related to complications of the index surgery
- Additional surgery required in at least 1 readmission; the denominators will be the number of subjects whose readmission was related to complications of the index surgery
- Been in an ICU in at least 1 readmission
- Not discharged; ongoing hospitalization

The numbers and proportions of responses in each category will be compiled for responses where more than 1 level may be selected, but no statistical comparisons will be performed because some subjects may appear in more than 1 level. "Other" will be retained as a category. This applies to the following:

- Indication for surgery
- Decolonization measures, limited to subjects with any such measures
- Incision site hair removal method
- Implanted devices
- Specialty of surgeon
- Vertebrae fused

The denominators for the proportions will be the number of subjects, except as noted.

Descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) will be compiled for numeric variables:

- Glucose value on the day of surgery, both fasting and nonfasting
- Glucose value on the day after surgery, both fasting and nonfasting
- Change in fasting glucose value
- Intraoperative blood loss
- Duration of surgery
- Number of vertebrae fused
- Duration of hospitalization
- Number of days in an ICU for the index visit (limited to subjects in intensive care)
- Number of days in an ICU during all readmissions (limited to subjects with at least 1 readmission)

Glucose statistics will be compiled by fasting status (fasting and nonfasting). If fasting status is unknown, then the value will be assumed to be nonfasting.

Vaccine groups will be compared using the van Elteren test, with site as the blocking factor, for duration of surgery, intraoperative blood loss, all glucose responses, duration of hospitalization, number of vertebrae fused, number of days in an ICU for the index visit, total number of days in an ICU during readmission, and total number of days hospitalized for readmissions.

Statistical analyses are summarized in Section 8.2.3.2.

8.1.3.3. Healthcare Utilization

Healthcare utilization variables whose units are days (eg, number of days in an ICU) will be summarized by sample size, mean, standard deviation, median, minimum, and maximum. Vaccine groups will be compared using the van Elteren test, with site as the stratification variable.

Categorical variables (eg, interventional procedures, discharge disposition) will be summarized by frequency counts and proportion of subjects in each category. If a subject has more than 1 hospital admission leading to more than 1 response, only the earliest hospital admission will be included in the analysis. Vaccine groups will be compared using the CMH method, with site as the stratification variable.

The number of service visits will be summarized by sample size, mean, standard deviation, median, minimum, and maximum. Vaccine groups will be compared using the van Elteren test.

The number of rehabilitation/physical therapy visits will be summarized by sample size, mean, standard deviation, median, minimum, and maximum. Vaccine groups will be compared using the van Elteren test.

Zeroes will not be imputed to subjects without the indicated response, eg, number of days in a skilled nursing facility.

All healthcare variables will be reanalyzed, but limited to subjects from the mITT efficacy population who have at least 1 PDI. Subjects with more than 1 PDI may be included in more than 1 analysis. There will be 6 additional analyses, 1 for each type of PDI (BSI and/or deep incisional or organ/space SSI, SSI, or ISA disease) by 2 windows (1-90 and 1-180 days). Vaccine groups will be compared in numeric variables using the Mann-Whitney test and compared in categorical variables using the Fisher exact test. The statistical tests will not incorporate site because the number of PDIs per site will be small or zero.

Statistical analyses for healthcare utilization are summarized in Section 8.2.3.3.

8.1.3.4. Colonization with *Staphylococcus aureus*

Colonization values obtained up to 7 days before vaccination until the day of admission for surgery will be considered eligible baseline values.

Colonization at each visit, acquisition, and clearance are binary endpoints, so they will be summarized with proportions. Vaccine groups will be compared using the CMH test. Site will be the stratification variable.

Carriage status will form a 3 × 2 table with 3 categories—"persistent," "intermittent," and "noncarrier"—and 2 vaccine groups. "Indeterminate" will be considered a missing value. Vaccine groups will be compared using the CMH test. Site will be the stratification variable.

Subgroup analyses will be performed by executing logistic regressions with the dependent variable of the proportion of subjects colonized. The model terms will be vaccine group, subgroup, and vaccine group–subgroup interaction. The colonization results for subgroups with significant interactions, defined as $p<0.10$, will be examined more closely.

One subgroup will be examined at a time. Anatomical sites will be examined separately. Only colonization at scheduled visits will be included. Only *S aureus* colonization will be analyzed because the purpose is to see if vaccine group has different effects within subgroup levels.

Vaccine groups will be compared using the Fisher exact test, 2-sided, for the proportion positive for *S aureus* from unscheduled visits. Stratification by clinical site is not proposed because the unscheduled visits will presumably be associated with possible BSI/SSI/ISA.

The number of these assessments per site should be low. Analyses will be performed by anatomical site and for anatomical sites combined.

Isolates from microbiologically confirmed cases: The association between the *S aureus* strain or strains identified as colonizing the subject and the *S aureus* strain or strains recovered from the PDI will be examined for subjects with microbiologically confirmed *S aureus* infection. Strains will be identified using whole-genome sequencing (WGS). These strains will be paired with the strain or strains from the previous scheduled visit and from the day-of-surgery visit. Matches will be scored as "yes" or "no." If there is no colonization result, then the match will be scored as "missing." The strain identified as colonizing the subject will be obtained from the unscheduled visit associated with the microbiologically confirmed infection. The infection-colonization match will be "yes" if the strain from the microbiologically confirmed case matches the strain from either anatomical site"; otherwise, it will be "no." Vaccine groups will be compared using the Fisher exact test, 2-sided. Comparisons will be performed separately for matches to the day of surgery and matches to a previous scheduled visit.

Colonization at unscheduled visits: Nose and throat swabs will be collected at unscheduled visits for BSI/SSI assessment. The proportions of subjects with at least 1 positive swab at any unscheduled visit will be compiled for each vaccine group. The proportions will be compiled for nose, throat, and any anatomical site. In the latter case, a positive swab in either anatomical site is sufficient to declare a positive result. This will be performed for any *S aureus*, MRSA, and MSSA. The denominators will be the number of subjects with any swabs at any unscheduled visit. Vaccine groups will be compared using the Fisher exact test, 2-sided.

Statistical analyses for colonization are summarized in Section 8.2.3.4.

8.1.3.5. Immunogenicity

On December 20, 2018, the STRIVE study met its pre-specified futility criteria at its Interim Analysis and ceased enrollment. The rationale for this change in immunogenicity testing and analysis is to focus efforts on improving our understanding of why the vaccine did not appear to prevent post-operative *S. aureus* infections. This analysis will replace all previously planned immunogenicity analyses.

The immunogenicity testing and analysis will focus on the four objectives listed below:

Primary objective:

1. To assess the association between CP5/8, ClfA, and MntC functional antibody levels and *S. aureus* infections among subjects within the STRIVE study, adjusting for demographics, health status, and surgical characteristics

Secondary objective:

2. To describe the CP5/8, ClfA, and MntC functional antibody levels among surgical subjects at selected time points by case and vaccination status

Exploratory objectives:

3. To compare the functional immune response to CP5/8, ClfA, and MntC among vaccinated surgical subjects without *S. aureus* infection with vaccinated healthy (non-surgical) subjects

4. To compare the functional immune response of CP5/8, ClfA, and MntC at unscheduled BSI/SSI visit among subjects with *S. aureus* infection with scheduled visits of similar time intervals among subjects without *S. aureus* infection

Study Design:

A nested case-control study within the STRIVE study will be conducted to assess the association of *S. aureus* infection and immunogenicity endpoints at select timepoints, adjusting for demographics, health status, and surgical characteristics. Cases and controls will be matched by study site.

Cases will be any subjects (per protocol or mITT) with adjudication-confirmed *S. aureus*-related SSI and/or BSI (includes deep, organ/space and superficial SSIs). Infections with positive *S. aureus* cultures deemed not clinically significant by adjudicators will be excluded. Other invasive *S. aureus* infections will have descriptive analysis separate from objective 1 and without matched controls. At this time, there are 5 such cases: additional cases may be identified prior to study closeout.

Controls will be per protocol subjects from same study site as cases who completed Visit 5 (post-op Day 90) but did not develop adjudication-confirmed *S. aureus* infections. A total of 7 controls will be selected per case (5 vaccinated and 2 unvaccinated controls per case).

At this time, the plan is to include a total of 51 cases of *S. aureus* infection (both SA4Ag vaccinated and placebo) and 357 controls will be included in this sub-analysis. For the few sites with a shortfall in available controls due to low enrollment, remaining controls will be randomly selected from other “case” sites (ie, sites with cases that have unselected controls remaining) to meet control targets. Extra controls will be associated with their actual site in the logistic regression analysis.

Data analysis:

All analyses will be performed for CP5/OPA, CP8/OPA, ClfA/cLIA, and MntC/cLIA.

Objective 1:

The key independent variables of interest are CP5 and CP8 functional antibody levels (as measured by OPA assay), and secondary independent variables include ClfA and MntC antibody levels (as measured by cLIA assay) assessed at the following time points.

The primary analysis will include the following immunogenicity variables (one value for each antigen for each specimen):

- Day of index surgery before surgery (V2 admission) log transformed titers
- V2 hospital discharge log transformed titers minus V2 admission log transformed titers [Quantify change from V2 admission]
- V5 post-op day 90 log transformed titers minus V2 hospital discharge log transformed titers [Quantify change from V2 discharge]

Multivariate conditional logistic regression will be conducted to model the odds of *S. aureus* infection based on functional antibody levels (ie, 3 immunogenicity variables based on V2 and V5 described above), adjusting for following covariates:

- Demographics (age, sex, race/ethnicity, BMI, ASA, CCI)
- Operative time (mins)
- Number of motion segments/intervertebral levels fused
- Proportion of blood loss (%)
- Units of transfusion
- Days from vaccination to surgery
- Days from surgery to discharge
- Primary versus revision spinal fusion surgery
- Season/year of surgery
- Colonization status (IHMA swab results)
- Diabetes
- Smoking status
- Intrawound antibiotic use (eg, vancomycin vs. other vs. none)

It is recognized that this is an extensive list of potential covariates with a limited sample size. Model selection will attempt forward or backward selection approaches to arrive at a parsimonious model. Results will be reported as odds ratios (ORs) with corresponding 2-sided 95% CIs. Effect modification/interaction will be tested for select variables (eg, vaccination status, colonization status, blood loss, units of transfusion). Results of the multivariable conditional logistical regression will be reported as odds ratios (ORs) with 95% confidence intervals (CIs). Frequency counts and summary statistics (n, mean, standard deviation, median, minimum, and maximum) will be compiled for serology results and all covariates listed above by case/control status.

Proportion of blood loss will be estimated from blood loss (ml) divided by the estimated preoperative blood volume.[11] Estimation of preoperative blood volume depends on sex:

- Male: volume (mL) = $(0.006012 \times H^3)/(14.6 \times W) + 604$
- Female: volume (mL) = $(0.005835 \times H^3)/(15 \times W) + 183$
- where H = height in inches and W = weight in pounds.

Additional sensitivity analyses include 1) repeating overall model for STRIVE primary endpoints only, 2) excluding controls with other non-*S. aureus* infections, and 3) limiting analysis to per protocol subjects.

Objective 2:

Tabular summaries and graphical displays of serological results by case/control status and vaccination status at each sampling time point will be generated. The immunogenicity baseline will be the Visit 1 prevaccination blood draw. Assay values from sera obtained up to 7 days before vaccination will be considered eligible for analysis.

The number and proportion of subjects achieving threshold values will be obtained at each scheduled time point for vaccine group. Threshold values are identified in Table 8. Two-sided 95% CIs will be compiled. Comparisons will be conducted by vaccination and case status (ie, placebo-case, placebo-noncase, SA4Ag-case, and SA4Ag-noncase) using the Fisher exact test, 2-sided.

Vaccine groups will be summarized by proportion of subjects achieving each of 4 thresholds simultaneously. The 4 antigens will be CP5/OPA, CP8/OPA, ClfA/cLIA, and MntC/cLIA. These proportions will be compiled for each scheduled visit. Two-sided 95% CIs will be compiled for each proportion. The vaccine groups will be compared using the Fisher exact test, 2-sided.

GMTs at select time points (V1, V2, V2 discharge, V5, and unscheduled BSI/SSI visits) are obtained by log transformations of indicated values, averaging the log values, then exponentiating the result. The associated 2-sided 95% CI will be obtained by calculating the CI in log scale, referencing the t-distribution, and then exponentiating the lower and upper limits.

GMFRs are geometric means of the titer from the indicated visit divided by the corresponding baseline titer. Subjects missing either baseline or the applicable visit titer are excluded from the calculation. GMTs, GMFRs, and their 2-sided 95% CIs will be calculated for each scheduled time point.

GMTs, GMFRs, and their associated 2-sided 95% CIs will be calculated again for each select time point, but the statistics will be obtained for vaccine group and case occurrence (yes/no), ie, placebo-case, placebo-noncase, SA4Ag-case, and SA4Ag-noncase. These time profiles will be compiled for BSI cases, SSI cases, and ISA cases. Subjects who have more than 1 type of case will appear in as many case categories as relevant. Profiles will be calculated for all time points up to 90 days. Titers obtained after case onset will be excluded, so sample sizes for cases will shrink over time.

GMTs and GMFRs will also be obtained for the following subjects with unscheduled BSI/SSI visits:

- Subjects with any postoperative *S aureus* BSI and/or deep incisional or organ/space SSI occurring within 90 days of the index surgery, limited to unscheduled blood draws within 90 days

- Subjects with any postoperative *S aureus* BSI and/or deep incisional or organ/space SSI occurring within 180 days of the index surgery, limited to unscheduled blood draws within 180 days

- Subjects with any postoperative *S aureus* SSI within 90 days of the index surgery, limited to unscheduled blood draws within 90 days

- Subjects with any postoperative *S aureus* SSI within 180 days of the index surgery, limited to unscheduled blood draws within 180 days

- Subjects with any adjudicated postoperative ISA disease within 90 days of the index surgery, limited to unscheduled blood draws within 90 days

- Subjects with any adjudicated postoperative ISA disease within 180 days of the index surgery, limited to unscheduled blood draws within 180 days

Subjects with more than 1 endpoint of unplanned BSI/SSI assessment will participate in each relevant analysis.

If a specific unscheduled assessment visit was not done, serology samples collected at scheduled visits occurring within 7 days before or after the onset of a PDI may be used in these analyses.

Two additional analyses using ANCOVA will be performed to assess the association between absolute functional antibody levels (at V1 versus V2, V2 versus V2 discharge) and possible interfering host factors, include interaction terms.

1) *ANCOVA comparing V1 versus V2 admission:* This sub-analysis will be limited to those with serological testing at V1 which includes 51 cases and a subset of ~50 controls (chosen from among the 2 vaccinated controls per vaccinated case matched by site). Least-squares means (LSMs) and associated CIs for both vaccine groups at each postbaseline visit will be estimated from an analysis of covariance (ANCOVA). The dependent variable will be log V2 admission titer minus log day-of-vaccination (V1) titer. The model terms will be vaccine group, site, demographics (age, sex, race/ethnicity, BMI, ASA, CCI), colonization status at V1, diabetes, smoking status and elapsed number of days from V1 to V2 admission. Colonization status will be based on any *S aureus* at V1 and will be defined as positive if either nose or throat is positive; otherwise, it will be defined as negative.

Type 3 sums of squares will be used. The difference between SA4Ag and placebo in LSMs will be obtained for V1 versus V2 admission. The exponentiated differences will be ratios of the vaccine groups' geometric means. The corresponding 2-sided 95% CIs will be obtained.

ANCOVA comparing V2 admission versus V2 discharge: An exploratory analysis of effect of blood loss on immunogenicity will be performed. This sub-analysis will include subjects with serological testing at both V2 admission and discharge which includes 51 cases and 357 controls. Loss of titer is assumed to be proportional to blood loss divided by the estimated preoperative blood volume. Assay titers will be log transformed. The dependent variable will be log day-of-discharge titer minus log day-of-surgery titer. If a subject has more than 1 set of relevant immunogenicity results after the index surgery, the earliest results will be used.

The statistical model will be ANCOVA. The model terms will be vaccine group, site, colonization status at V1/V2, , demographics (age, sex, race/ethnicity, BMI, ASA, CCI), diabetes, smoking status, elapsed number of days from the day of surgery to the day of discharge (calculated as the day of discharge minus the day of surgery, plus 1), proportion of blood loss (%), transfusion volume (mL), presence of specific types of transfusion (packed RBCs, platelets, whole blood and plasma), origin of blood product (heterologous or autologous, limited to subjects with packed RBCs or whole-blood transfusion), cell saver, case status and case status by vaccine group interaction. Colonization status will be based on any *S aureus* at V1 or V2 admission and will be defined as positive if either nose or throat is positive; otherwise, it will be defined as negative.

Other terms may be explored. Geometric means will be estimated for levels of statistically significant factors.

Objective 3:

To allow comparison of immunogenicity of surgical population to healthy non-surgical subjects, ~30 samples from B3451015 study (USA-only) and ~34 samples from B3451003 study (Japan only) will be included for OPA and cLIA testing of the four antigens.

For B3451015 which involved subjects aged 18 to <65 years, we will select the oldest available subjects with adequate serology sample remaining. For B3451003 study, we will select vaccinated subjects aged 65 to <86 years with adequate serology sample remaining.

Summaries of serological results will be generated comparing CP5/8, ClfA, and MntC GMTs between the following two groups: 1) Vaccinated surgical subjects without *S. aureus* infection from day of surgery before surgery (V2 admission) and 2) Vaccinated healthy subjects pooled from B3451015 and B3451003 at day 15 [visit window day 15 to 17].

As STRIVE surgical subjects' specimens range from 10 to 60 days after vaccination, comparison with healthy subjects will be limited to STRIVE subjects with specimens from Day 13 to Day 17 among the vaccinated controls selected for objective 1

We will perform ANCOVA controlling for demographics (age, sex, race, ethnicity, BMI), health status (diabetes, smoking status), days after vaccination, and country for this analysis. Additional secondary analysis for objective 3 will include comparison of healthy subjects with surgical subjects with *S. aureus* infection.

Least-squares means (LSMs) and associated CIs for both surgical and health non-surgical groups at their respective Day 15 visit window will be estimated from an ANCOVA. The dependent variable will be postvaccination titer at Day 15. The model terms will be country, demographics (age, sex, race, ethnicity, BMI), diabetes, smoking status and elapsed number of days after vaccination. Type 3 sums of squares will be used. Postvaccination titer at Day 15 will be log transformed. The difference between surgical and non-surgical groups in LSMs will be obtained for postvaccination visit Day 15. The exponentiated differences will be ratios of the surgical versus non-surgical groups' geometric means. The corresponding 2-sided 95% CIs will also be obtained.

Objective 4:

To compare the functional immune response of CP5/8, ClfA, and MntC at unscheduled BSI/SSI visit among vaccinated subjects with *S. aureus* infection with scheduled visits among vaccinated subjects without *S. aureus* infection, blood samples for immunogenicity will be collected at unscheduled visits for BSI/SSI assessment. Any subject with more than 1 unscheduled titer within a specified window will be summarized by the maximum titer.

An exponential decay curve estimating functional immune responses by days since vaccination of vaccinated STRIVE subjects at scheduled V2 hospital discharge visit not associated with *S. aureus* infection will be generated from among the 357 controls from objective 1 for comparison to vaccinated subjects with unscheduled BSI/SSI visits. Graphs of this decay curve will be generated and individual titers for vaccinated cases will be plotted at the appropriate days since vaccination.

ANOVA will be performed to compare serological results of functional antibody levels between the following 2 groups: 1) Unscheduled BSI/SSI visits among vaccinated subjects with *S. aureus* infection and 2) Scheduled V2 discharge visits of vaccinated subjects without *S. aureus* infection. The GMT ratio between cases and non-cases will be estimated, correcting for days since vaccination.

Table 12. Sample Table – Total ~1450 Specimens

Objective #	Sampling Plan	Number of Subjects	Number of Specimens
1-2	All 50 cases (+ 12 other SA infection) and 5 vaccinated controls & 2 unvaccinated controls per case	50 cases (+12 other) and 357 controls (Total 413)	50 x 5 time points = 250 12 x 5 time points = 60 350 x 3 time points = 1050 ~50 x 1 baseline = ~50 = 1410 specimens
3	SA4Ag vaccinated subjects without SA infection (from study above) and healthy non-surgical subjects from 1015 (US) and 1003 (Japan)	Additional 30 healthy vx controls (B3451015) and 34 healthy vx (B3451003)	Additional 64 specimens to above x 1 time point (15d) = 64 add'l specimens

Table 13. Sample Table by Time Point for Serological Testing (n=~1450)

Time points for testing	62 STRIVE case subjects	350 STRIVE control subjects	30 subjects from B3451015	34 subjects from B3451003
V1 Baseline	62	~50*		
V2 Admission	62	350		
V2 Discharge	62	350		
V5 (Day 90)	62	350		
All unplanned BSI/SSI visits	~30	~20		
Day 15 only from B3451015/B3451003			30	34
Total number of specimens	~278	~1120	30	34

* 50 selected vaccinated controls will get V1 testing.

Statistical analyses for immunogenicity are summarized in Section 8.2.3.5.

8.1.4. General Considerations

Day of surgery will be labeled Day 1. The day prior will be Day -1. However, in the analyses of local reactions and systemic events, the 10 days after vaccination will be referred to as Days 1-10.

All CIs for proportions will be 2-sided 95% intervals, obtained using the exact Clopper-Pearson method, as described by Agresti.[12]

Performance of the van Elteren test will use modified ridit scores (Stokes et al).[13]

Based on FDA recommendations, subgroup analyses will be performed for efficacy and selected immunogenicity analyses. The proposed subgroups will be defined by the following:

- Race
- Age group (18 to <50, 50 to 64, and 65 to <86 years)
- Sex
- Ethnicity (Hispanic/Latino and not Hispanic/Latino)
- Smoking status (current smoker, past smoker, and never smoked)
- Site (sites may be combined by similar rates of SSIs following surgery)
- Type of surgeon (as defined in Section 6.3.2)
- Country
- CCI score substituting for medical history; CCI levels will be 0, 1, 2, and ≥3
- Colonization status at the time of surgery
- Colonization status at the time of vaccination
- Timing of surgery after vaccination (1-14 days, 15-30 days, 31-45 days, 46-64 days, and 65 days or more after vaccination)

Analyses with limited numbers of cases per subgroup may not be performed.

Colonization status at the time of surgery is defined as follows:

- Positive for any subject with at least 1 positive *S aureus* colonization swab at 1 anatomical site or both anatomical sites on the day of surgery
- Negative for any subject with negative *S aureus* colonization swabs at both anatomical sites and not more than 1 missing colonization result on the day of surgery
- Missing for subjects without any colonization swab results from either anatomical site on the day of surgery

The definition of colonization status for "any anatomical site" is the same for colonization at the time of vaccination and colonization on the day of surgery:

- Positive for any subject with at least 1 positive *S aureus* colonization swab at either anatomical site or both anatomical sites
- Negative for any subject with negative *S aureus* colonization swabs at both anatomical sites and not more than 1 missing colonization result
- Missing for subjects without any colonization swab results from either anatomical site

All statistically significant results associated with subgroup analyses will be regarded as hypothesis generating.

8.2. Statistical Analyses

The day of surgery will be labeled Day 1. The days before surgery will be labeled Day -1, Day -2, etc.

Besides the analyses described in this document, other exploratory analyses may be performed.

8.2.1. Analyses for Efficacy

All primary and secondary endpoints will be subjected to subgroup analysis, where SA4Ag and placebo will be compared at each level of the subgroup. Fifteen subgroups will be examined:

1. Race.
2. Age group.
3. Sex.
4. Ethnicity.
5. Smoking status.
6. Country.
7. CCI score.
8. Colonization at the time of surgery (performed for any *S aureus*).
9. Colonization at the time of surgery (performed for MRSA).
10. Colonization at the time of surgery (performed for MSSA).
11. Timing of the surgery after vaccination.

12. Presurgery decolonization (any/none).

13. Intrawound application of antibiotic (yes/no).

14. High-enrolling investigator sites (yes/no); the number of mITT subjects will be compiled by site. Sites with ≥50 mITT subjects will be considered high-enrolling sites.

15. High–attack rate investigator sites (yes/no); the number of mITT subjects with any PDI (BSI, deep incisional or organ/space SSI, superficial SSI, or ISA disease) will be compiled by site. The median of the nonzero values will be computed (only nonzero values will be included because multiple sites are expected to not have any PDI). Sites with the median or higher will be considered high–attack rate sites.

The subgroup analysis by timing of surgery after vaccination in the interval 1-14 days should be understood as an interval of 9-14 days in the PP efficacy population, because subjects with timing of 1-8 days after vaccination will be excluded from the PP efficacy population.

Subgroup analyses by site are not proposed because of the expected sparseness of cases.

Subgroup analyses will be performed in both the PP and mITT efficacy populations.

All analyses for 1 endpoint in 1 population, ie, all subjects plus every level of each subgroup, will be included in the same table.

Subgroup analyses will not be performed for postoperative ISA disease based on baseline *S aureus* colonization, because these are exploratory endpoints. Instead, baseline *S aureus* colonization will be included as a subgroup in the analyses of postoperative ISA disease.

Efficacy analyses are summarized in Table 14.

Table 14. Summary of Efficacy Analyses in the Per-Protocol Population

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
Postoperative *Staphylococcus aureus* BSI and/or deep incisional or organ/space SSI occurring within 90 days	PP	Binomial	N/A	N/A	Primary efficacy endpoint
Postoperative *S aureus* BSI and/or deep incisional or organ/space SSI occurring within 180 days	PP	Binomial	N/A	N/A	Secondary endpoint
Postoperative *S aureus* SSI occurring within 90 days	PP	Binomial	N/A	N/A	Secondary endpoint
Postoperative *S aureus* SSI occurring within 180 days	PP	Binomial	N/A	N/A	Secondary endpoint
Postoperative ISA disease occurring within 90 days	PP	Binomial	N/A	N/A	Exploratory

Table 14. Summary of Efficacy Analyses in the Per-Protocol Population

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
Postoperative ISA disease occurring within 180 days	PP	Binomial	N/A	N/A	Exploratory
Postoperative *Staphylococcus aureus* BSI and/or deep incisional or organ/space SSI occurring within 90 days based on baseline *S aureus* colonization	PP	Binomial	N/A	N/A	Exploratory
Postoperative *Staphylococcus aureus* BSI and/or deep incisional or organ/space SSI occurring within 180 days based on baseline *S aureus* colonization	PP	Binomial	N/A	N/A	Exploratory
Postoperative BSI and/or deep incisional or organ/space SSI or superficial SSI, attributable to any pathogen, occurring within 90 days	PP	Binomial	N/A	N/A	Exploratory
Postoperative BSI and/or deep incisional or organ/space SSI or superficial SSI, attributable to any pathogen, occurring within 180 days	PP	Binomial	N/A	N/A	Exploratory

Abbreviations: BSI = bloodstream infection; ISA = invasive *Staphylococcus aureus*; N/A = not applicable; PP = per-protocol; SSI = surgical-site infection.

The analyses will be repeated in the mITT efficacy populations (Table 15).

Table 15. Summary of Efficacy Analyses in the mITT Efficacy Populations

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
Postoperative *Staphylococcus aureus* BSI and/or deep incisional or organ/space SSI occurring within 90 days	mITT Restricted mITT	Binomial	N/A	N/A	Secondary efficacy endpoint
Postoperative *S aureus* BSI and/or deep incisional or organ/space SSI occurring within 180 days	mITT Restricted mITT	Binomial	N/A	N/A	Secondary endpoint

Table 15. Summary of Efficacy Analyses in the mITT Efficacy Populations

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
Postoperative *S aureus* SSI occurring within 90 days	mITT, Restricted mITT	Binomial	N/A	N/A	Secondary endpoint
Postoperative *S aureus* SSI occurring within 180 days	mITT, Restricted mITT	Binomial	N/A	N/A	Secondary endpoint
Postoperative ISA disease occurring within 90 days	mITT, Restricted mITT	Binomial	N/A	N/A	Exploratory
Postoperative ISA disease occurring within 180 days	mITT, Restricted mITT	Binomial	N/A	N/A	Exploratory
Postoperative ISA disease occurring within 90 days based on baseline *S aureus* colonization	mITT, Restricted mITT	Binomial	N/A	N/A	Exploratory
Postoperative ISA disease occurring within 180 days based on baseline *S aureus* colonization	mITT, Restricted mITT	Binomial	N/A	N/A	Exploratory
Postoperative BSI and/or deep incisional or organ/space SSI or superficial SSI, attributable to any pathogen, occurring within 90 days	mITT, Restricted mITT	Binomial	N/A	N/A	Exploratory
Postoperative BSI and/or deep incisional or organ/space SSI or superficial SSI, attributable to any pathogen, occurring within 180 days	mITT, Restricted mITT	Binomial	N/A	N/A	Exploratory
Overrun	mITT, Restricted mITT	Binomial	N/A	N/A	Exploratory; includes cases arriving after the planned 48

Abbreviations: BSI = bloodstream infection; ISA = invasive *Staphylococcus aureus*; N/A = not applicable; SSI = surgical-site infection.

NNT analyses will be reported for each endpoint in both the PP and mITT populations (see Table 16). NNTs will not be compiled for subgroups. All NNT analyses for the same population will be presented in the same table.

Table 16. Summary of Number-Needed-to-Treat Analyses for Efficacy Endpoints

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
NNT for each efficacy endpoint	PP	Reciprocal of difference in proportions and associated 95% CI	N/A	N/A	One table for all NNT endpoint analyses
NNT for each efficacy endpoint	mITT	Reciprocal of difference in proportions and associated 95% CI	N/A	N/A	One table for all NNT endpoint analyses
NNT for each efficacy endpoint	Restricted mITT	Reciprocal of difference in proportions and associated 95% CI	N/A	N/A	One table for all NNT endpoint analyses

Abbreviations: N/A = not applicable; NNT = number needed to treat; PP = per-protocol.

Only the primary and secondary endpoints will be subjected to sensitivity analyses. Missing data sensitivity analyses will not be performed on subgroups. Sensitivity analyses will be performed for both the PP and mITT populations. There will be 12 sets of sensitivity analyses.

All sensitivity analyses for a given endpoint in a given population should appear on the same table. The corresponding efficacy analysis described in Table 14 (PP efficacy population) or Table 15 (mITT efficacy population) should be included for ease of review. Sensitivity analyses are summarized in Table 17.

Table 17. Summary of Sensitivity Analyses

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
Postoperative *Staphylococcus aureus* BSI and/or deep incisional or organ/space SSI occurring within 90 days	PP, mITT, Restricted mITT	Best case, worst case, observed, MAR, MNAR, follow-up time	N/A	N/A	Endpoint in PP population is primary endpoint
Postoperative *S aureus* BSI and/or deep incisional or organ/space SSI occurring within 180 days	PP, mITT, Restricted mITT	Best case, worst case, observed, MAR, MNAR, follow-up time	N/A	N/A	Secondary endpoint
Postoperative *S aureus* SSI occurring within 90 days	PP, mITT, Restricted mITT	Best case, worst case, observed, MAR, MNAR, follow-up time	N/A	N/A	Secondary endpoint
Postoperative *S aureus* SSI occurring within 180 days	PP, mITT, Restricted mITT	Best case, worst case, observed, MAR, MNAR, follow-up time	N/A	N/A	Secondary endpoint

Abbreviations: BSI = bloodstream infection; MAR = missing at random; MNAR = missing not at random; N/A = not applicable; PP = per-protocol; SSI = surgical-site infection.

8.2.2. Analysis of Safety Data

The tier method for AEs is described in Section 8.1.2.1.

All analyses will be performed on the safety population.

8.2.2.1. Adverse Events and Serious Adverse Events

Tier 1 AEs will be identified in the safety review plan.

Tier 1-3 methodology will be applied to analyses from 3 intervals: vaccination until surgery, vaccination until Day 42, and surgery until Day 42. Tier 3 methodology will be applied to newly diagnosed chronic medical disorders from Day 42 until Day 180.

Tier 1, Tier 2, and Tier 3 analyses will be repeated for each level of sex, race, age group, and ethnicity. All levels of the subgroup will be included in the same table, eg, males and females in the same table, in order to facilitate review.

Subgroup analyses are not proposed for newly diagnosed chronic medical disorders, SAEs, or deaths.

Subgroup analyses are not proposed for AE rates before surgery, because this is a sensitivity analysis.

All analyses will be performed in the safety population.

AE analyses are summarized in Table 18.

Table 18. Summary of Adverse Event Analyses

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
Proportions of subjects with AEs from vaccination until day of surgery	Safety	Tier 1-3	Vaccine group	N/A	Assess safety of SA4Ag until surgery
Proportions of subjects with AEs from vaccination until Day 42	Safety	Tier 1-3	Vaccine group	N/A	Assess safety of SA4Ag from vaccination until Day 42 after surgery
Proportions of subjects with AEs from day of surgery until Day 42	Safety	Tier 1-3	Vaccine group	N/A	Assess safety of SA4Ag for 42 days after surgery
Proportions of subjects with newly diagnosed chronic medical disorders from Day 42 until Day 180	Safety	Tier 2-3	Vaccine group	N/A	Assess safety of SA4Ag
Rates of AEs per 100 or 1000 days from vaccination until day of surgery	Safety	Poisson regression	Vaccine group	N/A	Surgery follows vaccination in 10-60 days, so this analysis will account for differing exposures among subjects

Table 18. Summary of Adverse Event Analyses

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
SAEs and deaths from vaccination until day of surgery	Safety	Tier 2-3	Vaccine group	N/A	Assess safety of SA4Ag
SAEs and deaths from vaccination until Day 180	Safety	Tier 2-3	Vaccine group	N/A	Assess safety of SA4Ag
SAEs and deaths from surgery until Day 180	Safety	Tier 2-3	Vaccine group	N/A	Assess safety of SA4Ag
Deaths associated with *Staphylococcus aureus*	Safety	Fisher exact	Vaccine group	N/A	Denominator will be number of deaths
Proportions of subjects with AEs from vaccination until day of surgery, by subgroup	Safety	Tier 1-3	Vaccine group	N/A	Assess safety of SA4Ag from vaccination until surgery
Proportions of subjects with AEs from vaccination until Day 42, by subgroup	Safety	Tier 1-3	Vaccine group	N/A	Assess safety of SA4Ag from vaccination until Day 42 after surgery
Proportions of subjects with AEs from day of surgery until Day 42, by subgroup	Safety	Tier 1-3	Vaccine group	N/A	Assess safety of SA4Ag for 42 days after surgery

Abbreviation: N/A = not applicable.

Tier 1 and Tier 2 AEs will have risk differences plotted. All risk differences will be calculated as SA4Ag minus placebo.

- Tier 1 figures will have the footnotes "95% confidence intervals are provided to help gauge the precision of the estimates for risk difference," "p-Values and confidence intervals are not adjusted for multiplicity and should be used for screening purpose only," and "Risk difference is computed as SA4Ag minus placebo."

- Tier 2 figures will have the footnotes "95% Confidence intervals are provided to help gauge the precision of the estimate for the risk difference," "They are not adjusted for multiplicity and should be used for estimation purpose only," and "Risk difference is computed as SA4Ag minus placebo."

The EAC will adjudicate events contributing to ISA disease. Events that are not primary or secondary endpoints associated with surgery will be included in the AE/SAE tables. However, since these AEs will be adjudicated, a separate compilation will be performed, further classifying each of these conditions as "associated with *S aureus*," with responses being "yes," "no," or "not determined." AE methodology will be Tier 3 because a small number of events is expected. Compilations will be performed over the same 3 time intervals as AEs.

8.2.2.2. Multiple-Organ Failure

The analyses for MOF are summarized in Table 19. All endpoints should be presented in the same table.

Table 19. Summary of Analyses for Multiple-Organ Failure

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
Organ systems that failed	Safety	Fisher exact	Vaccination group	N/A	
Meet criteria for MOF	Safety	Fisher exact	Vaccination group	N/A	
MOF associated with infection (PDI or non-PDI)	Safety	Fisher exact	Vaccination group	N/A	
MOF not associated with infection (other or unknown)	Safety	Fisher exact	Vaccination group	N/A	
Given MOF associated with PDI, confirmed *Staphylococcus aureus*, confirmed non–*S aureus*, not microbiologically confirmed	Safety	Fisher exact	Vaccination group	N/A	
MOF associated with non-PDI, confirmed *S aureus*, confirmed non–*S aureus*, not microbiologically confirmed	Safety	Fisher exact	Vaccination group	N/A	

Abbreviations: MOF = multiple-organ failure; N/A = not applicable; PDI = protocol-defined infection.

8.2.2.3. Local Reactions and Systemic Events

A listing will be created for local reactions and systemic events (including fever) still present on the last day that the subject's e-diary was completed. This will not include subjects with both redness and swelling <5 caliper units on the last day. The number of days since vaccination will be computed as the stop date minus the date of vaccination, plus 1. If the event is still ongoing, then the stop date will be set to missing, as will the number of elapsed days. The listing will include site, subject, injection site (for local reactions only), vaccine group, name of local reaction or systemic event, vaccination date, stop date, ongoing (yes/no), and number of elapsed days. The listing will be sorted by vaccine group, site, subject, and name of local reaction or systemic event. The listing will include the date when the reaction resolves, or ends as "ongoing."

Subjects reporting any severe local reaction and any severe systemic event on the same day will be identified. Their 10-day local reactions and systemic events will be listed, as well as resolution of events persisting beyond 10 days.

The proportions of subjects completing the e-diary each day will be compiled, as well as the categories "Days 1-4," "Days 1-7," and "All days (Days 1-10)." Subjects who fail to complete the e-diary only because of early surgery, ie, surgery on the 10th day after vaccination or earlier, will still be considered to have completed the e-diary as of the day before surgery.

For example, a subject who completes the e-diary on every day up to the 8th day, and then has surgery on the 9th day, will be considered to have completed "All days (Days 1-10)."

The proportions of subjects with at least 1 e-diary entry on or after the day of surgery will be compiled by vaccine group. Entries in e-diaries on or after the day of surgery will be excluded from analysis. These proportions will quantify what proportion of subjects may have additional local or systemic entries that were not included in the analysis.

Per protocol Section 4.1, the e-diary need not be completed on the day of surgery. The proportions of subjects with surgery on Days 1, 2, 3, etc, will be compiled to explain incomplete e-diaries.

All analyses will be performed in the safety population. No subgroup analyses are proposed.

Analyses for local reactions and systemic events are summarized in Table 20.

Table 20. Summary of Analyses for Local Reactions and Systemic Events

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
Proportions of maximum mild, moderate, severe, and any reaction over Days 1-10 after vaccination (redness, swelling, pain, plus any local reaction)	Safety	Fisher exact	Vaccine group	See Section 6.2.3	Assess reactogenicity
Proportions of subjects with the indicated reaction (redness, swelling, pain, plus any local reaction) on each day	Safety	Descriptive only	Vaccine group	N/A	No statistical test
Proportions of subjects with moderate or severe indicated reaction (redness, swelling, pain, plus any local reaction) on each day	Safety	Descriptive only	Vaccine group	N/A	No statistical test
Duration of local reaction in days, but only for pain, swelling, and redness	Safety	Mann-Whitney	Vaccine group	N/A	Assess reactogenicity
Onset day of local reaction for pain, swelling, redness, and any local reaction	Safety	Mann-Whitney	Vaccine group	N/A	Assess reactogenicity
Baseline assessment of fatigue, headache, muscle pain, and joint pain over the previous month	Safety	Fisher exact	Vaccine group	N/A	Assess reactogenicity

Table 20. Summary of Analyses for Local Reactions and Systemic Events

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
Proportion of maximum mild, moderate, severe, and any systemic event over Days 1-10 after vaccination (fever, vomiting, diarrhea, fatigue, headache, muscle pain, joint pain, plus any systemic event); also antipyretic/pain medication use	Safety	Fisher exact	Vaccine group	See Section 6.2.3	Assess reactogenicity
Proportions of subjects with the indicated systemic event each day, plus any systemic event	Safety	Descriptive only	Vaccine group	N/A	No statistical test
Proportions of subjects with moderate or severe indicated systemic event on each day	Safety	Descriptive only	Vaccine group	N/A	No statistical test
Duration of systemic event/fever, but not for any systemic event	Safety	Mann-Whitney	Vaccine group	N/A	Assess reactogenicity
Onset of systemic event/any systemic event/fever	Safety	Mann-Whitney	Vaccine group	N/A	Assess reactogenicity
Subjects reporting any severe local reaction and any severe systemic event on the same day	Safety	N/A	Vaccine group	N/A	Listing of Days 1-10, including day of interest
Listing of unresolved reactions still present on the last e-diary day	Safety	N/A	Vaccine group	N/A	
Completing the e-diary each day	Safety	N/A	Vaccine group	N/A	
Completing the e-diary on Days 1-10	Safety	N/A	Vaccine group	N/A	Categories for days completed are 100%, 75%-<100%, 50%-74%, 25%-49%, and <25%
One or more e-diary entries on or after the day of surgery	Safety	N/A	Vaccine group	N/A	
Proportion of subjects with surgery on Days 1, 2, 3, etc, after vaccination	Safety	N/A	Vaccine group	N/A	Days with zero proportions may be omitted; eg, there is no expectation of subjects' receiving surgery on the day

Table 20. Summary of Analyses for Local Reactions and Systemic Events

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/ Comments
					after vaccination
Number needed to harm	Safety	Reciprocal on 95% CI on difference between investigational products	Vaccine group	N/A	Performed for any local reaction and any systemic event

Abbreviations: e-diary = electronic diary; N/A = not applicable.

8.2.2.4. Concomitant Medications, Vaccinations, and Blood Products

Analyses for concomitant medications, nonstudy vaccines, and blood products are summarized in Table 21.

Table 21. Summary of Analyses for Concomitant Medications, Nonstudy Vaccines, and Blood Products

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
Antibiotics, systemic steroids	Safety	Fisher exact	Vaccine group	N/A	3 Time intervals: 1. The day of vaccination to the day before surgery 2. The day of surgery until study completion 3. The day of vaccination until study completion
Intraspinal steroids	Safety	Fisher exact	Vaccine group	N/A	2 Time intervals: 1. From 6 months before enrollment until the day before vaccination 2. The day of vaccination until study completion
Nonstudy vaccines	Safety	Fisher exact	Vaccine group	N/A	2 Time intervals: 1. From 6 months before enrollment until the day before vaccination 2. The day of vaccination until study completion

Table 21. Summary of Analyses for Concomitant Medications, Nonstudy Vaccines, and Blood Products

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
Days of antibiotic use	Safety	van Elteren	Vaccine group; site for van Elteren test	N/A	3 Time intervals: 1. The day of vaccination to the day before surgery 2. The day of surgery until study completion 3. The day of vaccination until study completion
Blood products: transfusions (blood product type, origin of blood product if packed RBCs or whole blood, use of cell saver, number of units, number of transfusions per subjects)	Safety	CMH for discrete variables, van Elteren for number of units and number of transfusions	Vaccine group; site for van Elteren test	N/A	3 Time intervals: 1. The day of vaccination until before surgery 2. Days 1-7 (where Day 1 is the day of surgery) 3. Days 8 or more

Abbreviations: CMH = Cochran-Mantel-Haenszel; N/A = not applicable; RBC = red blood cell.

All analyses will be conducted in the safety population. No subgroup analyses are proposed.

8.2.3. Analysis of Other Endpoints

8.2.3.1. Demographic and Baseline Characteristics

Statistics on demographic characteristics will be compiled for the PP efficacy, mITT efficacy, safety, mITT immunogenicity, and mITT colonization populations, except where noted.

Demographic and baseline characteristic analyses are summarized in Table 22.

Table 22. Summary of Demographic and Baseline Characteristic Analyses

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
Disposition	5 Populations	Descriptive	Vaccine group	N/A	Lines for enrolled, randomized, not vaccinated, vaccinated, surgery, no surgery, completed, and discontinued; discontinued further split by reason for discontinuation

Table 22. Summary of Demographic and Baseline Characteristic Analyses

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
Sex, race, racial designation, ethnicity, smoking status, age group, any alcohol use, colonization status, indication for surgery, age (in years), BMI, units of alcohol, Charlson Comorbidity Index score, Charlson mortality probability, ASA classification, vital signs, elapsed days from vaccination to index surgery	5 Populations	Descriptive	Vaccine group	N/A	
Medical history	Safety only	Descriptive	Vaccine group	N/A	
Physical examination	Safety only	Descriptive	Vaccine group	N/A	
Vaccine administration	mITT	Descriptive	Vaccine group	N/A	
Subjects with blood samples drawn for immunogenicity analysis	mITT	Descriptive	Vaccine group	N/A	

Abbreviations: ASA = American Society of Anesthesiologists; BMI = body mass index; e-diary = electronic diary; N/A = not applicable.

No subgroup analyses are proposed.

8.2.3.2. Index Surgery and Hospitalization

Vaccine groups will be compared in the safety, mITT efficacy, and PP efficacy populations.

The analyses will be performed for all subjects in the indicated populations, and then performed for the indicated subgroups:

- With and without postoperative BSI and/or deep incisional or organ/space SSI within 90 days
- With and without postoperative BSI and/or deep incisional or organ/space SSI within 180 days
- With and without postoperative SSI within 90 days
- With and without postoperative SSI within 180 days
- With and without postoperative SSI within 90 days

- With and without postoperative ISA disease within 180 days

These subgroup analyses will be limited to the mITT efficacy and PP efficacy populations. The tables with these analyses should include both levels of the indicated infection, eg, BSI yes/no, to facilitate identification of antagonistic or synergistic effects.

Index surgery and hospitalization analyses are summarized in Table 23.

Table 23. Summary of Index Surgery and Hospitalization Analyses

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
Variables where 1 level selected per subject, eg, *Staphylococcus aureus* carriage	Safety, mITT efficacy, PP efficacy	CMH	Site as stratification factor	N/A	
Variables where multiple levels may be selected per subject, eg, vertebrae fused	Safety, mITT efficacy, PP efficacy	Descriptive	Vaccine group	N/A	No statistical test because some subjects may report more than 1 level
Numeric, eg, glucose responses, duration of surgery, intraoperative blood loss	Safety, mITT efficacy, PP efficacy	van Elteren	Vaccine group, site as blocking factor	N/A	Glucose responses will be compiled by fasting and nonfasting status.
Variables where 1 level selected per subject, eg, *S aureus* carriage	mITT efficacy, PP efficacy	CMH	Site as stratification factor	N/A	Performed for each level of BSI yes/no, SSI yes/no, and ISA yes/no
Variables where multiple levels may be selected per subject, eg, vertebrae fused	mITT efficacy, PP efficacy	Descriptive	Vaccine group	N/A	Performed for each level of BSI yes/no, SSI yes/no, and ISA yes/no
Numeric, eg, glucose responses, duration of surgery, intraoperative blood loss	mITT efficacy, PP efficacy	van Elteren	Vaccine group, site as blocking factor	N/A	Performed for each level of BSI yes/no, SSI yes/no, and ISA yes/no. Glucose responses will be compiled by fasting and nonfasting status.

Variables are classified as single level, multiple levels, and numeric in Section 8.1.3.2.
Abbreviations: BSI = bloodstream infection; CMH = Cochran-Mantel-Haenszel;
ISA = invasive *Staphylococcus aureus*; N/A = not applicable; PP = per-protocol; SSI = surgical-site infection.

8.2.3.3. Healthcare Utilization

All analyses will be performed in the mITT efficacy population. No subgroup analyses are proposed.

Healthcare variables will be reanalyzed, but limited to subjects adjudicated to have 1 of 3 endpoints (BSI and/or organ/space or deep incisional SSI, SSI, or ISA disease) crossed with 2 windows (1-90 and 1-180 days after surgery).

Healthcare utilization analyses are summarized in Table 24.

Table 24. Summary of Healthcare Utilization Analyses

Endpoint	Population	Statistical Method	Stratification	Missing Data	Interpretation/Comments
Numeric healthcare utilization: • duration of hospitalization • number of readmissions related to complications of index surgery • number of days in the ICU • total hospitalizations from index surgery and any readmissions • total days in ICU from index surgery and any readmissions • number of rehabilitation/ physical therapy visits as an outpatient • number of service visits • number of inpatient days in rehabilitation or nursing facility	mITT	van Elteren	Site	N/A	Impact of vaccination on subsequent healthcare use

Table 24. Summary of Healthcare Utilization Analyses

Endpoint	Population	Statistical Method	Stratification	Missing Data	Interpretation/Comments
Categorical healthcare utilization: • location prior to hospital admission • ongoing hospitalization, yes/no • discharge disposition • proportion of readmissions to hospital • proportion of readmissions related to complications of index surgery • additional surgery • proportion in an ICU in readmission • proportion discharge after readmission	mITT	CMH	Site	N/A	Impact of vaccination on subsequent healthcare use
Numeric healthcare utilization: • duration of hospitalization • number of readmissions related to complications of index surgery • number of days in the ICU • total hospitalization from index surgery and any readmissions • total days in ICU from index surgery and any readmissions • number of service visits • number of inpatient days in rehabilitation or nursing facility	Any subject with PDI	Mann-Whitney	N/A	N/A	Impact of vaccination on subsequent healthcare use, limited to subjects with PDIs Performed for PDIs in 1-90 and in 1-180 days

Table 24. Summary of Healthcare Utilization Analyses

Endpoint	Population	Statistical Method	Stratification	Missing Data	Interpretation/Comments
Categorical healthcare utilization: • location prior to hospital admission • ongoing hospitalization, yes/no • discharge disposition • proportion of readmissions to hospital • proportion of readmissions related to complications of index surgery • additional surgery • proportion in an ICU in readmission • proportion discharge after readmission	Any subject with PDI	Fisher exact	N/A	N/A	Impact of vaccination on subsequent healthcare use, limited to subjects with PDIs Performed for PDIs in 1-90 and in 1-180 days

Abbreviations: CMH = Cochran-Mantel-Haenszel; ICU = intensive care unit; N/A = not applicable; PDI = protocol-defined infection.

8.2.3.4. Colonization with *Staphylococcus aureus*

All analyses will be performed using the mITT colonization population.

The proportions of subjects with positive swabs will be plotted against time point. The time points' labels on the figures will be "Day of Vaccination," "Day of Surgery," "Day of Discharge," "Day 42," "Day 90," and "Day 180." The values for the time points will be -15, 1, 2, 42, 80, and 180. The legend will identify anatomical site (nasal/oropharyngeal/combined). Plots will be created for "any *S aureus*," MSSA, and MRSA.

Subgroup analyses will be performed on colonization, carriage status, acquisition, and clearance. The subgroups will be age group, sex, baseline colonization status, and primary endpoint (case or noncase). The subgroup analysis of the primary endpoint will be limited to scheduled visits within 90 days, as per definition of the primary endpoint.

A further subgroup will be defined by subjects having any decolonization measures or preadmission antimicrobial agent(s) before surgery (response = yes/no). These analyses will be performed only for "any *S aureus*."

The analysis of matching isolates from microbiologically confirmed cases will be performed separately for nose and throat for each of the efficacy endpoints (BSI and/or deep incisional or organ/space SSI within 90 days, BSI and/or deep incisional or organ/space SSI within 180 days, SSI within 90 days, SSI within 180 days, ISA disease within 90 days, and ISA disease within 180 days), but limited to subjects in the mITT colonization population. If there may be more than 48 cases for the primary endpoint, ie, with BSI and/or deep incisional or organ/space SSI, these extra cases will be included in this analysis.

Colonization analyses and their statistical tests are summarized in Table 25.

Table 25. Summary of Colonization Analyses

Endpoint	Population	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
Proportions of subjects colonized with *Staphylococcus aureus* on each visit	mITT colonization	CMH	Site as stratification variable	N/A	Explore colonization patterns between vaccine groups longitudinally Perform for any *S aureus*, MRSA, and MSSA
Carriage status (persistent, intermittent, etc)	mITT colonization	CMH	Site as stratification variable	N/A	Explore effect of vaccination of carriage duration Perform for any *S aureus*, MRSA, and MSSA
Acquisition	mITT colonization	CMH	Site as stratification variable	N/A	Explore effect of vaccination on acquiring *S aureus* Perform for any *S aureus*, MRSA, and MSSA
Clearance	mITT colonization	CMH	Site as stratification variable	N/A	Explore effect of vaccination on clearing *S aureus.* Perform for any *S aureus*, MRSA, and MSSA
Colonization at unscheduled visits	mITT colonization	Fisher exact		N/A	Colonization in suspected BSI/SSI cases Perform for any *S aureus*, MRSA, and MSSA
Association between strain colonizing subject and strain recovered from infection	mITT colonization	Fisher exact	Nose and throat separately	N/A	Repeat for all efficacy endpoints
Subgroup analysis on proportions colonized	mITT colonization	Logistic regression	Vaccine group, subgroup, and interaction	N/A	Examine interaction of subgroup and vaccine. Perform for any *S aureus*. Nose and throat separately
Infection-colonization	mITT colonization	Fisher exact	N/A	N/A	Are subjects self-infecting?

Abbreviations: BSI = bloodstream infection; CMH = Cochran-Mantel-Haenszel;
MSSA = methicillin-sensitive *Staphylococcus aureus*; MRSA = methicillin-resistant *Staphylococcus aureus*;
MSSA = methicillin-sensitive *Staphylococcus aureus*; N/A = not applicable; PDI = protocol-defined infection;
SSI = surgical-site infection.

8.2.3.5. Immunogenicity

Immunogenicity analyses are summarized in Table 26.

Table 26. Summary of Immunogenicity Analyses

Endpoint	Populations	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
Odds of *S. aureus* infection based on functional antibody levels at time points	mITT immunogenicity	Multivariable conditional logistic regression	The model terms will be demographics (age, sex, race/ethnicity, BMI, ASA, CCI), operative time (mins), number of motion segments/ intervertebral levels fused, **proportion of** blood loss (**%**), units of transfusion**, days from vaccination to surgery, d**ays from surgery to discharge**,** primary versus revision spinal fusion surgery, season/year of surgery, colonization status, diabetes. smoking status, intrawound antibiotic use	N/A	Sensitivity analyses for primary endpoints only, excluding controls with other non-S. aureus infections, and limiting analysis to per protocol subjects.
Proportions of subjects achieving threshold at postvaccination time points	mITT immunogenicity	Fisher exact	Vaccine group	N/A	Describe immunogenicity

Table 26. Summary of Immunogenicity Analyses

Endpoint	Populations	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
Proportions of subjects achieving threshold to 4 assays (ClfA/cLIA, MntC/cLIA, CP5/OPA, CP8/OPA) simultaneously at postvaccination time points	mITT immunogenicity	Fisher exact	Vaccine group	Subjects missing 1 or more assays will be excluded	Describe immunogenicity
GMT and 95% CI at baseline and postvaccination time points	mITT immunogenicity	N/A	Descriptive only	Nonmissing values < LLOQ will be replaced with 0.5 × LLOQ	Describe immunogenicity
GMFR and 95% CI from baseline to postvaccination time points	mITT immunogenicity	N/A	Descriptive only	Nonmissing values < LLOQ will be replaced with 0.5 × LLOQ	Describe immunogenicity
Profiles of GMT by visit, compiled for cases and noncases	mITT immunogenicity	Descriptive	N/A	Nonmissing values < LLOQ will be replaced with 0.5 × LLOQ	Repeat for each type of case (BSI, SSI, and ISA)
Proportions with n-fold rise from baseline to each postvaccination time point	mITT immunogenicity	Descriptive		Nonmissing values < LLOQ will be replaced with 0.5 × LLOQ	Repeat for 2-, 4-, 8-, 16-, and 32-fold
GMTs and GMFRs from unscheduled visits for BSI/SSI assessment	mITT immunogenicity	2-Sample t-test	N/A	Nonmissing values < LLOQ will be replaced with 0.5 × LLOQ	Limited to subjects with any unscheduled visit

Table 26. Summary of Immunogenicity Analyses

Endpoint	Populations	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
LSMs at postvaccination time points V1 vs V2 admission	mITT immunogenicity	ANCOVA	The model terms will be vaccine group, site, demographics (age, sex, race/ethnicity, BMI, ASA, CCI), colonization status at V1, diabetes, smoking status, and elapsed number of days from V1 to V2 admission.	Nonmissing values < LLOQ will be replaced with 0.5 × LLOQ	Describe immunogenicity
Effect of blood loss on titer at time points V2 admission vs V2 discharge	mITT immunogenicity	ANCOVA	The model terms will be vaccine group, site, colonization status at V1/V2, demographics (age, sex, race/ethnicity, BMI, ASA, CCI), diabetes, smoking status, elapsed number of days from the day of surgery to the day of discharge, proportion of blood loss (%), transfusion volume (mL), type of transfusion (packed RBCs, platelets, whole blood and plasma), origin of blood product (heterologous or autologous, limited to subjects with packed RBCs or whole-blood transfusion), cell saver, case status and case status by vaccine group interaction.	N/A	Describe immunogenicity

Table 26. Summary of Immunogenicity Analyses

Endpoint	Populations	Statistical Method	Model/ Covariates/ Strata	Missing Data	Interpretation/Comments
LSMs at postvaccination time point Day 15 between surgical and non-surgical population	mITT immunogenicity	ANCOVA	The model terms will be country, demographics (age, sex, race, ethnicity, BMI), diabetes, smoking status and elapsed number of days after vaccination.	Nonmissing values < LLOQ will be replaced with 0.5 × LLOQ	Describe immunogenicity Non-surgical healthy subjects from B3451003 and B3451015 Secondary analysis with surgical subjects with *S. aureus* infection compared to healthy subjects
Comparison of GMTs between cases from unscheduled visits for BSI/SSI assessment versus non-cases from scheduled V2 discharge visits	mITT immunogenicity	ANOVA	N/A	Nonmissing values < LLOQ will be replaced with 0.5 × LLOQ	Describe immunogenicity. The GMT ratio between cases and non-cases will be estimated, correcting for days since vaccination.

Abbreviations: ANCOVA = analysis of covariance; BSI = bloodstream infection; ClfA = clumping factor A; cLIA = competitive Luminex immunoassay; CP5 = capsular polysaccharide serotype 5; CP8 = capsular polysaccharide serotype 8; GMFR = geometric mean fold rise; GMT = geometric mean titer; ISA = invasive *Staphylococcus aureus*; LLOQ = lower limit of quantitation; LSM = least-squares mean; MntC = manganese transporter C; N/A = not applicable; OPA = opsonophagocytic activity; SSI = surgical-site infection.

Descriptive Figures

Descriptive figures will be generated for immunogenicity. Each antigen-assay combination will be presented in a separate figure. The legend will identify SA4Ag and placebo, except as noted. All scales for antigen titers and geometric means will be log scales. Plots will be generated for the mITT immunogenicity population.

1. Reverse cumulative distribution curves (RCDCs) will be generated for the day of vaccination, the day of surgery, the day of discharge and Day 90.

2. Titers from the day of index surgery will be plotted against elapsed days from the day of vaccination to the day of the index surgery. The purpose is to profile titer distribution before surgery for a variety of days since vaccination.

3. GMTs and their 95% CIs will be plotted against time point to create kinetic plots. The time points will be labeled “Day of Vaccination,” “Day of Surgery,” “Day of Discharge,” and “Day 90,”.

9. REFERENCES

[1] Halperin M, Lan KKG, Ware J, Johnson NJ, DeMets DL (1982) An aid to data monitoring in long-term clinical trials. *Controlled Clinical Trials* **3**: 311–23.

[2] Whitehead J. Underrunning and overrunning. In: The design and analysis of sequential clinical trials. Revised 2nd ed. Chichester: John Wiley & Sons Ltd; 1997: p. 151-7.

[3] US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. Rockville, MD: US Department of Health and Human Services; September 2007.

[4] Charlson ME, Sax FL. The therapeutic efficacy of critical care units from two perspectives: a traditional cohort approach vs a new case-control methodology. J Chron Dis 1987;40(1):31-9.

[5] Kastner C, Armitage J, Kimble A, et al. The Charlson comorbidity score: a superior comorbidity assessment tool for the prostate cancer multidisciplinary meeting. Prostate Cancer Prostatic Dis 2006;9:270-4.

[6] American Society of Anesthesiologists. ASA physical status classification system. Available: http://www.asahq.org/Home/For-members/Clinical-Information/ASA-Physical-Status-Classification-System. 18 Jun 2014.

[7] Chow SC, Shao J, Wang H. Vaccine clinical trials. In: Sample size calculations in clinical research. New York: Marcel Dekker; 2003: p. 323-4.

[8] Blackwelder WC. Sample size and power for prospective analysis of relative risk. Stat Med 1993;12:691-8.

[9] Chan ISF, Zhang Z. Test based exact confidence intervals for the difference of two binomial proportions. Biometrics 1999;55:1201-9.

[10] Miettinen O, Nurminen M. Comparative analysis of two rates. Stat Med 1985;4:213-26.

[11] Nadler SB, Hidalgo JH, Bloch T. Prediction of blood volume in normal human adults. Surgery 1962;51:224-32.

[12] Agresti A. Exact small-sample inference. In: Categorical data analysis. 2nd ed. Hoboken, NJ: John Wiley & Sons, Inc; 2002: p. 18-20.

[13] Stokes ME, Davis CS, Koch GG. Sets of *2 x r* tables. In: Categorical data analysis using the SAS system. 2nd ed. Cary, NC: SAS Institute, Inc; 2000: p. 67-77.