STAPHYLOCOCCUS AUREUS 4-ANTIGEN VACCINE (SA4Ag)

VACCINES AND RELATED BIOLOGICAL PRODUCTS ADVISORY COMMITTEE (VRBPAC) MEETING BRIEFING DOCUMENT

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ABCs	(CDC's) Active Bacterial Core surveillance
AE	adverse event
BMI	body mass index
BSI	bloodstream infection
CBER	Center for Biologics Evaluation and Research
CC	clonal complex
CDC	(US) Centers for Disease Control and Prevention
CDP	clinical development plan
CFU	colony-forming units
ClfA	*S. aureus* clumping factor A (protein)
cLIA	competitive Luminex immunoassay
CP5	*S. aureus* capsular polysaccharide serotype 5
CP5-CRM_{197}	CP5 conjugated to CRM_{197}
CP8	*S. aureus* capsular polysaccharide serotype 8
CP8-CRM_{197}	CP8 conjugated to CRM_{197}
CRM_{197}	cross-reactive material 197 (nontoxic mutant form of diphtheria toxin)
DMC	data monitoring committee
EAC	event adjudication committee
ELISA	enzyme-linked immunosorbent assay
FBI	fibrinogen binding inhibition
FDA	(US) Food and Drug Administration
GMT	geometric mean titer
ISA	invasive *S. aureus*
IsdB	iron surface determinant B
metS	metabolic syndrome
MntC	*S. aureus* manganese transporter C
MRSA	methicillin-resistant *S. aureus*
MSCRAMM	Microbial Surface Components Recognizing Adhesive Matrix Molecules
MSSA	methicillin-sensitive *S. aureus*
NHSN	National Healthcare Safety Network
NSQIP	National Surgical Quality Improvement Project
OPA	opsonophagocytic assay
rClfA*m*	a truncated recombinant form of ClfA with a mutation (Y338A) that abolishes binding to human fibrinogen, and an 11 amino acid T7 epitope tag; component of SA3Ag vaccine
r*m*ClfA	a recombinant form of ClfA with a single amino acid substitution (Y338A) that abolishes binding to human fibrinogen; component of SA4Ag vaccine
rP305A	a recombinant nonlipidated form of the *S. aureus* manganese transporter C
SA3Ag	*S. aureus* 3-antigen vaccine, a predecessor of SA4Ag
SA4Ag	*S. aureus* 4-antigen vaccine

Abbreviation	Definition
SAE	serious adverse event
SCIP	Surgical Care Improvement Project
SSI	surgical-site infection
STRIVE	*STaphylococcus aureus* suRgical Inpatient Vaccine Efficacy (study)
TEST	Tigecycline Evaluation and Surveillance Trial
US	United States (of America)
VRBPAC	Vaccines and Related Biological Products Advisory Committee
Y338A	a site-directed amino acid substitution from tyrosine to alanine at position 338 in ClfA that abolishes binding to human fibrinogen

1. GENERAL INVESTIGATIONAL PRODUCT INFORMATION

Product Name: *Staphylococcus aureus* 4-antigen vaccine (SA4Ag)

Proposed Indication for Use: SA4Ag is indicated for prevention of invasive disease caused by *Staphylococcus aureus* in adults 18 years of age and older who are undergoing elective orthopedic surgery.

Dosage Form: SA4Ag is presented as a lyophilized powder for reconstitution with a diluent provided in a prefilled syringe. SA4Ag does not contain an adjuvant.

Administration: A single 0.5-mL dose of SA4Ag is to be administered by intramuscular injection 10 to 60 days prior to the elective orthopedic surgical procedure (instrumented or noninstrumented) to protect the patient from invasive *S. aureus* disease from the time of surgery throughout the period of postoperative infection risk. The vaccine is intended to be used in conjunction with existing surgical infection prevention efforts that are standard of care.

Manufacturer: SA4Ag is manufactured by Pfizer Inc.

2. EXECUTIVE SUMMARY

The Center for Biologics and Research (CBER) has called a public Vaccines and Related Biological Products Advisory Committee (VRBPAC) meeting scheduled for 07 November 2017 to discuss and make recommendations on the clinical development plan (CDP) for Pfizer's investigational *Staphylococcus aureus* vaccine intended for presurgical prophylaxis in elective orthopedic surgical populations. Pfizer's scientific rationale and proposed CDP to support future licensure of SA4Ag for use in elective orthopedic surgical populations (ie, persons undergoing surgery involving the musculoskeletal system) are provided herein.

Proposal:

It is Pfizer's proposal that if the ongoing pivotal STaphylococcus aureus suRgical Inpatient Vaccine Efficacy study (STRIVE) successfully demonstrates acceptable safety and efficacy of SA4Ag in adult patients undergoing elective open posterior multilevel instrumented spinal fusion surgery, then the safety and efficacy demonstrated in STRIVE should be representative of expected safety and efficacy in other elective orthopedic surgical populations 18 years of age and older. *(For the purposes of the VRBPAC discussion and in the absence of data, Pfizer assumes a vaccine efficacy of approximately 70%)*.

Rationale:

Despite the current standard of care, invasive disease caused by *S. aureus* (defined as isolation of *S. aureus* from a normally sterile site) is a serious condition and represents a high unmet medical need in adult elective orthopedic surgical populations. Pfizer is currently evaluating SA4Ag in a comprehensive global clinical development program designed to support the proposed indication. As no correlate of protection for invasive disease caused by *S. aureus* has been established, a clinical endpoint efficacy study demonstrating reduction of disease is currently required for vaccine licensure in the US and Europe. The current STRIVE efficacy study population (patients undergoing elective open posterior multilevel

instrumented spinal fusion surgery) is an elective orthopedic surgical subpopulation of spinal fusion surgery patients. Safety and efficacy of SA4Ag demonstrated in the STRIVE population is expected to be representative of the vaccine's safety and efficacy in other elective orthopedic surgical populations because of the common pathophysiology of invasive *S. aureus* disease and the similar risk factors for developing a postoperative surgical-site infection (SSI) across these elective surgical populations. These risk factors are:

- The primary risk for SSI is the surgical incision, when bacteria gain access to a normally sterile site, which is independent of the nature of the surgical site.
- Additional risk factors for developing an infection are similar and include patient-related factors (eg, age, health status, comorbidities, and colonization status) and procedure-related factors (eg, use of implanted instrumentation, size of the incision, wound characteristics, and perioperative care).

Because of these similar patient- and procedure-related factors, patients across elective orthopedic surgical populations are expected to be similarly capable of mounting a protective functional immune response to the SA4Ag antigens. In general, antibodies are present in blood, lymphoid, and synovial fluid; and therefore, functional immune responses elicited by vaccination with SA4Ag would have access to anatomical sites in elective orthopedic surgical procedures (irrespective of joint type). Thus, once SA4Ag is proven to be efficacious in STRIVE, SA4Ag is expected to provide protection against *S. aureus* disease in other elective orthopedic surgical patients.

Importantly, the elective open posterior multilevel instrumented spinal fusion surgical population has, on average, a higher rate of invasive *S. aureus* infection (1.44%) compared with the range of rates for other elective orthopedic surgical populations (~0.25-~0.50%) (Section 4.2). The higher infection rate is expected because the procedure is, on average, longer in duration and more complex than other elective orthopedic surgeries. Therefore, the STRIVE population is a stringent and representative elective orthopedic surgical population in which to evaluate a vaccine effect.

Moreover, given the lower rate of infection in other elective orthopedic surgeries, the conduct of additional clinical efficacy studies in other elective orthopedic surgical populations would require tens of thousands of patients and take many years to complete resulting in a delay in providing an additional preventative measure against *S. aureus* infections to at-risk populations. For example, Pfizer estimates that the conduct of an additional clinical endpoint efficacy study in another elective orthopedic surgical population (eg, hip arthroplasty) similar in design to STRIVE would take >10 years to complete and would require enrollment of 20,000 to 40,000 subjects (Section 4.4). Alternatively, adding other elective orthopedic surgical subjects with a lower *S. aureus* infection attack rate to STRIVE would significantly increase the study size and complexity. In addition, the interpretation of the study would be put at risk due to insufficient power to assess efficacy in the added subsets. Specifically, the small numbers of cases in other orthopedic surgical subsets would be more likely to lead to a type 2 error (ie, false-negative result).

Rationale for SA4Ag Development:

S. aureus is a gram-positive coccus and a commensal organism that colonizes the nares, axillae, pharynx, and other mucosal and skin surfaces of approximately 30% of humans at any given time. The organism causes a wide range of diseases, the most clinically serious being invasive disease involving normally sterile sites. An estimated 2 million major elective orthopedic surgeries are completed annually, and data from the Centers for Disease Control and Prevention (CDC) National Healthcare Safety Network (NHSN) indicate that invasive *S. aureus* SSIs represent a risk across all orthopedic surgical types, with rates of ~0.15% to 0.40% for the major orthopedic surgical types and as high as ~1% for revisional procedures (Table 1). *S. aureus* causes nearly half of invasive SSIs in orthopedic surgical populations (Table 1). Among these *S. aureus* infections, 41% are methicillin resistant. These invasive SSIs cause substantial morbidity, requiring surgical drainage and debridement, and prolonged intravenous antibiotic treatment. For infected prosthetic joints, a 2-stage exchange has been the traditional standard treatment modality used, involving debridement and removal of the infected prosthesis, followed by prolonged antimicrobial therapy (often up to 6–8 weeks) and subsequent re-implantation of a new prosthesis. Correspondingly, the economic burden associated with *S. aureus* infection complicating orthopedic surgical inpatient stays is high. These direct medical costs have been estimated to be $1.5 billion nationwide annually in the US (Section 3.1.1).

The broad effectiveness of antibiotic prophylaxis against SSIs supports similar pathogenesis of invasive *S. aureus* infection across elective orthopedic surgeries. Antibiotic prophylaxis is the core infection prevention strategy that has been shown to reduce SSIs in elective orthopedic surgeries (Section 3.1.2). To be effective, systemic antibiotic prophylaxis must achieve therapeutic concentrations at the time of the incision and maintain effective tissue levels while the wound is open. Accordingly, antibiotic use after the surgery is not effective. Antibiotic prophylaxis, therefore, is the proof of concept that infections can be prevented at the time of surgery (Section 5.1.7). Other preventative strategies include preoperative decolonization of the skin and nares, surgical-site preparation with combined antimicrobial and alcohol agents, and locally applied antibiotics during surgery. Routine use of antibiotic prophylaxis, locally applied antimicrobials such as vancomycin, and decolonization agents such as mupirocin have resulted in selection pressure on colonizing strains to develop resistance, including strains that are methicillin resistant or have high levels of mupirocin resistance. *S. aureus* is very adept at developing resistance to many antistaphylococcal agents to date, increasing the risk of failure of current SSI prophylaxis efforts. With the growing threat of antibiotic resistance, the long-term effectiveness of antibiotic prophylaxis is uncertain, and a vaccine approach would be an important additional preventative measure. Thus, despite existing preventative strategies and improved adherence to infection prevention practices, postoperative invasive *S. aureus* disease continues to be a large unmet medical need that is associated with significant patient morbidity and mortality as well as substantial healthcare costs.

In addition, the increasing age of the population has resulted in increasing demand for elective orthopedic surgeries and makes a preventative *S. aureus* vaccine even more important from the public health perspective. Pfizer has estimated that in the US over a 10-year period (2021-2030), a 70% effective *S. aureus* vaccine could prevent 56,783 *S. aureus*

infections among persons undergoing elective spinal surgery and 70,581 more among persons undergoing elective hip/knee arthroplasty (ie, >100,000 *S. aureus* infection in all) (Section 3.3).

SA4Ag Design:

SA4Ag contains 4 surface-expressed *S. aureus* antigens that target 3 key virulence mechanisms deployed by the pathogen early in the infection process. The vaccine was designed to stop the early infectious process by inducing high levels of functional antibodies that neutralize these virulence mechanisms and kill the bacteria, thus preventing bacterial growth and dissemination, and biofilm formation. The vaccine antigens are highly conserved across global disease isolates, expressed early in vivo by most global clinical isolates, and required by *S. aureus* to initiate invasive infection. The SA4Ag antigens are:

- CP5-CRM_{197} and CP8-CRM_{197}: *S. aureus* capsular polysaccharides serotype 5 (CP5) and serotype 8 (CP8), each conjugated to the nontoxic mutant form of diphtheria toxin, cross-reactive material 197 (CRM_{197}); anti-CP5 and anti-CP8 antibodies effectively facilitate opsonophagocytic killing of *S. aureus* (Appendix 1).
- r*m*ClfA: a recombinant form of *S. aureus* clumping factor A (ClfA) with a single amino acid substitution (Y338A); ClfA is responsible for bacterial adhesion to fibrinogen, a protein found at the surface of the host cell; anti-ClfA antibodies block *S. aureus* adhesion to the host cell surface (Appendix 1).
- rP305A: a recombinant nonlipidated form of the *S. aureus* manganese transporter C (MntC) protein; antibodies to this antigen impair survival of *S. aureus* in the host environment by limiting accessibility to manganese (Appendix 1).

SA4Ag Clinical Evaluation:

The candidate vaccine is being evaluated under a global clinical development program to support the proposed indication. The first clinical study in the *S. aureus* vaccine program was initiated in January 2010, evaluating the safety and immunogenicity of a first-generation 3-antigen vaccine (SA3Ag). Clinical development of SA4Ag began in August 2011 following preclinical analysis demonstrating that the vaccine was efficacious in a range of animal models including sepsis, bloodstream infection, and implanted devices. In February 2014, FDA granted Fast Track designation to SA4Ag for adults 18 years of age and older who are undergoing elective surgery. Results from the completed Phase 1/Phase 2a clinical trials conducted in healthy subjects confirmed that a single dose of SA4Ag elicits rapid, robust, functional immune responses to the 4 antigens, and has an acceptable safety profile in healthy nonsurgical subjects 18 through 85 years of age (Section 4.1). Both younger and older adults responded well to the vaccine, and the vaccine was well tolerated.

The currently ongoing STRIVE study is a 1:1 randomized, double-blind, placebo-controlled study evaluating the safety, immunogenicity, and efficacy of a single dose of SA4Ag administered to adults 18 through 85 years of age undergoing elective open posterior multilevel instrumented spinal fusion surgery (Section 4.3). STRIVE is a global study that was initiated in July 2015. At the time of preparation of this document, ~1650 subjects have been randomized at ~100 clinical sites in 8 countries. While originally designed as a

Phase 2b "proof of efficacy" case-accrual study to assess safety and efficacy in approximately 2600 subjects, Pfizer plans to convert STRIVE to a powered Phase 3 study involving approximately 6000 subjects (3000 SA4Ag, 3000 controls). With this new design, STRIVE would have 88% power to demonstrate vaccine efficacy with a 95% CI lower bound ≥20%, if the underlying placebo infection rate is 1.4% for the primary outcome and the true vaccine efficacy is ≥70%. (The final study design will be determined in consultation with the FDA). The Phase 3 STRIVE will contribute at least 3000 vaccinated surgical subjects to a total SA4Ag-vaccinated safety database of ~5400 (Table 13). Pfizer proposes that the safety and efficacy results from STRIVE can be applied to other elective orthopedic surgical populations. Therefore, STRIVE will serve as a pivotal study to support future licensure of SA4Ag for the proposed indication. Based on anticipated enrollment of ~6000 subjects and the currently estimated incidence rate, Pfizer anticipates that STRIVE's last subject visit may be completed in 2020.

Pfizer has considered the clinical development and regulatory pathways to support future indications for elective surgery, beyond elective orthopedic surgery. Upon demonstration of positive benefit-risk and licensure of SA4Ag for use in the elective orthopedic surgical population, Pfizer plans to evaluate the safety and immunogenicity of SA4Ag in patients undergoing other clean elective surgeries (eg, clean general, neurosurgery, abdominal surgery, and plastic surgery including breast reconstruction/augmentation) including those with high-risk comorbidities (eg, cardiovascular surgery), and possibly conduct a postapproval effectiveness study to confirm the benefit of the vaccine in these surgical populations after licensure for these indications. The design for these studies will be discussed with the FDA as part of the life cycle plans for SA4Ag. Furthermore, studies in immunocompromised persons (including patients undergoing dialysis) and subsets of the pediatric population are also considered as part of product life cycle.

The design of SA4Ag and STRIVE ensures a higher likelihood of success in demonstrating efficacy and a satisfactory safety profile compared with past vaccines that have failed in Phase 3 studies. Key factors supporting this likelihood of success are:

- SA4Ag targets 3 virulence mechanisms rather than a single virulence mechanism. These are expressed early in infection.
- SA4Ag includes 4 vaccine antigens that are conserved across *S. aureus* clinical isolates:
  - ClfA and MntC are novel antigens.
  - CP5 and CP8 are conjugated to CRM_{197} using proven experience and technology.
- SA4Ag antigens have demonstrated efficacy in preclinical models including invasive disease animal models.
- Clinically, SA4Ag induces functional antibodies to each of the 3 virulence mechanisms; these antibodies facilitate killing of *S. aureus* by opsonophagocytosis and neutralize the virulence pathways directly associated with the target antigens.

The STRIVE population has been chosen as the most stringent population in which to demonstrate vaccine efficacy and safety.

Why the STRIVE Population is Representative of Other Elective Orthopedic Surgical Populations:

Similar Early Pathophysiology of S. aureus SSI Across Elective Orthopedic Surgeries

For elective orthopedic surgeries, the primary risk for establishing infection is during the surgical procedure itself from the time of incision to wound closure, when bacteria can enter a normally sterile site that has been exposed by the incision (Section 5.1.1 and Section 5.1.7). The risk of inoculation is higher in patients colonized with *S. aureus*, as inoculation of patients' wounds occurs most often by their colonizing strain, present in the operative environment and entering the surgical incision (Section 5.1.2).

Early virulence factors, such as those targeted by SA4Ag, are required for *S. aureus* to initiate infection. Immediately upon entering the surgical site, the bacteria upregulate the expression of genes to adapt to the wound microenvironment and avoid immune-mediated killing. Upregulation of capsular polysaccharides (CP5 or CP8) helps the bacteria to evade neutrophil-mediated killing; ClfA upregulation promotes tissue adhesion; and nutrient transporter expression such as MntC allows the bacteria to obtain essential nutrients limited in the host (Section 3.4 and Appendix 1). Blocking these virulence mechanisms that are expressed early in the infection process prevents the establishment of a productive infection and subsequent processes such as biofilm formation, irrespective of the presence of implanted devices (Section 3.5 and Section 5.1.4).

There is no evidence linking specific *S. aureus* strains to specific surgical procedures that would suggest a strain- or surgery-associated pathogenesis. The same *S. aureus* strain types are isolated from SSIs irrespective of surgical type, and these are also the same strains that cause the majority of invasive disease and that are asymptomatically carried by the healthy population. Periodic disease outbreaks in hospitals caused by specific *S. aureus* isolates are not limited to particular surgical procedures. Rather, disease outbreaks are linked to a patient coming into contact with the outbreak strain either through colonization or from an exogenous source. Thus, the presence of *S. aureus* during the surgery and accessibility to a previously sterile site during surgery are prerequisites of SSI, but not the type of elective surgery or the *S. aureus* strain (Section 5.1.3).

Similar Immune Capabilities Across Elective Orthopedic Surgeries

The various orthopedic surgical sites (eg, spine, knee, hip,) are sterile under normal circumstances (no exposure to pathogens) but have full access to the human immune repertoire. Vasculature is found in all bones throughout the body, and joints are drained by lymphatics. Both vasculature and lymph ensure that bones and joints are connected to and protected by the immune system (Section 5.1.5 and Appendix 2). The pathophysiology of *S. aureus* infection in these joint spaces is similar. *S. aureus* will directly bind to host tissues or implants coated with host extracellular matrices, sequester nutrients, and elaborate several approaches to evade the immune system. These immune evasion tactics change as the infection progresses, which is also why it is critical to intercept the invading pathogen early during the infection process. Early intervention assures that vaccine-induced antibodies can prevent the bacteria from binding to the host extracellular matrices and obtain essential nutrients, and facilitate bacterial killing by opsonophagocytosis.

Similar Risk Factors Across Elective Orthopedic Surgeries

While the risk of postoperative invasive *S. aureus* disease is directly attributable to the surgical incision and duration of surgery, preoperative, intraoperative, and postoperative patient and procedural factors can influence the risk of developing a SSI. These risk factors for SSI are common among elective open posterior multilevel instrumented spinal fusion surgical and other elective orthopedic surgical populations.

Patient risk factors are largely driven by the health status of the patient and include older age, high body mass index (BMI), diabetes, and smoking status (Section 5.2.1). The percentage of patients with these risk factors and other comorbidities (ie, chronic obstructive pulmonary disease, congestive heart failure) is similar across elective orthopedic surgeries. Patients undergoing spinal surgery and other elective orthopedic surgeries have a similar Charlson Comorbidity Index (a validated prognostic indicator for factors that increase the risk of short-term mortality) affirming the similarity of the prevalence of comorbidities and the overall general health status among these populations. Additionally, rates of *S. aureus* nasal carriage, which has a well-established association with postoperative SSI, are similar in patients undergoing spinal procedures and other elective orthopedic surgeries (Section 5.2.2).

Procedural risk factors for postoperative infection include duration of surgery, wound characteristics, involvement of similar anatomical structures (eg, bone, cartilage, and joint spaces with synovial fluid), use of implanted devices and blood transfusions, and perioperative care (Section 5.2.3). Surgical techniques and procedural characteristics for elective open posterior multilevel instrumented spinal fusion surgeries have numerous commonalities shared with other elective orthopedic surgeries. Each of these surgical procedures involves disruption of the dermis, soft tissue, fascial and muscle layers, and bone, allowing possible introduction of infection through the wound. The recommended perioperative care is the same for patients undergoing spinal surgery and other elective orthopedic surgeries. The wounds of >93% of these surgeries are classified as clean or clean/contaminated. Many of these surgeries have similar median durations, with spinal surgery having the longest median duration; thus, infection rates in the spinal surgical population are at the higher end of the range for elective orthopedic surgeries. Many spinal surgeries and other major elective orthopedic procedures such as hip and knee arthroplasties commonly use implanted materials composed of titanium or cobalt chromium alloys, plastics, and stainless steel. Although implantation is considered a risk factor for SSI, the implant material itself is not relevant, as *S. aureus* mostly binds to the implanted device once primed with human extracellular matrix materials and not directly to the device material itself (Section 5.1.4). The rate of postsurgical medical complications and mortality after elective orthopedic surgeries including spinal surgery is low (0.18 to 0.35%). Complications such as pulmonary embolism and myocardial infarction are low and comparable between hip and knee replacements and spinal surgery, and have been observed at similar rates in the STRIVE patients who have already undergone their surgical procedure (Section 5.2.4).

Based on the similar pathophysiology and patient- and procedure-related risk factors for invasive *S. aureus* SSI across elective orthopedic surgeries described above, Pfizer therefore proposes that successful demonstration of safety and efficacy (prevention of postoperative

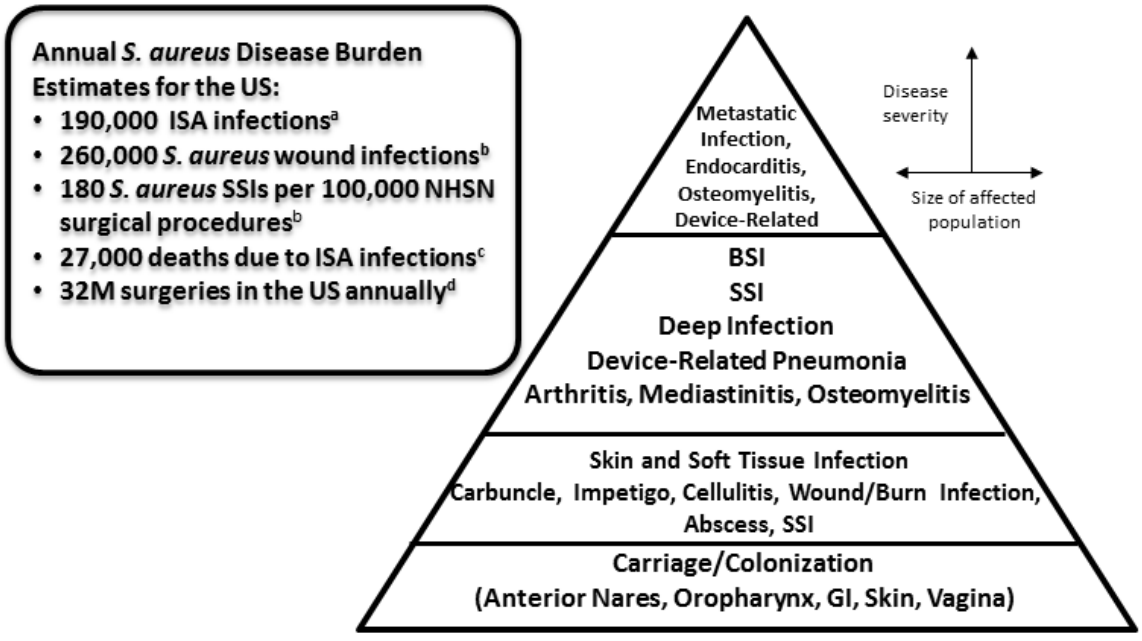
invasive *S. aureus* disease) of SA4Ag in STRIVE will be representative of safety and efficacy in other elective orthopedic surgical populations.

3. RATIONALE FOR SA4Ag DEVELOPMENT

3.1. Unmet Medical Need for *S. aureus* Disease Prevention

S. aureus is a Gram-positive coccus and a commensal organism that colonizes the nares, axillae, pharynx, and other mucosal and skin surfaces in about 30% of healthy individuals at any given time.[1,2,3] Intermittent colonization occurs in 60% of people. Under normal circumstances, *S. aureus* colonization does not present a medical problem; however, breaches of the skin or mucosa by wounds, trauma, or surgical interventions can allow *S. aureus* access to tissues or the bloodstream, resulting in tissue infection and/or bacteremia.[4]
S. aureus (MSSA and MRSA) causes a range of different diseases, from mild skin infections, SSIs, and deep wound infections to life-threatening bacteremia and sepsis (Figure 1).[5,6,7]

Figure 1. Diverse Manifestations of *S. aureus* Infection and Burden of Invasive *S. aureus* Disease



Abbreviations: BSI=bloodstream infection; GI=gastrointestinal tract; ISA=invasive *S. aureus*; NHSN=National Healthcare Safety Network; SSI=surgical-site infection.

Source: (a) ABCs, 2014[8] and See et al, 2015[9]; (b) Includes 37 CDC-selected NHSN procedures from orthopedic, abdominal, cardiac, ob/gyn, neurological, vascular, transplant, breast, neck, and other procedure categories, CDC, 2016[10]; (c) NIH Jordan Report, 2012[11]; (d) CDC Fast Stats on Inpatient Surgery, CDC/NCHS National Hospital Discharge Survey, 2010.[12]

Despite improved infection control strategies, invasive MRSA continues to be responsible for about 72,000 cases[8] and 11,000 deaths annually in the US.[7] Adding the number of invasive MSSA cases (61%),[9] the total US burden of invasive *S. aureus* (MRSA and MSSA) is

~190,000 cases a year. These numbers represent a large unmet medical need and substantial burden to the healthcare system, and justify the need for new strategies to prevent *S. aureus* infections.

3.1.1. Burden of Postoperative Surgical-Site Infections Remains High

SSIs are the most common and most costly healthcare-associated infections, accounting for ~20% of all infections in hospitalized patients and one third of total costs.[13,14,15,16,17] SSIs can be superficial (ie, involving the skin and subcutaneous tissue) or invasive (involving deep soft tissues below the fascial plane and other normally sterile organs and anatomical spaces).[17]

An estimated 2 million major orthopedic surgeries are completed annually in the US, and CDC's NHSN data indicate that invasive *S. aureus* SSIs represent a risk across all orthopedic surgical types, with rates of ~0.2% to 0.5% for the major orthopedic surgical types and as high as ~1% for revision procedures (Table 1). The STRIVE population (open posterior instrumented multilevel spinal fusion) is a higher risk subset of the spinal fusion/refusion population with an invasive *S. aureus* infection rate of ~1.4% (Section 4.2). *S. aureus* causes nearly half of invasive SSIs in orthopedic surgical populations. Among these *S. aureus* infections, 41% are methicillin resistant.[18] These invasive SSIs cause substantial morbidity, requiring surgical drainage and debridement, prolonged intravenous antibiotic treatment. For infected prosthetic joints, a 2-stage exchange has been the traditional standard treatment modality used, involving debridement and removal of the infected prosthesis, followed by prolonged antimicrobial therapy (often up to 6–8 weeks), and subsequent re-implantation of a new prosthesis.[19]

Revision/refusion procedures (ie, secondary procedures that often involve implant/hardware removal and replacement) carry a higher risk of infection than primary procedures. Early-onset prosthetic joint infections are frequently caused by *S aureus*, while delayed-onset infections typically involve inoculation with less virulent microorganisms at the time of surgery, such as coagulase-negative staphylococci.[20,21] Invasive *S. aureus* SSI rates are ~0.6% to 1.0% for revision/refusion procedures compared with ~0.2% to 0.5% for primary procedures (Table 1); however, revision/refusion procedures account for less than 10% of the procedure volume within a given orthopedic population. For example, 43,150 of 453,640 knee replacements performed annually are replacement procedures (Table 1). Furthermore, occult prosthetic joint infections can cause the symptoms that lead to joint replacement, complicating the interpretation of the SSI rates in these subpopulations.

Nearly all of the available national SSI data in the US are from CDC's NHSN system. This system is a passive reporting system that does not conduct routine auditing of reporters to identify unreported events, as is conducted with active surveillance systems like CDC's Active Bacterial Core surveillance (ABCs). On this basis, some underreporting of infections may be expected, and the SSI rates reported in Table 1 should be interpreted with that limitation in mind.

Table 1. Estimated Annual US Procedure Volume and NHSN-Based Invasive Surgical-Site Infection Incidences for Major Orthopedic Surgeries

Orthopedic Surgical Procedure	Annual Procedure Volume (N)	Invasive SSI Rate (all pathogens)	Proportion Due to *S. aureus*	Estimated *S. aureus*-Specific Invasive SSI Rate
Spinal fusion	451,232[a]	0.75%[a]	42.3%[c]	0.32%[d]
Primary	435,551[a]	0.71%[a]		0.30%[d]
Refusion	15,681[a]	1.40%[a]		0.59%[d]
Spinal decompression	402,927[a]	0.35%[a]		0.15%[d]
Hip arthroplasty	453,640[b]	0.89%[b]	45.2%[b]	0.40%[d]
Primary total hip	321,005[b]	0.69%[b]	44.7%[b]	0.31%[d]
Primary hemiarthroplasty	89,485[b]	0.99%[b]	46.4%[b]	0.46%[d]
Revision	43,150[b]	2.12%[b]	45.3%[b]	0.96%[d]
Total knee arthroplasty	695,875[b]	0.64%[b]	40.0%[b]	0.26%[d]
Primary	640,695[b]	0.54%[b]	40.9%[b]	0.22%[d]
Revision	55,180[b]	1.75%[b]	36.6%[b]	0.64%[d]

Abbreviations: AAOS=American Academy of Orthopedic Surgeons; HCUP=Healthcare Cost and Utilization Project; NHSN=National Healthcare Safety Network; NIS=Nationwide Inpatient Sample; SASD=State Ambulatory Surgery and Services Databases; SSI=surgical-site infection.

[a] Volumes derived from 2014 HCUP NIS (inpatient procedures) and HCUP SASD and AAOS data (outpatient procedures). These estimated spinal volumes are also adjusted to include only unique and elective procedures based on HCUP data. Breakdown of primary versus implant removal/replacement procedure estimates from the ratio of such procedure types in Premier Healthcare Database (96.5% primary and 3.5% revisional; Table 6).

[b] 2013 HCUP NIS (procedure volume) and 2011 NHSN data (SSI and *S. aureus* proportion) from Segreti et al, 2017[22]

[c] Procedure specific proportion not available; using results from all orthopedic from Solomkin et al, 2017[18] (2014 NHSN data) but similar to Pull ter Gunne, 2010[23] (46% for all spinal procedures).

[d] Overall invasive SSI rate and proportion due to *S. aureus* were multiplied to obtain the *S. aureus*-specific invasive SSI rate.

SSIs are associated with adverse clinical outcomes for patients.[24] Increased mortality has been documented in individuals with *S. aureus* infections.[3,25] In a 5% random sample of Medicare beneficiaries from 2004 through 2007, all-cause mortality rates were ~2- to 7-fold higher among orthopedic surgical patients with a billing code for *S. aureus* infection (any site) during the postoperative period compared with uninfected patients (Table 2). One limitation of this study is that it included patients undergoing elective and emergent procedures, and mortality rates due to *S. aureus* may be different for these 2 groups. Also, since the subjects are Medicare beneficiaries, most (89%) are ≥65 years of age, making these results less representative of the US as whole.

Table 2. Unadjusted All-Cause Mortality Rates Within 180 Days After Emergent and Elective Surgery by *S. aureus* Infection Status (Any Site) Among Medicare Beneficiaries

Orthopedic Procedure	Unadjusted 180-Day Mortality Rate (N=Patients) Without Postoperative *S. aureus*	With Postoperative *S. aureus*	Mortality Risk Ratio
Knee arthroplasty	0.7% (N=34,755)	4.7% (N=359)	6.9
Hip arthroplasty	8.1% (N=25,792)	20.0% (N=594)	2.5
Other arthroplasty[a]	11.8% (N=31,346)	19.0% (N=1061)	1.6

[a] Other arthroplasty includes fractures, partial/total hip/knee implant removal/replacement, and all other joints (shoulder, elbow, hand, ankle, and foot) arthroplasty.
Source: Razavi et al, 2014.[26]

SSIs following orthopedic surgery are also associated with important personal and societal impacts due to the complicated patient journey that ensues,[27,28,29] including lost work and productivity by patients and caregivers.[30] SSIs involving *S. aureus* are challenging to treat because the biofilm formed on implants is resistant to antibiotic treatment, making eradication of *S. aureus* infection difficult.[31] As a results, treatment of orthopedic *S. aureus* SSIs usually requires at least 1 implant removal/replacement surgery (in some cases multiple surgeries) and weeks or months of treatment with intravenous antibiotics with its associated negative impact on the patient's microbiome and consequences of long-term infusion therapy. Prolonged rehabilitation can be required, including inpatient rehabilitation stays. A patient's physical functioning, social/emotional status, financial/employment status, and energy/sleep are affected.[29]

The complex treatment for orthopedic SSIs also results in increased healthcare utilization and corresponding costs. Two recent reviews of published literature documented orthopedic SSIs increase readmission rates and length of stay (6-19 days longer; 2-3 times that of non-SSI controls), resulting in healthcare costs that are 1.5 to 4 times that of surgeries without SSIs.[32,33] Correspondingly, the economic burden associated with *S. aureus* infection complicating orthopedic surgical inpatient stays is high. These direct medical costs have been estimated to be $1.5 billion nationwide annually in the US.[34]

Furthermore, a rate of 0.25% to 0.50% is mathematically equivalent to 250 to 500 events per 100,000, which is well above the frequency of other serious infections considered to be important public health concerns, such as invasive pneumococcal disease. Based on the CDC ABCs, before the approval of the infant pneumococcal conjugate vaccine in 2000, the invasive pneumococcal disease incidence was 163 per 100,000 in children <1 year of age, 205 per 100,000 in children 1-2 years of age and 33 per 100,000 in children 2 to 4 years of age.[35]

3.1.2. Current Practices for Preventing Surgical-Site Infections Have Limitations

Current postoperative *S. aureus* infection prevention strategies for surgical patients include improved hygiene and aseptic surgical techniques, carrier screening, skin and nares decolonization,[36,37,38,39,40] application of antibiotics to the surgical site prior to wound closure,[41,42] and intravenous antibiotic prophylaxis.[43,44] Antibiotic prophylaxis is a core

intervention for SSI prevention in elective orthopedic surgery.[45,46,47] Meta-analyses have shown that antibiotic prophylaxis results in an 81% reduction in risk of infection compared with no antibiotics,[47] and, prior to the introduction of SSI prevention bundles, infection rates were 2.2% compared with 5.9% in orthopedic surgical patients with and without antibiotic prophylaxis, respectively.[45] To be effective, antibiotic prophylaxis has to achieve therapeutic concentrations at the surgical site at the time of incision and maintain effective tissue levels while the wound is open.[48,49] Despite the numerous strategies employed, the burden of *S. aureus* disease remains substantial, demonstrating the limitations of current infection control strategies.[50,51,52,53] Moreover, the growing threat of antibiotic resistance has led to recommendations to include vancomycin for patients with MRSA colonization,[43] while some institutions have added gentamycin to cefazolin prophylaxis regimens due to increasing gram-negative resistance to cefazolin.[54] The long-term effectiveness of antibiotic prophylaxis is thus uncertain, and a vaccine approach would be an important additional preventative measure.

Other preventative strategies such as appropriate hair removal techniques, maintenance of normal body temperature, and glycemic control have been shown to reduce SSIs in randomized controlled clinical studies and are often delivered as bundles to improve best practices.[55,56,57,58] Strategies to prevent SSIs recommended by the Surgical Care Improvement Project (SCIP) initiative have been widely adopted in the US.[59] Additional guidance has been used in Europe, including Epic Guidelines 1, 2, and 387 and National Institute for Health and Clinical Excellence (NICE) SSI quality standards.[60] However, no consensus exists on the key components of a successful preventative bundle. Adherence to bundles is labor intensive for clinical staff, and poor compliance may be one reason for lower than expected effectiveness in SSI reduction in some settings.[58]

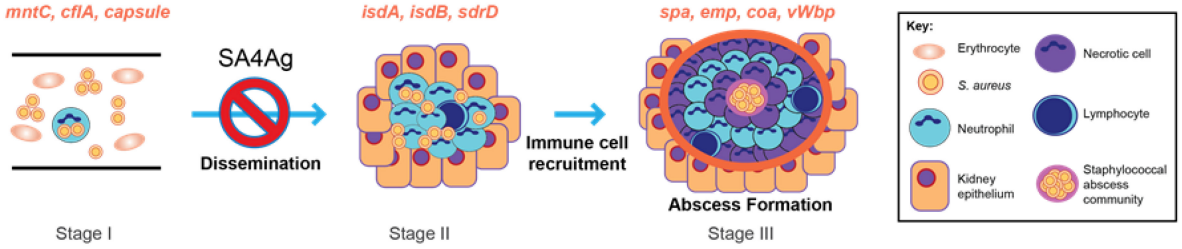
3.2. *S. aureus* Vaccination Could Prevent *S. aureus* Surgical-Site Infections

Vaccines offer the potential to lower the risk of *S. aureus* SSIs by eliciting a protective immune response in patients prior to the period of infection risk thereby preventing infection. An effective vaccine could augment existing preventative strategies. *S. aureus* utilizes several virulence mechanisms that are expressed at different stages of the infection process (Figure 2), including transporters, cell surface polysaccharides, cell surface proteins that bind to human ligands, and secreted antigens such as toxins.[61,62,63] Pfizer has designed a vaccine to prevent *S. aureus* SSIs by identifying antigens associated with key conserved virulence mechanisms that *S. aureus* utilizes early to initiate infection.[64]

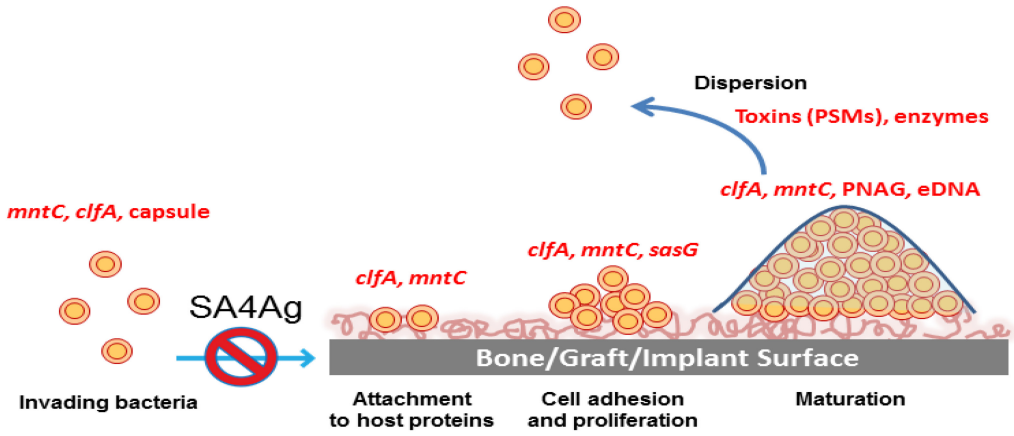
A *S. aureus* SSI is initiated as a direct result of the pathogen gaining access through the breach in the protective skin barrier. Immediately upon entering a surgical site, *S. aureus* upregulates expression of genes to adapt to the wound microenvironment and avoid immune-mediated killing.[61,65,66] Upregulation of capsular polysaccharides (eg, CP5 and CP8) that help the bacteria to evade neutrophil-mediated killing,[65,67,68,69] adhesins (primarily ClfA) that promote tissue adhesion and invasion of host cells,[70,71,72,73,74] and bacterial proteins (eg, MntC) that obtain essential nutrients limited in the host microenvironment (eg, manganese)[75,76] occurs early in the infection process in the presence or absence of implanted devices (Stage I in Figure 2 A and B).[65,66,77]

Figure 2. The Role of SA4Ag in Preventing Invasive *S. aureus* Surgical-Site Infections

A)



B)



A) Model for Invasive *S. aureus* Surgical-Site Infection Development With Foci:
Stage I: *S. aureus* survives innate and adapted (by natural exposure and colonization) immune responses in the wound and bloodstream. Stage II: After disseminating via the vasculature to peripheral organ tissues, *S. aureus* attracts an infiltrate of polymorphonuclear leucocytes and other immune cells. It changes its antigen repertoire expression to evade these immune defenses. Stage III: abscesses mature, with a central accumulation of the pathogen surrounded by a pseudocapsule of fibrin deposits (red rim), zones of necrotic and healthy polymorphonuclear neutrophils and, finally, a rim of eosinophilic material (orange rim). Genes involved at each stage are shown: *mntC*=manganese transporter protein C; *clfA*=*S. aureus* clumping factor A; capsule=CP5 and CP8; *isdA/B*=iron-regulated surface determinant proteins A/B; *sdrD*=serine–aspartate dipeptide repeat protein D; *spa*=polymorphic X region of protein A; *emp*=extracellular matrix protein-binding protein; *coa*=coagulase; *vWbp*=vonWillebrand factor-binding protein.

B) Model for Invasive *S. aureus* Surgical-Site Infection Development With Biofilm:
Upon coming into contact with an implant surface coated with host plasma proteins such as fibronectin and fibrinogen, invading *S. aureus* attaches through cell-wall-anchored (CWA) proteins including clumping factor A. Once attachment to matrix-covered devices is accomplished, *S. aureus* grows by proliferation, followed by biomass accumulation via cell division and the production of extracellular matrix leading to a mature biofilm. CWA proteins play a key role throughout the biofilm formation by mediating primary attachment and promoting intracellular adhesion and subsequent stages of biofilm accumulation and maturation. MntC protein was shown to be expressed on the cell surface and upregulated during *S. aureus* biofilms.[78] Environmental signals within the biofilm trigger the activation of dispersal mechanisms where the biofilm structure is disrupted by enzymatic degradation of matrix components by proteases, nucleases, and toxins. Genes and other bacterial components involved in each stage are shown: *sasG*=surface protein G; PNAG=poly-N-acetyl-glucosamine, exopolysaccharide; eDNA=extracellular DNA; PSMs=phenol-soluble modulins.

Source: Panel A is adapted from Cheng et al, 2011.[62] Panel B is adapted from Speziale et al, 2014[79] and Kırmusaoğlu et al, 2016.[80]

3.3. Potential Positive Public Health Impact of a *S. aureus* Vaccine Is Large

To assess the potential public health impact of an effective *S. aureus* vaccine on prevention of *S. aureus* infections after elective spinal surgeries or arthroplasties, Pfizer conducted a calculation that incorporated epidemiological and clinical data to predict outcomes over a 10-year time horizon from 2021 to 2030. For this analysis, only major elective orthopedic surgeries were considered (projected US annual procedure volumes for major elective orthopedic surgeries are listed in Table 3). The public health impact parameters with their calculation formulas, data inputs, and data sources are shown in Appendix 3.

Pfizer estimates that if all eligible patients undergoing elective spinal surgery in the 10-year time period received a 70% effective vaccine, vaccination could prevent 56,783 postoperative *S. aureus* infections, including 21,141 MRSA infections and 27,211 invasive infections (Table 4). Such a reduction in postoperative infections would also avert 1094 deaths, 27,211 hospitalizations, and 35,146 disability-adjusted life years. If additional patients undergoing elective arthroplasty were also vaccinated, Pfizer estimates that an additional 70,581 *S. aureus* infections could be prevented, and an additional 1149 deaths, 70,168 hospitalizations, and 33,596 disability-adjusted life years could be averted (Table 4). The total combined values would be the appropriate estimated positive impact of SA4Ag, if the STRIVE data could be applied to other major elective orthopedic surgical populations and the vaccine licensed for and used in all elective orthopedic surgical populations.

In fact, the potential positive vaccine impact on public health could be an underestimate, because the supporting epidemiological data were conservative and used 90-day postsurgical infection rates that did not account for later infections. In addition, only the first infection for one surgery was accounted for, while some patients might experience multiple surgeries during the year, which would increase the risk of an infection or possibility of a second infection. Also, the estimates are for infections formally diagnosed as involving *S. aureus*, and did not incorporate infections that were treated before microbiological culture was obtained or those not cultured at all. Lastly, we have only considered patients undergoing major orthopedic surgeries, and not patients undergoing other elective orthopedic surgeries, who would also have an indication for the vaccine.

In summary, elective orthopedic surgical populations would clearly benefit from a safe and effective vaccine to prevent invasive *S. aureus* infection. As outlined above, delay in access to a vaccine would have negative public health consequences. The large unmet medical need of *S. aureus* SSIs and the potential positive public health impact of an effective vaccine are important factors when considering the appropriate initial indication for SA4Ag, provided vaccine safety and efficacy is demonstrated in the STRIVE population.

Table 3. Projected US Annual Procedure Volume for Major Elective Orthopedic Surgeries (2021-2030)

	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	Total
Spinal fusion	549,625	561,807	574,260	586,989	599,999	613,299	626,893	640,788	654,991	669,509	6,078,160
Spinal decompression	329,702	337,010	344,480	352,115	359,920	367,898	376,052	384,387	392,907	401,616	3,646,087
Inpatient[a]	96,370	98,506	100,689	102,921	105,202	107,534	109,918	112,354	114,845	117,390	1,065,730
Outpatient[a]	233,332	238,504	243,790	249,194	254,717	260,363	266,134	272,033	278,063	284,226	2,580,357
Hip arthroplasty	534,674	546,525	558,639	571,021	583,678	596,615	609,839	623,357	637,173	651,297	5,912,817
Knee arthroplasty	1,008,230	1,030,578	1,053,421	1,076,770	1,100,637	1,125,033	1,149,970	1,175,459	1,201,514	1,228,145	11,149,757
Other arthroplasty	67,154	68,643	70,164	71,719	73,309	74,934	76,595	78,293	80,028	81,802	742,642

[a] Spinal decompressions are often conducted at the inpatient and outpatient settings, which could have an implication on the infection rate; therefore, they were also analyzed separately.

Source: Projected from Life Science Intelligence Report 2015, with adjustment based upon HCUP (2014) data analysis to eliminate overlapping multiple surgeries and emergent surgeries.

Table 4. Potential US Public Health Impact From a *S. aureus* Vaccine Assuming 70% Efficacy on Prevention of *S. aureus* Infections Following Major Elective Orthopedic Surgeries (2021-2030)

	Spinal Surgeries			Arthroplasty				Spinal Surgery/ Arthroplasty
Estimated 10-Year Vaccine Impact	Spinal Fusion	Spinal Decompression	Total	Hip Arthroplasty	Knee Arthroplasty	Other Arthroplasty	Total	Total
Surgical procedure volume	6,078,160	3,646,087	9,724,247	5,912,817	11,149,757	742,642	17,805,216	27,529,463
Total number of *S. aureus* infections averted	39,569	17,214	56,783	31,870	37,463	1,248	70,581	127,364
Total number of MRSA infections averted	15,377	5764	21,141	12,659	13,771	459	26,889	48,030
Total number of ISA infections averted	19,146	8065	27,211	15,728	18,732	624	35,084	62,295
Total number of deaths averted	938	156	1094	845	300	5	1149	2243
Total number of hospitalizations averted	19,146	8065	27,211	31,456	37,464	1248	70,168	97,379
Total number of disability-adjusted life years averted	29,257	5889	35,146	21,000	12,277	319	33,596	68,742

Abbreviations: ISA=invasive *S. aureus*; MRSA=methicillin-resistant *S. aureus*.

Note: Formulas and data for these calculations are shown in Appendix 3.

3.4. SA4Ag Design

The SA4Ag antigens, CP5, CP8, ClfA and MntC represent 3 major *S. aureus* virulence mechanisms (Table 5) that *S. aureus* deploys early for infection. These target antigens are all surface expressed, conserved, and globally represented in *S. aureus* invasive isolates. The majority of *S. aureus* invasive disease isolates express either CP5 or CP8. Mutations have been identified that affect the in vitro expression of CP5 by some strains belonging to the USA300 lineage; however, these mutations do not affect the ability of these strains to express capsule in vivo.[81,82] ClfA is well conserved with respect to amino acid sequence and was present in all clinical isolates from the Tigecycline Evaluation and Surveillance Trial (TEST; data on file) and contemporary collections.[83,84] The SA4Ag ClfA variant was the most common ClfA variant (37% of isolates), and the minimum pairwise identity of the most divergent sequence was 91.2% to the vaccine antigen. MntC is highly conserved; only 8 different protein sequence variants were identified from ~800 clinical *S. aureus* isolates, and the vaccine antigen variant was found in ~88% of clinical isolates.[75]

Table 5. SA4Ag Antigens and Their Virulence Mechanisms

Target Antigen	Antigen Composition	Virulence Mechanism
CP5 and CP8	CP5-CRM_{197} and CP8-CRM_{197}	antiphagocytic
ClfA	r*m*ClfA	adhesion to host factors
MntC	rP305A	divalent cation scavenging, neutrophil survival

Abbreviations: ClfA=*S. aureus* clumping factor A (protein); CP5=*S. aureus* capsular polysaccharide serotype 5; CP8=*S. aureus* capsular polysaccharide serotype 8; CRM_{197}=cross-reactive material 197 (nontoxic mutant form of diphtheria toxin); MntC=*S. aureus* manganese transporter C; r*m*ClfA=a recombinant form of ClfA with a single amino acid substitution that abolishes binding to human fibrinogen; rP305A=a recombinant nonlipidated form of MntC.

3.5. Proposed Mechanism of Action of SA4Ag

The proposed mechanism of action of SA4Ag is to induce functional antibodies that target *S. aureus* CP5, CP8, ClfA, and MntC and neutralize their virulence mechanisms. Antibodies that recognize the capsular polysaccharides (CP5 and CP8) recruit complement and facilitate the uptake of the bacteria into neutrophils and subsequent killing. ClfA-recognizing antibodies prevent the pathogen from binding to host fibrinogen, an essential first step in pathogenicity. Antibodies that recognize MntC prevent manganese uptake by *S. aureus*, thus diminishing the protective effect of *S. aureus* superoxide dismutases that rely on manganese as a cofactor and the ability of *S. aureus* to survive the killing mechanisms deployed by neutrophils.[75,76,85]

SA4Ag was designed to ensure the presence of functional antibodies at the time of surgery to prevent *S. aureus* from initiating infection and causing invasive disease. Each of the 4 antigens in SA4Ag was shown to elicit robust functional antibody responses with no safety concerns in clinical studies.[86,87,88] As part of the preclinical development, the expression of these 4 antigens was evaluated in wound and bacteremia models of *S. aureus* infection in mice. The results from these studies have demonstrated that all 4 antigens are expressed in these models, and expression of at least one of these antigens is detected within the first hours after wound and bacteremic infections.[75,81] The 4 antigens and the preclinical

evaluation of SA4Ag are further described in Appendix 1, and the validated serology assays used to measure the functional immune responses are described in Appendix 4.

4. SA4Ag CLINICAL EVALUATION

Following an initial first-in-human study involving the predecessor *S. aureus* 3-antigen vaccine (SA3Ag: CP5-CRM_{197}, CP8-CRM_{197}, and rClfA*m*), SA4Ag has been undergoing clinical evaluation with five Phase 1/2a studies completed to date (Table 13). These Phase 1/2a safety and immunogenicity studies included: 2 dose-escalation studies in healthy nonsurgical adults 18 through 64 years of age and 65 through 85 years of age (US), an antibody persistence study for up to 36 months after vaccination (US), a safety and immunogenicity study of the final clinical trial material for administration in STRIVE (US), and a study in Japanese subjects.

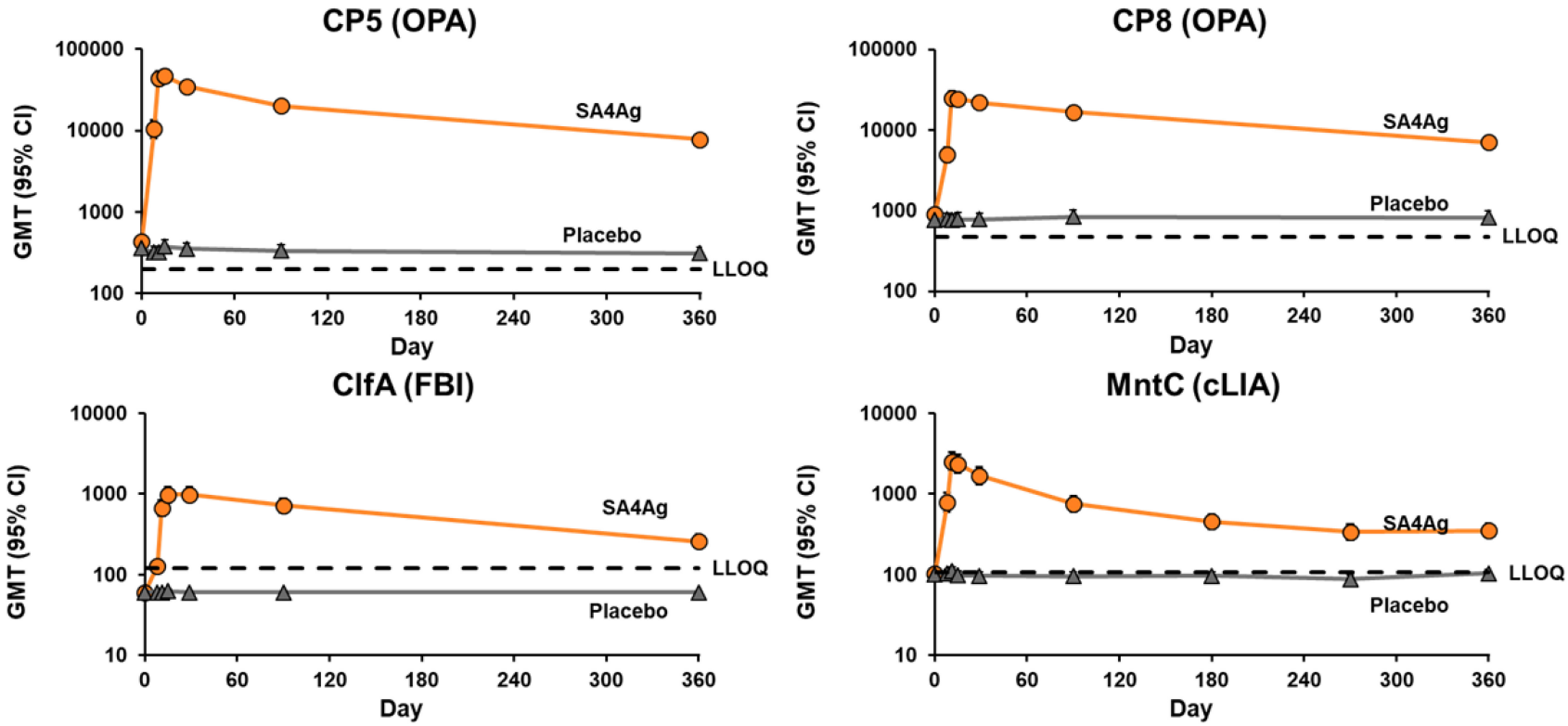
The currently ongoing STRIVE Phase 2b safety and efficacy study was originally positioned as a "proof of efficacy" study in approximately 2600 subjects. Pfizer plans to convert STRIVE to a pivotal Phase 3 study and increase its contribution to the safety database to at least 3000 vaccinated surgical subjects.

4.1. Results of Phase 1/2a Evaluation of SA4Ag

Phase 1/2a clinical studies of SA4Ag were conducted in healthy adults 18 through 85 years of age. The majority of subjects had a medical history of at least 1 disease/syndrome (>75% of subjects 18 through 64 years and >95% of subjects 65 through 85 years of age) with obesity common in these populations (approximately 40% of subjects in both age groups). Other common comorbidities included hypertension (~19% and ~55%, respectively), hypercholesterolemia/hyperlipidemia (~11% and ~42%, respectively), and diabetes (~4% and ~13%, respectively).

The Phase 1/2a studies demonstrated rapid, robust, functional antibody responses several times higher than baseline levels, with a safety profile consistent with that of other adult vaccines. Baseline antibody levels to *S. aureus* are a result of natural exposure and generally have limited functional activity (Appendix 5). Peak functional antibody responses to each of the 4 antigens were observed 10 to 14 days after a single vaccination and were sustained for over 12 months in healthy adults 18 through 85 years of age (Figure 3). Antibody responses were similar in healthy adults 18 through 64 years of age compared with those 65 through 85 years of age (Appendix 6).[87,88] These antibodies facilitate bacterial killing by opsonophagocytosis and neutralize the virulence pathways directly associated with the SA4Ag target antigens. Antibody kinetics showed robust and persistent immune responses to CP5, CP8, and ClfA through 36 months postvaccination; decay of antibodies to MntC was more pronounced but levels remained higher than baseline at 36 months (Appendix 6).

Figure 3. SA4Ag Rapidly Induces High and Persistent Levels of *S. aureus*-Killing Antibodies in Adults 18 Through 85 Years of Age



Abbreviations: CI=confidence interval; ClfA=clumping factor A; cLIA=competitive Luminex immunoassay; CP5=*S. aureus* capsular polysaccharide serotype 5; CP8=*S. aureus* capsular polysaccharide serotype 8; FBI=fibrinogen binding inhibition assay; GMT=geometric mean titer; LLOQ=lower limit of quantitation; MntC=manganese transporter C; OPA=opsonophagocytic assay.

Source: Pooled data from Creech et al, 2017[87] and Frenck et al, 2017.[88]

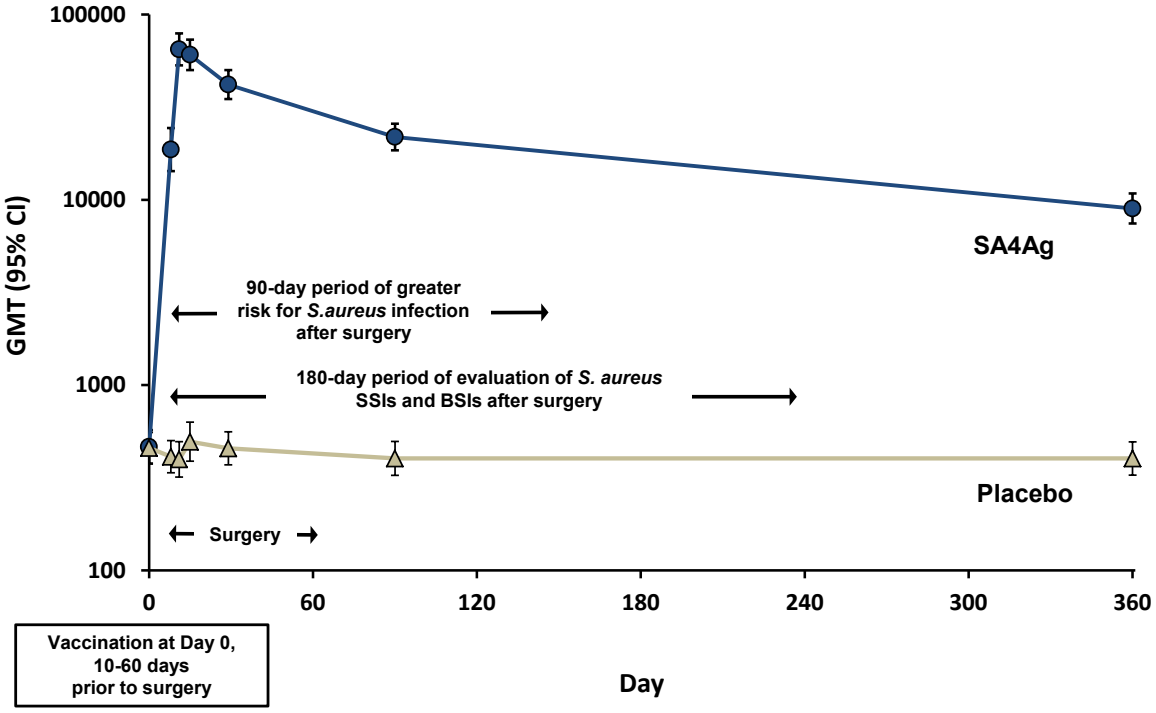
4.2. Selection of the STRIVE Population for Initial Efficacy Evaluation of SA4Ag

The STRIVE population was chosen for the initial clinical evaluation of vaccine safety and efficacy in the prevention of invasive *S. aureus* disease as it is a stringent and well-defined elective orthopedic subpopulation with the following prerequisites:

- competent immune system among most patients (including those with comorbidities such as diabetes, obesity, vascular disease, and other non-immunocompromising conditions)
- ability to be vaccinated prior to a known period of risk
- relatively high and predictable incidence of invasive *S. aureus* disease
- ability to have an invasive *S. aureus* clinical endpoint observed in a defined period of time

For patients undergoing elective orthopedic surgeries, the period of risk for *S. aureus* infection is initiated with the surgical site incision. The majority of SSIs occur through postoperative Day 180 (Section 4.3.2). Figure 4 illustrates that the optimal timing for vaccination with SA4Ag, based on the immune response profile elicited, is 10 to 60 days before surgery. This timing ensures robust antibody levels throughout the region of the surgical site (ie, tissue, fascia, and joints) at the time of incision. The antibody levels persist beyond the 180-day period of infection risk to prevent *S. aureus* infection. Antibody titers as a result of vaccination can be clearly differentiated from baseline responses with limited functional activity.

Figure 4. CP5 Antibody Levels Measured by Opsonophagocytic Assay After SA4Ag Vaccination and the Risk Period for *S. aureus* Infection After Surgery



Abbreviations: BSIs=bloodstream infections; CP5=*S. aureus* capsular polysaccharide serotype 5; GMT=geometric mean titer; SA4Ag=*S. aureus* 4-antigen vaccine; SSIs=surgical-site infections.

Note: Graph represents GMTs (95% CI) for CP5 in healthy adult subjects 18 through 64 years of age. Arrows illustrate the window of time for vaccination, surgery, maximum risk of infection, and efficacy endpoint evaluation in patients included in STRIVE. **Similar response curves were seen for CP8, ClfA, and MntC and among individuals 65 through 85 years of age**.[87]

Source: Adapted from Figure 3, Frenck et al, 2017.[88]

Patients undergoing elective open multilevel instrumented spinal fusion, a subpopulation of spinal fusion surgery recipients, were selected as the target for STRIVE. Published data on the incidence of invasive *S. aureus* SSI/bloodstream infection (BSI) rates in elective surgical patients in the US by specific surgical procedure type is very limited, and, on that basis, additional epidemiologic analyses to inform decisions regarding clinical study populations and appropriate sample size were undertaken. Analyses of linked administrative claims and microbiology data were conducted for Pfizer using the Premier, Inc. (Charlotte, NC) network of US hospitals database that captures 5% of all US admissions (Premier Healthcare Database). The invasive *S. aureus* infection rate in patients that approximated the patient characteristics in STRIVE was 1.44% within 90 days of surgery; invasive *S. aureus* infection rates in patients undergoing other elective orthopedic procedures (including other spinal surgeries) ranged from ~0.25% to ~0.50% within 90 days of surgery (Table 6). These rates are very similar to those based on NHSN data presented in Table 1 above.

The infection rates in the larger orthopedic populations (eg, hip and knee arthroplasty) are low to serve as a primary efficacy endpoint; thus, such a study would take >10 years to recruit and complete to demonstrate vaccine efficacy. Pfizer therefore sought to identify an elective orthopedic surgical subpopulation that, while representative of all other elective orthopedic surgical populations, had infection rates at the higher end of the range for elective orthopedic surgeries. The higher infection risk for the STRIVE population is primarily due to the longer incisions required and the longer time needed to perform these complex procedures. The infection rates permit the conduct of STRIVE in a manageable though extended period of time; the current estimate of study duration is ~5 years.

Table 6. Incidence and Timing of Invasive *S. aureus* Infections at 90 Days Postsurgery Among Adults (≥18 years of age) by Orthopedic Procedure Type - Premier Healthcare Database 01 July 2010 to 30 June 2015

	Total Surgical Discharges		Total *S. aureus* Infections			Total ISA Infections			Days to ISA Infection	
	N	% Inpatient	N	%	95% CI	N	%	95% CI	Median	Interquartile Range[a]
Spinal										
Primary spinal fusion	84,547	90.3	721	0.85	0.79, 0.92	351	0.42	0.37, 0.46	21	15.0, 36.0
Spinal refusion	3044	99.8	32	1.05	0.74, 1.49	15	0.49	0.29, 0.82	24	12.0, 47.0
STRIVE-like[b]	7519	100.0	143	1.90	1.61-2.24	108	1.44	1.18-1.73	23	15.0, 34.0
Spinal decompression	52,057	47.4	394	0.76	0.69, 0.84	190	0.36	0.32, 0.42	18	13.0, 32.0
Other Orthopedic										
Hip arthroplasty	83,335	99.9	642	0.77	0.71, 0.83	316	0.38	0.34, 0.42	25	18.0, 38.0
Knee arthroplasty	156,785	99.4	753	0.48	0.45, 0.52	380	0.24	0.22, 0.27	30	19.0, 49.5
Other joint fusion	14,290	19.6	145	1.01	0.86, 1.19	57	0.40	0.31, 0.52	29	21.0, 56.0

Abbreviations: CI=confidence interval; ISA=invasive *S. aureus*.

[a] Interquartile range=25 percentile to 75 percentile

[b] Data from a prior study of the same Premier Healthcare database (large claims database linked to microbiology data) and with slightly different time frame (01 January 2010 to 31 December 2014). STRIVE population was approximated by including index procedure requirements and all inclusion and exclusion criteria based on available claims and microbiology data. ISA outcomes were limited to the STRIVE primary outcome (ISA deep/organ/space SSI plus BSI) rather than all ISA infections.

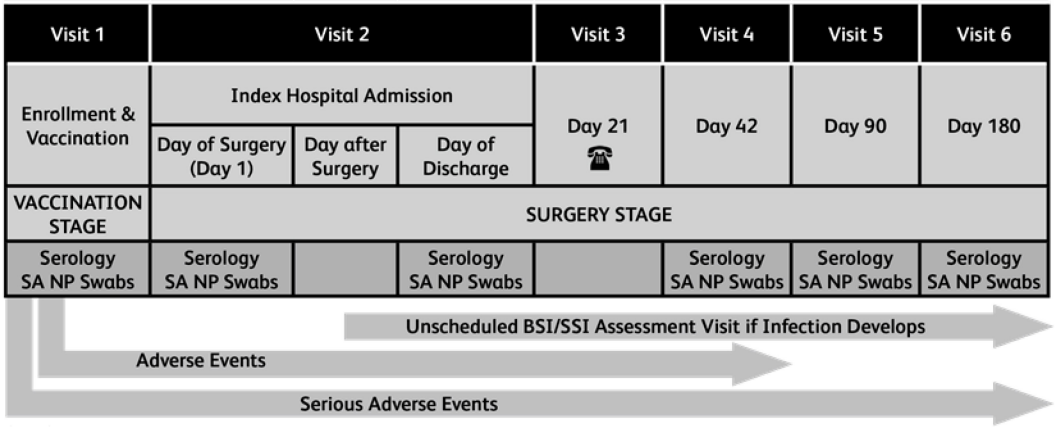
4.3. Phase 2b/3 Efficacy and Safety Evaluation - STRIVE

4.3.1. STRIVE Study Design

STRIVE (Study B3451002) is a multicenter, parallel-group, placebo-controlled, randomized, double-blind study evaluating the safety, tolerability, and efficacy of SA4Ag in the prevention of postoperative invasive *S. aureus* disease in adults 18 through 85 years of age who are undergoing elective open posterior spinal fusion procedures with multilevel instrumentation. The STRIVE study design is outlined in Figure 5. The study was initiated in July 2015, and enrollment and vaccination is ongoing at ~100 sites in the US, United Kingdom, France, Spain, Germany, Hungary, Canada, and Japan. Sites in Austria, Sweden, Bulgaria, and South Korea will be initiating soon. At the time of preparation of this document, STRIVE has been enrolling for 26 months with ~1650 subjects randomized and ~800 completed. More than 60% of subjects are from the US.

Over 1700 investigators have been contacted to identify these current ~100 study sites. Key challenges have been finding sites with adequate index surgery volume and the research infrastructure to conduct the trial.

Figure 5. STRIVE Study Design



Abbreviations: BSI=bloodstream infection; NP=nasopharyngeal; SA=*S. aureus*; SSI=surgical-site infection.

Notes: Visit 1 is 10 to 60 days before Visit 2 (index surgery hospitalization). Visit 3 is a telephone contact only. Day 1 serology swabs were collected before surgery. Day 1 for follow-up of BSIs and SSIs is the day of the index surgery.

Subjects are randomized in a 1:1 ratio to receive a single dose of SA4Ag or placebo 10 to 60 days prior to undergoing elective open posterior spinal fusion procedures with multilevel instrumentation (index surgical procedure). Subjects with comorbidities (eg, diabetes, obesity, and vascular disease) and those with a history of prior spinal surgery (if >6 months prior to study entry) are allowed in the study. Subjects with immunocompromising conditions, receiving active chemotherapy, or taking immune suppressant medications are excluded from participation in the study. Descriptive statistics on the demographics and comorbidities of subjects enrolled in STRIVE to date are available in Table 9.

Subjects are monitored closely for reactogenicity for 10 days after vaccination, all adverse events (AEs) through 6 weeks after the index surgery, and serious adverse events (SAEs) and newly diagnosed chronic medical disorders through Day 180 after the index surgery at 6 scheduled study visits/contacts.

To evaluate vaccine efficacy, subjects are also monitored for occurrence of protocol-defined infections, including BSIs, SSIs, and other invasive *S. aureus* infections, for 180 days after surgery, including at each visit after the index surgical procedure. All protocol-defined infections undergo adjudication by an independent external event adjudication committee (EAC) that includes infectious disease physicians and surgeons with specialized expertise in SSIs. Protocol-defined infections caused by other organisms are also referred to the EAC and adjudicated. Subjects with EAC-confirmed postoperative *S. aureus* BSI and/or deep incisional or organ/space SSI occurring within 90 days after the index surgical procedure contribute to the primary efficacy endpoint analysis.

Prior to vaccination, before surgery, at discharge from the hospital, and at each follow-up visit (including unscheduled visits for SSIs, BSIs, and other invasive *S. aureus* infections), blood samples are obtained for exploratory endpoint analysis of functional antibody levels to each of the 4 vaccine antigens, by using the assays described in Appendix 4. Pfizer will evaluate the immune responses observed in STRIVE to assess potential correlation with efficacy of SA4Ag. These STRIVE clinical assay results will be available as a reference for future immunobridging studies to other elective surgical populations.

STRIVE is a case accrual study, and, based on initial assumptions, it was expected that approximately 2600 subjects would be enrolled to accumulate 42 cases of BSI and deep/organ space SSI within 90 days of surgery. The initial assumptions in the power calculations included: 3% incidence of primary outcome in placebo arm, 80% power, true vaccine efficacy of ≥60%, and a lower end of 95% confidence interval >0% for vaccine efficacy. The study design included 3 conditional power-based futility assessments to be performed after accrual of approximately 10, 15, and 21 per-protocol cases meeting the primary endpoint. The external data monitoring committee (DMC) would perform these analyses in association with an unblinded independent statistical team, and review unblinded data during closed meeting sessions only. The sponsor, investigators, site staff, and subjects would remain blinded and not be permitted access to the randomization assignments until the database is locked. The study would continue if the prespecified criteria for conditional power are met as described in the statistical analysis plan. An interim efficacy analysis would be performed by the DMC in association with the independent statistical team after accumulation of 21 per-protocol cases that meet the primary endpoint. If the study was not stopped for either futility or efficacy at the interim analysis based on the prespecified criteria in the statistical analysis plan, then the study would continue to the accumulation of 42 cases.

Pfizer plans to convert the Phase 2b study to a Phase 3 study to support licensure of the vaccine for the elective orthopedic surgical population. The recent epidemiological study conducted by Premier, Inc. for Pfizer as well as feedback from STRIVE investigators have shown that the infection rate in this subpopulation of spinal fusion may be lower than initially anticipated at the time the study was designed (~1.4% vs 3.0%; Table 6). Based on this new information and FDA's request that at least 3000 vaccinated subjects from the target

indication population be included in the SA4Ag safety database prior to licensure, the plan is to convert STRIVE to a pivotal study for licensure involving ~6000 subjects (3000 SA4Ag, 3000 controls) and accrual of 48 cases. With this new design, STRIVE would have 88% power to demonstrate a vaccine efficacy with a 95% CI lower bound of ≥20%, if the underlying placebo infection rate is 1.4% for the primary outcome and the true vaccine efficacy is ≥70% (Table 7). (The final study design will be determined in consultation with the FDA). Futility would be conducted at 10 and 15 cases as initially planned, but the interim analysis would be performed after 24 cases rather than 21 cases. If STRIVE is stopped at the interim analysis for high level efficacy or reaches 48 cases before enrolling 6000 subjects, Pfizer is developing a plan to continue enrolling subjects undergoing spinal surgery to allow STRIVE to contribute at least 3000 vaccinated subjects to the prelicensure SA4Ag safety database.

Enrolling these additional subjects is expected to push the last subject visit to 2020 (~5 years). Pfizer will seek to increase the number of sites from 100 to 140, primarily by adding additional countries to the trial. A worldwide assessment of potential countries has been completed, and the regulatory process to add the selected additional countries is underway.

4.3.2. Efficacy Endpoint Assessment

In STRIVE, efficacy of SA4Ag is being evaluated throughout the primary period of infection risk. Efficacy endpoints in STRIVE are based on standard CDC definitions for BSI and SSI that are used to define postoperative infections across surgical populations.

The primary efficacy endpoint will assess 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 spinal fusion procedures with multilevel instrumentation, as confirmed by the EAC.

Secondary efficacy endpoints will assess postoperative *S. aureus* BSIs and/or deep incisional or organ/space SSIs occurring within 180 days of the index surgical procedures and superficial SSIs occurring within 90 days and 180 days of the index surgical procedures.

Exploratory endpoints include assessment of other invasive *S. aureus* infections (such as pneumonia, endocarditis, and other invasive disease not directly related to the index surgery), evaluation of healthcare utilization and outcomes, as well as evaluation of immune responses to each of the 4 antigens.

The primary efficacy endpoint in STRIVE evaluated through 90 days after the index surgery is consistent with the CDC NHSN recommendation of 90-day surveillance for deep or organ/space SSI following spinal fusion procedures.[89] This primary period for identification of clinical SSI presentation is supported by several studies showing that 75% to ~90% of deep and organ/space SSIs following spinal fusion surgery occur within the first 90 days after surgery.[90,91,92] In one large series of 1615 spinal fusions, the median time to deep SSI was 13.5 days, with a maximum time of 169 days;[92] 89% of SSIs occurred in the first 90 days. Data from an analysis of the Premier Healthcare Database conducted for Pfizer supports this published evidence. Among 7519 patients undergoing the STRIVE index procedure and

meeting other study inclusion/exclusion criteria, 84% of culture-confirmed *S. aureus* deep or organ/space SSI and/or BSI occurring in the year following surgery were identified within 90 days, and 93% occurred within 180 days.

4.3.3. Safety Assessment

STRIVE has been designed to include comprehensive safety assessments from the day of vaccination (10-60 days prior to index surgery) through 6 months after the index surgery; therefore, subjects are followed for safety up to 6 to 8 months after the single vaccination. Safety assessments include monitoring for acute reactions within 30 minutes of vaccination, local reactions and systemic events for 10 days via e-diaries, and AEs daily during the postsurgical hospital stay. Subjects are assessed for AEs and SAEs from the time of informed consent through Day 42 after the index surgery, and SAEs and newly diagnosed chronic medical disorders are collected through 6 months after the index surgery.

The duration of safety follow up for STRIVE is consistent with pivotal studies for licensed vaccines for use in healthy populations (eg, PREVNAR 13®, TRUMENBA®, and Gardasil 9), which typically include 6 months of safety follow-up after the last vaccine dose.[93] Six months has generally been considered by CBER to be a sufficient duration of safety follow-up for prophylactic, non-adjuvanted or aluminum-adjuvanted vaccines, along with routine postmarketing pharmacovigilance commitments.

Pfizer is aware that another *S. aureus* investigational vaccine (V710, Merck) was linked to a safety signal of multiple-organ failure in patients with postoperative *S. aureus* infections in the V710 vaccine arm. Pfizer's assessment based on the published literature is that the observed signal was a reflection of the limitations of the V710 composition and underlying comorbidities of the patient population rather than a more fundamental or general concern with *S. aureus* vaccines (Appendix 7). Nonetheless, as a precaution, Pfizer has included stringent prospective criteria in STRIVE to identify potential safety signals occurring at a rate of ~1 in 1000 or greater at study conclusion, including multiple-organ failure after vaccination and surgery.

4.3.4. Independent Verification of Events

Protocol-defined infections (primary and secondary endpoints and all other invasive *S. aureus* infections) and selected SAEs including multiple-organ failure and death are to be adjudicated by an event adjudication committee (EAC) ensuring independent assessment of study efficacy endpoints and important safety events. The list of events that are being adjudicated by the EAC in STRIVE includes:

Events Submitted for Adjudication
bloodstream infection
superficial surgical-site infection
deep incisional surgical-site infection
organ/space surgical-site infection
deep incisional surgical-site infection (not related to the index procedure)
osteomyelitis
joint or bursa infection
vertebral disc space infection
periprosthetic joint infection
meningitis

spinal abscess without meningitis
intra-abdominal infection
pneumonia
endocarditis
intracranial infection (brain abscess, subdural or epidural infection, encephalitis)
myocarditis or pericarditis
mediastinitis
mastoiditis
ventilator-associated probable or possible pneumonia
multiple-organ failure
death

In addition, independent ongoing safety monitoring of the study is performed by an external data monitoring committee (DMC), including review of all available safety data every 3 months, and ad hoc assessment of any SAEs related to vaccination or related AEs that may jeopardize further subject participation as determined by the sponsor clinician. In addition, the DMC is responsible for the assessment of vaccine efficacy/futility at the protocol-specified intervals as discussed in Section 4.3.1.

Safety data have been reviewed at regular intervals by the DMC, with the first meeting held on 10 December 2015. DMC meetings have subsequently been conducted every 3 months, with the most recent meeting held on 15 June 2017. At each DMC meeting, no specific safety concerns have been identified, and the DMC has recommended continuing the study without modification.

4.3.5. Collection of Surgical-Site Infection Risk Factors

Enrolled subjects receive usual standard-of-care intervention for infection prevention as per their facility's routine practices. Subjects are randomized at the site level to balance these practices between the SA4Ag and placebo group.

The STRIVE study design includes detailed assessment of SSI risk factors that are common to other elective orthopedic surgeries to ensure results are applicable across surgical subtypes.

In addition to key patient risk factors for infection, such as age, smoking status, *S. aureus* colonization status, and comorbidities (eg, obesity and diabetes), the following important procedural variables/risk factors identified from published literature and subject matter experts are collected in STRIVE:

Surgical Information Collected in STRIVE
wound site preparation method (clippers, shaving, etc)
skin preparation (eg, betadine, chlorhexidine)
surgical procedure performed, and number of spinal intervertebral levels fused
wound classification (eg, clean, clean contaminated, contaminated)
skin closure type (eg, staples, sutures, other)
implanted material (type and composition eg, rod, titanium)
graft type (eg, autograft, allograft, bone morphogenic protein)

duration of surgery (knife to skin closure)
surgical site drains, indwelling catheters
prophylactic antibiotic use (name, start and stop time, date, and dose)
estimated blood loss (mL)
blood product transfusion
postoperative patient temperature
decolonization strategies

The common patient and procedural risk factors for SSI across elective orthopedic surgical populations are described in further detail in Section 5, and, thorough collection and assessment of these variables in STRIVE will support that this subpopulation of patients undergoing elective spinal surgery represents other elective orthopedic surgical populations.

4.4. An Additional Phase 3 Efficacy Study in the Elective Orthopedic Surgical Population Would Substantially Delay Access to SA4Ag for At-Risk Patients

Conducting another randomized controlled efficacy study in addition to STRIVE in elective orthopedic surgical populations would require a large number of subjects to power the study adequately because of the lower risk of infection in these populations (~0.25-~0.50% within 90 days of surgery [Table 6]), thus substantially delaying access to SA4Ag for patients undergoing orthopedic surgery. Assuming an invasive *S. aureus* infection rate at the higher end of the range (0.50%), another efficacy study would require a sample size of 20,180 subjects to have 90% power to demonstrate vaccine efficacy with a ≥20% lower bound on the 95% confidence interval if the true vaccine efficacy is ≥70%, and 33,340 subjects if the true vaccine efficacy is ≥60% (Table 7). Assuming an enrollment rate of 100 to 150 subjects/month (consistent with STRIVE experience), this study would take longer than 10 years to complete enrollment. If the infection rate is at the lower end of the range, the sample size would substantially increase (Table 7), and timelines would be considerably longer. Because medical and surgical practices are constantly evolving, conducting an efficacy study over >10 year period would increase the risk of substantial heterogeneity among subjects enrolled at different time points potentially compromising the validity of results.

While revision surgeries could be considered as a clinical trial population due to their higher invasive *S. aureus* SSI rate (0.59–0.96%; Table 1), these populations are relatively small (~10% or less of any category). Thus, recruiting the needed number of subjects (10,090-20,180; Table 7) would be challenging due to this smaller population size and because 1) not all of these procedures are performed at facilities that have the infrastructure to participate in the clinical trial and 2) not all patients would want to participate and/or meet all of the inclusion/exclusion criteria. Furthermore, these surgeries can be confounded by unrecognized joint infections, making them a poor choice for evaluation of efficacy in a prophylactic vaccine trial. Since SA4Ag is designed to prevent infection, vaccine efficacy in treating an existing occult infection is unlikely. Finally, occult infections would complicate the interpretation of postoperative SSIs following revision procedures (ie, are the newly diagnosed SSIs truly newly occurring or just newly recognized with a chronic occult component?).

Table 7. Sample Size Estimations for Efficacy Studies in Elective Orthopedic Surgery

Placebo Infection Rate	Total Sample Size	Total Evaluable Subjects	Placebo Evaluable Subjects	SA4Ag Evaluable Subjects	Total Cases
≥70% Efficacy, 88% Power, ≥20% Lower Bound for 95% CI (STRIVE Scenario)					
1.40%	5870	5280	2640	2640	48
≥70% Efficacy, 90% Power, ≥ 20% Lower Bound for 95% CI					
0.25%	40,360	36,320	18,160	18,160	59
0.5%	20,180	18,160	9080	9080	59
1.00%	10,090	9080	4540	4540	59
1.50%	6740	6060	3030	3030	59
≥60% Efficacy, 90% Power, ≥20% Lower Bound for 95% CI					
0.25%	66,670	60,000	30,000	30,000	105
0.5%	33,340	30,000	15,000	15,000	105
1.00%	16,670	15,000	7500	7500	105
1.50%	11,120	10,000	5000	5000	105

Note: All sample size estimation scenarios include a dropout rate of 10%. The STRIVE scenario is shaded in grey.

Pfizer also considered opening the current STRIVE study to enroll other elective orthopedic surgical populations with infection rates at the lower end of the incidence range. However, a clear interpretation of the study efficacy could be put at risk, because lower numbers of cases (driven by lower infection rates in these additional populations) would be more likely to lead to a type 2 error (ie, false-negative result) for this added subset. The study would be insufficiently powered to assess efficacy in this subset. A small number of cases with an undifferentiated or adverse split could lead to erroneous assumption about efficacy for the added elective orthopedic surgical populations.

Recruitment is challenging in surgical studies due to the limited window of time to recruit, enroll, and vaccinate subjects prior to their scheduled surgery (ie, at least 10 days prior to the scheduled surgery, as in STRIVE). Also, unlike healthy nonsurgical subjects, many subjects scheduled for elective orthopedic surgeries are experiencing chronic pain and do not want to take on the extra travel/procedures that are part of being in a clinical study. Thus, while each invasive *S. aureus* infection following an orthopedic procedure can have catastrophic consequences for a patient, including re-operations, chronic pain, and increased risk of death, conducting a prelicensure randomized clinical study to reconfirm efficacy and safety among patients undergoing nonspinal elective orthopedic surgeries would substantially delay access to an effective vaccine. Consequently, if SA4Ag is demonstrated to be safe and effective in the STRIVE population, a representative (based on the rationale included in Section 5) and stringent population in which to demonstrate vaccine effect, Pfizer proposes STRIVE to be accepted as the pivotal efficacy study required for obtaining an indication in elective orthopedic surgical populations.

5. WHY THE STRIVE POPULATION IS REPRESENTATIVE OF OTHER ELECTIVE ORTHOPEDIC SURGICAL POPULATIONS

Pfizer's rationale for why the STRIVE elective open posterior multilevel instrumented spinal fusion surgical population is representative of other elective instrumented and noninstrumented orthopedic surgical populations is based on the common pathophysiology of invasive *S. aureus* disease and similar risk factors for developing a postoperative SSI across these elective orthopedic surgical populations. These risk factors are:

- The primary risk for SSI is the surgical incision, when bacteria gain access to a normally sterile site, which is independent of the nature of the surgical site.
- Additional risk factors for developing an infection are similar and include patient-related factors (eg, age, health status, comorbidities, and colonization status) and procedure-related factors (eg, use of implanted instrumentation, size of the incision, wound characteristics, and perioperative care).

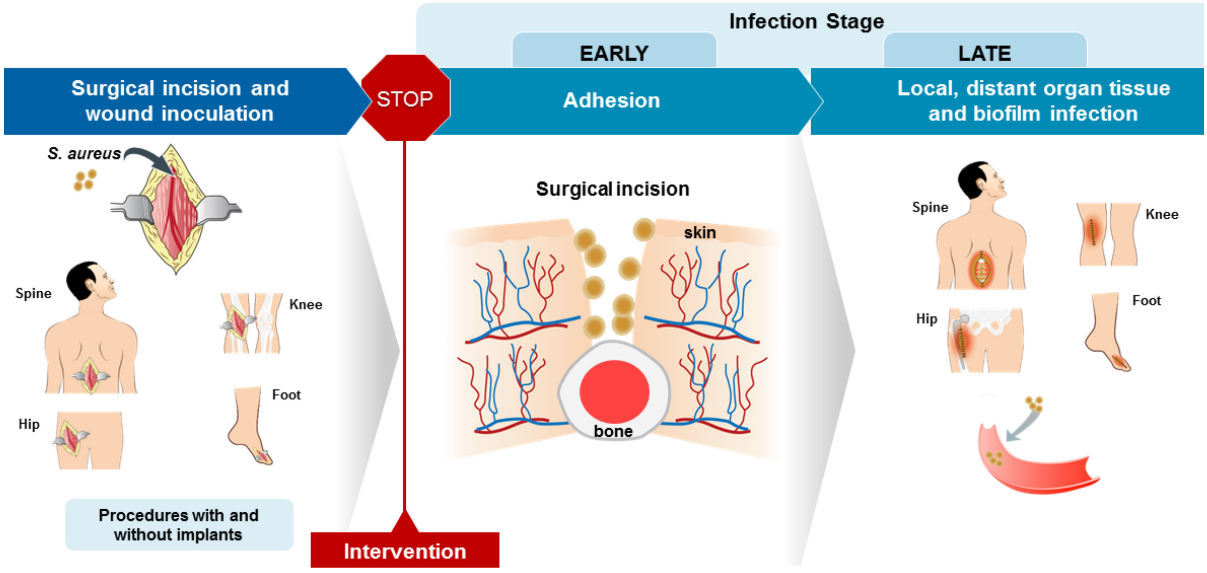
5.1. Elective Orthopedic Surgical-Site Infections Have Common Pathophysiology

SA4Ag is designed to provide protection against invasive disease established at the surgical incision site. The initial *S. aureus* SSI pathophysiology is the same regardless of the surgical type.

5.1.1. Site of Inoculation: The Surgical Wound

The surgical incision site provides the portal of entry for bacteria into a normally sterile site.[94,95,96,97,98,99,100] In all operative interventions, the primary risk period for establishing infection is during the surgical procedure itself. This is supported by data showing that mortality due to any hospital-acquired infection from amputations was estimated to be approximately 60% prior to the introduction of aseptic and antiseptic surgical techniques.[101] It is also supported by data showing that the timely perioperative administration of an appropriate prophylactic antibiotic can significantly reduce SSIs (irrespective of surgical procedure), whereas postoperative antibiotic use has limited utility.[59,102,103,104,105] Given that antibiotic effectiveness is dependent on having the antibiotic present at the time of surgery, prevention of bacterial adhesion and growth in the early stages of infection is likely to be the most important factor in protection against invasive *S. aureus* disease. These early events in invasive *S. aureus* disease include the introduction of bacteria into the wound during the surgical procedure and the elaboration of early virulence mechanisms by the bacteria as illustrated in Figure 6 and discussed below.

Figure 6. The Pathophysiology of Postoperative Surgical-Site Infections Is Consistent Across Elective Surgical Procedures



5.1.2. Source of Inoculation Is Similar Across Elective Orthopedic Surgeries

The main source of bacteria is from direct *S. aureus* inoculation from a colonized patient[106] or from contaminated healthcare personnel.[107,108] The association between *S. aureus* nasal carriage and *S. aureus* SSI risk is well established for both MRSA and MSSA.[106,109,110,111,112,113,114,115,116,117] *S. aureus* colonization density is also associated with infection risk; those with a higher load of *S. aureus* and persistent carriage are at a higher risk of *S. aureus* SSI.[106,113,114]

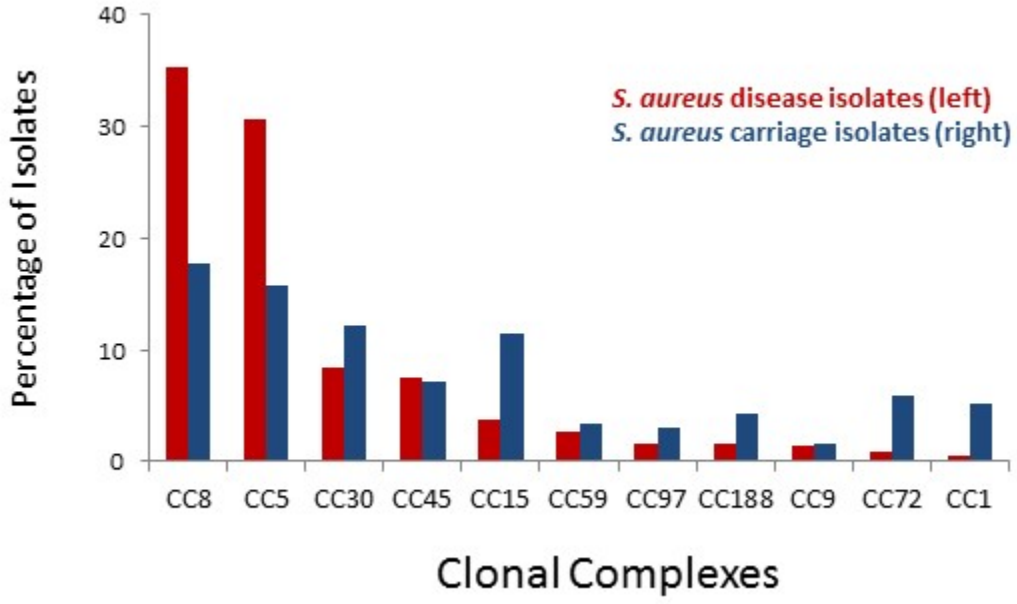
5.1.3. *S. aureus* SSI Isolates Mirror Carriage Isolates and Are Similar Across Elective Orthopedic Surgeries

S. aureus isolates are epidemiologically characterized by genotypic typing. Every isolate has near-identical genes that code for bacterial primary metabolites, which are known as "housekeeping genes." Comparing the nucleotide sequences of 7 of these (ie, multilocus sequence typing) permits related isolates to be grouped into clonal complexes.[118]

This approach has been useful in demonstrating that the source of the infecting strain is often from a patient's carriage and that there is no link between the type of surgery the patient is undergoing and the infecting isolate. In general, there are approximately 15 major *S. aureus* disease-causing clonal complexes globally.[119] In the US, 11 clonal complexes are responsible for 94% of disease-causing *S. aureus* isolated from patients ≥18 years of age in the general population (Figure 7, TEST data on file). In a separate clinical study of healthy US adults, *S. aureus* carriage isolates were characterized and found to be highly similar to those causing disease during a contemporary time period (Pfizer data on file) with 87% of the clonal complexes associated with disease detected in the general carriage population. Acknowledging that disease-causing isolates were collected from patients diagnosed with a

range of *S. aureus* infections, Pfizer assessed *S. aureus* isolates (n=146) from an orthopedic infection collection (Dr. Paul Fey, University of Nebraska Medical Center) and identified that over 97% of clonal complexes were shared between the TEST and Nebraska disease collections, providing further evidence that *S. aureus* strains that cause infections after orthopedic surgeries are not different than strains that cause *S. aureus* infections in the general population.

Figure 7. *S. aureus* TEST Disease Isolates From Patients in the US Mirror Carriage Isolates From the General Population



S. aureus disease isolates collected from patients ≥18 years of age in the US (2004-2010). These isolates were collected as part of the global collection of the Tigecycline Evaluation and Surveillance Trial (TEST).
S. aureus carriage isolates were collected from healthy subjects ≥18 through 85 years of age in the US (2009-2013).

Source: Hoban et al, 2015;[84] Pfizer data on file.

Skramm et al[120] used molecular typing to demonstrate that 6 of 7 patients who had undergone elective orthopedic surgery and developed *S. aureus* infections had identical isolates in their nares and wounds (Table 8).

Table 8. *S. aureus* Carriage Isolates From Patients Admitted for Elective Orthopedic Surgery Match Isolates From Patients Who Subsequently Developed *S. aureus* Surgical-Site Infections

Patient	SSI Isolate (CC)	Nasal Carrier Isolate (CC)
1	5	5
2	45	45
3	30	30
4	15	15
5	121	121
6	30	30
7	30	45
8	15	noncarrier
9	45	noncarrier
10	45	noncarrier

Abbreviations: CC=clonal complex; SSI=surgical-site infection.
Source: Skramm et al, 2014.[120]

Pfizer recently compared carriage isolates from 46 patients undergoing elective spinal surgery or hip or knee replacements, and no correlation was observed between the clonal complexes of nasal carriage strains and surgical types (Pfizer data on file). Moreover over 95% of these clonal complexes were identified in carriage in healthy adult populations (Figure 7) demonstrating that the epidemiology of nasal carriage of *S. aureus* among elective orthopedic patients mirrors that among healthy adults. Thus, both the accessibility to a previously sterile site during surgery and carriage of *S. aureus* are prerequisites of SSI, but not a particular surgical procedure or specific *S. aureus* strain, indicating that a *S. aureus* vaccine that is efficacious in one orthopedic surgical population would be expected to be efficacious in others as well.

In situations where hospitals periodically have disease outbreaks caused by specific *S. aureus* isolates, these isolates were not limited to particular surgical procedures. Rather, disease outbreaks are linked to transmission of the outbreak strain to additional patients via wound inoculation from carriage[117,121,122] or from an exogenous source.[123,124,125] Regardless of the source, the SA4Ag mechanism of action (the presence of high-titered functional antibodies throughout the period of infection risk) would still be effective at preventing infection.

5.1.4. Pathogenesis of *S. aureus*-Related Implant Infections Is Similar Across Elective Orthopedic Surgeries

Many orthopedic surgeries involve the use of implanted instrumentation, which is a risk factor for *S. aureus* disease. In all orthopedic SSIs, including those involving implanted devices, bacteria need to adhere to host tissue or the implanted device to establish infection. The pathophysiology of attachment is species specific. *S. aureus*, unlike coagulase-negative staphylococci (eg, *S. epidermidis*), binds to human proteins that have coated and primed the device, via Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs), eg, ClfA protein (an SA4Ag antigen) that binds to human fibrinogen deposited on the device surface.[126,127] *S. aureus* has limited ability to adhere to the surface of an implant in the absence of a conditioning film of host proteins. This film is formed rapidly

after implantation, offering a biologic substratum for adhesion to host tissue cells and bacterial cells.[128] Once *S. aureus* has made contact and formed an attachment with host proteins deposited on an implant, a biofilm can develop. This is independent of the type and material of implanted device.[129,130] SA4Ag-induced functional antibodies are expected to have the same access to the implant as the human matrix proteins and bacteria, and therefore antibodies present at the inoculation site should prevent bacterial survival, adhesion, and subsequent biofilm formation. In the absence of the implant, the infection is seeded as a result of the bacteria binding to host proteins, acquiring nutrients, and evading natural immune responses (the mechanisms that SA4Ag is designed to prevent). Thus, it is expected that the mechanism of action of SA4Ag for prevention of *S. aureus* SSIs is the same for orthopedic surgeries whether or not they include implanted materials.

5.1.5. Immunopathogenicity of *S. aureus* Infections Is Similar Across Elective Orthopedic Surgeries

The efficacy to be demonstrated in STRIVE is expected to be translatable to other elective orthopedic surgical sites including other anatomical joints and bone spaces, since these sites are accessible to the protective immune responses elicited by SA4Ag. The immunopathology of orthopedic bone and joint spaces is summarized here and described in more detail in Appendix 2. Joint spaces (bone, tissue, and synovial fluid) have access to both innate and adaptive immune factors, including humoral and cellular immune components.[131] Antibodies and phagocytes are both present in the synovial fluid of healthy joints, and immune cellular infiltrate increases during joint inflammation.[132] Joint bone and tissues are vascular and drain into the lymphatics. As in serum, antibodies to *S. aureus* surface antigens induced from natural infection can be detected in the synovial fluid of joints (Appendix 2). Although some anatomical differences exist between orthopedic surgical sites (spine, hip, knee, etc), there is no evidence that immune access is substantially different, which supports translation of vaccine efficacy across joint types. The components required for the proposed mechanism of action of SA4Ag (induction of functional antibodies and host phagocytes that kill the bacteria) are available at these different anatomical sites.[133,134] Additionally, as antibodies are a critical component for SA4Ag-mediated protection and as the amount of blood present in the wound may differ by surgical procedure depending on the surgery-related blood loss, Pfizer confirmed in unvaccinated subjects that natural antibody levels in subjects were similar before and after surgery despite differences in the amount of blood loss by surgical type (Appendix 2).

5.1.6. Median Time to *S. aureus* Infection Diagnosis Is Similar Across Elective Orthopedic Surgeries

Although it may take days to several weeks from initiation of infection to diagnosis, the median time of invasive *S. aureus* infection diagnosis after surgery is similar among orthopedic procedures. In a study involving 57,748 orthopedic procedures, Anderson et al[135] found that invasive *S. aureus* infections were detected a median of 24 days after surgery (interquartile range: 14-41 days) within a network of 11 US hospitals. The timing was similar for the subset of procedures with implants (n=16,549): median 29 days (interquartile range: 17-51 days). Campbell et al[136] found the median time to *S. aureus* infection to be 22 days among 11,786 orthopedic surgeries. A recent analysis of linked administrative claims and microbiology data from the Premier Healthcare Database found the median time

to invasive *S. aureus* infection to be similar (~20-30 days) among several orthopedic procedures (total knee arthroplasty: 30 days; total hip arthroplasty: 25 days; spinal refusion: 24 days; and spinal decompression: 18 days; Table 6). The median time to diagnosis was also similar for a STRIVE-like subpopulation examined (23 days).

5.1.7. The Broad Effectiveness of Antibiotic Prophylaxis Supports Similar Pathogenesis of Invasive *S. aureus* Infection Across Elective Orthopedic Surgeries

Prophylactic intravenous antibiotic intervention began in general surgery patients in the late 1950s and early 1960s.[137,138] Initially, "prophylactic" antibiotics were often administered after wound closure, and their effectiveness was questioned. However, careful experimental work elucidated that antibiotics are effective if present at the time of intrawound bacterial exposure (ie, during surgery).[139] Successful clinical studies in general surgery patients[137,138] and later orthopedic patients[140] confirmed this. Subsequently, formal guidelines recommended their use for a wide range of surgical types: cardiac, noncardiac vascular, orthopedic, neurosurgery, head and neck, thoracic, gastrointestinal, biliary, colorectal, appendectomy, Cesarean section, hysterectomy, and abdominal trauma.[141]

The established efficacy of prophylactic intravenous antibiotic administration within 1 hour of the surgical incision across spinal/orthopedic surgeries, with re-dosing recommended for longer duration surgeries, supports the common pathogenesis of disease.[45,46,142,143] Prolonged antibiotic prophylaxis beyond 24 hours after surgery generally has not been shown to provide additional benefit, again indicating that the critical time for establishment of infection is during the surgical procedure.[55,144]

Meta-analyses have demonstrated the efficacy of prophylactic antibiotics compared with placebo in reducing SSI for spinal procedures[45] and hip and knee arthroplasty.[47] Although no clear evidence for efficacy exists in shoulder, elbow, and ankle arthroplasty, the recommendations apply the same antimicrobial prophylaxis principles, due to the negative consequences of infections in procedures involving implanted materials.[43]

5.2. Elective Orthopedic Surgical-Site Infections Have Common Risk Factors

Numerous patient and procedural factors (microbial, preoperative, intraoperative, and postoperative) influence risk of developing SSI. The risk factors for SSI are common among the elective open posterior multilevel instrumented spinal fusion subpopulation and other elective orthopedic surgical populations.

5.2.1. Patient Risk Factors Are Similar Across Elective Orthopedic Surgeries

Patient risk factors are largely driven by the health status of the patient and include age, obesity, diabetes, and smoking status, which can result in frequent comorbid disorders in surgical populations. Obesity and diabetes are linked to metabolic syndrome, another marker for increased risk of postoperative SSIs.

Age: It is well established that aging negatively impacts numerous innate and adaptive immune functions resulting in immunosenescence. The incidence of *S. aureus* healthcare-associated infections increases with age, as demonstrated by an overall invasive MRSA disease incidence of 31.8/100,000 for the general US population, compared with

127.7/100,000 for those ≥65 years of age.[145] Advanced age is a consistent SSI risk factor among orthopedic surgeries, with those over 60 years of age nearly 3 times more likely to develop postoperative SSI after spinal surgery.[146,147,148]

Obesity: Obesity is consistently identified as a risk factor for postoperative invasive *S. aureus* infection across surgical practice. Obesity is associated with comorbidities such as insulin resistance, hyperglycemia, and metabolic syndrome and directly impacts surgical wound healing due to relative tissue hypoperfusion, local tissue ischemia, and impaired neutrophil oxidative killing.[149] Relative to non-obese patients, obese patients have twice the risk of postoperative infection for spinal, other orthopedic, general, cardiothoracic, and vascular surgical populations.[150,151,152,153,154,155]

Diabetes: Hyperglycemia negatively impacts neutrophil chemotactic, phagocytic, and bactericidal activity[156] and is associated with increased risk of postoperative infection in orthopedic populations [157] (almost 6× greater likelihood of developing deep SSI after spinal fusion surgery[92]), and across other populations such as patients undergoing cardiothoracic[158] and general surgeries.[159]

Smoking: In addition to noninfectious complications, smoking results in impaired immune cell function, poor wound healing, dehiscence (complication of wound rupture along the surgical line), and higher rates of wound infection.[160,161,162]

5.2.2. Patient Characteristics in STRIVE Are Representative of Patients Undergoing Other Elective Orthopedic Surgeries

Elective open posterior multilevel instrumented spinal fusion surgery and other elective orthopedic surgeries are well-defined surgical procedures performed in relatively healthy populations with similar patient-related risk factors. The important risk factors for SSI in patients undergoing open posterior multilevel instrumented spinal fusion surgery include older age,[90] high BMI,[90,163] diabetes,[163,164,165,166] and smoking.[90] Similarly, risk factors for SSI in adults undergoing other elective orthopedic surgeries (including hip and knee arthroplasty) include older age,[167] high BMI, diabetes, and smoking.[168]

Based on published literature, patient demographics (age, race, gender) of subjects enrolled in STRIVE who have undergone instrumented spinal surgery, are representative of other elective orthopedic surgical populations (Table 9). While the mean age differs slightly between patients undergoing spinal fusion compared with other elective orthopedic surgeries, the age ranges for these surgeries broadly overlap. Comorbidities (diabetes, chronic obstructive pulmonary disease, congestive heart failure, obesity) are generally similar across the elective orthopedic populations. Using the Charlson Comorbidity Index, a validated prognostic indicator for comorbid conditions that increase the risk of short-term mortality, patients undergoing spinal surgery (96.6%) and other orthopedic surgery (94.2%) had a Charlson Comorbidity Index <3. This further affirms the similar prevalence of comorbidities and the overall general health status among these populations.[169,170,171,172] *S. aureus* nasal carriage rates, another key attribute given the well-established association between *S. aureus* nasal/skin carriage and postoperative SSI,[37] are similar in patients undergoing spinal procedures (20%)[173] and other elective orthopedic surgeries (26%).[174]

Table 9. Patient Demographics and Risk Factors Are Similar Across Elective Orthopedic Surgical Populations

	Primary Total Knee Arthroplasty N=15,157[a]	Primary Total Hip Arthroplasty N=7791[a]	Spinal Fusion N=9719[b]	STRIVE N=1342[c]
Age (mean, years)	67.3	65.4	56.7	61.8
Male (%)	35.5	44.3	46.2	44.5
White (%)	79.3	80.5	82.7	83.6
Diabetes (%)	18.2	11.6	15.1	17.4[d]
Smoking (%)	8.6	13.8	26.4	16.2
COPD (%)	3.7	4.5	NR	4.5[d]
Congestive heart failure (%)	0.2	0.5	NR	0.8[d]
Peripheral vascular disease (%)	0.6[e]	0.5[f]	NR	0.9[d]
BMI (kg/m^2) [SD]	32.8 [7.3][e]	29.8 [6.5][f]	NR	29.5 [6.3]
BMI >30 (%)	NR	NR	42.9	NR
ASA: 1-2 (%)	51.0[e,g]	56.8[f]	56.4[h]	59.6
ASA: 3-4 (%)	48.9[e,g]	43.2[f]	43.6[h]	39.8

Abbreviations: ACS NSQIP=The American College of Surgeons National Surgical Quality Improvement Program; ASA=American Society of Anesthesiologists (adopted a 5-category physical score); BMI=body mass index; COPD=chronic obstructive pulmonary disease; NR=not reported; SD=standard deviation.

[a] ACS NSQIP: 2005-2010; Pugely et al, 2015[172]

[b] 70% lumbar spine; 66% posterior/posteriorlateral approach; 90% single level; ACS NSQIP: 2005-2011; McCutcheon et al, 2015[171]

[c] STRIVE data includes subjects who completed the index surgical procedure as of 01 August 2017.

[d] Out of a total population of N=1338

[e] Duchman et al, 2014[170]; the total population was N=27,745.

[f] ACS NSQIP: 2006-2011; Belmont et al. 2014[169]; the total population varied: BMI N=17,514; peripheral vascular disease/ASA N=17,628.

[g] Percentages were derived by adding individual ASA scores from Table 1 of Duchman et al, 2014[170]

[h] Percentages were derived by adding individual ASA scores from Table 1 of McCutcheon et al, 2015.[171]

An analysis of the Premier Healthcare Database was conducted for Pfizer and examined patient demographics across elective orthopedic surgical procedures (inpatient and outpatient) among adults ≥18 years of age (Table 10). While the proportion of adult patients in each age group differs across surgical types, the spinal fusion population has a substantial representation from all 3 age groups, including ~30% from the eldest age group that more frequently undergoes hip and knee replacements. Gender and race distributions were similar across surgical types. Although there were some differences in the percentages of individual comorbidities, the median Deyo-Charlson Comorbidity Index was the same across procedures (0), and the mean value for the index was similar: 0.5 to 0.7 with overlapping ranges.

Table 10. Patient Demographics and Comorbidities by Orthopedic Surgical Procedure (Inpatient and Outpatients Combined; Adults ≥18 Years of Age) - US Premier Healthcare Database: 01 July 2010-30 June 2015

Patient Characteristics	Spinal Fusion N=80,326	95% CI	Refusion of the Spine N=2948	95% CI	Spinal Decompression N=49,898	95% CI	Hip Prosthesis N=75,779	95% CI	Knee Prosthesis N=139,217	95% CI	Fusion Other Joint N=13,493	95% CI
Age (years)												
18-49 (%)	29.6	29.3, 29.9	29.8	28.1, 31.4	32.7	32.3, 33.1	10.4	10.2, 10.6	4.9	4.8, 5.0	26.2	25.5, 26.9
50-64 (%)	40.3	39.9, 40.6	40.9	39.1, 42.6	31.9	31.5, 32.3	39.6	39.2, 39.9	39.3	39.1, 39.5	42.8	42.0, 43.6
65+ (%)	30.1	29.8, 30.4	29.4	27.8, 31.0	35.4	35.0, 35.8	50.1	49.7, 50.4	55.8	55.5, 56.0	31.1	30.3, 31.8
Gender[a]												
Female (%)	55.4	55.0, 55.7	55.9	54.1, 57.6	44.2	43.7, 44.6	54.4	54.1, 54.8	62.1	61.8, 62.3	66.4	65.6, 67.2
Male (%)	44.6	44.3, 44.9	44.2	42.4, 45.9	55.8	55.4, 56.2	45.5	45.2, 45.9	37.9	37.9, 38.2	33.5	32.7, 34.3
Race												
Black (%)	9.5	9.3, 9.7	7.1	6.2, 8.1	6.4	6.2, 6.6	7.8	7.7, 8.0	8.8	8.7, 9.0	8.9	8.4, 9.4
Other (%)	8.0	7.8, 8.2	6.8	5.9, 7.7	9.3	9.1, 9.6	7.1	6.9, 7.3	9.3	9.1, 9.4	11.9	11.4, 12.5
White (%)	82.4	82.2, 82.7	86.1	84.8, 87.3	84.3	84.0, 84.6	85.1	84.8, 85.3	81.9	81.7, 82.1	79.2	78.5, 79.9
Deyo-Charlson Comorbidities[b]												
Myocardial infarction (%)	3.3	3.2, 3.5	3.5	2.9, 4.2	3.2	3.0, 3.3	3.7	3.6, 3.9	3.5	3.4, 3.6	2.2	1.9, 2.4
Congestive heart failure (%)	1.8	1.7, 1.9	1.9	1.5, 2.5	1.4	1.3, 1.5	2.7	2.6, 2.8	2.7	2.6, 2.8	1.5	1.3, 1.7
Peripheral vascular disease (%)	1.7	1.6, 1.8	1.9	1.5, 2.5	2.0	1.9, 2.1	2.0	1.9, 2.1	1.7	1.7, 1.8	1.1	0.9, 1.3
Chronic pulmonary disease[c] (%)	17.7	17.4, 17.9	21.7	20.2, 23.2	13.4	13.1, 13.7	15.0	14.8, 15.3	16.3	16.1, 16.5	13.0	12.5, 13.6
Rheumatologic disease (%)	3.0	2.9, 3.1	4.2	3.6, 5.0	2.0	1.9, 2.1	4.1	3.9, 4.2	4.2	41, 4.3	5.7	5.3, 6.0
Diabetes without chronic complications (%)	16.9	16.7, 17.2	16.5	15.2, 17.9	15.9	15.6, 16.3	13.8	13.5, 14.0	20.7	20.5, 20.9	11.2	10.7, 11.7
Diabetes with chronic complications (%)	1.6	1.6, 1.7	1.7	1.3, 2.3	1.5	1.4, 1.6	1.2	1.1, 1.3	1.9	1.8, 1.9	4.0	3.6, 4.3
Moderate or severe renal disease (%)	2.9	2.8, 3.0	3.2	2.6, 3.8	2.5	2.4, 2.7	4.9	4.8, 5.1	5.4	5.3, 5.5	3.0	2.7, 3.3
Charlson Comorbidity Index[b]												
Mean (SD)	0.6 (1)	0.59, 0.60	0.7 (1)	0.62, 0.69	0.5 (0.9)	0.51, 0.53	0.6 (1)	0.61, 0.62	0.7 (1)	0.68, 0.69	0.5 (1)	0.50, 0.53
Median (IQR)	0 (1)		0 (1)		0 (1)		0 (1)		0 (1)		0 (1)	
Min, max	0, 14		0, 8		0, 13		0, 11		0, 13		0, 9	

Abbreviations: CI=confidence interval; IQR=interquartile range; max=maximum; min=minimum; SD=standard deviation.

[a] 20 subjects had unknown gender.

[b] Individual comorbidities present in <1% of the population not listed separately, but are incorporated in Charlson Comorbidity Index.

[c] Chronic pulmonary disease includes chronic obstructive pulmonary disease (COPD) as well as other chronic pulmonary conditions.

Overall, key patient demographics and comorbidities were similar across spinal and other elective orthopedic surgical populations. Given this, Pfizer proposes that the instrumented spinal fusion population is representative of other elective orthopedic surgical populations.

As can be seen from Table 9 and Table 10, diabetes and obesity are common in elective orthopedic surgical populations. Diabetes, obesity, and the often concurrent condition metabolic syndrome increase the risk for postoperative SSIs. However, a comparison of neutrophil function in these groups with healthy controls indicates no impairment of intrinsic neutrophil function or antibody-mediated immune function. Supportive data are supplied in Appendix 8. Thus, it is anticipated that these patients will benefit from SA4Ag, if proven to be safe and efficacious.

5.2.3. Procedural Risk Factors Are Similar Across Elective Orthopedic Surgeries

Duration of surgery, wound characteristics, use of implanted instrumentation, blood transfusions, and perioperative care are all common procedural risk factors for infection.

Duration of Surgery: Duration of surgery is one of the most important procedural risk factors for development of SSIs in different surgical subgroups.[98,163,171,175,176,177] For example, in spinal surgery, an operative duration of 3 to 6 hours (odds ratio: 1.3) or >6 hours (odds ratio: 1.4) is a significant predictor of postoperative infection relative to procedures with a duration of <3 hours.[178]

Wound Characteristics: Several different wound characteristics can affect SSI rates including incision size, long stitch closure,[179] surgical wound class (clean, clean-contaminated, contaminated, or infected), presence of foreign material in the wound, and tissue trauma.[98,99]

Perioperative Care: Preoperative wound preparation (hair removal by shaving) and postoperative hypothermia and hyperglycemia have each been associated with several-fold increases in rates of SSI in the broad surgical population, including elective spinal surgery.[180] The SCIP initiative recommends preoperative hair removal using clippers rather than shaving, and maintenance of normal body temperature and euglycemia during the first 48 postoperative hours, based on the large body of evidence demonstrating preoperative shaving, hypothermia, and hyperglycemia as consistent risk factors for postoperative infection across surgical practice.[181] Maintenance of normal body temperature has been shown to significantly reduce the rate of SSI in comparison with that of patients experiencing postoperative hypothermia.

Use of Implanted Devices: In spinal fusion surgery, as in other orthopedic surgeries, the use of implanted instrumentation is associated with a higher risk of SSI.[178,182] In one series, the deep SSI rate in instrumented spinal fusion was 1.2% compared with 0.8% in spinal decompression.[182] As mentioned in Section 5.1.4, human cells coat the implant, which permits *S. aureus* to adhere and form infection foci.

Allogeneic Blood Transfusion: Due to altered function of macrophages, abnormal migration of cells, suppression of the lymphatic response to antigens, and decreased ratio of helper to suppressor T cells,[183,184] allogeneic blood transfusion is another important procedural risk factor for SSI in all surgeries.[185,186,187] A causal rather than associative relationship is

supported by the finding that allogeneic transfusion results in a 2- to 10-fold greater risk of infection compared with either no transfusion[188] or autologous transfusion.[189]

5.2.4. Surgical Procedures in STRIVE Are Representative of Other Elective Orthopedic Surgeries

Surgical techniques and procedural characteristics for elective open posterior multilevel instrumented spinal fusion surgeries have numerous commonalities shared with other elective orthopedic surgeries. Procedural characteristics of spinal and other elective orthopedic surgeries are summarized in Table 11. Each of these surgical procedures involves disruption of the dermis, soft tissue, fascial and muscle layers, and bone, allowing possible introduction of infection through the wound. The recommended perioperative care (SCIP initiative) is the same for patients undergoing spinal surgery and other orthopedic surgeries. Approximately 98% of spinal surgeries are clean or clean/contaminated.[190] Similarly, 93% to 99% of other elective orthopedic surgeries, including arthroplasty, are clean or clean/contaminated.[191] The median durations for many of these surgeries are similar (spinal surgery, 123 minutes; hip replacement, 82 minutes; knee replacement, 77 minutes).[192] Spinal surgery has the longest median duration, which corresponds to infection rates at the higher end of the range for elective orthopedic surgeries (see Section 4.2). For the more complex STRIVE spinal fusion surgeries (multilevel instrumented), the mean duration of surgery was 273 minutes (range: 55-858 minutes; number of vertebrae fused: 2-21) among subjects who had completed their index surgery at the time of preparation of this document. Implanted materials are commonly used in many spinal surgeries and other major orthopedic procedures such as hip and knee arthroplasties, and their use is associated with higher infection rates.[193] The implanted materials used in instrumented spinal surgeries and arthroplasties are similar, with both types of procedures commonly using implants composed of titanium or cobalt chromium alloys, plastics, and stainless steel.

Table 11. Procedural Characteristics of Spinal and Other Elective Orthopedic Surgeries

	Total Knee Arthroplasty	Total Hip Arthroplasty	Instrumented Spinal Surgery
OR time (mean minutes [SD])	96.9 (37.9)[a]	97.6 (42.9)[b]	196.6 (SD not provided)[c]
Incision length (mean [in])	~8-10	~8-12	~10-12 inches (larger incision if more vertebrae fused)[d]
Most common approaches	medial parapatella	anterior (lateral); posterior	PLIF, TLIF
Procedural overview	dermis → dissects between the muscles, tendons, and nerves to reach the joint	dermis → dissects between the muscles, tendons, and nerves to reach the joint	dermis → dissects between the muscles, tendons, and nerves to reach the vertebrae
Implant material	metal alloys (titanium or cobalt-chromium); plastics (ultra-high molecular weight polyethylene); ceramic; bone cement	plastic (polyethylene liner); metals (cobalt/chromium); ceramic; bone cement	plastics (PEEK), metals (titanium, stainless steel, cobalt); bone graft (autograft/allograft/BMP); bone cement

Abbreviations: ACS NSQIP=The American College of Surgeons National Surgical Quality Improvement Program; BMP=bone morphogenetic protein; in=inches; OR=operating room; PEEK=polyetheretherketone; PLIF=posterior lumbar interbody fusion; SD=standard deviation; TLIF=transforaminal lumbar interbody fusion.
Source: http://orthoinfo.aaos.org/topic.cfm?topic=a00405/a00406.

[a] ACS NSQIP: 2005-2011; Duchman et al, 2014[170]
[b] ACS NSQIP: 2006-2011; Belmont et al, 2014[169]
[c] ACS NSQIP: 2005-2011; McCutcheon et al, 2015[171]
[d] Incision length calculated based on data available on the AAOS website regarding spinal surgery http://orthoinfo.aaos.org/topic.cfm?topic=A00543 and adjusted for the mean vertebrae fused in STRIVE to date (5.1 vertebrae).

Orthopedic surgical procedures have similar rates of 30-day postsurgical complications in retrospective analyses of National Surgical Quality Improvement Project (NSQIP) data. NSQIP is a national voluntary surgical quality improvement system that utilizes a high quality chart review-based system for 30-day postoperative outcomes (Table 12). Overall, the 30-day mortality rate after these procedures is low (0.18-0.35%). Other major 30-day complications such as pulmonary embolism, myocardial infarction, and septic shock are also comparable between hip and knee replacements and spinal surgery. These complications are represented in the patients enrolled in STRIVE to date that have undergone the index surgical procedure.

Table 12. Similar Incidence of 30-Day Postoperative Complications Across Elective Orthopedic Surgeries

	Primary Total Knee Arthroplasty N=15,321[a]	Primary Total Hip Arthroplasty N=17,640[b]	Spinal Fusion N=9719[c]
Total events (n)	1058	1074	NR
Mortality (%)	0.18	0.35	0.35
UTI (%)	1.49	1.45	1.96
Superficial wound infection (%)	0.79	0.83	1.24
Deep venous thrombosis (%)	1.34	0.51	0.90
Postoperative sepsis (%)	0.44	0.47	1.08
Pneumonia (%)	0.37	0.42	0.84
Pulmonary embolism (%)	0.78	0.31	NR
Myocardial infarction (%)	NR	0.24	0.20
Septic shock (%)	0.13	0.12	0.30
Wound dehiscence (%)	0.27	0.14	0.35
Cardiac arrest requiring CPR (%)	0.09	0.12	0.20
Peripheral nerve injury (%)	0.10	0.11	0.23
Acute renal failure (%)	0.12	0.07	0.5

Abbreviations: ACS NSQIP=The American College of Surgeons National Surgical Quality Improvement Program; CPR=cardiac pulmonary resuscitation; NR=not reported; SSIs=surgical-site infections; UTI=urinary tract infection.

[a] ACS NSQIP: 2006-2010; Belmont et al, 2014[194]

[b] ACS NSQIP: 2006-2011; Belmont et al, 2014[169]

[c] 70% lumbar spine; 66% posterior; posteriorlateral approach; 90% single level; ACS NSQIP: 2005-2011; McCutcheon et al, 2015[171]

6. PROPOSED CLINICAL DEVELOPMENT PLAN

Pfizer proposes the CDP shown in Table 13 to support use of SA4Ag in the STRIVE population and in other elective orthopedic populations. The proposed CDP to support the initial BLA includes a safety database of at least 3000 subjects vaccinated in the target surgical group.

Pfizer has considered the clinical development and regulatory pathways to support future indications in elective surgical populations beyond elective orthopedic surgical populations. On demonstration of positive benefit-risk and licensure of SA4Ag in the elective orthopedic surgical population, Pfizer plans to evaluate SA4Ag in patients undergoing other clean elective surgeries (eg, clean general, neurosurgery, abdominal surgery, and plastic surgery including breast reconstruction/augmentation). As a reasonable next step, Pfizer proposes to demonstrate comparable safety and immunogenicity in at least one elective non-orthopedic surgical population at risk of invasive *S. aureus* disease, in order to extend the approved indication to these populations. The design of the additional safety and immunogenicity study(ies) will be informed by safety, immunogenicity, and efficacy data from STRIVE. The safety results of the additional study(ies) would supplement the growing safety database of elective orthopedic surgical patients who will have received SA4Ag as a licensed vaccine. Pfizer proposes the immunogenicity bridging study(ies) will support a future approval for an "all elective surgery indication." Pfizer is currently assessing if the clinical benefit of SA4Ag for use in other elective surgical populations (beyond orthopedics) could be confirmed postlicensure by conducting a well-designed noninterventional "effectiveness" study.

After an initial approval of SA4Ag for use in elective orthopedic surgical populations and a subsequent licensure for use in other elective surgical populations, it is reasonable to consider conducting further safety and immunogenicity bridging studies in populations with more complex underlying conditions (eg, immunocompromised populations including patients undergoing dialysis) as part of the life cycle plans for this vaccine.

Pfizer also plans to evaluate the safety and immunogenicity of SA4Ag in relevant subsets of the pediatric population in the future, once the benefit-risk of the vaccine has been established and the vaccine has been licensed for use in adults.

Table 13. SA4Ag Clinical Development Plan: Completed and Planned Studies

Study Number/ Subject Age	Study Description	Study Design and Type of Control	Healthy Subjects Receiving SA4Ag Final Formulation	SA4Ag-Vaccinated Subjects Undergoing Surgery	Placebo	Study Status
B3451001[88] Healthy nonsurgical adults 18-<65 years of age	Dose escalation; safety and immunogenicity	Phase 1/2a, multicenter, randomized, placebo-controlled, double-blind, sponsor-unblinded	112	0	112	Completed
B3451011[87] Healthy nonsurgical adults 65-<86 years of age	Dose escalation; safety and immunogenicity	Phase 1/2a, multicenter, randomized, placebo-controlled, double-blind, sponsor-unblinded	57	0	60	Completed
B3451014[195] Healthy nonsurgical adults 18-<86 years of age	Antibody persistence up to 36 months after vaccination	Phase 2a, multicenter, open-label	Subjects from 1001/1011	0	Subjects from 1001/1011	Completed
B3451015 (CTM Resupply)[86] Healthy nonsurgical adults 18-<65 years of age	Safety and immunogenicity of investigational CTM for STRIVE	Phase 1, single-arm, open-label	100	0	0	Completed
B3451003 (FIH in Japan); Clinicaltrials.gov: NCT02492958 Healthy nonsurgical adults 20-<86 years of age	Safety and immunogenicity in Japanese subjects	Phase 1/2a, placebo-controlled, randomized, double-blind, sponsor-unblinded	68	0	68	Completed
B3451002 (STRIVE)/ Adults 18-<86 years of age scheduled to undergo elective, open posterior, spinal fusion procedures with multilevel instrumentation	Efficacy and safety	Phase 2b/3[a], randomized, placebo-controlled, double-blind	0	3000	3000	Ongoing
B3451006 Healthy nonsurgical adults 18-<50 years of age	Clinical lot consistency	Phase 3	2061	0	687	Planned
Total Number			2398	3000	3927	

Abbreviations: CTM=clinical trial material (ie, investigational SA4Ag); FIH=first in human.
[a] Pfizer plans to convert STRIVE to a pivotal Phase 3 study.

7. CONCLUSIONS

Despite the current standard of care, invasive *S. aureus* disease represents a serious, unmet medical condition in adult patients undergoing elective orthopedic surgery.

SA4Ag is designed to stop the early *S. aureus* infection mechanisms by inducing high levels of functional bacterial-killing antibodies. The antibodies neutralize *S. aure*us virulence factors expressed early in the infection and required by the bacteria to establish a productive infection. Pfizer is currently evaluating SA4Ag in a comprehensive global CDP to support the following proposed initial indication:

"SA4Ag is indicated for prevention of invasive disease caused by *Staphylococcus aureus* in adults 18 years of age and older who are undergoing elective orthopedic surgery."

As no correlate or threshold of protection has been established for the immune response to a *S. aureus* vaccine, a clinical endpoint efficacy study is required for vaccine licensure in the US and Europe. The current STRIVE population (patients undergoing elective open posterior multilevel instrumented spinal fusion surgery) is a specific orthopedic surgical subpopulation of spinal fusion surgery recipients. Safety and efficacy of SA4Ag demonstrated in the STRIVE population are expected to be representative of the vaccine's safety and efficacy in other elective orthopedic surgical populations because of the common pathophysiology of invasive *S. aureus* disease; immune function at the surgical incision, wound, and in synovial fluid across orthopedic surgical sites; and similar risk factors for developing a postoperative SSI across these elective surgical populations. Therefore, assuming STRIVE is successful at achieving its pre-specified safety and efficacy endpoints, Pfizer proposes that the CDP is reasonable and appropriate to support an indication for use in the elective orthopedic surgical population. Additional indications in populations beyond elective orthopedic surgeries will be considered as part of the vaccine product life cycle plans.

Appendix 1. SA4Ag Antigens and Preclinical Evaluation

Capsular Polysaccharide Antibodies Provide Protection by Facilitating *S. aureus* Opsonophagocytosis and Killing

Capsular polysaccharides provide an effective strategy for bacteria to evade innate immune responses, enhance bacterial colonization and persistence on mucosal surfaces, and avoid opsonophagocytosis (ie, uptake and killing by neutrophils).[68] Encapsulated *S. aureus* strains are more virulent in bacteremia models compared with capsule-defective isogenic mutants. Strains that do not express capsular polysaccharides are readily killed in human serum.[67,69] The vast majority of *S. aureus* strains are encapsulated, and those that produce a serotype 5 (CP5) or serotype 8 (CP8) capsule predominate among clinical isolates.[196,197] Examination of large numbers of European and US clinical *S. aureus* isolates showed that all isolates were associated with a capsule genotype (CP5 or CP8).[83] Although CP5 and CP8 are composed of identical monosaccharides (L-FucNAc, D-FucNAc, and D-ManNAc), they are serologically distinct due to differences in the linkages between sugars and the sites of O-acetylation.[198]

To determine whether *S. aureus* capsular polysaccharides can induce complement-mediated opsonophagocytic antibody responses, Pfizer conducted preclinical studies with CP5 and CP8 conjugated to CRM_{197}, the same protein carrier used in PREVNAR® and PREVNAR 13®.[199] Efficacy of CP5/CP8 conjugate vaccination was demonstrated in preclinical animal models of *S. aureus* disease, such as murine pylonephritis, rat and rabbit endocarditis, and bacteremia models. The preclinical efficacy of conjugated capsular polysaccharides has also been demonstrated by other groups.[200,201,202] Using functional clinical assays such as opsonophagocytic assays (OPAs) (Appendix 4), Pfizer demonstrated that vaccination with both CP5 and CP8 conjugates in nonclinical and clinical studies resulted in induction of high levels of antibodies that facilitate killing of *S. aureus* clinical isolates by neutrophils.[69,87,88]

Clumping Factor A Antibodies Prevent *S. aureus* From Binding to Host Fibrinogen

The Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM) family, the most prevalent group of bacterial surface proteins, includes crucial virulence determinants that promote tissue adhesion and invasion of host cells. MSCRAMMs such as ClfA recognize the most prominent components of blood or extracellular matrix components, including fibrinogen, fibronectin, and collagens.[126,127]

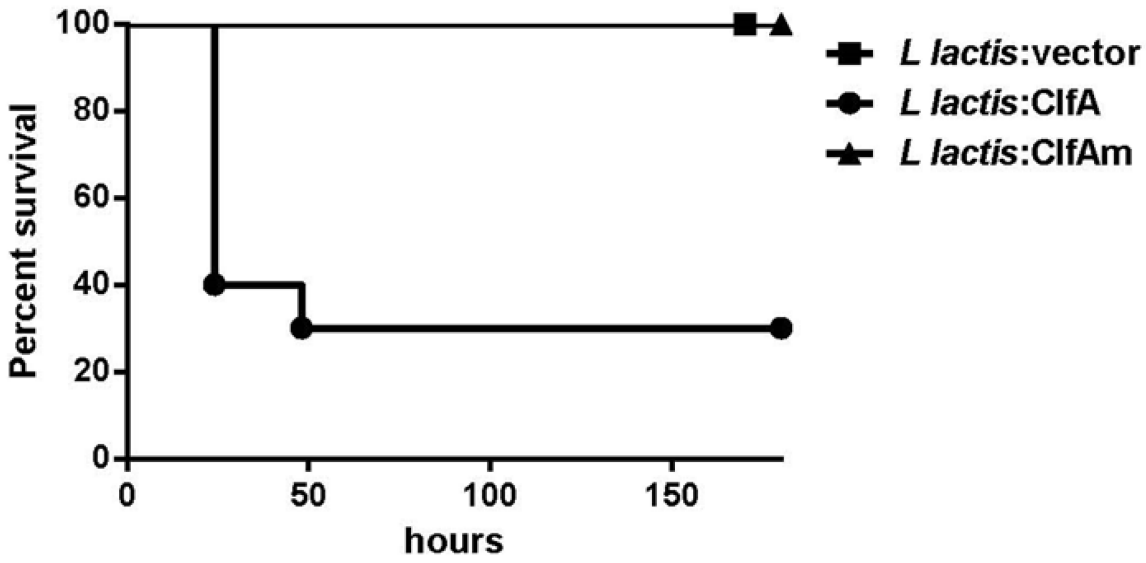
ClfA binds to the C-terminus of the plasma fibrinogen γ chain.[203,204] This interaction is central to the activity of this well-characterized virulence factor. Wild-type ClfA promotes fibrin cross-linking and mediates the binding of the pathogens to platelets[205,206] resulting in thrombus (blood clot) formation. It has also been shown to play a key role in the agglutination of staphylococci in the blood during infection, which can lead to thromboembolic lesions in heart tissue and sepsis.[74] The fibrinogen-binding activity of ClfA is linked to the ability of *S. aureus* to cause disease, as *S. aureus* strains with ClfA point mutations that prevent fibrinogen binding show reduced virulence.[71]

The SA4Ag ClfA antigen (r*m*ClfA) harbors a mutation (Y338A) that abolishes fibrinogen-binding activity and reduces the virulence of *S. aureus* strains carrying this mutation.[207] In

the context of the vaccine antigen, the mutation prevents r*m*ClfA from inhibiting the normal clumping of activated platelets and blood clotting.

Preclinical studies testing ClfA as a vaccine antigen showed antibody-mediated protection in several animal models including osteomyelitis and septic arthritis.[71] Vaccination of mice with SA4Ag results in anti-ClfA antibodies that prevent *S. aureus* from binding to fibrinogen.[208] This has also been modeled in a *Lactococcus lactis* model that specifically demonstrated ClfA-attributed virulence, which was reversed by mutating the fibrinogen-binding domain of the protein (ClfA*m*) (Figure 8). Virulence attributed to the native ClfA protein could only be prevented with antibodies that bound its fibrinogen-binding domain.

Figure 8. Heterologous Expression of *S. aureus* ClfA With Functional Fibrinogen Binding Activity Is Able to Confer a Lethal Phenotype to *L. lactis* in a Mouse Model of Infection



Abbreviations: CFU=colony-forming units; ClfA=*S. aureus* clumping factor A; ClfA*m*=mutated ClfA with impaired fibrinogen binding and virulence.

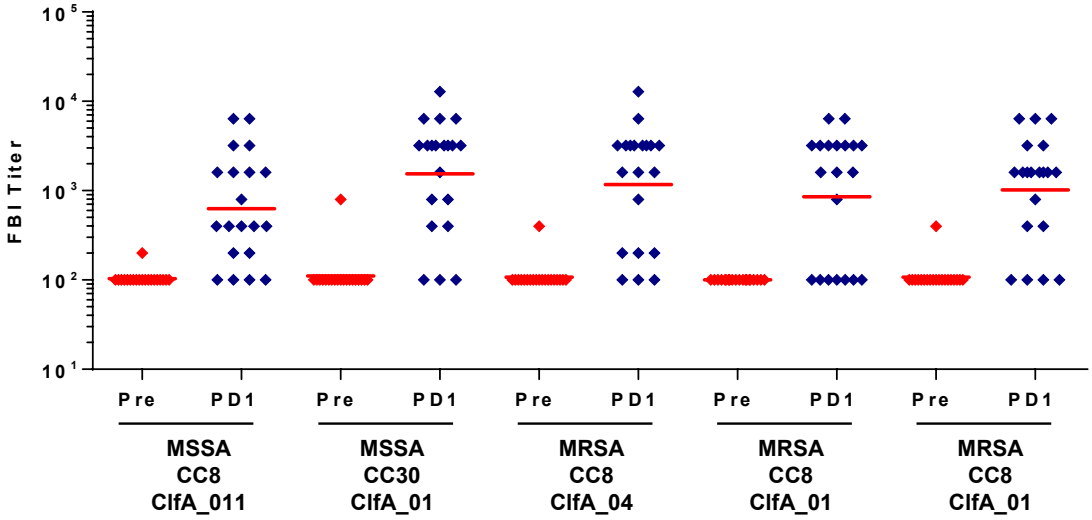
Note: Female BALB/c mice (n=10/group) were challenged intravenously with ~1×10^9 CFU *L. lactis*:ClfA, *L. lactis*:ClfA*m*, or the *L. lactis*:vector strains. Survival was monitored twice per day for 7 days.

Source: Scully et al, 2015,[208] adapted from Figure 2.

Based on this mechanism, Pfizer developed a stringent fibrinogen binding inhibition (FBI) assay that measures the anti-ClfA antibody-mediated inhibition of binding to fibrinogen of large numbers of live *S. aureus* clinical isolates that express diverse ClfA variants (Appendix 4).[209] Most humans do not have antibodies that can inhibit *S. aureus* adhesion to fibrinogen in this assay; however, Phase 1/2 studies demonstrated that SA4Ag induces robust antibody levels that can prevent *S. aureus* from binding to fibrinogen. A survey of *S. aureus* disease-causing isolates (n=399) in the US demonstrated that 99% of the *S. aureus* clinical

isolates (methicillin-sensitive *S. aureus* [MSSA] and methicillin-resistant *S. aureus* [MRSA]) contained *clfA*. Sequence analysis revealed that the overall minimum pairwise identities of ClfA variants to the vaccine antigen sequence ClfA 001 was 91.2%, based on the amino acid diversity to N1, N2, and N3 subdomains of ClfA A domain, the region included in the SA4Ag vaccine. ClfA-containing vaccines can induce anti-ClfA responses that can prevent *S. aureus* strains with diverse ClfA sequences from binding to fibrinogen.[209] Figure 9 illustrates that antibodies elicited by a recombinant ClfA-containing vaccine were capable of blocking the ClfA-dependent binding of a diverse and clinically relevant collection of *S. aureus* strains to fibrinogen.

Figure 9. Vaccination Generates Antibodies That Inhibit the Fibrinogen Binding of Invasive *S. aureus* Clinical Isolates



Abbreviations: FBI=fibrinogen binding inhibition; MRSA=methicillin-resistant *S. aureus*; MSSA=methicillin-sensitive *S. aureus*; CC=clonal complex lineage; PD1=2 weeks after SA3Ag vaccination; Pre=prevaccination.

Note: Sera from 20 subjects vaccinated with a single dose of the *S. aureus* 3-antigen vaccine (SA3Ag) were tested in the FBI assay with 5 *S. aureus* strains (MRSA and MSSA) representing diverse ClfA protein variants: 01 (vaccine antigen), 04 (94.5% identical to ClfA 001), and 011 (99.8% identical to ClfA 001). Prevaccination titers (Pre) were generally absent. Red horizontal lines in 2-week postvaccination (PD1) titers reflect geometric mean titers.

Source: Hawkins et al, 2012,[209] adapted from Figure 4.

Manganese Transporter C Allows *S. aureus* to Scavenge Key Nutrients in Vivo

S. aureus manganese transporter C (MntC) is a highly conserved (>98% sequence identity) lipoprotein that is the surface-exposed cation-binding subunit of MntABC, a heterotrimeric membrane transporter responsible for the acquisition of manganese. The sequestration of metal ions that are essential for bacterial survival is a primary host defense mechanism against bacterial invasion.[210] *S. aureus* and other bacteria have developed approaches to rapidly scavenge divalent cations like manganese and iron from the host when the bacterium establishes an infection. MntC is expressed in animal models very early upon infection,

when manganese is not freely available.[75] MntC expression transcripts have also been detected in *S. aureus* recovered from infected patients, further exemplifying its role during infection.[77,211]

As a cofactor for a number of diverse enzymes, manganese plays important roles in bacterial metabolism, cell wall synthesis, and virulence.[212,213] Most notably, it is the sole cofactor for superoxide dismutases, which neutralize superoxide radicals generated during the oxidative burst in the phagosome of activated macrophages and neutrophils (a key aspect of how neutrophils kill engulfed bacteria).[214,215,216] Engineered *S. aureus* strains that lack functional MntC display increased sensitivity to superoxide radicals.[76] A *S. aureus mntC* deletion mutant of the USA300 lineage was observed to be attenuated in a murine sepsis model.[217] Immunization with MntC-containing vaccines and polyclonal antibodies was capable of protecting mice against a lethal challenge with *S. aureus* in murine bacteremia and sepsis models.[75,218] MRSA biofilm infections were significantly cleared in rabbits using a combination of MntC-containing quadrivalent vaccine and antibiotic treatment.[78]

Thus, antibodies that target MntC have the potential to interfere with 2 distinct *S. aureus* virulence mechanisms: nutrient acquisition and phagosome survival. Anti-MntC antibodies deprive *S. aureus* of the ability to take up manganese and thus make the bacteria more vulnerable to the neutrophil respiratory burst (ie, intra-neutrophil killing).[75,219,220]

Conclusions

The initial pathophysiology of invasive *S. aureus* disease is expected to be the same across different elective surgical procedures, given that key virulence factors such as ClfA, MntC, and capsular polysaccharides (CP5 or CP8) are all expressed early in the infection process, irrespective of the bacterial strain or the animal model used (wound or bacteremic challenge). SA4Ag induces high levels of functional antibodies that neutralize these virulence factors and kill the bacteria. If shown to be effective in STRIVE, it is expected that SA4Ag-induced immune responses prior to surgical intervention and present at the time of infection risk (surgery) will, just like antibiotic prophylaxis, be efficacious for prevention of postoperative invasive *S. aureus* disease in any given elective orthopedic surgical procedure.

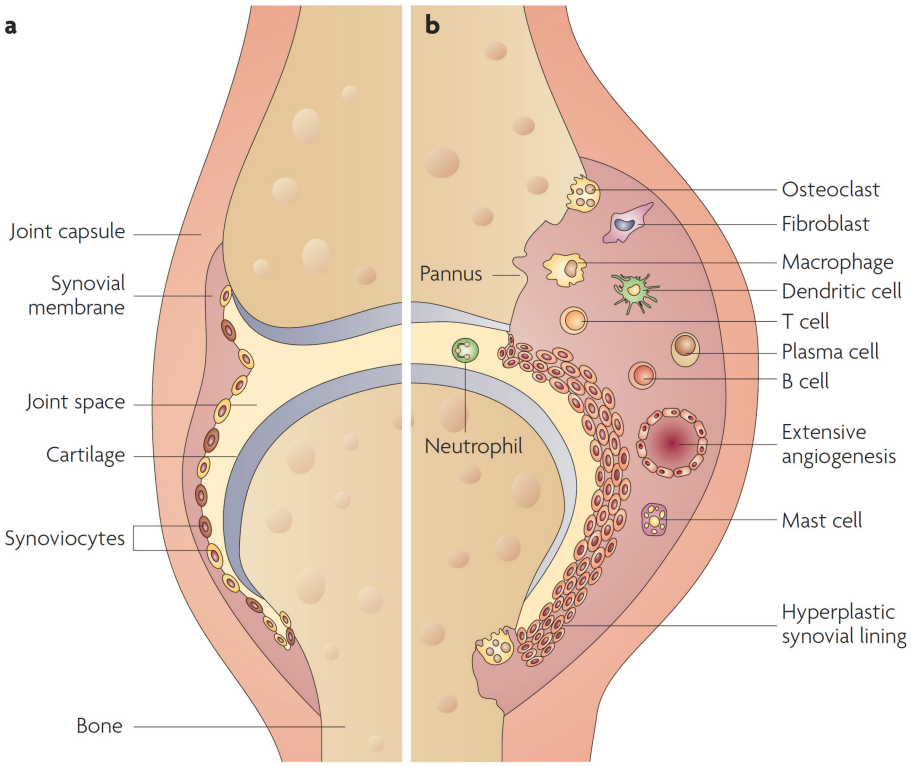
Appendix 2. Immunopathogenicity of *S. aureus* Infections Is Similar Across Elective Orthopedic Surgical Types

The human immune system provides protection against invading organisms through a series of physical barriers, such as the skin and mucosa, and through innate and adaptive immune responses. Cells and acellular components of the immune system are distributed across the human body wherever vital tissue, including bone, exists for the purpose of natural immune surveillance and disease prevention.[131] The availability of these immune components in the serum, mucosa, and tissues is well understood. The specifics of the immune repertoire in joint spaces are less well documented; however, the humoral and cellular components of the immune system have access to these sites. White blood cells including neutrophils are present in healthy synovial fluid. During inflammation, which will be the circumstance for many subjects undergoing orthopedic surgery, white blood cell numbers increase and are used as a diagnostic marker for inflammation (eg, rheumatoid arthritis) (Figure 10 and Figure 11). Cells and acellular components of the immune system are supplied to the joint space via the synovial fluid and the vasculature, especially in the context of inflammation.[132] Many of the soluble mediators of immune cells, including cytokines and growth factors, regulate the activities of bone osteoblasts and osteoclasts. This increased recognition of the complex interactions between the cells of the immune system and bone led to the development of the interdisciplinary osteoimmunology field to provide a better understanding of the pathogenesis of several diseases such as autoimmune arthritis.[221] Increasing evidence suggests a reciprocal interaction between the bone and the immune system.[222,223]

Orthopedic surgical sites (eg, knee, hip, spine) are sterile under normal circumstances (no exposure to pathogens) but are fully accessed by the human immune repertoire. Vasculature is found in all bones throughout the body. In addition, joints are perfused and drained by lymph. Both vasculature and lymph ensure that bones and joints are "connected" to and protected by the immune system. The presence of leukocytes (eg, neutrophils) in sterile inflamed tissue from patients undergoing implant removal/replacement surgery due to mechanical loosening shows that immune cells infiltrate surgical sites.[224]

Different immune microenvironments are observed for implant removal/replacement surgeries. Sterile inflammation can occur due to implant debris. This induces mainly an innate immune response (eg, activation of macrophages, infiltration of neutrophils, etc).[224,225] The result of the immune activation is infiltration of immune cells and increased permeability of the vasculature. This will enhance the immune response and not suppress it. An immune suppressive microenvironment at orthopedic surgical sites is usually only induced by immune suppressive medication.

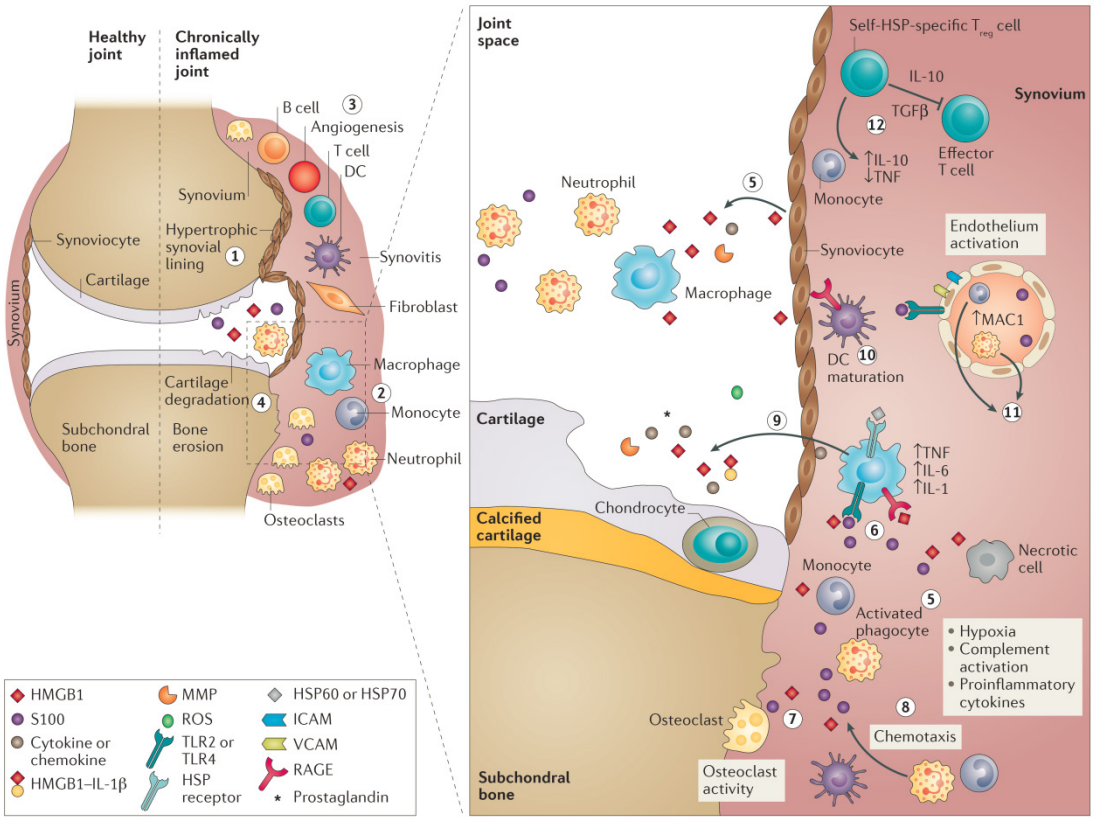
Figure 10. The Immunology of Joint Spaces



In the healthy joint (a) the thin synovial membrane lines the non-weight-bearing aspects of the joint. In rheumatoid arthritis (b) the synovial membrane becomes hyperplastic and infiltrated by chronic inflammatory cells. Ultimately, it develops into "pannus", which migrates onto and into the articular cartilage and underlying bone.

Source: Figure 1 of Strand et al, 2007.[226]

Figure 11. Synovial Inflammation and Joint-Tissue Damage During Chronic Inflammatory Arthritis



Synovial inflammation and joint-tissue damage are the main pathological processes that occur during chronic inflammatory arthritis. Hallmarks of chronic synovitis are hyperplasia of the synovial lining layer cells (1), infiltration of leukocytes (2), and angiogenesis (3). Pannus (the osteoclast-rich portion of the synovial membrane) destroys bone, whereas enzymes secreted by neutrophils, synoviocytes, and chondrocytes degrade cartilage (4). S100 proteins and high mobility group protein B1 (HMGB1) secreted from activated phagocytes and released from necrotic cells (5) activate other phagocytes through Toll-like receptor (TLR) and receptor for advanced glycosylation end products (RAGE) signaling to produce proinflammatory and catabolic factors (6), enhance osteoclastic activity (7), and lead to accumulation of other phagocytes through chemotaxis (8). HMGB1 amplifies the effect of other cytokines (eg, through formation of HMGB1–IL-1β complexes) (9) and induces maturation of dendritic cells (DCs) (10). S100 proteins activate endothelial cells and induce extravasation of leukocytes by intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) upregulation on endothelial cells and macrophage 1 antigen (MAC1; also known as integrin αMβ2) on leukocytes (11). By contrast, regulatory T cells (Treg cells) specific to self-heat-shock proteins (self-HSPs) inhibit effector T cells and dampen phagocyte activation (12).

Source: Figure 3 of Nefla et al, 2016.[227]

If *S. aureus* reaches the joint spaces, sufficient antibody levels will be required there to effectively prevent disease. Though Pfizer has not measured SA4Ag-specific antibody levels in vaccinated subjects, a study is ongoing to evaluate baseline titers for naturally occurring antibodies to the SA4Ag antigens in serum and synovial fluid samples collected from unvaccinated subjects following orthopedic surgeries (hip and knee). Because opsonophagocytic assays (OPAs) are traditionally conducted within a serum matrix, Pfizer first established that there were no inhibitory factors in synovial fluid to detect OPA titers by adding a known OPA positive immune serum to these samples.

After establishing that opsonophagocytic killing can occur in synovial fluid, Pfizer then assessed CP5 and CP8 OPA responses in a subset of serum and matched synovial fluid samples from unvaccinated subjects following orthopedic surgeries. Overall, the OPA responses in synovial fluid were positive, and titers were approximately one third of those observed in matched serum (Pfizer data on file). As mentioned previously, patients vaccinated with SA4Ag are expected to generate substantial-fold rises in antibody levels in their serum and synovial fluid.[88]

An additional consideration for SA4Ag is whether differences in blood loss during orthopedic surgeries would result in the patients having insufficient levels of antibodies present at the time of surgery to be protective. Pfizer has assessed this question in unvaccinated subjects undergoing orthopedic surgeries (hip, knee, and spine). First baseline antibody titers/concentrations were assessed in sera collected pre and postsurgery. Titers were determined using the CP5 and CP8 OPA assays, in addition to the ClfA and MntC cLIA. The results indicated that there was a moderate reduction in titers detected after surgery (Pfizer data on file). The ratio of antibodies detected presurgery compared with postsurgery was also assessed for hip, knee, and spinal surgeries. For all of these surgeries, the ratios were similar. For example, the CP5 OPA titer ratio was between 0.84 and 0.86 for the different surgical types (Pfizer data on file).

Appendix 3. Public Health Impact Parameters, Calculation Formulas, Data Inputs, and Data Sources

Public Health Impact	Basic Formula	Data Input	Data Source

Total number of *S. aureus* infections averted	Total number of elective surgical patient volumes × overall 90-day postsurgical *S. aureus* infection rate × vaccine efficacy	Overall 90-day postsurgical *S. aureus* infection rate:
		• Spinal fusion (0.93%)
		• Decompression
		  • Inpatient (1.00%)
		  • Outpatient (0.54%)
		• Hip (0.77%)
		• Knee (0.48%)
		• Other arthroplasty (0.24%)[a]
		Vaccine efficacy: 70% (assumed)
			Table 3 for total number of elective surgical patient volumes.
			Pfizer Premier Study, a retrospective study using the US Premier Healthcare Database that included patients of all ages with elective surgeries during 01 July 2010-30 June 2015. Patients were followed for up to 90 days for a positive *S. aureus* culture at the same facility through 30 September 2015.
			1. Overall postsurgical *S. aureus* infection was defined as any *S. aureus* positive culture found in the same hospital within 90 days postsurgery.
			2. Invasive postsurgical *S. aureus* infection was defined using an algorithm that combined test type, ICD-9 infection codes, and billing evidence for operations or deep tissue collection +/-1 day from the culture collection.
			3. MRSA rate was calculated as % of all *S. aureus* infections that were MRSA.
			4. Case fatality rate was defined as any death reported at the same hospital within 90 days postsurgery.

Total number of MRSA infections averted	Total number of *S. aureus* infections averted × 90-day MRSA rate	90-Day MRSA rate:
		• Spinal fusion (38.86%)
		• Decompression
		  • Inpatient (35.74%)
		  • Outpatient (31.76%)
		• Hip (39.72%)
		• Knee (36.76%)
		• Other arthroplasty (36.76%)[a]	

Total number of ISA infections averted	Total number of elective surgical patient volumes × overall 90-day postsurgical ISA infection rate × vaccine efficacy	Overall 90-day postsurgical ISA infection rate:
		• Spinal fusion (0.45%)
		• Decompression
		  • Inpatient (0.50%)
		  • Outpatient (0.24%)
		• Hip (0.38%)
		• Knee (0.24%)
		• Other arthroplasty (0.12%)[a]
		Vaccine efficacy: 70% (assumed)	

Total number of deaths averted	Total number of *S. aureus* infections averted × 90-day case fatality	90-Day case fatality rate:
		• Spinal fusion (2.37%)
		• Decompression
		  • Inpatient (1.20%)
		  • Outpatient (0.68%)
		• Hip (2.65%)
		• Knee (0.80%)
		• Other arthroplasty (0.40%)[a]	

Public Health Impact	Basic Formula	Data Input	Data Source
Total number of hospitalizations averted	Total number of ISA infections averted × 1 (for spinal surgery) or 2 (for arthroplasty) hospitalizations	Number of hospitalizations: • ISA infection would need 1 (for spinal surgery) or 2 (for arthroplasty) hospitalizations for revisional procedures. • No hospitalization needed for all noninvasive infection	Pfizer Health State Utilities Associated with Postsurgical *Staphylococcus aureus* Infection Study, a study designed to estimate the disutility of SSIs following either joint surgery or spine surgery in a time trade-off analysis with a 1-year time horizon. Health state descriptions for infection status and its associated recovery timeline (eg, no infection, superficial infection, deep wound infection) were drafted based on published literature and interviews with 7 clinicians who had experience related to SSIs. The potential number of hospitalization and extended recovery timeline in this study were used to estimate number of hospitalizations and days of disability. CDC Vital Statistics was used to estimate number of life year loss from the average age for spinal surgeries (56 years old) and arthroplasties (67 years old) due to early death.
Total number of disability-adjusted life years averted	Total number of ISA infections averted × 60 days/365 days (for spinal surgery) or 120 days/365 days (for arthroplasty) + [Total number *S. aureus* infections averted – total number of ISA infections averted] × 14 days/365 days + Total number of deaths averted × 27 years (for spinal surgery) or 18 years (for arthroplasty)	Disability-adjusted life years[b]: • ISA infection would need an average 60 days (for spinal surgery) or 120 days (for arthroplasty) to recover. • 14 days for all noninvasive infection • Patients who die from infection would lose ~27 years (for spinal) or ~18 years (for arthroplasty) of life expectancy.	

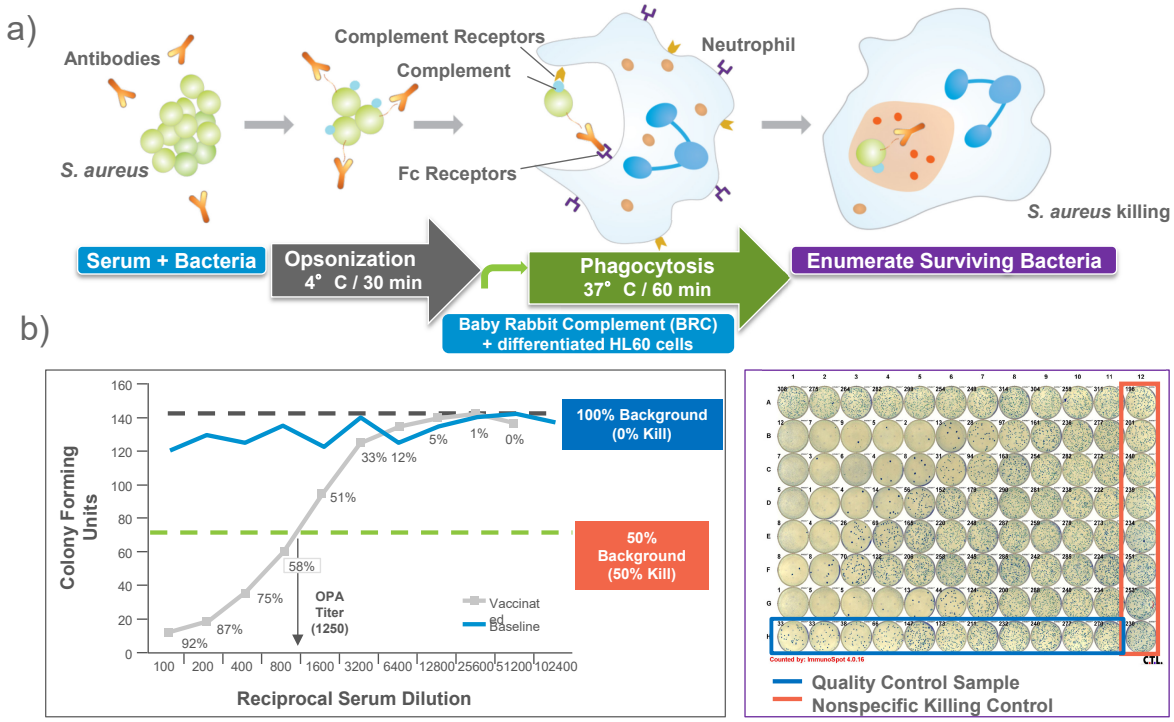
Abbreviations: CDC=US Centers for Disease Control and Prevention; ICD-9=International Statistical Classification of Diseases and Related Health Problems, 9th Revision; ISA=invasive S. aureus; MRSA=methicillin-resistant S. aureus; SSIs=surgical-site infections.

a. Assuming 'other' arthroplasty would have 50% of infection rate and mortality rate as knee arthroplasty and the same MRSA rate as knee arthroplasty.
b. Defined as the sum of the years of life loss due to disability (patients cannot work or attend to usual activities for treatment and recovery from the infection) plus premature death.

Appendix 4. Functional Clinical Assays That Demonstrate SA4Ag Mechanism of Action

To provide assurance that SA4Ag is inducing functional immune responses that target the key early *S. aureus* virulence mechanisms, Pfizer has developed several live cell-based functional assays using clinical *S. aureus* isolates that express the common *S. aureus* virulence factors: capsular polysaccharides, clumping factor A (ClfA), manganese transporter C (MntC), and protein A. For *S. aureus* capsular polysaccharide 5 (CP5) and 8 (CP8), an opsonophagocytic assay (OPA; Figure 12) has been developed to demonstrate functional anti-CP5 and anti-CP8 responses against clinical *S. aureus* isolates expressing each respective capsular polysaccharide.[69] Opsonophagocytic killing of live *S. aureus* by effector cells such as human peripheral blood mononuclear cells and the phagocytic cell line HL-60 by anti-CP5 or anti-CP8 antibodies has been observed with sera from animals and humans vaccinated with SA4Ag.[69,86,87,88] *S. aureus* has several mechanisms that attempt to impede the opsonic uptake of bacteria by neutrophils, including the surface expression of protein A. Protein A (SpA) binds to the Fc portion of IgG molecules, hindering the ability of *S. aureus*-specific antibody to bind the bacteria surface and initiate complement fixation.[228] To demonstrate that this mechanism does not interfere with the opsonophagocytic killing mediated by vaccine capsular polysaccharide antibodies, we have conducted OPA assays with clinical isolates that express SpA during the assay and have not observed interference with the capsular polysaccharide functional antibodies and opsonophagocytic activity.[69] Moreover, Pfizer's OPAs are conducted by lysing the phagocytes (HL-60 cells) at the end of the assay prior to enumeration of any live bacteria, thereby assuring that the robust opsonophagocytic killing observed with sera from SA4Ag-vaccinated subjects represents true killing of *S. aureus* inside of phagocytes.

Figure 12. Opsonophagocytic Assays Measure the Ability of Vaccine Antibodies to Kill CP5- and CP8-Expressing *S. aureus* Strains

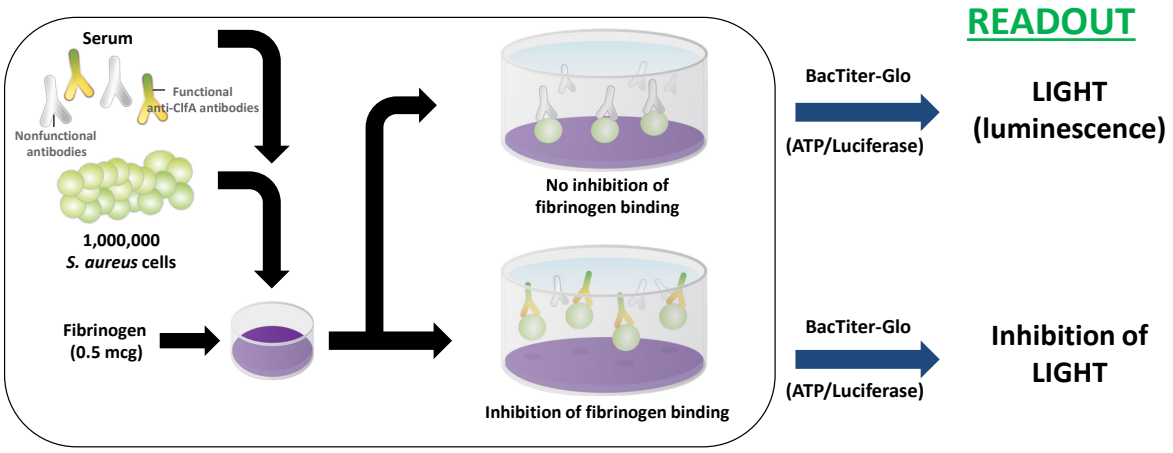


a) Opsonophagocytic assays (OPAs) are used to measure functional antibodies in human sera specific for CP5 or CP8 strains of *S. aureus*. These OPAs measure the ability of CP5- or CP8-specific immunoglobulin to opsonized bacteria and trigger complement deposition, thereby facilitating phagocytosis and killing of bacteria by phagocytes.

b) Human test serum is serially diluted and added to microtiter assay plates with live target bacterial strains, differentiated HL-60 cells (phagocytes), and baby rabbit serum (complement source). HL-60 cells are lysed to release any surviving bacteria within the phagocytes, and the assay reaction seeded on microcolony filter plates. Resulting microcolonies from surviving bacterium are counted on CTL Immunospot readers. The OPA titer is defined as the reciprocal dilution that results in a 50% reduction in bacterial count over control wells without test serum. The OPA titer is interpolated from the 2 dilutions that encompass this 50% killing cut-off. The reported OPA titer is the geometric mean of 2 replicate OPA titers.

For ClfA, the fibrinogen binding inhibition (FBI) assay was developed based on antibody-mediated prevention of *S. aureus* binding to fibrinogen (Figure 13).[209]

Figure 13. Fibrinogen Binding Inhibition Assay Measures Anti-ClfA Antibody Inhibition of *S. aureus* Fibrinogen Binding

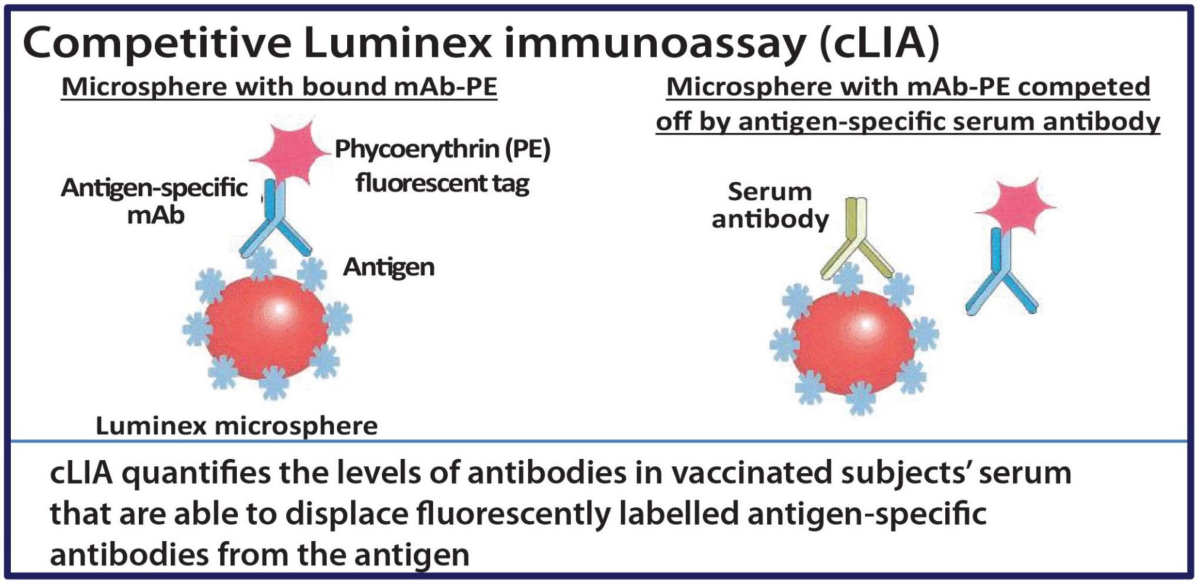


Abbreviations: ATP=adenosine triphosphate; ClfA=clumping factor A; FBI=fibrinogen biding inhibition.

Note: The FBI assay measures specific functional antibodies (in serum) blocking the binding of *S. aureus* (expressing ClfA on surface) to fibrinogen coated in 96-well plates. The FBI serum titer is the inverse of the serum dilution that inhibits ≥75% of binding (compared to bacteria without serum control).

A more high-throughput assay, a competitive Luminex immunoassay (cLIA), was also developed (Figure 14). The fibrinogen-binding cleft for ClfA has been mapped, and the cLIA measures antibodies that displace a functional monoclonal antibody binding to this cleft.[77]

Figure 14. Competitive Luminex Immunoassay (cLIA)



For MntC, the development of a robust, functional, live *S. aureus* in vitro assay is not possible given that MntC is not expressed using normal in vitro conditions. However, an anti-MntC monoclonal antibody has been identified that binds *S. aureus* cells and prevents

the acquisition of manganese.[220] This monoclonal antibody is also protective in a bacteremic infant rat passive protection model.[75] To assess anti-MntC responses, a cLIA was developed that measures functional vaccine-induced antibodies competing with this monoclonal antibody for binding to MntC. This MntC cLIA is multiplexed with the ClfA cLIA (2-plex cLIA).

Appendix 5. Natural Anti-*S. aureus* Immune Responses Compared With Vaccine-Induced Responses

Subjects who are colonized by *S. aureus* have a trend for increased *S. aureus*-associated antibody titers compared with subjects who are not colonized.[229,230,231,232] Likewise, patients with *S. aureus* infections have increases in their anti-*S. aureus* antibody levels as they recover from infection.[77,233,234,235] While naturally acquired antibodies can recognize the SA4Ag antigens, there are differences compared with vaccine-induced responses. First, the levels of antibody elicited after natural exposure due to colonization or disease are very low compared to vaccine-elicited responses (Table 14, healthy adults vs patients). In addition, antibodies generated through natural infection or exposure may not be effective at blocking the virulence mechanisms mediated by the antigen. For example, when nonhuman primates were vaccinated with killed *S. aureus* isolates, all generated antibodies that recognized ClfA. However, these antibodies were not effective at preventing the binding of *S. aureus* cells to fibrinogen. In contrast, when nonhuman primates were vaccinated with a ClfA subunit vaccine, they were able to induce antibodies that prevented the binding of *S. aureus* to fibrinogen (Figure 15). Likewise for humans vaccinated with SA3Ag or SA4Ag, little or no functional antibodies were observed prior to vaccination, yet high functional titers were observed within 2 weeks after vaccination (Figure 9). Thus, although humans do not generate significant functional antibodies through natural exposure, robust responses are observed in 18- through 64-year-old adults after vaccination (Table 14) and in healthy adults 65 through 85 years of age (Appendix 6).[87] Moreover, attempts for development of whole-cell vaccines have had limited success as they are expected to induce similar nonfunctional responses as seen post natural infection.

Table 14. Low Functional Antibody Titers to *S. aureus* Antigens in Previously Infected Patients or Unvaccinated Healthy Subjects Compared With SA4Ag-Vaccinated Subjects

Antigen	cLIA *S. aureus* Antigen GMTs			Fold Change
	S. aureus Patients[a] (n=51)	Healthy Adults[b] Prevaccination (n=105)	Healthy Adults[b] 1 Month After Vaccination (n=105)	
CP5	144	56	3526	63
CP8	120	163	4331	27
ClfA	39	231	4581	20
MntC	105	128	2352	18

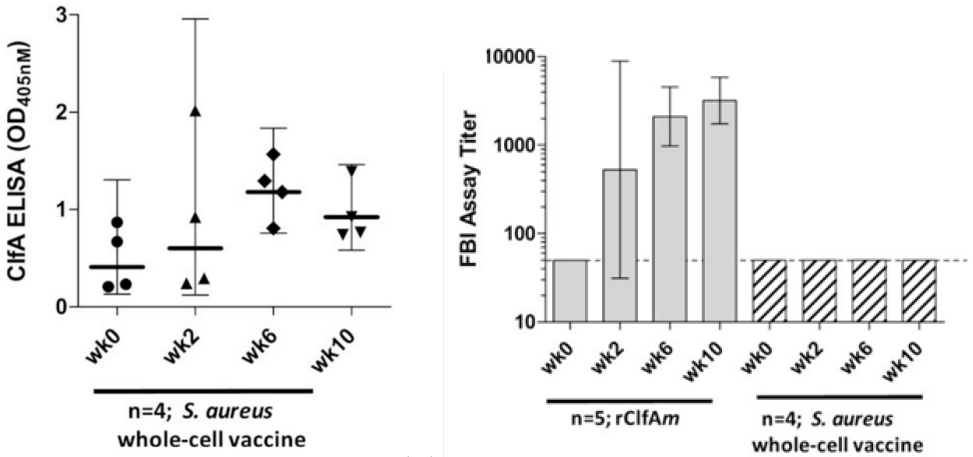
Abbreviations: ClfA=*S. aureus* clumping factor A (protein); cLIA=competitive Luminex immunoassay; CP5=*S. aureus* capsular polysaccharide serotype 5; CP8=*S. aureus* capsular polysaccharide serotype 8; GMTs=geometric mean titers; MntC=*S. aureus* manganese transporter C.

[a] Adults 18 through 64 years of age with previous invasive *S. aureus* infection

[b] Healthy adults 18 through 64 years of age enrolled in Study B3451001 and vaccinated with SA4Ag

Source: Adapted from Frenck et al, 2017[88] and Rozemeijer at al, 2015.[77]

Figure 15. Recombinant ClfA Antigen Vaccination of Nonhuman Primates Induces Functional Antibodies That Inhibit Binding of *S. aureus* to Fibrinogen but Whole-Cell Vaccination Does Not



(A) (B)

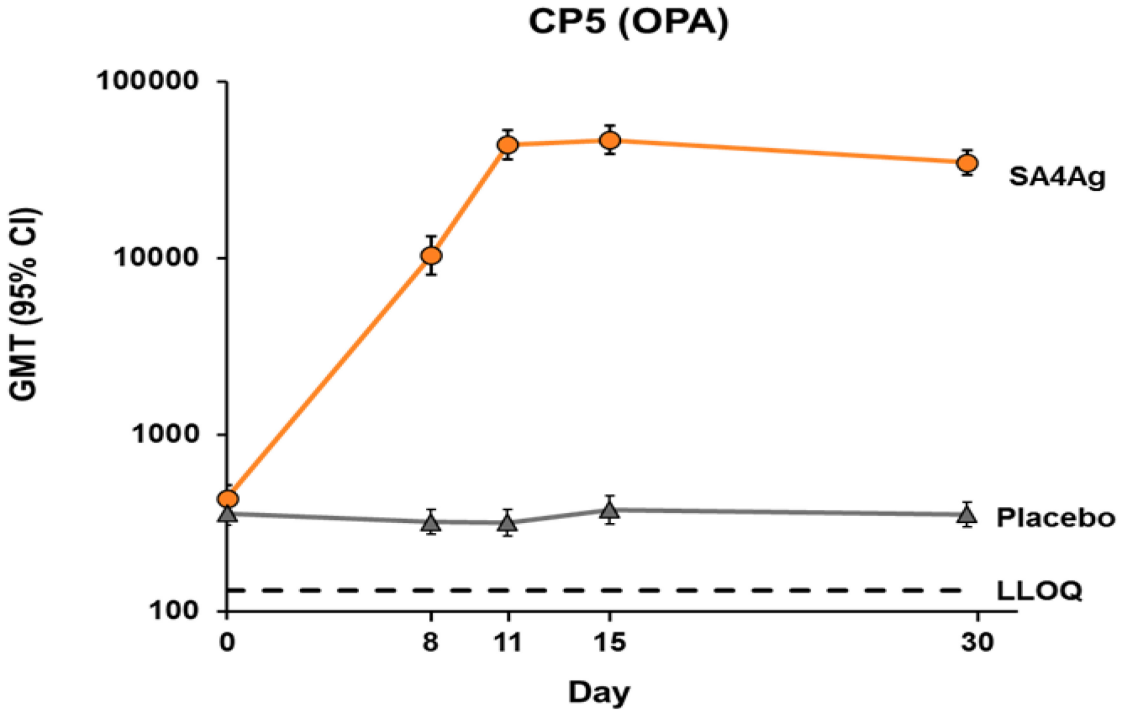
Abbreviations: ClfA=*S. aureus* clumping factor A; ELISA=enzyme-linked immunosorbent assay; FBI=fibrinogen binding inhibition; IgG=immunoglobulin G; rClfA*m*=recombinant ClfA; wk=week.

(A) ClfA ELISA activities of sera from monkeys vaccinated with heat-killed bacteria show the kinetics of IgG antibody responses. ClfA specificity of the rise in IgG (response) was demonstrated at all of the time points by complete inhibition in the presence of soluble vaccine antigen rClfA*m* (not shown).

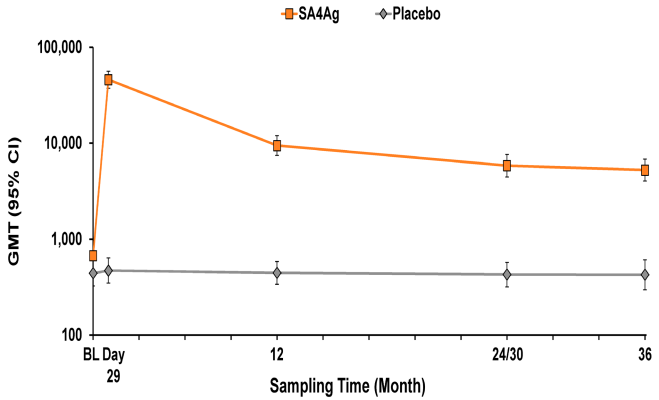
(B) Groups of 5 and 4 rhesus macaques were vaccinated with rClfA*m* antigen (200 µg) or heat-killed *S. aureus* strain PFESA0164 (2×10^8), respectively, and serum samples were collected at wk 2, 6, and 10. Animals were dosed at wk 0 and wk 4. Bars reflect fibrinogen binding inhibition titers (geometric mean titers) determined with *S. aureus* strain PFESA0237, and error bars show 95% confidence intervals. Vaccination titers elicited by rClfA*m* were significantly different from those elicited by heat-killed cells (wks 6 and 10) ($P<0.05$, 2-sided t-test). The dotted line marks 0.5 times the lower limit of detection.

Source: Hawkins et al, 2012.[209]

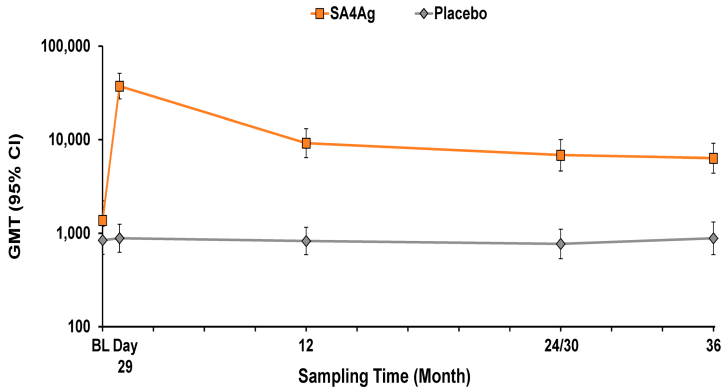
Appendix 6. SA4Ag Demonstrates Functional Immune Response Through 36 Months After Vaccination in Adults 18 Through 85 Years of Age



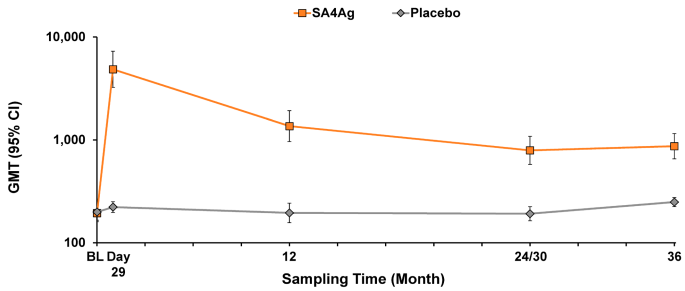
a) SA4Ag induces rapid functional antibodies that peak by 30 days after vaccination of adults 18 through 85 years of age.



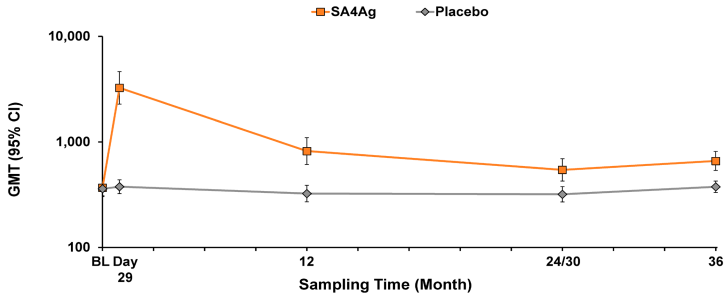
CP5 (OPA)



CP8 (OPA)

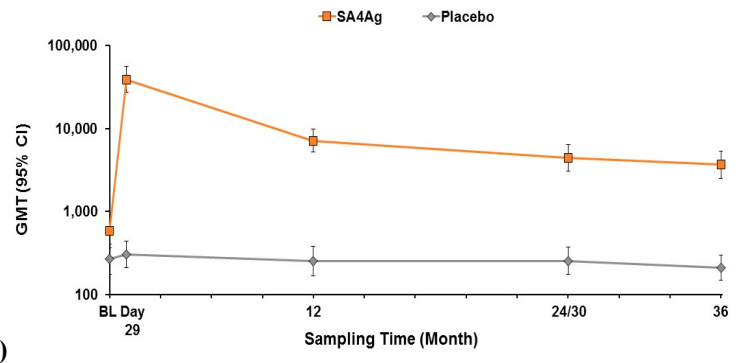


ClfA (FBI)

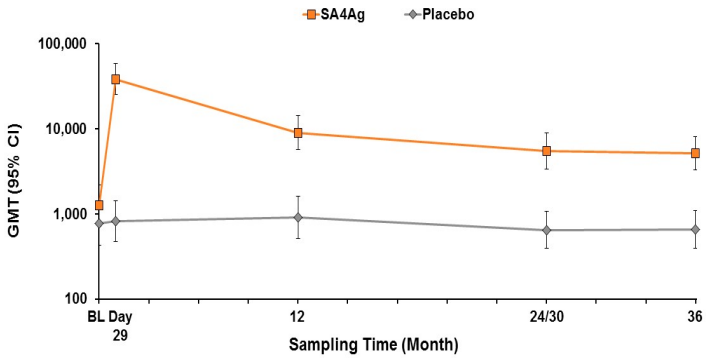


MntC (cLIA)

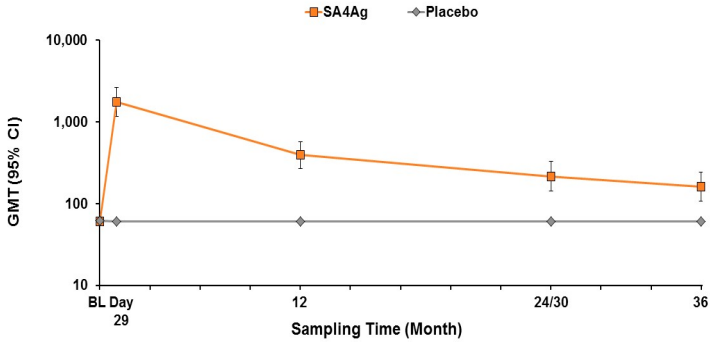
b) Functional antibody responses can be detected for a sustained period of time with levels exceeding baseline 3 years after vaccination of adults 18 through 64 years of age.



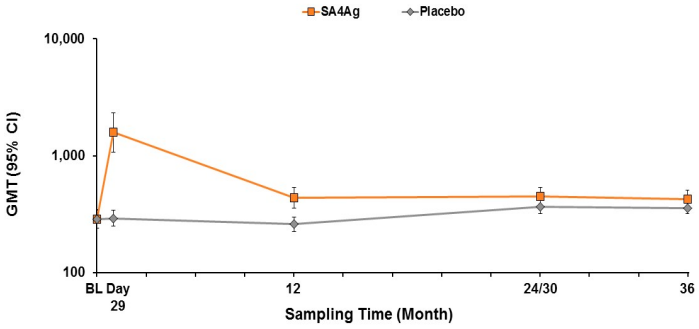
CP5 (OPA)



CP8 (OPA)



ClfA (FBI)



MntC (cLIA)

c) Functional antibody responses can be detected for a sustained period of time with levels exceeding baseline 3 years after vaccination of adults 65 through 85 years of age.

Abbreviations: BL=baseline; CI=confidence interval; ClfA=clumping factor A; cLIA=competitive Luminex immunoassay; CP5=*S. aureus* capsular polysaccharide serotype 5; CP8=*S. aureus* capsular polysaccharide serotype 8; FBI=fibrinogen binding inhibition assay; GMT=geometric mean titer; LLOQ=lower limit of quantitation; MntC=manganese transporter C; OPA=opsonophagocytic assay.

Source: a) Pooled data from Creech et al, 2017[87] and Frenck et al, 2017[88]; b) and c) adapted from Frenck et al, 2017.[195]

Appendix 7. Experience with Other Advanced Investigational *S. aureus* Vaccines: StaphVAX and V710

Vaccines are promising interventions and, assuming success, an anti-*S. aureus* vaccine will add to the armamentarium of infection control measures. To date however, successful antistaphylococcal vaccine development has proved to be difficult in humans. Two investigational *S. aureus* vaccines which completed Phase 2/3 testing in the past 2 decades were both unsuccessful and their development discontinued. The first, StaphVAX® (developed by Nabi Biopharmaceuticals), was a bivalent vaccine that contained capsular polysaccharides (CP5 and CP8) each conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A, and the second vaccine, V710 (developed by Merck), was a non-adjuvanted, single-antigen, iron-binding (iron surface determinant B [IsdB]) vaccine. StaphVAX and V710 each addressed a single virulence mechanism, and neither demonstrated consistent efficacy.

Nabi conducted 2 studies for StaphVAX in the end-stage renal disease (ESRD) population.[236,237] In the first study, 894 patients received a single vaccination. The study did not meet the primary efficacy endpoint of preventing *S. aureus* bacteremia 2 weeks after vaccination. However, a post hoc analysis revealed efficacy within the first 40 weeks of vaccination.[236] In the subsequent study, 1672 patients with ESRD received an initial vaccination followed by a booster dose at Week 35 postvaccination. No vaccine efficacy was observed. Further investigation of the lack of efficacy suggested "possible suboptimal vaccine quality (manufacturing) and a need to expand the antigen composition of the vaccine."[237] The safety profile of the vaccine ultimately tested in 2566 vaccinated patients with ESRD was unremarkable, with reactogenicity events (local and systemic) consistent with those observed with other adult vaccines. Most importantly, no differences were noted in the number of deaths or serious adverse events (SAEs) between vaccine and placebo arms when analyzed by body system. Multiple-organ failure was also evaluated in the booster study, and no cases were observed in patients experiencing the primary efficacy endpoint of *S. aureus* bacteremia.

Merck conducted a Phase 2b/3 efficacy study of V710 in patients scheduled to undergo cardiothoracic surgery. The vaccine was discontinued for futility at the planned 2nd interim analysis, when it was confirmed that the vaccine lacked efficacy and a potential safety signal was identified. Although no significant differences were noted in rates of SAEs, SAEs involving the diagnosis of *S. aureus*, or all-cause mortality (5.7 vs 5.0%; p=0.20), a post hoc analysis revealed postoperative multiple-organ failure developed more often among V710 recipients (31 events; N=3958) compared with placebo recipients (17 events; N=3967) (P=0.04), and the mortality rate in patients with any staphylococcal infections was approximately 5 times higher among V710 recipients (15 deaths out of 73 infections; mortality rate: 23.0) compared with placebo recipients (4 deaths out of 96 infections; mortality rate 4.2).[238] A standard definition of multiple-organ failure was not used across all sites. After a careful review of all available information and data, Pfizer has concluded that the potential safety signal of multiple-organ failure and lack of efficacy observed in the Merck study of V710 was due to limitations of the V710 design rather than reflective of a more fundamental or general concern with *S. aureus* vaccines for the following reasons:

- V710 was composed of a single surface protein, IsdB, which is one of several iron-binding proteins expressed by *S. aureus*. While iron acquisition is critical for *S. aureus* survival in the host, even if the function of IsdB were to be neutralized by V710-elicited immune responses, the other redundant iron acquisition mechanisms would allow the bacteria to acquire this important nutrient.[239] Thus, vaccine-elicited immune responses were unlikely to impair the growth of *S. aureus* in the host.

- Another potential cause for the failure of V710 was its inability to facilitate bacterial killing by opsonophagocytosis. Though antibodies were shown to facilitate the uptake of *S. aureus* into neutrophils, there was no evidence that *S. aureus* was killed inside the neutrophils.[238] In fact, it has been shown that nonfunctional antibodies present in most healthy individuals, while capable of facilitating uptake of *S. aureus*, are unable to promote killing of the bacteria in the neutrophil[240] and actually may facilitate the dissemination of the infection.

- The cardiothoracic surgical population evaluated in Merck's V710 safety and efficacy study has a relatively high rate of mortality, morbidity, and comorbidity,[238] making this population more challenging in which to assess vaccine safety. In this context it is important to reiterate that a safety signal was not observed for StaphVAX (even in patients that eventually became infected with *S. aureus* in the vaccine group), and to note that no safety signal was observed with V710 in a Phase 2a study conducted in patients with ESRD receiving hemodialysis.[241]

In the context of these vaccine failures, it is important to highlight the fundamental differences between these vaccines and SA4Ag. Unlike V710 and StaphVAX, SA4Ag targets 3 distinct and different virulence mechanisms expressed during the early infection phase, as described in Section 3.4. There are no known redundancies for the functions of capsular polysaccharide and MntC expression in *S. aureus*. Additionally, in contrast to V710 and StaphVAX, very robust functional killing responses were demonstrated in subjects 18 through 85 years of age after a single administration of SA4Ag (Section 4.1).

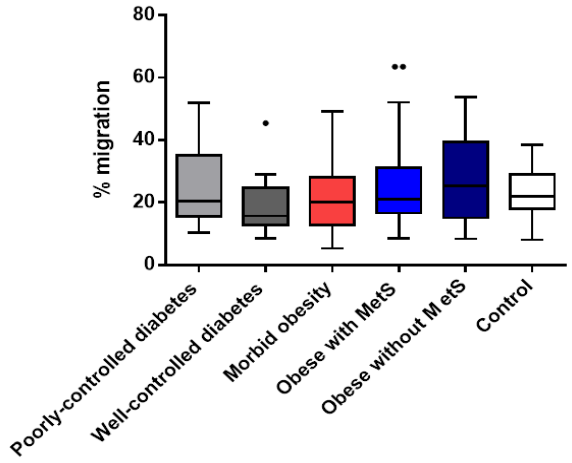
In studying SA4Ag and conducting STRIVE, Pfizer is applying diligence by use of extensive and appropriate measures to collect comprehensive safety and efficacy data prospectively, as described in Section 4.3.2 and Section 4.3.3. The size of STRIVE and standardized, prospective safety data collection and adjudication will provide the ability to identify safety signals of all types occurring at even infrequent rates (~1 in 1000).

Appendix 8. SA4Ag Is Expected to Be Safe and Effective in Obese and Diabetic Patients

Invasive *S. aureus* infections are more prevalent in patients with diabetes and obesity than in those without, and are associated with poor outcomes.[242,243,244] The underlying mechanisms linking these comorbidities to *S. aureus* infection are not fully defined, but may be linked to impairment in several aspects of the immune response to bacterial infections. The primary defense against Gram-positive pathogens such as *S. aureus* is engulfment and oxidative killing by neutrophils, which may be impaired in obese patients.[245,246] There are also reports of impaired bactericidal functions, including phagocytosis, adhesion to endothelium, and chemotaxis by neutrophils in patients with diabetes.[247,248] Conversely, other reports have failed to show significant differences in immunological function in patients with diabetes versus controls.[249] Impaired peripheral blood mononuclear cell (ie, lymphocyte and monocyte) function, decreased lymphocyte proliferation, and altered peripheral cytokine levels have also been reported in patients with obesity.[250] Distinct subsets of circulating neutrophils in peripheral blood, based on maturity, have been described during acute systemic inflammation. These cells may also differ in their functional capacities, such as chemotaxis and adhesion characteristics.[251,252] Manifestations of neutrophil dysfunction such as decreased phagocytosis, superoxide production, and killing activity of *S. aureus* have also been observed in diabetic db/db mouse models.[253]

Neutrophils provide an essential primary defense against *S. aureus*, and therefore contribute to vaccine-mediated, protective, immune responses. To provide a better understanding of the likelihood that a *S. aureus* vaccine is effective in subjects with diabetes, obesity, and metabolic syndrome, neutrophil functions in these patient populations were evaluated in a prospective serological and cellular surveillance study (Pfizer data on file). The primary objectives of the study were to descriptively compare immune function in: 1) adults without diabetes mellitus and with poorly controlled (HbA1c ≥10%) diabetes mellitus; 2) adults with normal body mass index (BMI 18.5 to 24.9 kg/m^2) and with morbid obesity (BMI ≥40 kg/m^2); and 3) in obese adults (BMI ≥30 kg/m^2) with and without metabolic syndrome. Neutrophil function was evaluated with regard to chemotactic migration, bacterial phagocytosis, and opsonophagocytosis (bacterial killing). The study demonstrated no observable impairment in intrinsic neutrophil function or antibody-mediated immune function in subjects with diabetes, obesity, and metabolic syndrome (Figure 16, Figure 17, Table 15). Importantly, neutrophils from all cohorts were able to kill *S. aureus* when provided sera containing functional antibodies. This implies that SA4Ag has the potential to be protective in these populations.

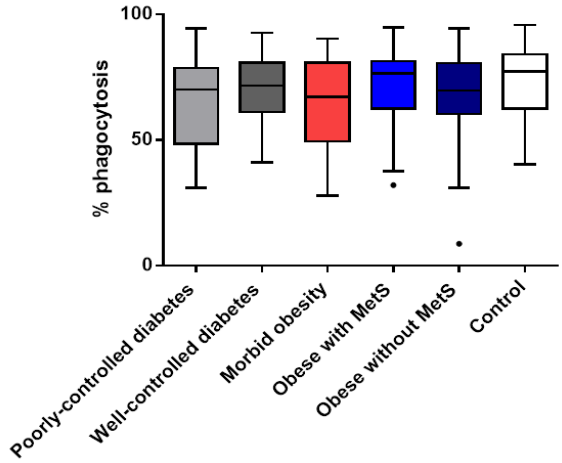
Figure 16. Mean Percent Neutrophil Migration Toward Chemoattractant Did Not Differ in Subjects With or Without Diabetes, Obesity, or Metabolic Syndrome



Abbreviations: Control=subjects without diabetes, obesity, or metS; metS=metabolic syndrome.

Source: Pfizer data on file.

Figure 17. Mean Percent Phagocytosis (Neutrophil Uptake of Opsonized *S. aureus*) With CP8 Immune Sera Did Not Differ in Subjects With Diabetes, Obesity, and Metabolic Syndrome



Abbreviations: CP8=*S. aureus* capsular polysaccharide serotype 8; metS=metabolic syndrome.

Source: Pfizer data on file.

Table 15. Average Opsonophagocytic Assay Titer by Cohort Did Not Differ in Subjects With Diabetes, Obesity, and Metabolic Syndrome

Subset	Cohort					
	Poorly Controlled Diabetes Mellitus (N=14)	Well Controlled Diabetes Mellitus (N=12)	Morbid Obesity (N=12)	Obese With Metabolic Syndrome (N=20)	Obese Without Metabolic Syndrome (N=20)	Control[a] (N=12)
High titer sera						
N, %RSD	14, 84.23	12, 54.00	12, 187.7	18, 197.2	20, 427.0	12, 89.48
Mean, Median	24968, 28580	27569, 30114	13422, 20032	17408, 21465	12036, 23405	13014, 13094
95% CI	5133, 121462	9056, 83928	898, 200545	1220, 248333	329, 440071	2406, 70391
Medium titer sera						
N, %RSD	14, 26.81	12, 85.08	12, 44.84	20, 81.37	20, 106.0	12, 25.00
Mean, Median	1262, 1222	1434, 1366	1109, 1135	1204, 1089	1224, 1363	1001, 1013
95% CI	714, 2230	283, 7276	432, 2844	271, 5351	199, 7529	582, 1722
Low titer sera						
N, %RSD	14, 88.09	12, 40.61	12, 26.80	20, 93.19	20, 54.46	12, 65.10
Mean, Median	87.3, 66.4	69.1, 63.4	59.7, 50.0	79.8, 55.4	77.5, 66.1	77.3, 63.8
95% CI	17.0, 448.9	29.2, 163.3	33.4, 106.5	15.2, 417.4	26.7, 225.1	20.9, 286.1

Abbreviations: CI=confidence interval; CP8=*S. aureus* capsular polysaccharide serotype 8; GMTs=geometric mean titers; OPA=opsonophagocytic assay; RSD=relative standard deviation.
Note: The table shows average OPA (titer) by cohort. OPA GMTs ranged from 13,014 to 27,569 for high-titered CP8 immune sera, from 1001 to 1434 for medium-titered CP8 immune sera, and from 59.7 to 87.3 for low-titered CP8 immune sera. The 95% CIs of the OPA titers in the 6 cohorts overlapped irrespective of titer level; therefore, there was no evidence for differences in opsonophagocytic killing activity among the neutrophils in all cohorts.
[a] Control cohort included 12 subjects without a diagnosis of diabetes mellitus and HbA1c <6.0%, without metabolic syndrome, and with normal body mass index (18.5 to 24.9 kg/m^2).
Source: Pfizer data on file.

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