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Safety, tolerability, and immunogenicity of a single dose 4-antigen or 3-antigen *Staphylococcus aureus* vaccine in healthy older adults: Results of a randomised trial

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ABSTRACT

Background: The decline in immune function with age is a challenge to vaccine development. Following an initial study in adults aged 18–64 years, this study evaluated the safety and immunogenicity of *Staphylococcus aureus* (*S. aureus*) 4-antigen (SA4Ag) and 3-antigen (SA3Ag) vaccine in older adults. SA3Ag included capsular polysaccharide serotypes 5 and 8 (CP5 and CP8) conjugated to the nontoxic mutant form of diphtheria toxin (CRM_{197}) and a recombinant version of clumping factor A (ClfA). SA4Ag included these antigens, with the addition of a recombinant manganese transporter C (rP305A or MntC). Both vaccines were unadjuvanted.

Methods: In this double-blind, sponsor-unblinded, placebo-controlled, phase 1/2 study, 284 healthy adults (aged 65 –85 years) were randomised to receive a single dose of one of three formulations of SA4Ag with escalating dose levels of rP305A, SA3Ag, or placebo. Functional immune responses were measured using opsonophagocytic activity (OPA) killing and fibrinogen-binding inhibition (FBI) assays; immunogenicity was also assessed using a competitive Luminex® immunoassay (cLIA). T-cell responses were measured in a small subgroup of subjects using intracellular cytokine staining (ICS) assays.

Results: The results demonstrated rapid and robust functional immune responses to all antigens in healthy older adults. A high proportion of active vaccine recipients met the pre-defined antibody thresholds for each antigen at Day 29. SA4Ag elicited a dose-level response to rP305A with up to a 13-fold rise in cLIA titres at Day 29. Opsonophagocytic activity (OPA) assays showed >50- and >20-fold rises in functional titres using *S. aureus* strains expressing CP5 and CP8, respectively, at Day 29. T-cell cytokine

Abbreviations: AE, adverse event; ClfA, clumping factor A; cLIA, four-plex competitive Luminex® immunoassay; CI, confidence interval; CP, capsular polysaccharide; CRM_{197}, diphtheria toxin; DMC, data monitoring committee; FBI, fibrinogen-binding inhibition; FDA, Food and Drug Administration; GCP, Good Clinical Practice; GMFR, geometric mean-fold rise; GMTs, geometric mean titres; ICS, intracellular cytokine staining; IFN, interferon; IL, interleukin; LLOQ, lower limit of quantification; MntC (or rP305A), manganese transporter protein; OPA, opsonophagocytic activity; PCR, polymerase chain reaction; rP305A, recombinant P305A; *S. aureus*, *Staphylococcus aureus*; SA4Ag, *S. aureus* four-antigen vaccine; SA3Ag, *S. aureus* three-antigen vaccine; SAE, serious adverse event; SSI, surgical site infection; TFN, tumour necrosis factor.

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responses were not substantially above background levels. There were no safety concerns in this study population and no increases in adverse events with higher rP305A dose levels.
Conclusions: Single-dose vaccination of SA4Ag and SA3Ag in healthy adults aged 65–85 years safely induced rapid and robust functional immune responses, supporting further development of SA4Ag for the prevention of *S. aureus* disease in adults up to age 85 years. Trial registration number: NCT01643941.

1. Introduction

Adults aged 65 years and older are at increased risk for invasive *Staphylococcus aureus* infection and poor clinical outcomes, particularly in the healthcare setting [1–3]. Surgical site infection (SSI) is a complication of between 2% and 5% of surgical procedures, with *S. aureus* being the most commonly implicated organism [4]. Although there are many patient and procedural factors that influence SSI risk, older age is an important risk factor associated with postoperative SSI [5–7]. Although numerous measures are used to reduce the incidence of infections in hospitals [8], a prophylactic *S. aureus* vaccine remains an elusive unmet medical need. A challenge to vaccine development in older populations is the negative impact of aging on innate and adaptive immune functions, including diminished responses to vaccinations and increased susceptibility to infection [9,10]. Development of a vaccine that induces a protective immune response against invasive *S. aureus* infections such as SSI, especially in these high-risk individuals, would be an important public health advance.

Dose-ranging studies of a first-generation *S. aureus* 3-antigen vaccine (SA3Ag) in healthy adults aged 18–24 and 50–85 years demonstrated safety, tolerability, and a robust functional immune response [11]. SA3Ag consisted of capsular polysaccharide serotypes 5 and 8 (CP5 and CP8) conjugated to the nontoxic mutant form of diphtheria toxin (CRM_{197}), and a recombinant version of the surface protein clumping factor A (ClfA). A dose level of 30 μg CP5-CRM_{197}, 30 μg CP8-CRM_{197}, and 60 μg r*m*ClfA was selected for further development. An additional antigen, recombinant P305A (rP305A), developed from a lipoprotein manganese transporter C (MntC), was included in an investigational *S. aureus* 4-antigen vaccine (SA4Ag). MntC facilitates *S. aureus* survival *in vivo*, and preclinical evaluations supported the addition of rP305A to target this important bacterial virulence factor [12].

Following initial determination of safety and immunogenicity from a companion study in healthy adults 18–64 years of age [13], a second study with single-dose vaccination with SA4Ag or SA3Ag was conducted in an older group of healthy adults, aged 65–85 years. The primary objectives were to evaluate the safety, tolerability, and immunogenicity of SA4Ag (three ascending dose levels of rP305A with fixed dose levels of CP5-CRM_{197}, CP8-CRM_{197}, and r*m*ClfA) at Day 29 after vaccination. Secondary objectives were to describe the kinetics of the immune response to SA4Ag for up to12 months post-vaccination, and to assess the safety, tolerability, and immunogenicity of a comparator formulation (SA3Ag [fixed dose levels of CP5-CRM_{197}, CP8-CRM_{197}, and r*m*ClfA without rP305A]). An exploratory objective to assess the T-cell cytokine response to both vaccines was also completed.

2. Methods

This multicentre, parallel-group, randomised, double-blind, placebo-controlled, sponsor-unblinded phase 1/2 trial was conducted at seven sites in the United States between 15 August, 2012 and 24 March, 2014 (ClincalTrials.gov identifier NCT01643941; https://clinicaltrials.gov/ct2/show/NCT01643941).

2.1. Participants

Healthy males and females aged 65–85 years were eligible for study participation. Males who were considered biologically capable of fathering children and who were sexually active with females of childbearing potential were instructed to use a highly effective method of contraception throughout the study. Exclusion criteria prohibited enrolling subjects with any chronic medical condition or disease requiring a significant change in therapy or hospitalisation within 3 months of study entry; serious chronic medical disorders; blood ($\geqslant$250 mL) or plasma donation within 3 months; bleeding diathesis or prolonged bleeding time that would be a contraindication for intramuscular injection or blood draw; immunocompromised subjects or those receiving immunosuppressive therapy; *S. aureus* infection within 6 months; receipt of blood products or immunoglobulin within 12 months; contraindication to the vaccine or vaccine components, or previous administration of *S. aureus* vaccine; Mini-Mental State Examination score $\leqslant$ 21; or residence in a nursing home or long-term care facility or skilled nursing care requirement.

No vaccines, other than influenza and pneumococcal vaccines, were permitted through the conclusion of the study unless medically indicated; where possible, non-study vaccines were given at least 28 days before or after study vaccination. With the exception of low-dose aspirin, any anticoagulant, antiplatelet, and/or antithrombotic agents were not permitted within 30 days of enrolment through 4 weeks after vaccination (Day 29).

The protocol, informed consent, and amendments were approved by the institutional review boards for each participating study site. Written informed consent was obtained from all subjects prior to study entry. This study was conducted in compliance with Good Clinical Practice (GCP) guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, the International Ethical Guidelines for Biomedical Research Involving Human Subjects, and the principles of the Declaration of Helsinki.

2.2. Study vaccine

Three dose levels (low, mid, high) of SA4Ag were evaluated; each dose level included 30 μg CP5-CRM_{197}, 30 μg CP8-CRM_{197}, and 60 μg r*m*ClfA, and one of three dose levels of rP305A (low, 20 μg; mid, 60 μg; or high, 200 μg). The SA3Ag comparator vaccine consisted of the same fixed-dose levels of 30 μg CP5-CRM_{197}, 30 μg CP8-CRM_{197}, and 60 μg r*m*ClfA without rP305A. Study vaccine or placebo was administered as a single 0.5 mL intramuscular injection into the deltoid muscle.

2.3. Study design and assessments

The phase 1 and phase 2 study design and assessments are the same as those described in the companion article published in this journal with the addition of SA3Ag as a comparator arm in phase 2 [13].

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