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A randomized phase I study of the safety and immunogenicity of three ascending dose levels of a 3-antigen *Staphylococcus aureus* vaccine (SA3Ag) in healthy adults

Michael Nissen[a,1], Helen Marshall[b,*,1], Peter Richmond[c], Sepehr Shakib[d], Qin Jiang[e], David Cooper[f], Denise Rill[e], James Baber[g], Joseph Eiden[f], William Gruber[f], Kathrin U. Jansen[f], Emilio A. Emini[e], Annaliesa S. Anderson[f], Edward T. Zito[e], Douglas Girgenti[f]

[a] *Queensland Paediatric Infectious Diseases Clinical Trials Centre, Royal Children's Hospital and Children's Health Queensland, Brisbane, QLD, Australia*
[b] *Vaccinology and Immunology Research Trials Unit, Women's and Children's Hospital and Robinson Research Institute and School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, SA, Australia*
[c] *University of Western Australia School of Paediatrics and Child Health & Telethon Kids Institute, Perth, WA, Australia*
[d] *CMAX, Adelaide, SA, Australia*
[e] *Pfizer Inc., Collegeville, PA, USA*
[f] *Pfizer Inc., Pearl River, NY, USA*
[g] *Pfizer Australia Pty Ltd, Sydney, NSW, Australia*

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ABSTRACT

Background: Staphylococcus aureus is a common cause of healthcare-acquired morbidity and mortality and increased healthcare resource utilization. A prophylactic vaccine is being developed that may reduce this disease burden.
Methods: Volunteers in good general health aged 50–85 ($n = 312$) and 18–24 ($n = 96$) years were randomized to receive a single intramuscular dose of one of three dose levels of a non-adjuvanted, 3-antigen *S. aureus* vaccine (SA3Ag) or placebo. SA3Ag antigens included capsular polysaccharides 5 and 8 (CP5 and CP8), each conjugated to cross-reactive material 197 (CRM_{197}), and recombinant clumping factor A (ClfA). Safety, tolerability, and immunogenicity were evaluated.
Results: At day 29 post-vaccination, robust immune responses were observed in both age cohorts at all three SA3Ag dose levels. In the primary analysis population, the 50- to 85-year age stratum, geometric mean-fold-rises in competitive Luminex® immunoassay antibody titers from baseline ranged from 29.2 to 83.7 (CP5), 14.1 to 31.0 (CP8), and 37.1 to 42.9 (ClfA), all ($P < 0.001$) exceeding the pre-defined two-fold rise criteria. Similar rises in opsonophagocytic activity assay titers demonstrated functionality of the immune response. Most injection-site reactions were mild in severity and there were no substantial differences (SA3Ag vs. placebo) with regard to systemic or adverse events.
Conclusions: In this study of healthy adults aged 50–85 and 18–24 years, SA3Ag elicited a rapid and robust immune response and was well tolerated, with no notable safety concerns.

Abbreviations: AE, adverse event; ClfA, clumping factor A; cLIA, competitive Luminex® immunoassay; CP, capsular polysaccharide; CP5, capsular polysaccharide type 5; CP8, capsular polysaccharide type 8; CRM_{197}, cross-reactive material 197; GMFR, geometric mean-fold-rise; GMT, geometric mean titer; mAb, monoclonal antibody; mITT, modified intention to treat; MntC, manganese transporter C; MRSA, methicillin-resistant *Staphylococcus aureus*; OPA, opsonophagocytic activity; rClfA*m*, recombinant clumping factor A mutant; SA3Ag, *S. aureus* 3-antigen vaccine; SA4Ag, *S. aureus* 4-antigen vaccine; SAE, serious adverse event.

* Corresponding author at: University of Adelaide, Vaccinology and Immunology Research Trials Unit, 72 King William Rd, North Adelaide, Adelaide 5006, SA, Australia. Tel.: +61 8 8161 8115/+61 7 3636 1260; fax: +61 8 8161 7031/+61 8 8313 6885.
E-mail addresses: theniss@uq.edu.au (M. Nissen), helen.marshall@adelaide.edu.au (H. Marshall).
[1] Helen Marshall and Michael Nissen contributed equally to the preparation of this manuscript.

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1. Introduction

Staphylococcus aureus is a leading cause of infection-related morbidity and mortality [1–3] and is the most frequent cause of post-operative infection [4,5]. The emergence of antibiotic-resistant strains has impeded *S. aureus* disease management [6,7]. During 2011–2012, 1734 cases of *S. aureus* bacteremia were reported in Australian public hospitals, of which 24% were methicillin-resistant (MRSA) [8]. In the USA, studies have shown the annual incidence of *S. aureus* bacteremia to be 15–17 per 100,000 population, of which nearly half are due to MRSA [9,10].

The complexity of *S. aureus* virulence mechanisms has made prophylactic vaccine development challenging. To date, two clinical vaccine programs that targeted single *S. aureus* virulence mechanisms have failed in development [11–16]. Based on the premise that a successful prophylactic *S. aureus* vaccine must address a combination of well-conserved virulence factors expressed by most strains, and generate antibodies that effectively kill the organism, a multi-antigen vaccine candidate targeting the bacterial capsular polysaccharide type 5 (CP5), capsular polysaccharide type 8 (CP8), clumping factor A (ClfA), and manganese transporter C (MntC) is under development [17]. *S. aureus* bacteria produce capsular polysaccharides (CPs) to evade the host's immune system [18]. All *S. aureus* strains possess the genetic pathway for synthesis of either CP5 or CP8 [19]. Analysis of CP expression shows that although some strains do not express CP under in vitro growth conditions, CP expression is detected in vivo [20,21]. ClfA is a well-conserved surface antigen that facilitates *S. aureus* infection by binding to fibrinogen, complement proteins, and platelets, thus mediating adhesion to host tissues [22]. MntC enables manganese acquisition, which is used by *S. aureus* to facilitate survival in neutrophils [23]. Preclinical studies have demonstrated efficacy of these antigens in several animal models of infection [24,25]. Antisera raised to these antigens generated robust antibody titers that killed *S. aureus* or neutralized the function of the antigen [26,27]. This first-in-human phase I study evaluated the safety, tolerability, and immunogenicity of a vaccine formulation comprising three of the four target antigens (3-antigen *S. aureus* vaccine [SA3Ag]): CP5 and CP8 individually conjugated to cross-reactive material 197 (CRM_{197}) – a non-toxic mutant form of diphtheria toxin (CP5-CRM_{197} and CP8-CRM_{197}) – and a recombinant mutant form of clumping factor A (rClfA*m*). In recognition of the increased burden of invasive *S. aureus* disease in older individuals [4] and lower immune responses to vaccines commonly observed with ageing [28], healthy adult volunteers aged 18–24, as well as those 50–85 years, were evaluated. The primary objectives of this study were to assess immunogenicity of escalating dose levels of SA3Ag in the older age stratum and safety and tolerability in both age strata.

2. Methods

2.1. Participants

Study participants were recruited from the community at five study centers in Australia. Exclusion criteria prohibited any major illness that increased risk associated with study participation, pregnancy (tested prior to vaccination), breastfeeding, any coagulation or bleeding time disorder (low-dose daily aspirin allowed), contraindication to vaccine components, known immunodeficiency, receipt of blood products or immunoglobulins within 12 months, previous *S. aureus* vaccination, and participation in any other investigational trial. Participants with stable chronic conditions were eligible for inclusion provided these conditions were being treated and were medically stable as determined by the investigator. The study was approved by the Human Research Ethics Committee of each participating institution. All participants provided written informed consent prior to undergoing any study-related procedures.

2.2. Study design

In this first-in-human, phase I, participant- and investigator-blinded, sponsor-unblinded, ascending dose level, randomized, placebo-controlled study, SA3Ag was administered as a non-adjuvanted, lyophilized vaccine containing 10 μg each of CP5- and CP8-CRM_{197} conjugates and 20 μg of rClfA*m* (low-dose level), 30 μg of CP5- and CP8-CRM_{197}, and 60 μg of rClfA*m* (mid-dose level), or 100 μg of CP5- and CP8-CRM_{197} and 200 μg of rClfA*m* (high-dose level), reconstituted with 60 mM NaCl. The saline placebo contained 150 mM (isotonic) NaCl. Randomization within each SA3Ag dose level cohort (low, mid, and high) was 3:1 active to placebo, such that overall, randomization was approximately 1:1:1:1 (low:mid:high:placebo). Dosing was administered in a step-wise manner, with sentinel safety cohorts of 12 participants dosed first, starting with the low-dose-level cohort (18–24- and 50–64-year age substrata). Subsequent dosing proceeded following evaluation of safety data of the sentinel safety cohorts by a project-independent safety review team. An unblinded dispenser randomized participants through use of an interactive website or voice-response system. Vaccine was administered to participants in a blinded manner by study staff.

2.3. Safety evaluation

Local injection-site reactions (erythema, induration, and pain) and systemic events (vomiting, diarrhea, headache, fatigue, muscle pain, joint pain, and fever) were recorded by participants in an electronic diary (e-diary) from day 1 through day 14 following vaccination. Laboratory assessments were performed in sentinel participants prior to vaccination and at days 5 and 15, including complete blood count, platelet aggregation assay, fibrinogen activity assay, prothrombin time, international normalized ratio, partial thromboplastin time, total hemolytic complement (CH50) and complement component 3 levels, and examination of urine sediment for white and red blood cell casts. Adverse events (AEs) were recorded for at least 28 days after vaccination, and serious adverse events (SAEs) and newly diagnosed chronic medical disorders for 6 months following vaccination. Investigators assessed each AE to determine severity and relatedness to vaccine.

2.4. Immunogenicity evaluation

Blood samples were collected prior to vaccination (day 1; the day of vaccination) and at each post-vaccination visit (days 5, 8, 11, 15, and 29; and months 2 and 3). Antigen-specific serum immune responses were measured using a multiplex competitive Luminex® immunoassay (cLIA) that measures the ability of serum immunoglobulin to compete with the binding of antigen-specific monoclonal antibodies (mAbs) to each antigen coated on beads [29]. The cLIA for ClfA utilized a functional competitive mAb previously shown to inhibit *S. aureus* binding to host fibrinogen [26]. The ability of SA3Ag to induce functional antibodies that kill *S. aureus* clinical isolates that express either CP5 (*S. aureus* PFESA0186) or CP8 (*S. aureus* PFESA0158) was measured using an opsonophagocytic activity (OPA) assay [27]. The OPA assay titer is calculated as the highest serum dilution at which serum antibodies facilitate the killing of 50% of the bacteria added to the assay in the presence of complement and phagocytes. Baby rabbit serum (Pel-Freez, USA)

was used as an exogenous complement source in the OPAs. HL-60 cells (Catalog no. CCL240, ATCC, USA) were differentiated with dimethylformamide and used as a source of phagocytic cells in the OPAs. Samples from all time points were tested using the cLIA assay, and samples from a randomly selected subset of participants in each dose level cohort and age substrata ($n = 22–32$) were tested using the OPA assay at baseline and post-vaccination days 15 and 29, and month 3.

2.5. Sample size and statistical analysis

Sample size for the 18–24-year age stratum was estimated based on safety evaluation. With 28 participants per SA3Ag vaccine group, there was at least 90% power to detect an event with 60% incidence in the vaccine group when the rate in the placebo group was no more than 10%, using a one-sided Fisher's Exact test at the 2.5% level. Allowing for a 12% dropout rate, 32 participants were needed per vaccine group.

Sample size for the 50–85-year age stratum was based on hypothesis testing of antibody geometric mean titers (GMTs) from preclinical animal studies. With 64 participants per SA3Ag vaccine group, there was an estimated 93.4% power per antigen to detect a two-fold increase in GMT relative to baseline using a one-sided paired *t*-test at the 2.5% level. Allowing for a drop-out of 18%, 78 participants were needed per vaccine group to achieve 81% power for all three antigens.

Analyses of the proportions of participants reporting local reactions and systemic events through post-vaccination day 14 were summarized descriptively. The primary immunogenicity analysis population was the modified intention to treat (mITT) population. The primary end point was antigen-specific geometric mean-fold-rise (GMFR) in cLIA titer from baseline (Visit 1) to post-vaccination day 29 (Visit 6) in the 50–85-year age stratum. The primary comparison of interest was a two-fold GMFR for each antigen. As the sample size is large enough to utilize parametric statistics, the one-sample (1-sided) *t*-test was used to compare GMFR with a two-fold increase in cLIA titer at a 2.5% level of significance. cLIA and OPA titers were descriptively summarized at each time point assessed.

3. Results

3.1. Participants

A total of 408 consenting participants were randomized into four groups (102 participants per vaccination group, each comprising 78 participants aged 50–85 years and 24 participants aged 18–24 years) (Fig. 1). Generally, demographic characteristics were comparable among vaccine groups in the 50–85-year age stratum (Supplementary data: Table 1).

All participants received study vaccination, except for one participant in the mid-dose-level group assessed as ineligible after randomization but prior to vaccination (due to receipt of a prohibited medication) and one participant in the high-dose-level group who withdrew consent prior to vaccination. In total, 406 participants were vaccinated. One additional participant withdrew before day 29 due to refusal to undergo additional blood draws. A total of 392 participants completed the month 6 visit.

3.2. Immunogenicity evaluation

Pre-vaccination cLIA GMTs ranged from 57 to 228 for CP5, 81 to 154 for CP8, and 25 to 27 for ClfA across all dose levels/age strata. For the majority of participants, cLIA titers to ClfA were below the limit of quantification at baseline (93.1–96.1% of participants across all dose levels/age strata). Substantial increases in cLIA GMTs from baseline to day 29 were observed for all three antigens at all vaccine dose levels and in each age stratum (Fig. 2). GMFRs from baseline to day 29 levels ranged from 29.2 to 83.7 for CP5, 14.1 to 31.0 for CP8, and 37.1 to 42.9 for ClfA in subjects aged 50–85 years. These results greatly exceeded the pre-specified two-fold increase in cLIA titer ($P < 0.001$). A dose–response relationship was observed for CP5 and CP8 in the 50–85-year age stratum as measured by both cLIA and OPA assays (Figs. 2 and 3). ClfA cLIA responses were similar across the SA3Ag vaccine dose levels.

The kinetics of the antigen-specific cLIA GMTs until the month 3 post-vaccination visit for the 50–85- and 18–24-year age strata are shown in Fig. 4. A rapid rise in GMTs was observed, with anti-CP5 and anti-CP8 antibodies reaching a peak between day 11 and day 15 for all dose levels and in both age strata. ClfA GMTs also rose rapidly,

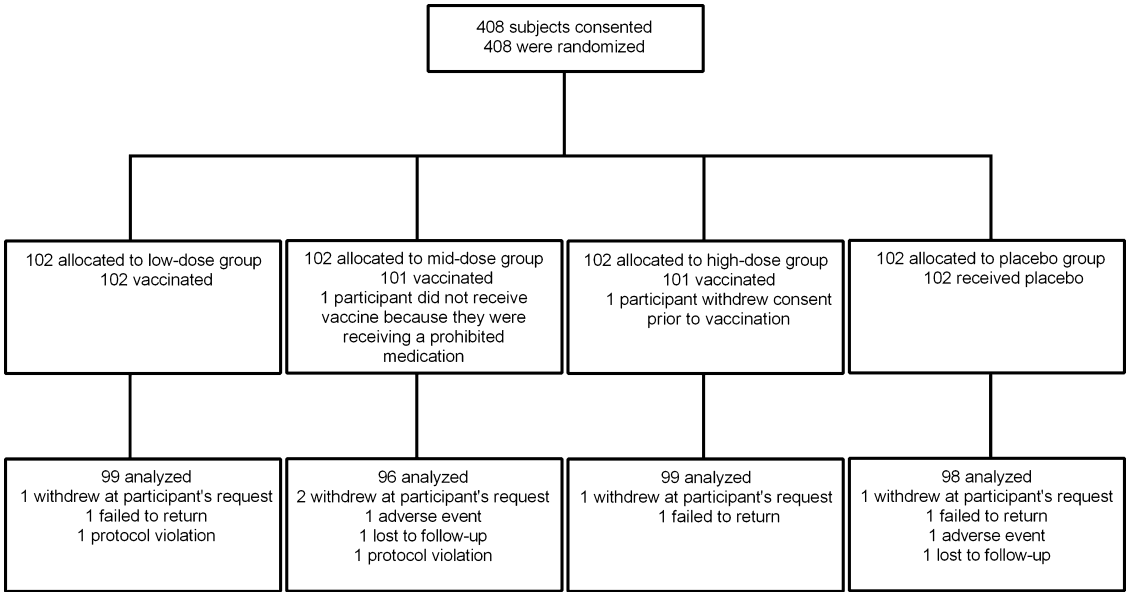

Fig. 1. Study profile.

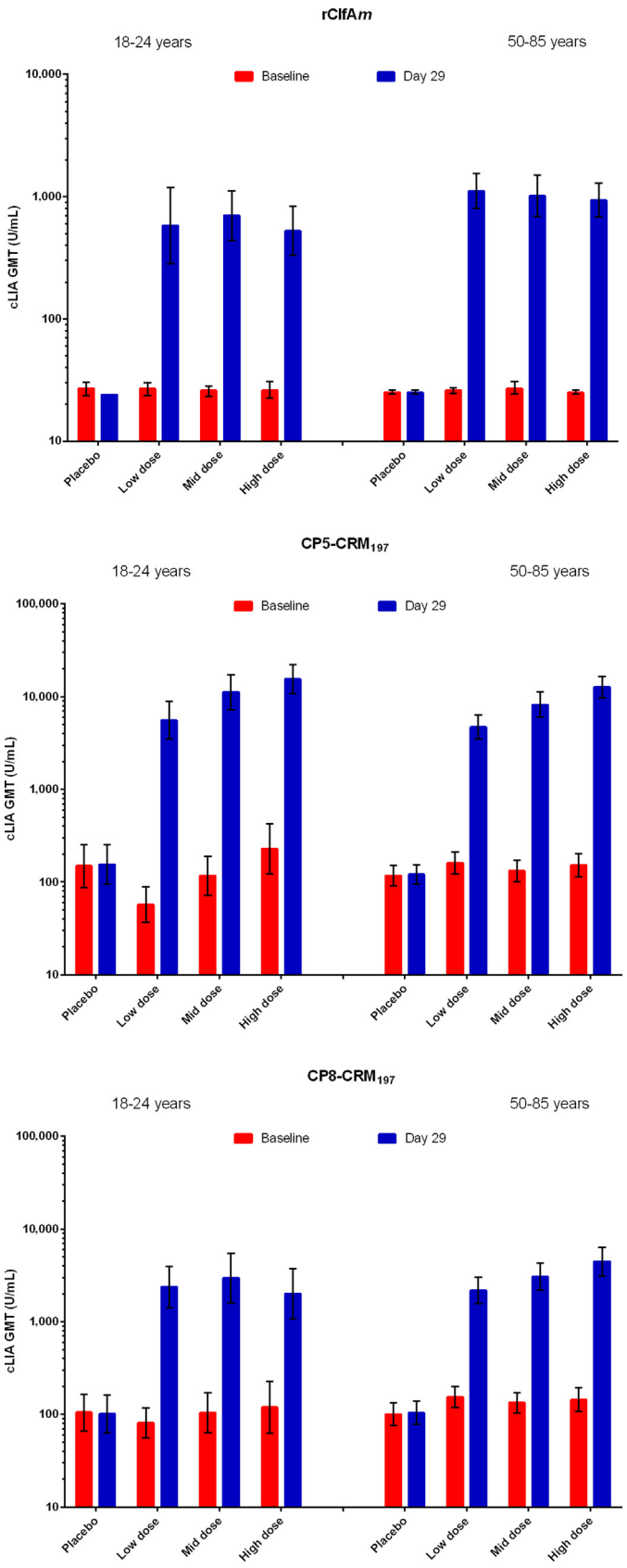

Fig. 2. Antigen-specific antibody titers at day 29 post-vaccination by age group using the cLIA assay.
cLIA: competitive Luminex® immunoassay; rClfA*m*: recombinant mutant form of clumping factor A; GMT: geometric mean titer; CP5-CRM_{197}: capsular polysaccharide type 5–cross-reactive material 197; CP8-CRM_{197}: capsular polysaccharide type 8–cross-reactive material 197.

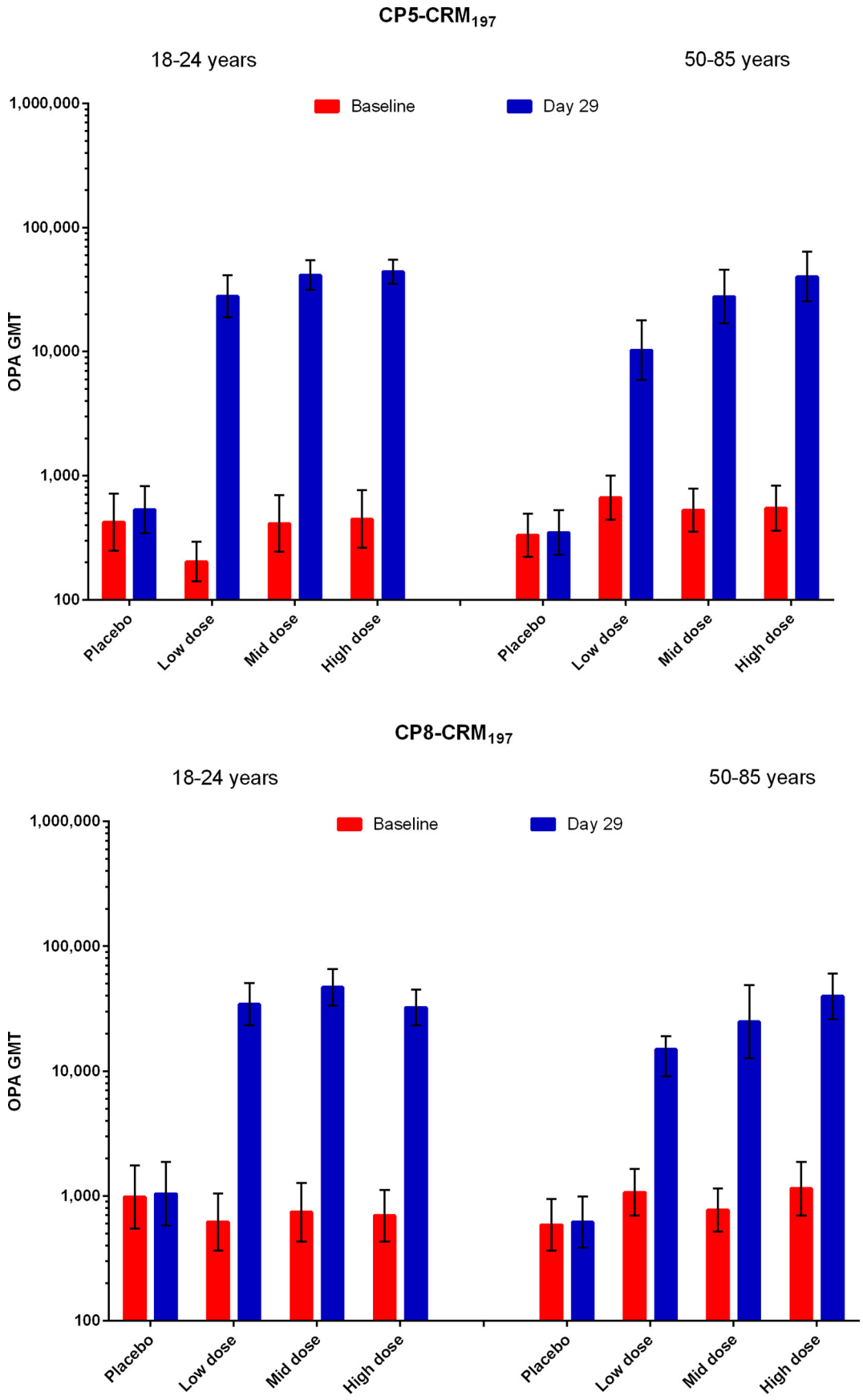

Fig. 3. Functional antibody titers at day 29 post-vaccination: OPA assay by age group.
OPA: opsonophagocytic activity; CP5-CRM$_{197}$: capsular polysaccharide type 5–cross-reactive material 197; GMT: geometric mean titer; CP8-CRM$_{197}$: capsular polysaccharide type 8–cross-reactive material 197.

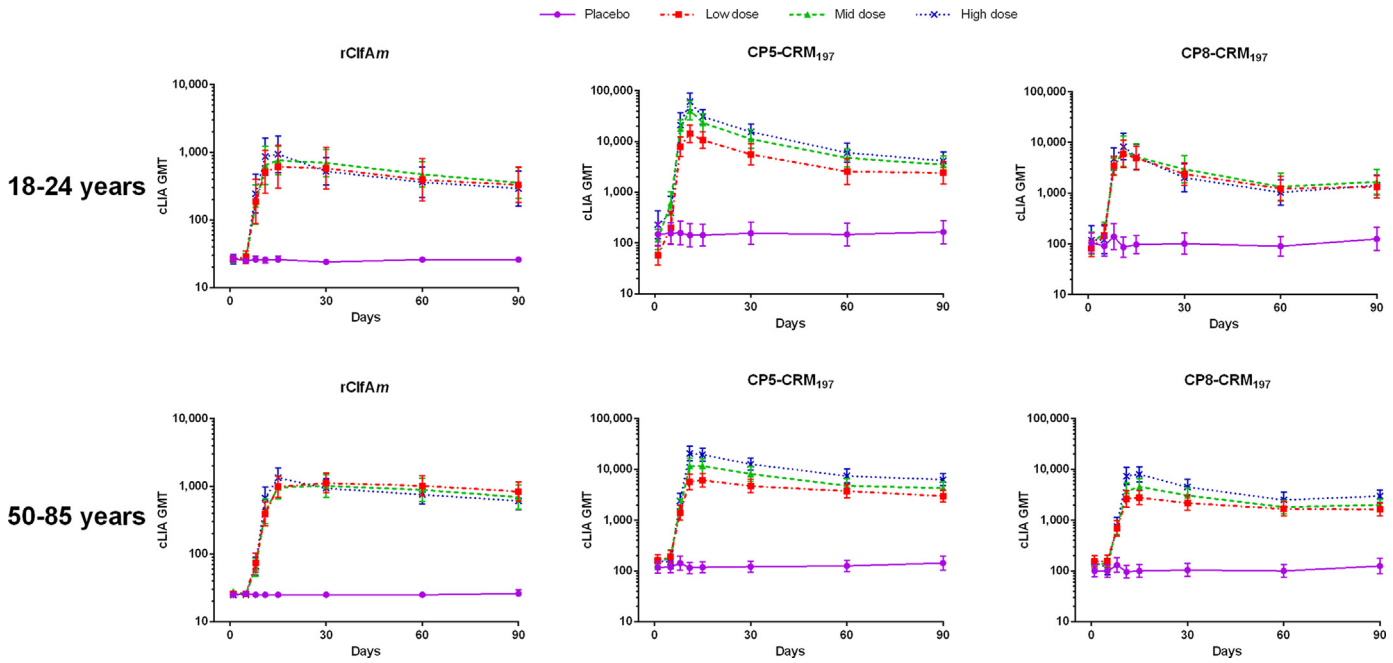

Fig. 4. Kinetics of immune response through 3 months using the cLIA assay.
cLIA: competitive Luminex® immunoassay; rClfA*m*: recombinant mutant form of clumping factor A; GMT: geometric mean titer; CP5-CRM_{197}: capsular polysaccharide type 5–cross-reactive material 197; CP8-CRM_{197}: capsular polysaccharide type 8–cross-reactive material 197.

reaching a peak between day 15 and day 29. GMTs waned gradually, remaining substantially higher at month 3 compared with baseline.

Pre-vaccination OPA GMTs ranged from 203 to 666 and from 589 to 1145 across all dose levels/age strata using OPA assays with the CP5- and CP8-expressing *S. aureus* clinical isolates, respectively. The majority of participants had measurable pre-existing OPA titers. Serum from participants vaccinated with either low-, mid-, or high-dose-level SA3Ag had substantially increased opsonophagocytic killing activity against CP5- and CP8-expressing *S. aureus* strains as demonstrated by post-vaccination day 29 OPA titers compared with baseline titers (Fig. 3). No increases in GMTs were seen in the placebo group. CP5 and CP8 OPA GMFRs in the 50–85-year age stratum at day 29 ranged from 15.4- to 73.5-fold and from 13.9- to 34.7-fold, respectively. OPA GMT responses through month 3 showed a similar pattern to those of cLIA GMT responses (data not shown).

3.3. Safety results

The most common local reaction in all age groups was injection-site pain, reported more frequently by participants in the high-dose-level SA3Ag vaccine group (Fig. 5), and by participants aged 18–24 years. Injection-site reactions were generally mild in severity. In the 50–85-year age stratum, incidences of redness and swelling were higher with increasing SA3Ag dose level. Onset of local reactions tended to occur within the first 2 days post-vaccination among participants aged 18–24 years, whereas older participants in the mid- and high-dose-level SA3Ag groups more often reported local reactions with onset between 6 to 8 days post-vaccination.

Frequencies of systemic events reported among the 50–85-year age stratum were generally comparable between the placebo (52.6%) and SA3Ag vaccine groups (44.9–61.0%), with no dose relationship observed (Fig. 6). Frequencies of systemic events reported among participants aged 18–24 years were generally higher than for the older age stratum, but the incidences were again comparable between the placebo and SA3Ag vaccine groups. The majority of systemic events among both age strata were mild in severity, with the duration being generally comparable among dose-level groups.

Proportions of participants reporting AEs were comparable among placebo (52.9%) and the low- (52.0%), mid- (51.5%), and high-dose-level (61.4%) SA3Ag vaccine groups. High-dose-level SA3Ag recipients reported significantly more related AEs (28.7%), according to the investigator's causality assessment than participants in the placebo group (11.8%; $P<0.01$). These largely comprised local reactogenicity events not captured via e-diary, and therefore reported as AEs. Most AEs were mild or moderate in severity. The proportion of participants reporting severe AEs was low overall, and there were no apparent differences among placebo (3.9%) and SA3Ag vaccine groups (4.0–5.0%). None of the SAEs reported during the study were considered related to the investigational vaccine. Two participants, both in the 65–85-year age substratum, reported SAEs up to day 29 after vaccination: a placebo recipient reported pyelonephritis, and a mid-dose-level SA3Ag vaccine recipient with a background history of recurrent elbow bursitis developed cellulitis of the elbow; no causative agent of cellulitis was identified. Twelve participants reported SAEs between day 29 and month 6. All SAEs occurred in the 50–85-year age stratum, with the exception of one SAE reported after day 29 (tonsillitis in a placebo recipient). No deaths occurred during the study.

One high-dose-level SA3Ag vaccine recipient was withdrawn due to an AE considered related to the investigational product (injection-site erythema and swelling on day 2 measuring 14.5 cm and 13 cm, respectively). Swelling resolved by day 7; however, redness took 36 days to completely resolve.

A total of six participants were withdrawn due to unrelated AEs or SAEs: two mid-dose-level SA3Ag vaccine recipients due to non-small-cell lung cancer and chronic obstructive pulmonary disease, respectively, and four placebo recipients.

Laboratory test results were comparable at baseline, day 5, and day 15. Reported laboratory abnormalities were sporadic and generally similar among placebo and SA3Ag vaccine groups. No

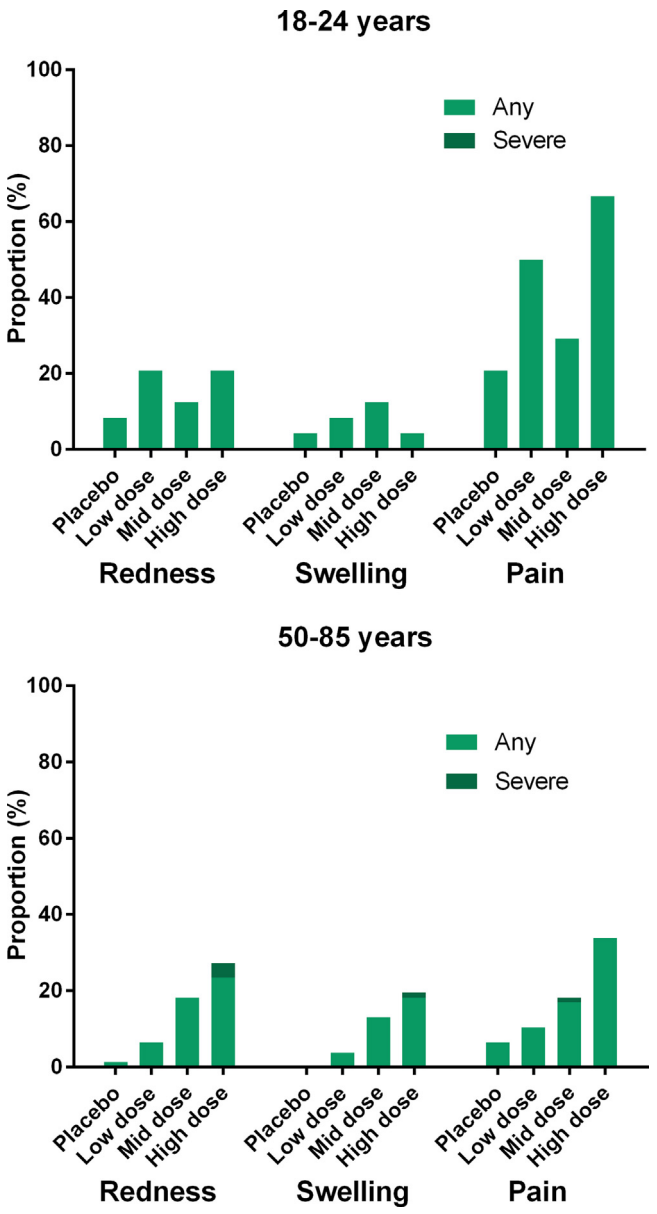

Fig. 5. Local injection-site reactions within 14 days of vaccination.

increase in frequency or severity of laboratory abnormalities with increasing SA3Ag vaccine dose level was identified.

4. Discussion

The development of a safe and efficacious vaccine could reduce the substantial burden of *S. aureus* disease; however, so far, several attempts to do so have been unsuccessful [30]. In contrast to previous vaccine candidates, SA3Ag targets a combination of *S. aureus* virulence mechanisms involved in *S. aureus* pathogenicity. First-in-human study results for this investigational vaccine demonstrate well-characterized functional immune responses to the three antigens. By comparison, previous candidate vaccines have only shown generation of anti-staphylococcal binding antibodies using ELISA [11], and only demonstrated bacterial uptake by phagocytic cells [14], rather than killing of *S. aureus* in OPA assays [14,15]. Both antibody- and cell-mediated mechanisms were demonstrated in protection against recurrent *S. aureus* infection in a murine model of skin and soft tissue infection [31]; however, the importance of bacterial killing following phagocytosis is supported by the observation that opsonophagocytic killing by neutrophils serves as a primary clearance mechanism for *S. aureus* infection, as evidenced by the high incidence of *S. aureus* disease in neutropenic patients and those with genetically-impaired neutrophil function (e.g., leukocyte adhesion deficiency or chronic granulomatous disease that result in defective neutrophil killing of *S. aureus*) [32].

OPA assays that measure the killing of *S. aureus* clinical isolates were, therefore, developed to demonstrate that SA3Ag could elicit antibodies that kill *S. aureus* clinical isolates expressing either CP5 or CP8 [27]. Humans are constantly exposed to *S. aureus* through colonization and in some cases disease. Consequently, most individuals have positive binding antibody responses to many *S. aureus* cellular components [33]. In most cases these responses are not functional but some individuals can exhibit low functional antibody titers to some antigens [34] as observed in our study and as exemplified by the low levels of functional antibodies to capsular polysaccharides that are measured in OPA. After vaccination with SA3Ag, OPA GMTs increased >10-fold at day 29. Although a recent preclinical study hypothesized that CP-specific antibodies are not effective at killing *S. aureus* in the presence of other antibodies that bind to *S. aureus* cell surface components [35], this finding was not observed in our study. Likewise, we sought to demonstrate that rClfA*m* could generate functional antibodies. As the mechanism for ClfA virulence is via binding to host fibrinogen [36], a mAb previously shown to inhibit *S. aureus* binding to fibrinogen [26] was utilized in the ClfA cLIA assay, to determine whether SA3Ag-generated antibodies could compete with this inhibitory mAb. While most participants did not demonstrate ClfA cLIA titers prior to vaccination, substantial responses were observed after SA3Ag vaccination. The existence of background ClfA-binding antibodies due to natural *S. aureus* exposure has been well documented [26,33,34,37,38]. In this study, we demonstrated using an indirect functional assay that humans do not have pre-existing functional titers to ClfA that can inhibit the binding of *S. aureus* to fibrinogen. However, a ClfA containing vaccine can elicit such a functional response after a single dose.

Single-dose administration of this non-adjuvanted SA3Ag vaccine was well tolerated, with an acceptable safety profile at all three dose levels. Local reactions following vaccination were reported more frequently with increasing dose level; however, most were mild or moderate and generally resolved within 3 days. The onset of local reactions was notably different between the two age strata, with reactions generally reported within 2 days in participants aged 18–24 years, and within 6 to 8 days in participants aged 50–85 years. Systemic events following vaccination were comparable among placebo and SA3Ag vaccine groups, the majority of which were mild in severity.

Importantly, acceptable safety and tolerability, and comparably robust immune responses, were seen among older and young adults. The rapid rise to peak antibody titers following SA3Ag vaccination suggests an anamnestic response to the *S. aureus* antigens, possibly as a result of prior natural exposure to the organism. The kinetics of the immune response through at least 3 months following SA3Ag vaccination, targeting a combination of pathogenic mechanisms, give confidence for potential vaccine utility in patients entering a period of high risk of *S. aureus* disease, such as those preparing to utilize healthcare systems [39].

The substantial immune responses, taken in combination with the acceptable safety and tolerability, led to selection of the mid-dose-level SA3Ag vaccine antigens (30 μg CP5-CRM_{197}, 30 μg CP8-CRM_{197}, and 60 μg rClfA*m*) for further clinical development. A 4-antigen vaccine formulation (SA4Ag) currently under development includes the SA3Ag antigens at this selected dose level in

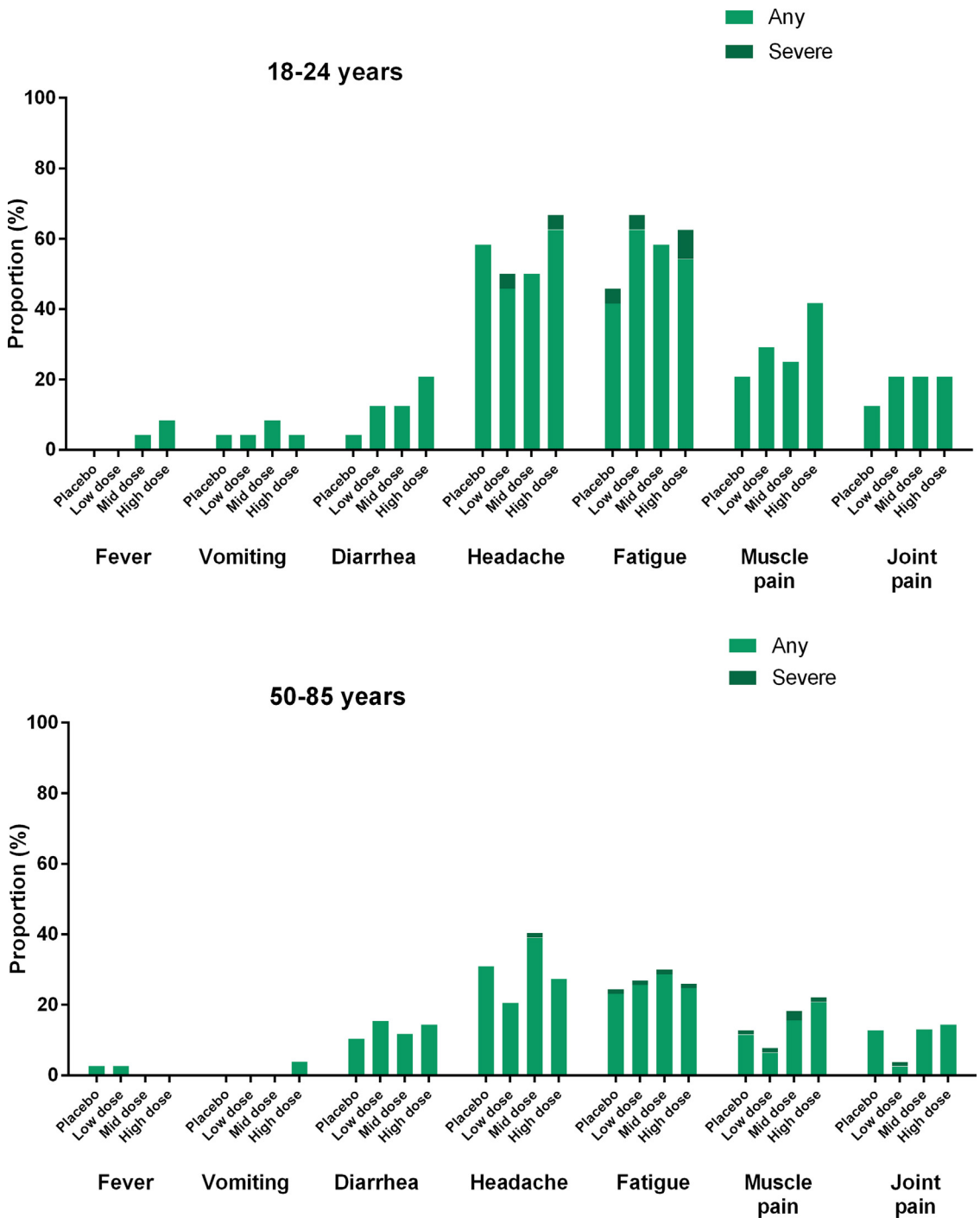

Fig. 6. Participants reporting systemic events within 14 days of vaccination.

combination with an additional antigen, the bacterial manganese transport protein (MntC) [17,40].

Contributors

HM, MN, and PR are study investigators and participated in study design, patient recruitment, acquisition of data, and analysis and interpretation of the findings. SS is a consultant to CMAX, Adelaide, Australia, who provided clinical trial support services, including data acquisition and analysis. QJ provided statistical support. DC, DR, JB, JE, WG, KUJ, EAE, ASA, ETZ, and DG participated in study design and analysis and interpretation of the data. The first draft of the manuscript was written by HM and MN. All authors participated in the development of the manuscript through subsequent stages and approved the final version for journal submission.

Role of the funding source

This study was funded by Pfizer Inc. Employees of the study sponsor, Pfizer Inc., are named as authors on this manuscript and contributed to the design of the study, the collection and interpretation of the data, and the preparation of this manuscript for submission.

Conflict of interest statement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.02.024.

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