Status of vaccine research and development of vaccines for *Staphylococcus aureus*☆

Birgitte K. Giersing [a],[*], Sana S. Dastgheyb [b], Kayvon Modjarrad [c], Vasee Moorthy [a]

[a] *Initiative for Vaccine Research, World Health Organization, CH-1211 Geneva 27, Switzerland*
[b] *Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA*
[c] *Walter Reed Army Institute of Research, Bethesda, USA*

ARTICLE INFO

ABSTRACT

Staphylococcus aureus is a highly versatile gram positive bacterium that is resident as an asymptomatic colonizer on the skin and in the nasopharynx of approximately 30% of individuals. Nasopharyngeal colonization is a risk for acquiring *S. aureus* infections, which can cause a range of clinical symptoms that are commonly associated with skin and soft-tissue infections. The emergence of *S. aureus* strains that are highly resistant to antimicrobials has recently become a major public health concern. In low-income countries the incidence of *S. aureus* disease is highest in neonates and children up to one year of age and mortality rates are estimated to be up to 50%. In the United States, *S. aureus* infection accounts for approximately 300,000 hospitalizations per year. A vaccine against multi-drug resistant *S. aureus*, therefore, is urgently needed. Two vaccine candidates have previously been evaluated in late-stage clinical trials but have not demonstrated efficacy. At present, one vaccine candidate and two monoclonal antibody are undergoing clinical evaluation in target groups at high risk for *S. aureus* infection. This review provides an overview of current vaccine development efforts and presents the major technical and regulatory challenges to developing a licensed *S. aureus* vaccine.

1. About the disease and pathogen

Staphylococcus aureus is a bacterium that is both an asymptomatic colonizer and frequent cause of disease in humans [1]. It is a highly versatile pathogen that causes a range of clinical manifestations of varying severity, and is the most commonly isolated pathogen in the setting of skin and soft-tissue infections, septic arthritis, pneumonia, endovascular infections, osteomyelitis, foreign-body associated infections, septicemia and toxic shock syndrome [2]. *S. aureus* infections strike people of all ages and backgrounds, but are most severe in young children, the elderly, the immunosuppressed and other individuals with major co-morbidities [3,4]. The incidence of *S. aureus* infection in low income countries is highest in neonates and children up to one year of age with mortality rates of up to 50% [5], in contrast to high income countries where the disease appears to increase with age, or is most prevalent at the extremes of the age spectrum. However, there have been very few epidemiological studies in low and middle income countries, and it is likely that there is an under-reporting of *S. aureus*-associated disease generally, and particularly in the elderly in these settings [6,7].

S. aureus is known to be highly adaptable, and in recent history has shown a remarkable epidemiologic transition. Since 1959, when methicillin was first introduced, strains of methicillin-resistant *S. aureus* (MRSA) have been documented at a rapid and increasing rate. Hospital-associated MRSA (HA-MRSA) clones are now recognized to be the leading cause of nosocomial infections in low-, middle-, and high-income countries [5,8,9]. The emergence of community-associated MRSA (CA-MRSA) in the past several decades has also become a point of concern, as virulent strains of CA-MRSA are fast-spreading and can affect seemingly healthy individuals [10]. Vancomycin is currently the first-line treatment for severe CA-MRSA and HA-MRSA infections, however strains with reduced susceptibility to this antimicrobial (vancomycin-intermediate *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA)) have been reported with increasing frequency [11]. Since

* Corresponding author at: World Health Organization, Initiative for Vaccine Research, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland. Tel.: +41 22 791 2816.
E-mail address: giersingb@who.int (B.K. Giersing).

nasal carriage is a well-defined risk factor for infection, decolonisation protocols have had some moderate success in reducing the incidence of infection in specific hospitalized groups. However, clearance is not consistent or long-lived, and this strategy is not applicable to community settings. Efforts to prevent MRSA infection in high-income countries are well-resourced, but risk factors and infection are poorly monitored in low- and middle-income settings, where in some cases over-the-counter antibiotics are available and frequently self-administered [12]. MRSA's status as a global public health threat will only be exacerbated by inappropriate anti-microbial use and will further accelerate the spread of CA-MRSA and other resistant microorganisms.

S. aureus, which is constitutes the normal flora in up to 30% of individuals (either persistently or intermittently), is typically spread *via* close contact from carriers to non-carriers. Transmission has been found to be most efficient within healthcare and athletic facilities [2,13]. In high income countries, target groups for potential *S. aureus* vaccination would be at-risk healthcare workers, the elderly ≥65 years, and the immunocompromised, as well as patients with recurrent invasive staphylococcal infection [3,14]. The global burden and spread of *S. aureus* infection is currently unknown and more data from low- and middle-income countries are needed, but in the US alone, *S. aureus* infection is reported as a discharge diagnosis for around 300,000 hospital stays per year. *S. aureus* infection is also associated with a five-fold increased risk of in-hospital death and three-fold higher cost of hospital stay compared to inpatients without infection [15]. A US study in 2003 estimated that *S. aureus* infections accounted for $14.5 billion in all inpatient hospital stays and $12.3 billion for surgical stays [16].

2. Overview of current efforts

A. Either vaccines currently available and their limitations or biological feasibility for vaccine development

Prior *S. aureus* infection does not provide protection against subsequent infection, but infections among carriers are less severe, suggesting that some form of immunity does develop during prolonged colonization. Although all adults have pre-existing binding antibodies to *S. aureus* antigens, including capsule and clumping factor A (ClfA), these do not typically include functional antibodies that have opsonophagocytic or neutralizing properties, and therefore do not provide protection against infection.

There is precedence for the development of safe and effective bacterial vaccines that target single antigens or toxins, particularly capsular polysaccharides. The most prominent examples are the tetanus toxoid and pneumococcal conjugate vaccines. Application of these technologies to *S. aureus* is complicated by the bacterium's complex mechanisms of pathogenesis. *S. aureus* can comprise the normal human flora and, as such, has evolved a number of strategies to colonize and evade the host immunity, including polymorphic expression of surface antigens and release of multiple redundant virulence factors [17,18]. These include iron acquisition factors such as IsdB, manganese uptake receptors such as MntABC, fibronectin binding proteins (ClfA, ClfB), polysaccharide capsule molecules (CP5 and CP8) and toxic shock syndrome toxin (TSST). To date, vaccine candidates have targeted individual cell surface components, such as the polysaccharide capsule, extracellular polysaccharides or cell wall associated proteins that aid attachment, invasion or act as a receptor (*e.g.*, hemoglobin for iron utilization). Although multiple vaccine candidates have shown promise through pre-clinical development in a range of animal models, those that have reached late stage clinical testing have failed to demonstrate efficacy in human trials [19,29].

B. General approaches to vaccine development for this disease for low and middle income country markets

S. aureus has not been viewed as a high-priority pathogen in low-income countries. However, based on the limited data available, the incidence and mortality from multidrug-resistant *S. aureus* in these regions is likely significant, To date, only two vaccines have completed human efficacy, and neither have contemplated target populations or indications that are prevalent in low- and middle-income countries [20]. StaphVAX is a bivalent polysaccharide- and protein-conjugated vaccine, directed against *S. aureus* capsular polysaccharide types 5 and 8 (CP5 and CP8), which are associated with approximately 80% of *S. aureus* clinical infections. The candidate was evaluated in two phase III studies to prevent bacteremia in end-stage renal dialysis patients in the 3–54 weeks following immunization. In the first 40 weeks, bacteremia was reduced by 57% but efficacy dropped to 26% by week 54 [21]. A confirmatory Phase III study involving 3600 heamodialysis patients who were evaluated for bacteremia showed no difference between vaccinated individuals and the placebo controls. The functional antibody titers induced by the vaccine in this second follow-up phase III study have not yet been made publicly available. Currently, then, the main reason for the second trial's failure is being attributed to manufacturing inconsistencies between different vaccine lots used for the two studies [22]. Development of the candidate has been discontinued.

Another candidate, V710, elicits immunity against the cell-wall anchored iron scavenger protein IsdB, and was evaluated in a Phase III randomized controlled trial in approximately 8000 adults scheduled for cardiac surgery. This trial was terminated when an interim analysis showed a statistically significant increase in mortality rate due to *S. aureus* infection and a significantly higher rate of other adverse events [23].

Passive immunization strategies utilizing both polyclonal and monoclonal antibodies (mAbs) have been targeted for those who are immunocompromised and cannot mount an independent, robust immune response and for those at immediate risk of infection and do not have time to for an active immunization to take effect. Five antibody candidates have been developed and evaluated in late stage clinical studies; none have demonstrated efficacy [24].

The focus has been on development of a prophylactic product that will protect against life-threatening *S. aureus* infections, but it is hoped that such a vaccine would also protect against all *S. aureus* infections including more commonly encountered skin and soft tissue infections. To date, however, no product has been shown to protect against any tested outcome.

3. Technical and regulatory assessment

Active and passive immunization approaches have been based on increasing the concentration of opsonic antibodies to single surface antigens, and all have failed to demonstrate protection. Antigenic variation and the multiple invasion and colonization mechanisms of *S. aureus*, absence of representative preclinical models and lack of immune correlates or surrogates of protection all present significant obstacles to the rational design, development and evaluation of potentially successful vaccine candidates.

Leaders in the field have been speculating about the future directions for *S. aureus* vaccine development, particularly in the aftermath of the failed efficacy trials [25–28]. First and foremost, a multi-antigen approach that targets both cell surface components and secreted virulence factors, and potentially responses against the disease causing toxins, have been hypothesized as essential to the success of a vaccine. Because *S. aureus* causes

a broad range of diseases, preclinical testing should involve the use of multiple models that approximate the different diseases that the pathogen causes in humans. Many candidates have been evaluated in murine models, due to convenience and low cost, and have demonstrated proof-of-concept in terms of preclinical protection through the elicitation of opsonic antibodies. However, these data have not translated into human efficacy [19,29]. In addition to developing a number of representative preclinical models, it is also important to evaluate protection against diverse *S. aureus* clinical isolates, and to assess development of *in vitro* assays that measure functional responses through the induction of both humoral and T-cell mediated responses, particularly toxin neutralization, opsonophagocytic killing by neutrophils, and IL-17 secretion.

The optimal population for clinical proof-of-concept or efficacy studies is challenging to identify. Generally speaking, the more immune compromised the patient, the greater the severity of *S. aureus* infection. However, these individuals are less likely to mount an effective immune response after vaccination. Patient groups who are generally healthy but elect to undergo surgery or some other invasive procedure are typical target populations for efficacy studies, as vaccination can be administered prior to a known period of increased risk (approximately 90 days) and be compared to a placebo. Regardless of the population in which vaccine proof-of-concept is demonstrated, an efficacious vaccine will ultimately be targeted to patients at who are already at high risk of infection and are not in good health at baseline (immunocompromised or with co-morbidities). Prospective studies across a number of elective surgery populations that define the risk, characteristics of surgical procedures, co-morbidities and infections rates are needed to accurately identify the optimal population in which to test *S. aureus* vaccines, as well as to plan how such a vaccine may be most impactful.

Recent analysis of the data from V710 vaccine recipients indicates that the combination of low pre-vaccination IL-2 levels, vaccination with V710, and post-operative *S. aureus* infection were associated with increased mortality following cardiothoracic surgery [30]. Nine of ten V710 recipients with undetectable pre-operative IL17a levels and postoperative *S. aureus* infections died. Investigation into the pathophysiologic mechanism of this outcome is ongoing.

A vaccine that offers sterilizing protection from *S. aureus* may be an overly ambitious target, but would be ideal. The alternative to a vaccine that protects against all clinical *S. aureus* syndromes is to develop vaccines against each or a subset of specific clinical manifestations, however this would be very costly, and certainly challenging for use in low income countries. A more achievable end point may be reduction in the severity of infection, as this may still have a very important impact on mortality.

S. aureus vaccines could also be considered a high priority within the antimicrobial resistance (AMR) agenda together with, for example, Group A Streptococcal, *P. falciparum* malaria and tuberculosis vaccines. However, the AMR research agenda has not yet fully incorporated vaccine R&D perspectives. A discussion about incentivizing vaccine development to address AMR concerns is needed at national and international policy levels.

Table 1
Development status of *Current* vaccine candidates.

Candidate name/Identifier	Developer	Vaccine approach	Pre-clinical	Phase I	Phase II	Status
Active prophylactic vaccines						
PF-06290510/SA4Ag	Phizer	ClfA/MntC/CP5/CP8 conjugated to CRM$_{197}$			X	Safety and Efficacy of SA4Ag Vaccine in Adults Having Elective Posterior Instrumented Lumbar Spinal Fusion Procedure (STRIVE): NCT01827358
					X	SA4Ag Safety, Tolerability, and Immunogenicity Study in Japanese Adults: NCT02492958
GSK2392103A	GSK	CP5/CP8/TT/AT/ClfA plus AS03B		X		No longer under active development. A Study to Evaluate the Safety, Reactogenicity and Immunogenicity of GSK Biologicals' Staphylococcal Investigational Vaccine in Healthy Adults: NCT01160172 [34]
NDV3	NovaDigm Therapeutics	rAls3p-N (C.albicans surface protein that cross reacts with *S. aureus*) plus Alum		X		Under development. Safety and Immunogenicity Study of a Recombinant Protein Vaccine (NDV-3) Against *S. aureus* and Candida: NCT01273922. Clinical development for Vulvovaginal candidiasis (VVC) ongoing: NCT01926028
Glycosylated CP5, CP8, and Hla$_{H35L}$	GSK (Glycovaxyn)		X			[33]
SA75	Vaccine Research International	Whole cell vaccine		X		No longer under active development http://www.vri.org.uk/PhaseITrial.pdf
4C-Staph	Novartis	FhiD2, EsxAB, Hla, Sur-2	X			[35]
SAG	Pan Chai University	*S. aureus* ghosts	X			[36]
various	IBT/NIAID	Multi-valent attenuated toxoid	X			[37]
Passive prophylactic immunization						
MEDI4893	Medimmune	mAb binding to S. aureus toxin			X	Dose-ranging efficacy and safety in mechanically Ventilated Adults: NCT02296320
AR-301	Aridis	mAb			X	Phase I/II Safety, Pharmacokinetics and Efficacy of KBSA301 in Severe Pneumonia (*S. aureus*) as an adjunctive therapy to standard of care antibiotics in hospital-acquired pneumonia (HAP) and ventilator associated pneumonia (VAP) patients: NCT01589185

4. Status of vaccine R&D activities

Following the failure of single antigen vaccine approaches, most development efforts are now focused on multiple antigen approaches. Pfizer's SA4Ag candidate is currently the most advanced and is comprised of four antigens: the adhesion molecule ClfA, the manganese transporter MntC and anti-phagocytic capsular polysaccharides 5 and 8 [31]. This combination is designed to elicit broad humoral and cellular immune responses against multiple virulence mechanisms involved in the establishment and maintenance of infection. Results from a phase I study in which healthy adults of ages 50–85 ($n = 312$) or 18–24 ($n = 96$) years randomly received a one of three dosages of SA4Ag as a single intramuscular injection or placebo showed that the 50- to 85-year age stratum elicited robust immune responses to all component antigens, as well as functional responses as measured by an opsonophagocytic activity assay. The rise in functional antibody titers against *S. aureus* was maintained through at least 12 months. A phase IIb placebo-controlled safety and efficacy study in adults undergoing elective spinal fusion surgery is underway (referred to as STRIVE: **ST**aphylococcus aureus Su**R**gical **I**npatient **V**accine **E**fficacy). SA4Ag was granted Fast Track designation by the U.S. Food and Drug Administration (FDA) in February 2014.

Other multi-antigen preclinical candidates targeting conserved capsular polysaccharide conjugates and/or protein antigens, some including toxins such as alpha toxin, TSST, and SEB are in development (Table 1). In the face of past failures, current efforts are also focused on further characterizing the immunopathology and immunity of *S. aureus* infections to identify new antigenic targets, and developing more representative preclinical [32] models in which opsonising and/or neutralizing immune responses are measured.

Passive immunization with mAbs is an alternative strategy to manage *S. aureus* infection in the context of increasing antimicrobial resistance. Questions about affordability and access of mAb interventions for low- and middle-income countries need to be clarified. As with previous vaccine candidates, clinical trials examining the efficacy of human antibodies have failed to meet their study end points and have been discontinued [19,29]. A new generation of mAbs is now undergoing clinical evaluation. Aridis Pharmaceuticals' candidate AR-301 (Salvecin™) is a fully human monoclonal IgG1 antibody targeting *S. aureus* alpha-toxin that is undergoing evaluation as an adjunctive therapy to standard-of-care antibiotic treatement for hospital-acquired pneumonia (HAP) and ventilator associated pneumonia (VAP) [38]. Medimmune's MEDI4893 long half-life mAb is also targeting the alpha toxin, and is currently in a Phase 2 dose-ranging study to evaluate safety and efficacy in mechanically ventilated adult subjects [39].

5. Likelihood for financing

The relatively well-characterized incidence of *S. aureus* in high-income countries and the market potential for a vaccine has meant that development, particularly through late-stage clinical testing, has to date been advanced by pharmaceutical companies. Basic research into *S. aureus* pathogenesis, development of improved preclinical models and functional assays is being supported by government funding agencies such as the European Commission and the National Institutes of Health. However, there are no efforts to develop a vaccine against *S. aureus* infections in low- and middle-income countries as no market has yet been established there.

Acknowledgements

References

[1] Lowy FD. *Staphylococcus aureus* infections. N Engl J Med 1998;339:520–32.
[2] David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010;23:616–87.
[3] Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 2007;298:1763–71.
[4] Farley JE, Hayat MJ, Sacamano PL, Ross T, Carroll K. Prevalence and risk factors for methicillin-resistant *Staphylococcus aureus* in an HIV-positive cohort. Am J Infect Control 2015;43(4):329–35.
[5] Nickerson EK1, West TE, Day NP, Peacock SJ. *Staphylococcus aureus* disease and drug resistance in resource-limited countries in south and east Asia. Lancet Infect Dis 2009;9(2):130–5.
[6] Nickerson EK, Wuthiekanun V, Wongsuvan G, Limmathurosakul D, Srisamang P, Mahavanakul W, et al. Factors predicting and reducing mortality in patients with invasive *Staphylococcus aureus* disease in a developing country. PLoS ONE 2009;4(8):e6512.
[7] Allegranzi B, Bagheri Nejad S, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet (Lond, Engl) 2011;377:228–41.
[8] Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K, Jernigan JA, et al. Methicillin-resistant *Staphylococcus aureus* disease in three communities. N Engl J Med 2005;352:1436–44.
[9] Carleton HA, Diep BA, Charlebois ED, Sensabaugh GF, Perdreau-Remington F. Community-adapted methicillin-resistant *Staphylococcus aureus* (MRSA): population dynamics of an expanding community reservoir of MRSA. J Infect Dis 2004;190:1730–8.
[10] Kourbatova EV, Halvosa JS, King MD, Ray SM, White N, Blumberg HM. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA 300 clone as a cause of health care-associated infections among patients with prosthetic joint infections. Am J Infect Control 2005;33: 385–91.
[11] Mandal SM, Ghosh AK, Pati BR. Dissemination of antibiotic resistance in methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *S. aureus* strains isolated from hospital effluents. Am J Infect Control 2015. pii:S0196-6553(15)00932-3.
[12] Van Duong D, Binns CW, Van Le T. Availability of antibiotics as over-the-counter drugs in pharmacies: a threat to public health in Vietnam. Trop Med Int Health 1997;2(12):1133–9.
[13] Mostofsky E, Lipsitch M, Regev-Yochay G. Is methicillin-resistant *Staphylococcus aureus* replacing methicillin-susceptible *S. aureus*? J Antimicrob Chemother 2011;66:2199–214.
[14] Lucero CA, Hageman J, Zell ER, Bulens S, Nadle J, Petit S, et al. Evaluating the potential public health impact of a *Staphylococcus aureus* vaccine through use of population-based surveillance for invasive methicillin-resistant *S. aureus* disease in the United States. Vaccine 2009;27:5061–8.
[15] Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Smulders M, et al. The burden of *Staphylococcus aureus* infections on hospitals in the United States: an analysis of the 2000 and 2001 Nationwide Inpatient Sample Database. Arch Intern Med 2005;165:1756–61.
[16] Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Jacobson C, et al. National trends in *Staphylococcus aureus* infection rates: impact on economic burden and mortality over a 6-year period (1998–2003). Clin Infect Dis 2007;45:1132–40.
[17] Golubchik T, Batty EM, Miller RR, Farr H, Young BC, Larner-Svensson H, et al. Within-host evolution of *Staphylococcus aureus* during asymptomatic carriage. PLOS ONE 2013;8(5):e61319.
[18] Dreisbach A, van der Kooi-Pol MM, Otto A, Gronau K, Bonarius HPJ, Westra H, et al. Surface shaving as a versatile tool to profile global interactions between human serum proteins and the *Staphylococcus aureus* cell surface. Proteomics 2011;11(14):2921–30.
[19] Verkaik NJ, van Wamel WJB, van Belkum A. Immunotherapeutic approaches against *Staphylococcus aureus*. Immunotherapy 2011;3(9):1063–73.
[20] Schaffer AC, Lee JC. Vaccination and passive immunisation against *Staphylococcus aureus*. Int J Antimicrob Agents 2008;32(Suppl. 1):S71–8.
[21] Fattom AI, Horwith G, Fuller S, Propst M, Naso R. Development of StaphVAX, a polysaccharide conjugate vaccine against *S. aureus* infection: from the lab bench to phase III clinical trials. Vaccine 2004;22(7):880–7.
[22] Fattom A, Matalon A, Buerkert J, Taylor K, Damaso S, Boutriau D. Efficacy profile of a bivalent *Staphylococcus aureus* glycoconjugated vaccine in adults on hemodialysis: phase III randomized study. Hum Vaccin Immunother 2015;11:632–41.

[23] Fowler VG, Allen KB, Moreira ED, Moustafa M, Isgro F, Boucher HW, et al. Effect of an investigational vaccine for preventing *Staphylococcus aureus* infections after cardiothoracic surgery: a randomized trial. JAMA 2013;309:1368–78.

[24] Fowler Jr VG, Proctor RA. Where does a *Staphylococcus aureus* vaccine stand? Clin Microbiol Infect 2014;20(Suppl. 5):66–75.

[25] Pier GB. Will there ever be a universal *Staphylococcus aureus* vaccine? Hum Vaccin Immunother 2013;9(9):1865–76.

[26] Jansen KU, Girgenti DQ, Scully IL, Anderson AS. Vaccine review: *Staphyloccocus aureus* vaccines: problems and prospects. Vaccine 2013;31(25):2723–30.

[27] Botelho-Nevers E, Verhoeven P, Paul S, Grattard F, Pozzetto B, Berthelot P, et al. Staphylococcal vaccine development: review of past failures and plea for a future evaluation of vaccine efficacy not only on staphylococcal infections but also on mucosal carriage. Expert Rev Vaccines 2013;12(11):1249–59.

[28] Daum RS, Spellberg B. Progress toward a *Staphylococcus aureus* vaccine. Clin Infect Dis 2012;54:560–7.

[29] Salgado-Pabón W, Schlievert PM. Models matter: the search for an effective *Staphylococcus aureus* vaccine. Nat Rev Microbiol 2014;12(8):585–91.

[30] McNeely TB, Shah NA, Fridman A, Joshi A, Hartzel JS, Keshari RS, et al. Mortality among recipients of the Merck V710 *Staphylococcus aureus* vaccine after post-operative *S. aureus* infections: an analysis of possible contributing host factors. Hum Vaccin Immunother 2014;10(12):3513–6.

[31] Anderson AS, Miller AA, Donald RG, Scully IL, Nanra JS, Cooper D, et al. Development of a multicomponent *Staphylococcus aureus* vaccine designed to counter multiple bacterial virulence factors. Hum Vaccin Immunother 2012;8(11):1585–94.

[32] Kim HK, Missiakas D, Schneewind O. Mouse models for infectious diseases caused by *Staphylococcus aureus*. J Immunol Methods 2014;410:88–99.

[33] Wacker M, Wang L, Kowarik M, Dowd M, Lipowsky G, Faridmoayer A, et al. Prevention of *Staphylococcus aureus* infections by glycoprotein vaccines synthesized in *Escherichia coli*. J Infect Dis 2014;209(10):1551–61.

[34] Levy J, Licini L, Haelterman E, Moris P, Lestrate P, Damaso S, et al. Safety and immunogenicity of an investigational 4-component *Staphylococcus aureus* vaccine with or without AS03B adjuvant: results of a randomized phase I trial. Hum Vaccin Immunother 2015;11(3):620–31.

[35] Torre A, Bacconi M, Sammicheli C, Galletti B, Laera D, Fontana MR, et al. Four-component *Staphylococcus aureus* vaccine 4C-staph enhances Fcγ receptor expression in neutrophils and monocytes and mitigates *S. aureus* infection in neutropenic mice. Infect Immun 2015;83(8):3157–63.

[36] Vinod N, Oh S, Park HJ, Koo JM, Choi CW, Kim SC. Generation of a novel *Staphylococcus aureus* ghost vaccine and examination of its immunogenicity against virulent challenge in rats. Infect Immun 2015;83(7):2957–65.

[37] Adhikari RP, Kort T, Shulenin S, Kanipakala T, Ganjbaksh N, Roghmann M-C. Antibodies to *S. aureus* LukS-PV Attenuated subunit vaccine neutralize a broad spectrum of canonical and non-canonical bicomponent leukotoxin pairs. PLOS ONE 2015;10(9):e0137874.

[38] http://www.aridispharma.com/ar301.html.

[39] Hua L, Cohen TS, Shi Y, Datta V, Hilliard JJ, Tkaczyk C, et al. MEDI4893* promotes survival and extends the antibiotic treatment window in a *Staphylococcus aureus* immunocompromised pneumonia model. Antimicrob Agents Chemother 2015;59(8):4526–32.