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TITLE PAGE

Staphylococcus aureus vaccine preclinical and clinical development: current state of the art

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SUMMARY

Staphylococcus aureus is a relevant pathogen both in community and in hospital settings. It is the etiological agent of significant to treat health care related infections due to both its ability to cause invasive infection as well as to form biofilm on biomaterials and high prevalence of resistance to first line antibiotics. The most challenging preventive strategy is the vaccine development to guarantee a full and durable protection from staphylococcal diseases in all different high-risk populations, even if the lack of a known correlate of protection from *S. aureus* is a relevant hindrance to this effort. We aimed to review the most recent advances in the field of vaccinology against *S. aureus*, highlighting the potential for future application of the different experimental vaccine types. Several vaccines have completed their preclinical phase of development and others have been tested in humans, however no successful phase III clinical trial has been completed yet.

KEY WORDS: *Staphylococcus aureus*, vaccine, prevention, immunogenicity, antigens

INTRODUCTION

Staphylococcus aureus is a Gram positive bacterium commonly colonizing humans. It can cause localized and serious invasive infections, as well as a severe septic shock syndrome (Krismer *et al.*, 2017; Que and Moreillon, 2015). Its clinical relevance is also related to its ability to adhere and to form biofilms, mainly on biomaterials (e.g. orthopaedic joint prostheses, artificial heart valves, intravenous devices), causing difficult to treat infections (Figueiredo, 2017; Oliveira *et al.*, 2018). *S. aureus* is one of the most important etiologic agents of post-surgical complications and hospital acquired or health-care associated infections and, moreover, it frequently develops resistance to beta-lactams agents. Prevalence of methicillin resistant *S. aureus* (MRSA) in Europe ranges from < 1% to over 50% and multidrug-resistant isolates have been demonstrated both in the community and in the health care settings (Hassoun *et al.*, 2017; Que and Moreillon, 2015; Reddy *et al.*, 2017; March *et al.*, 2017). The high prevalence of antibiotic resistance makes it difficult to prescribe an effective empiric therapy. Moreover, in sub-chronic infections, bacterial culture may be difficult to obtain: in these cases, molecular diagnostic approaches may be required to improve sensitivity and to achieve a rapid diagnosis (Sambri *et al.*, 2017), failing the goal to switch to a specific therapy after an *in vitro* chemosusceptibility test. Glycopeptides can be considered the cornerstone of antibiotic therapy for MRSA infections and the first-choice in patients with beta-lactams allergy, although resistance to this class is emerging in several countries, and toxicity issues may represent a limitation. Alternative anti-MRSA antimicrobials are available, but resistance to these newer molecules has already been reported in clinical *S. aureus* isolates and it is increasing (Que and Moreillon, 2015; Foster, 2017; Musumeci *et al.*, 2016).

To overcome problems in the clinical management of staphylococcal infections, several newer approaches and their possible application using different preventive or therapeutic strategies are being evaluated (e.g. biocidal nano-molecules, passive immunotherapy) (Oliveira *et al.*, 2018; Siddiqi *et al.*, 2018; Sause *et al.*, 2016). The most challenging preventive strategy is the vaccine development whose objective is to obtain a full and durable protection from staphylococcal diseases in all different populations at risk. The lack of a known correlate of protection from *S. aureus* infection is a major hindrance to vaccine development (Proctor, 2012). Since many years, efforts are therefore ongoing to gain a vaccine candidate, using recombinant or subunit antigens of *S. aureus* or antigens delivering system, with promising results in pre-clinical development (Adhikari *et al.*, 2012; Wacker *et al.*, 2014; Becherelli *et al.*, 2013; Colonna et al., 2013; Veloso *et al.*, 2015, Bagnoli *et al.*, 2015; Delfani *et al.*, 2015).

We aimed to review the most recent advances in the field of vaccinology against *S. aureus*, highlighting the potential for a future application of the different experimental vaccine types.

METHODS

We selected articles from Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/) using the following key words: 'vaccine', 'recombinant antigen', 'vaccination', 'immunization'. Matching each term with '*Staphylococcus aureus*' we found 2,229 articles. We selected review articles (326 results) and further selected those starting from January 2016 up to February 2018, thus obtaining 45 articles. We made a further critical selection based on the content of the abstracts, finally finding 7 reviews really arguing about active immunization against *Staphyloccoccus aureus.* With the same key words and in the same temporal interval, original articles regarding new vaccine approaches and not included in the previous selected reviews, were also selected and analysed. A total of 17 papers were eventually included in our review. Criteria of articles selection are summarized in Figure 1.

In summarizing Tables, original studies reporting preclinical and clinical trials (where available) have been mentioned.

PRECLINICAL STUDIES

About half of the analysed papers describe preclinical phases of *S. aureus* vaccine candidates mainly using the murine model. This is a crucial stage in the development of immunization strategies, because a failure in this phase obviously threatens any further research. GlaxoSmithKline (GSK) company approached active immunization in mice and rabbits using the capsular polysaccharide antigens serotype 5 and 8 (respectively CP5 and CP8), responsible of cellular adhesion, and detoxified α-hemolysin (Hla_{H35L}) that plays a crucial role in invasive infections (Giersing *et al.*, 2016, Reddy *et al.*, 2017). The vaccine was produced by recombinant technology in *Escherichia coli*, obtaining a bioconjugated and N-glycosilated protein (Wacker *et al.*, 2014). Even though elicited antibodies in immunized animals were protective against bacteraemia and pneumonia, there was no further development of this study (Reddy *et al.*, 2017). Nabi biopharmaceutical and Uniformed Services University of the Health Sciences (USUHS) evaluated the PentaStaph vaccine, still based on CP5, CP8 and Hla antigens, with the addition of the toxin Panton Valentine Leukocidin S (LukSPV) and wall teichoic acids (Reddy *et al.*, 2017). The efficacy was evaluated separately for each antigen component and studies seem ongoing regarding the penta-valent formulation: in 2009 PentaStaph was sold to GSK for further possible application (https://www.sec.gov/Archives/edgar/data/72444/000119312509167192/dex992.htm, last accessed February 28, 2018) but no final reporting paper is yet available.

CRM_{197} (a nontoxic recombinant mutant of diphtheria toxin)-conjugated polysaccharide antigens CP5 and CP8 have been recently valuated as vaccine candidates by Cheng *et al.* in a murine model of bacteraemia, lethal sepsis, and skin infection: even if a good antibody response was elicited and active immunization protected against staphylococcal bacteraemia, only CP8-CRM component protected against dermonecrosis and neither CP5-CRM nor CP8-CRM protected against mortality in the sepsis model (Cheng *et al.*, 2017).

A multicomponent surface protein (SdrE, IsdA, SdrD, IsdB) target vaccine was developed by Novartis (now GSK) and revealed a protection from lethal doses of *S. aureus* strains in mice (Reddy *et al.*, 2017). The same company has recently created an alum adjuvated vaccine, named 4C-Staph. It was targeted on four different antigens: the previously described Hla_{H35L} in combination with EsxAB, FhuD2, Csa1A. EsxAB is a fusion of two virulence secreted factors involved in abscess formation, FhuD2 is a lipoprotein involved in iron uptake, while the role of lipoprotein Csa1A is still not clearly understood (Mancini F, *et al.*, 2016; Dayan *et al.*, 2016). The beneficial effects of this quadrivalent vaccine have been shown in a murine model of joints and lung infections, with robust antibody response and CD4+ T lymphocyte activation (Corrado *et al.*, 2016). To date, there is no information about a further development (Reddy *et al.*, 2017; Giersing *et al.*, 2016).

Another potential vaccine *S. aureus* antigen is the surface protein Clamping factor A (ClfA) that allows the adhesion to several human tissues by fibrinogen binding. The successful preclinical study on ClfA opened the way to its application in multiple antigen vaccines, which are in advanced stages of development (Lacey *et al.*, 2016; Dayan *et al.*, 2016). An equally successful preclinical performance was not achieved by a recombinant vaccine (AT62, by National Institute of Allergy and Infectious Diseases, USA) based on the α-hemolysin (Hla) subunit, that showed a weak activity in preventing murine surgical wound infections, despite a robust antibody response. Hla subunit seems nevertheless to be suitable for the development of multivalent vaccines (Adhikari *et al.*, 2016). An interesting immunization target under evaluation, by the Pasture Institute of Iran and Pharmaceutical Sciences Branch of Islamic Azad University, is the Penicillin Binding Protein 2A (PBP2a) that is involved in beta-lactams resistance due to target mutation. Vaccine based on PBP2a reduced mortality rate and protected mice against lethal MRSA challenge (Haghighat *et al.*, 2017). Other possible vaccine candidates are a mutant live *S. aureus*, unable to synthetize cell wall D-alanine (Moscoso *et al.*, 2018) and a bivalent fusion vaccine based on the D domain of staphylococcal protein A (SpA) and the A domain of fibronectin-binding protein A (FnBPA), by the National Natural Science Foundation of China (Yang *et al.*, 2018). Vaccination with the mutant live *S. aureus* resulted in a protective effect against *S. aureus* bacteremia in mice (Moscoso *et al.*, 2018).

The bivalent fusion vaccine showed a protective efficacy in murine pneumonia and skin abscess model (Yang *et al.,* 2018).

CLINICAL STUDIES

Phase I

Despite the efficacy obtained in the preclinical studies, some of the evaluated vaccine candidates did not undergo further development. A composed target vaccine (conjugated to tetanus toxin CP5/CP8 polysaccharides *plus* recombinant Hla/ClfA proteins) was developed by GSK, and it completed the phase I clinical trial (Dayan *et al.,* 2016; Mohamed *et al.,* 2017). This vaccine elicited an increase in functional humoral antibody responses that could kill CP5-expressing strains in opsonophagocytic assays after a single dose, but an inefficient T-cell activation. No safety concerns arouse during this study but this vaccine was not further developed (Levy *et al.,* 2015; Giersing *et al.,* 2016; Reddy *et al.,* 2017). A hypothetically promising immunization strategy was proposed by NovaDigm Therapeutics with the so called NDV3 vaccine. This vaccine consists of an alum adjuvated, recombinant antigen rAls3p-N (agglutinin like sequence 3 protein), a C. *albicans* surface protein that cross reacts with *S. aureus* (Lacey *et al.,* 2016). NDV3 previously demonstrated a preclinical efficacy in reducing murine skin abscesses, so it was carried on phase I, showing safety and immunogenicity (Dayan *et al.,* 2016). NDV3 is currently under study for the prevention of *Candida* vaginitis (Giersing *et al.,* 2016). A cell wall vaccine, SA75 by Vaccine Research International, has shown good tolerability and safety during phase I, but it was not further developed (Giersing *et al.,* 2016). Indeed, preclinical studies on similar types of cell wall vaccines showed controversial results, showing sufficient immunogenicity only after intravenous injection, even if an efficient cellular and humoral response was observed in the murine model of skin and soft tissues infections (Selle *et al.,* 2016, Zhang *et al.,* 2017).

Secreted virulence factors have also been evaluated in phase I trials. Recombinant staphylococcal enterotoxins A and C1 by Integrated BioTherapeutics showed a safe profile (Roetzer *et al.,* 2017). Moreover, Integrated BioTherapeutics, in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), demonstrated a production of functional toxin-neutralizing antibodies in adults after immunization with STEBVax, an alum adjuvated recombinant enterotoxin B (rSEB) (Chen *et al.,* 2016).

The SA4Ag vaccine by Pfizer is composed by four *S. aureus* virulence factors: CP5 and CP8 conjugated with diphteric toxoid *plus* recombinant-mutated ClfA and recombinant-mutated MntC (manganese transporter protein C). A previous use of an SA3Ag vaccine (lacking of MntC) and of SA4Ag showed an acceptable safety for both, but SA4Ag showed a more robust humoral immune

response. (Xu *et al.*, 2018, Esposito *et al.*, 2016; Begier *et al.*, 2017; Creech *et al.*, 2017, Mohamed *et al.*, 2017).

One of the most recent phase I trials was conducted on the bivalent recombinant α-toxin and Panton Valentine Leukocidin vaccine (rAT/r rLukS-PV) produced by Nabi. It was investigated on healthy militaries obtaining positive results in terms of safety and long-term immunogenicity (Landrum *et al.*, 2017).

Phase II

There are no ongoing phase II studies.

Phase II of the previously described NDV3 by NovaDigm Therapeutics was stopped due to enrolment difficulties (Lacey *et al.*, 2016). The use of the previously described recombinant staphylococcal enterotoxins A and C1 by Integrated BioTherapeutics is under evaluation for a phase II trial (Roetzer et al., 2017).

SA4Ag (PF-06290510) is the only candidate tested in an ongoing phase IIb trial: the STRIVE (*STaphylococcus aureus* suRgical InpatientVaccine Efficacy) study aims to confirm the phase I results in a wider target population of adults receiving spinal surgery (Begier *et al.*, 2017, *et al.*, 2016, Mohamed *et al.*, 2017).

Phase III

Two phase III trials testing a purified CP5/CP8 conjugated with recombinant pseudomonal exotoxin A, StaphVax, by Nabi as well as a purified surface protein IsdB, V710 by Merck, were interrupted. The reason was the absence of difference in the primary endpoint between vaccine and placebo for StaphVax and an increased mortality in exposed subjects for V710 (Giersing *et al*., 2016; Dayan *et al.*, 2016; Reddy *et al.*, 2017; Missiakas and Schneewind, 2016; Mohamed *et al*., 2017; Pozzi *et al*., 2017; Lacey *et al.*, 2016). No other clinical phase III trial is ongoing or under evaluation. Possible manufacturing matters causing failure of StaphVax were hypothesized (Fattom *et al.*, 2015; Dayan *et al.* 2016), its capsular polysaccharide antigens are however further being evaluated within the PentaStaph vaccine, as previously described.

CONCLUSIONS

Development of an effective vaccination against *S. aureus* seems to be a relevant priority in terms of prevention at the individual patient level and as a public health measure, with the additional aim to reduce economic impact of these infectious complications.

Despite the plethora of preclinical studies during the last years, clinical trials are still far from approaching a potential application into clinical practice.

The multiple staphylococcal antigens and different pathogenic pathways make it difficult to imagine a single and universal anti *S. aureus* vaccine. Some authors referred to the bacterial complexity the failure of tested vaccine candidates (Lacey *et al.,* 2016; Dayan *et al.* 2016). Vaccines targeting each different type of staphylococcal infection have been proposed as a possible future approach (Lacey *et al.,* 2016).

Differences in staphylococcal pathogenic mechanisms in humans, as compared to those in animal models, could represent another relevant problem to translate results from the preclinical development into the clinical phases. Animals, ad in particular mice, may be a suboptimal model to study staphylococcal infections (Proctor, 2012): "humanized" mice, rabbits and guinea pigs have been proposed to be used as a more reliable animal model (Parker, 2017; Malachowa, 2016; Kim, 2015). Other intriguing and advanced experimental studies explore the potential of reverse vaccinology or immunoproteomics (Holtfreter *et al.,* 2016; Stentzel *et al.,* 2016).

More studies and clinical trials are warranted to reach the objective of an effective and widely employable anti-staphylococcal vaccine.

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CONFLICT OF INTEREST

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