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Novel targeted immunotherapy approaches for staphylococcal infection

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Abstract

Importance to the field—*Staphylococcus aureus* is a leading human pathogen in the hospital and the community. Many *S. aureus* strains are resistant to antibiotics, making treatment of *S. aureus* infections often very complicated. In contrast to many other bacterial pathogens, a working vaccine has never been found for *S. aureus* despite considerable efforts in academia and pharmaceutical companies.

Areas covered in this review—The latest strategies aimed to find a working vaccine against *S. aureus*, including active and passive immunization efforts in pre-clinical and clinical stages, and the molecular reasons for why it may be difficult to develop a vaccine will be discussed.

What the reader will gain—In addition to receiving an overview over current efforts in *S. aureus* vaccine research, the reader will understand that vaccine development for *S. aureus* may be difficult owing to the facts that *S. aureus* is a commensal microorganism and produces toxins that lyse white blood cells, thereby undermining a vaccine's role as a facilitator of opsonophagocytosis.

Take home message—As a result of failed clinical trials with monovalent traditional vaccines, recent development in the field includes a shift toward the potential use of polyvalent formulas, therapeutic antibodies, and more systematic selection of optimal antigens.

Keywords

Staphylococcus aureus; vaccine; antibody; antibiotic; immune evasion; pathogenesis

1. Introduction

Staphylococcus aureus is a leading pathogen that may cause a variety of diseases ranging from moderate to severe skin and soft tissue infections to very serious diseases such as septic shock, toxic shock syndrome, or necrotizing pneumonia¹. Some of these infections may be fatal. *S. aureus* is particularly notorious for causing hospital-associated infections, which are often complicated by the fact that many hospital strains are resistant to antibiotics, most notably methicillin². Methicillin is a penicillinase-resistant derivative of penicillin that was developed in the middle of the last century, because many *S. aureus* strains had acquired penicillinase-based resistance to penicillin^{3–4}. However, methicillin-resistant *S. aureus* (MRSA) strains occurred rapidly⁵. Ever since, MRSA has been a serious threat for hospitalized patients and immune-compromised or otherwise predisposed individuals. By now, it is so widespread that in many countries, methicillin is not regarded as an antibiotic of choice for *S. aureus* infections anymore^{6–8}. In addition, strains of MRSA have emerged recently that combine resistance to methicillin with exceptional virulence and capacity to infect healthy individuals outside

hospital settings⁹. These strains, so-called community-associated (CA-) MRSA, have spread sustainably in the population. In an astoundingly short time, one specific clone of CA-MRSA called USA300 became the by far most frequent source of skin and soft tissue infections reporting to U. S. emergency rooms¹⁰ and is also beginning to spread all over the globe^{6, 11-12}. Of note, it was estimated that MRSA causes more deaths annually in the U.S. than AIDS¹³, making MRSA one of the most important problems for the public health system.

Treatment of MRSA infections is further complicated by non-specific antibiotic resistance mechanisms, such as biofilm formation on indwelling medical devices¹⁴, and specific resistance to a wide variety of antibiotics⁹. While multi-drug resistance has so far been observed mainly among hospital-associated MRSA, it is now also found among CA-MRSA¹⁵. Outbreaks of infections with multi-drug-resistant CA-MRSA have been endemic up to now, but these strains are believed to spread further¹⁵. Successful treatment of infections caused by multi-drug resistant *S. aureus* is only possible with a few antibiotics, such as vancomycin and linezolid. In addition, this difficult situation is aggravated by the fact that not many companies are investing in the development of novel antibiotics. This is quite understandable from a marketing point of view given the high costs and lack of success in antibiotic discovery. Furthermore, antibiotic overuse, which leads to the spread of resistant strains, is common in many countries. However, there are a few noticeable exceptions with low MRSA rates, such as Scandinavian countries¹⁶⁻¹⁷. France, which has an especially high MRSA rate, recently started programs to limit the wide prescription of antibiotics¹⁸, but it will probably take a while until this will result in a re-emergence of antibiotic susceptible strains.

As a consequence of the problems related to antibiotic resistance and the development of new antibiotics, researchers have recently intensified the search for alternatives particularly in the field of vaccines and therapeutic antibodies. Companies have joined this quest for *S. aureus* vaccines, mostly because the development of vaccines or antibodies is deemed easier and less costly than that of novel antibiotics. While attempts to develop an *S. aureus* vaccine date back almost a century, they have never been successful. Here, I will present current clinical and pre-clinical efforts in the field of anti-staphylococcal immunotherapy. Furthermore, I will discuss how recent findings in staphylococcal pathogenesis research may help explain why finding a traditional vaccine against *S. aureus* may be difficult and more refined novel immunotherapy approaches may be needed to combat this important human pathogen.

2. *S. aureus* vaccines

Notwithstanding the potential difficulties to find a working vaccine against *S. aureus* infections, one first has to consider who would receive such a vaccine. There are certainly several populations that are at a very high risk for *S. aureus* infections and who might thus benefit from vaccination, such as dialysis patients or patients at risk of endocarditis. One may also extend vaccination to all patients with risk of HA-MRSA infection, such as those undergoing surgery, suffering from chronic illnesses, and experiencing prolonged stays in hospitals. Furthermore, vaccination of health care personnel as potential sources of HA-MRSA may prevent distribution of these strains in the hospitals and to patients. Finally, vaccination of larger subsets of the population may reduce infection rates by reducing colonization, and may be indicated for example in groups in which outbreaks of CA-MRSA were observed, such as sport teams or prison inmates.

In contrast to other bacterial and viral pathogens, a simple vaccine based on heat-killed or otherwise attenuated bacteria has never worked for *S. aureus*. This may in part be due to the fact that *S. aureus* is a permanent or transient colonizer at least in part of the population¹⁹⁻²⁰. The lack of a strong natural immune response to *S. aureus* may thus be due to the permanent inflammation that such a response would cause. Accordingly, there is no protective memory

against *S. aureus* infection. Evidence that pre-existing antibodies protect from *S. aureus* disease is only available for toxins such as toxic shock syndrome toxin (TSST) that occur but in a low percentage of *S. aureus* strains²¹⁻²². In contrast, circulating antibodies against core genome-encoded *S. aureus* proteins, such as alpha-toxin, do not appear to prevent from infection²³. Hence, current immunotherapy approaches undertaken to prevent or treat *S. aureus* infection primarily aim to boost the immune system by increasing the titer of pre-existing antibodies. In order to specifically combat virulent strains, active and passive immunization efforts in clinical trials or pre-clinical investigation are often targeted at molecules involved in pathogenesis. Recent advances in our understanding of *S. aureus* pathogenesis thus also caused a recent surge in novel immunotherapy approaches against *S. aureus*. Notably, owing to the facts that (i) *S. aureus* is a human commensal, (ii) there is host specificity among staphylococcal strains²⁴, and (iii) the interaction of the human immune system with *S. aureus* often differs from that in test animals²⁵, the predictive power obtained from animal experiments for vaccine development against *S. aureus* is limited. Despite the clear necessity for in-depth pre-clinical tests, valuable information about the efficacy of an *S. aureus* vaccine candidate is thus often only obtained in clinical trials.

2.1 Active immunization

2.1.1 Current and finished clinical vaccine trials using active immunization—

Several active immunization approaches to develop *S. aureus* vaccines against a variety of different antigens have been undertaken during the last decade and at least one is still in progress (Table 1). Except for during the development of the Merck V710 vaccine, these approaches have not used a specific selection process for the best antigen, but based their selection on information obtained from the literature on criteria such as surface exposition, involvement in pathogenesis, or distribution in virulent strains.

The Merck V710 vaccine is based on a study in which *S. aureus* peptide libraries were screened with patient sera and the surface protein IsdB was selected as antigen²⁶⁻²⁷. IsdB is involved in heme acquisition and thus believed to play a major role in the organism's need for iron uptake²⁸. Pre-clinical studies by Merck indicated high immunogenicity of IsdB and showed that an anti-IsdB vaccine is protective in animal infection studies against most *S. aureus* strains tested²⁷. Phase 2 clinical trials in patients undergoing cardiothoracic surgery and hemodialysis patients are underway.

StaphVax developed by Nabi is a vaccine against the type 5 and 8 *S. aureus* capsular polysaccharides. The selection of capsular polysaccharides in general was based on the promising results from other bacterial pathogens, in which capsules proved valuable vaccine targets²⁹. Types 5 and 8 were selected as these are widely distributed in the most prominent clinical strains of *S. aureus*³⁰. Pre-clinical data were promising, as passive immunization against type 5 capsular polysaccharide protected from lethality due to *S. aureus* infection and dissemination in a mouse model of bacteremia³¹, and from *S. aureus* infection in a rat device-associated infection model³². A first phase 3 trial in hemodialysis patients between 1998 and 2000 failed as there was no significant protection after 54 weeks, the chosen endpoint of the study. However, significant protection was achieved at earlier time points³³. In a second trial, again no significant protection was achieved, after which Nabi stopped further development of the StaphVax vaccine. It has been suggested that the failure of a capsular polysaccharide-targeted vaccine in *S. aureus* – in contrast to the success with similar vaccines in *Haemophilus influenzae* and *Streptococcus pneumoniae* – stems from the fact that the contribution of capsular polysaccharides to virulence of *S. aureus* was found to be minor^{30, 34}.

2.1.2 Vaccines in pre-clinical development using active immunization

2.1.2.1 Alpha-toxin: The pore-forming toxin alpha-toxin is a potent cytolysin for many eukaryotic cells³⁵. It also interferes with adhesion³⁶. Notably, alpha-toxin is encoded on the *S. aureus* core genome and thus produced in a majority of *S. aureus* strains. Wide distribution and significant contribution to *S. aureus* virulence³⁷⁻⁴⁰ make alpha-toxin a promising vaccine target for a broadly efficacious *S. aureus* vaccine. Earlier studies using alpha-toxin as vaccine target were not very successful. A phase 3 clinical trial using alpha-toxin and whole killed *S. aureus* as antigens did not show efficacy in preventing infection in dialysis patients⁴¹. In another study, immunization with an alpha-toxin-based toxoid protected rabbits from *S. aureus* mastitis, but did not prevent abscess formation⁴². However, more recent studies appear more promising. Particularly the production of a non-toxic and non-hemolytic variant of alpha-toxin, H35L, by site-directed mutagenesis has proven extremely valuable for vaccine development⁴³. Bubeck Wardenburg and Schneewind used H35L for active immunization in a mouse lung infection model and reduced lethality with *S. aureus* was observed⁴⁴.

2.1.2.2 Panton-Valentine leukocidin (PVL): The CA-MRSA epidemic has put the Panton-Valentine leukocidin (PVL), a toxin that forms pores in human leukocytes⁴⁵, in the focus of attention of pathogenesis and vaccine research⁴⁶. The epidemiological correlation of PVL gene presence with CA-MRSA infections suggested an important role of this toxin in CA-MRSA pathogenesis⁴⁷⁻⁴⁸. However, increasing numbers of virulent CA-MRSA strains lacking PVL have been found in the meantime^{6, 49-52} and studies evaluating PVL's contribution in animal infection models are highly controversial^{37, 46, 53-55}. Failure to find a considerable effect of PVL to pathogenicity of CA-MRSA in mouse infection models may be due to the relative insensitivity of mouse neutrophils to PVL⁵⁶. The role of PVL in CA-MRSA pathogenesis thus requires further evaluation. Notably, PVL production among MRSA strains is rare and virtually limited to CA-MRSA strains⁶. The use of a PVL vaccine would thus lack broad applicability and not be appropriate to treat hospital-associated infections.

Studies evaluating the protective efficacy of PVL as a vaccine are as controversial as those that analyzed its contribution to pathogenesis. Bubeck Wardenburg et al. found no protective effect of PVL against the most widely distributed CA-MRSA strain USA300 in a mouse lung infection model, in contrast to a strong protective effect by alpha-toxin immunization in the same model⁴⁴, while Brown et al. reported significant protection in a similar lung infection study⁵³.

2.1.2.3 PentaStaph (Nabi): After the failure of StaphVax in phase 3 clinical trials, Nabi decided to include further antigens in its vaccine, which is now called PentaStaph, owing to the 5 components included in the formula. This vaccine contains wall teichoic acid and non-toxic variants of alpha-toxin and PVL in addition to the original type 5 and 8 capsular polysaccharides. This vaccine is still in pre-clinical development, but enrollment in a clinical trial to test the novel tri-toxoid part of the vaccine has begun. A similar combination vaccine based on type 5 and type 8 capsules in addition to clumping factor A is being developed by Pfizer.

2.1.2.4 Multi-component adhesin vaccine: Stranger-Jones et al. recently systematically evaluated surface-anchored *S. aureus* proteins for their vaccine potential⁵⁷. Surface proteins are deemed valuable antigens due to their surface exposure, covalent linkage to the staphylococcal cell wall, and significant contribution to adhesion as a primary step in staphylococcal pathogenesis. The authors included 19 proteins that were known by sequence analysis to be cell-wall anchored as they contained the motifs for sortase-catalyzed anchoring to the staphylococcal cell wall⁵⁸. After analyzing initial data on the bacterial burden in kidneys in a murine infection model, in which mice were challenged with *S. aureus* strain Newman,

the authors selected 4 surface proteins (SdrE, IsdA, SdrD, and IsdB) for immunization. The multi-component surface protein vaccine protected from *S. aureus* lethality in 4 of 5 *S. aureus* strains. Of note, the authors also showed that the multi-component vaccine was superior in preventing lethality when compared to vaccines that contained only one of the 4 antigens⁵⁷. This is interesting particularly as the multi-component vaccine also contained IsdB, which is the sole antigen in Merck's V710 vaccine.

2.1.2.5 Poly-N-acetylglucosamine (PNAG): Poly-N-acetylglucosamine (PNAG, also called PIA, polysaccharide intercellular adhesin) is a component of the extracellular biofilm matrix in many staphylococcal strains and also found in several other bacteria¹⁴. It is generally believed to be crucial for staphylococcal biofilm formation, although strains of *S. aureus* and *S. epidermidis* have been detected that may form biofilms without PNAG⁵⁹. In addition, PNAG protects from neutrophil phagocytosis and antimicrobial peptides, thus contributing to immune evasion⁶⁰. PNAG has been evaluated as a potential vaccine candidate for some time⁶¹. More recent studies emphasized the importance of PNAG de-acetylation for immunogenicity and protection in a murine bacteremia model⁶². Naturally occurring PNAG is partially de-acetylated enzymatically⁶³, a modification crucial for surface retention of the polymer and its function in pathogenesis.

2.1.2.6 Als3p (Novadigm): The N-terminus of the candidal adhesion protein Als3p has structural resemblance to the *S. aureus* surface protein clumping factor A (ClfA), an *S. aureus* MSCRAMM (short for microbial surface component recognizing adhesive matrix molecule) also targeted by passive immunization strategies (see below). An Als3p vaccine protected mice from lethal challenge with *S. aureus*⁶⁴. Als3p is being investigated by Novadigm Therapeutics as a potential *S. aureus* vaccine.

2.1.2.7 Iron-regulated proteins (Syntiron/Sanofi Pasteur): Syntiron has patented a technology (SRP®) that is based on a multivalent vaccine targeting iron-regulated bacterial proteins. Iron acquisition is essential for all bacterial pathogens (except *Borrelia burgdorferi*⁶⁵). The Syntiron approach assumes that proteins responsible for iron acquisition are under iron-dependent regulation in *S. aureus*, which has received scientific support by recent studies⁶⁶⁻⁶⁷. Hypothetically, the multivalent vaccine would target all bacterial iron acquisition systems, thus making it impossible for the bacteria to circumvent opsonization. So far no data of an *S. aureus* infection model using the technology have been published. Of note, IsdB, the vaccine target of the Merck V710 vaccine, also is an iron-regulated protein.

2.2 Passive immunization/therapeutic antibodies

Lack of success with active immunization strategies against *S. aureus* has led to a recent shift in *S. aureus* immunotherapy research toward (re-)consideration of passive immunization. These efforts benefit from recent advances in *S. aureus* pathogenesis research. Most are aimed to eliminate major *S. aureus* virulence determinants such as toxins (Table 2).

2.2.1 Passive immunization strategies in clinical trials

2.2.1.1 Altastaph: Altastaph is a polyclonal antibody derived from sera obtained from individuals treated with Nabi's StaphVax, containing antibodies against capsular polysaccharides of types 5 and 8. Two clinical phase 2 trials with AltaStaph failed, one investigating bacteremia in neonates⁶⁸ and one death in children and adults with documented bacteremia⁶⁹. Thus, both active and passive immunization efforts based on staphylococcal capsular polysaccharides failed, indicating that approaches using this antigen - at least alone - are insufficient to provide protection against *S. aureus* infection.

2.2.1.2 Clumping factor (ClfA) targeted antibodies: Clumping factor A (ClfA) is a staphylococcal surface protein involved in binding to fibrinogen⁷⁰. It promotes attachment to various host tissue and cell types as well as biomaterials⁷¹. Of note, ClfA is one of the sortase-anchored surface proteins analyzed in the Stranger-Jones et al study that selected for efficacy in active immunization⁵⁷. However, ClfA was not among those with highest efficacy.

Pre-clinical studies had suggested that anti-Clf A antibodies have potential to protect from *S. aureus* infection⁷²⁻⁷³ and on that basis, were selected by Inhibitex as candidates for passive immunization efforts. This company developed Veronate, a pooled human antibody preparation from donors with high titers against fibrinogen- and fibrin-binding adhesins (*S. aureus* ClfA and *S. epidermidis* SdrG), in an attempt to develop a product with efficacy against *S. epidermidis* in addition to *S. aureus*. While positive trends were observed in a phase 2 clinical trial⁷⁴, a phase 3 clinical trial that analyzed differences in late onset sepsis in *S. aureus* and *S. epidermidis* bacteremia in neonates failed⁷⁵.

The same company also developed tefibazumab (Aurexis), a monoclonal antibody against ClfA. In a rabbit endocarditis model, tefibazumab given in combination with vancomycin resulted in fewer animals with bacteremia and a smaller bacterial load than in animals given vancomycin alone⁷⁶. Importantly, these conditions reflect those often encountered in patients with multi-drug resistant *S. aureus* that can only be treated with an antibiotic of last resort such as vancomycin. The additional antibody would thus be meant to assist the immune system and the antibiotic in clearing the infection. However, a phase 2 clinical study in bacteremia patients, also giving tefibazumab in addition to standard therapy, failed to achieve statistically significant differences in a composite clinical endpoint⁷⁷. Inhibitex then halted further development of the drug.

2.2.1.3 Aurograb: Aurograb is an antibody fragment against an *S. aureus* ABC transporter with unknown function that proved immunogenic when *S. aureus* extracts were tested with human sera⁷⁸. The further development of this antibody, first initiated by NeuTec Pharma and put in clinical trials in Europe, was stopped after Novartis had acquired NeuTec and reported disappointing results in clinical studies.

2.2.1.4 Pagibaximab: Lipoteichoic acid (LTA) is an anionic surface polymer characteristic of Gram-positive bacteria. It has been reported to have significant pro-inflammatory activity⁷⁹⁻⁸⁰, although it is controversial whether these activities are attributable to LTA rather than lipopeptide impurities⁸¹. LTA has also been implicated in staphylococcal biofilm formation⁸². Biosynexus developed an anti-LTA humanized mouse chimeric antibody called Pagibaximab designed to prevent neonatal bloodstream infections. These infections are often caused by coagulase-negative staphylococci in addition to *S. aureus*, in both of which LTA is present and believed to contribute to pathogenesis. Published results of a phase 1/2 clinical trial involving low birth-weight neonates focused on pharmacokinetic behavior and safety of the drug, while not addressing the efficacy to protect from *S. aureus* infection⁸³.

2.2.2 Passive immunization strategies in pre-clinical development

2.2.2.1 Alpha toxin: In addition to approaches using alpha-toxin for active immunization, anti-alpha-toxin antibodies have been used successfully to protect from experimental *S. aureus* pneumonia, caused by both MRSA and methicillin-susceptible strains⁴⁴. Recently, monoclonal alpha-toxin antibodies were developed that have high potential to protect from this type of *S. aureus* infection⁸⁴. The efficacy of anti-alpha toxin antibodies in other manifestations of *S. aureus* disease remains to be evaluated.

2.2.2.2 PVL: The potential use of anti-PVL antibodies against infection by CA-MRSA is similarly controversial as the contribution of PVL to CA-MRSA pathogenesis and PVL's use as an antigen for active immunization. In the only study published to date directly evaluating the efficacy of anti-PVL antibodies, Bubeck Wardenburg and Schneewind reported no statistically significant difference in CA-MRSA induced pneumonia in mice when anti-PVL antibodies were given⁴⁴, compared to the control. Notably, in the same model, anti-alpha toxin antibodies were highly protective.

2.2.2.3 Superantigens: Superantigens potently stimulate T cells without the need of cognate antigen exposure, leading to massive inflammation, such as during toxic shock syndrome caused by TSST⁸⁵. Further superantigens of *S. aureus* include more than a dozen enterotoxins, including enterotoxin B (SEB). Antibodies to SEB prevented severe symptoms and death in monkeys⁸⁶. Active immunization using a non-toxic SEB derivative had similar effects⁸⁷, indicating the potential of such antibodies or vaccines mainly to protect from aerolized superantigens that may be used in biological warfare. However, the distribution of superantigens in *S. aureus* varies widely and is limited to a subset of strains, making such approaches hardly appropriate for treatment of *S. aureus* infection in the hospital or community, unless patients are rapidly diagnosed with TSST or enterotoxin-caused disease. Antibodies to superantigens are developed by Integrated BioTherapeutics. A recombinant SEB vaccine has entered phase 1 clinical trials. Other vaccines developed by that company target enterotoxins A and C1 and TSST.

2.2.2.4 Targeting virulence regulators: Targeting global virulence regulators is a frequently discussed way to possibly eliminate the production of a series of virulence factors at a time, without the need to separately block the production of every single regulated component⁸⁸. The most intensively studied and arguably most pivotal virulence regulator in *S. aureus* is the accessory gene regulator Agr⁸⁹. This system controls virtually all secreted *S. aureus* toxins as well as a series of degradative exoenzymes. Furthermore, Agr regulates adhesins that facilitate the establishment of an infection by ensuring adhesion to host tissues. However, the latter are usually regulated by Agr in a fashion contrasting Agr's control of toxins. Therefore, it has been stressed that blocking Agr – while eliminating toxin production – would increase tissue adhesion. The use of Agr blockers may thus be limited to certain types or stages of *S. aureus* infection⁹⁰. Furthermore, interfering with Agr function was demonstrated to increase staphylococcal biofilm formation in vitro and in vivo⁹¹⁻⁹². Nevertheless, Agr blockers may be of benefit owing to their dramatic impact on toxin production as the most important contributors to acute *S. aureus* infection.

Methods to interfere with Agr function are mainly based on the so-called autoinducing peptides (AIPs), the extracellular signals of Agr⁹³. Altering the structure of these molecules led to derivatives that block Agr function in many *S. aureus* strains⁹⁴. More recently, antibodies that eliminate AIP function were developed⁹⁵. These antibodies have the advantage of higher stability compared to the AIP derivatives, which require a labile thiolactone structure for biological activity. However, so far only an antibody that reacts with one of the many structurally different AIPs has been produced. This antibody protected mice from abscess formation and death after a challenge with an *S. aureus* strain of the same Agr subgroup⁹⁵. A broadly reactive antibody eliminating all AIPs or a cocktail of antibodies with different AIP specificity would be required in a broadly efficacious formula targeting all *S. aureus* strains⁹⁶.

2.2.2.5 Heteropolymers: Elusys Therapeutics developed a bi-component antibody consisting of an anti-protein A monoclonal antibody and a monoclonal antibody to human complement receptor 1 (CR1)⁹⁷⁻⁹⁸. The bi-component antibody is meant to bridge *S. aureus* (via protein A) and bind to CR1, after which the complex would be eliminated by macrophages⁹⁹.

Pretreatment or administration of the antibody to infected mice showed promising results, as it proved protective or lowered the bacterial burden, respectively³⁴. This heteropolymer approach is further pursued by Elusys and Pfizer.

2.3 Colonization

Many people are permanent or transient carriers of *S. aureus*, with nasal colonization playing a predominant role¹⁰⁰. Furthermore, most *S. aureus* infections originate from strains carried by the patient¹⁰¹. It has thus been suggested to eliminate *S. aureus* in carriers to prevent infection. In addition to the antibiotic mupirocin often evaluated for that purpose with contradictory results¹⁰², antibodies to the surface proteins ClfB, IsdA, and IsdH have been studied for the prevention of *S. aureus* nasal colonization¹⁰³⁻¹⁰⁴. Immunization with any of these three proteins resulted in reduced colonization in mouse or cotton rat colonization models. However, decolonization strategies and their experimental evaluation are often criticized, because colonization models hardly predict the dynamics in a large human population in which re-colonization from other individuals may occur rapidly.

3. Expert opinion

It is evident that the development of novel immuno-therapeutics against *S. aureus* infections will benefit significantly from intensified basic research efforts in selecting the best targets for active and passive immunization. Already there is a clear trend toward more sophisticated selection strategies. In the case of *S. aureus* surface proteins, it is remarkable that the selective screen in the Stranger-Jones et al. study⁵⁷ did not identify ClfA, a previously widely investigated antigen, as an optimal target. We can expect to learn much from such screens for future vaccine development.

Attempts to develop vaccines based on active immunization are underway and will hopefully ultimately be successful. However, we know that many such attempts have failed in the past. Traditional vaccines that work by active immunization enhance bacterial uptake into phagocytes by opsonization using bacteria-specific antibodies in concert with serum complement. The antibody-scavenging and B cell-depleting function of protein A¹⁰⁵⁻¹⁰⁶, which is present in many *S. aureus* strains, may inhibit this process. In addition, the many mechanisms by which *S. aureus* can evade elimination by phagocytes after ingestion may help to explain why *S. aureus* vaccines have failed and anti-staphylococcal antibodies present in many people are not protective¹⁰⁷. These mechanisms include scavenging or elimination of reactive oxygen species and other bactericidal components produced by phagocytes¹⁰⁸. More importantly perhaps, many *S. aureus* strains produce leukocidins such as PVL or phenol-soluble modulins that lyse white blood cells^{45, 109}. Together, these mechanisms may allow bacterial escape largely independent of a vaccine's efficacy to promote opsonic uptake. Nevertheless, these considerations should not make us give up the important endeavor to search for a working *S. aureus* vaccine. Notably, in addition to its use for acute infection, such a vaccine would have extreme potential in preventing the otherwise difficult-to-tackle problem of biofilm formation on indwelling medical devices.

As an alternative, passive immunization to directly block *S. aureus* virulence by sequestering secreted virulence factors does not depend on opsonization and would thus circumvent the potential problems of *S. aureus*-triggered lysis of phagocytes. Basic research has helped much in endeavors aimed to select targets for passive immunization. However, given the functional redundancy of *S. aureus* toxins and other virulence determinants, a working antibody preparation would likely have to combine antibodies against several different virulence determinants. As such a cocktail may only contain antibodies to a limited number of antigens, ranking *S. aureus* virulence factors for their relative contribution to *S. aureus* pathogenicity represents a crucial task for *S. aureus* pathogenesis research. Such research should best

investigate the virulence determinants in question simultaneously in at least one clinically important strain such as USA300, to be able to directly compare their relative contribution to virulence.

Article highlights

- Current strategies for *S. aureus* vaccine development in clinical and pre-clinical stages are focused on antigens with a role in *S. aureus* pathogenesis.
- Lessons from pathogenesis research point to the use of surface proteins and toxins as promising targets for vaccine development.
- Possible reasons for the failure of traditional, active immunization strategies include immune evasion mechanisms of *S. aureus*, for example the killing of phagocytes by leukolytic toxins.
- In contrast to previous approaches, the more recent systematic selection of antigens attempts to select those antigens with the highest vaccine potential in pre-clinical experiments.
- The use of therapeutic antibodies represents a novel, alternative strategy to specifically target toxins with a demonstrated role in *S. aureus* virulence.

Acknowledgments

Declaration of interest

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Table 1*S. aureus* vaccine strategies (active immunization)

Target	Name	Company	Status	Remarks
<i>Single target</i>				
IsdB (surface protein)	V710	Merck	Phase 2	
Capsular polysaccharides types 5 and 8	Staph Vax	Nabi	Phase 3 failed.	
Alpha-toxin (non-toxic derivative H35L)			Pre-clinical (reduced lethality in mouse lung infection)	
Panton-Valentine leukocidin (PVL)			Pre-clinical (controversial results on efficacy in mouse lung infection)	for PVL-positive CA-MRSA
PNAG (PIA)			Pre-clinical (protection in murine bacteremia)	
Enterotoxin B (SEB)		Integrated Biotherapeutics	Phase 1 (protects monkeys from infection by SEB-positive strain)	possible anti-biological warfare drug
Enterotoxins A and C1, TSST		Integrated Biotherapeutics	Pre-clinical	
<i>Composite targets</i>				
Capsular polysaccharides types 5 and 8, non-toxic derivatives of alpha-toxin and PVL, wall teichoic acids	Penta Staph	Nabi	Pre-clinical	
Capsular polysaccharides types 5 and 8, ClfA		Wyeth/Pfizer	Phase 1	
Multi-component surface proteins (SdrE, IsdA, SdrD, IsdB)		Novartis	Pre-clinical (protects from lethality in mouse infection model)	Systematic antigen selection process
Iron-regulated proteins		Syntiron	Pre-clinical	
Candidal adhesion protein fragment Als3p		Novadigm	Structural similarity to ClfA	

Table 2Therapeutic antibodies/antisera against *S. aureus* infection (Passive immunization)

Target	Name	Company	Status	Remarks
<i>Single target</i>				
Capsular polysaccharides types 5 and 8	AltaStaph	Nabi	Phase 2 failed	Polyclonal serum from individuals treated with StaphVax
ClfA (surface protein)	Aurexis	Inhibitex	Phase 2 failed	mAb
ABC transporter	Aurograb	NeuTec/Novartis	Development stopped.	Ab fragment
Lipoteichoic acid	Pagimaximab	Biosynexus	Phase 2 finished	Humanized mouse chimeric Ab
Alpha-toxin (non-toxic derivative H35L)			Pre-clinical (protective in mouse lung infection)	Polyclonal Ab, mAb
PVL			Pre-clinical (no protection in mouse lung infection)	Polyclonal
Enterotoxin B (SEB)			Pre-clinical (protects monkeys from infection by SEB-positive strain)	possible anti-biological warfare drug
Agr AIP 4			Pre-clinical (protects mice from abscess formation, death)	Specific for <i>S. aureus</i> Agr subgroup 4
Protein A		Elusys/Pfizer		Heteropolymeric Ab against protein A and human CR1
<i>Composite targets</i>				
ClfA, SdrG	Veronate	Inhibitex	Phase 3 failed	Serum from donors with high titers against ClfA and SdrG